Microglia: sculptors of the polycystic ovary syndrome (PCOS)-like brain?

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Abstract

In brief: Neuroendocrine dysfunction and transgenerational susceptibility associated with polycystic ovary syndrome (PCOS) suggest that programmed changes within the brain contribute to adult development of the syndrome. This review discusses a potentially important role for microglia in mediating prenatal androgen-programmed changes in the female brain that contribute to PCOS-like features.

Abstract: Several lines of evidence support a role for the brain in both the development and maintenance of polycystic ovary syndrome (PCOS), the most common cause of anovulatory infertility worldwide. Persistently elevated luteinizing hormone secretion and impaired gonadal steroid hormone feedback in PCOS patients suggest impairments within the neuronal networks that regulate the reproductive axis. Evidence from preclinical models has linked androgen excess during prenatal life with altered structure and function of the developing female brain that might underpin syndrome development in adulthood. Studies investigating the mechanisms by which excess androgens program changes in the female brain have highlighted an important role for microglia. This review discusses how these non-neuronal cells shape the developing female brain in response to excess androgens and focuses on how microglia may be involved in the development of the neuroendocrine dysfunctions associated with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility worldwide, affecting 5–20% of women of reproductive age (Lizneva et al. 2016, Belenkaia et al. 2019). The etiology of PCOS is complex and remains poorly defined, resulting in largely symptom-based treatment. Neuroendocrine and reproductive impairments associated with PCOS suggest a role for the brain and the central circuits regulating fertility in the development of PCOS ((Pastor et al. 1998) and reviewed in (McCartney & Campbell 2020)). A large body of research using preclinical models of PCOS have been focused on identifying the mechanisms underlying the neuroendocrine and reproductive dysfunctions (Sullivan & Moenter 2004, Moore et al. 2015, Silva et al. 2018, Tata et al. 2018, Porter et al. 2019, Stener-Victorin et al. 2020). This review briefly discusses PCOS pathophysiology and highlights the role of the brain in PCOS pathogenesis. This is followed by a discussion of the potential mechanisms involved in shaping the PCOS-like brain, focusing on a proposed role for microglia. Microglia are the innate immune cells of the brain, also well characterized for establishing and refining neuronal connections through synaptic pruning.

PCOS etiology: a polygenic, transgenerational disorder

PCOS is a heterogeneous disorder, making diagnosis complicated. Current evidence-based guidelines endorse the Rotterdam diagnostic criteria, which requires at least two of three cardinal features to be present in the absence of other disorders that can present similar features (Rotterdam 2004, Teede et al. 2018). These features include androgen excess (hyperandrogenism), menstrual dysfunction (oligo- or amenorrhea) and a polycystic appearance of the ovaries. Despite the prevalent nature of PCOS, the etiology of the disorder remains poorly understood. A number of factors have been implicated in PCOS development that highlight the importance of both genetic polymorphism and environmental exposures that may contribute to epigenetic regulation of the genome. Briefly introduced here for context, these components are reviewed in detail elsewhere (Stener-Victorin & Deng 2021b, Dapas & Dunaif 2022, Silva & Campbell 2022).
Genetic, epigenetic and environmental contributors

PCOS is highly heritable. The incidence of PCOS in first-degree female relatives of PCOS patients is high (Kahsar-Miller et al. 2001), suggesting familial factors may contribute to the transgenerational nature of PCOS. Twin studies confirm that PCOS etiology has a genetic component (Vink et al. 2006), and several genome-wide association studies report polymorphisms in loci mapped to genes associated with PCOS symptoms (Park et al. 2004, Jamnongjit et al. 2005, Georgopoulous et al. 2013, Daan et al. 2015, Hayes et al. 2015, Gorsic et al. 2017). However, the loci identified in these genome-wide studies have a low heritability rate (<10%) that does not fully account for the high rate of heritability of PCOS previously identified in monozygotic twins (Kahsar-Miller et al. 2001, Vink et al. 2006). Such reports suggest the involvement of additional factors beyond genetics in PCOS development, including environmental and epigenetic factors.

Changes in epigenetic factors such as non-coding RNAs, DNA methylation and histone acetylation patterns are strongly associated with PCOS pathogenesis (Stener-Victorin & Deng 2021a). In a preclinical mouse model of PCOS, modifications to DNA methylation within ovarian, fat and hypothalamic tissue were found to be transmitted across three generations (Mimouni et al. 2021). The same study showed the identified dysregulated genes were mirrored in blood samples from PCOS patients, supporting that PCOS may be transmitted through DNA modifications of key genes. Epigenetic modifications can result from lifestyle factors and environmental exposures, including metabolic state and the in utero environment of development.

Exposure to excess androgens in critical windows of development is implicated in PCOS pathogenesis. Daughters of PCOS patients are more likely to develop PCOS (Sir-Petermann et al. 2002), exhibiting hyperandrogenemia as the most prevalent symptom (Risal et al. 2019). This transgenerational increased susceptibility to PCOS and associated comorbidities are likely to be a combination of genetic and epigenetic factors interacting with environmental (in utero androgen and anti-müllerian hormone (AMH) exposure) components (Stener-Victorin & Deng 2021a). Although direct evidence for in utero exposure to excess androgens is limited (Hickey et al. 2009, Caanen et al. 2016, Daan et al. 2017), PCOS daughters exhibit a longer anogenital distance at birth when compared to daughters of healthy women (Barrett et al. 2018). While the placenta is normally protected from elevated maternal androgens by enzymes that metabolize and synthesize sex steroids (i.e. P450 aromatase metabolizes androgens to estrogens), the activity of placental P450 aromatase is reduced in placentomal tissue of PCOS patients compared with unaffected women (Maliqueo et al. 2013). Additionally, the placenta of PCOS patients exhibits increased activity of 3β-hydroxysteroid dehydrogenase type 1 (3β-HSD-1) that converts sex steroids to androgen androstenedione (Maliqueo et al. 2013). Taken together, these studies suggest that an imbalance in placental enzyme activity in PCOS mothers can lead to a hyperandrogenic in utero environment that contributes to developmental programming of the PCOS phenotype in future generations.

Supporting the involvement of both genetic and environmental factors in the development of PCOS, brothers and sons of PCOS patients are reported to have PCOS-related symptoms, including hyperandrogenemia (Legro et al. 2002, Karthik et al. 2019) and a metabolic phenotype (Sam et al. 2008, Yilmaz et al. 2018, Karthik et al. 2019). Similar to PCOS daughters, sons of PCOS patients are also more susceptible to metabolic and cardiovascular diseases (Crisosto et al. 2017, Risal et al. 2019, Makrinou et al. 2020).

Neuroendocrine dysfunction associated with PCOS

Neuroendocrine dysfunction is common in PCOS patients, suggesting that the brain is a key player in the pathology and, likely, the development of PCOS (summarized in Fig. 1). PCOS patients exhibit elevated luteinizing hormone (LH) pulse frequency and amplitude and an elevated LH to follicle-stimulating hormone (FSH) ratio (Waldstreicher et al. 1988, Taylor et al. 1997). Gonadotropin release that persistently favors LH secretion leads to altered ovarian function, including theca cell hyperplasia and excess androgen production (Hughesdon 1982, Mortensen et al. 2009). This is accompanied by arrested follicular maturation due to low FSH levels, cystic follicles and anovulation driven by elevated AMH levels and androgen actions (Jonard et al. 2003, Pigny et al. 2006, Webber et al. 2007). Resulting hyperandrogenism contributes to the hirsutism, androgenic alopecia and acne that are often associated with PCOS (Ehrmann et al. 1992, Azziz et al. 2004, 2006).

Elevated LH pulse frequency in PCOS is likely to reflect elevated gonadotropin-releasing hormone (GnRH) secretion from GnRH neurons in the rostral forebrain, at the top of the hypothalamic–pituitary–gonadal (HPG) axis. Typically, the activity and secretion from GnRH neurons and downstream gonadotropin release are carefully governed by hormonal cues such as estradiol and progesterone. This steroid hormone feedback is largely indirect through the network of neurons that regulate GnRH neurons (Herbison & Theodosius 1992, Wintermantel et al. 2006). In PCOS patients, exogenous estradiol and progesterone administration fail to restore normal pulse frequency and amplitude (Daniels & Berga 1997). PCOS patients, in fact, require higher concentrations of estradiol and progesterone to slow LH pulse frequency and lower LH amplitude compared...
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Preclinical animal models of PCOS have been extensively used to identify the underlying mechanisms driving PCOS development and have been particularly important for understanding how the brain is involved. Most models of PCOS are generated by exogenous androgen delivery during different stages of development (Stener-Victorin et al. 2020). Some of the models recapitulate the human PCOS phenotype more closely than others; however, none is a complete representation of the disorder. Nonetheless, the majority of the preclinical models mimic the three main features used in the Rotterdam criteria for diagnosing PCOS, making them an invaluable tool in understanding PCOS pathophysiology and identifying therapeutic targets (Stener-Victorin 2022).

Various prenatally androgenized (PNA) models of PCOS including the PNA mouse (from DHT or AMH exposure) and the PNA sheep exhibit elevated LH pulse frequency and impaired steroid hormone negative feedback to the HPG axis (Robinson et al. 1999, Moore et al. 2013, Tata et al. 2018). Alterations to the HPG axis in these PNA models are associated with changes in the brain circuits modulating fertility (Moore et al. 2015, 2021, Tata et al. 2018, Porter et al. 2019, Jamieson et al. 2022). Together, these findings suggest that exposure to androgen excess early in development can program changes in the brain circuits mediating steroid hormone feedback to GnRH neurons which may ultimately result in elevated GnRH/LH release and downstream reproductive dysfunction in adulthood.

Neuronal abnormalities implicated in PCOS-associated neuroendocrine dysfunction

GnRH neuronal activity is regulated by a number of classical neurotransmitters (i.e. glutamate and gamma-aminobutyric acid (GABA)) and distinct neuronal populations (i.e. kisspeptin) that play important, yet not completely understood roles in relaying sex steroid mediated feedback. Several of these neuronal populations have been implicated in the impaired steroid hormone feedback and hyperactive HPG axis associated with PCOS.

Abnormalities in kisspeptin associated with PCOS

Kisspeptin neurons are critical mediators of the negative and positive steroid hormone feedback that shapes GnRH/LH release (Dubois et al. 2015, Clarkson et al. 2017, McQuillan et al. 2019) and obvious suspects in the neuroendocrine pathology of PCOS. Kisspeptin is elevated in the circulation of some PCOS patients (Gorkem et al. 2018, Umayal et al. 2019) and oligomenorrheic PCOS patients lack temporal coupling of kisspeptin and LH pulses in the blood (Katulski et al. 2018), but the source of circulating kisspeptin in these studies is unknown. Kisspeptin neurons that are located in the arcuate nucleus of the hypothalamus and co-express the peptides kisspeptin, neurokinin B and dynorphin (known as KNDy neurons) are of particular interest given their role in GnRH/LH pulse generation (Moore et al. 2018a). Neurokinin B receptor antagonist treatment in PCOS patients is reported to restore LH pulse frequency and subsequently

Figure 1 Alterations in the hypothalamic–pituitary–gonadal axis in the PCOS-like condition. Increased luteinizing hormone (LH) levels from the anterior pituitary gland, suggestive of increased gonadotropin-releasing hormone (GnRH) neuronal activity and GnRH release upstream. LH contributes to arrested estrous cyclicity and ovarian overproduction of androgens and other steroid hormones. Steroid hormone receptor-expressing afferent neurons that normally convey feedback signals to GnRH neurons are desensitized to steroid hormones and send increased depolarizing GABAergic inputs onto GnRH neurons. PCOS features are depicted in red. ER, estrogen receptor; FSH, follicle-stimulating hormone; PR, progesterone receptor. Figure is made using Biorender.com.

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circular LH and testosterone levels (George et al. 2016), implicating KNDy neuron signaling in PCOS pathogenesis. In preclinical models of PCOS, the role of kisspeptin and related neuropeptides in the PCOS-like state is not clear. Some studies report changes in kisspeptin gene expression and cell number within the arcuate nucleus (Kondo et al. 2016, Aliabadi et al. 2017, Matsuzaki et al. 2017) while others contradict those findings (Cheng et al. 2010, Brown et al. 2012). In the PNA sheep model of PCOS, KNDy neurons are differentially innervated and send fewer inputs to GnRH neurons (Cernea et al. 2015, Porter et al. 2019). In the PNA mouse model, both the rostral population of kisspeptin neurons in the pre-optic area and the KNDY neurons are reported to have altered afferent input (Moore et al. 2021, Jamieson et al. 2022), suggesting that prenatal androgen excess can program changes in these circuits. While these findings implicates kisspeptin neurons in mediating the neuroendocrine dysfunction of PCOS and as potential therapeutic targets, the exact mechanisms by which kisspeptin neurons account for elevated GnRH/LH release remains to be determined (McCartney et al. 2022).

Abnormalities in glutamate and GABA associated with PCOS

Glutamate and GABA are the two major neurotransmitters in the brain. While there is limited evidence to support modified glutamatergic transmission in PCOS pathology, several clinical and preclinical studies implicate GABA signaling in the dysregulation of the PCOS HPG axis.

Glutamate levels are unchanged in the cerebrospinal fluid of PCOS patients compared to controls and in contrast to GABA (Kawwass et al. 2017). Preclinical studies of glutamate signaling in PCOS-like states are sparse and inconsistent. While in the PNA sheep model of PCOS GnRH neurons receive fewer kisspeptin-sensitive glutamatergic inputs (Cernea et al. 2015), the PNA mouse model of PCOS shows no alterations in putative glutamatergic inputs onto GnRH neurons (Moore et al. 2015). However, arcuate nucleus glutamatergic neurons in the PNA mouse show reduced progesterone receptor expression, suggestive of reduced sensitivity to sex steroid-mediated feedback in this population (Moore et al. 2021). Such disparities may be due to anatomical differences between species assessed (Lehman et al. 2010) or indicate that glutamate signaling does not play a major role in centrally mediated PCOS pathology.

The majority of evidence in clinical and preclinical studies implicates GABA signaling in the dysregulation of the PCOS HPG axis. GABA is one of the major regulators of GnRH neurons (reviewed in (Herculano-Houzel et al. 2013), and are well-recognized as mediators of neuronal circuit development and modulators of neuronal activity (reviewed in (Allen & Lyons 2018)). Non-neuronal cells have also been shown to play a major role in the development and regulation of GnRH neurons, implicating them in the known brain wiring modifications associated with PCOS (reviewed in (Desroziers 2022)). Microglia, the innate immune cells of the brain, are one type of non-neuronal cell that has been hypothesized to mediate androgen excess-induced programming of abnormal hypothalamic circuit wiring associated with PCOS-like features.

Microglia: key players in PCOS pathogenesis?

Non-neuronal cells are abundant in the brain, accounting for 50% of cells in most regions of the brain (Herculano-Houzel et al. 2013), and are well-recognized as mediators of neuronal circuit development and modulators of neuronal activity (reviewed in (Allen & Lyons 2018)). Non-neuronal cells have also been shown to play a major role in the development and regulation of GnRH neurons, implicating them in the known brain wiring modifications associated with PCOS (reviewed in (Desroziers 2022)). Microglia, the innate immune cells of the brain, are one type of non-neuronal cell that has been hypothesized to mediate androgen excess-induced programming of abnormal hypothalamic circuit wiring associated with PCOS-like features.

Microglia shape the developing brain

Microglia are involved in an array of physiological processes within the central nervous system across
development and in adulthood, including immune surveillance, homeostasis and neuronal health, sexual differentiation, synaptic plasticity and pruning synapses. During development, microglia are involved in the establishment and refinement of brain connections through mediating developmental processes such as synaptic pruning (Schafer et al. 2012), synaptogenesis (Miyamoto et al. 2016) and modulating the number of cells in certain brain regions (VanRyzin et al. 2019). Interestingly, these microglial functions are performed in a sex-specific manner throughout development (Lenz et al. 2013, Lenz & McCarthy 2015), resulting in a brain that is wired in a male-typical or female-typical pattern, in part, dependent upon exposure to sex steroids in specific developmental windows. These findings suggest that microglia play an important role in mediating sex steroid-induced changes in the developing brain. Specifically, microglia are involved in shaping brain circuits that drive male and female-typical sex behaviors later in life (Lenz et al. 2018, VanRyzin et al. 2019). Microglia-mediated changes in brain circuitry in response to androgens are governed by local cues and are thus remarkably distinct in different brain regions. Specifically, testosterone, following its local aromatization to estradiol, increases glutamatergic synapses within the preoptic area, leading to male-typical sex behavior in adulthood (Lenz et al. 2018). Estrogen receptor-mediated effects of aromatized androgens are initiated through the degranulation and release of histamine from mast cells that are resident within the medial preoptic area (mPOA). In response to histamine, microglia increase prostaglandin E2 release which induces dendritic spine formation on neurons in the mPOA during early development. Elevated dendritic spine density, likely associated with a greater number of excitatory synapses, which is increased in response to androgen exposure, is required for male-typical copulatory behavior in adulthood. Microglia are also involved in androgen receptor-mediated mechanisms to modulate other sexually differentiated behaviors (VanRyzin et al. 2019). Testosterone increases endocannabinoid release in the developing amygdala through an androgen receptor-dependent mechanism. Endocannabinoids, in turn, act on microglia to increase their phagocytic capacity to remove newborn cells that are destined to become astrocytes. This mediates increased neuronal activity in the androgen-exposed-male amygdala compared to females and is associated with sex-related differences in juvenile play behavior in rats. Thus, androgens act indirectly via microglia to program changes in brain wiring early in development through both estrogen and androgen receptor-mediated mechanisms to influence sex-specific phenotypes in adulthood. These findings raise questions about whether prenatal androgen excess in females, associated with the development of PCOS features in adulthood, involves microglia-mediated changes in brain circuit development.

**Refinement of synapses via microglia phagocytosis**

Synaptic pruning, or the refinement of neuronal inputs on both the pre- and post-synaptic side (Faust et al. 2021), is one of the classical functions of microglia during early development. Developing neuronal networks begin with excessive synaptic inputs that are subsequently refined, often in an activity-dependent manner, to produce eventual adult connections. Microglia refine excessive synapses through a number of processes including phagocytosis, which is the engulfment of synaptic components (Schafer et al. 2012). This was first demonstrated by Paolicelli et al. who utilized a knock-in transgenic mouse line expressing GFP in microglia (CX3CR1-GFP) and confocal imaging to reveal that microglia phagocytose excitatory postsynaptic and presynaptic components (Paolicelli et al. 2011). This study also demonstrated that deficient microglia function results in excessive immature synapses as revealed by an increased ratio of spontaneous excitatory postsynaptic currents (sEPSC) to miniature excitatory postsynaptic currents (mEPSC) using single-cell recordings. These results indicate that microglia function is required for the refinement of synaptic inputs early in development. Direct evidence that microglia phagocytose weak synapses in an activity-dependent manner was demonstrated later for the retinogeniculate system (Schafer et al. 2012). Using electron and confocal microscopy, this study showed that functionally weak synapses are tagged with complement proteins (i.e. C3), signals for microglia complement receptors and induce microglia phagocytosis. Pharmacological studies have provided further evidence supporting the role of microglia in pruning synapses. Organotypic hippocampal slices pharmacologically depleted of microglia using clodronate liposomes show an increased frequency of sEPSC and mEPSC, suggestive of increased synaptic inputs (Ji et al. 2013). Increased postsynaptic excitatory currents were likely due to an increase in the density of dendritic spines and glutamatergic synapses into hippocampal pyramidal neurons. Additionally, excessive neuronal inputs onto the medial nucleus of the trapezoid body neurons of the brainstem were found after depleting microglia during early postnatal development (Milinkeviciute et al. 2019). Impaired synaptic pruning is associated with a number of neurological disorders such as schizophrenia and autism (Sellgren et al. 2019). Taken together, disruption to the microglia-mediated refinement of synapses may play a role in the development of numerous disease states. Reduced microglia engulfment of GABA synapses in the developing brain of PCOS-like mice excessive excitatory GABAergic inputs to GnRH neurons are established in prenatally androgenized PCOS-like...
Figure 2  GABAergic synaptic content engulfed within microglia in the prenatally androgenized (PNA) model of polycystic ovary syndrome (PCOS). (A–G) Representative images of microglia (GFP) from the rPOA of control and prenatally androgenized (PNA) mice and their 3D reconstructions showing engulfed GABAergic synaptic material (vesicular GABA transporter, vGAT; purple) within lysosomes (CD68; blue). Scale bar = 5 μm (2 μm in small boxes). Adapted from Sati et al. 2021. (A) Representative images of microglia (GFP) from the ARN of control and PNA mice and their 3D reconstructions showing engulfed GABAergic synaptic material (vesicular GABA transporter, vGAT; purple) within lysosomes (CD68; blue). Scale bar = 5 μm (2 μm in small boxes). (B) Percentages of the four microglia morphologies were not different between PNA and controls. Pearson Chi-square. (C) Average microglia volume (μm$^3$). Mann–Whitney U test. (D) Percentage lysosomal content was not affected by PNA treatment. Unpaired t-test. (E) Percentage of microglia engulfing vGAT was not different between PNA and control mice. Mann–Whitney U test. (F) Percentage of vGAT engulfed per microglia was also not affected by PNA treatment. Mann–Whitney U test. (G) Volume of vGAT engulfed per microglia was not different between PNA and controls (μm$^3$). Unpaired t-test. Data are shown as mean ± s.e.m. for control (n = 7) and PNA mice (n = 7).
models prior to puberty (Sullivan & Moenter 2004, Berg et al. 2018, Silva et al. 2018), suggesting modified synaptic pruning in early development. An assessment of microglia populations over development in the PNA mouse model of PCOS identified a specific and significant decrease in microglia number in the rPOA at postnatal day 25, just prior to puberty (Sati et al. 2021). Reduced numbers of microglia in the hypothalamus have been correlated with changes in the GnRH neuronal network and downstream disruptions in reproductive functions (Cohen et al. 2002). Previous reports in the hippocampus have found that reduced microglia engulfment during early development is associated with excess excitatory synapses (Paolicelli et al. 2011). Reduced microglia in the PNA brain may similarly drive impaired refinement of GABA synapses within the rPOA, where the excess GABA wiring associated with PCOS was detected. Three-dimensional reconstructions of confocal images demonstrated rPOA microglia engulfing vesicular GABA transporter labeled tissue, likely indicative of GABAergic synaptic material, at 7 and 15 days postnatally (Fig. 2A). However, in brain sections from postnatal day 15 PCOS-like PNA animals, the amount of GABAergic synaptic material engulfed by microglia was significantly reduced. Reduced GABAergic material evident in microglia of the rPOA, where pathological GABA wiring is detected in the PCOS-like state, may be indicative of reduced pruning of GABAergic synaptic terminals in the PNA brain (Fig. 3).

This indication of impaired synaptic pruning was only detected in the rPOA of PNA mice. In the ARN, microglia refinement of GABAergic synapses was comparable between PNA and control mice (Fig. 2B, C, D, E, F and G), suggesting that prenatal androgen excess does not affect microglia refinement of GABAergic inputs within the ARN. GnRH neurons have specialized dendrons that extend to the median eminence through the ARN (Herde et al. 2013). Distal regions of GnRH dendrons receive extensive inputs as shown using electron microscopy (Moore et al. 2018b); however, whether these inputs are altered in the PCOS-like brain remains unknown. Microglia refinement of GABAergic synapses is not affected by PNA treatment within the ARN suggesting that androgen actions on microglia are localized to the rPOA, where GnRH neuron cell bodies and proximal dendrites are located. While it remains to be determined whether the reduced terminal pruning is specific to GnRH neurons, microglia have been found in close association with GnRH neurons within the rPOA (Sati et al. 2021). These results suggest a potential mechanism that could underpin increased GABAergic inputs and neurotransmission associated with elevated GnRH/LH release in the PNA PCOS-like state.

Questions remain regarding mechanism

These findings suggest that microglia are involved in shaping the PCOS-like brain during early development. As noted previously, microglia are recognized downstream mediators of androgen actions in sexual differentiation of the brain, involved in an intricate pathway that leads to sex-specific wiring of different brain regions (summarized in Fig. 4) (Lenz et al. 2018, Cohen et al. 2018, Paolicelli et al. 2011, Paolicelli et al. 2007). This microglia-mediated pathway influences androgen actions and may be central to the development of sex-specific brain circuits in the PNA mouse model of PCOS. While the mechanisms by which androgens drive changes in the brain remain unknown, studies suggest that microglia-mediated refinement of GABAergic inputs is reduced within the rPOA and is likely to drive the excessive wiring associated with the PCOS-like phenotype. Figure has been made made using Biorender.com.

Figure 3 The impact of androgen excess exposure on prenatally androgenized (PNA) mouse model of polycystic ovary syndrome (PCOS). Excess prenatal androgen program changes in microglia prior to the development of PCOS-like features in females that contribute to the development of aberrant brain wiring that drives the PCOS-like state in the PNA mouse model. Specifically, microglia-mediated engulfment of GABAergic inputs is reduced within the rPOA and is likely to drive the excessive wiring associated with the PCOS-like phenotype. Figure has been made made using Biorender.com.

Figure 4 Microglia program brain circuits in response to androgens through distinct region-specific pathways. Androgens lead to sex-specific changes in hypothalamic regions including the medial preoptic area (mPOA) and the medial amygdala (meA) through microglia-mediated mechanisms. The mechanism by which androgens drive the pathological changes in the GABAergic wiring of gonadotropin-releasing hormone (GnRH) neurons within the rostral preoptic area (rPOA) is yet to be determined. Androgens, however, could drive such alterations through a histamine-mediated or endocannabinoids-mediated pathway similar to the male responses in the mPOA and the meA. Figure is made using Biorender.com.
Whether prenatal androgen excess that drives a PCOS-like phenotype in females acts through similar mechanisms remains unknown. There is no evidence to suggest that microglia are direct targets of androgens. Microglia isolated from the adult hippocampus have been reported to lack the expression of AR mRNA (Sierra et al. 2008). It remains to be determined, however, whether microglia lack the androgen receptor during earlier developmental stages, which may allow androgens to act directly on the cells to drive the previously reported changes in the refinement of neuronal inputs within the PNA brain. The exact cellular and molecular pathways that induce these androgen-mediated changes in microglia within the PCOS-like brain remain unknown (Fig. 4). Androgens could drive an intricate histamine-mediated chain of events similar to the male-typical response in the developing preoptic area (Lenz et al. 2018).

Whether metabolic perturbations associated with PCOS drive changes in microglia that contribute to differences in brain structure remains largely unexplored. Accumulating evidence suggests microglia are responsive to changes in metabolic status and insulin levels (Haas et al. 2020). Insulin promotes the expression of a number of pro-inflammatory markers in microglia, altering the state of the cells and potentially their function (Haas et al. 2020). A recent study found that microglia are activated in a letrozole-treated rat model of PCOS (Wang et al. 2023), known to exhibit both a reproductive and metabolic phenotype (Kauffman et al. 2015) and that this was reduced with aerobic exercise, likely mirroring changes in hypothalamic inflammation.

Supporting an important role for other non-neuronal cells in PCOS development and pathology, a recent proton magnetic resonance spectroscopy study in women with PCOS indicated increased axonal/glial signaling associated with increased AMH signaling (Barbotin et al. 2023). This was supported by electron microscopy work in a preclinical prenatal-AMH treated model showing a retraction of tanyocytes, specialized glial cells, in the median eminence (Barbotin et al. 2023).

Concluding remarks

We are just beginning to understand how microglia and other non-neuronal cells of the brain contribute to the development and pathology of PCOS. Models of female androgen excess suggest that early exposure to a modified hormone environment can interrupt the important sculpting work of microglia in the developing brain. Future studies are required to determine the mechanisms by which androgen excess modifies microglia behavior and whether this pipeline can be interrupted once initiated. Understanding the specific mechanisms by which androgen excess impacts the female brain, both in development and in adulthood, will lead us toward a better understanding of the pathological mechanisms underpinning PCOS.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

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