The scientific body of knowledge – whose body does it serve? A spotlight on oral contraceptives and the brain

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Abstract

Women constitute half of the world's population, yet neuroscience research does not serve the sexes equally. Fifty years of preclinical animal evidence documents the tightly-coupled relationship between our endocrine and nervous systems, yet human neuroscience rarely considers how endocrine factors shape the structural and functional architecture of the human brain. Here, we quantify several blind spots in human neuroscience research, which overlooks aspects of the human condition that impact women's health (e.g. the menstrual cycle, hormonal contraceptives, pregnancy, menopause). Next, we illuminate one of the public health ramifications of this bias: today over 100 million women use oral hormonal contraceptives, but few investigations examine whether disrupting endogenous hormone production impacts the brain. We close by presenting a roadmap for progress, highlighting the *University of California Women's Brain Initiative* which is addressing unmet needs in women's health research.

Key Words: sex hormones | neuroimaging | birth control | women's health

Highlights

- The brain is an endocrine organ whose structure and function is influenced by neuromodulatory hormones.
- Human neuroscience research rarely considers endocrine factors: over the past 25 years only 0.5% of neuroimaging articles focus on endocrine-related variables.
- This oversight obscures basic knowledge of endocrine–brain function and undermines women's health.
- 100 million women use oral contraception, yet we lack basic knowledge of its influence in the CNS.
- Future studies should shine a much-needed spotlight on women's health in human neuroscience.

Neuroscientists have plumbed the depths of the mind and brain to extraordinary lengths, but occasionally we forget that the brain is part of a larger, integrated biological system. The brain is an endocrine organ, one whose structure and function is intimately tied to the action of neuromodulatory hormones (Frick et al., 2015; Galea et al., 2017; Hara et al., 2015; Pritschet et al., 2019; Taylor et al., 2020; Woolley and McEwen, 1993). The brain coordinates the release of hormones from peripheral endocrine glands and, in turn, is a major target of these signaling molecules. Fifty years of accumulating evidence from animal studies documents the tightly-coupled relationship between our endocrine and nervous systems (Frick and Kim, 2018; Galea et al., 2017; Hara et al., 2015; Woolley and McEwen, 1993). Yet human neuroscience rarely considers how endocrine factors shape the structural and functional architecture of the human brain.

Human neuroscience has almost entirely ignored how the brain responds to major changes in sex hormone production (e.g. during the menstrual cycle, pregnancy, menopause, or andropause). During the average human menstrual cycle, women experience a ~12-fold increase in estradiol and an ~800-fold increase in progesterone. During pregnancy, production of sex hormones surge throughout the gestational window. Later in life, women experience a steep decline in sex hormone production during the transition to menopause. For men, testosterone production shows a protracted decline beginning in the mid-30s and continuing throughout life. The field has also overlooked the neuronal effects of disrupting sex hormone production via common exogenous hormone manipulations (e.g. oral hormonal contraceptives, androgenic anabolic steroids, and gonadotropin releasing hormone agents). For example, sex hormone production is chronically suppressed in the 100 million women worldwide who use oral hormonal contraceptives. How do these shifts in gonadal hormone production shape the brain?

The field of human neuroscience has not adequately addressed these factors (Hampson, 2020). Beyond obscuring basic knowledge about the endocrine basis of brain function, this oversight places a disproportionate burden on women's health.

In this review, we highlight seminal findings from the animal and human literature establishing the neuroendocrine basis of brain structure and function. Next, we take stock of how often endocrine factors are considered in human brain imaging studies, revealing major blind spots in the field. We illuminate the public health ramifications of this oversight using oral hormonal contraceptives as an example. We close by presenting a roadmap for progress, highlighting efforts from the *University of California Women's Brain Initiative* to address unmet needs in women's health research.

1. Brief review of sex hormone action in the central nervous system

Hormones are chemical messengers that travel through the circulatory system, coordinating the activity of major organ systems. Sex steroids (androgens, estrogens, progestins) are one major class of hormones that are synthesized from cholesterol and produced primarily by the gonads (testes, ovaries). Sex steroid hormones coordinate the physiological transformations that occur during puberty, pregnancy, and menopause. Within the central nervous system, estrogen and progesterone receptors are expressed throughout the brain (McEwen, 2002; McEwen and Alves, 1999; Rossetti et al., 2016), including enriched expression in extra-hypothalamic regions like the hippocampus and prefrontal cortex (PFC) (Almey et al., 2015; Brinton et al., 2008). Estradiol and progesterone signaling are critical components of cell survival and plasticity, exerting excitatory and inhibitory effects that are evident across multiple spatial and temporal scales (Frick and Kim, 2018; Galea et al., 2017). Below, we highlight major discoveries from the past

20 years establishing estrogen and progesterone action in higher-order cognitive regions of the brain (for a comprehensive review of sex hormone action in memory circuitry, see Frick, 2019).

1.1 Sex hormones regulate hippocampal/prefrontal cortex morphology across species

Animal studies offer unambiguous evidence that sex steroid hormones shape the synaptic organization of the brain, particularly within the hippocampus and PFC (Frick et al., 2015; Frick and Kim, 2018; Galea et al., 2017; Hara et al., 2015; Woolley and McEwen, 1993). Rodent (Frick et al., 2015; Frick and Kim, 2018; Mahmoud et al., 2016; Woolley and McEwen, 1993) and non-human primate (Hao et al., 2003) studies have established 17β-estradiol and progesterone as powerful regulators of hippocampal morphology. At the epigenetic level, sex hormones induce chromatin modifications that promote hippocampal plasticity (Fortress and Frick, 2014). At the synaptic level, sex hormones regulate spine proliferation in the hippocampus (Hara et al., 2015). Dendritic spine density in CA1 neurons varies by ~30% over the 4–5-day rodent estrous cycle (Woolley et al., 1990; Woolley and McEwen, 1992). Hormone deprivation (via gonadectomy) in the rat (Gould et al., 1990; Woolley and McEwen, 1993) and African green monkey (Leranth et al., 2002) leads to a pronounced loss of spines on CA1 neurons, which is reversed by hormone replacement.

At the macroscopic level, total hippocampal volume is regulated by sex hormones in the meadow vole (Galea et al., 1999) and fluctuates across the estrous cycle in the mouse (Qiu et al., 2013). In-vivo magnetic resonance imaging (MRI) in mice demonstrates hormone-mediated hippocampal changes are detectable within a 24-hour period (Qiu et al., 2013). In humans, progesterone dynamically shapes medial temporal lobe morphology across the ~28-day menstrual cycle, with volumetric changes in CA2/3, parahippocampal cortex, entorhinal cortex

and perirhinal cortex—effects that are blocked by progesterone suppression (Taylor et al., 2020). During pregnancy, the rise in sex hormone production throughout gestation modulates hippocampal plasticity in rodents (Galea et al., 2014; Kinsley and Lambert, 2008; Workman et al., 2012) and likely mediates the decline in hippocampal volume observed in humans post-pregnancy (Hoekzema et al., 2017). Finally, the abrupt hormonal changes associated with surgical menopause lead to structural changes in the medial temporal lobe, including thinning of the parahippocampus/entorhinal cortex (Zeydan et al., 2019), while hormone supplementation in postmenopausal women increases hippocampal volume (Albert et al., 2017). Together, these findings provide converging cross-species evidence that sex hormones induce structural changes in the hippocampus on rapid and protracted timescales.

Nonhuman primate studies have established similar relationships within the PFC (Hao et al., 2006; Morrison et al., 2006). In female rhesus macaques, ~50% of PFC pyramidal neurons express estrogen receptors (ER-α) and those with enriched PFC ER-α expression show stronger working memory performance (Wang et al., 2010). At the synaptic level, cyclic estradiol administration in ovariectomized rhesus macaques leads to increased spine density in PFC neurons (Hao et al., 2006) and improved working memory performance relative to estradiol-depleted controls (Rapp et al., 2003).

1.2 Sex hormones regulate hippocampal/prefrontal cortex function across species

Human neuroimaging studies have started to establish sex hormones' role in the regulation of memory circuitry (Duff and Hampson, 2000; Dumas et al., 2010; Jacobs and D'Esposito, 2011; Shanmugan and Epperson, 2014). This research builds on pioneering work from Berman (1997) and Shaywitz (1999), who used pharmacological blockade and hormone replacement

techniques to illustrate the influence of estradiol and progesterone on regional activity in memory circuitry. A series of recent studies offers additional evidence that functional changes in ER-rich regions of the brain are tied to ovarian status. Across the human menstrual cycle, endogenous fluctuations in estrogen and progesterone exert a powerful influence on intrinsic brain networks (Pritschet et al., 2019). Later in life, the depletion of sex hormones during the menopausal transition impacts PFC and hippocampal responses when participants engage in working memory and episodic memory tasks (Jacobs et al., 2016, 2017). Research targeting the midlife menopausal transition has revealed the neurobiological consequences of *neuroendocrine* aging, above and beyond more well-established effects of chronological aging (Jacobs and Goldstein, 2018; Rentz et al., 2017; Taylor et al., 2019).

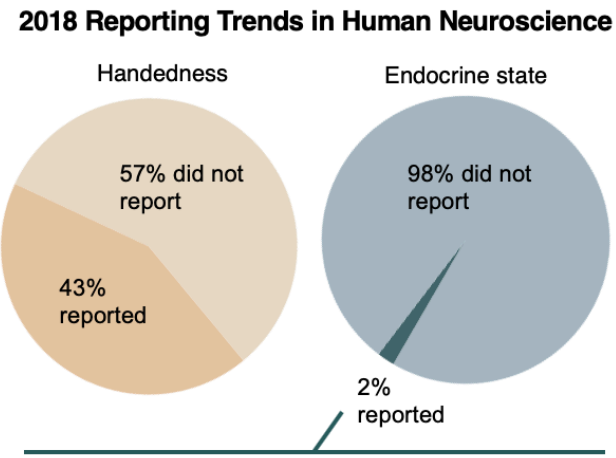
An emerging theory from the human literature is that estradiol increases the efficiency of cortical circuits within the PFC. In young women performing a working memory task, PFC activity is exaggerated under low estradiol conditions and reduced under high estradiol conditions (Jacobs and D'Esposito, 2011). The same pattern is observed decades later in life: as estradiol production declines over the menopausal transition, working memory–dependent PFC activity becomes exaggerated despite no deficit in performance (Jacobs et al., 2017). In a recent dense-sampling study, Pritschet and colleagues (2019) used time-lagged methods from dynamical systems analysis to show that day-to-day changes in estradiol enhance the global efficiency of large-scale functional networks, with pronounced effects in networks (e.g. Default Mode and Frontal Control) with hubs in PFC (Schaefer et al., 2018; Yeo et al., 2011). Thus, one principle of estradiol action may be that it helps generate efficiency in cortical circuits, particularly within PFC, and one intriguing possibility is that these effects are mediated through dopamine signaling pathways (Becker, 1990; Williams and Goldman-Rakic, 1995; Cai and

Arnsten, 1997; Jacobs and D'Esposito, 2011).

2. Identifying blind spots in human cognitive neuroscience

While animal studies have documented the role of sex hormones in the brain for decades, human neuroscience has not kept pace. Given a groundswell of evidence that sex hormones regulate the structure and function of the mammalian brain, we sought to document the frequency with which human neuroscience research considers endocrine factors. We approached this in two ways. First, to capture a contemporary state of the field, we analyzed every empirical human neuroimaging paper published in five leading neuroscience journals in 2018: *Nature Neuroscience*, *Neuron*, *Journal of Neuroscience*, *Neuroimage*, and *Human Brain Mapping*. Articles (n=1,066) were coded based on a range of women's health factors, including whether the article mentioned participants' menstrual cycle phase, pubertal stage, hormonal contraceptive use, pregnancy, menopause status, endocrine disorders, direct hormone assays and more (a full description of the methods are provided in **Supplementary Material**). Two percent (n=29) of the articles surveyed mentioned a women's health factor (**Figure 1** – *top*). Of those that did, 20% (n=6) used the information to *exclude* women (i.e. to justify conducting a male-only study); 52% (n=15) did so to characterize the general study population (e.g. reporting hormonal contraceptive use) but did not use the data further; and only 17% (n=5) of the subset focused some aspect of their study design or analysis on a women's health research question. In short, while ~2% of brain imaging articles surveyed mentioned an endocrine factor, far fewer — *less than half of one percent* —investigated the relationship between a women's health factor and the brain.

Next, a historical survey of neuroimaging papers published from 1995–2018 revealed the persistence of this oversight across all journals indexed on PubMed. Women's health factors are severely understudied in human neuroscience. Of the ~43,000 human neuroimaging articles published over the last 25 years, fewer than 300 were focused on women's reproductive health (including the menstrual cycle, pregnancy, menopause, birth control, and more; see **Supplementary Material**). **Figure 1** (*bottom*) illustrates the magnitude of this disparity. The number of articles dedicated to understanding the neuronal effects of a broad range of women's health factors barely registers on a plot of neuroimaging articles published over time—accounting for ~0.5% of total publications—and is dwarfed by papers on 'reward processing' (shown for comparison). Women constitute half of the world's population, yet the brain imaging community rarely considers basic aspects of women's health.



Use of endocrine data	Count of papers
Used to exclude women	6
Sample characterization	15
Study covariate	3
Variable of interest	5

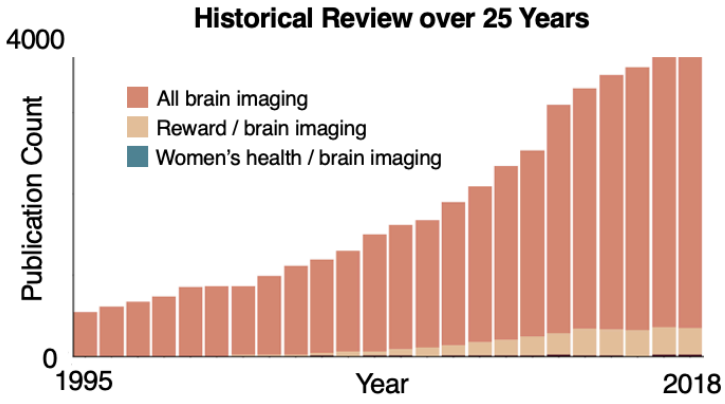


Figure 1. Women's health factors are severely understudied in human neuroscience. **Top** | In 2018, only 2% of neuroimaging articles published in leading neuroscience journals mentioned endocrine/women's reproductive health factors. Of those, 20% merely did so to exclude women and justify conducting a male-only study. Less than 0.5% of articles directly studied sex hormones or a sex hormone–related topic. **Bottom** | Publication count of human neuroimaging studies from 1995–2018. The number of brain imaging articles that consider women's reproductive health is dwarfed by other research categories, such as 'reward processing.'

Since the mid-1990s the number of human neuroimaging studies has exploded, and the number of neuroimaging studies addressing women's health has not kept pace.

3. A spotlight on oral hormonal contraceptives

Perhaps one of the most striking illustrations of this oversight is neuroscience's neglect with respect to one of the largest uncontrolled medical experiments in human history: over the past half-century, women have used oral hormonal contraceptives without full knowledge of their influence on the central nervous system. Few rigorous neuroimaging studies of oral hormonal contraception (OC) have been conducted in humans. The public health ramifications of this oversight are vast. Here, we use OC to highlight our historical failure to consider the brain in its endocrine context. We close by presenting a roadmap for how to address these oversights as quickly and effectively as possible.

First introduced in the U.S. in 1960, "the pill" revolutionized women's reproductive health and was quickly adopted as the first widespread hormonal method of birth control. By 1967, 13 million women were using the pill, by 1984 those numbers rose to 50-80 million (Knowles and Correia, 2015), and today OC is used by more than 100 million women worldwide (Christin-Maitre, 2013; Petitti, 2003). In the US alone, 10 million women currently take OC and 60 million have done so over their lifetime (Daniels et al., 2015; Daniels and Jones, 2013; Jones et al., 2013).

3.1 Oral contraception's mechanism of action

"The pill" is sold under ~100 different brand names with more than 40 different formulations. Almost all consist of a combination of two synthetic sex hormones, estrogen and progestin, that

act predominantly on endogenous sex steroid hormone receptors (Louw-du Toit et al., 2017; Sitruk-Ware and Nath, 2013). OC has been described as "mimicking pregnancy," though this mechanistic explanation is misleading. During pregnancy, women experience a ~900-fold increase in estradiol and ~400-fold increase in progesterone between the 1st and 3rd trimesters (in addition to a host of other endocrine changes) (Berg and Kuss, 1992; Schock et al., 2016; Tal et al., 2000). Oral contraceptives prevent ovulation by mimicking the negative feedback effects of estradiol and progesterone. The exogenous hormones introduced by the pill limit gonadotropin releasing hormone secretion from the hypothalamus, in turn inhibiting follicle stimulating hormone (FSH) and luteinizing hormone (LH) release by the anterior pituitary. The reduction in FSH prevents follicle growth, the mid-cycle surge in estradiol, and the LH surge that would trigger ovulation (Bronson, 1981; Jones and Lopez, 2013). By inhibiting hypothalamic and pituitary hormones, OC chronically suppresses ovarian production of estradiol and progesterone. In women using OC, endogenous sex hormone concentrations are on par with levels observed during the early follicular phase of freely cycling women (De Bondt et al., 2013b). Some formulations of OC can suppress progesterone concentrations by ~97% (Taylor et al., 2020) and suppression of sex hormones can persist after pill use is discontinued (Fleischman et al., 2010).

Fifty years have now passed since the widespread adoption of the pill, yet very few studies have investigated the impact of chronic sex hormone suppression on brain regions that are densely populated with sex hormone receptors and modulated by sex hormones. It is unclear whether long-term ovarian hormone suppression has consequences at the macroscopic level of brain morphology and function in humans, but emerging evidence from a handful of small-scale human studies raises the possibility.

3.2 Effects of oral contraceptives on brain structure and function

Despite the striking change in endocrine status that occurs in response to OC use, neuroscientists lack even a basic understanding of how estrogen receptor–rich brain structures like the hippocampus and PFC respond to chronic suppression of sex hormone production. Observational studies have started to lay the groundwork for understanding OC's effects on the central nervous system. Preliminary evidence suggests OC users have reduced gray matter volume in the amygdala and parahippocampal gyrus relative to naturally cycling women (Lisofsky et al., 2016; Pletzer, 2019; but see Pletzer et al., 2019, 2010). Other studies report differences in white matter mean diffusivity (De Bondt et al., 2013b), prefrontal GABA concentrations (De Bondt et al., 2015a), hypothalamic choline/N-acetyl-aspartate (NAA) ratio (Baroncini et al., 2010) and resting-state functional connectivity (De Bondt et al., 2015b; Engman et al., 2017; Petersen et al., 2014). For a recent review of brain imaging literature on OC see Beltz and Moser, 2020.

While these studies reflect early efforts to characterize the neuronal effects of OC, research in this arena is nascent. Oral contraceptive use varies across multiple dimensions (age of initiation, duration of use, hormone formulation, schedule), any of which could influence the magnitude of OC's impact on the structural and functional architecture of the brain (Hampson, 2020). Well-powered, systematic, quasi-experimental approaches that take these factors into account are essential for making meaningful scientific progress. Below we highlight some of the most pressing questions for future research.

3.3 Formulation and regimen

There are currently more than 40 OC formulations on the market, yet few studies differentiate between them (see Engman et al., 2017 and Pletzer et al., 2015 for exceptions). Most OC formulations pair an estrogen with a progestin, though progestin-only pills are available. The

estrogen component can vary by type (e.g. estradiol, ethinyl-estradiol or mestranol) and dose (ranging from "ultra-low dose" formulations of 0.01 mg to higher doses of 0.05 mg). The progestin component also varies by type (with effects ranging from strongly anti-androgenic to strongly androgenic) and dose (0.1–3.0 mg/pill). Hormonal regimens also vary based on whether the drug dose is constant or variable across a pill pack (e.g. monophasic versus multi-phasic doses).

These variations likely alter the downstream neurobiological effects of the pill. For example, in preclinical studies, 17β-estradiol's neuronal effects depend on whether the hormone is administered cyclically, continuously, and with or without progestin. In ovariectomized animal models, hormone replacement regimens that consistent of *cyclic* estradiol unopposed by progesterone enhance PFC spine density (Tang, 2004; Wang et al., 2010). However, regimens containing continuous estradiol (with or without progestin) and cyclic estradiol paired with cyclic progestin fail to induce similar synaptogenic effects (Hara et al., 2015; Nagahara et al., 2010; Ohm et al., 2012).

3.4 Direct hormone assays

Serum assessments of circulating sex hormones are essential for characterizing the endocrine effects of a particular OC formulation, yet these data are rarely acquired. While it is generally assumed that OC chronically suppresses the ovarian production of estradiol and progesterone, the magnitude of suppression may vary by OC formulation. In one study of OC users (of various formulations), mean estradiol and progesterone concentrations were suppressed to levels at or

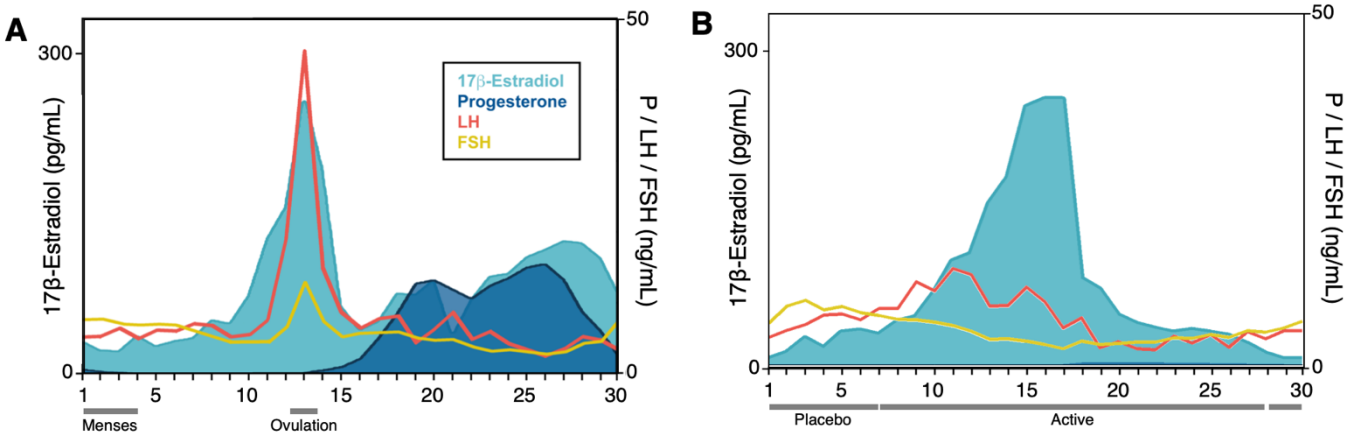


Figure 2. Endocrine profile of a woman across a menstrual cycle and on oral hormonal contraception. A. Pituitary gonadotropins (LH, FSH) and gonadal hormones (estradiol, progesterone) across 30 days of a complete menstrual cycle. Estradiol exhibits a 12-fold increase prior to ovulation. Progesterone concentrations increase 800-fold during the luteal phase. **B.** Hormone concentrations during 30 days on a combined oral hormonal contraceptive (0.02 mg ethinyl-estradiol, 0.1 mg levonorgestrel). In response to this OC formulation, progesterone was suppressed by 97% on average while estradiol concentrations were unmodified. Note that exogenous hormone concentrations (not shown here) were very low: ethinyl estradiol, M=0.01 ng/mL; levonorgestrel, M=0.91 ng/mL. Abbreviations: *P*, progesterone; *LH*, luteinizing hormone; FSH, follicle-stimulating hormone (see *Taylor et al., 2020)*

below those observed in the early follicular phase of naturally cycling controls (De Bondt et al., 2013b). In contrast, a hormone regimen of low-dose ethinyl estradiol (0.02 mg) and levonorgestrel (0.1 mg) had strong suppressive effects on progesterone, with serum concentrations reduced by ~97% over a 28-day period, but no detectable suppressive effect on estradiol (**Figure 2**). Under this OC regimen (Aubra, Afaxys Pharmaceuticals), dynamic changes in estradiol mimicked those observed under naturally cycling conditions (Taylor et al., 2020). Thus, when forming hypotheses about neuronal effects of OC it is critical to classify OC formulations based on the downstream endocrine effects of each regimen. Failing to do so will make findings uninterpretable and hinder efforts at reproducibility. For a complete picture, studies should also assess serum concentrations of *exogenous* hormones, i.e. those attributable to the hormone regimen itself.

Finally, to fully understand the neurobiological effects of OC, we need preclinical animal studies that interrogate the extent to which ovarian hormone suppression alters hormone

concentrations locally in the CNS. Rodent studies suggest some congruity between central and peripheral levels. For example, concentrations of sex hormones from serum are correlated with levels acquired from cerebral cortex and hippocampal tissue (Caruso et al., 2013) and a 4-week OC regimen of ethinyl estradiol/levonorgestrel suppressed concentrations of progesterone in the hippocampus by 65% (Porcu et al., 2012). In contrast, a recent study in marmosets reported opposing effects of an aromatase inhibitor on peripheral and central estradiol concentrations (Gervais et al., 2019). Peripheral hormone suppression could induce compensatory upregulation of hormone synthesis *de novo* in the brain, and this should be clarified in future studies addressing the complex relationship between peripheral and central hormone levels.

3.5 Defining a control group

In the current literature, comparisons are often drawn between women currently using OC versus those not using OC. However, this comparison group conflates women who are naturally cycling now but have used OC in the past ("ever users") with women who have never used OC ("never users"). The hormonal milieu of past OC users may not be the same as women who have never used hormonal contraception. Some evidence suggests that the suppression in endogenous ovarian hormone levels induced by OC persists after pill discontinuation (Balogh et al., 1981; Panzer et al., 2006). Given our limited knowledge of long-term effects of OC use, control groups that mix "ever" and "never" users may obscure findings.

3.6 Age of initiation and duration of use

Finally, two additional understudied factors that may shape OC's influence on the brain are *age of initiation* and *duration of use*. Up to one-third of OC users begin OC use in early adolescence, yet we know relatively little about how hormone suppression impacts the developing brain

(Cahill, 2018). While the hippocampus and basal ganglia typically reach maturity in late childhood or early adolescence (Gogtay et al., 2006; Segawa, 2000), the development of the PFC is protracted, with cortical volumes stabilizing in the mid-20s (Lenroot and Giedd, 2006). The neuroendocrine changes that accompany puberty produce a second 'window of opportunity' or sensitive period in brain development (see Fuhrmann et al., 2015, for review).

In girls, the pubertal transition typically begins at 10–11 years of age and ends between the ages of 15 and 17. Many women begin OC use during this pubertal period. In a US study, 36% of 13–18-year-olds filled a prescription for OC (Ehrlich et al., 2011), and in a population Danish study, ~28% of 15–19-year-olds used OC (Skovlund et al., 2016). Given the early age of first exposure, OC use in adolescence has the potential to alter the organizational effects of endogenous sex hormones via chronic ovarian hormone suppression. However, to our knowledge, no large-scale prospective study has examined the impact of age of initiation and duration of OC use on neuronal development. Further, the short-term and long-term effects of OC may differ. In adults, even short-term OC use is associated with gray matter volume changes (Lisofsky et al., 2016; Pletzer et al., 2015), however it is unclear whether these changes persist over time (Pletzer et al., 2019), or whether the magnitude of change tracks with total duration of use over longer timescales (e.g. years, decades).

4. A roadmap for the future: harnessing new methodological and technological approaches to bolster women's health research

Below we propose three programmatic initiatives to advance knowledge on women's health in neuroscience. We describe "Big Data" approaches, such as the *University of California Women's Brain Initiative*, that are beginning to address unmet areas of women's health research at the

population level. Next, we describe innovations in methodological and computational approaches in human neuroimaging that capture the *dynamic* properties of the endocrine system. We end with a vision for cross-species translational studies that capitalize on emerging technologies from systems neuroscience to decipher estrogen and progesterone's influence on populations of neurons recorded chronically at subcellular resolution. Our hope is that together these approaches generate novel discoveries about hormone action in the mammalian brain and stimulate research efforts, particularly within the human neuroimaging community.

4.1 The University of California Women's Brain Initiative: Using 'Big Data' to benefit women's health

Over the last ten years human neuroimaging has witnessed a remarkable growth in "Big Data" initiatives that are mapping the structural and functional connectome of the human brain at the population level. Large-scale, multi-site, "population neuroscience" approaches like the Human Connectome Project (HCP) (Van Essen et al., 2013) have transformed our understanding of brain organization and variability across disease states. Sister studies such as HCP-Aging (Bookheimer et al., 2019) and HCP-Development (Somerville et al., 2018) bring a lifespan perspective, while UK Biobank (Sudlow et al., 2015) merges brain phenotyping with extensive electronic health records in midlife and older adults. These initiatives offer an invaluable resource for probing fundamental questions about the human brain, yet it is striking that none were designed with women's health in mind.

To address this, in 2019 we launched a population-based neuroimaging database dedicated specifically to strengthening women's health research. The *University of California Women's Brain Initiative* (UC-WBI) leverages the activity of the University of California's brain imaging community. Although still in its infancy (data collection has rolled out at UC Santa Barbara and

UC Berkeley, with a current n=400), our goal is to expand to the nine UC campuses with a research-dedicated MRI facility, targeting the ~10,000 unique individuals scanned across sites each year. In addition to pooling standard MRI sequences and demographic/behavioral data, the UC-WBI provides extensive life-history data across a range of women's health factors via a Women's Reproductive Health History battery.

One driving question for the UC-WBI is to leverage the population neuroimaging approach to understand how oral hormonal contraceptives impact the human brain. OC use is the kind of multifactorial problem that would benefit from a large-scale dataset that captures normal variability in OC use among the population. Using data generated from the UC-WBI database, we are investigating the association between OC use and brain morphology with respect to a person's age of initiation, duration of use, and OC formulation, with participants matched across a broad range of demographic variables. This approach will set a new standard for OC–brain research, help define a path forward for rigorous, controlled follow-up studies, and represents one of a multitude of research questions that can be asked within the broader UC-WBI framework. Ultimately, our goal is to provide an open-access dataset that the neuroimaging community can draw upon to ask questions at the intersection of women's health and the brain.

4.2 Dense-sampling neuroimaging studies capture the dynamic properties of the endocrine system

A central feature of the mammalian endocrine system is that hormone secretion varies over time. Circadian, infradian, and circannual rhythms are essential for sustaining many physiological processes. However, the study of brain–hormone interactions in human neuroscience relies heavily on cross-sectional designs that, by nature, cannot capture dynamic changes in hormone

production. In network neuroscience, an emerging trend is to flip the cross-sectional design by densely sampling individuals over timescales of weeks, months, or years to provide greater insight into the dynamic properties of the human brain. Applying these dense-sampling approaches to probe brain–hormone interactions could reveal organizational principles of the functional connectome previously unknown, transforming our understanding of how hormones influence brain states.

For example, in a series of dense-sampling studies we probed the dynamic properties of the brain over a complete menstrual cycle (30 consecutive days) and throughout an oral contraceptive regimen (30 consecutive days) (Pritschet et al., 2019; Taylor et al., 2020). Using high-resolution imaging of the medial temporal lobe (MTL) and daily serum hormone measurements, we discovered that intrinsic fluctuations in progesterone across the menstrual cycle are associated with volumetric changes in CA2/3, entorhinal, perirhinal, and parahippocampal cortex. Chronic progesterone suppression induced by the OC (**Figure 2**) abolished these cycle-dependent effects. These results suggest that progesterone can rapidly and dynamically shape MTL morphology across the human menstrual cycle over unprecedented time-scales (Taylor et al., 2020). We then used resting-state functional MRI to investigate how sex hormones modulate day-to-day changes in the brain's intrinsic functional network architecture. Estradiol facilitated tighter coherence across broad swaths of cortex while progesterone had the opposite, inhibitory effect (Pritschet et al., 2019). These effects were pronounced in functional network hubs populated with estrogen receptors and offer compelling evidence that sex hormones modulate widespread patterns of connectivity in the human brain. Moving forward, these dense-sampling approaches could be applied to brain imaging studies of

other major neuroendocrine transitions, such as pubertal development and reproductive aging (e.g. menopause).

4.3 Systems Neuroscience Approaches

To fully understand hormone action in the mammalian brain, research efforts should be harmonized across rodent, nonhuman primate and human studies using translational and back-translational approaches. In particular, emerging technologies from systems neuroscience could be leveraged to decipher estrogen and progesterone's influence on populations of neurons via chronic recording in awake behaving animals. Despite powerful evidence that sex steroid hormones influence spine structure and synaptic plasticity in rodents (Frick et al., 2015; Frick and Kim, 2018; Galea et al., 2017; Hara et al., 2015; Woolley and McEwen, 1993), hormonal influences on neural processing at the cellular and microcircuit level in intact animals is poorly understood. For example, an open question is whether estradiol-driven spine turnover in the hippocampus induces functional changes in hippocampal neuron activity during the performance of a cognitive task (e.g. navigation).

Historically, it has been technically difficult to chronically record neural activity from the same neurons across many sessions (e.g. over a 4–5-day rodent estrous cycle). However, recent developments in genetically encoded sensors and physiology instrumentation have greatly improved researchers' ability to measure activity in the same neurons over long timescales. Genetically-encoded calcium indicators (Chen et al., 2013; Tian et al., 2009) combined with 2-photon imaging enable the chronic measurement of activity in large neural populations over several weeks in the hippocampus (Hainmueller and Bartos, 2018; Kaufman et al., 2020) and cortex (Driscoll et al., 2017; Huber et al., 2012; Pho et al., 2018). Moreover, genetic

identification of particular cell types can indicate exactly how sex steroid hormones modulate neural microcircuitry, and has already been used effectively to investigate estrous cycle regulation of social touch (Clemens et al., 2019). This approach could be leveraged to measure changes in functional properties (e.g. hippocampal place fields) across the estrous cycle and disambiguate the specific cell types that are modulated. Measuring changes in large-scale neural activity across the estrous cycle or in response to pharmacological manipulation would offer a powerful approach for understanding how gonadal hormones influence neural responses and cognitive processing at the systems level.

5. Conclusion

Fifty years of basic science research has established a critical role for sex hormones in higher-order brain regions, including the hippocampus and prefrontal cortex. Yet, human brain imaging studies overlook basic elements of endocrinology and women's reproductive health. Moving forward, large-scale population-based studies, targeted dense-sampling studies, and translational research will provide novel insight into sex hormone action in the mammalian brain. Finally, without gender equality in scientific leadership positions, scientists will continue to overlook aspects of the human condition that are relevant to half of the world's population (Jacobs et al., 2020). Applying a women's health lens to the study of the human brain is long overdue. Doing so may be critical for understanding basic principles of brain function and for women's health at large.

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Supplementary Information

The scientific body of knowledge – whose body does it serve? A spotlight on oral contraceptives and the brain

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Background

Human neuroscience systematically overlooks aspects of the human condition that impact women's health. Here, we quantify this oversight within human neuroscience research in two distinct ways. We first take a deep dive into typical human neuroscience investigations to assess the frequency of reporting on and consideration of women's health factors in a recent year (2018). Then, to capture the history of this oversight in a larger context, we conducted a Pubmed (PM) search to estimate the relative frequency of women's health and brain imaging publications over time relative to all brain imaging publications. The combination of these approaches allows us to better understand both the history and current state of the field of women's health research in human neuroscience.

Human Neuroscience and Women's Health Factors - 2018

All research articles from 2018 published in five top human neuroscience journals *(Nature Neuroscience, Neuron, Journal of Neuroscience, Neuroimage,* and *Human Brain Mapping*) were downloaded and evaluated by a trained research associate. An article remained in the analysis if it fit the following criteria: 1) study involved human participants, and 2) used one of the following non-invasive brain imaging techniques: structural or functional magnetic resonance imaging (MRI), diffusion-weighted imaging (DWI), transcranial magnetic stimulation (TMS), electroencephalogram (EEG), or magnetoencephalography (MEG). A total of 1,066 papers fit these criteria and were further evaluated.

Two research assistants extracted author gender, institution, funding sources, and various participant demographics (gender, ethnicity, handedness, education levels) from each article and recorded this information in an excel spreadsheet. To examine whether a paper considered or

recognized any of a range of women's health factors, a keyword search for the following terms was conducted: "hormon", "menstr", "meno", "endo", "oral", and "cycle". These keywords were chosen to direct the coder's attention to any mention of women's health–related topics, such as hormonal status/disorders, menstrual cycles, endocrine-related conditions, menopause, oral contraceptive use, pregnancy, etc. If an article mentioned and considered any of these keywords in the main text (for examples, see **Supplementary Table 1**), it was coded as "1". Articles that used endocrine variables to exclude participants were also coded as "1" (e.g. "*Women were excluded from the study as changes in ovarian hormones may influence cortical excitability in humans…"*). However, if the keyword was identified in the document but was only referenced with respect to previous literature, future directions, or within the references, it was coded as "0".

Reliability assessments revealed a 98.8% agreement on keyword identification between the two coders; an investigator (LP) then analyzed the discrepancies and made final coding decisions. Further, a research assistant examined the first 20 articles from each journal to assess whether online supplementary materials might contain additional relevant information. No new or relevant information regarding any of the keywords was obtained from this search and therefore supplementary material of the remaining articles were not evaluated further. A final count of 29 papers were identified as using one or more of these keywords (**Supplementary Table 2**).

It is important to note that while there was high inter-coder reliability and a systematic search into the collection and keyword identification of these articles, there are likely discrepancies, albeit minimal, in the true number of articles to be included in this analysis. Therefore, this survey offers an estimate of the state of the field but does not represent an absolute accounting.

Table S1. Text examples of keywords coded as '1'

keyword	*text excerpt*
"hormon"	"Further exclusion criteria were…**hormon**al treatment…"
"menstr"	"The **menstr**ual cycle of naturally cycling women was recorded"
"meno"	"Female participants self-reported **meno**pausal status"
"endo"	"Participants…reported…no history of or current **endo**crine treatment"
"oral"	"None of the female participants had been using **oral** contraceptives"
"cycle"	"did not have resources to include females from different **cycle** phases…restricted study to only male participants"

Human Neuroscience and Women's Health Factors – 1995–2018

A broader survey of the literature was conducted using the PubMed search engine. This search was limited to articles published between 1995 (signifying the rise and use of MRI) and 2018, the most recent year to be fully indexed. Search results were downloaded as a .csv file that listed the article count per year.

To quantify the number of brain imaging papers on women's health, we conducted an advanced search with the keywords "estrogen", "progesterone", "pregnancy", "menopause", "menstrual cycle", "contraceptives", or "birth control", paired with "MRI" and "brain", while excluding the term "fetal". Further inclusion criteria were: human investigations, case studies, clinical trials, meta-analyses, observational studies, technical reports, and journal articles. This search yielded a total of 286 papers.

Two additional searches were conducted to compare the rate of women's health publications against the backdrop of more general human brain imaging publications. First, we

quantified the number of all brain imaging papers published between 1995-2018 using the keywords ‘brain” and “MRI”. A total of 41,379 papers were identified using this advanced search. We then quantified the number of human brain imaging papers dedicated to reward processing, to provide an additional discipline-specific publication rate comparison. An advanced search with keywords “reward”, “reward processing”, or “reward circuit” was paired with “MRI” and “brain” yielded a total of 2,995. These additional searches were also narrowed to human investigations, case studies, clinical trials, meta-analyses, observational studies, technical reports, and journal articles.

Table S2. Articles reporting on endocrine status

Paper	DOI
01	10.1016/j.neuroimage.2018.01.012
02	10.1016/j.neuroimage.2017.12.061
03	10.1016/j.neuroimage.2018.01.010
04	10.1016/j.neuroimage.2018.01.024
05	10.1016/j.neuroimage.2017.12.092
06	10.1016/j.neuroimage.2018.01.027
07	10.1016/j.neuroimage.2018.01.043
08	10.1016/j.neuroimage.2018.08.058
09	10.1016/j.neuroimage.2018.08.043
10	10.1016/j.neuroimage.2018.07.058
11	10.1016/j.neuroimage.2018.08.004
12	10.1016/j.neuroimage.2018.08.040
13	10.1016/j.neuroimage.2018.03.055
14	10.1016/j.neuroimage.2018.04.013
15	10.1016/j.neuroimage.2018.04.072
16	10.1016/j.neuroimage.2018.02.038
17	10.1016/j.neuroimage.2018.07.050
18	10.1523/jneurosci.2097-17.2017
19	10.1002/hbm.23850
20	10.1002/hbm.23888
21	10.1002/hbm.23908
22	10.1002/hbm.23916
23	10.1002/hbm.23942
24	10.1002/hbm.24003
25	10.1002/hbm.24019
26	10.1002/hbm.24030
27	10.1002/hbm.24069
28	10.1002/hbm.24336
29	10.1002/hbm.24345