Oral Contraceptives and Ovarian Steroid Hormones: The Effects on the Brain and Behaviour in Young Adult Women

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Abstract

Puberty is a critical period of development characterized by rapid physiological changes and significant brain reorganizing and remodeling in women. These rapid changes render the developing brain particularly vulnerable to stress and exogenous factors. According to recent trends, women are increasingly likely to begin using exogenous/synthetic hormones (i.e., oral contraceptives; OCs) as early as during puberty. While millions of women worldwide use OCs, the extent of OC-related structural, functional, and behavioral changes and the role of ovarian steroid hormones remain elusive. Thus, we hypothesized that hormonal modulation through OC use during puberty would result in unique neural and behavioural outcomes compared to OC use later in life.

The current thesis examined the effects of OC use, especially during puberty, on cortisol reactivity, brain structure, and brain function during a working memory cognitive task (Article 1). Next, we examined whether the task-related functional differences between OC users and non-OC users could be informed by resting state functional connectivity in the default mode, salience, central executive, and, reward networks (Article 2). Lastly, we examined the roles of progesterone and estradiol on emotion processing during the cognitive task in all women (Article 3). The current dissertation has addressed OC-related differences to the brain and behaviour and the influence of ovarian steroid hormones in these differences. These studies have demonstrated that OC use is related to specific structural and functional differences, with some findings being dependent on the age of OC onset and duration of OC use. The findings offer critical insight into women's brain health and bring awareness to the potential behavioural and neural consequences of OC use and role of ovarian steroid hormones.

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General Introduction: Conceptual and Theoretical Background

Pubertal development and sexual maturation

The terms puberty and adolescence are often used interchangeably. While they do overlap in time, they represent two fundamentally distinct phases of development. In humans, puberty occurs at the beginning of adolescence and involves a transition from a non-reproductive to a reproductive-state (Sisk & Foster, 2004). Puberty is initiated by the peptide hormone kisspeptin (KISS), which is encoded by the Kiss-1 gene (West et al., 1998; Kotani et al., 2001). KISS initiates puberty by increasing the pulsatile secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus when it binds to a G-protein coupled receptor called GPR54 (Millar et al., 2010; Rhie et al., 2013). More than 75% of GnRH neurons in the mammalian hypothalamus co-express GPR54 mRNA (Rhie et al., 2013). Modulation of the GnRH pulse frequency is the primary mechanism through which the body alters its reproductive status during puberty giving rise to the hypothalamic-pituitary-gonadal (HPG) axis (Sisk & Foster, 2004). GnRH enters the pituitary gland to signal the synthesis and release of two pituitary gonadotropins: luteinizing hormone (LH) and follicle stimulating hormone (FSH). Peripheral LH and FSH act on target cells in the testes and ovaries to direct the production of sperm and eggs, as well as the secretion of gonadal steroid hormones (estrogens, progestins, and androgens). The rise in these circulating gonadal steroid hormones influences GnRH secretion via feedback loops in the brain and participates in spermatogenesis and follicle maturation within the gonads (Baerwald et al., 2011). Puberty is accompanied by several physiological changes including rapid physical growth and the development of secondary sex characteristics. A reliable staging system of these characteristics was developed by James Tanner in the 1960s (Marshall & Tanner

1969; 1970). Tanner staging categorizes boys and girls along an ordinal scale (1 to 5) of pubertal development (Marshall & Tanner, 1969; 1970).

In boys, puberty begins around 11 to 12 years of age, and males can have ongoing physical changes up until 16 to 17 years of age (Marshall & Tanner, 1970). Reproductive competence is characterized by spermaturia and first ejaculation, which typically occurs from 13 to 14 years of age (Marshall & Tanner, 1970; Patton & Viner, 2007). Tanner stage I is the prepubertal stage. The first major sign of puberty for boys is when the skin on the scrotum thins, reddens, and enlarges in stage II (~9 to 11 years old). During this time, the testes also enlarge. At stage III, testicular volume continues to increase to greater than 4 mL, the scrotum enlarges further, and penis length begins to increase (~11 to 12.5 years old). Testicular volume increases up to 20 mL during stage IV, and the scrotum enlarges further and thickens while penis length continues to increase (~12.5 to 14 years old). Finally, in stage V, testicular volume increases to greater than 20 mL and adult scrotum and penis sizes are reached (~15+ years old) (Marshall & Tanner, 1970).

Compared to boys, pubertal maturation begins earlier in girls at around 10 to 11 years of age and is fully completed by 15 to 16 years of age (Marshall & Tanner, 1969). In girls, Tanner stage I is typically the prepubertal stage (10 years old and younger) during which there is no glandular tissue or pubic hair. The first signs of pubertal onset in girls are the appearance of breast buds and pubic hair in stage II (~10 to 11.5 years old). During stage III, the breasts begin to grow and the texture of pubic hair changes (i.e., coarser/curlier) while extending laterally (~11.5 to 13 years old). Breast size and elevation continue to increase during stage IV and adult-like pubic hair quality appears (~13 to 15 years old). Finally, in stage V, the breasts reach their final size and pubic hair extends to the medial surface of the thighs (~15+ years old). The

culminating event of female pubertal development is menarche, which is the first occurrence of menstruation that is reached on average at the age of 13 years old (Marshall & Tanner, 1969). While the age of menarche varies, it typically occurs sometime between Tanner stages II and IV (Marshall & Tanner, 1969).

The menstrual cycle has an average length of 28 days (Buffet et al., 1998), and it is characterized by fluctuations in the concentration of ovarian steroid hormones, estrogens (mostly 17β-estradiol) and progestins (mostly progesterone), that are regulated by the HPG axis. The menstrual cycle is divided into 5 phases: early follicular, follicular, late follicular/pre-ovulatory, ovulatory, and luteal (Fauser & van Heusden, 1997). The early-follicular phase is marked by menstruation and a low concentration of ovarian steroid hormones. FSH secretion begins to rise during this phase, leading to the maturation of ovarian follicles. As FSH and LH levels continue to rise during the follicular phase, the dominant developing follicle causes a rise in estradiol levels. The hypothalamus recognizes these rising levels and further releases GnRH. The late follicular/pre-ovulatory phase constitutes the period during which the surge in FSH and LH levels causes estradiol to peak. Ovulation typically lasts from 24 to 36 hours and results in the rupture of an ovarian follicle, causing the ovum (i.e., egg) to be released from the ovary. After ovulation, the ruptured follicle continues to grow for some time and transforms into the corpus luteum, which produces significant amounts of progesterone and estradiol as well, but to a lesser extent. This estradiol production after ovulation is known as a secondary estradiol surge (Weschler, 2002). The hormones produced by the corpus luteum also suppress the production of LH and FSH. With continued low levels of LH and FSH, the corpus luteum undergoes gradual atrophy, and the death of the corpus luteum results in falling levels of estradiol and progesterone. These falling levels of ovarian steroid hormones trigger the end of the luteal phase and stimulate

menstruation, which begins the onset of the next menstrual cycle (Fauser & van Heusden, 1997). Variability in menstrual cycle length is the greatest 1 to 2 years following menarche (Treloar et al., 1967) and approximately 80% of young girls are often anovulatory in the first year after menarche (Apter et al., 1978; Apter, 1980) (Figure 1). Thus, puberty consists of an influx in endogenous gonadal steroid hormones in boys and girls that triggers physiological development across adolescence.

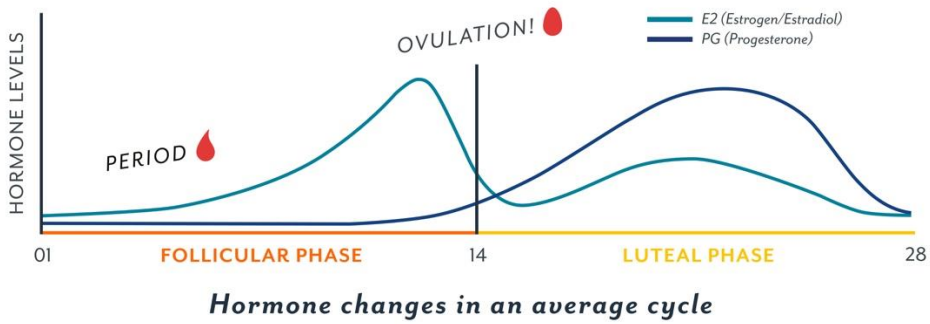

Figure 1. Schematic representation of the hormonal fluctuations across the menstrual cycle.

Brain development during puberty

The influx of gonadal steroid hormones during puberty also induces changes to regional brain volume. The brain undergoes dynamic and complex changes in volume and cortical thickness across puberty and adolescence, with the most prominent region-specific increases and decreases occurring between 9 and 13 years of age in boys and girls (Lenroot et al., 2007; Shaw et al., 2008). The brain is composed of two different types of structural tissue. The grey matter (GM) in the brain is composed of cell bodies, dendrites, and non-myelinated axons, as well as glial cells and capillaries. White matter (WM) is composed of nerve fibers (i.e., axons) that are covered by myelin (Blakemore et al., 2010). The current literature reports somewhat contradictory findings in structural volume changes, which may be attributed to methodological

differences between studies. Some studies solely use Tanner staging to describe pubertal development, whereas other studies include both Tanner staging and a direct measurement of gonadal steroid hormones. The studies in the current literature are also discrepant in the age range of participants examined, cross-sectional versus longitudinal research designs, hormonal analyses (i.e., separated by sex or collapsed), and MRI correction factors. There are greater inconsistencies with regards to GM than WM development across puberty and adolescence.

Grey matter changes. GM volume follows an inverted U-shaped developmental trajectory, initially increasing in volume during childhood, reaching a peak in early adolescence, and declining steadily into adulthood (Brain Development Cooperative Group-NIH, 2012). The inverted U-shaped developmental trajectory in GM volume is due to dendritic outgrowth and synaptogenesis early in development, followed by synaptic pruning during adolescence (Giedd et al., 1999). Non-linear patterns of GM development have been identified across the frontal, temporal, and parietal cortical lobes along with consistent sex differences (Giedd et al., 1999; Lenroot et al., 2007). More specifically, in the frontal lobe, GM volume peaks at 9.5 years of age in girls and at 10.5 years of age in boys. In the parietal lobe, GM volume peaks at age 7.5 in girls and at age 9 in boys. Finally, in the temporal lobe, GM volume peaks at age 10 in girls and at age 11 in boys, before undergoing a sequence of thinning into adulthood (Lenroot et al., 2007).

The organization of GM development by gonadal steroid hormones during puberty contributes to numerous sex differences. As boys and girls age and advance in Tanner staging (up to 15 years of age), a decrease in regional GM volume is prominent in the anterior brain regions (i.e. lateral and frontal medial, anterior cingulate, precuneus, and parietal) (Sullivan et al., 2011). Similarly, advanced Tanner staging in children from 7 to 20 years of age is related to decreased GM volume in the nucleus accumbens, caudate, putamen, and globus pallidus but

increased GM volume in the amygdala and hippocampus in both boys and girls (Goddings et al., 2014). Gonadal steroid hormones also have different effects on GM development when boys and girls are examined separately, which invalidates the findings from studies examining both sexes together. For example, girls with advanced Tanner staging show larger GM volumes in the amygdala, hippocampus, putamen, and caudate in late childhood/early adolescence, an earlier peak in global (i.e., total) GM volume, and then a smaller GM volume until the end of puberty compared to their age-matched peers (Blanton et al., 2012; Goddings et al., 2014; Neufang et al., 2009). Moreover, in a cross-sectional study, global GM volume negatively correlates with estradiol levels in girls, but positively correlates with testosterone levels in boys (Peper et al., 2009a). However, in a longitudinal study with adolescents aged 10 to 14 years old, girls with lower estradiol levels at the time of the first scan display more robust decreases or pruning in GM volume across adolescence compared to their peers with higher estradiol levels (Herting et al., 2014). To add, in girls aged 10 to 15 years old, high levels of estradiol are associated with decreases in prefrontal, parietal, and middle temporal GM volumes, but are associated with increases in GM volumes in middle frontal-, inferior temporal-, and middle occipital gyri (Peper et al., 2009a). In age-matched boys (10 to 15 years old), both estradiol and testosterone levels are unrelated to regional GM volume (Peper et al., 2009a). However, there are sexually dimorphic differences in GM volume with boys showing greater volume in the putamen, insula, and amygdala than girls irrespective of gonadal steroid hormone levels (Peper et al., 2009a). Therefore, ovarian steroid hormones partly contribute to GM volume development in both boys and girls.

However, when both sexes are examined together, gonadal steroid hormones have slightly different effects on GM volume. In boys and girls aged between 8 and 15 years old,

increased circulating estradiol levels are related to increased GM volume in the parahippocampal gyrus. Higher circulating testosterone levels are related to increased GM volume in diencephalic brain structures and are related to decreased GM volume in parietal regions in both boys and girls (Neufang et al., 2009). The discrepancies between Peper et al. (2009a) and Neufang et al. (2009) could be due to the aforementioned methodological differences. Nonetheless, these findings show that there are important differences in GM development across puberty and adolescence that are mediated by gonadal steroid hormones, which result in adult structural differences between men and women.

Cortical thickness. Pubertal development also affects cortical thickness. In girls, increases in estradiol levels predict greater middle temporal lobe thinning at a two-year follow-up in girls aged 10 to 14 years old (Herting et al., 2015). In similarly aged girls, testosterone levels are inversely correlated with cortical thickness in the occipital lobe compared to boys matched on Tanner staging (Bramen et al., 2012). In boys aged 11 to 14 years old, testosterone levels are positively correlated with cortical thickness in limbic and primary sensory cortices of the occipital lobes (Bramen et al., 2012). It is thought that the opposing effects of testosterone levels on cortical thickness in boys and girls are due to differences in androgen receptor signaling efficiency. Higher androgen receptor signaling efficiency is related to region-specific increases or decreases in cortical thickness in boys, whereas it predicts only cortical thinning in girls (Raznahan et al., 2010; Herting et al., 2015). Therefore, testosterone levels have opposing effects on cortical thickness in boys and girls across puberty and adolescence.

White matter. In comparison to GM development, global WM typically follows a steady linear increase between childhood and adolescence, and slowly stabilizes in adulthood (Giedd et al., 1999). During early puberty, LH levels predict WM volume densities in both boys and girls

(Peper et al., 2008). More specifically, larger changes in total WM volume occur in boys and girls between 10 to 14 years old, during early pubertal maturation (Tanner stage I), as indexed by lower gonadal steroid hormone levels, followed by less robust changes during late puberty (Herting et al., 2014). However, the rate of change in regional WM volume differs between boys and girls across adolescence. Boys show considerably steeper age-related increases and decreases in regional WM volume and integrity compared to age-matched girls (Lenroot et al., 2007; Perrin et al., 2009; Herting et al., 2011). Tanner staging and gonadal hormone levels also interact with sex to predict regional WM volume. For example, advanced Tanner staging and higher levels of testosterone in boys are associated with decreased WM volume in the right amygdala (Herting et al., 2014) but increased WM integrity in previously identified sexually dimorphic regions such as the superior and inferior frontal gyri, superior temporal gyrus, thalamus, and midbrain (Herting et al., 2011). In contrast, higher testosterone and estradiol concentrations in girls are associated with increased WM volume in the right amygdala (Herting et al., 2014). Estradiol, on its own, shows inconsistent associations with regional WM integrity in girls (Herting et al., 2011). The difference between boys and girls may be attributed to the sex-specific mechanisms that underlie the development of WM during adolescence. For example, in boys, increases in WM volume with age is associated with increases in axonal caliber. However, in girls, increases in WM volume is associated with increased myelination (Perrin et al., 2009).

Functional changes. Pubertal gonadal steroid hormones mediate functional changes that are associated with socio-emotional development throughout adolescence. In girls aged 11 to 14 years old, increasing levels of gonadal steroid hormones (i.e., testosterone, estradiol, and dehydroepiandrosterone) are associated with higher brain activity in the anterior temporal lobe during a socio-emotional processing task that requires the representation of mental states (i.e.,

understanding others' mental states are different from oneself) (Goddings et al., 2012). This region has been previously implicated in the processing of social emotions such as guilt and embarrassment (Zahn et al., 2007). Furthermore, pubertal maturation results in altered brain function at rest (i.e., resting state functional connectivity). For example, advanced pubertal maturation in girls at 14 years of age results in decreased functional connectivity in the anterior cingulate cortex within the default mode network. This decreased connectivity is associated with higher mood dysregulation symptoms at a two-year follow up (Ernst et al., 2019). In contrast to functional connectivity changes in late pubertal girls, increased functional connectivity in the salience network is associated with higher levels of depression-like behavior in early pubertal girls (Ordaz et al., 2017). Thus, pubertal changes in brain function at rest and during task induction impact socio-emotional development, which then affects mood.

Cognitive changes. The hormonal changes during puberty also contribute to cognitive changes throughout adolescence. For example, there is evidence for a pubertal "dip" with regards to certain cognitive processes like facial emotion processing. Pubertal girls and boys, between the ages of 11 and 12 years old, show an increase in reaction time when matching emotional words to emotional facial expressions. The increase in reaction time, which reflects a relative inefficiency in frontal circuitry, declines slowly over the following 2 to 3 years and stabilizes by 15 years of age (McGivern et al., 2002). Thus, the cognitive changes mediated by pubertal brain development can bias early adolescent trajectories in potentially negative directions, and in ways that may begin as small influences but could spiral into large-scale effects over time (Peper & Dahl, 2013), such as increased vulnerability to stress- and mood-related disorders, especially in girls (Kessler et al., 2005).

Overall, the influx of gonadal steroid hormones during puberty regulates the structural and functional brain changes that give rise to social, emotional, and cognitive development across adolescence in a sexually dimorphic manner.

Ovarian steroid hormones influence brain and behaviour

Hormonal fluctuations and an undue sensitivity to such fluctuations in brain systems that mediate mood-related states are also thought to be involved in the predisposition to mood disorders in women. Fluctuations in ovarian steroid hormones alter brain structure and physiology across the natural menstrual cycle. For example, bilateral increases in hippocampal GM volume and WM integrity have been observed in the late follicular/pre-ovulatory phase relative to the late luteal/early follicular phase (Protopopescu et al., 2008; Lisofsky et al., 2015a; Barth et al., 2016). In the preovulatory phase, there is also more functional connectivity between the hippocampus and surrounding regions (Lisofsky et al., 2015a). Accordingly, this suggests a mediating role of estradiol on hippocampal volume and brain activation at rest (Lisofsky et al., 2015a). Hormonal variations across the menstrual cycle also affect task-induced brain function and behaviour. Several limbic, frontal, and hypothalamic regions show significantly reduced brain function to negative stimuli processing during the late follicular/pre-ovulatory phase, when estrogen levels are high, compared to the early follicular phase when estrogen levels are low (Goldstein et al., 2005). Progesterone, on the other hand, stimulates the neural circuitry involved in arousal during emotion processing. Previous research consistently depicts increased amygdala activation in response to negative emotional stimuli and increased ACC activation in response to positive emotional stimuli when progesterone levels are high in the luteal phase (Protopescu et al., 2005; Amin et al., 2006; Andreano and Cahill, 2010; Gingnell et al., 2012; Bayer et al., 2014). Lastly, functional lateralization of the hippocampus and amygdala changes across the

phases of the menstrual cycle (Lisofsky et al., 2015b). However, few studies have used regression analyses to identify relationships between endogenous hormone concentrations and brain activity in NC women (Andreano & Cahill, 2010; Miedl et al., 2018).

The cyclical changes in ovarian steroid hormones across the menstrual cycle also give rise to mood-related changes. While there is significant intra-individual variance in mood across the menstrual cycle (Lorenz et al., 2017), a majority of women (61%) experience less negative mood symptoms between menstruation onset and ovulation. These women then experience an increase in negative mood symptoms during the late-luteal period prior to the onset of the next menstrual cycle (Kiesner, 2011; Kwan & Onwude, 2015). The aforementioned mood pattern characterizes the classic premenstrual syndrome (PMS), which can be described as a constellation of adverse psychological symptoms that include worsening of mood (e.g., sadness or irritability), often triggered by relatively minor stressors related to everyday life (O'Brien et al., 2011). To contrast the classic PMS pattern, a small percentage of women (~13%) show a mid-luteal increase in psychological symptoms, like negative affect, low motivation, and aggression, peaking around ovulation with the least symptoms occurring during the pre-menstrual and menstrual periods. On the other hand, some women demonstrate no cyclical pattern of mood symptoms (Kiesner et al., 2011). While it is unclear how and why hormones during the menstrual cycle affect mood, these cyclical changes in hormones and mood are initiated at puberty. Thus, it is clear that puberty is a highly programmed event that shapes brain-behaviour interactions and may then set the stage for the onset of mood-related disorders, especially in girls later in life.

Development of the stress response during puberty

The hormonal changes during puberty also contribute to the maturation of the hypothalamic-pituitary-adrenal (HPA) axis. Maturation of the HPA axis often begins earlier than sexual maturation, typically between 6 and 9 years of age in girls and between 7 and 10 years of age in boys (Dorn et al., 2006). The HPA axis is involved in "allostasis", which means that it is a mechanism by which adaptive changes are initiated to maintain physiological stability in an organism (Korte et al., 2005; McEwen, 2000; McCormick et al., 2010). The HPA axis requires the perception of external and internal challenges that are typically not predictable. Signals of external and internal stressors are then relayed to the paraventricular nucleus (PVN) of the hypothalamus. The HPA axis controls the release of corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) from the PVN into the hypophysial portal veins. Activation of the PVN causes adrenocorticotrophic hormone (ACTH) to be released from the anterior pituitary gland into the bloodstream (Whitnall, 1993). ACTH, in turn, causes the synthesis and release of glucocorticoid hormones from the adrenal cortex into the bloodstream. Adrenal release of glucocorticoids is moderated by diverse factors other than ACTH from the pituitary, such as paracrine factors within the adrenal cortex, and a multi-synaptic pathway from the suprachiasmatic nucleus of the hypothalamus to the adrenal cortex (Bornstein et al., 2008). The HPA axis therefore regulates the production of glucocorticoid hormones (primarily cortisol in humans and corticosterone in rodents).

The autonomic nervous system (ANS) is another system involved in the peripheral physiological stress response. The ANS is composed of two coordinated and often opposing systems: the sympathetic nervous system (mediates activating processes) and the parasympathetic nervous system (mediates vegetative processes), which interact to produce

various degrees of arousal. Activation of the sympathetic nervous system leads to increased release of epinephrine and norepinephrine from the adrenal medulla. Activation of the sympathetic nervous system also results in physiological symptoms of the stress response such as increased heart rate and blood pressure (Porth, 2011). ANS activity in humans can be inferred by levels of salivary α-amylase (AA), an enzyme produced in the oral mucosa via α- and β-adrenergic mechanisms (Nater & Rohleder, 2009; Nater et al., 2006; Granger et al., 2007).

Puberty and basal stress output. Pubertal maturation results in changes to basal stress output, as reflected by the puberty-HPA stress hypothesis, which stipulates that there is increasing activity of the HPA axis at baseline with increased sexual maturation (Gunnar et al., 2009). Numerous studies show that basal cortisol levels increase during adolescence (Gunnar & Vazquez, 2006). However, whether basal cortisol levels increase gradually over time or rise more markedly at some point during adolescent development remains to be further understood. Legro et al. (2003) examined urinary free cortisol in young girls every 6 months for a four-year period. They noted little change in the initial stages of sexual maturation, but higher levels of cortisol were found around Tanner Stage III and continued to increase through Tanner Stage V. This hormonal change around Tanner Stage III suggests a peak in basal cortisol levels around mid-puberty. Other studies examining both sexes have revealed pubertal increases in basal cortisol in girls (Netherton et al., 2004; Scheifbein & Susman, 2006), although cortisol increases in boys have been reported in a subset of published findings (Adam, 2006; Shirtcliff et al., 2005; Walker et al., 2001). Consistent with pubertal increases in basal HPA axis activity in girls, Stroud et al. (2004) used CRH to stimulate ACTH and cortisol activity in 7- to 16-year-old participants. They noted that with increasing sexual maturation via Tanner staging, girls displayed higher total cortisol output in response to CRH compared to age-matched boys (Stroud

et al., 2004; Hollanders et al., 2017), suggesting that girls consistently display more age-associated increases in basal HPA axis activity during puberty.

Puberty and stress reactivity. Changes in stimulated stress reactivity occur throughout puberty as well, but research in this area remains limited. One common and widely standardized method used in laboratory settings to stimulate a stress response is the Trier Social Stress Test (TSST; Kirschaum et al., 1993), as it contains all necessary elements (unpredictability, uncontrollability, and social-evaluative threat) to induce increases in both the HPA axis and the sympathetic nervous system (Birkett, 2011). The TSST includes a public speaking task and a mental arithmetic task. Following exposure to the TSST for children (TSST-C), cortisol reactivity differs in age- and sex-specific manners. During the adaptation period (i.e., baseline) of the TSST-C, 15 years old boys and girls display greater cortisol secretion than do 9- and 11-yearolds. Following the TSST-C, 13-year-old girls, in particular, exhibit greater cortisol reactivity than do age-matched boys. However, sex differences are not significant at 9, 11, and 15 years of age (Gunnar et al., 2009). Similarly, adolescents that are 13 to 17 years of age show greater neuroendocrine (i.e., cortisol and alpha-amylase levels) and cardiovascular responses to performance and social rejection stressors compared to children between 7 to 12 years of age (Stroud et al., 2009; Sumter et al., 2010). As a result, stress reactivity increases throughout puberty in both boys and girls, but the stimulated stress response is stronger in girls.

Sex and age differences in the pubertal stress response. Hormonal changes during puberty lead to sex differences in the adult HPA stress response. Sex differences in HPA axis regulation are well-documented in animal (Handa et al., 1994; Rhodes & Rubin, 1999; Minni et al., 2014) and in human studies (Kajantie & Phillips, 2006; Kudielka & Kirschbaum, 2005). Both basal and stimulated HPA axis activities are generally greater in female compared to male

rodents (Rhodes and Rubin, 1999; Minni et al., 2014). In humans, sex differences in the HPA axis response are inconsistent, as they appear to be influenced by a variety of factors such as hormonal shifts associated with the menstrual cycle or oral contraceptive use (Kudielka et al., 2009; Young & Korszun, 2010). For example, estradiol levels in the late follicular phase decrease the glucocorticoid response in adult women (Lindheim et al., 1994). Progesterone has opposite effects on HPA axis functioning. Women in the luteal phase of the menstrual cycle consistently show increased basal cortisol levels as well as higher ACTH and cortisol release compared to early- and late-follicular women (Bouma et al., 2009; Tersman et al., 1991; Altemus et al., 1997; Kirschbaum et al., 1999; Wolf et al., 2001; Andreano et al., 2008; Kajantie & Phillips, 2006). Additionally, synthetic estradiol (i.e., ethinyl estradiol) that is commonly found in oral contraceptives results in blunted cortisol levels (Moore et al., 1978; Kajantie & Phillips, 2006; Kumsta et al., 2007).

Sex and age differences in the pubertal and adolescent stress responses also vary depending on the nature of the stressor. Adolescent girls show increased adrenergic activity in response to a peer-rejection challenge compared to a performance stressor; however, pre-pubertal girls show heightened hypothalamic and adrenergic activities in response to a performance stressor (Stroud et al., 2017). Boys, on the other hand, show similar biological responses across puberty with higher cortisol output following a performance task. As a result, girls are more sensitive to rejection-related stressors and display greater stress reactivity across development, whereas boys display a more stable stress response (Stroud et al., 2017). Consistent with humans, animal models also reveal consistent increases in HPA axis reactivity in response to pubertal/adolescent stress. For example, studies in rats and mice have indicated that pubertal rodents exhibit prolonged stress-induced corticosterone responses, particularly in response to

stressors with both physical and psychological attributes (≥ 65 days of age; Romeo 2010a; 2010b; Romeo et al., 2013). The stress response is also particularly greater in pubertal females compared to age-matched males and adult females (Viau et al., 2005; Romeo et al., 2013). Thus, the increasing biological stress sensitivity across adolescence is related to both age and pubertal maturation. Girls appear to show an increasing basal HPA axis set point, increasing sensitivity to HPA axis stimulation, and different sensitivity depending on the type of stressor across development, whereas boys show fewer alterations in HPA axis functioning.

Vulnerability of puberty/adolescence and the onset of mental illnesses

Adolescence has been described as a time of "storm and stress" (Arnett, 1999). This transitional period can be characterized as a period of stress and resilience, but paradoxically, it can also mark a time of risk and vulnerability. The hormonal changes during puberty and maturation of the stress system make adolescence particularly vulnerable to the onset of mental health disorders, which often affect girls more than boys.

The incidence of psychiatric mental illnesses, including anxiety, mood disorders, eating disorders, personality disorders, psychosis and substance abuse, increases substantially during adolescence (Ge et al., 2001; Kessler et al., 2005; Newman et al., 1996; Silverman et al., 1996). It is also suggested that half of all lifetime cases for various mental health disorders emerge during adolescence and peak at 14 years old (Kessler et al., 2005; Paus et al., 2008). One possible explanation for the heightened emergence of mental illness is the significant neurodevelopment occurring during puberty, which could result in a heightened sensitivity to psychosocial, physiological, and emotional stressors (Dahl, 2004; Steinberg et al., 2004). Exposure to these stressors can alter important structural and functional changes in the brain, which can then affect an individual's cognitive, emotional, and social development.

There are also sex differences in the prevalence of various mental health disorders like depression, that emerge specifically during puberty/adolescence. Epidemiological studies show that stress-related mental illnesses often onset during puberty with a higher prevalence rate in females than in males at about a 2:1 ratio (Shors & Leuner, 2003; Angold & Costello, 2006). During childhood, depression rates are comparable between boys and girls, whereas in adulthood, women account for the majority of diagnosed cases (Stroud et al., 2004). The female preponderance in depression emerges around 13-14 years of age (Stroud et al., 2004), which corresponds to the midpoint of pubertal development. Thus, the role of the HPG axis on depression in girls is a great focus of attention, with research suggesting that declines in endogenous ovarian steroid hormones render the female brain more vulnerable to depression (Angold & Costello, 2006).

Oral contraceptives

One stressor that decreases endogenous ovarian steroid hormones is the usage of oral contraceptives (OCs) (i.e., "the birth control pill"). OCs have been in the market for over 60 years and contain synthetic forms of the ovarian steroid hormones, estradiol and progesterone. The first OC pill was introduced in North America in the 1960s. American biologist, Gregory Goodwin Pincus, proved in a large-scale study that oral administration of hormonal contraceptives prevents pregnancy in women. In 1960, the Food and Drug Administration in the USA legalized the first contraceptive (Enovid®), a combination pill containing 10 mg of synthetic progesterone, norethyndrel, and 150 μg of mestranol, a synthetic estradiol. Nowadays, the combined OC pill typically contains 0.02-0.04 mg of ethinyl estradiol and varying levels of synthetic progestins, which can be classified by their generation. Importantly, older generation (i.e., second generation) progestins (e.g., levonorgestrel and desogestel) are derived from 19-

nortestosterone, a common anabolic steroid able to activate androgen receptors and exert androgenic actions (Brueggemeier, 2006; Sitruk-Ware, 2006; Wiegratz & Kuhl, 2006). Newer progestins (e.g., drospirenone) are classified as third generation and bind very specifically to the progesterone receptor and are anti-androgenic (Wiegratz & Kuhl, 2006). OCs containing synthetic progestins like levonorgestrel, norgestimate, and gestodene are mostly used in Italy (Bezemer et al., 2016; Montoya & Bos, 2017), Canada, and the USA (Hall & Trussell, 2012).

Millions of women worldwide use OCs, and often start using them during puberty when their brain is undergoing extensive remodeling and reorganizing. The trend of early OC onset may be attributed to their off-label use as a "regulator" – that is, OCs can regulate a woman's menstrual cycle, mood, and skin. OCs suppress the production and release of endogenous gonadotropins and ovarian steroid hormones preventing women from experiencing a natural menstrual cycle and subsequent pregnancy. Specifically, synthetic estradiol inhibits the secretion of FSH, and synthetic progestin mainly inhibits the secretion of LH, leading to less ovarian activity and release of endogenous estradiol and progesterone from the ovaries. As a result, the hormonal profile of OC users differs substantially from that of naturally cycling (NC) women in that endogenous levels are lowered, and fluctuations are suppressed (Andreano & Cahill, 2010; Pletzer & Kerschbaum, 2014; Petersen et al., 2014). While OCs are among the best-researched drugs with regards to their link to breast cancer and blood clots (Cobey & Buunk, 2012), there is remarkably limited research on the effects of OCs on brain function and behaviour. Emerging evidence shows that OC intake causes structural and functional differences in a woman's brain, but the direction of the effects is inconsistent, and the role of ovarian steroid hormones is unclear.

Impact of oral contraceptives on the brain and behaviour

Oral contraceptives and GM volume. The investigation of the effects of OCs on GM volume has been inconsistent. Some studies show an increase (Pletzer et al., 2010; Pletzer et al., 2015), while others show a decrease (Lisofsky et al., 2016; Hertel et al., 2017; Pletzer et al., 2019) in regional GM volumes. In a structural brain analysis, OC use has been shown to significantly increase GM volume in pre- and post-central gyri, the parahippocampal gyrus, fusiform gyrus, and the superior, middle, and inferior temporal gyri (Pletzer et al., 2010). A follow-up study by the aforementioned researchers shows that progestin androgenicity in the OC pill may affect GM volume differences. Users of OCs with anti-androgenic progestins display relatively larger regional GM volume while users of OCs with androgenic progestins display relatively smaller regional GM volume compared to NC women (Pletzer et al., 2015). Despite the differing effects of anti-androgenic vs. androgenic OCs, Lisofsky et al. (2016) showed decreased GM volume in the left amygdala and anterior parahippocampal gyrus among OC users relative to NC women, regardless of the progestin androgenicity. Similarly, Hertel et al. (2017) and Pletzer et al. (2019) showed that OC users have less GM volume in the hippocampus and parahippocampal/fusiform gyrus compared to NC women. Although such inconsistencies in the literature are likely due to methodological differences (e.g., within-subjects [Lisofsky et al., 2016] versus between-subjects analyses [Pletzer et al., 2010; 2015; 2019]), duration of OC use has not been readily accounted. The importance of factoring in duration of OC use was highlighted by Pletzer et al. (2015; 2019) who found that duration of OC use was positively linked to higher global and regional GM volumes especially among users of androgenic progestins.

Oral contraceptives and WM volume. WM volume changes have also been observed but have been less investigated than GM volume. In fact, to date, only one study has examined the effects of OCs on WM volume in the brain. De Bondt et al. (2013b) found that OC users show lower WM integrity in the fornix, an area of the brain that regulates emotional processes, compared to NC women. These findings emphasize the need to examine WM changes following OC use, as research in this area is lacking.

Oral contraceptives and cortical thickness. OC use has been associated with decreases in cortical thickness in the orbitofrontal cortex and posterior cingulate cortex, two regions implicated in response to rewards and self-reflection (Petersen et al., 2015). Thus, the regional structural differences among OC users appear to be linked to symptoms such as negative affect or rumination, which are risk factors for stress- and mood-related disorders.

Oral contraceptives and brain function. Functional MRI studies reveal that OC intake causes functional changes in users at rest and during task induction. At rest, there are region-specific increases and decreases in the functional connectivity of the default mode, central executive, salience, and reward networks of OC users compared to NC women (Petersen et al., 2014; Pletzer et al., 2016; Engman et al., 2018). Similarly, the functional connectivity between the dorsolateral prefrontal cortex and the left amygdala/anterior parahippocampal gyrus is altered in OC users (Lisofsky et al., 2016). Taken together, the functional changes of OC users at rest suggest consequences for emotion regulation abilities. In contrast to functional changes at rest, during a task or exposure to various stimuli, OC users display decreased amygdala reactivity in response to negative emotional images (Petersen & Cahill, 2015) and reduced activity in insular and frontal regions following an emotional face matching task (Gingnell et al., 2013). However, other studies report increased brain activity during emotion processing. One study reported

increased activity of the fusiform face area towards angry and neutral facial expressions in OC users, which could reflect increased attention to specific features of the face relative to emotional aspects (Mareckova et al., 2014). Similarly, OC users show more brain activity in the insula, anterior cingulate cortex, precuneus, and thalamus during traumatic film viewing, which could be related to heightened emotion processing (Miedl et al., 2018). The higher brain activity observed among OC users in response to emotional stimuli may be related to stronger ratings of emotional valence (Spalek et al., 2019). While these OC-induced functional differences during emotion processing tasks are postulated due to lower endogenous ovarian steroid hormone concentrations, the existing literature has not observed consistent suppression of endogenous estradiol and progesterone in OC users (Andreano & Cahill, 2010; Petersen et al., 2014). Furthermore, no study to date has examined whether there are relationships between hormone concentrations and brain activity in OC users. Overall, the task-induced functional differences, as well as those during rest, may represent altered regulation of positive and negative affect symptoms in OC users, leading to certain mental health outcomes.

Oral contraceptives and cognition. The presence of structural and functional differences in OC users may give rise to altered cognition and behaviour such as emotional memory. OC users display enhanced memory for gist, but not for details of an emotional story, whereas NC women exhibit enhanced emotional memory for details, but not for gist (Nielsen et al., 2011). OC users also display an altered relationship between stress hormones and emotional memory. More specifically, a post-learning cortisol release modulates emotional memory for positive images in OC users, but not NC women. However, a post-learning cortisol release does not affect memory for gist or peripheral details in OC users, but it boosts recall in NC women (Nielsen et al., 2013ab). While the structural, functional, and behavioural differences identified

among OC users appear slightly scattered in the current literature, the findings, overall, point to differences in reward sensitivity and emotional processing, and general mood regulation. Thus, the various structural and functional differences following OC intake may predispose users towards mood-related symptoms that may result in psychiatric conditions, namely depression.

Role of oral contraceptives in stress. OC users demonstrate blunted HPA axis reactivity (Rohleder et al., 2003; Bouma et al., 2009). The interaction between OC use and the HPA axis is due to the ability of OCs to regulate peripheral stress markers. OC use stimulates the synthesis of corticoid binding globulin (CBG), the transport protein that binds to cortisol in the bloodstream making it biologically inactive (Wiegratz et al., 2003; Kumsta et al., 2007). This then leads to lower ACTH and cortisol levels in adolescent and adult users (Kirschbaum et al., 1999; Rohleder et al., 2003; Kumsta et al., 2007; Bouma et al., 2009; Roche et al., 2013). In addition, cortisol administration increases hippocampus activation to a learned fear stimulus in OC users, but decreases it in men and NC women, suggesting that women taking OCs are more sensitive to emotional information during stressful situations (Merz et al., 2012).

Oral contraceptives and mental health. Given that OC use modulates endogenous hormone production, an examination of the influence of OCs on women's mood is warranted. However, inconsistent findings on the link between OC use and mental health have been reported. While certain studies have identified associations between OC intake and use of anti-depressants or mood-related symptoms (Wirehn et al., 2010; Lindberg et al., 2012; Skovlund et al., 2016), other studies have found either no association (Duke et al., 2007) or a positive association between OC intake and better mood (Toffol et al., 2011; 2012; Keyes et al., 2013). A Danish study that made international headlines in 2016 suggests that the combined OC pill increases the relative risk of first anti-depressant use and first diagnosis of depression, especially

amongst adolescents. This study was the first of its kind, as it used a large-scale prospective cohort design to identify depression as a potential adverse outcome of OC use (Skovlund et al., 2016). More recently, Anderl et al. (2020) suggest that OC use during adolescence results in a lasting vulnerability to depression in adulthood.

In women who are susceptible to the adverse effects of OCs such as irritability and depression (Poromaa et al., 2012), suppressing endogenous ovarian steroid hormones might affect emotional reactivity differently. Altered structural and functional differences in regions regulating mood and other executive functions and blunted stress reactivity could constitute neural and neuroendocrine mechanisms for the link between OC use and depression. Given that puberty is a time when there is a surge of ovarian steroid hormones that drive cognitive, social and emotional development, perhaps OC intake during this early period of neurodevelopment could further exacerbate the risk of mood-related disorders in women compared to OC intake later on in adolescence or adulthood. Since OC-related differences have rarely been examined, let alone during puberty, it is important to understand the neural outcomes of OC use to advance our understanding of women's brain health.

Current thesis and objectives

In this thesis, we examined the effects of OC use and the age of OC onset (i.e., puberty and adulthood) on brain structure (i.e., regional GM and WM volume and WM integrity), and brain function during an emotional working memory task, and cortisol reactivity compared to NC, non-OC users (Article 1). Furthermore, we investigated OC-related differences in resting state functional connectivity in various limbic and executive networks (Article 2). Lastly, we examined the regulatory role of ovarian steroid hormones on emotional processing from the functional task in all women (Article 3). We hypothesized that OC intake would be related to

structural differences in regional GM and WM volumes and in WM integrity relative to NC women and altered activity in limbic areas when processing the emotionally arousing images in the working memory task. Additionally, we hypothesized that OC users would show blunted cortisol reactivity following a psychosocial stress test and region-specific increases and decreases in resting state functional connectivity compared to NC women. We also hypothesized that estradiol and progesterone concentrations would regulate activity in different brain regions during the emotional working memory task.

Results of the current studies will provide a better understanding of the neural outcomes of ovarian steroid hormone regulation and OC use. The relevance of sex hormone and OC research in today's world is manifold given that over 150 million women worldwide use OCs. First and foremost, the impact of OCs on the brain and behaviour is relevant at the individual level because users need to know the consequences of pill use and be better informed about the age at which they should start using OCs. This research also has implications at the societal level and the broad scientific community. Behavioural and neuroscientific insights on OC use may impact socio-emotional processes such as relationship satisfaction, friendships, and parenting. Finally, OC research will encourage other researchers to incorporate women's hormonal status (i.e., OC use or endogenous hormonal concentrations) in neurobiological research.

Article 1: Use of the birth control pill affects stress reactivity and brain structure and function.

Publication

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Abstract

Millions of women worldwide use oral contraceptives (i.e., birth control pill; OCs), often starting during puberty/adolescence; however, it is unknown how OC use during this critical period of development affects the brain, especially with regards to emotional working memory. Here, we examined stress reactivity, and brain structure and function in OC users using the Trier Social Stress Test and structural and functional magnetic resonance imaging (MRI). Our results show that OC use during puberty/adolescence gives rise to a blunted stress response and alters brain activation during working memory processing. OC use, in general, is also linked to increased prefrontal brain activation during working memory processing for negatively arousing stimuli. OC use is also related to significant structural changes in brain regions implicated in memory and emotional processing. Together, these findings highlight that OC use induces changes to brain structure and function and alters stress reactivity. These findings may provide a mechanistic insight for the increased vulnerability to mood-related mental illness in women after OC use.

Keywords: Puberty; Adolescence; Oral Contraceptives; Stress Reactivity; Voxel Based Morphometry; fMRI; N-Back; DTI; Working Memory; Emotion

1. Introduction

Millions of women worldwide use oral contraceptives (i.e., the birth control pill; OCs) and, although they have been commercially available for over 60 years, remarkably little is known about their behavioural and neurophysiological effects (Pletzer & Kerschbaum, 2014; Montoya & Bos, 2017). Many women begin using OCs during puberty/adolescence, not solely for reproductive purposes, but also for their off-label use (e.g., to regulate the menstrual cycle, mood, and skin). OCs work by suppressing endogenous gonadotropins and ovarian steroid hormones to prevent pregnancy (McGuire et al., 1975). The heightened sensitivity of the pubertal/adolescent brain to the organizational effects of ovarian steroid hormones (Levitt et al., 2003; Schulz et al., 2009; Vigil et al., 2016) raises concerns about the effects of using this hormone-regulating drug during this critical period of development.

Emerging evidence suggests that OC use during adulthood elicits significant behavioural and neurophysiological changes. Compared to naturally cycling (NC) women, OC users generally show blunted stress reactivity (Kirschbaum et al., 1999; Rohleder et al., 2003; Kumsta et al., 2007; Roche et al., 2013), region-specific increases and decreases in grey matter (GM) volume (Pletzer et al., 2010; 2015; Lisofsky et al., 2016) and decreases in white matter (WM) integrity (De Bondt et al., 2013) and cortical thickness (Petersen et al., 2015). OC users also display differences in brain function at rest (Petersen et al., 2014; Lisofsky et al., 2016; Pletzer et al., 2016) and during tasks related to memory, reward, and emotion compared to NC women (Marečkovà et al., 2012; Bonenberger et al., 2013; Gingnell et al., 2013; Petersen & Cahill, 2015; Miedl et al., 2018).

The OC-induced structural and functional changes to the brain may also increase the risk of stress-related disorders, namely depression. For example, OC users show reduced WM

integrity in the fornix (De Bondt et al., 2013) and less functional connectivity in the executive control network (Petersen et al., 2014; Pletzer et al., 2016). These changes are implicated in depressive symptoms such as altered emotion regulation and cognition (Hollocks et al., 2015; Ellard et al., 2018). There is also a sex bias in the pathogenesis of depression since women are twice as likely as men to develop it following adolescence (Angold et al., 1998; Shors et al., 2003). There are certain psychosocial factors such as interpersonal stressors and body image issues that disproportionately affect women (Keita, 2007; Sides-Moore et al., 2011). In addition to these psychosocial factors, hormonal fluctuations across the reproductive life cycle, including puberty and the premenstrual period, increase the risk of depression (Soares & Zitek, 2007). Thus, ovarian steroid hormones have a partial mediating role in the pathogenesis of stress-related mental disorders. As a result, the emerging trend towards younger OC use may exacerbate vulnerability to mental health conditions in women by influencing normal brain development. However, the current literature has rarely accounted for the age of OC onset and duration of use.

Given the oversight in the current literature regarding the age of OC onset/duration of use, the objective of the current study was to examine structural, functional, and behavioural differences in women who began using OCs either during puberty (i.e., pubertal-onset OC users) or adulthood (i.e., adult-onset OC users), and in NC women. We hypothesized that OC use would be related to region-specific increases and decreases in GM volume, WM integrity, and brain activity to follow up on previous inconsistent findings regarding structural and functional differences (Gingnell et al., 2013; Pletzer et al., 2010; Petersen & Cahill, 2015; Lisofsky et al., 2016; Miedl et al., 2018). We also hypothesized that OC use would be related to blunted stress reactivity and that pubertal-onset OC use would result in structural, functional, and behavioural differences compared to shorter OC use during adulthood.

2. Material and Methods

2.1 Participants. Women between the ages of 18 and 26 years old were recruited through the University of Ottawa's Integrated System of Participation in Research (ISPR) and in the community. A total of 75 women (mean age 19.6; SD = 1.96), including both OC users and NC women, participated in the MRI session (Part 1) and a total of 140 women (mean age = 19.1; SD = 1.96) participated in the stress testing (Part 2). OC users self-identified as pubertal/adolescent-onset or adult-onset users. Pubertal-onset OC users began using OCs within the first 6 months following menarche (first occurrence of menstruation), while adult-onset OC users began using OCs after 18 years of age and had been taking OCs for a minimum of 3 months at the time of testing. All users reported using OCs continuously and were tested in the "active" phase of their OC regimen. The MRI session consisted of 27 OC users (12 pubertal-onset users and 15 adult-onset users) whereas the stress testing consisted of 58 OC users (26 pubertal-onset users and 32 adult-onset users). NC women reported never having used hormonal contraceptives (including emergency hormonal contraception) and had regular menstrual cycles (ranging from 24 to 32 days). NC women were tested either during the early follicular phase (days 2 to 6 following the onset of menstruation), pre-ovulatory phase (days 10 to 14), or the luteal phase (days 18 to 24), which was established via a forward day count (Pletzer et al., 2010; Petersen et al., 2014). The MRI session consisted of 48 NC women whereas the stress testing consisted of 82 NC women. All participants were right-handed, had normal visual acuity, and were screened for histories of any psychiatric or medical illnesses. Participants with contra-indications to MRI (e.g., non-removable/implanted metal, back problems, loss of consciousness, pregnancy, or claustrophobia) were excluded. No brain tissue abnormalities were observed. Both studies were approved by the University of Ottawa's research ethics board and the Royal Ottawa Mental Health Centre's

ethics panel, and informed written consent was obtained from each participant. A full description of demographic factors such as age, race, and type of OCs for participants in both parts of the study can be found in Tables 1 and 2.

2.2 Hormonal assessment

2.2.1 Salivary 17β-estradiol and progesterone quantification. 1 mL saliva samples were obtained immediately prior to the MRI session and stress testing to examine endogenous ovarian steroid hormone levels in OC users and NC women. High sensitivity 17β-estradiol and progesterone enzyme-linked immunosorbent assay (ELISA) kits were used according to the supplier's instructions (Salimetrics, Kit Lot numbers 1901540 & 1810501, respectively). The sensitivity of the ELISA kits was the following: 19.08 pg/mL ± 4.77 (high control range) and 5.49 pg/mL ± 2.20 (low control range) for 17β-estradiol and 820.13 pg/mL ± 205.03 (high control range) and 47.32 pg/mL ± 18.93 (low control range) for progesterone. All samples were plated in duplicate for the hormone assays. All plates were read with Biotek Powerwave XS2 and analyzed with the Gen 5 V2.0 software with inter- and intra-assay precisions of less than 15 %. One-way ANOVAs were used to detect differences in ovarian steroid hormone levels (salivary 17β-estradiol and progesterone) between sub-groups of the OC users and NC women in SPSS 25. The Tukey HSD test was used for pairwise analyses when appropriate.

2.2.2 Trier social stress test (TSST). A subset of participants was exposed to the TSST, which consists of public speaking (5 min) and mental arithmetic (5 min) tasks performed in front of a panel of evaluating judges. Experimental sessions started between 1500 and 1700 hours to reduce variability in the circadian cortisol rhythm. Participants were instructed to refrain from physical exercising, smoking, eating, and drinking anything but water 1 h before the test session (Kirschbaum et al., 1993; Birkett et al., 2011; Allen et al., 2017).

2.2.3 Salivary cortisol quantification. During the experimental stress testing procedure, 1 mL saliva samples were obtained at 4 time-points via the passive drool method: baseline (Time 1), anticipating the speech task (Time 2), following the arithmetic task (Time 3), and 20 minutes following the end of the TSST (Time 4). (Figure 1). Salivary cortisol enzyme-linked immunosorbent assay (ELISA) kits were used (Salimetrics, Kit Lot number: 1701506). The sensitivity of the ELISA kits was the following: 0.985 μg/dL ± 0.246 (high control range) and 0.097 μg/dL ± 0.024 (low control range) for cortisol. All samples were plated in duplicate. All plates were read with Biotek Powerwave XS2 and analyzed with the Gen 5 V2.0 software with inter- and intra-assay precisions of less than 15 %. Mixed ANOVAs were used to examine cortisol reactivity across the 4 time-points between OC users and NC women, as well as between pubertal-onset OC users and their adult-onset counterparts in SPSS 25. The Tukey HSD test was used for pairwise analyses when appropriate. Exploratory analyses based on previous cortisol reactivity publications were also conducted, which separate participants based on whether they are cortisol responders or cortisol non-responders. This criterion was established by Nielsen et al. (2014) due to large variability within sample groups. Participants who exhibited an increase of at least 0.05 μg/dL from Time 1 to Time 3 were classified as cortisol responders.

2.4 Subjective stress quantification. A visual analog scale (VAS) was also used to measure subjective stress responses. The VAS consisted of a 100 mm bipolar line on which participants were asked to estimate their stress level on a continuum from 0 equaling to “not at all” to 100 equaling to “very much” based on the statement “I feel stressed”. Scores were determined by measuring the distance from the left end to the appraisal mark. The VAS measurements were concurrent with saliva sampling. Mixed ANOVAs were used to examine subjective stress responses across the 4 time-points between OC users and NC women, as well as between

pubertal-onset OC users and their adult-onset counterparts in SPSS 25. The Tukey HSD test was used for pairwise analyses when appropriate.

2.5 Questionnaires. Participants were asked to complete the State Trait Anxiety Inventory (STAI) and the Beck Depression Inventory (BDI) prior to their scanning session and stress testing. The STAI was implemented to assess levels of state and trait anxiety (i.e., STAI-S and STAI-T, respectively). It consists of 2 sub-scales to assess each type of anxiety. Each scale consists of 20 items and is based on a 4-point Likert scale (Spielberger et al., 1970). The BDI was administered to measure affect. It contains 21 items with each response being scored on a scale value from 0 to 3 (Beck & Steer, 1996). The scores on each item of the BDI and STAI questionnaire were summed and the total score was used in a two-sample t-test to compare differences in questionnaire scores between OC users and NC women and between the two OC sub-groups.

2.6 Memory task

2.6.1 *N*-back test. Participants were scanned as they engaged in an emotional picture *n*-back task. The block design task consisted of images from the International Affective Picture System (IAPS) database and were grouped by emotional valence (i.e., neutral, positive, and negative) (Lang et al., 2008). Each stimulus image was presented for 1000 ms with an interval of 500 ms followed by the next stimulus image. In total, 18 blocks were included with 16 stimulus images per block. The blocks were divided into 6 main conditions based on emotional valence and task type (i.e., 1-Back Negative, 1-Back Positive, 1-Back Neutral, 2-Back Negative, 2-Back Positive, & 2-Back Neutral) resulting in 3 blocks per *n*-back condition. Each block consisted of 6 target images and 10 non-target images (37.5% target images per condition). No responses were required for the non-targets. The order of presentation of each block was counterbalanced.

Participants were instructed to 'Press for 1-Back' (baseline memory condition) or 'Press for 2-Back' (working memory test condition) when they recognized an image that they saw *n*-steps (i.e., 1-Back and 2-Back) in the sequence. Brain activity was calculated as the average activity of each condition, including both target and non-target images. Participants viewed the task on a mirror attached to the head-coil reflecting a projection screen, and all images were presented on a black background. Lights were off during task completion.

2.6.2 Performance/Errors. During the memory task, performance data were screened for omission and commission errors (i.e., response for a non-target image) and the average reaction time was calculated for correct target responses occurring within 900 ms of stimulus presentation. Two sample t-tests were used to compare omission and commission errors between OC users and NC women and between the two OC sub-groups. These data were analyzed with SPSS 25.

2.7 MRI data acquisition. Structural MRI and diffusor tensor imaging (DTI) and functional MRI (fMRI) data were acquired using a Siemens Biograph 3 Tesla Magnetom MR scanner (Siemens, Erlangen, Germany) equipped with a 32-channel head coil at the Royal Ottawa Mental Health Centre's Brain Imaging Centre. A conventional T1-weighted spin echo localizer was acquired to confirm that the anterior commissure-posterior commissure (AC-PC) line in the sagittal view was at right angles to the slice select direction. A high-resolution structural image was acquired for each participant with a T1 weighted MPRAGE sequence (TR/TE 1650/2.89 ms, flip angle 9°, field of view (FOV) 256 × 256 mm2, slice thickness = 1 mm, 176 sagittal slices, band-width 170 Hz). For the DTI data, 56 axial slices were obtained using a single-shot echo-planar imaging (EPI) sequence (TR/TE 10200/80 ms, field of view (FOV) 256 × 256 mm2, slice thickness = 2 mm, band-width 1776 Hz/Px). Whole brain echo planar fMRI was performed using

a gradient echo pulse sequence (TR/TE 3000/34 ms, flip angle 90°, FOV 200 × 200 mm2, voxel size 1.6 mm × 1.6 mm × 3 mm, 48 axial slices, slice thickness 3 mm, band-width 2894 Hz). The entire functional scan had a duration of 11 min and 12 s with a total of 221 functional images.

2.8 MRI data analysis

2.8.1 Structural voxel-based morphometry analysis. T1 structural imaging data were analyzed using Matlab (R2013b) (Mathworks Inc., Sherborn, MA, USA) and the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) in Statistical Parametric Mapping software (SPM8) (http://www.fil.ion.ucl.ac.uk/spm). The VBM8 default preprocessing involves intra-subject realignment, intra-subject bias correction, tissue classification, segmentation, and normalization. The baseline (or mean) of the realigned images were used as a reference for realignment and signal-inhomogeneity correction. The normalization parameters of the baseline image were applied to the segmented, bias-corrected images, which were then again realigned (Ashburner & Friston, 2005). Following realignment and segmentation, all resulting GM and WM images were registered to a template provided by the International Consortium of Brain Mapping, and a diffeomorphic image registration algorithm (DARTEL) was used for spatially normalizing tissue maps into stereotactic Montreal Neurological Institute (MNI) space. Then the normalized GM and WM images were smoothed with a full-width half maximum (FWHM) kernel of 8 mm. Parameters were set as follows: warping regularization = 4, bias regularization = 0.0001, bias FWHM cutoff = 60 mm, sampling distance =3, MRF weighting = 0.15. The final smoothed images were entered in second-level group comparisons between OC users and NC women for GM and WM volumes using a two-sample t-test in SPM8.

Total GM and WM volumes were used as covariates of no interests to correct the global volume effect. All reported results were derived from an uncorrected threshold of $p_{uncorr} < 0.005$ at the

whole brain level and deemed significant at a threshold of $p < 0.05$ after family wise error (FWE) correction for multiple comparisons at the cluster level. All reported clusters consisted of ten or more contiguous voxels.

2.8.2 Structural DTI analysis. All MRI data were preprocessed using the FMRIB Software Library (FSL). First, all original data were processed for brain extraction. Next, the FMRIB Diffusion Toolbox (FDT; version 6) was used to correct for eddy current distortion and head motion artifacts. A brain mask was then created using the script BET based on the b0 image. The fractional anisotropy (FA) was calculated by reconstructing a tensor matrix from the raw diffusion data using the FDT in the FSL. The tract-based spatial statistics (TBSS) processing technique was then performed to analyze the FA images. All FA images were aligned to a Montreal Neurological Institute 152 (MNI152) standard space through non-linear registration. The mean FA maps of all participants were projected onto the FMRIB58_skeleton, and the FA threshold was set at 0.2. After full alignment with the common skeleton, the data were in the form of a four-dimensional image. The Johns Hopkins University (JHU) white-matter tractography atlas and the JHU International Consortium of Brain Mapping (ICBM)-DTI-81 white-matter labels were utilized to identify specific fiber tracts. The FSLview enabled visualization of the resulting statistical maps for the FA measures.

We conducted a whole brain search for differences in FA (i.e., WM integrity) between OC users and NC women. FA images were entered into a two-sample t-test design matrix in the FSL. The permutation-based approach was conducted in our statistical analysis using the Threshold-Free Cluster Enhancement option in the FSL randomize tool. The random permutation was set at 500. A level of $p < 0.05$ indicated statistical significance.

2.8.3 fMRI data analysis. Functional imaging data were analyzed using Matlab (R2013b) and Statistical Parametric Mapping (SPM12) software. The functional scans were normalized to the standard SPM Montreal Neurological Institute template and spatially smoothed with a 6 mm FWHM Gaussian kernel following SPM12 standard preprocessing procedures (i.e., realignment, normalization, and smoothing). The final images were corrected for field inhomogeneity. Six first-level contrast images were constructed at the individual participant level: (i) 2-Back Neutral > 1-Back Neutral; (ii) 2-Back Positive > 1-Back Positive; (iii) 2-Back Negative > 1-Back Negative; (iv) 2-Back Negative > 2-Back Positive; (v) 2-Back Positive > 2-Back Neutral; (vi) 2-Back Negative > 2-Back Neutral. The contrasts represent working memory, and positive and negative emotionality. Contrasts constructed at the single participant level were then entered into a second-level group analysis using two-sample t-tests in SPM12 to compare differences in brain activation between OC users and NC women. Whole-brain multiple regression analyses were conducted to explore the relationship between duration of OC usage and brain activity, while controlling for the age of OC onset, in response to the 2-Back Positive > 1-Back Positive, 2-Back Negative > 1-Back Negative, and 2-Back Neutral > 1-Back Neutral contrasts separately in pubertal- and adult-onset OC users. Similarly, whole-brain multiple regression analyses were conducted to explore the relationship between endogenous progesterone levels and brain activity in response to the 2-Back Positive > 1-Back Positive and 2-Back Negative > 1-Back Negative contrasts separately in OC users and NC women. After getting the correlated regions from the whole-brain analyses, independent anatomical ROIs of these regions were defined using WFU_pickatlas AAL template (http://fmri.wfubmc.edu/software/PickAtlas), and the parametric estimated activation values of the two contrasts' SPM-T maps were extracted for these ROIs individually using the Marsbar toolbox (Brett et al., 2002). Next, Pearson correlations were

conducted between the endogenous progesterone levels and the brain activation values to generate the correlation scatter plots using SPSS (Brett et al., 2002; Miedl et al., 2018). Furthermore, region of interest (ROI) analyses for the left and right insula were conducted based on previous literature depicting altered insular reactivity in OC users (Abler et al., 2013; Bonenberger et al., 2013). All reported results were derived from an uncorrected threshold of $p_{uncorr} < 0.005$ at the whole brain level and deemed significant at a threshold of $p < 0.05$ after FWE correction for multiple comparisons at the cluster level. All reported clusters consisted of ten or more contiguous voxels.

2.8.4 Quality assessment. Both structural and functional MRI data were visually inspected and reviewed for artifacts. The final functional images were screened for head motion (> 3 mm in any direction [absolute maximum]). One participant was detected by this criterion and outlier images (greater than 3 mm of movement) were excluded from further analysis.

3. Results

3.1 17β-estradiol and progesterone hormone levels. No significant differences were observed between any of the OC users and NC women for salivary estradiol levels. However, NC women in the luteal phase of the menstrual cycle showed higher salivary progesterone levels in both parts of the study compared to pubertal- (MD = 82.80, SE = 20.00, p = 0.001; MD = 153.53, SE = 20.17, p < 0.001, respectively) and adult-onset (MD = 53.63, SE = 18.82, p = 0.044; MD = 108.25, SE = 19.11, p < 0.001, respectively) OC users, and higher salivary estradiol levels only in part 2 of the study compared to pubertal-onset OC users (MD = 0.53, SE = 0.13, p = 0.001). (Figure 1).

3.2 Stress reactivity. No significant differences in subjective stress reactivity (i.e., VAS scores) and cortisol concentration were observed between OC users to NC women and pubertal- to adult-

onset OC users across the four time-points. However, exploratory pairwise analyses using the Least Significant Differences (LSD) test identified differences in stress reactivity between pubertal-onset OC users and their adult-onset counterparts. Pubertal-onset OC users displayed a significant decrease in cortisol concentration at Time 3 (following arithmetic task) (MD = 0.073, SE = 0.030, p = 0.019) and a marginally significant decrease at Time 4 (20 minutes post-TSST) (MD = 0.090, SE = 0.047, p = 0.063) compared to their adult-onset counterparts. (Figure 1). Previous publications on stress reactivity have separated participants based on whether they are cortisol responders or cortisol non-responders (Nielsen et al., 2014). In the current study, 53% of adult-onset OC users can be classified as cortisol responders, whereas only 19% of pubertal-onset OC users can be considered cortisol responders.

3.3 Questionnaires. No significant differences in STAI-S, STAI-T, and BDI scores were observed between OC users to NC women and pubertal- to adult-onset OC users in both parts of the study.

3.4 Structural differences. OC users showed less GM volume in the right putamen ([27 14 -2], $t(1,71) = 3.94$, $p = 0.005$) compared to NC women. No brain regions were identified with larger GM volumes in OC users compared to NC women. However, OC users showed more WM volume in the right putamen ([26 18 -3], $t(1,71) = 4.57$, $p < 0.001$), right rectus ([18 14 -12], $t(1,71) = 4.57$, $p < 0.001$), right amygdala ([29 -4 -18], $t(1,71) = 4.57$, $p < 0.001$), left parahippocampal gyrus ([-29 -22 -21], $t(1,71) = 4.03$, $p = 0.010$), and left hippocampus ([-30 -33 -9], $t(1,71) = 4.03$, $p = 0.010$) compared to NC women. (Figure 2). Consistent with differences in WM volume, OC users also showed increased WM integrity in the left hippocampus compared to NC women ($p < 0.05$). (Figure 3).

3.5 Functional differences. OC users showed greater brain activity in the right inferior frontal gyrus ([46 30 26], t(1,72) = 4.42, p = 0.046), right mid-frontal gyrus ([36 40 16], t(1,72) = 4.11, p = 0.046), and right insula ([-44 6 -4], t(1,72) = 3.71, p = 0.017) during the 2-Back Negative > 1-Back Negative contrast compared to NC women. We also observed greater brain activity in the right supplementary motor area (SMA) ([10 -12 72], t(1,72) = 4.16, p = 0.035), right superior frontal gyrus ([18 -10 72], t(1,72) = 4.16, p = 0.035), left paracentral lobule ([-10 -14 66], t(1,72) = 4.16, p = 0.035), and the left lingual gyrus ([-18 -66 -8], t(1,72) = 4.06, p = 0.007) in OC users compared to NC women during the 2-Back Negative > 2-Back Positive contrast. (Figure 4). No significant differences in brain activity between OC users and NC women were identified during the following contrasts: 2-Back Positive > 1-Back Positive, 2-Back Neutral > 1-Back Neutral, 2-Back Positive > 2-Back Neutral, and 2-Back Negative > 2-Back Neutral. Additionally, no significant differences in omission and commission errors were identified in any of the contrasts between OC users and NC women.

3.6 Hormone correlations. Among the OC users, there was a positive correlation between progesterone levels and brain activity in the temporal areas of the brain ((i.e., bilateral superior temporal pole ([-52 10 -4], t(1,24) = 4.57, p = 0.001; [52 4 -6], t(1,24) = 6.71, p < 0.001, respectively), bilateral mid-temporal gyrus ([-46 -68 16], t(1,24) = 4.15, p < 0.001; [44 -66 2], t(1,24) = 5.04, p < 0.001, respectively), left inferior temporal gyrus ([-44 -4 -30], t(1,24) = 4.85, p = 0.035), left superior temporal gyrus ([-60 -6 0], t(1,24) = 4.57, p = 0.001) as well as the bilateral SMA ([-6 4 64], t(1,24) = 6.08, p < 0.001; [8 4 66], t(1,24) = 4.25, p < 0.001, respectively), bilateral mid-occipital gyrus ([-20 -92 10], t(1,24) = 4.15, p < 0.001; [36 -80 14], t(1,24) = 5.04, p < 0.001, respectively)), and the left superior ([-28 38 40], t(1,24) = 4.96, p = 0.049) and mid-frontal gyri ([-28 44 20], t(1,24) = 5.32, p = 0.003) during the 2-Back Negative >

1-Back Negative contrast. Progesterone concentration also correlated with higher brain activity in the left putamen ([-26 2 -2], t(1,24) = 6.11, p = 0.013), left pallidum ([-22 -6 4], t(1,24) = 6.11, p = 0.013), left lingual gyrus ([-18 -52 -10], t(1,24) = 5.87, p < 0.001), and left fusiform gyrus ([-34 -8 -28], t(1,24) = 4.85, p = 0.035) in OC users. (Figure 5). This relationship was observed in NC women but only for the positively arousing images. In the NC group, a positive correlation between progesterone levels and brain activity in the right superior frontal gyrus ([22 48 18], t(1,46) = 5.57, p = 0.001) and the right middle frontal gyrus ([22 48 30], t(1,46) = 5.57, p = 0.001) was observed during the 2-Back Positive > 1-Back Positive contrast. (Figure 6). No significant correlations were identified between estradiol levels and brain activity among OC users or NC women.

3.7 Group differences within OC users. No structural and functional differences were observed between pubertal- and adult-onset OC users. However, exploratory analyses between the two sub-groups of OC users were conducted from an uncorrected threshold of $p_{uncorr} < 0.05$ at the whole brain level and deemed significant at a threshold of $p < 0.05$ after FWE correction for multiple comparisons at the cluster level. Results showed that pubertal-onset OC users have more WM volume in the left fusiform gyrus ([-27 -31 -27], t(1,24) = 5.20, p = 0.001) and right precuneus ([3 -73 34], t(1,24) = 5.20, p = 0.001) compared to their adult-onset counterparts. Similarly, pubertal- and adult-onset OC users showed differences in brain activity during the *n*-back task. Pubertal-onset OC users showed more brain activity in the left fusiform gyrus ([-38 -38 -18], t(1,24) = 4.43, p < 0.001) and right cuneus ([14 -80 18], t(1,24) = 4.43, p < 0.001) during the 2-Back Positive > 1-Back Positive contrast and made more commission errors during the 1-Back Positive block (MD = 0.429, SE = 0.123, p = 0.038) compared to their adult-onset counterparts. (Figure 7). Pubertal-onset OC users also showed greater brain function in the

bilateral lingual gyrus ([-24 -66 -10], t(1,24) = 4.32, p = 0.003; [22 -72 -8], t(1,24) = 4.32, p = 0.003, respectively), left mid-temporal gyrus ([-42 -52 10], t(1,24) = 4.32, p = 0.003), right pre-central gyrus ([36 0 34], t(1,24) = 4.79, p < 0.001), and right insula ([38 22 -2], t(1,24) = 4.79, p < 0.001) during the 2-Back Neutral > 1-Back Neutral contrast. (Figure 8). Adult-onset OC users, on the other hand, showed greater brain activity in the left lingual gyrus ([-22 -72 -12], t(1,24) = 4.94, p < 0.001), right fusiform gyrus ([30 -64 -10], t(1,24) = 4.94, p < 0.001), right putamen ([24 12 -6], t(1,24) = 4.75, p = 0.010), and the right inferior frontal gyrus ([54 36 12], t(1,24) = 4.75, p = 0.010) during the 2-Back Negative > 1-Back Negative contrast compared to pubertal-onset OC users.

3.8 Correlations between OC use and brain activity. Regression analyses between duration of OC use and brain activity were conducted from an uncorrected threshold of $p_{uncorr} < 0.05$ at the whole brain level and deemed significant at a threshold of $p < 0.05$ after FWE correction for multiple comparisons at the cluster level within the two OC sub-groups. Adult-onset OC users demonstrated a positive association between duration of OC use and brain activity in the right inferior frontal gyrus ([44 28 18], t(1,12) = 8.37, p < 0.001), right SMA ([2 -4 54], t(1,12) = 11.57, p < 0.001), and left lingual gyrus ([-26 -88 -16], t(1,12) = 6.97, p = 0.005) during the 2-Back Negative > 1-Back Negative contrast. Among the pubertal-onset OC users, there was a positive correlation between duration of OC use and brain activity in the left fusiform gyrus ([-26 -60 -8], t(1,10) = 10.08, p = 0.012) during the 2-Back Negative > 1-Back Negative contrast. Duration of OC use also correlated with brain activity in the right SMA ([14 -6 48], t(1,10) = 8.36, p = 0.001), and the right superior medial ([6 30 40], t(1,10) = 8.36, p = 0.001) and middle frontal gyri ([22 12 50], t(1,10) = 8.36, p = 0.001) during the 2-Back Positive > 1-Back Positive contrast among pubertal-onset OC users. During the 2-Back Neutral > 1-Back Neutral contrast,

duration of OC use correlated with brain activity in the right angular gyrus ([40 -52 24], $t(1,10) = 8.01$, $p = 0.009$) among pubertal-onset OC users. Within both pubertal- and adult-onset OC users, duration of OC use correlated negatively with brain activity in the right superior medial frontal gyrus ([6 36 50], $t(1,10) = 6.88$, $p = 0.002$; [10 58 18], $t(1,12) = 8.89$, $p < 0.001$, respectively) during the 2-Back Neutral > 1-Back Neutral contrast.

4. Discussion

Our findings demonstrate that OC use is related to behaviour and neurophysiology. Some findings demonstrate a relationship with the age of OC onset while others are related to the duration of OC use. Pubertal-onset OC users show more regional WM volume and higher brain activity during working memory compared to their adult-onset counterparts. In general, all OC users show region-specific differences in structural brain volumes and more brain activity during working memory for negative pictures compared to NC women.

No differences in endogenous estradiol concentration and stress reactivity were observed between OC users and NC women. While some researchers report lower endogenous estradiol concentration in OC users compared to NC women (Petersen et al., 2014), others do not (Andreano & Cahill, 2010). Given that some OC formulations have very low ethinyl estradiol concentrations, it is possible that these types of OCs allow endogenous estradiol to escape suppression, while progesterone is uniformly suppressed across all formulations and generations. Similarly, we did not observe any group difference in cortisol concentration to the TSST while others have (Kirschbaum et al., 1999; Rohleder et al., 2003; Kumsta et al., 2007; Roche et al., 2013). Although our standardized TSST protocol was similar to the one used by previous researchers, our study was limited to salivary measures whereas others have examined both salivary and plasma cortisol concentrations (Kirschbaum et al., 1999; Rohleder et al., 2003;

Kumsta et al., 2007). Our estradiol and cortisol detection protocols were also limited to a colorimetric immunoassay while others have noted that immunoassays using a fluorometric end point may have greater detection sensitivity (Dressendorfer et al., 1992; Samineni et al., 2006). Additionally, we did not account for the time that OC users took their pill; thus, it is possible that the timing of OC intake in relation to the TSST and different OC formulations have different effects on estradiol concentration and stress reactivity (i.e., blunted or heightened cortisol response) resulting in no group difference between OC users and NC women.

Within OC users, however, we did find a blunted cortisol response after the TSST with a quicker recovery to baseline in pubertal-onset OC users relative to adult-onset OC users. Despite a lack of statistical correction factors, this suggests that there may be long-term effects of prolonged OC use during puberty on stress reactivity, which could involve a variety of mechanisms. For example, OCs naturally stimulate the production of corticoid binding globulin (CBG), the transport protein that renders cortisol biologically inactive (Wiegratz et al., 2003; Kumsta et al., 2007). Given that puberty is also a period of HPA axis maturation (Sisk & Foster, 2004), the higher percentage of cortisol non-responders within the pubertal-onset OC group may be due to HPA axis alteration resulting in higher CBG production. This could then explain the blunted cortisol response after the TSST in the pubertal-onset OC users compared to their adult-onset counterparts. While we found no difference in mood-related symptoms between pubertal-onset OC users and their adult-onset counterparts, there are reports suggesting that a blunted stress reactivity can be a risk factor for depression and that adolescent OC use is related to greater vulnerability to depression diagnosis in adulthood (Fiksdal et al., 2019; Anderl et al., 2020).

In addition to the effects on stress reactivity, OC users show differences in GM and WM volume, as well as WM integrity, compared to NC women. Our results show that OC users display less GM volume in the putamen compared to NC women. While we are the first to show an OC-induced volumetric difference in this region, others have noted a sex difference (Pletzer et al., 2010). Previous publications also show that OC use decreases GM volume in the amygdala and the hippocampus (Lisofsky et al., 2016; Pletzer et al., 2019). GM volume development typically follows an inverted U-shaped trajectory reaching a peak volume in late childhood before gradually declining across adolescence/early adulthood (Blakemore et al., 2010). It is possible that the disruption in endogenous hormones caused by OC use accelerates GM volume decline in the putamen.

Additionally, women show a compensatory GM:WM volume ratio (Allen et al., 2003; Chen et al., 2007). This ratio may be altered in the current study, as OC users show more WM volume in the putamen as well as in the amygdala and hippocampus. Only two studies to date have examined the role of the menstrual cycle and OC use on WM integrity (De Bondt et al., 2013; Barth et al., 2016). NC women show a positive correlation between estradiol levels and WM integrity in the hippocampus during the follicular phase, reaching a peak around ovulation (Barth et al., 2016). However, in comparison to NC women, OC users display reduced WM integrity in the fornix, a region involved in emotion regulation (De Bondt et al., 2013). While our results are not consistent with these findings, we suggest that the higher WM volume in the hippocampus of OC users may be a consequence of increased myelination in this area, as identified by higher FA in the OC users relative to NC women. The higher WM volume and integrity among OC users may be due to a delay or suppression of the natural synaptic pruning process that occurs in early adulthood (Giedd et al., 1999; Tamnes et al., 2010; Blakemore et al.,

2010). Some researchers have also identified associations between increased WM volume and integrity in the hippocampus and aspects of cognitive decline and depressive symptom severity (Lyons et al., 2004; Chao et al., 2015). We also observe that pubertal-onset OC users show more WM volume in the fusiform gyrus and precuneus compared to their adult-onset counterparts. This suggests that prolonged OC use during puberty may further disrupt synaptic pruning. Overall, our findings point to OC-related differences in brain structures that may be due to altered neurodevelopment. The structures identified are implicated in emotion regulation and memory (Andreasen 1997; Nee et al., 2011).

In addition to the structural findings from this study, the fMRI results also revealed a relationship between brain activity during emotional working memory and OC use. The novel version of the *n*-back task was developed since past research suggests that brain activity in response to emotional images is altered by OC use (Petersen et al., 2015) and that cognitive processing of an emotional story is modulated by ovarian steroid hormones and OC use (Nielsen et al., 2011). No difference was observed in brain activity during working memory and performance for neutral images between OC users and NC women; however, pubertal-onset OC users show more brain activity during working memory for neutral images in the bilateral lingual gyrus, mid-temporal gyrus, pre-central gyrus, and insula compared to adult-onset OC users. While there is no correlation between brain activity in these regions and the duration of OC use in pubertal-onset OC users, there is a positive association between brain activity in the right angular gyrus, a critical brain area for memory retrieval (Hutchinson et al., 2012), and duration of OC use during the neutral memory processing. Duration of OC use is also negatively associated with brain activity in the superior medial frontal gyrus in both pubertal- and adult-onset OC users. Despite the lenient whole brain threshold, our findings, suggest that pubertal-

onset OC use and a longer duration of OC use differentially impact brain structure and function. Given these results, there is the possibility that the differences observed between pubertal- and adult-onset OC users might partially be due to time on OCs rather than the age of OC onset. On the other hand, OC-related effects on frontal activity during neutral working memory may be most apparent earlier on in OC use, regardless of the age of OC onset. However, OC-related impacts on frontal brain activity can be altered when working memory is engaged for emotionally arousing stimuli.

In general, OC users show more frontal and insular brain activity during the working memory task for negatively arousing images compared to NC women. In this component of the *n*-back task (i.e., 2-Back Negative), we also observe greater brain activity in the SMA, superior frontal gyrus, paracentral lobule, and the lingual gyrus compared to the working memory task for positively arousing images (i.e., 2-Back Positive). While our results are consistent with some publications showing heightened brain activation among OC users (Marečkovà et al., 2012; Miedl et al., 2018), they are inconsistent with others (Gingnell et al., 2013; Petersen & Cahill, 2015); thus, the altered brain activity of OC users appears to be task-dependent. Since there was no difference in reaction time and performance, the increased brain activation in response to the negatively arousing images in OC users compared to NC women suggests that OC use may predominantly affect an early emotional processing stage of working memory for negative pictures. This is supported by previous studies that identify increased brain activity in OC users in response to emotional tasks as a fear response (Merz et al., 2012; Miedl et al., 2018). The higher brain activity could also be attributed to duration of OC use since adult-onset OC users show a positive association between duration of OC use and frontal brain activity. Pubertal-onset OC users, on the other hand, demonstrate a positive association between duration of OC use and

brain activity in the fusiform gyrus, which could be due to their higher WM volume in this region.

Our findings also suggest that OC use alters the mediating role of endogenous progesterone on stimuli sensitivity when working memory is engaged. Previous research suggests different associations in various brain regions between salivary progesterone concentration and brain activity when processing positively or negatively arousing stimuli, demonstrating its mixed anxiolytic and anxiogenic properties (Andreano and Cahill, 2010; Gingnell et al., 2012; Bayer et al., 2014; Montoya & Bos, 2017). The association between endogenous progesterone concentration and brain activity has not been previously examined in OC users. We observe a positive relationship between progesterone levels and brain activity in the SMA, dorsolateral PFC, and putamen during the working memory task for negatively arousing images; however, this positive relationship is only present in the dorsolateral PFC of NC women during the working memory task for positively arousing stimuli. Thus, it appears that OC use results in a hormonally induced sensitivity to negatively arousing stimuli. Given that OC formulations range in progestin androgenicity from androgenic to anti-androgenic, it is possible that progestin androgenicity contributes to differences in stimuli sensitivity. For example, OC users of anti-androgenic progestins show enhanced face recognition performance compared to androgenic OC users (Pletzer et al., 2015). Regardless of progestin androgenicity, OC users display higher emotional valence ratings, which may be a direct result of a hormonally mediated sensitivity to negative stimuli (Spalek et al., 2019). This sensitivity may then lead to symptoms that can exacerbate the risk for stress-related disorders (Skovlund et al., 2016).

While the current study demonstrates OC-related differences in stress reactivity and brain structure and function, it does have some limitations. First, all comparisons were between rather

than within subjects. A replication of these effects in a single group of women, especially with placebo controls for OC use and a longitudinal design, would provide much stronger evidence that these effects are observable in the general population. A longitudinal design would also help clarify if OC-related changes in brain structure and function are due to the age of OC onset, duration of OC usage, or both factors. Second, given the vast diversity in OC formulations, including generation, hormone concentrations, and progestin androgenicity, the inclusion of OC participants using a specific formulation would provide clearer results. While our findings have implications for cognitive or mood-related consequences following OC use, they should not be seen as negative effects since we did not specifically observe any group differences in task performance measures or mood-related questionnaire scores. In addition, the age of OC onset effects on stress reactivity and neurophysiology were based on lenient thresholds compared to the more stringent thresholds used to examine differences in neurophysiology between all OC users and NC women. Finally, given the lack of a group difference in endogenous estradiol concentration between OC users and NC women observed in our study, future studies should incorporate multiple immunoassay techniques (e.g., liquid chromatography-mass spectrometry) to confirm hormonal data (Rosner et al., 2013).

5. Conclusions

Despite the limitations of this study, it provides valuable information to an under-researched field riddled with inconsistent findings. This study is the first to examine the age-dependent effect of OC use on brain structure and function during working memory tasks. It highlights the need for further investigation into the age-specific OC effects on stress reactivity and neurophysiology, as well as general effects on brain structure and function. Research is just beginning to elucidate the effects of OCs on behaviour and neurophysiology. Even less is known

about the impact of OCs during puberty/adolescence, a sensitive period of neurodevelopment with a high emergence of psychiatric disorders. Thus, the current study is of immense importance because it offers critical insight into women's health and brings awareness to the potential behavioural and neural influences of OC use.

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Part 1: MRI Session	***OC Users (N = 27) M (SD)***	***NC Women (N=48) M (SD)***
Demographics		
Age at Testing	20.15 (2.25)	19.23 (1.70)
Age at Menarche	12.87 (1.15)	12.29 (1.30)
Race (%)		
White (non-Hispanic)	85.2	35.4
Black	0	20.8
Asian	3.7	27.1
Other	7.4	8.3
Mood Questionnaires		
BDI	7.62 (7.48)	6.79 (4.82)
STAI (State)	30.48 (8.49)	30.90 (6.46)
STAI (Trait)	38.56 (12.65)	37.40 (8.79)
Part 2: Stress Testing	***OC Users (N = 58) M (SD)***	***NC Women (N=82) M (SD)***
Demographics		
Age at Testing	19.38 (1.81)	19.08 (2.16)
Age at Menarche	13.11 (1.69)	12.12 (1.23)
Race (%)		
White (non-Hispanic)	80.6	35.4
Black	3.2	27.7
Asian	16.1	23.1
Other	0	13.8
Mood Questionnaires		
BDI	10.79 (6.47)	11.74 (6.31)
STAI (State)	34.33 (8.44)	37.38 (11.04)
STAI (Trait)	40.71 (10.62)	42.21 (10.12)

Table 1. Participant Characteristics.

Part 1: MRI Session	***Pubertal-Onset (N = 12) M (SD)***	***Adult-Onset (N=15) M (SD)***
Demographics		
Time on OC (months)	73.00 (28.58)	20.40 (11.61)
Age of Onset (years)	13.79 (1.27)	19.07 (1.53)
OC Name		
Tri-Cyclen	5	3
Alesse	1	3
Alysena	3	1
Yasmine	1	1
Marvelon	0	2
Other	2	5
Progestin Type		
Levonorgestrel	5	7
Norgestimate	5	3
Desogestrel	1	2
Drospirenone	1	1
Cyproterone	0	1
Norethindrone	0	1
Part 2: Stress Testing	***Pubertal-Onset (N = 26) M (SD)***	***Adult-Onset (N=32) M (SD)***
Demographics		
Time on OC (months)	65.31 (26.25)	15.32 (16.58)
Age of Onset (years)	13.73 (1.48)	18.56 (1.27)
OC Name		
Tri-Cyclen	2	5
Alesse	6	7
Alysena	8	6
Yasmine	1	2
Marvelon	3	1
Other	6	11
Progestin Type		
Levonorgestrel	16	14
Norgestimate	5	4
Desogestrel	2	5
Drospirenone	1	3
Cyproterone	0	1
Norethindrone	2	5

Table 2. Oral Contraceptive Characteristics.

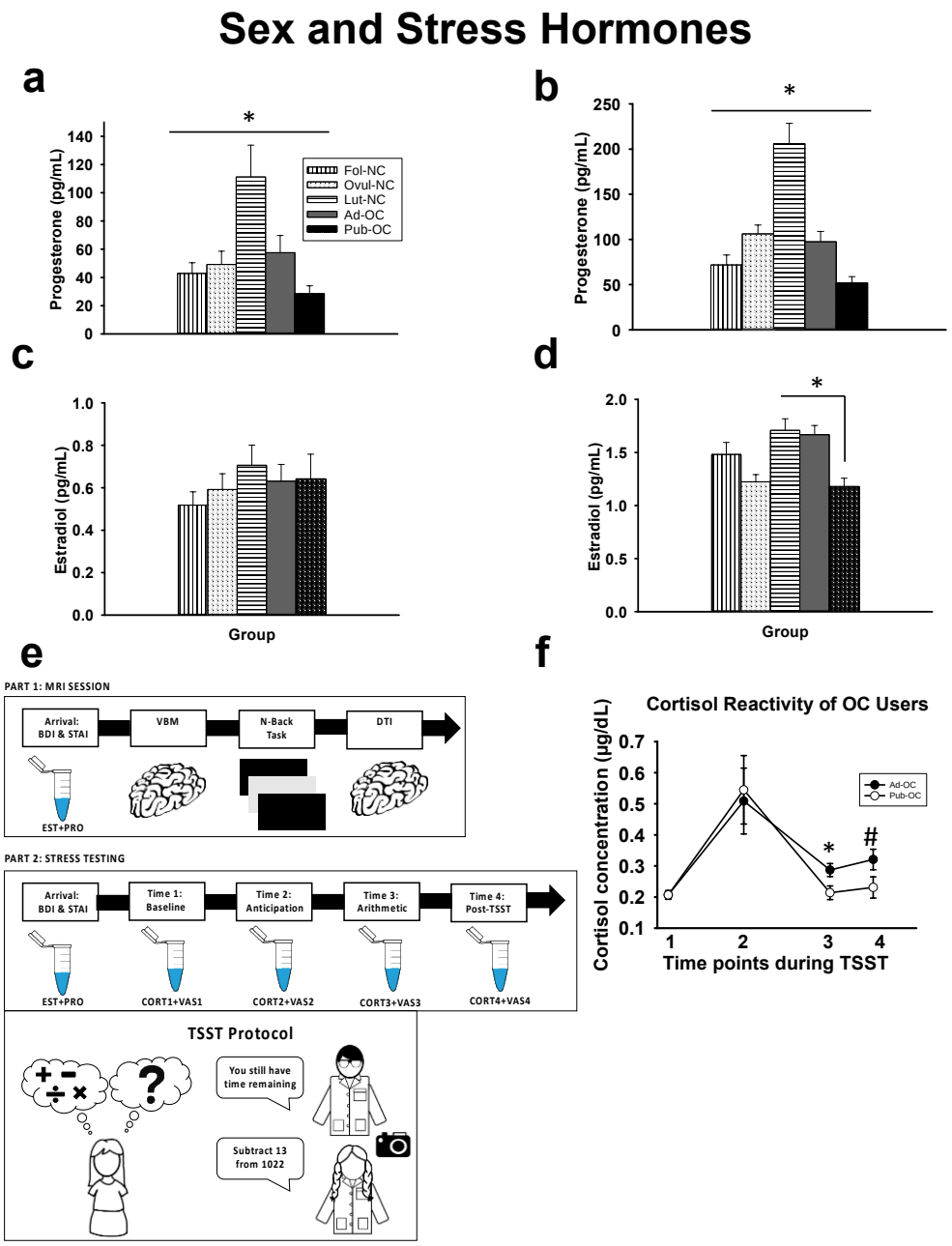

Figure 1. Pubertal- and adult-onset OC use suppresses endogenous salivary a & b) progesterone concentration ($p < 0.001$ respectively) compared to NC women in the luteal phase of the menstrual cycle (prior to MRI session & stress testing). d) Pubertal-onset OC users display less endogenous salivary estradiol concentration compared to NC women in the luteal phase of the menstrual cycle at baseline. The asterix (*) represents a significant difference between groups and the hashtag (#) represents a marginal difference between groups. e) Graphical representation of the MRI and TSST protocols with f) the mean (± S.E.) salivary cortisol concentration in pubertal-onset and adult-onset OC users in response to the TSST. Pubertal-onset OC users (N = 25) displayed less salivary cortisol concentration compared to adult-onset OC users (N = 30) following the arithmetic task of the TSST ($p = 0.019$).

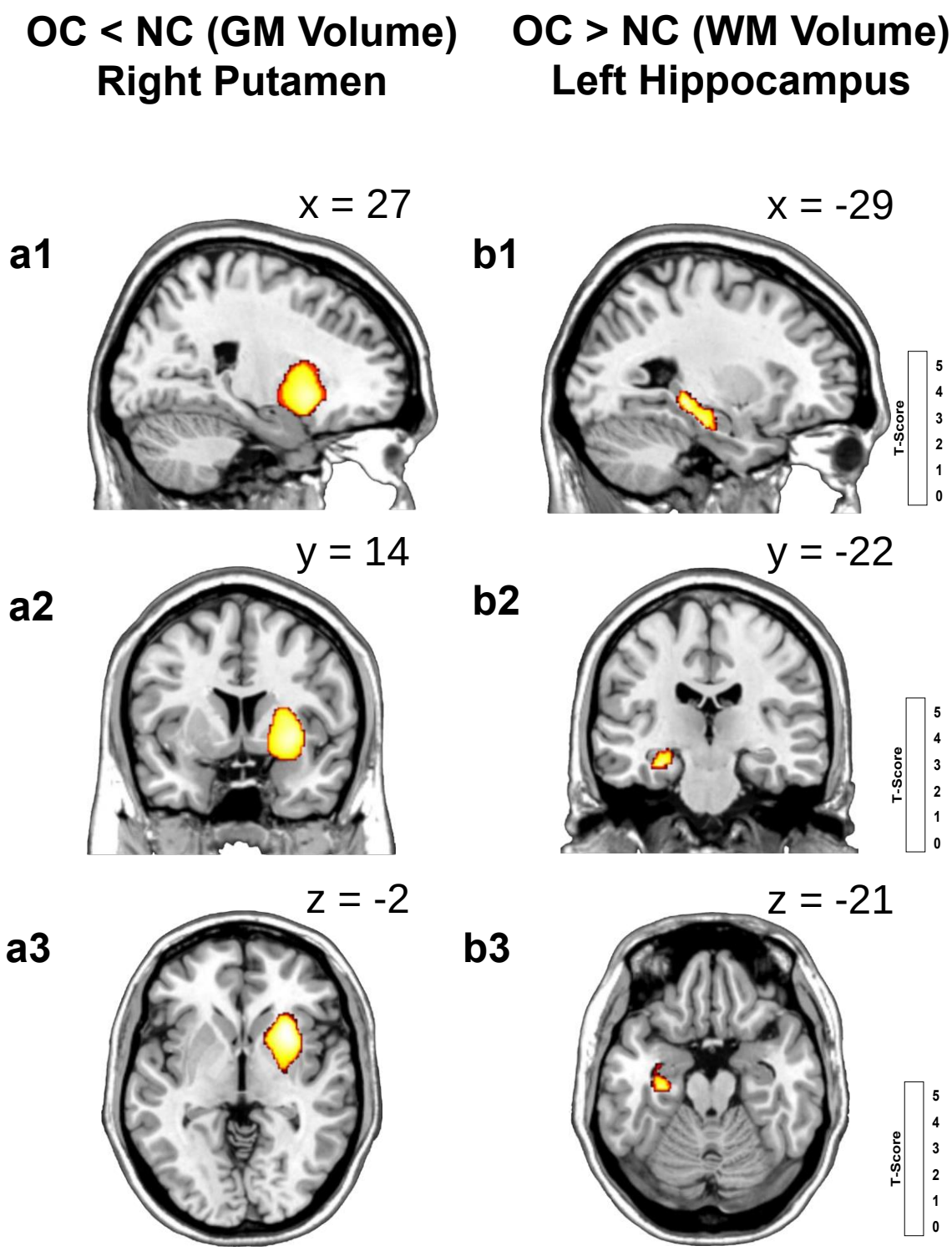

Figure 2. OC users (N = 27) display less GM volume in the right putamen and more WM volume in the left hippocampus compared to NC women (N = 48). Regions of GM (a1-3) and WM (b1-3) volume differences are overlaid on coronal, sagittal, and transverse sections ($p < 0.005$ uncorrected, $k \geq 20$, T value scale presented on the right). MNI coordinates presented at the top of each section. Statistical analyses controlled for global GM and WM volume effects.

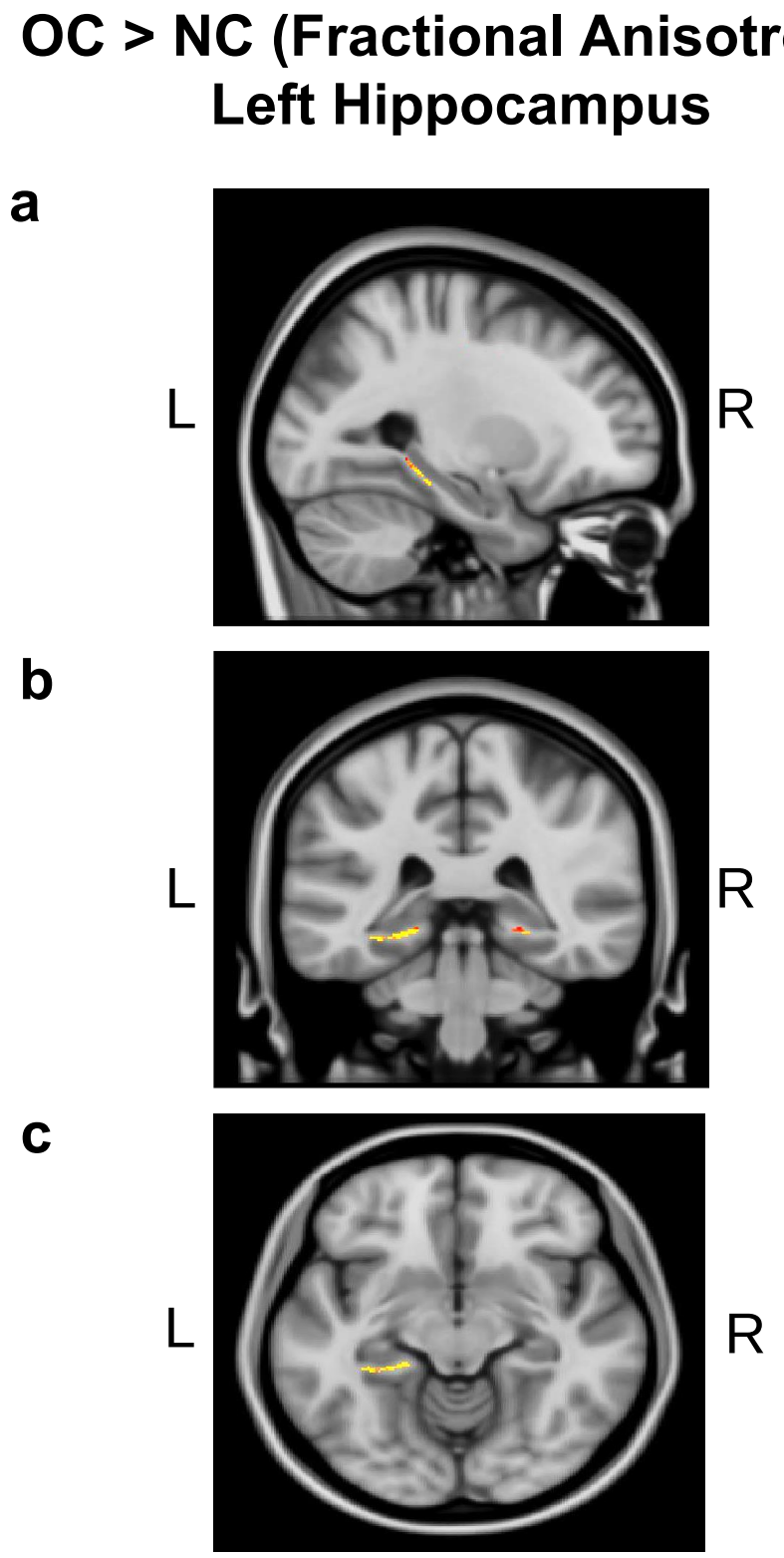

Figure 3. OC users (N = 27) display more FA (i.e., white matter integrity) in the left hippocampus compared to NC women (N = 48). The region of FA difference is overlaid on coronal, sagittal, and transverse sections ($p < 0.05$).

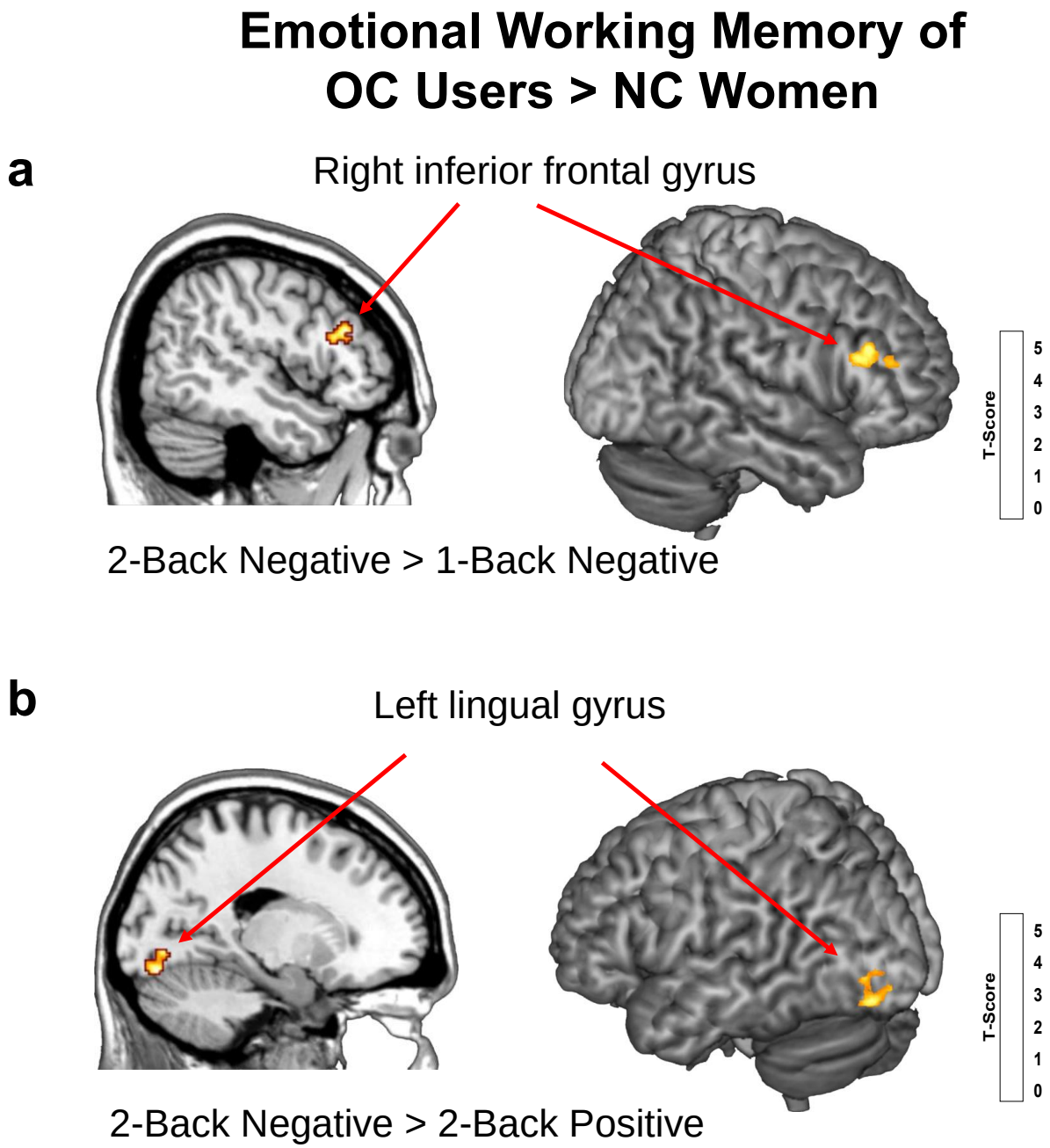

Figure 4. OC users (N = 26) display more brain activity in the a) right inferior frontal gyrus during the 2-Back Negative > 1-Back Negative contrast and b) more brain activity in the left lingual gyrus during the 2-Back Negative > 2-Back Positive contrast compared to NC women (N = 48). Regions of interest are overlaid on sagittal sections ($p < 0.005$ uncorrected, $k \geq 20$, T value scale presented on the right).

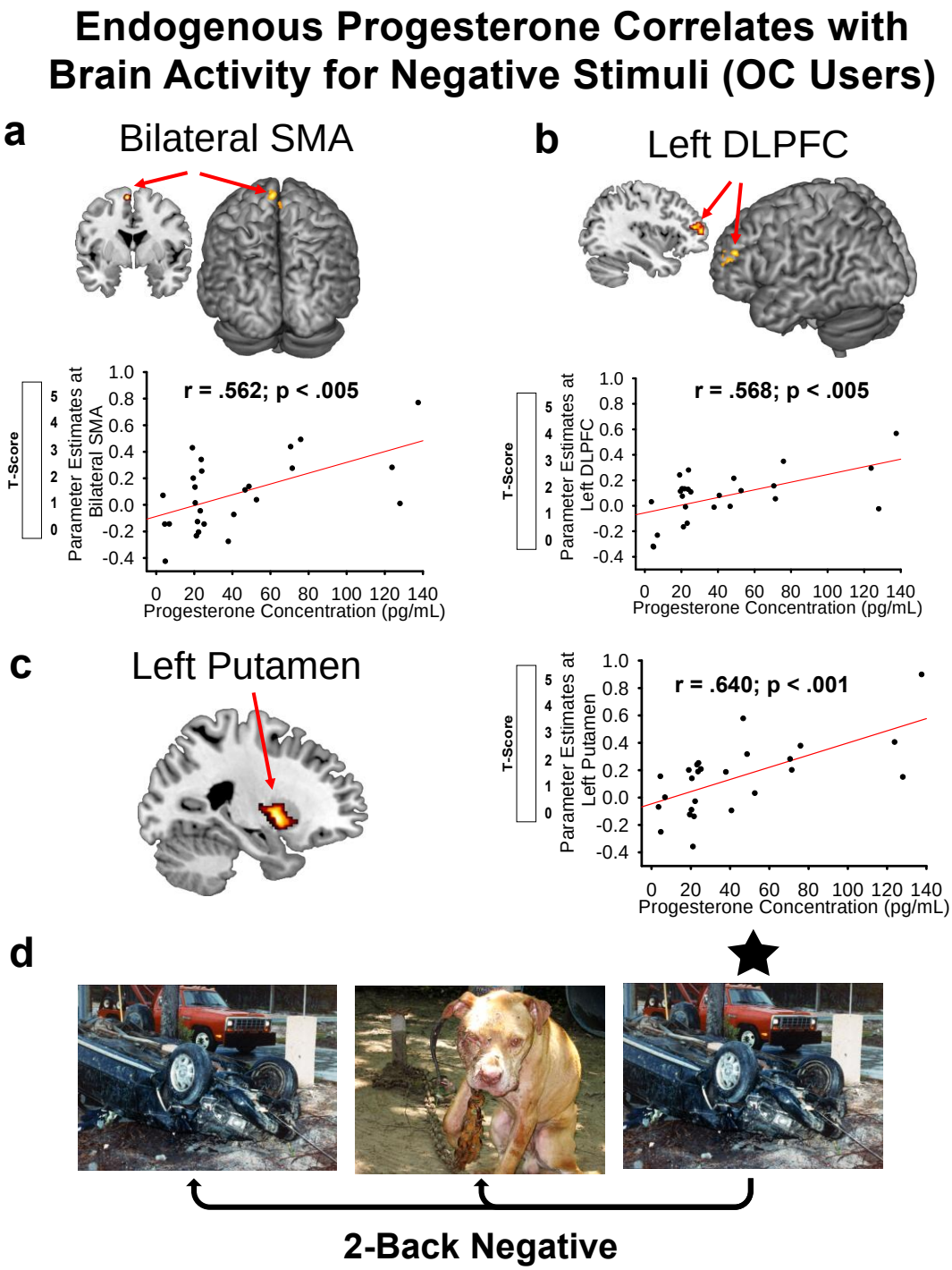

Figure 5. Endogenous progesterone correlates with working memory for negative pictures in OC users (N = 26). Positive correlation between progesterone levels and brain activity in the a) bilateral supplementary motor area (r = .562, p = 0.003), b) left dorsolateral PFC (r = .568, p = 0.002), and c) left putamen (r = .640, p < 0.001) within OC users during the 2-Back Negative > 1-Back Negative contrast ($p < 0.005$ uncorrected, $k \geq 20$). Scatterplots showing the strength of brain activation and salivary progesterone levels are displayed with the brain images. All associations were detected using Pearson correlation coefficients. d) Graphic representation of the 2-Back Negative > 1-Back Negative contrast.

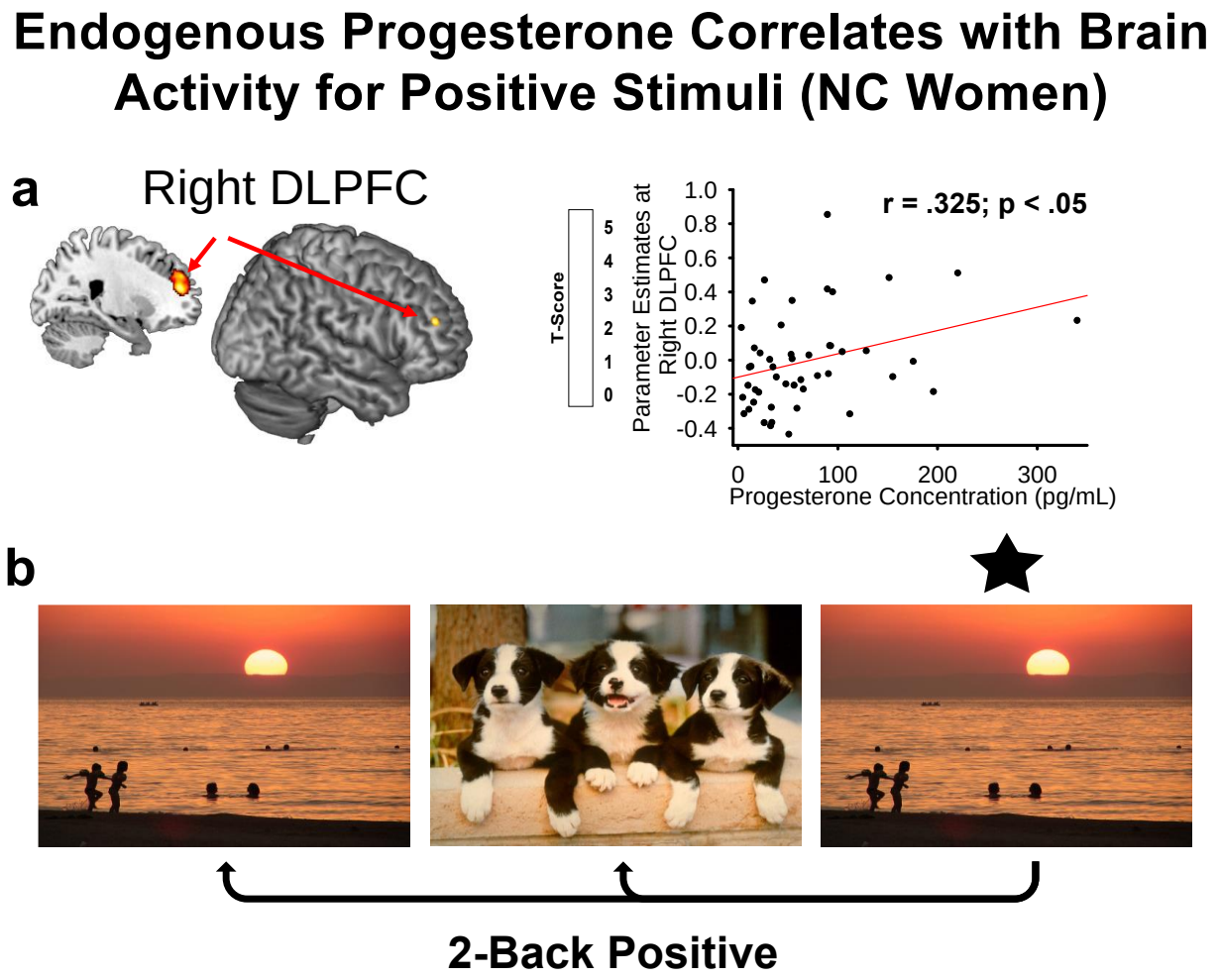

Figure 6. Endogenous progesterone correlates with working memory for positive pictures in NC women (N = 48). Positive correlation between progesterone levels and brain activity in the a) right dorsolateral PFC ($r = .325$, $p = 0.024$) within NC women during the 2-Back Positive > 1-Back Positive contrast ($p < 0.005$ uncorrected, $k \geq 20$). Scatterplot showing the strength of brain activation and salivary progesterone levels is displayed with the brain image. The association was detected using Pearson correlation coefficients. b) Graphic representation of the 2-Back Positive > 1-Back Positive contrast.

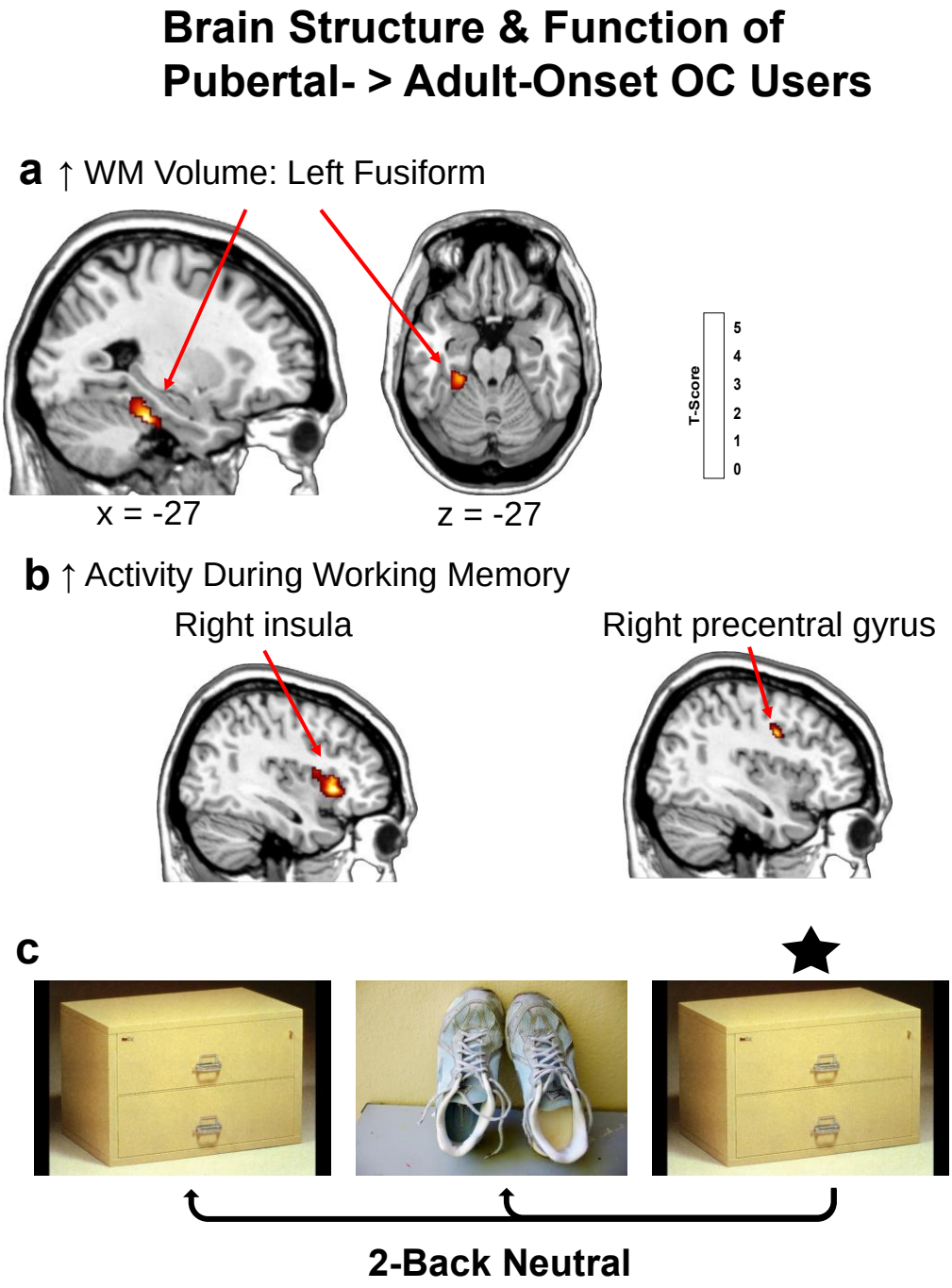

Figure 7. Exploratory analyses between pubertal- and adult-onset OC users. a) Pubertal-onset OC users (N = 12) display more WM volume in the left fusiform gyrus compared to their adult-onset counterparts (N = 15). Region of interest is overlaid on sagittal and transverse sections ($p < 0.05$ uncorrected, $k \geq 20$, T value scale presented on the right). MNI coordinates presented at the bottom of each section. b) Pubertal-onset OC users also show more brain activity in the right insula and right pre-central gyrus during the 2-Back Neutral > 1-Back Neutral contrast compared to adult-onset OC users. c) Graphic representation of the 2-Back Neutral > 1-Back Neutral contrast.

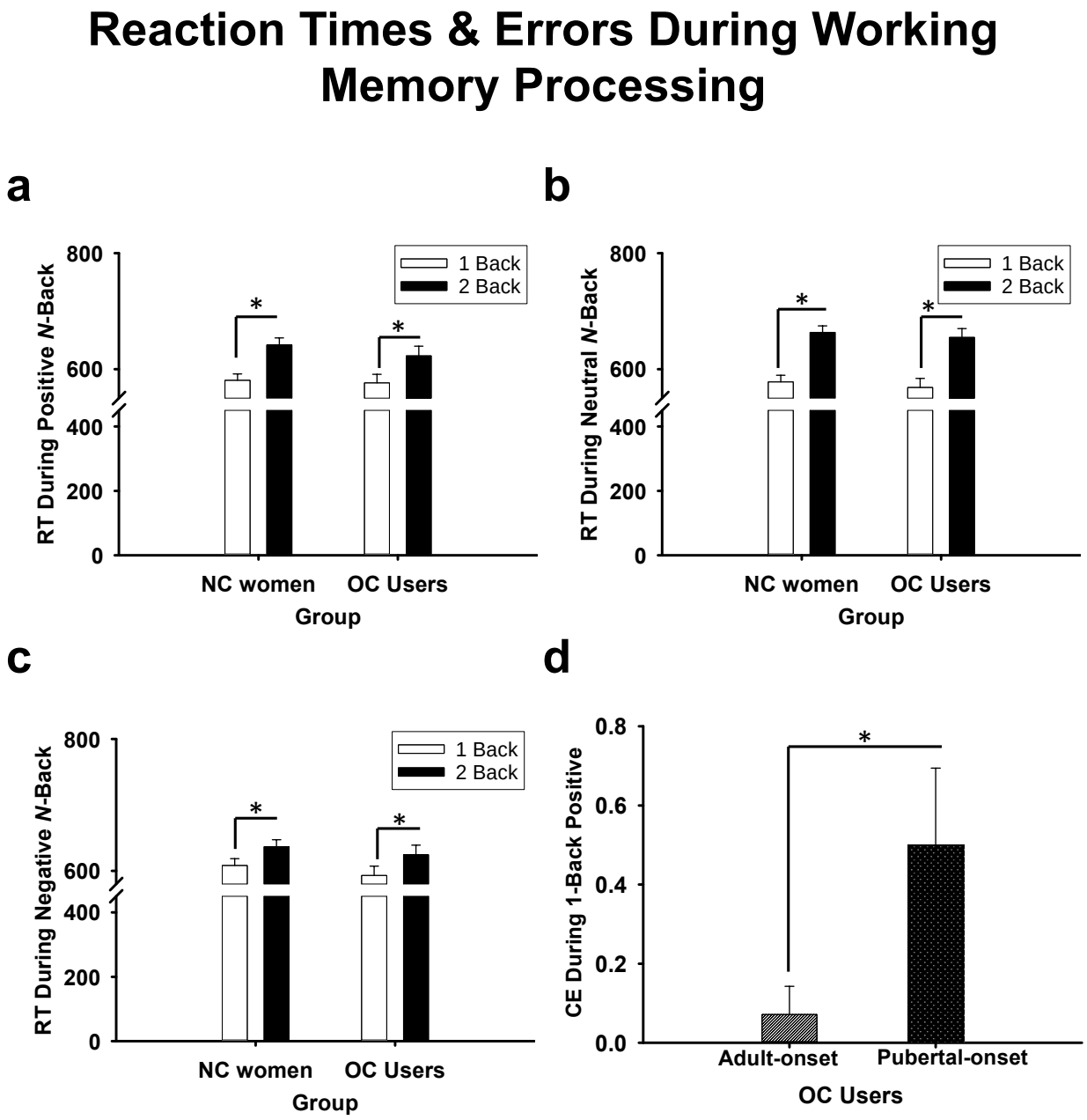

Figure 8. Both OC users (N = 26) and NC women (N = 48) show greater reaction time (RT) during the a) 2-Back Positive, b) 2-Back Neutral, and c) 2-Back Negative blocks of the *n*-back task ($p < 0.05$, respectively). d) Pubertal-onset OC users (N = 12) display more commission errors during the 1-Back Positive block compared to adult-onset OC users (N = 14) ($p < 0.05$, respectively).

Article 2: Oral contraceptive use, especially during puberty, alters resting state functional connectivity.

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Abstract

Millions of women worldwide use oral contraceptives (OCs), often starting during puberty/adolescence. It is, however, unknown how OC use during this critical period of development affects the brain. The objective of the current study was to examine resting state functional connectivity (FC) in the default mode network (DMN), central executive network (CEN), salience network (SN), and reward network (RN) of the brain using independent component analysis (ICA) between pubertal- and adult-onset OC users (n = 27) and naturally cycling women (n = 48). It was hypothesized that OC use would result in network-specific increases and decreases in FC and that pubertal-onset OC use would result in differences to the aforementioned networks compared to adult-onset OC use. Pubertal-onset OC use is related to heightened FC in the SN compared to adult-onset OC users. In general, OC use also increases connectivity in the SN, RN, and CEN compared to NC women. No significant differences in connectivity were observed in the DMN between OC users and NC women. These findings provide a mechanistic insight for the altered executive functioning and emotion/reward processing previously seen in OC users, which may then increase their vulnerability to mental health conditions.

Keywords: Puberty; Oral Contraceptives; Resting State fMRI; Central Executive Network; Salience Network; Reward Network; Independent Component Analysis

1. Introduction

Oral contraceptives (OCs) have been in the market for over 60 years and are used by millions of women worldwide, making them one of the most widely prescribed medications among women (Pletzer & Kerschbaum, 2014). OCs work by suppressing the hypothalamic-pituitary-gonadal axis through negative feedback, resulting in lower levels of endogenous gonadotropins and ovarian steroid hormones (McGuire et al., 1975). The suppression of these endogenous hormones and the intake of exogenous (i.e., synthetic) hormones via OCs have also been shown to produce negative emotional side effects (Skovlund et al., 2016; Lundin et al., 2017). These side effects may exacerbate women's vulnerability to stress-related mental health disorders by changing brain structure and function (Montoya & Bos, 2017; Lewis et al., 2019). However, the mechanisms mediating this hormone-related vulnerability remain unclear.

Emerging evidence suggests that OC use induces significant structural and functional changes in the brain. Compared to naturally cycling (NC) women, OC users, in their early to mid-20s, show region-specific increases and decreases in grey matter (GM) volume (Pletzer et al., 2010; 2015; Lisofsky et al., 2016; Hertel et al., 2017; Pletzer et al., 2019; Sharma et al., in press), white matter (WM) volume and integrity (De Bondt et al., 2013; Sharma et al., in press) and cortical thickness (Petersen et al., 2015). OC users also generally display several functional differences on tasks that primarily involve memory and emotion, although the direction of the effects appears to be task-dependent. OC users show increased blood-oxygen-level-dependent (BOLD) response in frontal brain regions (inferior, superior, and middle frontal gyri), as well as in the insula, fusiform gyrus, lingual gyrus, and supplementary motor area when exposed to an emotionally arousing picture *n*-back task (Sharma et al., in press). While this heightened BOLD response is consistent with other OC-related functional tasks such as following traumatic film

exposure (Miedl et al., 2018), OC users show less BOLD signal in frontal brain regions and in the insula compared to NC women during an emotional face matching task (Gingnell et al., 2013). Thus, OC use alters task-dependent brain activity in areas involved in memory and emotion regulation.

In addition to changes in brain structure and function, other studies have examined resting state functional connectivity (FC) in OC users and NC women. At rest, the brain has a characteristic pattern of synchronous low-frequency oscillations (Biswal et al., 1995; 1997). A number of functional networks show correlated patterns of activity at rest, with one being the default mode network (DMN). The DMN strongly corresponds to theory of mind and social cognition tasks (Laird et al., 2011). Other networks, such as the central executive network (CEN) and the salience network (SN), also correspond to specific behavioural outcomes. The CEN is involved in numerous cognitive processes, including reasoning, working memory (e.g., *n*-back task), and divided attention, whereas the SN is known to link cognition with emotion/interoception (Laird et al., 2011).

Emerging evidence suggests that OCs may alter the resting state FC of various networks in the brain; however, the findings are inconsistent. For example, Petersen et al. (2014) report that OC users show less FC in the angular gyrus of the DMN compared to NC women. In contrast, Pletzer et al. (2016) found more resting state FC in the post-central gyrus of androgenic OC users, but less resting state FC in the superior frontal gyrus of anti-androgenic OC users compared to NC women. While both studies compared OC users to NC women in the follicular phase of the menstrual cycle, Pletzer et al. (2016) also compared users of androgenic OCs separately from users of anti-androgenic OCs. They found that users of anti-androgenic OCs show less resting state FC in the middle prefrontal cortex (mPFC) compared to NC women in the

SN (Pletzer et al., 2016). Nevertheless, regardless of pill type, OC users show less resting state FC in the anterior cingulate cortex (ACC) in the SN (Petersen et al., 2014). While Petersen et al. (2014) and Pletzer et al. (2016) both used cross-sectional designs, there is variability in the acquisition of the resting state data between the two studies, which may contribute to inconsistencies in findings (Wang et al., 2015). For example, Pletzer et al. (2016) instructed all participants to keep their eyes closed, whereas Petersen et al. (2014) did not. Furthermore, differences in study designs (e.g., within-subjects compared to between-subjects) can also lead to differences in resting state FC (Engman et al., 2018). In a randomized control trial, women showed greater FC in the ACC of the SN after OC intake compared to before use. Yet, in comparison to placebo users, OC users showed reduced FC between the amygdala and the cuneus and the post-central gyrus (Engman et al., 2018). Overall, these findings show that OCs affect the resting state dynamics across multiple networks, such as the SN and CEN, which are highly implicated in memory and emotion regulation.

While the direction of the effects is mixed due to differences in participant inclusion/exclusion criteria and study design, alterations in FC in these networks have been related to negative affect (Brakowski et al., 2017; Engman et al., 2018), which could exacerbate vulnerability to depression among OC users. The associations between OC use and mood disruptions have received considerable attention in the last few years. OC users show a greater risk of first depression diagnosis and use of psychotropic drugs (e.g., antidepressants, anxiolytics, etc.) than non-OC users (Skovlund et al., 2016; Zettermark et al., 2018). They also report significantly lower well-being and increased anxiety, irritability, and mood swings in comparison to non-OC users (Bengtsdotter et al., 2018; Lundin et al., 2017; Zethraeus et al., 2017). Finally, OC users display increased incidence of suicide attempts and suicides compared

to non-OC users (Skovlund et al., 2017). The differences between OC users and non-OC users in resting state dynamics may provide a neural mechanism for the increased vulnerability to mood-related disorders following OC use (Pletzer et al., 2014; Engman et al., 2018). However, there is a limited number of studies investigating OC-dependent effects on resting state FC, which warrants further research into the area. The current study aims to contribute to the emerging literature and expand it by incorporating the age of OC onset and duration of use.

The effects of OC use during early adolescence (i.e., puberty) on resting state FC have not been investigated. Puberty is a critical period of development during which gonadal steroid hormones reorganize and remodel the brain (Levitt et al., 2003; Schulz et al., 2009; Vigil et al., 2016). There is also a high prevalence of psychiatric disorders during this time, namely depression and anxiety (Angold et al., 1998; Shors et al., 2003). Emerging evidence suggests that the pubertal female brain undergoes changes to resting state FC in the DMN and SN that are associated with mood-related symptoms (Ordaz et al., 2016; Ernst et al., 2019). Many women also begin using OCs during puberty for not only reproductive purposes, but for their additional off-label use as "regulators" (e.g., to regulate the menstrual cycle, mood, and skin). The heightened sensitivity of the pubertal/adolescent brain to the organizing effects of circulating gonadal steroid hormones raises concerns about the effects of OC use during this sensitive period. Current literature on OC-related mood symptoms shows that adolescent OC use results in a lasting vulnerability to depression in adulthood (Anderl et al., 2020). Adolescents are also more sensitive than older women to the influence of OCs with regard to first suicide attempt (Skovlund et al., 2017). To date, studies have yet to examine the influence of adolescent OC use on post-adolescent brain function at rest (Cahill, 2018). Additionally, recent findings from our laboratory show that pubertal-onset OC use results in more WM volume in the fusiform gyrus

and cuneus and an increased BOLD response in the bilateral lingual gyrus, mid-temporal gyrus, pre-central gyrus, and insula when working memory is engaged compared to OC use in adulthood (Sharma et al., in press). However, the FC in the DMN, CEN, and emotional networks (i.e., SN and reward network [RN]) at rest remains unknown.

Therefore, the objective of the current study was to examine FC differences in the DMN, CEN, SN, and RN in women who began using OCs either during puberty (i.e., pubertal-onset OC users) or in adulthood (i.e., adult-onset OC users), and in NC women. These networks were determined a priori due to their involvement in memory and emotion regulation. Similarly, based on our previous publication depicting a strong OC-related volumetric difference, we also sought to examine regional FC with the putamen using a seed-to-voxel based approach (Sharma et al., in press). Based on the OC-related functional differences during an emotional working memory task observed in our previous study (Sharma et al., in press), we hypothesized that OC users would show more FC in the SN and less FC in the middle, inferior, and superior frontal gyri in the CEN compared to NC women (Engman et al., 2018; Petersen et al., 2014; Pletzer et al., 2016) demonstrating altered emotion regulation and working memory. We also hypothesized that pubertal-onset OC use would result in altered FC compared to shorter OC use during adulthood.

2. Material and Methods

2.1 Participants

Women between the ages of 18 and 26 years old were recruited through the University of Ottawa's Integrated System of Participation in Research (ISPR) and in the community. A total of 75 women (mean age 19.6; SD = 1.96) participated in the functional magnetic resonance imaging (fMRI) study. Twenty-seven of them were OC users, who self-identified as either pubertal/adolescent-onset or adult-onset users. There were 12 pubertal-onset users and 15 adult-

onset users. Pubertal-onset OC users began using OCs within the first 6 months following menarche (first occurrence of menstruation), while adult-onset OC users began using OCs after 18 years of age and had been taking OCs for a minimum of 3 consecutive months at the time of testing. On average, pubertal-onset OC users had been taking OCs for approximately 6 years whereas adult-onset OC users had been taking OCs for approximately 1.5 years (Sharma et al., in press). All users reported using OCs continuously and were tested in the "active" phase of their combined OC pill regimen (i.e., containing both ethinyl estradiol and progestin). Additionally, the study included 48 NC women, who reported never having used OCs (including emergency hormonal contraception) and had regular menstrual cycles (ranging from 24 to 32 days). NC women were tested either during the early follicular phase (days 2 to 6 following the onset of menstruation), pre-ovulatory phase (days 10 to 14), or the luteal phase (days 18 to 24), which was established with a forward day count (Petersen et al., 2014). All participants were right-handed, had normal visual acuity, and were screened for histories of any psychiatric or medical illnesses. Participants with contra-indications to MRI (e.g., non-removable/implanted metal, back problems, loss of consciousness due to head injury, pregnancy or claustrophobia) were excluded. No clinically significant brain abnormalities were observed. This study was approved by the University of Ottawa's research ethics board and the Royal Ottawa Mental Health Centre's ethics panel, and informed written consent was obtained from each participant. A full description of demographic factors such as age, race, and type of OCs for participants can be found in Tables 1 and 2 of our previous publication (Sharma et al., in press).

2.2 fMRI acquisition and preprocessing

Resting state fMRI data was acquired using a Siemens Biograph 3 Tesla Magnetom MR scanner (Siemens, Erlangen, Germany) equipped with a 32-channel head coil at the Royal Ottawa Mental

Health Centre's Brain Imaging Centre. Functional echoplanar image sequencing was used (TR = 3000 ms, TE = 32 ms, FOV = 200 mm, flip angle = 90°, slice thickness = 3.0 mm, voxel size 3.1 mm × 3.1 mm × 3.0 mm, 48 axial slices). Ninety-seven image volumes were collected. Structural T1-weighted data was collected in 176 slices with a 1.0 mm × 1.0 mm × 1.0 mm voxel size. Once positioned in the scanner, participants were instructed, "Relax and try not to think of anything in particular. Keep your eyes closed until the scan is over. The scan will take about 5 minutes."

The first three volumes were discarded to allow the magnetization to approach a dynamic equilibrium. The data pre-processing, including slice timing, head motion correction, and spatial normalization to the Montreal Neurological Institute (MNI) template were conducted using the SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) modules via the toolbox Data Processing Assistant for Resting-State fMRI (DPARSF; http://www.restfmri.net; Yan and Zang, 2010) implemented in MATLAB R2012b. A spatial filter of 4 mm FWHM was used and head motion scrubbing parameters were applied (FD Power > 0.05). The fMRI data were then temporally bandpass filtered (0.01 – 0.1 Hz) to reduce low-frequency drift and high frequency respiratory and cardiac noise (Biswal et al., 1995). To reduce the effect of physiological artifacts, we removed effects of the following nuisance covariates: global mean signal and the signal from cerebrospinal fluid and WM.

2.3 ICA

Preprocessed data was entered in the Group ICA Toolbox v.3.0e (GIFT; http://mialab.mrn.org/software/gift) to compute network connectivity. Twenty independent components were extracted by independent component decomposition using the Infomax algorithm. Multiple runs (20 iterations) were performed using ICASSO to increase robustness of

the results (Petersen et al., 2014; Pletzer et al., 2016). The components containing the DMN, CEN, SN, and RN were initially identified by visual inspection then compared to each of the 20 intrinsic connectivity networks (ICNs) identified by Laird et al. (2011) through spatial correlation (http://www.brainmap.org/icns/). The Laird networks were chosen as a reference for two main reasons. First, these networks have been used as a reference in a variety of resting state studies, including previous publications examining OC-dependent effects (Petersen et al., 2014; Pletzer et al., 2016), which maintains comparability between studies. Second, they are based on the ICA approach, utilized in this study. Individual subject maps were back-reconstructed using GICA and converted into z-score maps representing the degree of correlation between voxel signal and the overall time course of the network (Erhardt et al., 2011; Petersen et al., 2014; Pletzer et al., 2016).

2.4 Seed-based FC analysis

We conducted a seed-based resting state FC analysis with the right putamen on the preprocessed data. The anatomical putamen seed region was selected from the WFU pickatlas AAL template in SPM12. Using the DPARSF toolbox, the mean time course of all voxels in the seed were used to calculate voxel-wise linear correlations (Pearson's correlations) for the whole brain.

2.5 Statistical analysis

Individual z-score component images from the ICA and individual Fisher's z transformed FC maps from the seed-based analysis were entered into an independent samples t-test analysis in SPM12 to generate contrast files. All reported results were derived from an uncorrected threshold of $p_{uncorr} < 0.005$ at the whole brain level and deemed significant at a threshold of $p < 0.05$ after family wise error (FWE) correction for multiple comparisons at the cluster level. All reported clusters consisted of fifteen or more contiguous voxels.

3. Results

3.1 Default mode network (DMN)

The DMN (component 20) showed the strongest correspondence to Laird's ICN13 (Laird et al., 2011), with a high spatial correlation ($r = 0.58$). It included the precuneus, PCC, middle and anterior cingulate gyri, and parts of the middle and superior frontal gyri. No significant differences in resting state FC were identified in the DMN between OC users and NC women. In addition, a sub-division of the DMN (component 8) was identified with a high spatial correlation with ICN13 ($r = 0.44$). This sub-division included the precuneus, rectus, PCC, middle and anterior cingulate gyri, supplementary motor area, and parts of the middle temporal and middle and inferior frontal gyri, as well as the cerebellum. No significant differences in resting state FC were identified in this sub-division of the DMN between OC users and NC women. (Table 1).

3.2 Salience network (SN)

The SN (component 2) showed strong correspondence to Laird's ICN4 ($r = 0.43$) (Laird et al., 2011). The identified component included the bilateral insula, ACC, midcingulate cortex, superior temporal gyrus, and parts of the dorsolateral PFC (Laird et al., 2011). (Figure 1A). OC users displayed increased connectivity in the left superior medial frontal gyrus ([-9 51 15], $t(1,71) = 3.99$, $p = 0.013$) and the left anterior cingulum ([0 39 6], $t(1,71) = 3.99$, $p = 0.013$) compared to NC women. (Figure 1B). In relation to OC users, NC women did not show increased connectivity in the SN; however, pubertal-onset OC users showed more connectivity in the left supramarginal gyrus ([-63 -42 36], $t(1,25) = 4.41$, $p = 0.031$) and inferior parietal gyrus ([-48 -60 48], $t(1,25) = 4.41$, $p = 0.031$) compared to their adult-onset counterparts. (Figure 1C). (Table 1).

3.3 Central executive network (CEN)

The CEN (component 18) showed a strong correspondence to Laird's ICN15 ($r = 0.44$), also described by Laird et al. (2011) as the frontoparietal network (Laird et al., 2011). The identified component included various sub-regions of the bilateral frontal (e.g., left precentral gyrus, inferior frontal gyrus, etc.) and parietal (e.g., bilateral angular gyrus) lobes. (Figure 2A). OC users showed more connectivity in the left angular gyrus ([-51 -72 36], $t(1,71) = 4.88$, $p = 0.001$) and in the right middle frontal gyrus ([45 48 21], $t(1,71) = 4.72$, $p < 0.001$) compared to NC women. (Figure 2B). NC women did not show increased connectivity relative to OC users in the CEN. (Table 1).

3.4 Reward Network (RN)

The RN (component 5) showed correspondence to Laird's ICN3 ($r = 0.37$). This network included the thalamus, lateral ventricle, and caudate as well as portions of the superior parietal lobule, precuneus, and dorsolateral PFC (Laird et al., 2011). (Figure 3A). In relation to NC women, OC users showed more connectivity in the left middle frontal gyrus ([-42 33 36], $t(1,71) = 4.86$, $p = 0.009$). (Figure 3B). NC women did not show more connectivity in the RN compared to OC users. (Table 1).

3.5 Seed-based connectivity

OC users showed greater FC between the right putamen seed and the right middle frontal gyrus compared to NC women ([30 12 60], $t(1,66) = 4.24$, $p = 0.048$). NC women did not show increased connectivity with the seed relative to OC users. (Table 1).

4. Discussion

In the current study, we used resting state fMRI to investigate OC-dependent changes on FC within various networks. We found that OC use is associated with increased connectivity in

predominantly frontal regions of the SN, RN, and CEN. Connectivity in the SN was also dependent on the age of OC onset.

Pubertal-onset OC users showed increased connectivity in the supramarginal gyrus and inferior parietal lobule compared to their adult-onset counterparts. Given that puberty is a period of neuronal plasticity (Vigil et al., 2016), our results suggest that the use of synthetic steroids during puberty may result in more neuronal reorganization and remodeling in the brain. Adolescent development is characterized by neuronal loss and dendritic pruning, with females exhibiting more substantial neuron loss compared to males (Willing & Juraska, 2015). It is possible that these naturally occurring processes are altered in pubertal OC users, which could then increase the resting state dynamics of the supramarginal gyrus and inferior parietal lobule of the SN. The supramarginal gyrus is a central region involved in interpersonal emotional processing and empathy (Spies et al., 2016). Increased connectivity within this region may relate to changes in emotion recognition (Cui et al., 2018). While we did not find any difference in mood-related symptoms between pubertal-onset OC users and their adult-onset counterparts (Sharma et al., in press), there is evidence suggesting that increased connectivity in the supramarginal gyrus and inferior parietal lobule is implicated in depression and social anxiety disorder (Pannekoek et al., 2013; Cui et al., 2018).

In addition to the differences in SN connectivity related to the age of OC onset, all OC users showed more connectivity in the superior medial frontal gyrus and the ACC within the SN. Similar to our results, Engman et al. (2018) showed that OC users demonstrate higher connectivity between the superior medial frontal gyrus and the ACC-seed of the SN. However, contrary to our results, Petersen et al. (2014) found that NC women in the follicular phase show greater connectivity in the ACC and middle frontal gyrus within the SN compared to OC users.

Since the follicular phase is often considered the low hormone phase, it is common to observe similar endogenous estradiol concentration between NC women in the follicular phase and OC users (Petersen et al., 2014; Petersen & Cahill, 2015). However, because we did not observe differences in endogenous estradiol concentration between NC women in the pre-ovulatory or luteal phases compared to the OC users (Sharma et al., in press), our results cannot be attributable to the inhibitory effects of OCs on endogenous ovarian steroid hormone production. Rather, our results suggest that the intake of ethinyl estradiol in the presence of similar endogenous estradiol concentration likely contributes to the higher connectivity within the SN, which could then have task-related functional implications. For example, the ACC plays a critical role in mediating the interaction between emotion perception and executive control, as is required for working memory. In an *n*-back task of emotionally arousing faces, participants display more activity in the ACC when comparing fearful faces against neutral faces (Luo et al., 2014). The higher connectivity in the ACC of the SN may also provide mechanistic insight for the increased brain activity in OC users following a traumatic video task and emotional *n*-back picture task (Miedl et al., 2018; Sharma et al., in press). In our previous study, we found that OC users demonstrate increased BOLD signal in the inferior frontal gyrus and middle frontal gyrus of the brain and in the insula, lingual gyrus, and supplementary motor area during working memory processing for negatively arousing images compared to NC women (Sharma et al., in press). Overall, our findings indicate that OC use changes resting state FC within the SN, with some regional differences being dependent on age of OC onset. It is unclear, however, whether the differences related to the age of OC onset are specifically due to pubertal-onset OC use, a longer duration of OC usage, or both factors. Nonetheless, the regions identified with altered

connectivity in the SN may relate to differences in brain activity observed among OC users during emotion processing.

Moreover, we observed more resting state FC in the RN, which may relate to functional differences during reward processing between OC users and NC women (Bonenberger et al., 2013). OC users displayed heightened connectivity in the dorsolateral PFC of the RN and with the putamen-seed. Only one study to date has examined the effects of the menstrual cycle and OC use on connectivity in the RN. Pletzer et al. (2016) found decreased connectivity in the basal ganglia of this network but the effect was only observed in users of anti-androgenic OCs compared to NC women. Consistent with these findings, Hidalgo-Lopez et al., (2020) observed stronger connectivity between the middle frontal gyrus and caudate during the pre-ovulatory phase when estradiol levels are typically high compared to the early follicular phase. Since we did not separate our analyses based on anti-androgenic vs. androgenic OCs, our findings reflect a general difference in OC users, regardless of pill type, compared to all NC women. It is possible that the ethinyl estradiol in the OC pill further enhances frontal-striatal connectivity in comparison to endogenous estradiol, as previous literature has identified increased dopaminergic production in the brain following OC use (Algeri et al., 2004). Therefore, the altered connectivity observed in the dorsolateral PFC of the RN and with the putamen may be attributed to the ethinyl estradiol in the OC pill and could then provide neurophysiological insight for the greater sensitivity to reward processing in OC users.

Similar to the heightened connectivity within the RN, OC users showed more connectivity in the dorsolateral PFC and angular gyrus of the CEN. While we are the first to show increased connectivity in these regions, Pletzer et al. (2016) report increased connectivity in the bilateral basal ganglia to the CEN in OC users compared to NC women. Although we did

not perform hormone assays for salivary testosterone levels or include men in this study, it is possible that synthetic hormone intake through OC use results in a masculinization effect on regional connectivity within the CEN. For example, a sex difference has been observed such that men demonstrate greater connectivity in the middle, inferior, and superior frontal gyri of the CEN compared to women (Smith et al., 2014).

Progestin androgenicity could also contribute to the higher connectivity within the dorsolateral PFC. While progestins bind to progesterone receptors at different levels to suppress ovulation, they can interact with other steroid receptors such as the androgen receptor (AR) and estrogen receptor (Germain et al., 2006). Progestins that exhibit a relatively high affinity to the AR are generally derived from testosterone (Stanczyk et al., 2013) and could potentially increase androgen metabolism. However, the role of progestins in androgen metabolism is complex. While a systematic review found that all combined OCs, including different types of progestins, have suppressive effects on free and total circulating testosterone levels, OCs with a lower ethinyl estradiol dose (20 – 25 μg) combined with first or second generation progestins (e.g., levonorgestrel) have less suppressive effects (Zimmerman et al., 2013). This type of hormonal composition could thereby contribute to higher resting state dynamics in the dorsolateral PFC. In comparison to the progestins found in OCs, endogenous progesterone has been shown to increase connectivity within the dorsolateral PFC during the luteal phase of the menstrual cycle compared to the follicular phase (Arelin et al., 2015). Similarly, NC women in the luteal phase show more connectivity in the mPFC compared to NC women in the follicular phase (Pletzer et al., 2016). In our previous study, we observed decreased progesterone concentration in OC users compared to NC women in the luteal phase but similar concentrations between OC users and the other NC sub-groups (Sharma et al., in press). Thus, it is possible that decreased endogenous progesterone

concentration coupled with the interaction between synthetic progestin/ethinyl estradiol increases bilateral connectivity within the dorsolateral PFC of the CEN and RN in OC users compared to NC women.

The similarity between the CEN and RN was noteworthy in the current study. The CEN is involved in multiple cognitive processes (i.e., reasoning, attention, memory during an *n*-back task) whereas the RN is involved in processes related to reward tasks (Laird et al., 2011). Sheline et al. (2010) report increased connectivity in the bilateral dorsal medial PFC within three separate resting state networks and suggest a "hot-wiring" of these systems meaning that the networks are connected through a critical mediating region. The authors also show that the degree of increased connectivity in the dorsal medial PFC correlates with depression severity in their participants. It is possible that a similar hot-wiring pattern exists between the RN and CEN via the dorsolateral PFC in OC users. In addition, alterations in connectivity within the CEN are implicated in various psychiatric disorders such as bipolar (Goya-Maldonado et al., 2016) and obsessive-compulsive disorders (Chen et al., 2016). Therefore, our findings point to OC-dependent changes in regional connectivity within the CEN and a possible hot-wiring effect of the CEN and RN through the dorsolateral PFC.

There are nonetheless some limitations to the study. First, the lack of significant differences in endogenous estradiol concentration could be attributed to methodological limitations. Using the forward day count to establish menstrual cycle phase, especially in younger women who have more intraindividual variability in cycle length, is less reliable than using other hormone measures (e.g., serum samples) or ovulation kits (Hampson, 2020). We also did not account for the time that OC users took their pill prior to the fMRI session. Mean concentration of ethinyl estradiol typically peaks within two hours post-ingestion followed by a

rapid clearance. This could explain the variability in endogenous estradiol concentration within OC users resulting in no group difference in comparison to NC women (Hampson, 2020). Future studies should incorporate multiple immunoassay techniques (e.g., liquid chromatography-mass spectrometry) to confirm hormonal data (Rosner et al., 2013) and use antibodies with high specificity for ethinyl estradiol to quantify synthetic vs. endogenous hormones (Hampson, 2020). The incorporation of radioligand positron emission tomography could be included to provide essential insight into how OC-induced hormonal states (low endogenous but high synthetic hormones) may directly influence resting state FC (Lewis et al., 2019). Moreover, our findings should be interpreted with caution. While they may provide critical insight regarding why some women experience greater vulnerability to psychiatric disorders following OC use, they should not be taken to represent these consequences. Additionally, the cross-sectional design of this study does not allow us to establish causation of resting state dynamics following OC use. Finally, given the vast diversity in OC formulations and the complex interplay between ethinyl estradiol doses and synthetic progestins, the inclusion of OC participants using a specific formulation would provide clearer results.

5. Conclusions

Nonetheless, this study is the first to examine the age-dependent effect of OC use on resting state FC within the DMN, SN, CEN, and RN. This work highlights that pubertal-onset OC use results in higher regional connectivity in the SN compared to shorter OC use during adulthood. In addition, general OC-dependent effects were observed such that OC use is related to greater connectivity in the SN, CEN, and RN and that the CEN and RN may be hot-wired via the dorsolateral PFC. Research is beginning to elucidate the effects of OCs on resting state FC. The impact of OCs during puberty/adolescence, a sensitive period of neurodevelopment with a

high emergence of psychiatric disorders, is also unknown. To conclude, the current study offers critical insight into the potential impact of OC use at an early age on women's brain activity and related mental health.

Declaration of Interest

We declare no conflict of interest.

Author Contribution Statement

Rupali Sharma, Andra Smith, and Nafissa Ismail designed the current study. Rupali Sharma was also responsible for data collection, data analysis, and manuscript preparation. Zhuo Fang contributed to data analysis and manuscript preparation. Zhuo Fang, Andra Smith and Nafissa Ismail revised and edited the manuscript.

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Age of OC onset differences (Pubertal-onset > Adult-onset OC users)				
Network	Regions in component	FC differences	MNI coordinates	$p_{FWE,\ cluster\ level}$
SN	Bilateral insula, ACC, midcingulate cortex, superior temporal gyrus, and dorsolateral PFC	↑ L-supramarginal gyrus ↑ L-inferior parietal gyrus	-63 -42 36 -48 -60 48	0.031 0.031
General OC-related differences (all OC users > NC women)				
Network	Regions in component	FC differences	MNI coordinates	$p_{FWE,\ cluster\ level}$
SN	Bilateral insula, ACC, midcingulate cortex, superior temporal gyrus, and dorsolateral PFC	↑ L-superior medial frontal gyrus ↑ L-ACC	-9 51 15 0 39 6	0.013 0.013
CEN	Bilateral frontal (e.g., left precentral gyrus, inferior frontal gyrus, etc.) and parietal (e.g., bilateral angular gyrus) lobes	↑ L-angular gyrus ↑ R-dorsolateral PFC	-51 -72 36 45 48 21	0.001 0.001
RN	Thalamus, lateral ventricle, caudate, superior parietal lobule, precuneus, and dorsolateral PFC	↑ L-dorsolateral PFC	-42 33 36	0.009
Seed-based connectivity	Seed = Putamen	↑ R-middle frontal gyrus	30 12 60	0.048

Table 1. Summary of OC-related differences in resting state FC.

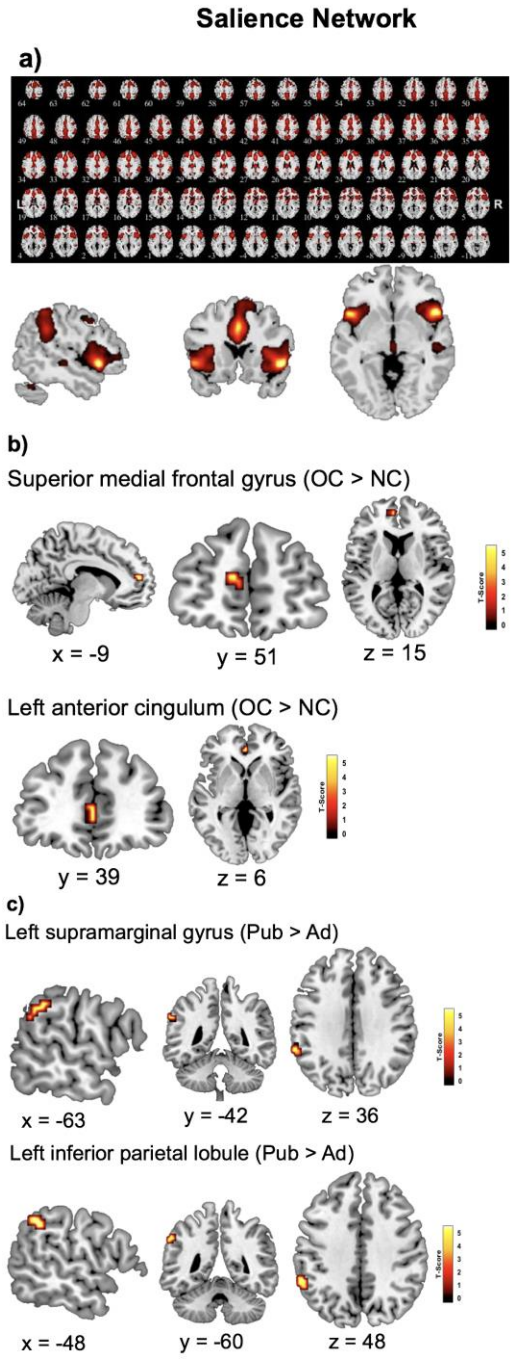

Figure 1. (A) Structure of the SN. This component corresponds to ICN4, identified by Laird et al. (2011), with a spatial correlation of 0.43. (B) OC users (N = 27) showed more resting state FC in the superior medial frontal gyrus ([-9 51 15], t(1,71) = 3.99, p = 0.013) and anterior cingulum ([0 39 6], t(1,71) = 3.99, p = 0.013) in the SN compared to NC women (N = 48). (C) Pubertal-onset OC users (N = 12) showed more resting state FC in the supramarginal gyrus and inferior parietal lobule compared to their adult-onset counterparts (N = 15). All regions are represented on coronal, sagittal, and transverse sections ($p < 0.005$ uncorrected, $k \geq 20$, T value scale presented on the right). MNI coordinates presented at the bottom of each section.

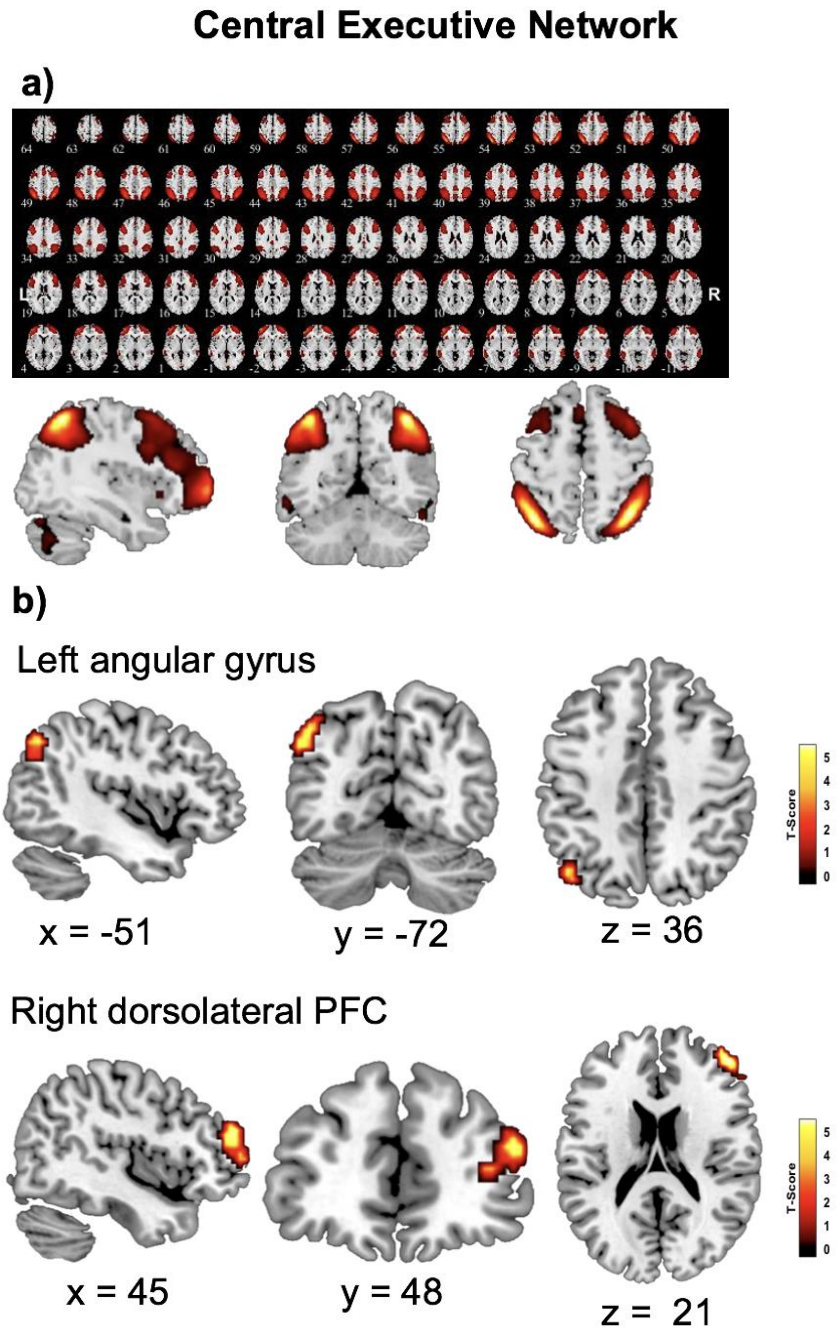

Figure 2. (A) Structure of the CEN. This component corresponds to ICN15, identified by Laird et al. (2011), with a spatial correlation of 0.44. (B) OC users (N = 27) showed more resting state FC in the angular gyrus ([-51 -72 36], $t(1,71) = 4.88$, $p = 0.001$) and in the DLPFC ([45 48 21], $t(1,71) = 4.72$, $p < 0.001$) in the CEN compared to NC women (N = 48). All regions are represented on coronal, sagittal, and transverse sections ($p < 0.005$ uncorrected, $k \geq 20$, T value scale presented on the right). MNI coordinates presented at the bottom of each section.

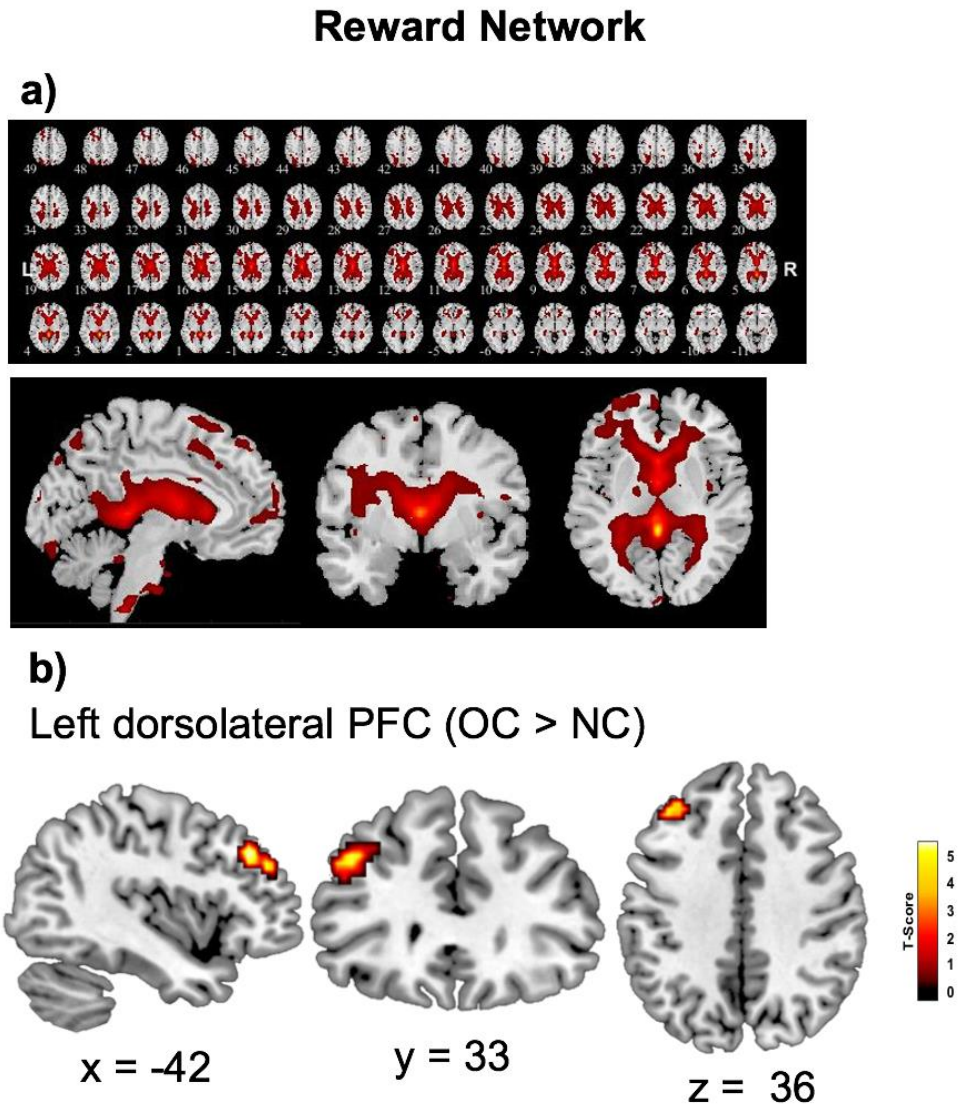

Figure 3. (A) Structure of the RN. This component corresponds to ICN3, identified by Laird et al. (2011), with a spatial correlation of 0.37. (B) OC users (N = 27) showed more resting state FC in the DLPFC ([-42 33 36], $t(1,71) = 4.86$, $p = 0.009$) in the RN compared to NC women (N = 48). All regions are represented on coronal, sagittal, and transverse sections ($p < 0.005$ uncorrected, $k \geq 20$, T value scale presented on the right). MNI coordinates presented at the bottom of each section.

Article 3: The regulatory roles of progesterone and estradiol on emotion processing in women.

*Under review; submitted: Cognitive, Affective, and Behavioural Neuroscience

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Abstract

Emotion processing is known to interact with memory. Ovarian steroid hormones such as progesterone and estradiol modulate emotion processing and memory. However, it is unclear how these hormones influence brain activity when emotion processing is integrated with working memory. Therefore, the objective of this study was to examine the relationship between endogenous hormonal concentration and brain activity during emotion processing in the context of a working memory *n*-back task in 74 young women using functional magnetic resonance imaging (fMRI). Results show that positive emotion processing activates reward-related areas such as the caudate and putamen whereas negative emotion processing activates a corticolimbic network including the amygdala and hippocampus. Furthermore, our findings provide evidence that progesterone modulates more bottom-up brain activation during both positive and negative emotion processing whereas estradiol activates lateralized, top-down regulation. These findings provide insight on the neural correlates of emotion processing during an *n*-back task in young women and highlight how important it is to consider women's endogenous hormonal concentration in neurobiological and cognition research.

Keywords: Progesterone; Estradiol; Emotional Processing; Working Memory; fMRI; *N*-Back Task

1.Introduction

Emotional processes are known to interact with executive control (Lindstrom & Bohlin, 2011). One important aspect of executive control is the ability to retain relevant information and update it according to task demands. This is referred to as working memory and it is prefrontal cortex-dependent (Miyake et al., 2000). There are shared and valence-specific neural correlates of emotion processing such that changes in brain activity in the left prefrontal cortex (PFC) and striatum are related to positive emotion processing whereas changes in the right PFC and anterior cingulate cortex (ACC) are associated with negative emotion processing (Mak et al., 2009; Grimm et al., 2012; Grosse et al., 2019). However, processing of emotional stimuli in conjunction with a working memory task has not been shown to have a consistent impact on brain activity especially since different types of working memory tasks exist. For example, both positive and negative emotional stimuli, compared to neutral stimuli, in a verbal working memory task elicit more brain activity in the dorsolateral PFC in healthy men, and this is associated with longer reaction times (Grimm et al., 2012). Comparatively, in a non-verbal working memory task that is limited to emotional faces, positive stimuli processing results in more brain activity in the medial PFC and only negative stimuli processing leads to more brain activity in the dorsolateral PFC compared to neutral stimuli in a clinical population (Becerril & Barch, 2010). Few, if any, studies to date have used a working memory paradigm such as an *n*-back task encompassing a wide array of emotional images. While some attempts have been made, the majority of these tasks introduce images of emotional faces surrounding the stimulus images as distractors, rather than the focus of the task (Bertocci et al., 2012; Tavitian et al., 2014).

One factor shown to modulate the integration of emotion processing and memory is the fluctuation of ovarian steroid hormones (Sundstrom & Gingnell, 2014; Catenaccio et al., 2016). This fluctuation occurs throughout the menstrual cycle and can be suppressed through the use of oral contraceptives (OCs). Women experience menstrual cycles that are characterized by fluctuations of estrogens (mostly 17β-estradiol) and progestins (mostly progesterone) due to regulation of the hypothalamic-pituitary-gonadal (HPG) axis. The menstrual cycle can be generally divided into the follicular (low hormone), ovulatory (peak estradiol), and luteal (high progesterone/high estradiol) phases (Fauser & van Heusden, 1997). Menstrual cycle studies show that ovarian steroid hormone modulation results in structural and functional plasticity in the central nervous system. For example, brain regions that commonly exhibit follicular/luteal structural plasticity include the hippocampus, parahippocampal gyrus, fusiform gyrus, ACC, insula, middle frontal gyrus, thalamus, and cerebellum (Catenaccio et al., 2016). Moreover, several studies report changes in brain activity associated with emotion processing and memory across the menstrual cycle. Estradiol demonstrates predominantly anxiolytic properties while progesterone displays mixed anxiolytic and anxiogenic properties (Catenaccio et al., 2016). The most consistent finding so far appears in the luteal phase when progesterone levels are high resulting in an increased amygdala response to negative emotional stimuli and an increased ACC response to positive emotional stimuli (Protopescu et al., 2005; Amin et al., 2006; Andreano and Cahill, 2010; Gingnell et al., 2012; Bayer et al., 2014). On the other hand, estradiol levels during the follicular and pre-ovulatory phases result in better emotion recognition accuracy independent of emotional stimuli (Pearson & Lewis, 2005; Derntl et al., 2008a,b) and more brain activity in the ACC and dorsolateral PFC during response inhibition to positive stimuli (Amin et al., 2006). In contrast to emotion processing, the effects of ovarian steroid hormones on emotional memory

are mixed. While a longitudinal study reports decreased recognition for negative items during the luteal phase (Bayer et al., 2014), cross-sectional studies have demonstrated no difference (Felmingham et al., 2012) or enhanced memory for emotional items during this phase (Ertman et al., 2011).

Suppression of endogenous ovarian hormones through OCs has also been linked to altered emotion processing and memory. OC users display both increased and decreased regional brain activity compared to naturally cycling (NC) women depending on the type of negative emotional stimulus (Petersen & Cahill, 2015; Miedl et al., 2018). Similarly, OC users display enhanced memory for gist, but not for details of an emotional story, compared to NC women (Nielsen et al., 2011). Recent findings from our laboratory demonstrate increased brain activity in OC users, compared to NC women, when working memory is engaged for negatively, but not positively, arousing images in an emotional *n*-back picture task (i.e., 2-Back Negative > 1-Back Negative) (Sharma et al., in press). However, OC users and NC women show no significant differences in brain activity during positive and negative emotion processing of the working memory conditions in the same task (i.e., 2-Back Positive > 2-Back Neutral and 2-Back Negative > 2-Back Neutral), suggesting that OC users and NC women share similar neural responses during emotion processing (Sharma et al., in press). It remains unclear whether positive and negative emotion processing recruit different brain regions in the context of a visual working memory picture task with respect to endogenous ovarian steroid hormones in women.

Therefore, the objective of the current study was to examine the global role of endogenous progesterone and estradiol on positive and negative emotion processing in the context of a picture working memory task that is specifically designed for women. We hypothesized that estradiol would show a positive relationship with PFC activation during both

positive and negative emotion processing (Catenaccio et al., 2016). Furthermore, we hypothesized that progesterone would have a positive relationship with ACC activation during positive emotion processing and the amygdala during negative emotion processing.

2. Material and Methods

2.1 Participants

Women between the ages of 18 and 26 years old were recruited through the University of Ottawa's Integrated System of Participation in Research (ISPR) and in the community. A total of 74 women (mean age 19.6; SD = 1.96), including both OC users and NC women, participated in the fMRI session. Twenty-six participants were OC users who were using a combined OC formulation at the time of the fMRI session. There were 48 NC women who were in either the early follicular phase (days 2 to 6 following the onset of menstruation), pre-ovulatory phase (days 10 to 14), or the luteal phase (days 18 to 24) of the menstrual cycle, which was established via a forward day count. Since the objective of the current study was to examine the global role of ovarian steroid hormones, OC use was controlled for as a covariate of no interest. The number of participants was also selected based on previous literature (Amin et al., 2006; Andreano & Cahill, 2010; Pletzer et al., 2019) and recommendations by Desmond and Glover (2002) for sufficient power in fMRI studies. All participants were right-handed, had normal visual acuity, and were screened for histories of psychiatric or medical illnesses. Participants with contra-indications to MRI (e.g., non-removable/implanted metal, back problems, loss of consciousness, pregnancy or claustrophobia) were excluded. The experimental protocol was approved by the University of Ottawa's research ethics board and the Royal Ottawa Mental Health Centre's ethics panel, and informed written consent was obtained from each participant.

2.2 Questionnaires

Participants were asked to complete the Beck Depression Inventory (BDI) prior to the fMRI session. The BDI was administered to measure individual differences in affect. It contains 21 items with each response being scored on a scale value from 0 to 3 (Beck et al., 1996). The scores on each item of the BDI were summed and the total score was used in the analyses.

2.3 Assessment of ovarian steroid hormones

1 mL saliva samples were collected before the fMRI experimental procedure. These samples were used in high sensitivity progesterone and 17β-estradiol enzyme-linked immunosorbent assay (ELISA) kits according to the supplier's instructions (Salimetrics, Kit Lot numbers 1901540 & 1810501, respectively). The sensitivity of the ELISA kits was the following: 19.08 pg/mL ± 4.77 (high control range) and 5.49 pg/mL ± 2.20 (low control range) for 17β-estradiol and 820.13 pg/mL ± 205.03 (high control range) and 47.32 pg/mL ± 18.93 (low control range) for progesterone. To obtain a measure of free estrogenic activity, an estradiol-to-progesterone (E/P) ratio was calculated since estradiol actions are often counteracted by progesterone (Pletzer, 2019).

2.4 Memory task

2.4.1 *N*-back test

Participants were scanned as they engaged in an emotional picture *n*-back task. The block design task was developed by our laboratory and consisted of stimulus images from the International Affective Picture System (IAPS) database (Lang et al., 2005). The images were selected based on the 5-cluster solution proposed by Constantinescu et al. (2016). Using a model-based clustering technique, the aforementioned researchers were able to create discernable and discrete groups within the IAPS dataset, classifying the images into 5 statistically supported clusters

based on emotional valence (i.e., neutral, positive, and negative). However, their clusters were created based on combined male and female valence scores from the IAPS dataset. Since our task was to be used on a female population, the combined male and female valence scores of each image in each respective cluster were compared to their female only valence scores. The difference between these two valence scores was taken, and the images were re-ordered from the smallest to largest difference. The first 60 images from clusters 2, 3, and 4 were selected to be incorporated in the current task since these images had minimal differences between the combined and female only valence scores. Cluster 2 represented positive emotion (valence = 7.27; arousal = 4.69) whereas cluster 3 represented negative emotion (valence = 2.27; arousal = 5.87). Cluster 4 represented neutral emotion (valence = 5.05; arousal = 3.31) (Constantinescu et al., 2016). Following the stimulus selection, a sorting algorithm was applied to create a balanced block design. E-prime was used to create the final computerized task. In each block, a stimulus image was presented for 1000 ms with an interval of 500 ms followed by the next stimulus image. In total, there were 16 stimulus images per block with 3 blocks per *n*-back condition. The 6 main conditions were based on emotional valence and task type (i.e., 1-Back Negative, 1-Back Positive, 1-Back Neutral, 2-Back Negative, 2-Back Positive, 2-Back Neutral). Each individual block consisted of 6 target images and 10 non-target images (37.5% target images per condition). No responses were required for the non-targets. The order of presentation of each block was counterbalanced. Participants were instructed to 'Press for 1-Back' (baseline memory condition) or 'Press for 2-Back' (working memory test condition) when they recognized an image that they saw *n*-steps (i.e., 1-Back and 2-Back) in the sequence. Brain activity was calculated as the average activity of each condition, including both target and non-target images. Participants viewed the task on a mirror attached to the head-coil reflecting a projection screen, and all

images were presented on a black background. Lights were off during task completion. (Figure 1).

2.4.2 Performance

During the memory task, average reaction time (RT) was calculated for accurate responses occurring within 900 ms of stimulus presentation. Performance data were also screened for omission errors. A one-way repeated measures ANOVA was used to assess whether there were statistically significant differences in RTs between the 2-Back Positive, 2-Back Negative, and 2-Back Neutral blocks. Post-hoc testing used the Bonferroni correction factor.

2.5 fMRI data acquisition

Whole brain echo planar fMRI data were acquired using a Siemens Biograph 3 Tesla Magnetom MR scanner (Siemens, Erlangen, Germany) equipped with a 32-channel head coil at the Royal Ottawa Mental Health Centre's Brain Imaging Centre. A gradient echo pulse sequence was used (TR/TE 3000/34 ms, FA 90°, FOV 200 × 200 mm2, voxel size 1.6 mm × 1.6 mm × 3 mm, 48 axial slices, slice thickness 3 mm, band-width 2894 Hz). The duration of the functional scan was 11 min and 12 s with a total of 221 functional images.

2.6 fMRI data analysis

Functional imaging data were analyzed using Matlab (R2013b) and Statistical Parametric Mapping (SPM12) software. The functional scans were normalized to the standard SPM Montreal Neurological Institute template and spatially smoothed with a 6 mm FWHM Gaussian kernel following SPM12 standard preprocessing procedures (i.e., realignment, normalization, and smoothing). The final images were corrected for field inhomogeneity. Two first-level contrast images were constructed at the individual participant level: (i) 2-Back Positive > 2-Back Neutral; (ii) 2-Back Negative > 2-Back Neutral. The contrasts represent positive and negative

emotionality in working memory. Contrasts constructed at the single participant level were then entered into second-level multiple regression analyses. Whole-brain multiple regression analyses were conducted to examine the relationship between endogenous progesterone and estradiol levels and brain activity in response to the two contrasts. Individual variability in factors that could affect brain activity were controlled for in the analyses, which included the participants' total score on the BDI and RTs for the 2-Back Positive or 2-Back Negative blocks. The analyses also accounted for OC use to examine the global effects of endogenous progesterone and estradiol concentration. All reported results were derived from an uncorrected threshold of $p_{uncorr} < 0.005$ at the whole brain level and deemed significant at a threshold of $p < 0.05$ after family wise error (FWE) correction for multiple comparisons at the cluster level. All reported clusters consisted of ten or more contiguous voxels.

3. Results

3.1 Ovarian steroid hormones and BDI

OC users and NC women were hormonally similar since no significant differences were observed in endogenous progesterone and estradiol concentration between the two subgroups ($p = 0.100$ and $p = 0.702$, respectively). The mean progesterone concentration was 59.42 (±*58.42*) pg/mL and the mean estradiol concentration was 0.616 (±*0.326*) pg/mL in all participants. The mean total BDI score was 7 (±*6*) in all women.

3.2 *N*-back task performance

The mean RT for the 2-Back Positive block was 607.5 (±*75.5*) ms with a mean of 16 (±*2*) correct responses and 2 omission errors in all women. During the 2-Back Negative block, the mean RT was 606.2 (±*69.7*) ms with a mean of 15 (±*2*) correct answers and 2 omission errors. Finally, the mean RT for the 2-Back Neutral block was 629.8 (±*74.0*) ms with a mean of 15 (±*2*) correct

responses and 2 omission errors. A main effect of emotion was identified in the repeated measures ANOVA ($p < 0.001$, $n_p^2 = 0.130$, power = 0.988). Post-hoc testing revealed that all participants displayed a significantly slower RT for the 2-Back Neutral block compared to the 2-Back Positive and 2-Back Negative blocks (MD = 23.4, SEM = 5.4, $p < 0.001$; MD = 23.7, SEM = 6.2, $p = 0.001$, respectively).

3.3 Positive emotionality (2-Back Positive > 2-Back Neutral)

Results from the regression of the 74 participants showed bilateral brain activity in the frontal regions of the brain and insula as well as in the right lingual gyrus, right calcarine, left caudate, left putamen, and left inferior occipital gyrus during the positive emotionality contrast. (Figure 2A). Progesterone was positively associated with brain activity in the right insula, right postcentral gyrus, right mid-cingulum, and right supplementary motor area (SMA). (Figure 2B). Estradiol, on the other hand, was positively related to brain activity in the right superior medial frontal gyrus (SFG), right precentral gyrus, and right superior parietal lobule. (Figure 2C). While no significant relationship was observed between the E/P ratio and regional brain activity at the whole brain uncorrected threshold of $p_{uncorr} < 0.005$, estradiol was positively associated with brain activity in the bilateral superior parietal lobule and the left precuneus. This association was identified from a regression conducted at an uncorrected threshold of $p_{uncorr} < 0.05$ at the whole brain level and deemed significant at a threshold of $p < 0.05$ after FWE correction for multiple comparisons at the cluster level. (Table 1). No significant negative associations between hormone concentration and brain activity were identified for progesterone, estradiol, or the E/P ratio.

3.4 Negative emotionality (2-Back Negative > 2-Back Neutral)

Results from the regression of the 74 participants showed bilateral brain activity in the SFG, SMA, insula, and hippocampus as well as in the right inferior and medial occipital gyri, right inferior temporal gyrus, right cingulum, left mid-temporal gyrus, and left amygdala during the negative emotionality contrast. (Figure 3A). Progesterone was positively related to brain activity in the right rolandic operculum, right putamen, right insula, left pallidum, and left thalamus. (Figure 3B). While no significant relationship was observed between estradiol or the E/P ratio and regional brain activity at the whole brain uncorrected threshold of $p_{uncorr} < 0.005$, estradiol was positively related to brain activity in the right postcentral gyrus, left ACC, and left SFG. This regression was conducted at an uncorrected threshold of $p_{uncorr} < 0.05$ at the whole brain level and deemed significant at a threshold of $p < 0.05$ after FWE correction for multiple comparisons at the cluster level. (Figure 3C). In contrast, the E/P ratio was negatively associated with brain activity in the right parahippocampal gyrus, right inferior temporal gyrus, right lingual gyrus, left calcarine, and left precuneus. (Table 2). No significant negative associations between hormone concentration and brain activity were identified for progesterone and estradiol, and no significant positive association was identified between the E/P ratio and brain activity.

4. Discussion

In the current study, we used fMRI to investigate the role of ovarian steroid hormones on brain activation during positive and negative emotion processing in a working memory task. This task consisted of images from the IAPS database that were grouped by emotional valence (i.e., neutral, positive, and negative) and were specific to female emotion processing. We identified the neural correlates of positive and negative emotion processing during working memory and the regions specific to ovarian steroid hormone regulation. Participants showed brain activity in

reward-related areas during positive emotion processing while activation in corticolimbic areas was more prominent during negative emotion processing. Participants also reacted quicker during positive and negative emotion processing than to neutral stimuli. Similarly, during processing for both types of emotion, progesterone appeared to modulate more posterior regions of the brain whereas estradiol was more related to activation in anterior brain regions.

The RT results from this study are consistent with the emotional enhancement hypothesis, which predicts that emotional stimuli attract additional neural resources relative to neutral stimuli, thereby making it easier to encode and store information in working memory capacity (Droit-Volet, 2016). While studies at a behavioural level depict mixed findings for this hypothesis (Crowell & Schmeichel, 2016; Garrison & Schmeichel, 2019), there is evidence for it at a neural level. More specifically, processing affective versus neutral material during working memory tasks frequently recruits the PFC, amygdala, and temporo-occipital cortex in healthy participants (Schweizer et al., 2019). Brain activity predominantly in the right PFC has been associated with improvements in working memory performance in women (Joseph et al., 2012).

During the current *n*-back task, participants showed bilateral frontal and insular activity during both positive and negative emotion processing, including additional regions that were either left or right lateralized. The two current leading hypotheses regarding emotion processing are the "right hemisphere hypothesis" (RHH; Levy et al., 1983; Prete et al., 2015) and the "valence hypothesis" (VH; Davidson et al., 1987; Prete et al., 2015). According to the RHH, the right hemisphere is superior to the left hemisphere in the processing of both positive and negative emotions. On the other hand, according to the VH, the right hemisphere specializes in negative emotion processing and the left hemisphere specializes in positive emotion processing. The current literature suggests that the two hypotheses are not mutually exclusive, resulting in a

"modified valence hypothesis" (MVH). In this model, hemispheric superiority depends on prefrontal specialization (in which the left PFC specializes in positive emotion processing and the right PFC specializes in negative emotion processing), with posterior areas showing right hemispheric superiority during both positive and negative emotion processing (Killgore & Yurgelun-Todd, 2007). Our results are somewhat consistent with the MVH, which could be attributed to the design of our *n*-back task. For example, participants predominantly activated right-lateralized posterior regions such as the right occipital and temporal gyri during negative emotion processing. While we observed left-lateralized activity in sub-regions of the frontal cortex during positive emotion processing, activation of right-lateralized frontal sub-regions may reflect the non-verbal nature of the *n*-back task, which has been previously identified (Smith and Jonides, 1999; Rottschy et al., 2012). Furthermore, the visual stimuli in the task were not limited to specific sub-groups of positive or negative emotions (e.g., sexually arousing, fear, disgust, etc.).

Contrary to the MVH, participants showed brain activity in left dorsal and ventral striatal regions such as the putamen and caudate. These regions have previously been associated with emotional response inhibition (Amin et al., 2006), reward processing, and positive affect including the processing of positive verbal stimuli (Hamann & Mao, 2002; Li et al., 2018). However, it was unique to identify these brain regions during emotion processing in the context of working memory. Additionally, amygdala activation has been identified during positive emotion processing (Hamann & Mao, 2002; Li et al., 2018). While this activation was not seen in the current study, we observed left-lateralized amygdala activity during negative emotion processing. This is consistent with the lateralization effect identified in women in other studies (Stevens & Hamann, 2012). The amygdala, hippocampus, insula, and ACC form a hypothesized

corticolimbic emotion processing network (Davidson et al., 2000) with the amygdala and insula activated by bottom-up emotional processes, and the ACC involved in top-down regulation (Bush et al., 2000; Shin & Liberzon, 2010). Previous publications report amygdala reactivity in response to emotional words and faces throughout the menstrual cycle (Protopopescu et al., 2008; Gingnell et al., 2012). Comparatively, women using OCs show greater or blunted amygdala activity relative to NC women (Marečkovà et al., 2014; Petersen et al., 2015). Our study identifies a global amygdala response irrespective of menstrual cycle phase and OC use, which has been thought to be mediated by endogenous progesterone.

While we did not observe any relationship between progesterone levels and amygdala activity during negative emotion processing, progesterone levels correlated with brain activity in the thalamus and insula. The thalamus is densely localized with the classic nuclear progesterone receptor (Brinton et al. 2008). This region also projects to the orbitofrontal cortex (OFC) and ACC during emotion processing similar to the amygdala and parahippocampal gyrus (Catani et al. 2013; Catenaccio et al., 2016). The rostral ACC within the ventral medial PFC governs amygdala activity when processing threat-related stimuli (Rauch et al., 2006). Thus, it is possible that the relationship between progesterone and amygdala activity was not detected during negative emotion processing due to possible top-down amygdala inhibition (Rauch et al., 2006). However, amygdala activity may be mediated indirectly via progesterone's bottom-up effects on connected regions such as the thalamus and insula. The thalamus serves as an integrative center that relays information from sensory organs or subcortical structures to the cortex. It is also activated by tasks that require multiple cognitive functions (Hwang et al., 2017). Similarly, the insula is activated during emotional tasks with cognitive demands (Phan et al., 2002). Both regions show a strong functional connectivity to the central nucleus of the amygdala at rest

(Gorka et al., 2018). Consistent with the bottom-up modulation of integrative regions by progesterone during negative emotion processing, we identified a positive relationship between progesterone levels and sensory areas during the positive emotion processing contrast. Progesterone levels were associated with brain activity in the postcentral gyrus and SMA, which are regions known to be sensitive to sensory/premotor processing and acute progesterone administration (van Wingen et al., 2008; Luo et al., 2015). Thus, during emotion processing, progesterone levels may predominantly activate bottom-up brain activation and a heightened attention to relevant emotional cues. This is in contrast to what was observed for estradiol.

In comparison to progesterone, estradiol levels were positively related to brain activity in the right SFG during positive emotion processing and in the left SFG and ACC during negative emotion processing, which is inconsistent with the VH. While the association between estradiol and brain activity during negative emotion processing was identified at a liberal whole brain threshold, it was important to examine because previous literature has not yet shown consistent findings in the context of emotion processing during working memory. However, in other types of working memory tasks, such as verbal processing, estradiol has been associated with more brain activity in the left PFC (Berent-Spillson et al., 2015). Estrogen receptors are widely expressed in several brain regions including the PFC, amygdala, and hippocampus (Osterlund et al., 1999). Previous research also routinely shows increased activation during cognitive processing in frontal and cingulate cortical regions of the working memory circuitry with increasing estradiol levels (Dumas et al., 2010; Joffe et al., 2006; Shaywitz et al., 1999). Our findings indicate that estradiol modulation has a lateralization effect (right vs. left) during emotion processing. Similar to our findings, Miedl et al. (2018) also reported a positive correlation between estradiol and ACC activity during traumatic vs. neutral film viewing in NC

women. Other researchers propose that estradiol may influence top-down regulation of emotion processing (Zeidan et al., 2011), which is reflected in the current study and suggests that estradiol acts on regions with connections to both cognitive and limbic influences. For example, women in the early and late follicular phase show more brain activity in the SFG during a figure comparison task compared to women in the mid-luteal phase of the menstrual cycle (Weis al., 2008). Women with high estradiol levels also show more brain activity in the ACC along with other brain regions relative to men (Hwang et al., 2015). Thus, it appears that estradiol predominantly activates top-down brain activation during positive and negative emotion processing in the context of a working memory task, which benefits cognitive processes (Joffe et al., 2006; Berent-Spillson et al., 2015). Our findings also add newer evidence to existing studies.

While our results from the E/P ratio do not necessarily reflect this top-down regulation, activation of the precuneus shows opposing associations with the ratio during positive and negative emotion processing. The precuneus responds to a wide variety of cognitive processes including visual processing and recollection and memory (Kraft et al., 2015). Taken together, our results add newer evidence to existing studies on the regulatory roles of ovarian steroid hormones on emotion processing during working memory.

However, there are some limitations in the study. First, all scans were based on a single visit. While this was necessary to avoid ceiling effects on task performance, a longitudinal design would provide additional evidence that brain activation during emotion processing fluctuates as a function of hormone concentration. Second, the relationship between estradiol or the E/P ratio and brain activity during the emotion processing contrasts were based on lenient whole brain thresholds compared to the more stringent thresholds used for progesterone. However, in light of the large number of covariates included in the analysis, this thresholding could be considered

justified. Additionally, using the forward day count to establish menstrual cycle position and not accounting for the timing of OC intake in relation to the fMRI session results in less reliable hormone concentrations compared to using other hormone measures (e.g., serum samples) (Hampson, 2020).

5. Conclusions

Despite these limitations, this is an under-researched area that lacks consistent findings and the current study shows that ovarian steroid hormones modulate brain activity during positive and negative emotion processing in the context of working memory. Since our analyses controlled for OC use, our findings suggest that there are global neural responses across women, in general, during emotion processing tasks. Progesterone appears to predominantly regulate bottom-up regulation by activating posterior, sensory-processing regions during both positive and negative emotion processing. On the other hand, estradiol appears to modulate top-down regulation by activating frontal regions during emotion processing. In summary, a novel *n*-back picture task was designed that provides insights into the neurophysiology of emotion processing during working memory in a large sample of young women and that can be used in women's cognition/emotion research. Our findings also underline that women's endogenous hormonal status should be considered in future cognition research on healthy adults as well as when men and women are grouped together in clinical settings.

Declaration of Interest

We declare no conflict of interest.

Author Contribution Statement

Rupali Sharma, Andra Smith, and Nafissa Ismail designed the current study. Rupali Sharma was also responsible for data collection, data analysis, and manuscript preparation. Andrew Cameron

created and piloted the *n*-back task. Zhuo Fang contributed to data analysis and manuscript preparation. Nafissa Ismail and Andra Smith revised and edited the manuscript.

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Brain region	MNI coordinates			p_{FWE}
	X	Y	Z	
Activation during positive emotion processing				
Right calcarine	8	-90	2	<0.001
Right lingual gyrus	22	-80	-10	
Left inferior occipital gyrus	-42	-80	-4	
Right superior frontal gyrus	28	10	62	<0.001
Left superior frontal gyrus	-38	54	6	
Right mid-frontal gyrus	48	30	34	
Left mid-frontal gyrus	-32	60	2	
Right inferior frontal gyrus	46	32	24	
Left inferior frontal gyrus	-46	14	30	
Left precentral gyrus	-42	20	26	
Left caudate	-10	10	8	0.007
Left putamen	-16	4	16	
Right insula	32	26	0	<0.001
Left insula	-30	22	2	
Progesterone-associated activation				
Right SMA	12	-4	50	<0.001
Right mid-cingulum	4	-18	50	
Right postcentral gyrus	38	-26	54	<0.001
Right insula	42	-6	-2	<0.001
Estradiol-associated activation				
Right precentral gyrus	40	-24	66	<0.001
Right superior parietal lobule	22	-48	48	
Right superior frontal gyrus	22	48	22	<0.001
Right superior medial frontal gyrus	4	50	28	
E/P ratio-associated activation				
Right superior parietal lobule	18	-60	54	0.033
Left superior parietal lobule	-14	-70	46	
Left precuneus	-4	-64	56	

Table 1. Brain activation during positive emotion processing.

Brain area	MNI coordinates			p_{FWE}
	X	Y	Z	
Activation during negative emotion processing				
Right inferior occipital gyrus	42	-78	-2	<0.001
Right mid-occipital gyrus	36	-82	6	
Right inferior temporal gyrus	44	-60	-12	
Left superior medial frontal gyrus	0	62	26	<0.001
Right superior medial frontal gyrus	-2	58	34	
Left mid-temporal pole	-48	8	-32	<0.001
Left mid-temporal gyrus	-54	0	-20	
Right SMA	8	16	50	<0.001
Left SMA	-2	16	50	
Right mid-cingulum	12	14	42	
Left hippocampus	-20	-30	-4	<0.001
Right hippocampus	28	-8	-18	
Left amygdala	-26	-34	0	
Right insula	36	28	0	<0.001
Left insula	-26	26	0	
Progesterone-associated activation				
Left pallidum	-2	2	0	0.008
Left thalamus	-16	-16	10	
Right rolandic operculum	50	-2	6	0.014
Right putamen	32	0	6	
Right insula	42	2	10	
Estradiol-associated activation				
Right postcentral gyrus	46	-18	34	<0.001
Left anterior cingulum	-4	28	18	
Left superior medial frontal gyrus	-8	32	50	
E/P ratio-associated activation				
Right parahippocampal gyrus	18	-42	-10	0.002
Right inferior temporal gyrus	42	-64	-8	
Right lingual gyrus	20	-78	-6	
Left calcarine	-14	-54	4	0.020
Left precuneus	-8	-50	14	

Table 2. Brain activation during negative emotion processing.

Figure 1. Illustration of the experimental *n*-back task.

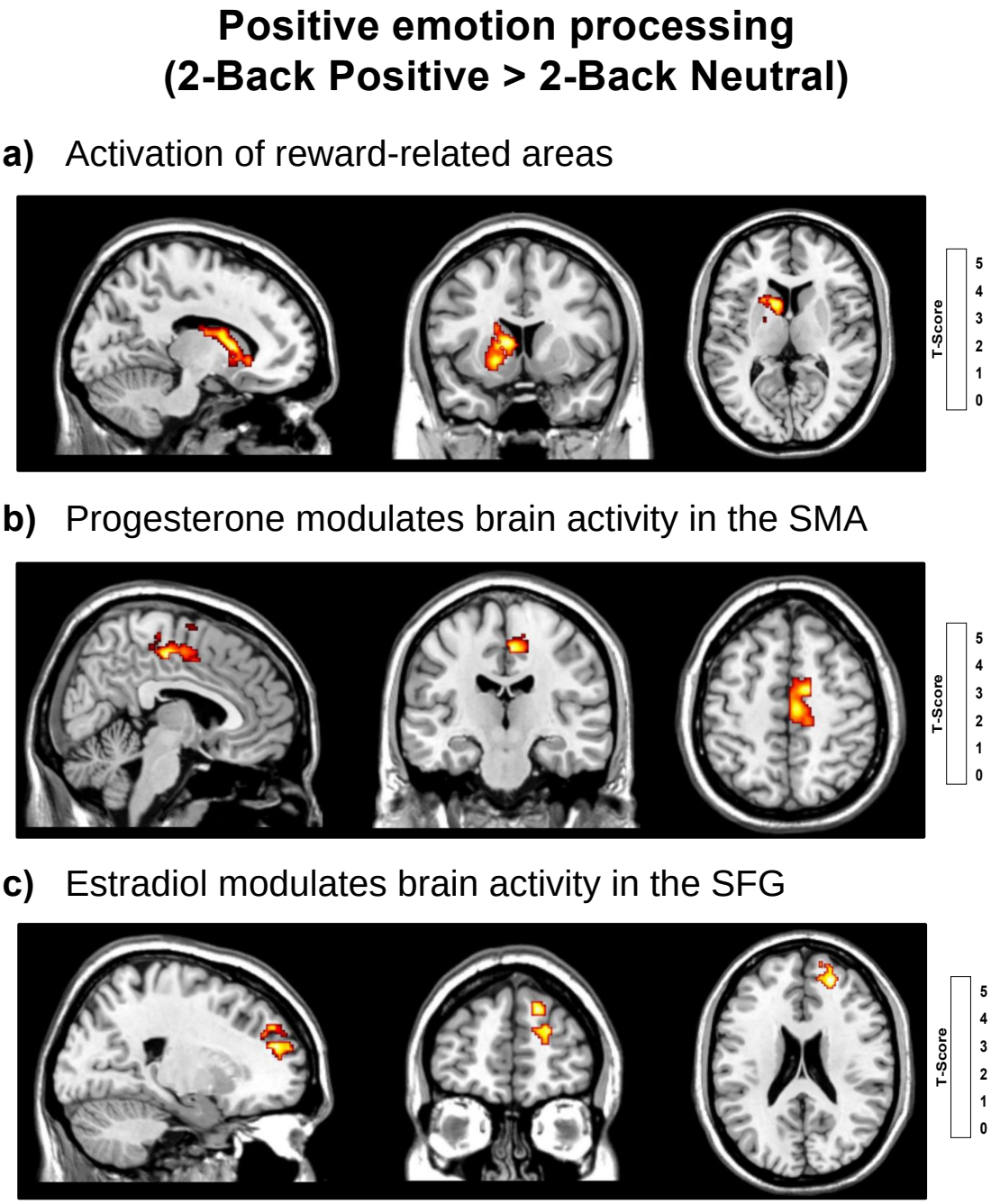

Figure 2. (A) Activation of reward-related brain areas, including the caudate [-10 10 8] and putamen [-16 4 16], from the regression analysis of all participants during positive emotion processing (2-Back Positive > 2-Back Neutral). (B) Progesterone positively correlated with brain activity in the SMA [12 -4 50]. (C) Estradiol positively correlated with brain activity in the SFG [4 50 28]. All regions are represented on coronal, sagittal, and transverse sections ($p < 0.005$ uncorrected and $p < 0.05$ after FWE correction, $k \geq 20$, T value scale presented on the right).

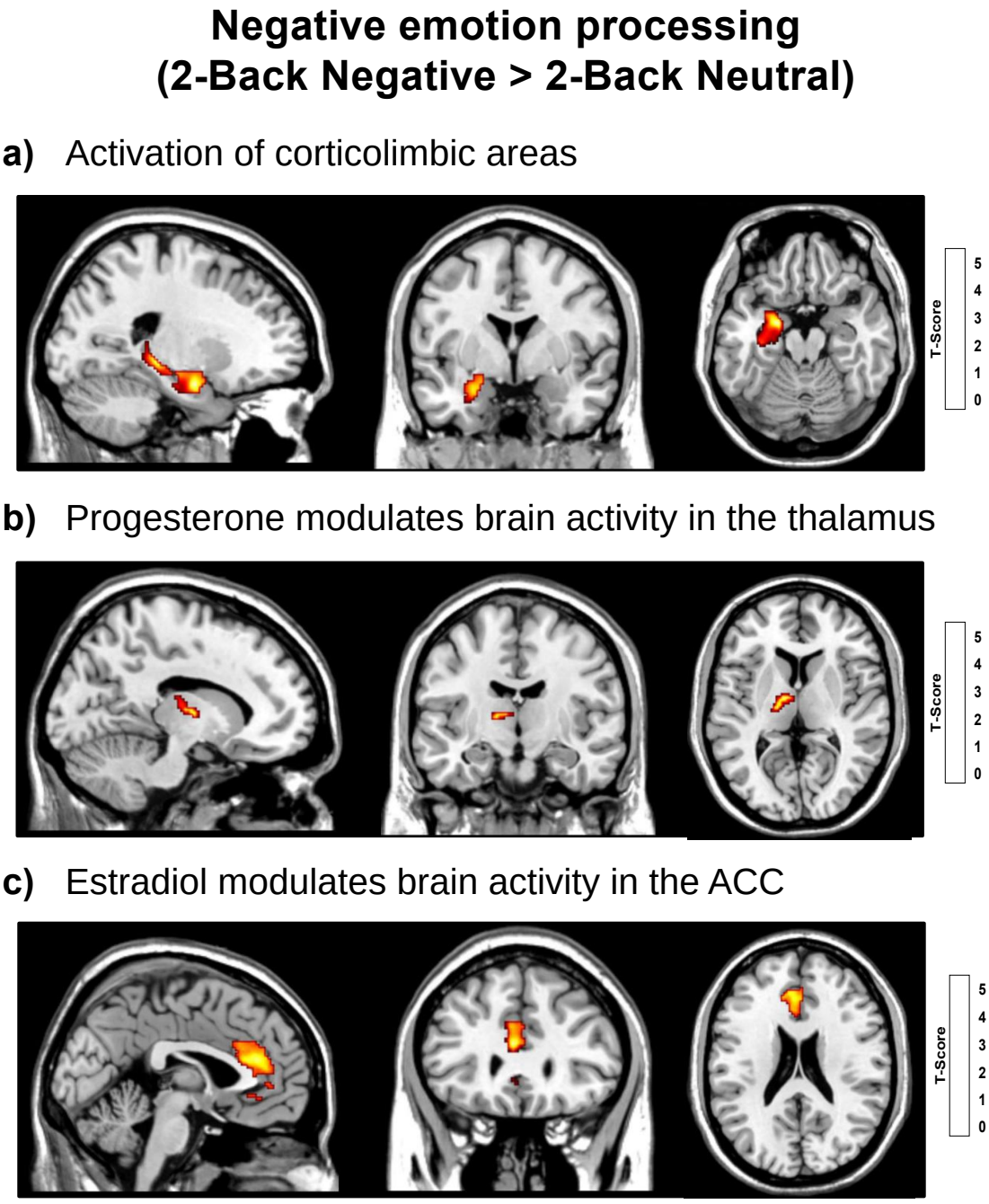

Figure 3. (A) Activation of corticolimbic brain areas, including the hippocampus [-20 -30 -4 and 28 -8 -18] and amygdala [-26 -34 0], from the regression analysis of all participants during negative emotion processing (2-Back Negative > 2-Back Neutral). (B) Progesterone positively correlated with brain activity in the thalamus [-16 -16 10]. (C) Estradiol positively correlated with brain activity in the ACC [-4 28 18]. All regions are represented on coronal, sagittal, and transverse sections ($p < 0.005$ or $p < 0.05$ uncorrected and $p < 0.05$ after FWE correction, $k \geq 20$, T value scale presented on the right).

General Discussion

The influx of ovarian steroid hormones during puberty induces brain remodelling and reorganization (Levitt et al., 2003; Sisk & Foster, 2004; Schulz et al., 2009). These hormones also cause changes in brain structure and function, and various behaviours across the menstrual cycle. Suppression of these endogenous hormones with the synthetic compounds found in the oral contraceptive (OC) pill (i.e., ethinyl estradiol and progestin) also changes brain structure and function and likely affects behaviour (Pletzer & Kerschbaum, 2014; Montoya & Bos, 2017; Lewis et al., 2019). However, the extent of OC-related structural, functional, and behavioral changes and the role of ovarian steroid hormones remain elusive. Additionally, it is unclear how these hormonal modulations, specifically during puberty, a critical period of neurodevelopment, affect brain structure and function (Blakemore et al., 2010; Peper & Dahl, 2013).

Therefore, we sought to examine the effects of OC use, especially during puberty, on neural and behavioural outcomes. More specifically, the studies included in this thesis addressed the following research questions: 1) Does OC use, especially during puberty, affect cortisol reactivity, regional gray matter (GM) and white matter (WM) volumes, and WM integrity in the brain? 2) Does OC use during puberty affect brain activity during a working memory cognitive task? 3) Could resting state functional connectivity in limbic and executive networks explain task-induced functional differences between OC users and NC, non-OC users? 4) Do salivary progesterone and estradiol concentrations in women (OC users and non-OC users) affect emotion processing during a cognitive task?

Blunted cortisol reactivity is dependent on the age of OC onset

The basal and stimulated activities of the hypothalamic-pituitary-adrenal (HPA) axis rapidly change during puberty due to the influx of ovarian steroid hormones in women (Legro et

al., 2003; Gunnar & Vazquez, 2006). These stress responses also fluctuate across the menstrual cycle (Kudielka & Kirschbaum, 2005) and permanently change following OC usage (Kirschbaum et al., 1999). Estradiol and progesterone generally have opposing effects on HPA axis activity with estradiol decreasing (Lindheim et al., 1994) and progesterone increasing stress reactivity and cortisol concentration in the blood and in the saliva of adult women (Altemus et al., 1997; Kirschbaum et al., 1999; Wolf et al., 2001). In contrast to endogenous estradiol, synthetic ethinyl estradiol decreases stress reactivity and cortisol concentration in the blood of adult OC users (Moore et al., 1978; Kajantie & Phillips, 2006; Kumsta et al., 2007).

However, we found no significant difference in salivary cortisol concentration between OC users and NC women across four distinct timepoints during the Trier Social Stress Test (TSST) (Article 1, Study 2). Our results are inconsistent with previously published findings showing that OC users display a reduced salivary cortisol concentration in response to the TSST compared to NC women (Kirschbaum et al., 1999; Rohleder et al., 2003; Kumsta et al., 2007; Roche et al., 2013). Although our standardized TSST protocol was similar to the one used by other researchers (Kirschbaum et al., 1999), the lack of significant difference between OC users and NC women in our study could be attributed to higher basal cortisol levels among some OC users, which has also been observed in previous studies (Nielsen et al., 2013; Mordecai et al., 2017).

Additionally, the type of progestin used in OC formulations might contribute to differences in stress reactivity across studies. Levonorgestrel was the most commonly used progestin by participants in our study (52%), while norgestimate (16%) was the second commonly used progestin. Notably, the combination of ethinyl estradiol plus norgestimate increases cortisol-binding globulin (CBG) in the blood (Wiegratz et al., 1995; 1998; Mordecai et

al., 2017). Norgestimate has low androgenic activity, particularly compared with levonorgestrel, suggesting that lower progestin androgenicity might increase CBG regulation than OCs with higher progestin androgenicity (Wiegratz et al., 2003). Levonorgestrel, in particular, could also have a binding affinity for CBG resulting in more cortisol release (Wiegratz et al., 2003). CBG is a transport carrier protein found in the blood and brain (Sivukhina & Jirikowski, 2014). CBG binds to cortisol and renders it biologically inactive, reducing the amount of free cortisol (Kumsta et al., 2007; Henley & Lightman, 2011). As CBG concentration increases, there is less cortisol uptake by the tissues (Bright, 1995; Henley & Lightman, 2011). Higher CBG concentration in the blood is associated with lower adrenocorticotropic hormone (ACTH) concentration in the blood and lower cortisol concentration in the saliva (Kumsta et al., 2007). While salivary cortisol concentration is used as a reliable estimate of free, biologically active cortisol concentration in the blood (Dorn et al., 2007), there is variability between methods of quantifying this concentration. A strong correlation has been identified between salivary cortisol concentration and free serum cortisol concentration in studies using mass spectrometry. On the other hand, immunoassays, such as the ELISAs used in the current thesis, show a lack of specificity for cortisol concentration (Perogamvros et al., 2012). Thus, the non-significant differences between OC users and NC women in response to the TSST may be due to higher basal cortisol concentration among OC users and variability in cortisol regulation through different OC formulations, or methodological limitations.

Despite the lack of significant difference between OC users and NC women in salivary cortisol concentration, the current thesis found that pubertal-onset OC users displayed lower cortisol concentration in response to the TSST and a quicker recovery to baseline compared to their adult-onset counterparts (Article 1, Study 2). There was also a higher percentage of cortisol

non-responders within the pubertal-onset OC group (81%) compared to the adult-onset OC group (47%) (Article 1, Study 2). Given that puberty is a period of maturation for the HPA axis (Sisk & Foster, 2004), the higher percentage of cortisol non-responders within the pubertal-onset OC sub-group may be due to altered cortisol regulation by CBG or down-regulation of pituitary corticotropin releasing factor (CRF) receptors (Kumsta et al., 2007). While the OC formulations used were similar between pubertal- and adult-onset OC users, it is possible that the time of OC onset or duration of use have different effects on HPA axis functioning. Only one study to date has examined the effects of OCs on stress reactivity in adolescents. Between the ages of 15- and 17-years old, adolescent OC users show no change in salivary cortisol concentration compared to non-OC users following a similar psychosocial stress task to the TSST (Bouma et al., 2009). While the researchers did not account for the age of OC onset, the pubertal-onset OC users in the current thesis had a mean age of onset of approximately 14 years old. It is possible that OC use in late adolescence/early adulthood results in a hyperresponsive HPA axis whereas OC use initiated during puberty before the age of 15 years old results in a hyporesponsive HPA axis due to the extensive brain remodelling and reorganization during this time.

While the blunted cortisol concentration among pubertal-onset OC users did not relate to group differences in mood-related symptoms, there are reports suggesting that a blunted stress response can be a risk factor for depression in women (Fiksdal et al., 2019; Hodes & Epperson, 2019; Lewis et al., 2019). The questionnaires used to examine mood-related symptoms in the current thesis are typically meant to serve diagnostic purposes. These questionnaires may not have been specific enough to detect mild or subtle differences in mood-related symptoms, since we excluded all participants diagnosed with a psychiatric condition. However, our findings, nonetheless, demonstrate that a longer duration of OC use, with a pubertal onset, results in a

blunted cortisol concentration in response to the TSST. Additionally, adolescent OC use has been associated with a greater vulnerability to depression diagnosis in adulthood (Anderl et al., 2020). OC-related differences in brain structure and function may provide further insight into changes in behaviour.

General OC use and the age of OC onset are related to structural differences

The investigation of the effects of OCs on GM and WM volumes has been inconsistent. While some studies report an increase in regional GM volume in OC users compared to non-OC users (Pletzer et al., 2010; Pletzer et al., 2015), others show a decrease (Lisofsky et al., 2016; Hertel et al., 2017; Pletzer et al., 2019). Moreover, progestin androgenicity and duration of use has also been shown to contribute to region-specific differences in GM volume among OC users (Pletzer et al., 2015; 2019). In contrast to GM volume, WM volume and integrity changes have been less investigated in OC users compared to non-OC users. In fact, only a single study to date has examined the effect of OCs on regional WM integrity (De Bondt et al., 2013).

In the current thesis, we observed general OC-related structural differences with some differences being related to pubertal-onset OC use. All OC users displayed less GM volume in the putamen and more WM volume in the putamen, rectus, amygdala, parahippocampal gyrus, and hippocampus compared to NC women (Article 1, Study 1). Similarly, OC users showed more WM integrity in the hippocampus compared to NC women (Article 1, Study 1). While we are the first to report an OC-induced volumetric difference in the putamen, others have noted a sex difference with men showing more GM volume in this region compared to NC women in both the follicular and luteal phases (Pletzer et al., 2010). The structure of the putamen is also modified by hormonal fluctuations across the menstrual cycle. Women display less GM volume in the putamen during the follicular phase compared to the luteal phase (Protopopescu et al.,

2008). Since the follicular phase is often referred to as the low hormone phase of the menstrual cycle, it is possible that a similar endogenous hormonal state coupled with OC use further decreases GM volume in the putamen. Moreover, women show a compensatory GM:WM volume ratio (Allen et al., 2003; Chen et al., 2007). However, in the case of OC users, this ratio could be altered resulting in more WM volume in the putamen. OC use is also related to less GM volume in the amygdala, parahippocampal gyrus, and hippocampus (Lisofsky et al., 2016; Hertel et al., 2017; Pletzer et al., 2019). While we did not observe GM volume differences in these regions, we found more WM volume in these regions in OC users compared to NC women, which may further support alterations to the GM:WM volume ratio. The differences in GM and WM volumes between OC users and NC women may present structural risk factors in women for neuropsychiatric and neurodegenerative disorders later in life. For example, less GM volume in the putamen has been identified in Alzheimer's disease and dementia, Parkinson's disease, and major depression (Luo et al., 2019). Similarly, increased WM volume and integrity in the hippocampus has been associated with aspects of cognitive decline and depressive symptom severity (Lyons et al., 2004; Chao et al., 2015). The differences between OC users and NC women could be attributed to altered neurodevelopmental trajectories since declines in GM and increases in WM occur up to early adulthood (Giedd et al., 1999; Tamnes et al., 2010; Blakemore et al., 2010).

Consistent with the plausible altered neurodevelopment of OC users, we observed that pubertal-onset OC users presented more WM volume in the fusiform gyrus and precuneus compared to their adult-onset counterparts (Article 1, Study 1). The differences among pubertal-onset OC users can partially be explained by the fact that puberty is a period of neuronal plasticity (Vigil et al., 2016) and that hormone levels induce changes in neurons and affect brain

structure and function (Del Rio et al., 2018). For example, higher estradiol levels decrease myelination and enhance plasticity in females compared to estradiol suppression (Yates & Juraska, 2008; Laube et al., 2020). While we did not observe less estradiol concentration in pubertal-onset OC users compared to their adult-onset counterparts or between all OC users and NC women, it is possible that a longer duration of ethinyl estradiol use in the OC pill, with a pubertal onset, may alter this plasticity. This could then result in more regional WM volume. Additionally, the progestin androgenicity could influence neuronal plasticity. Androgenic progestins are derived from testosterone and exhibit a relatively high affinity to the androgen receptor (AR). Transcriptional activity of the AR is also linked to an increase in WM volume in males (Perrin et al., 2008). While there were no major differences in the OC formulations used by pubertal- and adult-onset OC users, it is possible that OC use with androgenic properties specifically during puberty increases regional WM volume via AR signaling. This higher WM volume could then have functional implications, especially in the fusiform gyrus. Post-pubertal OC users between the ages of 13- and 15-years old show more brain activity in the fusiform gyrus when viewing video clips of neutral faces compared to non-OC users (Mareckova et al., 2014). Activity of the fusiform gyrus also increases as a function of duration of OC use (Mareckova et al., 2014). Similarly, the current thesis identified a positive association between duration of OC use and brain activity in the fusiform gyrus, only in pubertal-onset OC users, during the working memory component for negatively arousing images in our cognitive task (Article 1, Study 1). Thus, our findings indicate that OC use during puberty and early adulthood may alter naturally programmed neuronal mechanisms resulting in more regional WM volume and less GM volume. These structural differences could then have implications for task-related differences in brain activity.

General OC use and the age of OC onset are related to functional differences during a working memory cognitive task and at rest

The current literature reports several functional differences between OC users and non-OC users during cognitive tasks and at rest. Some studies report heightened brain activation among OC users compared to non-OC users (Marečkovà et al., 2014; Miedl et al., 2018), while others show reduced brain activation (Gingnell et al., 2013; Petersen & Cahill, 2015). OC users also display network-specific increases and decreases in resting state functional connectivity compared to non-OC users (Petersen et al., 2014; Pletzer et al., 2016). No study to date has examined the effects of pubertal-onset OC use on long-term brain function during an emotional working memory cognitive task and at rest.

The current thesis demonstrates that general OC use and the age of onset induce functional differences to a uniquely designed emotional picture *n*-back task. We found that pubertal-onset OC users displayed more brain activity in the bilateral lingual gyrus, mid-temporal gyrus, pre-central gyrus, and insula during working memory for neutral pictures compared to their adult-onset counterparts (Article 1, Study 1). Duration of OC use was also positively associated with brain activity in the angular gyrus in pubertal-onset OC users during neutral memory processing (Article 1, Study 1). Higher brain activity among pubertal-onset OC users in the absence of significant performance differences might suggest greater brain remodeling and reorganization due to delayed or suppressed neural pruning (Vigil et al., 2016). During neural pruning, frequently used synaptic connections are reinforced and conserved, while seldom activated synapses degenerate. One aspect of neural pruning is the change in dendritic spine density such that less density is associated with more efficient connectivity and a potentially more stable system (Dahl et al., 2018; Laube et al., 2020). A peak in dendritic spine

density appears at pubertal onset followed by a decrease after puberty and across adolescence. Suppression of ovarian steroid hormones inhibits the change in dendritic spines over the course of adolescence, suggesting a role of these hormones in neural pruning (Meyer et al., 1978; Laube et al., 2020). It is possible that the suppression of ovarian steroid hormones and prolonged use of synthetic hormones slows down or inhibits this natural decline in dendritic spine density, which results in an inefficient neural circuitry (i.e., more brain activation) during neutral memory processing. While pubertal-onset OC users showed distinct differences compared to their adult-onset counterparts, the current thesis also found general OC-related differences during working memory for emotional pictures.

All OC users showed more brain activity in the inferior frontal gyrus, mid-frontal gyrus, insula, supplementary motor area, superior frontal gyrus, paracentral lobule, and the lingual gyrus during working memory for negative pictures (Article 1, Study 1). Since OC users did not exhibit significant differences in reaction time and performance compared to NC women, their higher brain activity may be indicative of a compensatory mechanism or a heightened fear response to negative emotional subject matter. The higher brain activity in OC users compared to NC women could also be attributed to the duration of OC use since frontal brain activity increased as a function of duration of OC use in a sub-group of OC users (Article 1, Study 1). The current literature consistently reports a negativity bias in emotion recognition and memory in OC users compared to non-OC users (e.g., an attentional bias to negative emotions) (Lewis et al., 2019). Our results may provide a neural mechanism for this bias, which can also be induced by epigenetic biomarkers. OC users who are carriers of the mineralcorticoid receptor (MR) haplotype 1 or 3 have a more pronounced negativity bias and may be more vulnerable to the psychiatric side effects of OCs (Hamsta et al., 2015; 2016; 2017; Lewis et al., 2019).

Additionally, OC users show significantly higher valence ratings of emotional stimuli (Spalek et al., 2019). It is possible that the increased brain activity observed among OC users during working memory for negative pictures is a result of epigenetic factors or greater valence ratings of the images used in the *n*-back task.

Moreover, the functional differences between OC users and NC women at rest could also explain the task-induced differences in the current thesis. We found that OC users displayed more resting state functional connectivity in the salience, central executive, and reward networks (Article 2). The central executive network is involved in numerous cognitive processes, including reasoning, working memory (e.g., *n*-back task), and divided attention, whereas the salience network is known to link cognition with emotion/interoception (Laird et al., 2011). OC users showed more connectivity in the superior medial frontal gyrus and the anterior cingulate cortex within the salience network and the dorsolateral prefrontal cortex of the central executive and reward networks. These regions have been shown to be activated by our task as well as other emotional cognitive tasks (Luo et al., 2014; Miedl et al., 2018). Overall, our results indicate that the age of OC onset and duration of OC use affects working memory for neutral pictures, whereas general OC use affects working memory for negative pictures. The higher brain activity for negative pictures could be due to a variety of factors such as epigenetic mechanisms, valence ratings, and functional connectivity of limbic and executive networks at rest. Our results may also provide neural insight into the negativity bias, which could be attributed to changes in the hormonal milieu following OC use.

Ovarian steroid hormones affect brain function during the cognitive task

Endogenous ovarian steroid hormones regulate emotion processing and memory (Sundstrom & Gingnell, 2014; Catenaccio et al., 2016). Several studies report changes in brain

activity associated with emotion processing and memory across the menstrual cycle. The literature consistently demonstrates anxiolytic properties of estradiol but mixed anxiolytic and anxiogenic properties of progesterone (Andreano and Cahill, 2010; Gingnell et al., 2012; Bayer et al., 2014; Montoya & Bos, 2017). The use of OCs may also change the role of endogenous ovarian steroid hormones.

The current thesis identified a positive association between progesterone concentration and working memory for emotional pictures. We observed a positive relationship between progesterone concentration and brain activity in the supplementary motor area, dorsolateral prefrontal cortex, and putamen during working memory for negative pictures in OC users (Article 1, Study 1). In comparison to OC users, NC women displayed a positive association between progesterone concentration and brain activity in the dorsolateral prefrontal cortex during working memory for positive pictures (Article 1, Study 1). We are the first to examine the association between progesterone concentration and brain activity during working memory for emotional pictures separately in OC users and NC women. Our results suggest that OC use results in a hormonally induced sensitivity to negatively arousing stimuli. While this sensitivity could be attributed to progestin androgenicity, our results suggest that the intake of synthetic hormones alters the mediating role of endogenous progesterone leading to greater sensitivity/preference for negative emotional subject matter.

Despite the higher brain activity and hormonal mediation of OC users during working memory for negative pictures, OC users and NC women did not present differences in brain activity during positive and negative emotion processing of the working memory conditions in the same task (Article 1, Study 1). This suggests that OC users and NC women share similar

neural responses during emotion processing. Thus, we sought to investigate the roles of ovarian steroid hormones during emotion processing in all women.

The current thesis found that women displayed brain activity in reward-related areas, such as the putamen and caudate, during positive emotion processing. We also observed that activation in corticolimbic areas, such as the amygdala and hippocampus, was more prominent during negative emotion processing (Article 3). While progesterone concentration is associated with amygdala activation during negative emotion processing (Andreano & Cahill, 2010), we observed a positive association between progesterone concentration and brain activity in the thalamus and in the insula (Article 3). It is possible that amygdala activity may be mediated indirectly via progesterone's bottom-up effects on these connected regions since the thalamus and insula show a strong functional connectivity to the amygdala at rest (Gorka et al., 2018). Progesterone concentration was also associated with brain activity in the postcentral gyrus and supplementary motor area (Article 3), which are regions known to be sensitive to sensory/premotor processing and acute progesterone administration (van Wingen et al., 2008; Luo et al., 2015;). Thus, our results suggest that progesterone levels may predominantly activate bottom-up brain activation during emotion processing.

In contrast to progesterone, estradiol concentration was associated with brain activity in the right superior frontal gyrus during positive emotion processing and in the left superior frontal gyrus and anterior cingulate cortex during negative emotion processing (Article 3). Our findings indicate that estradiol has a lateralization effect (right vs. left) during emotion processing, and that estradiol may influence top-down regulation by acting on regions with connections to cognitive and limbic mechanisms (Zeidan et al., 2011). Since we did not examine subjective fear/anxiety ratings to the images in the *n*-back task, it is unclear if the associations between

estradiol concentration and brain activity in anterior regions of the brain during negative emotion processing serve a protective function. However, our findings provide insights into the neurophysiology of emotion processing during working memory in a large sample of young women and underline the role of ovarian steroid hormones, which could have implications for anxiety disorders (Lebron-Milad & Milad, 2012). Our findings also emphasize the consideration of women's endogenous hormonal concentration in neurobiological and cognition research.

Limitations

The current studies have certain limitations. While we selected a narrow window of pubertal-onset OC use (i.e., up to 6 months post-menarche) to maximize the differences between pubertal- and adult-onset OC users, the cross-sectional design of the studies cannot establish causation of the effects of OC use. It is also unclear whether the differences between the sub-groups of OC users are specifically due to pubertal-onset OC use, a longer duration of OC usage, or both factors. Second, our studies included a vast diversity of OC formulations and we did not account for the time at which OC users took their pill prior to the brain scanning and stress testing. Additionally, our neuroimaging and stress reactivity results were not paralleled by behavioural outcomes (i.e., differences in *n*-back performance and mood-related questionnaires); thus, our findings need to be interpreted with caution. It is possible that OC use specifically impacts a very early stage of emotion processing during working memory. Finally, we were limited by the detection sensitivities of the hormonal assays since we did not observe a group difference in endogenous estradiol concentration between OC users and NC women.

Future directions

Future studies should focus on incorporating longitudinal designs or randomized control trials (e.g., Engman et al., [2018]) to establish causation of OC-related effects. A longitudinal

design would also help researchers clarify whether OC-related differences are due to the age of onset, duration of usage, or an interaction of both factors. Future studies could also focus on specific OC formulations to provide clearer results such as those containing only levonorgestrel. These studies could also focus on certain brands such as Tri-Cyclen, Alesse, or Alysena, which were the most commonly used OCs in the current studies. Furthermore, men should be added as an additional control group. Additional physiological (e.g., genetic stress markers, triglycerides, etc.) and hormonal data (e.g., testosterone, dehydroepiandrosterone, oxytocin, etc.) could be examined to identify the mechanisms of OC-related structural and functional differences. Furthermore, other questionnaires could be included to detect subtle differences in mood-related symptoms (e.g., The Positive and Negative Affect Schedule). Finally, future studies should incorporate ovulation kits to account for within-person variability in menstrual cycle phase and more specific immunoassay techniques (e.g., liquid chromatography-mass spectrometry) to quantify hormone concentration (Rosner et al., 2013; Hampson, 2020). Other types of cognitive tasks could also be used such as spatial memory tasks to examine whether OC-related functional differences and the regulatory roles of ovarian steroid hormones extend to other cognitive domains or are limited to emotional working memory.

Summary

The current studies filled an important knowledge gap in our understanding of the effects of OC use on the brain and behaviour. This dissertation established that there are important OC-related differences to the brain and behaviour with some findings being dependent on the age of onset and duration of OC use. We demonstrated that OC use is related to less GM volume and more WM volume and integrity. OC use is also related to more brain activity during working memory for negative pictures and higher functional connectivity of limbic and executive

networks at rest. Ovarian steroid hormones also affect different brain regions during emotion processing in all women and induce lateralized effects. The findings of the current dissertation greatly contribute to women's brain health. They also provide neural evidence to previous research depicting an increased vulnerability to certain mood-related disorders following OC use. It is critical that we continue to investigate the impact of OCs, especially on the developing brain.

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