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Estrogens and androgens in the prefrontal cortex: Relevance for cognition and decision-making

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ESTROGENS AND ANDROGENS IN THE PREFRONTAL CORTEX:

RELEVANCE FOR COGNITION AND DECISION-MAKING

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Abstract

Organizational and activational effects of reproductive steroids regulate many aspects of brain function in nonhuman species, including certain cognitive functions. These actions are often exerted in a region- or pathway-specific manner in the CNS to promote reproductive objectives. Similar effects are thought to occur in human brains. Growing evidence suggests that 2 major families of hormones, estrogens and androgens, may influence cognitive and motivational processes. This chapter will briefly review findings from humans and nonhuman primates suggesting that the prefrontal cortex is an important target for estrogens and androgens. Hormonal regulation in the frontal cortex is discussed in terms of its possible adaptive significance from an evolutionary perspective.

Introduction

Reproductive hormones have wide-ranging effects in the central nervous system (CNS). In the past 50 years, basic science has revealed effects at all levels from molecular to behavioural. Once thought to be important only in the body periphery, androgens and estrogens during early brain development sculpt the architecture of the nervous system in a sex-dependent manner, and in reproductively mature adults these families of hormones intricately modulate the physiology and function of the CNS, outfitting both body and mind for reproductive function. The CNS effects of sex steroids were first discovered in other species, but they operate in the human CNS too. Importantly, hormone-driven processes include sex-typed modifications in certain cognitive functions. Although men and women do not differ in general intelligence, modest sex differences are seen in a range of more specific cognitive functions and it is the latter where the footprint of sex steroids is most readily found. Until recently, human work focused largely on the role of estrogens in episodic memory, with post-menopausal women the most common population studied. However, the CNS effects of reproductive steroids extend well beyond the medial temporal lobe. Newer work involves regions of the prefrontal cortex (PFC) that participate in social decision-making such as the ventromedial/orbitofrontal cortex (VMPFC/OFC) and dorsolateral prefrontal cortex (DLPFC). It is these socially-relevant functions that are the subject of the present chapter.

What are Organizational and Activational Effects?

Androgens (e.g., testosterone) and estrogens (e.g., 17β-estradiol, the dominant estrogen in women of reproductive age) are potent modulators of CNS function. Briefly, their actions fall into 2 major classes, called *organizational* and *activational* effects (Phoenix et al., 1959). *Organizational effects* are long-term changes in the morphological and functional differentiation of the CNS caused by exposure to specific steroids during critical periods in early brain development, which occur mainly in the prenatal or early postnatal period. (Some organizational effects might also occur at puberty; Schulz & Forrester-Fronstin, this volume). *Activational effects*, on the other hand, are reversible and occur in reproductively mature adults. They are caused by androgens or estrogens currently in the bloodstream and reflect their available concentrations. Examples of activational effects include changes in the numbers of synaptic contacts or changes in neurotransmitter availability in a particular brain region. Both organizational and activational effects have repercussions for brain function, and both require the presence of specific hormone receptors in a target brain region in order for effects to be seen. Many actions of testosterone or its metabolite dihydrotestosterone are mediated by androgen receptors, while at least 3 forms of the

estrogen receptor have been identified, 2 of which (ERα, ERβ) act by regulating gene transcription upon binding to estrogens, allowing for the endocrine control of a vast array of CNS-related gene products.

Organizational and activational effects were first demonstrated in guinea pigs in the context of mating behaviours (Phoenix et al., 1959), establishing that steroids are able to modify the behavioural output of the CNS. Applicability of these principles to *cognitive* functions (including memory) was slower to be recognized. However, research accelerated in the 1990s, triggered by data showing a remarkable 30% change over the female rat's 5-day estrous cycle in the density of axospinous synapses (synapses that occur on dendritic spines--tiny projections found on dendrites) in the hippocampus (Woolley & McEwen, 1992), and data demonstrating that neonatal steroid exposure could produce enduring (organizational) changes in spatial memory in the rat (Williams et al., 1990). These findings reinforced parallel work on cognitive function already underway in humans and other primates. In humans, work by Resnick et al. (1986) in people exposed to excess androgens *in utero,* and our own work on variations in estradiol over the menstrual cycle (Hampson, 1990; Hampson & Kimura, 1988), provided some of the first evidence for organizational and activational effects, respectively, on cognitive functions. They also underscored the selectivity of these effects--not all aspects of cognitive function are similarly affected (or affected at all) by reproductive steroids. Around the same time, Clark and Goldman-Rakic (1989) discovered that manipulating testosterone levels in rhesus monkeys during the prenatal or early postnatal infant period influenced their ability to perform an object reversal task dependent on the orbitofrontal cortex at later ages—a first indication of the possible hormone-responsivity of the PFC. Together, these early studies supported the view that sex steroids can affect higher cortical function. Only in the past decade or so has attention turned in earnest to the PFC as a potential site of steroid action.

Hand in hand with the possibility of regulatory actions of steroids in the PFC is the idea that the PFC may be a sexually differentiated region of the primate brain. Historically, sex differences in the PFC have not been widely entertained, despite occasional reports of sex-related volumetric or biochemical differences (e.g., Schlaepfer et al., 1995). Thus, the discovery of regulatory actions of sex steroids in the PFC pushes outward the boundaries of our current understanding of this highly complex brain region. The mapping between sex differences at the functional level and hormonal effects is not a simple one, however. On the one hand, the presence of a qualitative or quantitative sex difference in overt performance is often a signal that an organizational or activational effect of sex steroids is acting (or has acted) upon underlying neural pathways. Recognizing that many of the effects of sex steroids occur at the neurochemical level, a morphological sex difference will not always be seen. On the other hand, steroid-generated effects

sometimes serve to help equalize overt outcomes in the 2 sexes, by compensating for brain differences generated through other mechanisms (de Vries, 2004). Therefore, the presence of an observable sex difference in a specific cognitive process may signal a hormone effect, but the absence of an observable sex difference does not guarantee that hormone effects are absent (see also McCarthy & Konkle, 2005). Obsolete expectations premised on morphometric sex differences underestimate the true pervasiveness of sex steroid effects in the CNS.

The Prefrontal Cortex and Decision-Making

Over the past decade, cognitive neuroscience has devoted increasing attention to the study of decision-making and the complex cognitive and motivational processes that underlie it (see Fellows, 2013). The frontal cortex has long been acknowledged to play a key role in adaptive decision-making, providing behavioural flexibility in response to environmental context and its ever-changing demands. Indeed, early studies revealed impaired decision-making in neurological patients with acquired lesions of the PFC or conditions that involve perturbed development of the frontal cortex (Milner & Petrides, 1984). What's new in the past decade, though, is a greater recognition that 'decision-making' is not unitary. It is in fact a complex outcome that represents an emergent property of many fundamental subprocesses that must be finely orchestrated and are exquisitely tuned to current contingencies in the environment (Kennerley & Walton, 2011). Together, these processes yield the surface behaviour that we observe ('adaptive decision-making'). Changes in any of the latent processes may alter the surface behaviour we see expressed.

Two regions of the PFC of particular importance for social and non-social decision-making are the DLPFC, a region critical to the executive control processes of planning and working memory (the ability to temporarily hold information 'on-line' and manipulate it over short timeframes of up to a few minutes, Funahashi, 2017) and the VMPFC/OFC which, together with its connections to the amygdala and ventral striatum, is important in reward-based learning, valuation of reinforced outcomes and their anticipated probabilities in light of recent experience (Kringelbach & Rolls, 2004). VMPFC/OFC is also thought to help regulate negative affect via the top-down inhibition of brain regions involved in processing negative emotion, particularly the amygdala (Motzkin et al., 2015). It has been proposed that the DLPFC and VMPFC/OFC represent significant nodes in two distinct but interacting functional-anatomical control networks that provide non-emotive and affective guidance, respectively, over decision-making and its expression in overt behaviour (e.g., Baumgartner et al., 2011; Gläscher et al., 2012; Krawczyk, 2002). While not yet on the radar of most cognitive neuroscientists, a body of neuroendocrine evidence has

emerged over the past 10-15 years to suggest that these segments of the PFC may be modulated by sex steroids.

[Put Table 1 about here]

Estrogens and the DLPFC

Our own work on estrogens and the frontal cortex began in 1997. In 2000 we were the first to show that circulating levels of estrogens may enhance the frontal executive components of working memory (Duff & Hampson, 2000). In a sample of 96 healthy post-menopausal women, we found that women receiving estrogen replacement performed significantly better than untreated women on 3 tests of working memory (Figure 1), regardless of whether or not a progestin was simultaneously being used[1]. No significant difference was observed between the 2 treated groups, suggesting that estrogens not progestins were the primary source of the effect. Our tests included standard working memory tasks (Digit Ordering, Petrides et al., 1993; Digits Backward, Wechsler, 1987; see Table 1 for task overview), plus a newly developed test of spatial working memory (the "SPWM"; Duff & Hampson, 2001) known to correlate well with established working memory tasks having a high cognitive load (see e.g., Table 2). The effects in our study were seen robustly only on memory tasks that required the active manipulation of items within working memory, a process that recruits the DLPFC (Owen et al., 1999; Petrides et al., 1993). At the same time, no significant group differences were found on control tasks that required only the immediate passive recall of short sequences, i.e., tasks that rely on more posterior brain regions.

Multiple sources now support the idea that circulating estrogen levels do influence frontally-mediated elements of the working memory system. In the first study subsequent to ours, Keenan et al. (2001) found superior performance on an auditory *N*-back task of working memory (see Table 1) in a small group of post-menopausal women taking estrogens compared with control women not on treatment. Later, using a randomized double-blind cross-over design, Krug et al. (2006) confirmed an effect of 17β-estradiol using the Digit Ordering task (as in our own study) and showed that the effects generalized to a task involving memory for temporal order (another potential indication of a frontal lobe effect, Romine & Reynolds, 2004). The short latency of the effects (improvement in memory was seen after just a 3-day treatment with estradiol versus placebo) is consistent with the hypothesized activational nature of the hormone action. Early tests of the estrogen hypothesis focused on post-menopausal women, but the

[1] Unless the uterus is removed, progestins are prescribed to reduce the risk of endometrial carcinoma in women using estrogen replacement.

effect on working memory has been confirmed recently using a broader range of research paradigms (see below).

Cognitive studies illustrate the functional impact of higher estrogen levels, and speak to the efficiency of recruitment of the working memory network during everyday decision-making. However, functional magnetic resonance imaging (*f*MRI) has been a valuable tool to confirm a PFC locus of estrogen action. Half a dozen studies have now demonstrated increased neuronal activity at prefrontal sites during the performance of working memory tasks in women treated with estrogens compared with placebo. Smith et al. (2006), for example, in a placebo-controlled crossover trial found increased blood oxygen level-dependent (BOLD) activation under 17β-estradiol treatment in the PFC during a working memory task emphasizing active maintenance. In a monkey study, positron-emission tomography (PET) found that endogenous estradiol correlated positively with resting metabolism in the DLPFC and anterior cingulate cortex (Rilling et al., 2008). Sample sizes used in most imaging studies tend to be small, therefore the effects of estrogens on working memory are not always significant at a behavioural level in such studies, particularly in light of the effect sizes that can realistically be expected. However, imaging has been an important tool to visualize the precise loci of significant change and, as expected, implicates sites in PFC classically associated with working memory processes.

Post-menopausal women have been the most heavily studied population to date vis-à-vis the impact of estrogens on working memory. Endogenous estradiol production is negligible after menopause, but 17β-estradiol or other forms of estrogen may be given exogenously and the outcomes studied using various research designs. Existing work includes observational studies and placebo-controlled trials. In post-menopausal women, the question of whether estrogens positively influence working memory is of clinical not just theoretical importance. Following menopause, women commonly self-report modest reductions in memory function. Efforts to study *episodic* memory, the assumed substrate for women's self-impressions, have produced very equivocal results (Hogervorst & Bandelow, 2010). It is possible that these impressions might instead reflect a reduction in the working memory system. Interestingly, post-menopausal rhesus monkeys make higher numbers of working memory errors on the classical delayed-response task of working memory than female monkeys who are matched on chronological age but still retain a regular menstrual cycle (Roberts et al., 1997). This observation suggests generalizability to other female primates, and supports an estrogen- not age-dependent mechanism.

[Put Figure 1 and Table 2 about here]

Working memory has been studied extensively in monkeys using the spatial delayed-response task since the pioneering work of Jacobsen (1936) (see Table 1 for task description). The performance of delayed-response is impaired by lesions of DLPFC (Passingham, 1985), demonstrating a direct anatomical parallel with the human working memory system (Barbey et al., 2013). The monkey is thus considered a useful model of human working memory (whereas in rats working memory is sometimes conceptualized in a slightly different manner and may be operationalized by laboratory tasks that recruit the hippocampus). As we might predict based on the estrogen hypothesis, female monkeys display reduced accuracy on delayed-response after menopause (Roberts et al., 1997) or after ovariectomy (Tinkler & Voytko, 2005) and, consistent with estradiol as the causal agent, their performance can be restored substantially by exogenous treatments with estradiol (Kohama et al., 2016; Rapp et al., 2003).

Besides offering convergent support for the human cognitive data, nonhuman primates have afforded preliminary insights into molecular mechanisms. When our lab's work began, the existence of estrogen receptors in the adult PFC was still uncertain. But the presence of the ERα receptor in DLPFC has now been confirmed in both humans and other primate brains (Montague et al., 2008; Perlman et al., 2005; Wang et al., 2004). Montague et al. (2008) specifically targeted the DLPFC in a comparative anatomical study examining human, monkey, and rat brains. Immunohistochemistry showed abundant ERα-positive cells throughout all layers of DLPFC in the monkey and human specimens. In female monkeys, dendritic spine densities have been found to vary within DLPFC as a function of both age and estradiol levels (Hao et al., 2007), and the density of a particular type of spine, the 'thin' spine, is associated with accuracy on delayed-response in aged ovariectomized monkeys and is up-regulated by estradiol (Hara et al., 2015), making it a possible candidate mechanism to explain estradiol's effects on working memory. However, several other mechanisms also have been proposed, including regulatory effects of estradiol on serotonergic (Epperson et al., 2012), cholinergic (Dumas et al., 2012), and dopaminergic pathways (e.g., Duff & Hampson, 2000; Jacobs & d'Esposito, 2011), all of which are implicated in working memory processes (Arnsten & Robbins, 2002), and are also subject to modulation by circulating estradiol levels (e.g., Bethea et al., 2002).

It is important to realize that the effects of estrogens on working memory are not confined to *deficiency* conditions. Past work on post-menopausal women (or animal models of aging and menopause, e.g., Rapp et al., 2003) has focused on conditions characterized by extremely low estrogen availability and its remediation with estrogens given exogenously. But recent work in younger women conversely asks whether *high* estrogen states (e.g., pregnancy, higher-estradiol stages of the menstrual cycle) that occur

under natural conditions, may be associated with *superior* working memory. If, in fact, high estradiol promotes the functioning of frontal control elements of the working memory system, positive effects on working memory can be hypothesized. (Estradiol is higher at all phases of the menstrual cycle, including the lowest-estradiol phase (menses), than after menopause). Knock-down of estradiol in young women by drugs that inhibit estrogen synthesis has been shown to reduce women's performance on the *N*-back task of working memory (Grigorova et al., 2006). More importantly, higher estradiol concentrations do seem to be associated with *improvements* in working memory performance. This has been found, for example, at high-estradiol phases of the menstrual cycle, in pregnant women evaluated during the third trimester of pregnancy, and in women actively taking oral contraceptives containing ethinyl estradiol (Hampson, in press; Hampson et al., 2015; Hampson & Morley, 2013; but see Leeners et al., 2017). One small *f*MRI study has reported a possible estradiol x genotype interaction (Jacobs & D'Esposito, 2011). These studies suggest greater cognitive control via the dorsal frontocortical network is available during high-estrogen conditions.

To summarize, it is increasingly clear that at least some working memory processes are hormonally modulated, but the ancillary question of whether sex differences in working memory performance occur as a result of this hormonal modulation (and if so, under which conditions) has seldom been addressed. Present findings are heterogeneous, reflecting diversity in task demands and likely also uncontrolled hormonal variation. In adults of reproductive age, we discovered that men and women performed equivalently on the SPWM task of working memory if women were explicitly tested at menses (lowest estradiol levels), but when tested at high-estradiol phases of the natural menstrual cycle then women performed superiorly (Hampson & Morley, 2013). The same pattern was observed in women using oral contraceptives when they were tested during the "on" phase of the contraceptive cycle where ethinyl estradiol is given exogenously versus the "off" phase where ethinyl estradiol is not given (Hampson, in press; Figure 2). Older work not controlling for cycle-related differences in sex steroid concentrations showed an overall female advantage in groups of unselected young adults on the SPWM, Digit Ordering, or other working memory tasks having similar cognitive demands (Duff & Hampson, 2001; Lejbak et al., 2009; Voyer et al., 2017; see also Anderson et al., 2001). (Note that under random conditions only a minority of women would be expected by chance to be tested in their lowest estradiol state). Even older data suggest a female advantage can be seen on the delayed-response task in primates (McDowell et al., 1960).

Findings for the *N*-back task are mixed, but are often an exception to this pattern and even show a male advantage in a few studies (Voyer et al., 2017; but see Speck et al., 2000) for reasons that are not well-understood. Because the *N*-back is often used in the context of functional imaging, inadequate sample size combined with a potentially smaller effect size on the *N*-back (Cohen's $\underline{d} \approx 0.20$ versus $\underline{d} \approx 0.60$-$0.75$ for the SPWM; see Duff & Hampson, 2001; Voyer et al., 2017) might explain some of the inconsistencies. But features of the *N*-back task that vary from study to study (e.g., whether the stimuli are auditory or visual; the level of cognitive load assessed), and differences in the brain regions it recruits, might also be relevant. A sex difference would be expected only if working memory demands are sufficiently high, not under low-load conditions that are insensitive to individual differences in performance (e.g., 0-back, 1-back). Theoretical models of working memory suggest that the active maintenance versus manipulative processes of working memory may recruit slightly different anatomical regions within the PFC (Owen et al., 1999), with maintenance/monitoring (of the sort required by the *N*-back) recruiting ventrolateral regions but less prominently recruiting the DLPFC than tasks that emphasize manipulation (Barbey et al., 2013; Hoshi et al., 2003). Given that ERα is densely expressed in the DLPFC whereas ventrolateral PFC appears to be more lightly populated, it might help to explain the discrepant *N*-back findings.

[Put Figure 2 about here]

In this chapter, working memory is emphasized because it is a core process important for cognitive control, sustained representation of context, planning, and goal-directed decision-making. Working memory, though, is unlikely to be the only process mediated by the PFC that is influenced by estrogens, given confirmation of estrogen receptor expression in the dorsolateral cortex and the multiple cognitive processes that rely upon the region. We investigated working memory in our studies as a representative frontocortical function, but the hypothesis that 17β-estradiol is active in adult female PFC is theoretically broader and encompasses other functions too, that likewise depend on the DLPFC. While our own initial work was underway, PET imaging showed that estradiol treatment normalized regional activation seen in the DLPFC during the performance of an attentional set-shifting task (Berman et al., 1997), whereas ovarian suppression produced by a gonadotropin-releasing hormone analog decreased it. Effects at the cognitive/behavioural level were not found, but might be observable with more refined tasks or greater statistical power. The presence of a sex difference in other frontally-mediated control processes might signal their receptivity to hormonal modulation by estrogens (e.g., set-shifting, Kuptsova et al., 2015), but functions other than working memory have rarely been studied. Going forward, it will be important

to begin to test the range and limits of estrogen's effects on functionality within the dorsal frontal cortex.

Androgens and the VMPFC/OFC

DLPFC is not the only region of prefrontal cortex that is subject to hormonal regulation by sex steroids. A growing body of work suggests that testosterone (and possibly other androgens) plays a role in the VMPFC/OFC. Because VMPFC/OFC participates in the representation and updating of anticipated rewards, risks, and emotional regulation (Bechara et al., 2000; Kennerley & Walton, 2011; Krawczyk, 2002; Sinha et al., 2016), testosterone too may influence decision-making processes, especially in males where it is present in the greatest abundance.

Testosterone is the primary endocrine signal that drives sexual differentiation of the male CNS. In many species, programmed release of testosterone by the testes during a defined prenatal or early postnatal period permanently masculinizes certain features of the brain via genomic actions of testosterone or its active metabolites (for a review see Breedlove & Hampson, 2002). During adulthood, testosterone may also modify specific neurochemical/anatomical parameters to promote a male phenotype. Activational effects do not always follow organizational ones, however, and may be independent. In humans, some organizational effects of testosterone are thought to occur prenatally, mediated by androgen receptors expressed in selective regions of the CNS during that time.

Regarding the VMPFC/OFC, structural imaging has revealed a regional sex difference in the volume of the VMPFC/OFC in adult male and female brains (e.g., Welborn et al., 2009). The male brain on average is larger but after correcting for whole-brain volume, further sex differences in grey and white matter organization are evident. For instance, men possess stronger fiber connections between the striatum and OFC and ventrolateral PFC than women do; conversely, women have stronger fiber connections between the striatum and DLPFC (Lei et al., 2016). A volumetric sex difference in lateral OFC is already visible in childhood, and prenatal testosterone concentrations predict local grey matter volumes among boys (Lombardo et al., 2012). Testosterone at puberty too has been found to relate predictively to grey and especially white matter volumes in male adolescents (Paus et al., 2010), including the orbital cortex, and an association with a functional polymorphism in the androgen receptor has been observed. While these findings are only correlational, they suggest the operation of an androgen-dependent mechanism (and further CNS changes at puberty when testosterone begins its climb toward adult levels).

Consistent with an organizational effect of early testosterone, monkey studies confirm that androgen receptors are in fact expressed in the primate OFC during prenatal and early postnatal life (Clark et al., 1988). Male but not female OFC also displays a right hemisphere bias in receptor density (Sholl & Kim, 1990). In human adults undergoing PET imaging, a lateralized sex difference may be seen in activation of the VMPFC/OFC (Bolla et al., 2004) during the Iowa Gambling Task (IGT; Bechara et al., 1997), a widely used measure of decision-making that recruits a network of brain regions including, importantly, VMPFC/OFC (Bechara et al., 1998; Lawrence et al., 2009). Specifically, PET showed significantly greater task-related activation in the right lateral OFC in men's than women's brains during the IGT (Bolla et al., 2004). In principle, a sex difference in adult brains could be caused by *activational* effects of hormones. But differences between men and women in the clinical symptoms of lateralized brain lesions (damage to the right VMPFC/OFC causes severe impairment in socioemotional functioning and decision-making in men, whereas it is *left* VMPFC/OFC lesions that most adversely affect women) favor a true sex difference in the local functional organization of the cortex (Reber & Tranel, 2017).

Currently the best evidence for an organizational effect of testosterone in VMPFC/OFC is behavioural. Clark and Goldman-Rakic (1989) found that female monkeys treated with testosterone prenatally or in early infancy displayed masculinized performance on an object reversal task (see Table 1) that relies on the OFC, when later tested at 75 days of age. (Reversal tasks require efficiently switching between two choice options as the rewards or payouts associated with each option change and alternate throughout the task). The importance of OFC for reversal learning is well-established (e.g., Fellows 2013; O'Doherty et al., 2001). Male monkeys outperform females on object reversal at this young age (Clark & Goldman-Rakic, 1989), whereas females outperform males on a different reward-based implicit learning task that depends on other brain pathways. The male advantage in object reversal is seen in human children too (Overman et al., 1996). Only recently, Evans and Hampson (2015a) found that the sex difference can be demonstrated in *adults*, providing a sufficiently demanding reversal task is used. Extension of these observations to adults is important because it reinforces the probability that a true organizational effect is present, not simply a transient developmental delay in the maturation of the female primate OFC relative to the male, as proposed by Clark and Goldman-Rakic (1989).

VMPFC/OFC has been implicated in a number of different processes, but progress in exploring whether androgens organizationally modify other aspects of VMPFC/OFC function has been slow. This reflects the limited paradigms currently available to study organizational effects in humans (prenatal exposure to sex steroids cannot ethically be manipulated for research purposes). In principle, the second-to-

fourth digit ratio (so-called 2D:4D ratio), a putative somatic biomarker of testosterone concentrations present prenatally (Manning et al., 1998), could be used to study organizational questions and in fact, associations between individual differences in 2D:4D ratio and risk-taking propensity (for example) have been identified in a few studies (e.g., Evans & Hampson, 2014; Stenstrom et al., 2011). However, these findings are difficult to interpret because of lingering doubts about the validity of the 2D:4D ratio as a legitimate reflection of prenatal testosterone activity (for discussion see Hampson & Sankar, 2012).

Growing evidence suggests that adult testosterone levels exert a modulatory influence on processes governed by the VMPFC/OFC too. Existing data are largely correlational, but support the view that one or more latent processes that underlie performance on the IGT or other laboratory tasks may be androgen-dependent in an *activational* sense. One recent study reported that adult testosterone levels positively predicted the ability to flexibly adjust responding on a reversal learning task (Diekhof & Kraft, 2017). Taking the IGT as a more often-studied example, healthy adults of either sex can learn the task in a moderate number of trials but on average men learn the deck contingencies more rapidly than women, and adjust their decision-making patterns accordingly (Reavis & Overman, 2001; van den Bos et al., 2013). However, among adult men, a high endogenous testosterone level measured in serum or saliva predicts *poorer* not better performance on the IGT (Evans & Hampson, 2014; Reavis & Overman, 2001; Stanton et al., 2011). In women too, higher testosterone predicts *poorer* performance (Stanton et al., 2011). The direction of the association is consistent across both sexes, but is surprising in light of the male advantage seen on the task as a whole, reinforcing the concept that on a complex task like the IGT, composed of multiple converging subprocesses, a sex difference does not always straightforwardly predict activational control by sex steroids. More than one dimension of the IGT task might be subject to hormonal regulation. Although current evidence is largely correlational, a double-blind crossover trial (van Honk et al., 2004) showed that women treated acutely with a high dose of sublingual testosterone chose more cards from disadvantageous decks, confirming that high testosterone is disadvantageous to IGT decision-making. In favour of the view that VMPFC/OFC is responsive to adult testosterone levels, androgen receptors have now been identified in the adult OFC (Finley & Kritzer, 1999).

Which cognitive process is subject to regulation by testosterone? The IGT has been a fruitful tool and starting point for studying decision-making, but is a complex task that evokes multiple latent processes, including decision-making under uncertainty, reinforcement-based learning, and inhibitory control (for discussion see Evans & Hampson, 2015a,b). Exactly which process(es) are modulated by testosterone is not presently understood. In keeping with current conceptualizations of the VMPFC/OFC, prominent

ideas regarding the source of the testosterone effect (and the male advantage on the IGT as a whole) include risk evaluation/updating, risk-averseness, sensitivity to reward and/or punishment, or emphasis on immediate versus long-term outcomes (e.g., Evans & Hampson, 2014; 2015a,b; Overman et al., 2011; Stanton et al., 2011; van den Bos et al., 2013)--processes that also apply to decisions made under many everyday conditions.

Motivational processes are likely to be involved. Functional imaging studies reveal that testosterone levels modify activity in neural circuitry important for the regulation of emotion and reward, including activity in the VMPFC/OFC, amygdala, and ventral striatum. Under resting state conditions, VMPFC-amygdala functional connectivity is enhanced in men compared with women (Engman et al., 2016). A review of functional imaging studies suggested that high endogenous testosterone concentrations are associated with greater activation in the amygdala and VMPFC/OFC in reaction to stimuli signaling threat or reward (van Wingen et al., 2011). In placebo-controlled trials, testosterone administration increased amygdala reactivity (and in some studies activation in OFC; e.g., Hermans et al., 2008) elicited under conditions of social threat or vigilance, but at very high levels such as those produced by a supraphysiological dose of testosterone, testosterone decreased amygdala connectivity with the OFC (Bos et al., 2012; van Wingen et al., 2010), indicating functional decoupling. Exogenous testosterone increases the motivation to seek rewards (van Honk et al., 2004) and heightens the BOLD response in the ventral striatum during anticipation of reward (Hermans et al., 2010). Although fewer studies have examined VMPFC/OFC, the level of BOLD activation observed in VMPFC/OFC in anticipation of reward, in response to social threat, or during risky decision-making, all show graded correlations with endogenous levels of testosterone measured in saliva or plasma (e.g., Op de Macks et al., 2016; Stanton et al., 2009; see also Mehta & Beer, 2009). In short, imaging studies suggest that adult testosterone levels do influence activity in VMPFC/OFC under certain conditions.

Our own early work on testosterone and decision-making began by studying associations between endogenous testosterone levels and entrepreneurial business ventures (White et al., 2006). We found that risk-taking was one mediator of the associations we observed. Neurons exist in the OFC that code a risk signal (O'Neill & Schultz, 2015), but whether these cells are modulated by testosterone is currently unknown. Opinions diverge on whether testosterone's influence on risk-taking is organizational or activational or both (Brañas-Garza & Rustichini, 2011; Stenstrom et al., 2011; but see Apicella et al., 2014; Goudriaan et al., 2010) and on how (or if) a willingness to incur risk is conceptually separable from VMPFC/OFC's role in the anticipatory evaluation of punishment and reward contingencies. Holding sex

constant, Evans and Hampson (2014) suggested that the organizational and activational effects of testosterone might act on different latent processes relevant to decision-making on the IGT--whereas androgen exposure during early development may act to increase risk-taking, at least part of the effect of adult testosterone may be independent of risk and due to an activational influence on the weights afforded to different reinforcement outcomes (Evans & Hampson, 2014). Related though not identical concepts regarding reinforcement processing have been advanced by others to explain *sex differences* on the IGT (Overman & Pierce, 2013; van den Bos et al., 2013). Alternative hypotheses concerning the underlying processes responsible for sex- and testosterone-dependent differences in performance have received less empirical support (e.g., impulsivity).

VMPFC/OFC is not completely devoid of estrogen receptors. But if estradiol plays any role, preliminary evidence suggests its effect at high levels is to promote *decreased* not increased risk-taking (Barel et al., 2017; Op de Macks et al., 2016). Present findings with regard to estradiol must be treated with caution because few studies currently exist and small sample sizes can give rise to inaccurate findings (Button et al., 2013; Schultheiss & Mehta, this volume). However, the picture emerging so far suggests that high estradiol unopposed by progesterone[2] is associated with increased activation in subcortical regions responsive to reward, but also increased top-down control via VMPFC/OFC (e.g., Thomas et al., 2014). High estradiol seems to be associated with increased behavioural restraint and decreased reactivity to negative emotional stimuli compared with low estradiol conditions. For example, brain responses in the mesocorticolimbic reward circuit to certain primary and secondary reinforcers vary across the menstrual cycle in women (Dreher, 2015) including responses to men's faces as sexual stimuli, which elicit a larger BOLD response in the medial OFC during the late follicular (fertile) phase of the cycle (Rupp et al., 2009). Response magnitude in the OFC was positively correlated with serum estradiol concentration, peaking at that time (also see Zeidan et al., 2011). Conversely, activation in the amygdala and OFC in response to stressful or negative stimuli is *lower* during the late follicular phase (Goldstein et al., 2005; Goldstein et al., 2010; Jacobs et al., 2015; Protopopescu et al., 2005), indicating reduced reactivity of the stress circuitry. Both men, and women tested at the beginning of the menstrual cycle when estradiol is low, had a greater BOLD signal increase in OFC and amygdala in response to negative emotional stimuli than women tested at high estradiol levels (Goldstein et al., 2010). Despite suggestions that reward salience increases under high estradiol conditions, the tendency for women to choose immediate rewards over

[2] Progesterone conceivably plays a role in adaptive functioning, perhaps through synergistic motivational effects (but also antagonistic ones; e.g. Dreher et al., 2007). Its relevance for processes in the PFC is largely unknown.

larger delayed rewards is *reduced* at midcycle when estradiol is high, with the magnitude of reduction correlating directly with the circulating concentration of estradiol (Smith et al., 2014). In short, present evidence suggests that estradiol's actions differ considerably from testosterone's: high estradiol appears to promote a lower-risk, controlled mode of thinking, while high testosterone is associated with increased risk-taking and reward-seeking. Animal studies tell us that testosterone sometimes acts in the CNS via intracellular conversion to estradiol, but the data reviewed here suggest that binding to ER after conversion to estradiol is not the basis for testosterone's actions in the VMPFC/OFC.

A Working Model of Sexual Differentiation in the PFC

The frontal association cortex is often overlooked as a site of sexual differentiation or modulation by reproductive steroids because the cognitive and socioemotional functions it governs do not on the surface seem relevant to reproductive function. However, growing evidence supports the idea that sex steroids are important modulators of two partially distinct but interfacing regulatory control systems vested in the dorsal and ventral PFC (Gläscher et al., 2012) that assist in decision-making by reference to external contextual cues and internal body-related or motivational cues, respectively, and that may be differentially sensitive to estrogens and androgens.

Given that sex hormones generally act in the CNS to promote reproductive outcomes, why is endocrine control present in the PFC? This can be understood within an evolutionary framework, by considering the classes of cognitive functions that are modulated, and when. Past arguments have often been based on the assumption of a sex difference, but many cognitive sex differences reported for the PFC appear to depend on reproductive states. Therefore, the proper question to ask is not why is there a sex difference, but why is there state-dependent endocrine control? What is it about high levels of estradiol or testosterone that requires sharpening of specific cognitive processes in one sex more than the other?

Among other theoretical speculations, it's been suggested that steroid-driven changes in the reactivity of the reward network might facilitate procreation by increasing sexual receptivity or desire (Caldú & Dreher, 2007). Existing theories, however, are narrowly focused and don't adequately explain the full spectrum of hormonal effects beginning to emerge. We propose, alternatively, that regulation of the PFC by sex steroids during the prime reproductive years evolved as a mechanism to promote a type of decision-making bias in each sex that best facilitates reproductive success ('fitness'). The targets of this modulation are reproductively-relevant decisions such as acquiring suitable sexual partners, mates, raising offspring that survive and thrive, but might extend to non-social decisions that recruit the same

neuronal pathways. According to parental investment theory (Trivers, 1972), males can increase their chances of reproductive success by acquiring resources and pursuing mating opportunities (both require willingness to engage in risk and are motivated by anticipated rewards), while not being easily deterred when faced with short-term negative outcomes. Males must defend their acquired social status against competitors and accurately discern when it is necessary. This behavioural phenotype may be promoted by testosterone, a hormonal signal of both reproductive maturity and viability. Females, on the other hand, have greater obligatory parental investment so may benefit more than males (especially at times of increased conception risk or if reproductive effort is already underway, as signaled by a high estradiol level) from increased deliberative top-down cognitive control. Optimal gating by the dorsal and ventral control networks may optimize outcomes for both sexes.

Consistent with the idea of enhanced dorsal network function when estradiol is high, a growing body of evidence suggests that working memory, a central element of planning, is improved during high estradiol conditions. Data for functions other than working memory are scarce, but reports of greater inhibitory control when estradiol is highest (Amin et al., 2006; Hjelmervik et al., 2012), greater delay of gratification (Diekhof, 2015; Smith et al., 2014), or facilitated attentional-switching (Colzato et al., 2012), are consistent with amplified dorsal network function, and are core cognitive processes important for child-rearing. Sex differences have been reported intermittently for these same processes (e.g., Bjorklund & Kipp, 1996), but further study is required to establish if the magnitude of the sex difference is dependent upon endocrine state (i.e., upon activational effects of steroids). High testosterone during the organizational period of the CNS and high (but not excessive) testosterone at reproductive maturity may enhance ventral network function in the male brain and promote regulatory control by VMPFC/OFC over linked subcortical structures. Heightened estradiol production by the ovaries and/or placenta may serve as a biological signal to the CNS that elicits heightened dorsal network function in women.

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Table 1. Glossary of Cognitive Tasks (with Example References)

Name of Task	Brief Description
Delayed Response (Jacobsen, 1936)	A type of task originally used to study working memory in nonhuman primates. Each trial involves the presentation of a simple stimulus that is then removed or hidden from view. Its location (or identity) must be remembered following a short delay. Typically only a small pool of items is used (e.g., 2 possible locations), and the choice that is the correct one is randomized from trial to trial. On each trial, therefore, the animal must keep track of the *current* location in order to receive a food reward.
Digit Ordering (Petrides et al., 1993)	Using a set of digits provided by the experimenter, each trial involves generating aloud a re-ordered sequence of the digit set, arranged according to some pre-specified criterion. Because the original stimuli must be mentally re-arranged and temporarily held in mind, the task requires working memory. Ordering constraints might include, for example, saying the digit set aloud in ascending or descending numerical order, arranging them into a random string, or repeating them in backwards order relative to their position in the original set (as in the Digits Backward task, for example).
SPWM (Duff & Hampson, 2001)	Involves finding matching pairs of coloured tokens, which are hidden beneath the identical-appearing flaps of a response board. In order to locate correct matches, the spatial locations of the hidden tokens must be temporarily held in mind until all of the pairs have been successfully discovered and matched. Thus spatial working memory is required.

N-Back Task (Kirchner, 1958)	The participant is asked to monitor a series of rapidly presented auditory or visual stimuli and indicate (usually by a buttonpress) whenever the identity (or location) of the current stimulus is the same as the stimulus that appeared "N" stimuli back in the series (e.g., 1-back, 2-back). This working memory task is often used in functional imaging studies.
Self-Ordered Pointing (Petrides & Milner, 1982)	Each trial involves a small array of stimuli, which are shown on successive cards (or successive computer screens) in differing spatial arrangements. The participant is asked to point to one item on each card (or screen) so that by the end of the set, each item in the array has been pointed to once and only once, without skipping or repeating any of the items. Working memory is required, in that a participant must keep track of which stimuli have already been pointed to, and which remain to be pointed to, within a given trial. Array size can be varied to increase or decrease the load on working memory.
Attentional Set-Shifting (Roberts et al., 1988)	A type of cognitive task that involves switching attention to a formerly irrelevant feature of a complex stimulus after having learned previously over a set of trials to pay attention to a different feature.
Reversal Learning (Clark & Goldman-Rakic, 1989)	A type of task that Involves learning over a set of trials to choose the one of two paired stimuli that is most consistently associated with receiving a reward, then, when the reward contingencies switch without warning, efficiently switching to the other member of the pair.
Delay Discounting (Reynolds & Schiffbauer, 2004)	Temporal discounting tasks typically involve establishing how long a participant is willing to wait to receive a larger delayed reward rather than an immediate but smaller reward, or how rapidly the

	perceived value of a reward falls off as a function of the duration of time to its receipt.
Iowa Gambling Task (Bechara et al., 1994)	A decision-making task that involves choosing from four decks of 'cards' which vary in their payoffs, penalties, and the probabilities of each, with the goal of maximizing one's winnings by the end of the task.

Table 2. Pearson's Correlations Between Working Memory Errors on the SPWM and Working Memory Errors on Self-Ordered Pointing (SOPT) (*n* = 38 adult females; from Hampson et al., 2015)

	SOPT 6-item	8-item	10-item	12-item	SOPT Total Errors
SPWM Total Errors	*r* = .37*	*r* = .34*	*r* = .48**	*r* = .65**	*r* = .57**

Note. Correlations represent the Pearson's correlations between the total number of WM errors committed on the SPWM (summed over two trials; only two trials were administered in Hampson et al., 2015) and the total number of WM errors committed on the SOPT by the same women at each set size used, ranging from 6-item sets to the maximum 12-item sets. Also shown is the grand correlation between WM errors on the two tasks as a whole. Both tasks are thought to measure WM. Note that the size of the correlation increases with greater cognitive load on the SOPT.

* $p < .05$; ** $p < .01$, two-tailed

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Figure Captions

Figure 1. Working memory performance in postmenopausal women (*n* = 96) from Duff and Hampson (2000). Healthy women not using replacement estrogens (Non-HRT, *n* = 35) made significantly greater numbers of working memory errors on the Digit Ordering task (top) and SPWM task (middle) compared with women taking either estrogen alone (E-Only, *n* = 38) or estrogen plus a progestin (E+P, *n* = 23), and showed a poorer score on Digits Backward (bottom). Thus on all 3 tests of working memory, superior performance was observed among estrogen users. In contrast, there was no effect of estrogen use on Digits Forward ($p = 0.563$) or on the Corsi Blocks ($p = 0.588$) (data not shown). The latter tasks require only passive immediate recall, not active maintenance or manipulation of information within working memory.

Figure 2. Working memory errors on the SPWM, a test of spatial working memory, in oral contraceptive users (OC users) and a group of demographically-matched male controls. Three trials were given. The total number of working memory errors is shown separately for each trial. All study participants were tested on a brief set of cognitive tasks, including the SPWM, during active OC use (On OC; *n* = 40) or during the one-week interval of the contraceptive cycle when no active hormone is taken (Off OC; *n* = 20). For the male controls, *n* = 96. All the women shown here were regular users of combined OCs that consisted of ethinyl estradiol (15-35 ug/day, depending on the brand of OC being used) combined with a progestin. Participants were tested blind (without experimenter knowledge of OC use, brands used, or phase of the contraceptive cycle), and then were classified retrospectively into groups based on OC details provided by the participants at the end of the test session. ANOVA revealed that women using exogenous estradiol in the form of OCs at time of testing made significantly fewer WM errors on the SPWM than either women who were tested during the washout week of the contraceptive cycle, or matched male controls. Women on OCs also showed more rapid improvement over the 3 trials. Among naturally-cycling women (non-OC users) who participated in the same study (not shown in figure), a similar pattern was found--higher levels of estradiol, measured in saliva, were associated with superior WM performance on the SPWM (Hampson & Morley, 2013). See Hampson, in press, for further details of the data plotted here (figure adapted from Hampson, in press).