Title
Estrogen shapes dopamine-dependent cognitive processes: Implications for women's health

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Publication Date
2010

Peer reviewed|Thesis/dissertation

Estrogen shapes dopamine-dependent cognitive processes: Implications for women's health

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A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Neuroscience

in the

Graduate Division

of the

University of California, Berkeley

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Spring 2010

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Implications for women's health

by Emily C. Jacobs

Abstract

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Emily Christine Jacobs

Doctor of Philosophy in Neuroscience

University of California, Berkeley

Professor Mark D'Esposito, M.D, Chair

The prefrontal cortex (PFC) is exquisitely sensitive to its neurochemical environment. Minor fluctuations in cortical dopamine (DA) can profoundly alter working memory (WM), a PFC-dependent cognitive function that supports an array of essential human behaviors, from problem-solving to fluid intelligence. Dopamine's action in the PFC follows an inverted U-shaped curve, where an optimal DA level is necessary for maximal function and both insufficient and excessive DA activity impairs PFC processes. In animals, estrogen has been shown to increase dopaminergic activity, yet this relationship has not been demonstrated in humans. This suggests that working memory performance might be affected by estrogen's rhythmic changes throughout the menstrual cycle, and that baseline DA levels will influence the direction of estrogen's effect.

In a series of cognitive genomic, neuroendocrine studies in healthy young women, we examined estrogen's impact on the performance of DA-dependent tasks as a function of COMT Val^{158}Met genotype and COMT enzyme activity (indices of baseline DA). The results demonstrate that estrogen status impacts working memory function and, crucially, that the direction of the effect depends on an individual's COMT genotype and, at a finer scale, COMT enzyme activity, demonstrating a dependence on baseline DA. At a neural level, functional MRI revealed that cortical dopamine (shaped by a balance of genetic and hormonal factors) is associated with a broadly 'efficient' pattern of sustained activity (that which occurs across WM blocks), and a selective, event-related enhancement of activity during episodes of high interference (e.g. lures), when the demand for cognitive control is greatest. Furthermore, the extent to which an individual enhances PFC activation during the demanding lure trials is predictive of their performance.

Next, we used a visual selective attention paradigm to probe the effects of estrogen and COMT genotype on top-down, goal-directed modulation of neural activity in visual association cortices (VAC). We used a recently established metric of goal-directed 'enhancement' and 'suppression' that is sensitive to identifying group differences in VAC

modulation. Scene-selective regions of interest (bilateral PPA) showed robust suppression and enhancement effects at the group level, which were dependent on task goals, but further analyses revealed an important difference between low and high estrogen groups. While both groups successfully enhanced PPA activity during the *Remember Scenes* condition above a perceptual baseline, only the high estrogen subjects were able to appropriately attenuate the processing of task-irrelevant scenes in the *Ignore Scenes* condition. This effect of estrogen on distracter filtering parallels the suppression deficit observed in older adults, and young adults when attentional resources are taxed.

Furthermore, when attentional resources were imposed upon (during a dual-task condition in which two stimuli from different object categories must be attended to and maintained over a delay) low estrogen subjects succumbed to an 'enhancement deficit', which has been shown to occur in young adults when attentional resources are limited. High estrogen subjects, however, were resilient to the high load condition. Thus, even when attentional/working memory resources were taxed, if estrogen levels were high women showed no evidence of strained top-down, goal-directed processing. When estrogen levels dropped (during the beginning of the cycle) the enhancement deficit emerged.

Multivariate functional connectivity data assessing coherence between frontal control regions and visual association cortices revealed an estrogen*genotype interaction. Subjects with naturally reduced prefrontal DA (*val/val* genotype) showed greater top-down coherence when estrogen levels were high versus low; but subjects with naturally elevated prefrontal DA (*met/met* genotype) showed the opposite pattern, with the most robust coherence when estrogen levels were low. These data parallel the interaction observed in the N-back task, which both follow the theoretical inverted-U shaped DA model.

In humans there has been a strong effort to understand the effects of estrogen on cognition, but the data have been inconsistent. This study establishes that taking baseline DA into account is pivotal to detecting the direction of estrogen's effect on working memory. The results carry direct ramifications for women's health, as the response to DA medications (e.g. Ritalin for attention-deficit disorder and l-DOPA for Parkinson's disease) may differ between men and women, and within women in different endocrine states. A man and woman's milieu differ; until we understand how we cannot fully understand neural processes as they unfold in the healthy state, less still in the diseased state.

Dedication

To Jo Ellen, whose life approaches Eudemonia more than any life I know.
To Gary, who is a model of how to love and excel at a profession so naturally.
To Megan, who taught me how to see the beauty of a broken cicada shell.
To Michael, who joins me in this mind-bending quest to plumb the depths of the brain, and each other, hand in hand.

This, humbly, is for you.

§

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Abbreviations

COMT:	catechol-o-methyltransferase
DA:	dopamine
ER:	estrogen receptor
ERT:	estrogen replacement therapy
MAO:	monoamine oxidase
OVX:	ovariectomy
PFC:	prefrontal cortex (dl: dorsolateral)
SN:	substantia nigra
ROI:	region of interest
VAC:	visual association cortex
VTA:	ventral tegmental area
WM:	working memory
WCST:	Wisconsin card sorting task

Acknowledgments

I'm grateful for the help of so many. In particular, Mark D'Esposito – for his indefatigable efforts to push students towards new ways of advancing the study of brain function; for his willingness to let me approach that topic from a woman's health perspective; and, consequentially, for his rather kind acceptance of hearing the words 'estrogen' and 'menstrual cycle' uttered ad nauseam.

My thanks to current and former lab members, too many to name, for support in all those tiny crevices and massive calderas that, together, make up lab-life.

Thank you.

§

1

INTRODUCTION

1.1 *Background*

The motivation of this work is two-fold: first, this project explores the extent to which endocrine and genomic processes impact the brain's dopaminergic system and, in turn, cortical functions that are exquisitely dependent thereon. A second, loftier goal was (and remains) to promote the importance of considering the role of hormones and sex-differences in cognition. From a basic science perspective an intimate understanding of how the endocrine system impacts neural and cognitive processes is interesting; from a women's health perspective it is *fundamental*. The estrogen-cognition literature (including large-scale population health studies on hormone replacement therapy) is famously inconsistent. We can ask, at the level of the brain, *why?*

I begin in Chapter 1 by examining the relationship between dopamine, the prefrontal cortex (PFC) and working memory. Next, I explore how an evolutionarily recent genetic polymorphism shapes PFC function. I then turn to endocrinology to examine estrogen's impact on the dopamine system. Finally, in Chapters 2-4, I weave these three scientific threads together within the context of my own work.

1.2 *Dopamine, the prefrontal cortex and working memory*

The human brain contains three main ascending dopaminergic projections that enable distinct, but highly integrated, neural functions (**Fig. 1.1**). The nigrostriatal tract originates from the substantia nigra (SN) and projects to the striatum, guiding activity in the basal-ganglia motor system. The mesocortical and mesolimbic tracts originate from the ventral tegmental area (VTA) and project, as their names suggest, to frontal and limbic regions. It has been just over fifty years since Arvid Carlsson first defined dopamine (DA) as a neurotransmitter; in the late 1950's dopamine was thought to be simply a precursor in the biosynthetic pathway of norepinephrine. Carlson's research focused on dopamine's function in the nigrostriatal pathway, work that led to the development of dopaminergic drugs to treat Parkinson's Disease, a neurodegenerative disorder marked by degradation of the substantia nigra cells that produce dopamine.

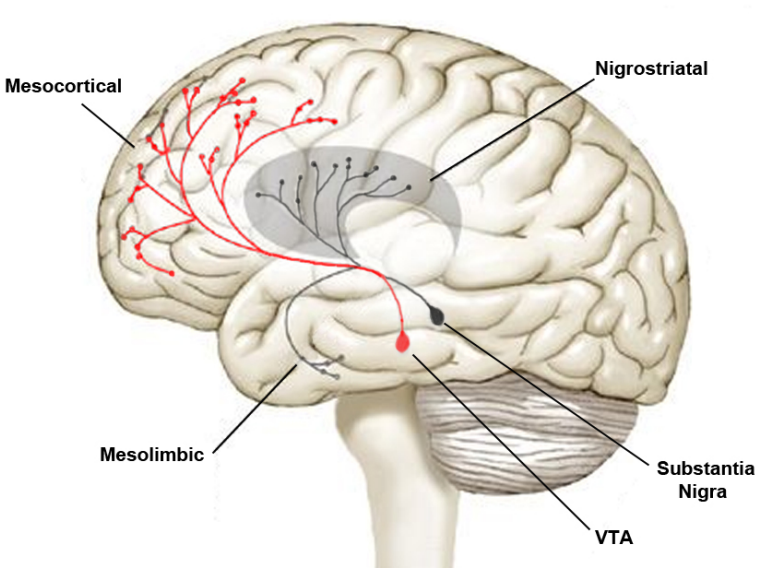

Figure 1.1: Ascending dopaminergic pathways in the human brain. The nigrostriatal tract originates from the substantia nigra and projects to the striatum (gray region). The mesolimbic and mesocortical tracts originate from dopaminergic cell bodies in the ventral tegmental area (VTA) and project to the nucleus accumbens and prefrontal cortex, respectively. A fourth tract, the tuberoinfundibular, is not shown. (*Figure by E.J & M. Goard*).

Twenty years later, in the late 1970's, the definitive link between DA and frontal lobe function was established. Brozoski et al (1979) showed that regional depletion of DA in the frontal lobe of rhesus monkeys led to severe working memory impairments. DA depleted animals performed no better than monkeys with full ablation to the dorsolateral prefrontal cortex (PFC). The impairment was reversed following DA replacement. Their discovery catalyzed the study of the mesocortical tract and significantly strengthened our understanding of the intimate relationship between the prefrontal cortex, dopaminergic signaling and working memory.

Working memory (WM), the ability to temporarily maintain and manipulate information that is no longer accessible in the environment, is a basic cognitive ability that supports an array of complex processes from problem solving to reading comprehension to fluid intelligence (Barrett et al., 2004). The importance of the PFC to working memory is well known. As Brozoski first demonstrated, DA transmission within this region is critical to successful WM performance (Brozoski et al., 1979; Sawaguchi & Goldman-Rakic, 1991; Williams & Goldman-Rakic, 1995). Later work established the importance of a subset of DA receptors, D1, in mediating the link between DA transmission and WM performance (Sawaguchi and Goldman-Rakic, 1991; Muller et al., 1998; Seamans et al, 1995). In humans DA agonists modulate WM performance and the efficiency of prefrontal cortical networks (Mehta et al., 2000). Electrophysiological data, regional depletion studies, pharmacological manipulations and patient studies provide converging evidence that prefrontal DA is critical for working memory.

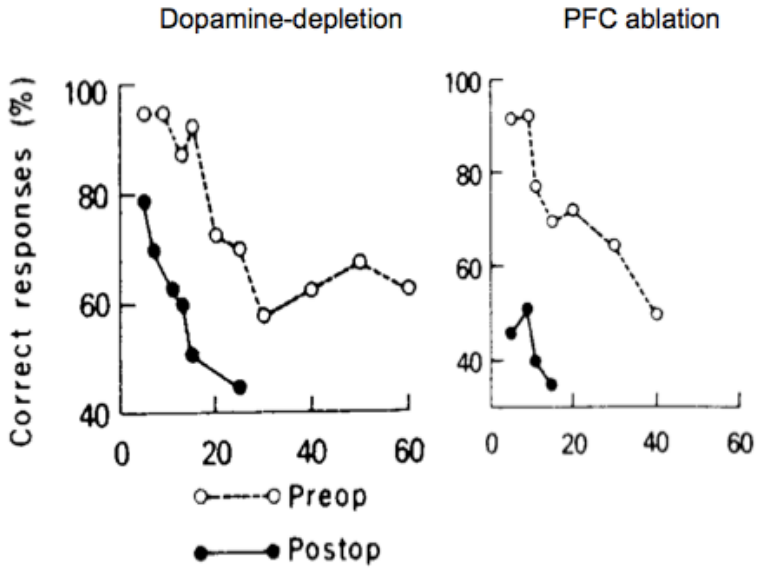

Figure 1.2: Dopamine depletion leads to WM deficits in monkeys. *Left:* intact animals (white trace) show a typical decline in performance on a delayed-response task as the duration of delay period increases. Following 6-OHDA lesioning (black-trace), animals are impaired at nearly all delays. *Right:* performance of DA-depleted animals is on par with animals that experienced direct structural ablation to the frontal lobe. (Figure adapted from Brozoski et al, 1979)

1.3 *The dopamine paradox*

While the general relationship between dopamine and PFC function is clear, the intricacies of this association are not straightforward: in humans the drug response to a DA agonist may vary between individuals, improving performance for some and impairing performance for others. DA's relationship to performance on frontally-mediated cognitive tasks is not linear—dopamine follows an 'inverted U-shaped' curve, where an optimal DA level is necessary for maximal function and both insufficient and excessive levels lead to PFC dysfunction (Vijayraghavan et al, 2007; Arnsten & Goldman-Rakic, 1986; Williams & Goldman-Rakic, 1995; Kimberg et al., 1997; Cools et al., 2004). Consistent with this model, DA D1 receptor agonists improve working memory performance in monkeys at low doses but impair performance at high doses (Cai and Arnsten 1997).

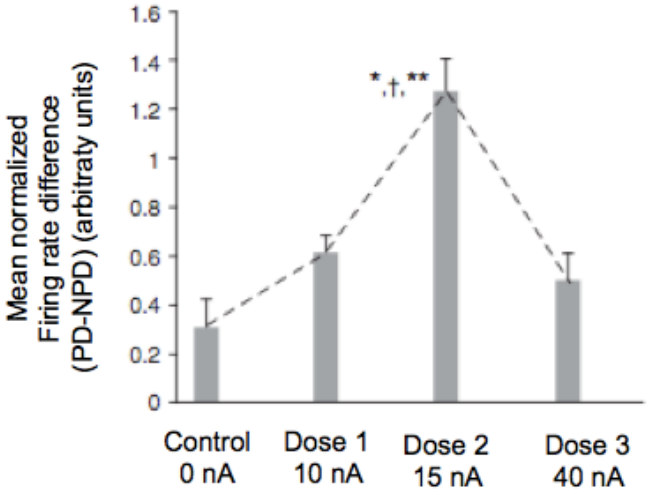

Figure 1.3: Dopamine's inverted-U shaped function at the single unit level. Spatial tuning of PFC neurons is enhanced at low to moderate levels of D1 DA-receptor stimulation, but degraded at extreme levels of DA stimulation. (Figure from Vijayraghavan et al, 2004)

Similar baseline dependent effects are evident in humans. The effect of dopaminergic drugs, like the D2 agonist bromocriptine, depends on subjects' baseline level of working memory performance (Kimberg et al., 1997), a predictor of basal dopamine (Cools et al., 2008). Thus, taking baseline DA into account (i.e. the "law of initial value") is essential for predicting how DA augmentation (e.g. via a drug) will affect cognitive performance (Egan et al., 2001; Cools et al., 2004) (**Appendix A**).

Kimberg and colleagues (1997) found that subjects with low WM spans (arguably a reflection of *low* PFC DA) demonstrated cognitive improvement after administration of a DA agonist, while high span subjects (*high* PFC DA) were impaired ON-drug. The results appear paradoxical only when considered under a linear model of DA action. In accordance with the 'inverted U' model, low span subjects benefited from the DA agonist because their DA levels were elevated to a more optimal position on the dose- response curve (symbolically speaking). Hence, working memory performance improved ON-drug. Conversely, high span subjects (with typically higher DA levels OFF drug) performed well to begin with but did worse after administration of the agonist, presumably because DA levels were pushed beyond the optimal range ("overdosed"). Similarly, in children with ADHD there is an inverse relationship between subjects' WM capacity and the extent to which they benefit from methylphenidate. Subjects with a weaker digit span show greater levels of task and clinical improvement ON-drug compared to high span subjects (Mehta et al., 2004).

1.4 *COMT regulates prefrontal dopamine*

What contributes to individual differences in baseline DA levels? Some inter-subject variability stems from genetic polymorphisms that alter the DA system. The catechol-O-methyltransferase (COMT) gene on chromosome 22q11 codes for an enzyme that metabolizes released dopamine. The enzyme is crucial for regulating DA transmission in the PFC where DA transporters (the primary mechanism of sub-cortical dopamine degradation) are scarce. COMT accounts for over 60% of total DA turnover in the PFC (compared to 15% in the striatum) (Karoum et al., 1994) and COMT knockout mice show a two to three-fold increase in DA levels only in the PFC (with no change in noradrenaline levels) (Gogos et al., 1998).

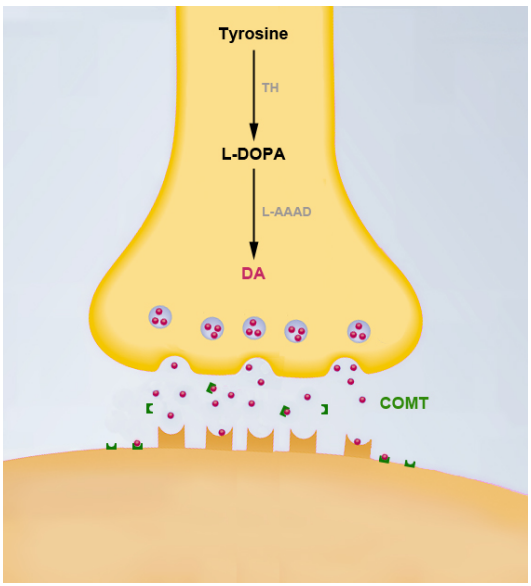

Figure 1.4: COMT's action in the synapse. COMT (green bar) is expressed peri-synaptically in membrane-bound and soluble forms. COMT degrades DA (pink circles) by placing a methyl group on the catecholamine, thus rendering it inactive. (Figure by E.J. and M. Goard).

An evolutionarily recent single nucleotide polymorphism at locus 158 in the COMT gene ($Val^{158}Met$) alters the efficiency of the COMT enzyme, which in turn affects how long DA remains active in the synaptic cleft. The two alleles –"val" (ancestral allele) and "met" (human allele)—are co-dominant, giving rise to three allelic variants (val/val, val/met, met/met) with a trimodal distribution of COMT activity (Mannisto & Kaakkola, 1999). Met/met individuals have decreased COMT activity owing to a less efficient enzyme, leading to increased cortical DA relative to val/val individuals.

Functionally, the met allele is associated with enhanced PFC-dependent cognitive function and greater cortical efficiency (Egan et al., 2001; Tunbridge et al., 2006). Egan and colleagues (2001) found that COMT genotype correlates with working memory span: val/val subjects show poorer performance on Wisconsin Card Sorting Task (WCST) and reduced neural efficiency during an n-back compared to val/met and met/met subjects. These results have been replicated in children (Diamond et al., 2004). More recently, pharmacogenomic studies have been able to predict the effects of DA drugs on performance based on an individual's COMT genotype. Val/val subjects make more perseverative errors on the WCST at baseline but improve on amphetamine; the opposite pattern holds true for met/met participants (Mattay et al., 2003; **Fig. 1.5**).

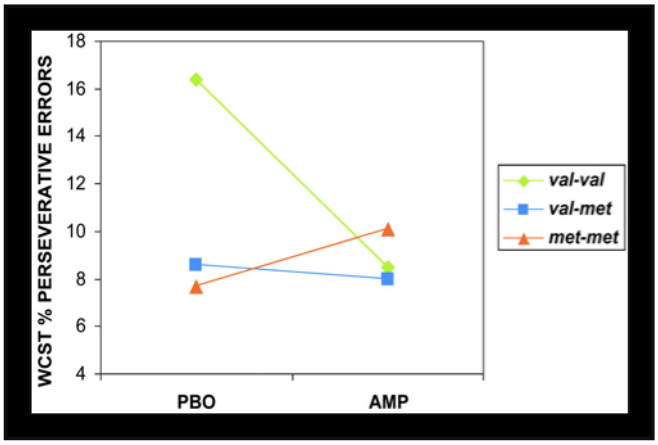

Figure 1.5: Effects of amphetamine on a classic frontal lobe task are dependent on COMT genotype. *Val/val's* make more errors on placebo compared to met/mets and val/mets; their performance improves following amphetamine administration. *Met/met* subjects show a trend toward the reverse pattern (worse on drug vs. placebo). Figure adapted from Mattay *et al* (2001).

1.5 *Estrogen impacts the dopamine system*

Effects of estrogen on the DA system have been observed in the nigrostriatal, mesolimbic and mesocortical pathways in rats, monkeys and humans. In animals, strong evidence indicates that estrogen enhances DA activity on rapid and protracted timescales, both genomic and nongenomic mechanisms are evident (Becker, 2000). There are many ways to experimentally modulate DA—drugs, patients, gene knockout models—but each comes with disadvantages. One goal of this series of studies was to ask whether estrogen offers an informative, noninvasive method for studying the role of DA in higher cognitive function. The relationship between estrogen and DA at the genomic, cellular, network and cognitive levels is reviewed below.

1.5.1. Estrogen's rapid (non-genomic) effects

The classic view of estrogen centers on the hormone's role in regulating genomic activity in tissues throughout the body. Estrogen's ability to promote rapid, nongenomic effects (on the order of seconds to minutes) in the CNS has been recognized more recently. Estrogen enhances DA synthesis (Pasqualini et al., 1995), release and turnover and modifies basal firing rates of dopaminergic neurons via membrane estrogen receptors (Chiodo & Caggiula, 1980) via membrane estrogen receptors (Becker, 2000; Pasqualini et al., 1995; Becker, 1990; Xiao & Becker, 1994). Thompson et al (1997) used in vivo voltammetry to measure potassium- stimulated DA release in the nucleus accumbens of cycling female rats. DA release and reuptake fluctuated in synchrony with hormonal changes during the estrous cycle, rising when estrogen levels were high and falling when levels were low. Similarly, amphetamine stimulated DA release is potentiated when estrogen levels are high and attenuated when estrogen levels are low (Becker, 1990).

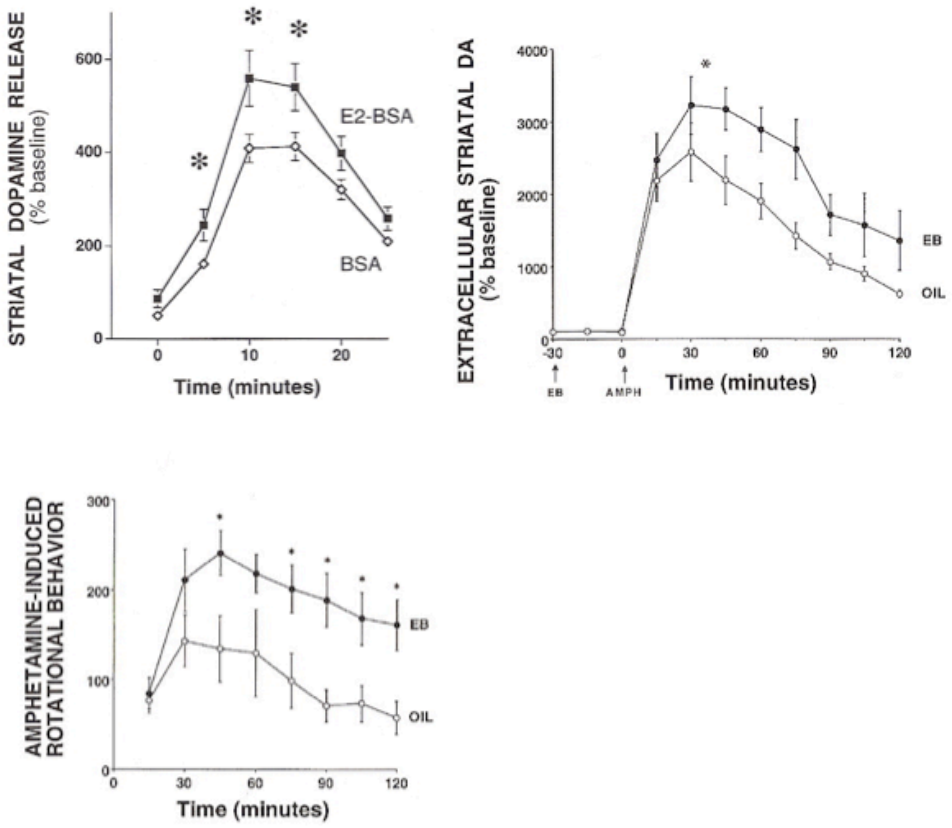

Figure 1.6: Estrogen potentiates activity in the DA system (in vitro, in vivo and behavioral evidence). *Left:* amphetamine (white trace) or amphetamine plus estrogen (black trace) was superfused on to striatal

tissue slices from female overiectomized rats. Estrogen treatment enhanced drug-stimulated dopamine release. *Right:* an in vivo microdialysis study of ovariectomized female rats showed that extracellular striatal DA is increased by amphetamine stimulation as expected (white trace) and pretreatment with estrogen potentiates this effect (black trace). *Bottom:* estrogen increases dopamine-dependent rotational behavior (black trace) above the effects of amphetamine alone (white trace). (Figure adapted from Becker, 1990 and 1998.)

Focusing specifically on the striatum in cycling female rats, Morissette et al (1993) found that the highest level of basal DA (and the peak density of DA uptake sites) occurs during proestrus, the stage of rats' cycle when estrogen levels peak. These results were replicated by Xiao and colleagues (1994), who used quantitative microdialysis to measure extracellular striatal DA levels at multiple time points across the estrous cycle. Finally, tyrosine hydroxylase (a key enzyme in the DA synthetic pathway) and DA turnover rates fluctuate in step with hormonal changes during the estrous cycle in rats (Fernandez-Ruiz et al., 1991).

1.5.2. Estrogen's protracted (genomic) effects

Estrogen's delayed genomic action is well characterized: estrogen binds to intracellular estrogen receptors, which translocate to the nucleus. The hormone-receptor complex binds to estrogen response elements on a gene's promotor region and ultimately regulates gene expression. Genomic effects related specifically to PFC DA are discussed in **Box 1** below. Peripherally administered estrogen increases the number of postsynaptic DA receptors by 20% in the striatum of male rats (Hruska et al., 1980). Estradiol also decreases MAO (McEwen 1984) and downregulates COMT (Jiang et al., 2003)—actions that are likely to increase DA tone.

1.5.3. Estrogen's effects examined via ovariectomization

Data from rodent, primate and human ovariectomy studies offer further insight into estrogen's role in maintaining the integrity of the DA system. These studies have the potential to provide important information about the risks and benefits of hormone replacement for menopausal women. The relevant findings are:

i. Aged female OVX monkeys perform better on working memory tasks after estrogen replacement compared to placebo.
ii. Ovariectomization profoundly decreases the density and distribution pattern of DA (TH-immunoreactive) axons innervating dorsolateral pPFC in monkeys, and estrogen coupled with progesterone replacement restores this deficit (Leranth et al., 2000; Kritzer et al., 1998).
iii. OVX decreases and estrogen replacement restores the rate of DA release and turnover in the striatum.

iv. Direct infusion of estradiol into the nucleus accumbens of OVX rats enhanced K+ stimulated DA release by 138% (over controls) within 2 minutes of administration (Thompson, 1994), an effect that lasted more than 2 hours. Similar results are reported in the striatum (Becker, 1990).

Box 1. Estrogen up-regulates gene expression in PFC neurons

Given reports that estrogen replacement therapy improves working memory performance in menopausal women and primates (Roberts et al., 1997; Duff et al., 2000) and given that estrogen levels are higher in the PFC than any other cortical area (or the hippocampus) (Bixo et al., 1995), Wang et al (2004) sought to study estrogen's affect on gene expression in the PFC.

The nature of the experiment
Female macaques were implanted with vehicle or 17B-estradiol sustained release capsules three weeks after being ovariectomized. Three days later tissue samples from the PFC were obtained.

Results and their significance
The estradiol treated group had 60% greater c-FOS immunoreactivity in the dlPFC, most notably in layer II, V and VI pyramidal neurons. Estrogen receptor alpha (ER- a) showed a similar expression pattern throughout the PFC, with the highest concentrations in layers IV-VI. This co-localization of estradiol-induced cFOS expression with ER-a positive neurons suggests an active role of estrogen in regulating gene expression in the dlPFC.

1.5.4. Estrogen influences neuronal activation patterns: PET, fMRI

Dietrich and colleagues (2001) investigated the effects of estrogen on cerebral blood flow. In the study, men and women performed a mental rotation and word-stem completion task while undergoing fMRI scanning. Female subjects were scanned twice, in the low and high estrogen stage of their cycle. Performance across all three groups was similar, but the imaging data show a dramatic increase in task-related activity in women when estrogen levels were high vs. low ($p < 0.0001$). There were no activational differences between low-estrogen women and men. Estrogen's effect was largely specific to activation size as opposed to lateralization or localization of activation patterns. Whether these effects are due to primary blood flow increases or increased blood flow secondary to increased neuronal activity is open to question (**Fig. 1.7**).

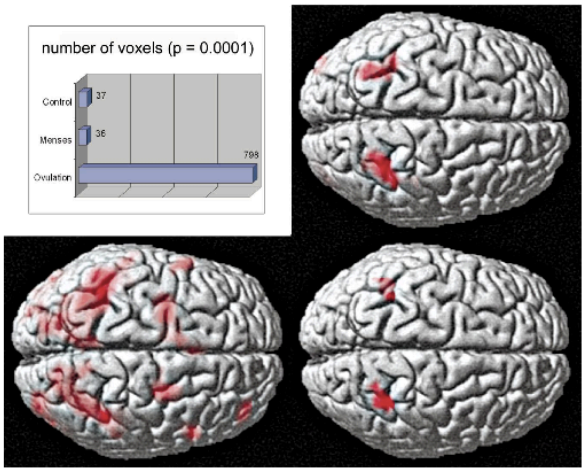

Figure 1.7: Effects of estrogen on cerebral blood flow. Activation patterns during a mental rotation task are shown for men (upper right), female- low estrogen (lower right) and female-high estrogen (lower left). Voxel counts for each condition are also noted. The size of activation shows a dramatic increase during the peak estrogen phase of the cycle. Figure from Dietrich *et al* (2001).

Estrogen's modulation of cortical activation patterns was also observed in a PET study of women performing the Wisonsin Card Sorting Task (WCST), a task that typically evokes strong dlPFC activation. Subjects were scanned once on Lupron, a steroid hormone suppressor, and again on Lupron + estrogen replacement. The Lupron-alone condition resulted in dramatically reduced task related dlPFC activity (7 activated voxels). Estrogen replacement reversed this effect, prompting a return to typical levels of activation (590 voxels).

1.5.5. Effects of estrogen on cognition and behavior

Much of the estrogen-cognition literature focuses on postmenopausal women undergoing estrogen replacement therapy (ERT). Some randomized control trials of perimenopausal women report improved memory and verbal fluency for patients on ERT compared to placebo. When treatment stops, the advantages disappear (e.g. Wolf, 1999; Sherwin, 2003). There is some evidence linking estrogen and WM function: for example, improvements in WM have been observed in postmenopausal women on estrogen compared to nonusers, but the data are inconsistent (Sherwin, 2005; Duff & Hampson, 2000).

Weak evidence suggests that WM span fluctuates throughout the cycle (Rosenberg & Park, 2002), during which estrogen levels naturally rise and fall (there is a four-fold increase in estrogen at the end of the follicular phase compared to the first few days of

menses) (**Fig 1.8**) but here too the data are inconsistent (Gasbarri et al., 2008). No human study to date has systematically examined whether dopamine mediates estrogen-related effects on prefrontal function.

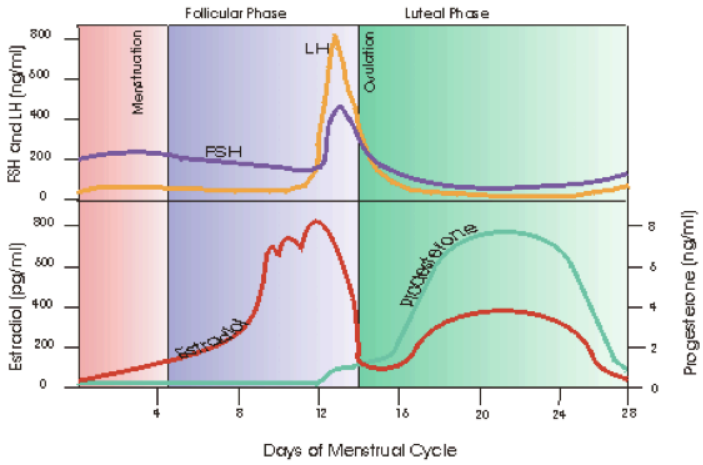

Figure 1.8: Ovarian hormones fluctuate throughout the menstrual cycle. Estradiol levels are low at the start of menses and peak in the late follicular phase. Progesterone rises in the luteal phase. FSH and LH levels also shown. (Figure from McGill University: http://sprojects.mmi.mcgill.ca/menstrualcycle/physiology.html)

1.5.6. Behavioral effects of dopaminergic stimulation depend on rats' estrous stage

A key study in rats (Shansky & Arnsten, 2004) wove together the link between estrogen, DA and WM by discovering that administration of a DA-like agonist (pharmacological stressor FG7142) had very different effects on WM performance depending on the rats' estrous stage. WM performance was maintained after drug administration if rats' estrogen levels were low, but performance dropped dramatically if estrogen levels were high, in keeping with the 'inverted-U' model of DA function (**Fig. 1.9**). It is possible that when DA levels were heightened from the drug, rats in proestrus exhibited a DA "overdose" effect when high levels of circulating estrogen further increased DA levels beyond the optimal range. For rats in estrus, DA levels probably remained within the optimal range for task performance.

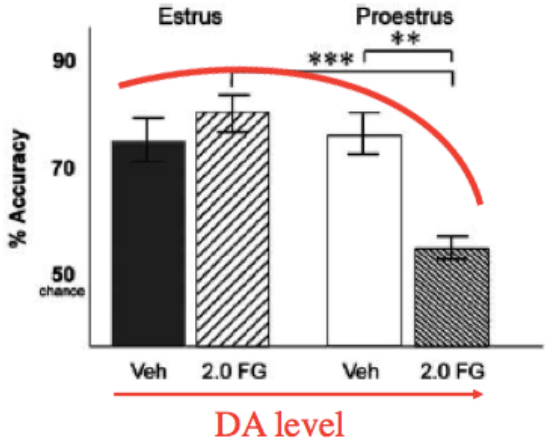

Figure 1.9: Effects of a pharmacological stressor on WM performance depend on female rats' estrous stage. The pharmacological stressor and DA-like 'agonist' FG7142 impairs T-maze accuracy when estrogen levels are high (proestrus), but not when levels are low (estrus). The results follow the shape of a DA response curve, overlaid in red. Modified from Shansky *et al* 2004.

2

ESTROGEN SHAPES DOPAMINE-DEPENDENT WORKING MEMORY PROCESSES IN HUMANS

2.1 *Background*

While evidence for an estrogen-DA link in animals is strong, no human study has systematically examined whether DA mediates estrogen-related effects on PFC function. The PFC is exquisitely sensitive to fluctuations in dopamine—both insufficient and excessive dopaminergic activity impairs PFC function. Given that estrogen levels are higher in the PFC than any other cortical area (or the hippocampus) (Bixo et al., 1995), it is likely that estrogen's effects on WM function are mediated through modulation of PFC DA activity. Thus, we predicted that performance on tasks that depend on precise levels of PFC DA would vary throughout the menstrual cycle. Importantly, estrogen's effects may not be measurable at the behavioral level unless individual variation in baseline DA is accounted for. Here, we examine the effects of estrogen on the performance of a DA-dependent task and on task-related neural activity as a function of COMT genotype and COMT enzyme level (indices of baseline DA).

2.2 Predictions

We predicted that val/val subjects (low baseline DA) would show improved WM performance when estrogen levels were high versus low, while met/met subjects (high DA) would show the opposite pattern, with the best performance when estrogen levels were low (**Fig. 2.1**). These predictions are informed by pharmacological COMT studies showing that *val/val* individuals improve on PFC-dependent tasks after administration of a DA agonist, while *met/met* individuals are impaired by the same drug (Egan et al., 2001).

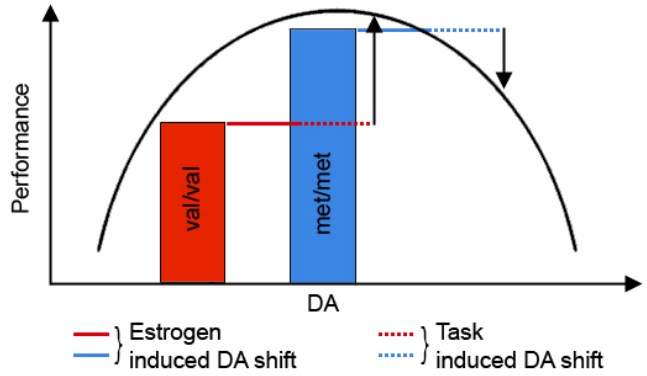

Figure 2.1: Theoretical inverted-U model of cortical DA function. Both insufficient and excessive DA-receptor stimulation leads to poor performance on DA-dependent tasks. The *val/val* allele of the COMT Val[158]Met polymorphism is associated with low basal PFC DA (red bar), relative to *met/met* carriers (blue bar). Estrogen (solid line) and WM load (dotted line) potentiate DA, which is expected to have beneficial effects for val homozygotes and unfavorable effects for met homozygotes. Peak levels of circulating estrogen, symbolically speaking, shifts *val/val* subjects to an elevated position on the DA dose-response curve. This shift toward optimal DA levels is reflected at the neurobiological level (more efficient processing) and behavioral level (better performance). In contrast, high estrogen levels/high task demands shifts prefrontal DA in *met/met* subjects beyond the optimal range (to a hyper-dopaminergic or 'overdosed' state) (c.f. *Fig. 1.3* and *Appendix A*).

Given evidence that increased WM demands leads to increased synaptic DA release in PFC (Phillips et al., 2004; Watanabe et al., 1997; Aalto et al., 2005), a further prediction was that the most pronounced gene/hormone effects would occur during lure trials, or during high load blocks, when cognitive control demands are highest (see **2.3.5**). At a neural level, we predicted that increased synaptic DA would be associated with greater neural efficiency (i.e. at an equal level of performance a "high" DA state would increase the SNR of cortical processing, decreasing the extent of PFC activity), in keeping with previous COMT and patient findings (Egan et al., 2001; Tunbridge et al., 2006). The dopamine-efficiency relationship is further explored in **Appendix F.**

2.3 *Methods*

2.3.1 Subjects

Seventy-nine healthy female participants (age M = 21.7 ± 2.4) were recruited via advertisements on the UC Berkeley campus and prescreened for COMT $Val^{158}Met$ polymorphism. Exclusionary criteria included any history of neurological or psychiatric disorders, an episode of loss of consciousness, use of psychotropic drugs, a history of substance abuse, MRI contraindications, abnormal or infrequent menstrual cycle and use of a hormonal birth control. Blood samples were collected by a licensed phlebotomist and sent for DNA extraction and genotyping. The menstrual cycles of homozygous participants were tracked for 3-4 months (across 3 or 4 menstruation periods) to select subjects with the most highly regular cycles, who were then invited for inclusion in the central study. A final count of 24 women were enrolled: 13 val/val, 8 met/met and 3 val/met (the 3 heterozygotes were enrolled before their genotype was known; their data are included in analyses assessing the effects of estrogen irrespective of genotype). Subjects underwent both behavioral and fMRI testing on two occasions (see below), resulting in the acquisition of 44 total datasets. (Two val/val and two val/met subjects did not return for the second session; three subjects moved from the Bay Area and one chose not to continue due to uneasiness with the blood draw, hence 44 and not 48 datasets). Genotype groups were not significantly different with respect to age, education or background neuropsychological measures (Supplementary Table 1). Of the 24 subjects, 21 had no history of smoking; three reported having previously (> 4 months prior to study-enrollment) smoked, albeit infrequently (<4 cigarettes/week). The study was approved by the UC Berkeley Committee for the Protection of Human Subjects. All volunteers gave written informed consent and were paid for their participation.

2.3.2 Experimental design

Following inclusion in the main study, subjects were seen on three occasions. On the first visit exclusionary criteria were reviewed, consent for the main study was obtained and the participant completed an introductory neuropsychological exam (see Supplementary Results). The second and third visits were time-locked to the subject's menstrual cycle. One test session occurred on or near Day 1/2 of the subject's cycle (during menses, when estrogen levels are low – 'low estrogen') and the other session occurred on Day 11/12[1] (late follicular phase when estrogen levels peak – 'high estrogen'), order counter-balanced. Subjects' menstrual cycles were tracked (including start and duration of menses) for 3-4 months prior to testing through 1 month post-testing to assure cycles were predictable and regular. Blood samples were acquired on both test-days to measure estradiol and COMT enzyme levels.

[1]assuming a 28 day cycle. The period between the start of menses and ovulation (follicular phase) can vary in duration, contributing to a longer of shorter overall cycle duration. The period from ovulation to the start of the next cycle is less variable, with an average duration of 14 days.

2.3.3 Test day measures

During the low and high estrogen test sessions, subjects completed an N-back verbal working memory task during fMRI scanning. To assess mood subjects completed a Positive and Negative Affect Scale (PANAS) (Watson et al., 1988) and Visual Analogue Scales (VAS) for the items *anxious, happy, sad, nauseous, drowsy, jittery, fatigued* and *dizzy*, pre and post fMRI scan. Motor speed was measured with a box completion task and vigilance was measured with a numeral cancellation task (Lewis & Kupke, 1977).

2.3.4 N-back paradigm

Subjects completed three loads, 0- 2- and 3-back, of an N-back verbal working memory task (**Fig 2.2**). Subjects were presented with a series of single non-vowel letters that appeared on the screen every 2 sec one after the other. Using two buttons, subjects responded to every letter to indicate whether it matched or did not match the letter seen *n* previously. A 'target' is defined as a letter that matched the letter seen *n*-previously; for example, in a 3-back condition the second R in the sequence R-T-K-R-D is a target. 'Lure' trials match a recently seen but non-target letter. For example, in a 3-back condition the second D in the sequence D-T-K-R-D is a lure; it matches the letter seen *4* letters earlier but not *3* as the condition specifies. All other non-target trials were categorized as non-lures. In contrast to targets and non-lures, lures create high interference given a "spurious" sense of familiarity and place the greatest demand on cognitive control mechanisms (Gray et al., 2003). Previous data suggest that lure trials are uniquely sensitive to identifying differences in PFC-dependent processes within and between groups (Perstein et al., 2001; Perlstein et al., 2004) and individual differences in fluid intelligence (Gray et al., 2003). Lure interference effects are also more pronounced in older adults, while target performance is unimpaired (Schmiedek et al., 2009). On 0-back blocks subjects indicated whether or not a target letter ('X') appeared. 0-back blocks consist of targets and nontargets; 'lure' trials are not relevant in this condition. The task was set up into 16 blocks of trials (eight 0-back blocks, four 2-back blocks and four 3-back blocks) ordered in a latin squares sequence. Subjects were instructed on the condition before each block of trials. A block consists of 20 trials plus 5 randomly placed null events to introduce temporal jitter (necessary for event-related fMRI analyses.) A trial consists of a stimulus presented for 1 sec followed by a 1 sec delay. A null event is a 2 sec blank period (the same duration as a regular trial). Trial types include targets (20%), lures (15%) and nonlures (65%). Each block of trials (50 sec) is preceded by instructions (6 sec) and fixation (10 sec) and followed by a brief period of rest (20 sec).

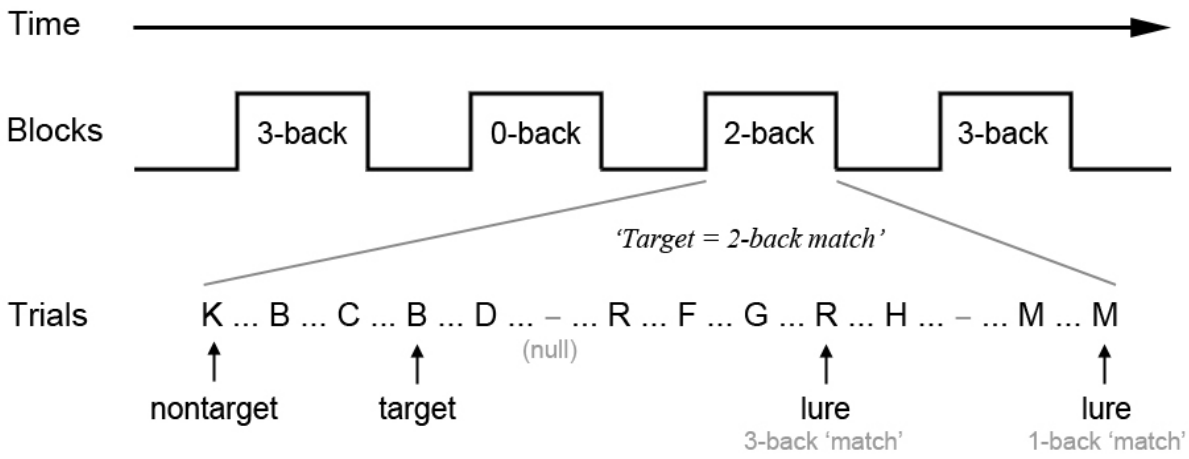

Figure 2.2 Task protocol: hybrid block/event-related design. Subjects perform blocks of WM trials with loads of 0, 2 and 3. Within load blocks, trial types include targets (an n-back match), lures (a recently seen but non-target stimulus—e.g in the 2-back condition, a 1- or 3-back "match" is a lure) and non-targets (all other non-lure, non-target trials). Stimuli (uppercase consonants) are presented for 1 second followed by a 1 sec delay. Subjects respond to every trial, indicating whether the currently presented letter does or does not match the letter seen n-previously. The 0-back condition consists of target-detection (the letter 'X'). Null events provide temporal jitter for event-related analyses.

2.3.5 Genetic analysis

DNA extraction and analysis was conducted according to standard methods on samples obtained from informed consenting subjects. The Ernest Gallo Clinic and Research Center Genomics Core (Emeryville, CA) carried out genotyping of the COMT Val^{158}/Met (rs4680) polymorphism with polymerase chain reaction using TaqMan® technology (Applied Biosystems, Foster City, CA). The COMT genotype frequencies of pre-screened subjects were in Hardy-Weinberg-Equilibrium ($CHI^2 = 2.01$, df = 1, n. s). The genotype distribution was as follows *val/val* = 26 subjects, *val/met* = 33 subjects, *met/met* = 20 subjects).

2.3.6 COMT enzyme analysis

One 4-5 ml blood sample was collected in a vacutainer EDTA tube (BD Diagnostic Systems, Franklin Lakes, NJ) on each test day by a licensed phlebotomist to measure COMT enzyme levels. Genotyping the Val^{158}Met polymorphism provides a static determination of genotype. COMT *function* is determined by the extent to which the gene

gets translated into an active protein—the COMT enzyme. Enzymatic activity is strongly related to COMT genotype but can vary even within the static designations of *val/val, val/met* and *met/met*. Assaying blood COMT enzyme levels provides an additional, more direct measure of COMT activity than assaying genotype alone and reflects brain COMT levels (Chen et al., 2004). COMT enzyme activity was determined in whole peripheral blood collected on each scan day. The COMT enzyme activity assay is based on the organic solvent extraction method that separates the radioactive product, the methylated catechol, and the free radioactive co-enzyme, ^{3}H-S-adenosyl-methionine (SAM) as described elsewhere (Apud et al., 2007). Determination of whole-blood COMT activity confirmed that *met/met* samples (N=14) had greatly reduced COMT activity ((*M* ± *s.e.m*) 23,408 ± 2465 cpm mg protein) compared to *val/val* samples (N=23) (51,474 ± 3688 cpm mg protein; $t(35) = 5.480$, $p < .0001$). Variance in the amount of active COMT protein is evident within genotypic designations (see **Fig. 2.3D** below) and is likely due to epigenetic or epistatic interactions.

2.3.7 Estradiol Assay

One 4-5 ml blood sample was collected in a vacutainer SST tube (BD Diagnostic Systems, Franklin Lakes, NJ) on each test day by a licensed phlebotomist to measure serum estradiol levels. Samples were stored on ice until centrifugation, after which serum was collected and stored at -20°C until assayed. 17β-estradiol concentrations were determined using a commercially available enzyme immunoassay (ELISA; Calbiotech, Spring Valley, CA). The assay sensitivity was 10 pg/ml and the intraassay coefficient of variation (CV) was 2.26%. All samples were run in a single assay and all instructions provided by the company were followed without modification. Menses/early follicular phase testing was first confirmed from cycle records: all 'low' estrogen test sessions occurred within three days of the start of menses (duration of menses across subjects ranged from 3-7 days). Determination of serum estradiol levels confirmed that subjects tested during the early follicular phase had reduced levels of circulating estradiol (137.5 ± 58.9 pg/ml) compared to the late-follicular phase (251 ± 178 pg/ml). Thirty-seven samples were assayed. Samples from 7 test sessions (4 'low estrogen' sessions and 3 'high estrogen' sessions) were not assayed due to subject constraints (difficulty acquiring an adequate blood sample) or the sample being unusable (problems extracting serum from whole blood). A record of those subjects' menstrual cycles confirmed that all 4 "low" sessions for which blood samples were not assayed occurred while the subject was in menses.

2.3.8 fMRI acquisition and analysis

MR data were acquired with a Siemens 3T Trio Magnetom scanner. Functional data were

obtained using a T2* weighted echo-planar imaging (EPI) sequence sensitive to BOLD contrast (TR = 2000 ms, TE = 28 ms, FOV = 230 mm, flip angle 90°, voxel size 2.0 x 1.8 x 3.0). Each functional volume consisted of twenty-nine 3-mm oblique axial slices separated by a 0.45 mm inter-slice gap. A high-resolution T1 (MPRAGE) anatomical scan was also acquired. Following acquisition MRI data were converted to Nifti format; processing included correction for slice-timing and motion artifacts, spatial normalization and spatial smoothing with a 3D Gaussian kernel (8 mm FWHM). For spatial normalization, the individual subject's MP-RAGE was coregistered to the mean functional image and normalized to the Montreal Neurological Institute (MNI) structural template. Normalization parameters were then written to the functional images. Time series were high-pass filtered (240 s cutoff). Statistical parametric maps of BOLD activation were calculated in SPM5 (http://www.fil.ion.ucl.ac.uk/spm) using the general linear model approach (Worsley et al., 1995).

The main analysis focused on BOLD activity in load sensitive regions within prefrontal cortex. Statistical maps representing areas that increase activity linearly across load (3>2>0-back) were generated at the random effects level ($p_{FWE} < 0.00001$) (**Appendix B**). The most significant load-sensitive PFC region (that which exceeded a threshold of T=8) was area 9/46 (see **Fig. 2.4**). Thus this region was the focus of our current analyses, in keeping with previous COMT n-back findings (Egan et al., 2001; Mattay et al., 2003), which localize COMT genotype effects to dorso-lateral PFC. To capture the true linear relationship between load conditions, the conditions were weighted in the contrast as follows: 0-back (-5/3), 2-back (1/3), 3-back (4/3). Bilateral PFC regions of interest (ROIs) were defined as 10 mm spheres around peak loci. The statistical model was then reapplied to the average signal within the ROI using MarsBar (www.mrc-cbu.cam.ac.uk/imaging/marsbar.html). Mean beta weights from the regions of interest (ROI) defined from the load contrast were extracted and compared directly between genotype (val, met) and estrogen (low, high) groups and entered into an ANOVA to examine estrogen x genotype effects. Event-related analyses modeled only correct trials to avoid error-related confounds.

To obtain additional evidence for a relationship between DA and sustained PFC function, we entered COMT enzyme as a covariate of interest at the group-level. Whole brain maps revealed a specific, significant relationship between COMT enzymatic activity and left MFG activation, with no other association across the brain. To probe this finding further in an unbiased manner, we used an independently defined left and right area 9/46 dlPFC ROI from a meta-analysis of N-back activations observed across 24 fMRI studies (Owen et al., 2005), and reapplied the statistical model (parametric load contrast) to the average signal across the ROI (10 mm sphere around reported coordinates) for each subject's session (see Supplementary Material). Data from the left and right ROI were extracted and the mean signal values were submitted to a Pearson's correlation.

2.4 Behavioral Results

2.4.1 Subjective Effects

No differences were observed between genotypic groups (val, met) or estrogen status (low, high) on background demographic or neuropsychological measures (including age, education, Beck's Depression Inventory, Montreal Cognitive Assessment, Barratt Impulsiveness Scale, letter fluency and North American Adult Reading Test) (**Table 2.1**), or measures of mood (Visual Analogue Scales for the items *anxious, happy, sad, nauseous, drowsy, jittery, fatigued* and *dizzy*), affect (Positive and Negative Affect Scale), motor speed and vigilance, administered pre and post MRI on each test day.

Table 2.1 Demographic and neuropsychological measures by genotype group.

	Met/met	**Val/val**	***P***
Age (years)	22.0 (2.2)	22.1 (2.4)	0.9
Education	15.8 (2.2)	15.7 (2.2)	0.9
NAART	36.1 (3.0)	35.4 (6.8)	0.8
BDI	3.5 (4.1)	4.1 (5.4)	0.8
MoCA	28.4 (1.2)	28.6 (1.3)	0.7
CWAT	16.5 (3.9)	16.7 (2.9)	0.9
Stroop	52.4 (15.3)	58.4 (16.8)	0.4
Barratt	55.6 (10.6)	62.1 (13.8)	0.3
L-SPAN			
span	3.4 (0.9)	3.9 (1.4)	0.3
corr mc	83.4 (0.7)	82.7 (1.7)	0.2
R-SPAN			
absolute score	45.5 (14.5)	49.5 (13.0)	0.6
accuracy errors	2.3 (1.5)	5.3 (3.1)	0.05

2.4.2 N-back Behavioral Results

Parametric effects of N-back WM load (**Fig. 2.3A**) and trial-type (**Fig. 2.3B**) were strongly reflected in performance. No group differences were observed for overall task performance (i.e block-level performance). Overall mean accuracy (response time): *val/val*, 75.9% (546.3 ms); *met/met,* 75.6% (560.8 ms); low estro, 76.1% (561.9); high estro, 75.4% (547.4 ms). At the trial level, group differences in performance were not significant when examining estrogen status (low, high) and genotype (val, met) alone.

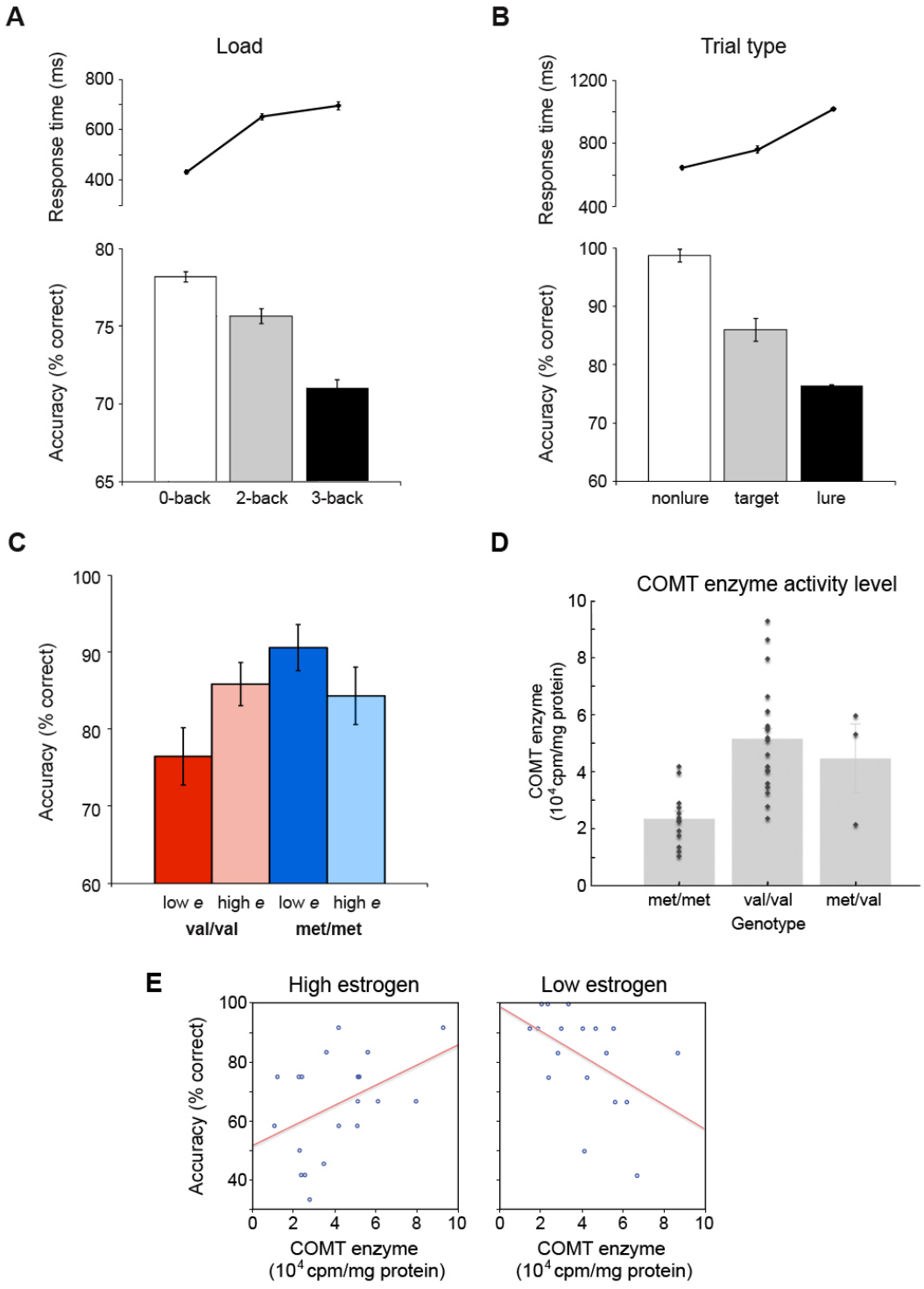

Figure 2.3 (A) Repeated measures one-way ANOVA shows a significant difference in load accuracy ($F(2,86) = 93.98$, $p<0.0005$, effect size: eta-squared = .69) and reaction time (RT) ($F(1,43) = 401.63$, $p<0.0005$, eta-squared = .90). Posthoc Bonferroni corrected comparisons showed that all three means for both accuracy and RT were significantly different: subjects performed faster and more accurately on 0-back

(accuracy, RT) 78%, 436) compared to 2-back (75.6%, 653) compared to 3-back trials (71%, 696). Error bars represent s.e.m. **(B)** Similar analyses revealed significant differences in trial type accuracy ($F(1,43) = 81.14$, $p<0.0005$, effect size: eta-squared = .65) and RT ($F(1,43) = 362.78$, $p<0.0005$, eta-squared = .89): nonlure (99%, 644), target (86%, 759), lure (76%, 1019). **(C)** Two-back lure trial performance as a function of genotype (val, met) and estrogen (high, low). Met/met + low E subjects (91% ± 2.9% correct (mean ± s.e.m.)) performed significantly better than val/val + low E subjects (77% ± 3.7%; $p=0.041$, 2-tailed t-test). **(D)** COMT enzyme activity level by genotype, depicting variance within met/met and val/val genotypes (overlaid on mean values; pale grey bars). (Blood samples from three heterozygous val/met samples were analyzed and are included here for display.) **(E)** For low estrogen subjects (right panel), COMT enzyme correlates negatively with 2-back lure accuracy ($r= -.482$; $p=.043$). For high estrogen subjects (left panel), COMT enzyme correlates positively with 3-back lure accuracy (approached significance $r=.433$; $p=.056$).

Performance differences emerged when examining the impact of estrogen and genotype together, as predicted. On the high interference/high control lure trials during the 2-back condition, *val/val* women performed poorly at the start of their cycle when estrogen is low and improved when estrogen levels were high. *Met/met* women showed the opposite pattern, with strong performance under low estrogen conditions and impaired performance under high estrogen conditions (**Fig. 2.3C**). Comparing the 'low' DA (val, low estrogen) and 'optimal' DA (met, low estrogen) groups shows a significant difference in 2-back lure accuracy. In the 3-back condition lure performance was poor across all subjects (mean accuracy: 2-back lure, 84%; 3-back lure, 67%) and the effects were not significant as a function of genotype. Of note, the 3-back lure data (**Appendix C**) appear to fit the theoretical model (see **Fig. 2.1**) if the predicted task-induced shift in DA[29, 30, 31] is weighted more strongly (i.e. for the condition that places the greatest demand on cognitive control, 3-back lures, val and met groups are shifted further along the DA axis).

We also examined lure accuracy within low and high estrogen groups as a function of COMT enzyme level (which provides a measure of the biologically active protein above and beyond 'static' genotype) (**Fig. 2.3D**). For high estrogen subjects COMT enzyme activity was positively correlated with lure accuracy; for low estrogen subjects enzyme levels were negatively correlated with lure accuracy (**Fig. 2.3E**), providing a depiction of the inverted-U model at the individual subject level. That is, just prior to ovulation, when estrogen levels are elevated, the best performers were women with high COMT activity (indicative of low baseline prefrontal DA). Conversely, towards the beginning of the cycle, when estrogen levels are reduced, the best performers were women with low COMT activity (high baseline prefrontal DA), in keeping with DA's inverted U-shaped action.

2.5 Functional MRI results

2.5.1 Group effects – estrogen, genotype

To investigate the neural effects underlying hormone—genotype interactions, we examined BOLD activity in load sensitive PFC regions, which reflects areas involved in WM maintenance processes. Statistical maps representing regions that increase activity linearly across load (3>2>0-back) were generated. Mean beta weights from the regions of interest (ROIs) defined from this contrast (bilateral middle frontal gyrus (MFG); area 9/46) (**Fig. 2.4A**) were extracted and group differences compared. Despite the observation that behavioral performance across loads did not differ between groups, by examining PFC activity sustained across task blocks we found that bilateral MFG ROI activity (mean signal across 10-voxel ROI) markedly declined as inferred PFC DA increased. Higher DA (high estrogen, met genotype or both) was associated with lower sustained PFC activation, while lower DA (low estrogen, val genotype or both) was associated with greater sustained PFC activation (**Fig. 2.4B-C**), in keeping with a neural efficiency hypothesis (Egan et al., 2001; Tunbridge et al., 2006).

2.5.2 Group effects – COMT enzyme

To obtain additional evidence for a relationship between prefrontal DA and PFC function, mean signal was extracted from independently defined left and right MFG ROI's (as reported in a meta-analysis of N-back activation) (Owen et al., 2005) and directly compared with COMT enzymatic activity. The average signal magnitude in left MFG correlated with COMT activity ($r = .513$, $P < 0.001$; **Fig. 2.4D**) (right MFG did not reach significance), suggesting that as the amount of DA available in PFC declines (that is, as COMT activity increases), activity in the frontal regions supporting WM processes becomes more exaggerated.

2.5.3 Neuroimaging data: Event-related lure activity

We examined event-related activity during high interference/high control lure trials and lower interference target trials within the WM load-sensitive PFC ROI's. Target-related activity showed the same pattern of decreased bilateral MFG activity with increased PFC DA as observed at the sustained-activity block level (**Fig. 2.5A**). Lure trials showed a different pattern (**Fig. 2.5B**), where the magnitude of MFG activation mirrored the inverted U-like performance curve (i.e. there was a selective increase in activity for

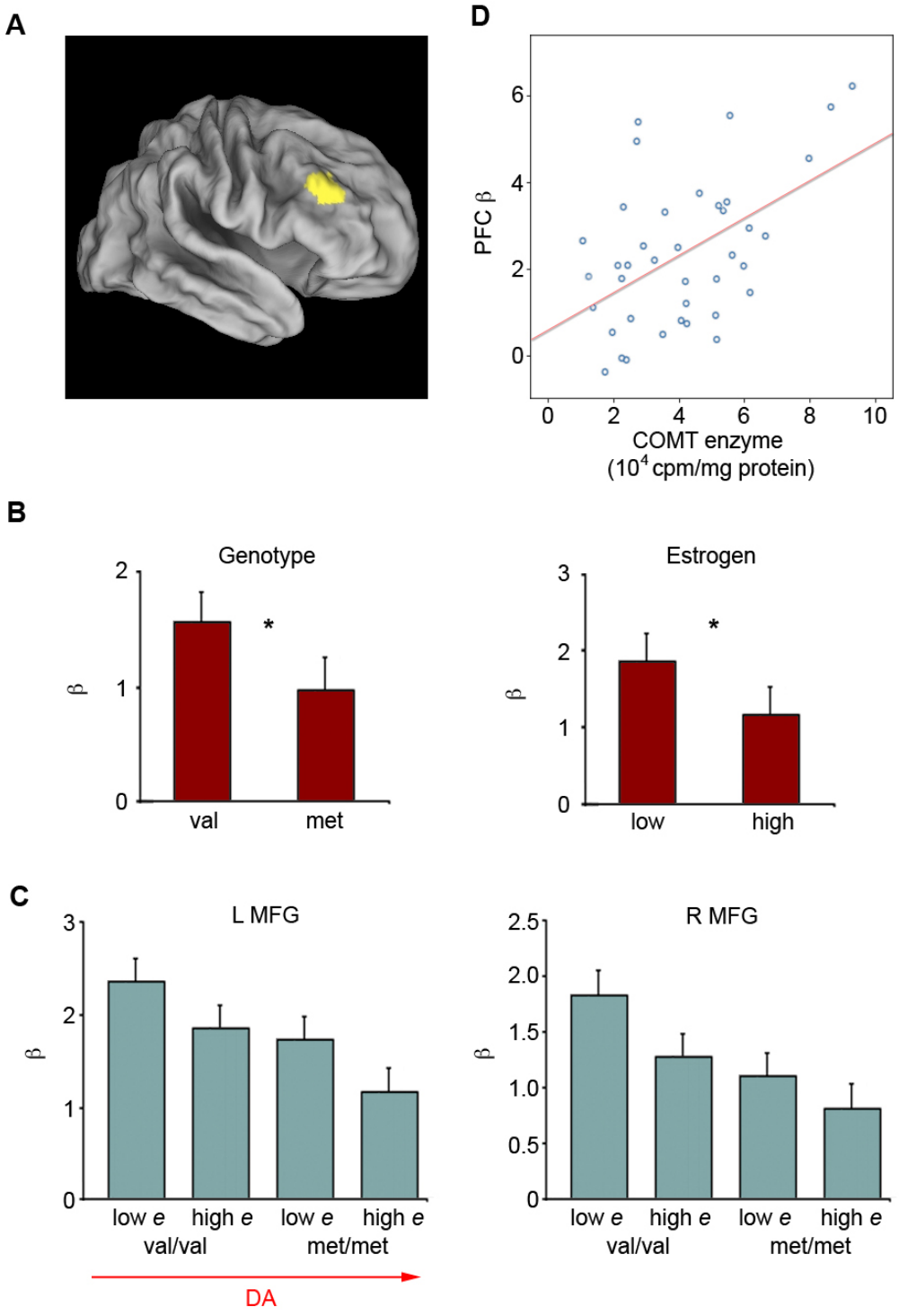

Figure 2.4 Across blocks, sustained PFC activity decreases as PFC DA increases across groups. (A) Load-sensitive suprathreshold PFC cluster (right hemisphere shown at $P_{FWE} < 0.0001$; T=7.5). Left and right MFG were identified as ROI's (10 contiguous voxels; MNI coordinates of peak locus, left: -45, 32, 30; right: 44, 32, 32; defined at $P_{FWE} < 0.00001$). **(B)** Parameter estimates, or mean beta values, in right

MFG between genotypic groups (val, met) (p = 0.038) and estrogen status (low, high) (p = 0.011). Similar results were observed in left MFG. **(C)** Parameter estimates in bilateral MFG shown together as a function of genotype and estrogen status (lowest vs. highest DA groups: *val* + low estrogen vs. *met* + high estrogen; left MFG p = 0.021; right MFG p = 0.019) . **(D)** Mean signal in left MFG versus COMT enzymatic activity. As COMT activity increased (indicating a probable concomitant decrease in PFC DA), PFC activity became more exaggerated.

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higher, more 'optimal' dopaminergic groups: val+low *e* compared with met + low *e*; p = 0.028). The greater a subject's MFG response to lure trials, the better their lure performance (2back lures: r = 0.532, $P < 0.005$; **Fig 2.5C**; total lures: r = 0.392, $P = 0.009$). To further probe the connection between PFC DA, MFG activity and lure performance at the single subject level, we compared COMT enzyme with lure activity within the PFC ROI's. In right MFG, activity during lure trials was negatively correlated with COMT enzyme (r = 0.425, $P = 0.006$; **Fig. 2.5D**) (the association did not meet significance within the left MFG ROI). In right MFG the relationship was significant for both 2back (r = 0.334, $P = 0.035$) and 3back (r = 0.4, $P = 0.01$) lure trials.

These data suggest that cortical DA is associated with a broadly 'efficient' pattern of sustained activity (that which occurs across WM blocks), and a selective, event-related enhancement of activity during episodes of high interference (e.g. lures), when the demand for cognitive control is greatest. Furthermore, the extent to which an individual enhances PFC activation during the demanding lure trials is predictive of their performance.

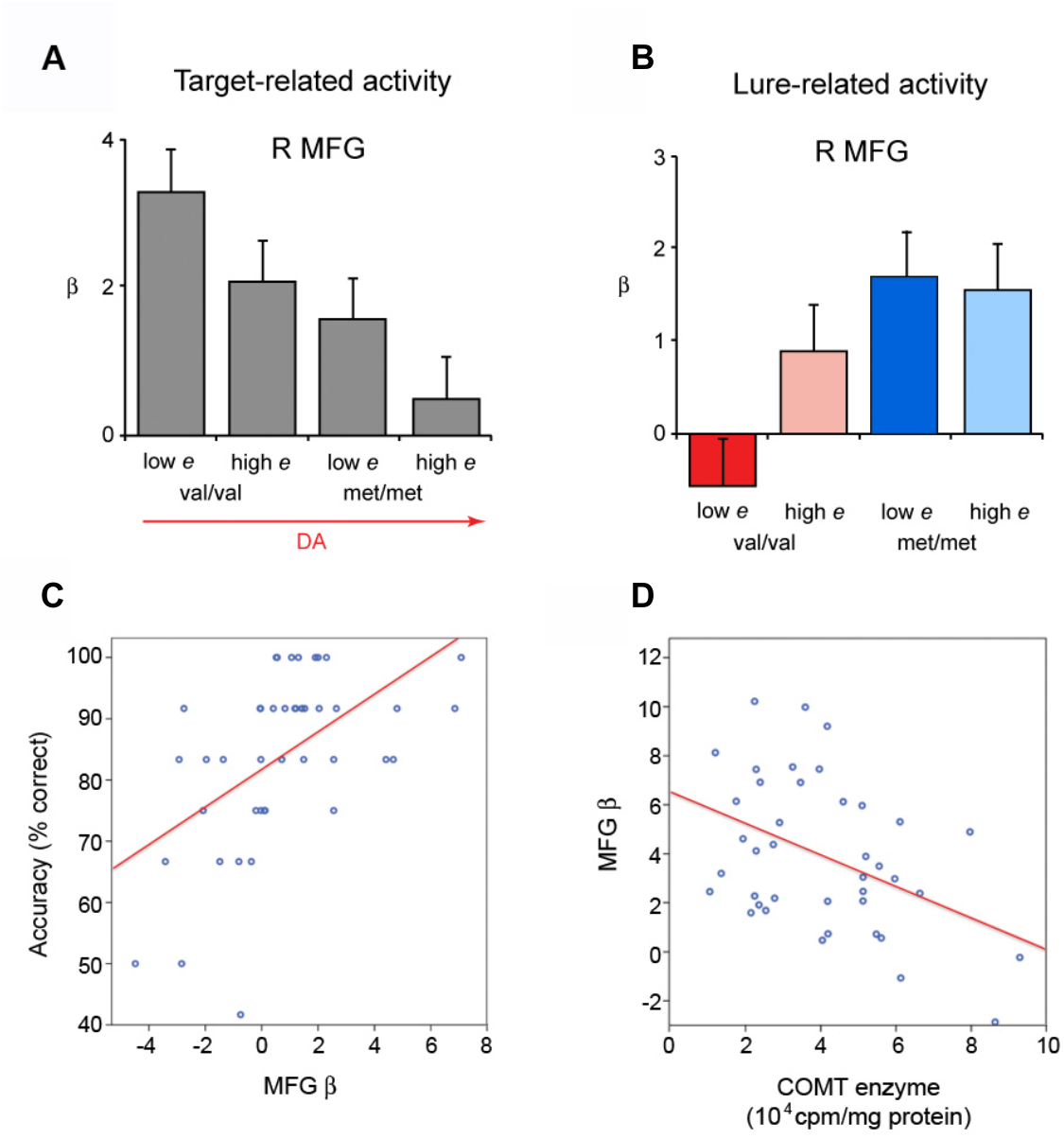

Figure 2.5 (A) Target- and **(B)** lure- related neural activity within low (val, low estrogen) and high (met, high estrogen) dopaminergic groups (*A-B* represent event-related activity on correct trials within two and three-back WM blocks; note that 0-back blocks do not, by necessity, contain lure trials) **(C)** Two-back lure performance versus the magnitude of correct lure-trial activity in right MFG (mean signal within the ROI). **(D)** COMT enzyme versus the magnitude of correct lure-trial activity in right MFG (mean signal).

2.7 Interim summary and discussion

In humans there is a strong effort to understand the effects of estrogen on cognition, but the data have been inconsistent. This study suggests that taking baseline DA into account is pivotal to detecting the direction of estrogen's effect on WM. Specifically, the results establish that estrogen can be beneficial *or* detrimental to WM and, crucially, the direction of the effect depends on COMT genotype and, at a finer scale, COMT enzymatic activity (proxies of baseline PFC DA). At the behavioral level, the effects of estrogen and COMT genotype emerged on trials that require a high demand of cognitive control. Neurally, hormonal and genotypic differences were apparent even in the absence of significant behavioral differences. 'Suboptimal' DA subjects showed exaggerated task-related PFC activity across blocks but weak PFC activity on the high interference lure trials. 'Optimal' dopamine subjects showed the opposite pattern, with efficient sustained PFC activity accompanied by selectively robust PFC activity when task demands were high. Conceptually similar results were observed in a study of the neural mechanisms of general fluid intelligence (gF) (Gray et al., 2003), in which low gF subjects showed exaggerated sustained PFC activity during a WM task but paradoxically weak activity during high-control trials. High gF individuals showed the opposite pattern, with an efficient, reduced BOLD response at a block level, and a selective event-related enhancement of PFC BOLD response to lure trials (**Fig. 2.6**).

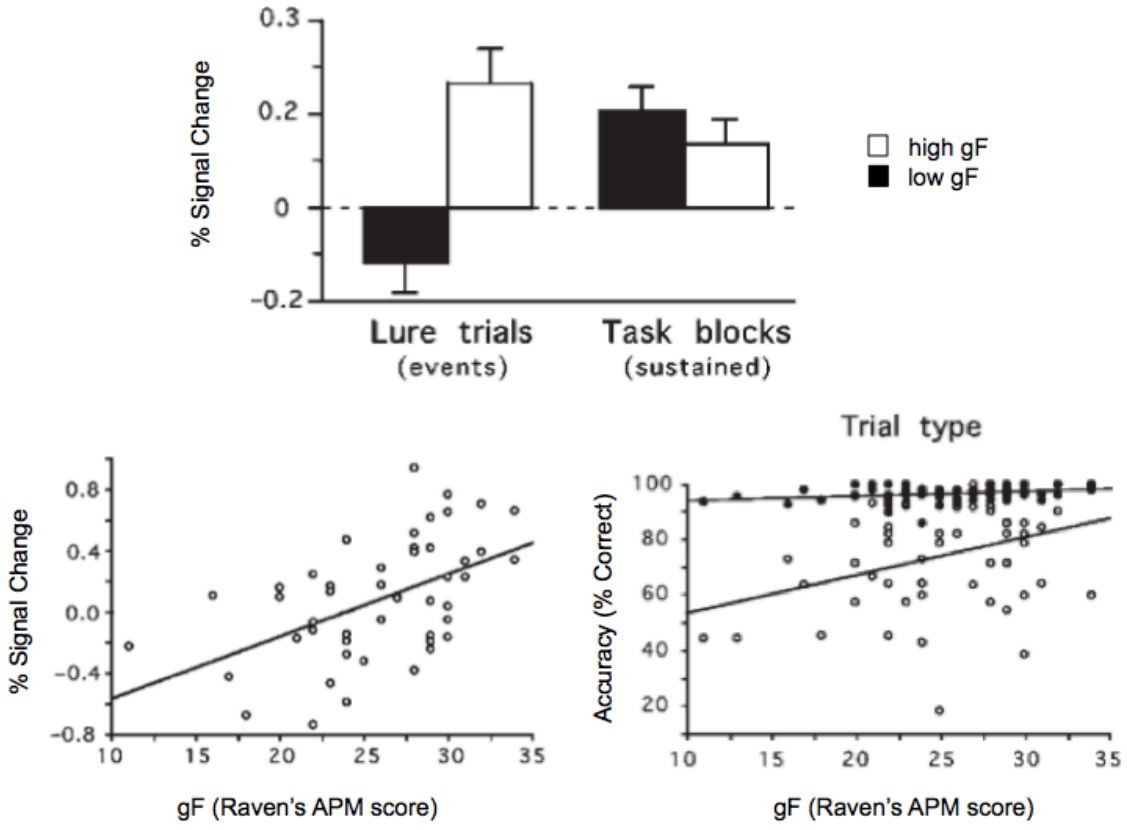

Figure 2.6 gF predicts the magnitude of PFC activity during a cognitive control task. Top: low gF subjects show greater sustained PFC activity across WM blocks but a deficit of lure-related activity when cognitive control demands are greatest. High gF subjects show the opposite effect. Bottom-left: lure-related PFC activity predicts gF. Bottom-right: lure trials (white circles, high cognitive control demands) but not target trials (black circles, low control demands) are related to gF. (Figure from Gray et al, 2003).

The data are in keeping with work from Egan *et al* 2001, who demonstrated that Met allele load predicts the efficiency of PFC physiology (for more on the relationship between COMT and cortical efficiency see **Appendix F**). While all genotypic groups in their sample (*val/val, val/met, met/met*) showed similar levels of performance on a 2-back WM task, task-related PFC BOLD response was greatest for *val/val* subjects, reduced for *val/met*, and lowest for *met/met* subjects. In a related pharmacogenomic study, Mattay *et al* (2003) found that amphetamine administration improved WM performance and enhanced cortical efficiency for *val/val* subjects. *Met/met* subjects, however, became cortically inefficient on drug, presumably because near-optimal basal DA levels were heightened beyond the optimal range. Similarly, Mehta *et al* (2000) showed that the indirect catecholamine agonist methylphenidate (Ritalin) improves WM performance and reduces task-related regional cerebral blood flow in the PFC. These beneficial drug effects were most pronounced for subjects with lower baseline WM capacities (a behavioral index of dopamine synthesis capacity) (Cools et al., 2008). Similar results were found using the D2 receptor agonist bromocriptine (Gibbs & D'Esposito, 2005). Together, these findings contribute to a body of evidence demonstrating that optimal dopaminergic stimulation is associated with greater cortical efficiency (i.e. at a given level of performance, dopamine optimizes the signal-to-noise ratio of cortical processing, decreasing the extent of task-related PFC activity as measured by BOLD) (Winterer & Weinberger, 2004; Durstewitz & Seamans, 2002) (**Appendix F**).

While the relationship between dopamine, working memory and cortical efficiency has been demonstrated in humans using pharmacological manipulation of dopamine, we observe a strikingly similar relationship when considering the natural hormonal fluctuations that occur over the coarse of a woman's menstrual cycle each month. Further, we found that estrogen's 'DA agonist-like' effect on behavioral and neural processes depends on baseline dopamine (COMT $Val^{158}Met$ genotype and, at an individual level, COMT enzymatic activity). The results carry direct ramifications for women's health, as the response to DA medications (e.g. Ritalin for attention deficit disorder and l-DOPA for Parkinson's disease) may differ between men and women, and within women in different endocrine states.

3

ESTROGEN ALTERS TOP-DOWN MODULATION OF SENSORY PROCESSING

3.1 *Background*

The PFC is well-known to be involved in stabilizing the representation of items in working memory (e.g. via persistent activity of PFC neurons during the delay period of a WM task). The PFC is also important for guiding selective-attention processes, biasing attention toward goal-relevant items and away from irrelevant, potentially distracting items in the environment. Previous work demonstrates that visual association cortices (VAC) are modulated during both selective attention (stimulus related) and working memory (stimulus absent / delay period) paradigms. Neural activity is enhanced in VAC regions encoding the goal-relevant representation and suppressed in regions encoding the irrelevant/ignored stimuli (Gazzaley et al, 2005; Kanwisher & Wojciulik, 2000; Kastner & Ungerleider, 2001). The PFC is thought to be a significant source of this 'top-down' goal-directed modulation of activity in sensory cortex (Fuster et al, 1985; Gazzaley et al, 2007). Reciprocal cortico-cortical connections between PFC and VAC (Ungerlieder et al, 1989) provide neuroanatomical support for the PFC's role as 'top' in the top-down signal.

Selective-attention, like working memory, is a ubiquitous aspect of human behavior. Imagine a scenario in which paying attention to faces is required to achieve an immediate goal; for example, you're scanning the faces of people in a park in hopes of locating a friend. While you search the crowd, inspecting each face, activity in VAC regions that respond preferentially to faces is heighted, while activity in regions that code, for example, houses or landscapes is suppressed (compared to a "passive-viewing" baseline when no goal biases your attention toward or away from a particular class of objects). This suppression effect is thought to be an important neural signature of our ability to keep out distracting, irrelevant representations.

Gazzaley and colleagues (2005) showed that both the magnitude of neural activity in VAC and the speed of neural processing are modulated by goal-directed selection in a healthy sample of young adults (age 19-30) (**Fig. 3.1**). In older adults (age 60-77), they found a suppression deficit—neurally, older adults did not show the expected suppression effect in VAC in response to items they were asked to ignore and behaviorally, this manifested as better incidental long-term memory of the irrelevant items. The authors characterize the suppression deficit as a likely source of the working memory impairments that accompany normal aging.

Here, using a selective attention task designed to probe top-down, goal-directed modulation of neural activity in visual association cortices, we examined the impact of estrogen and COMT on cortical suppression and enhancement.

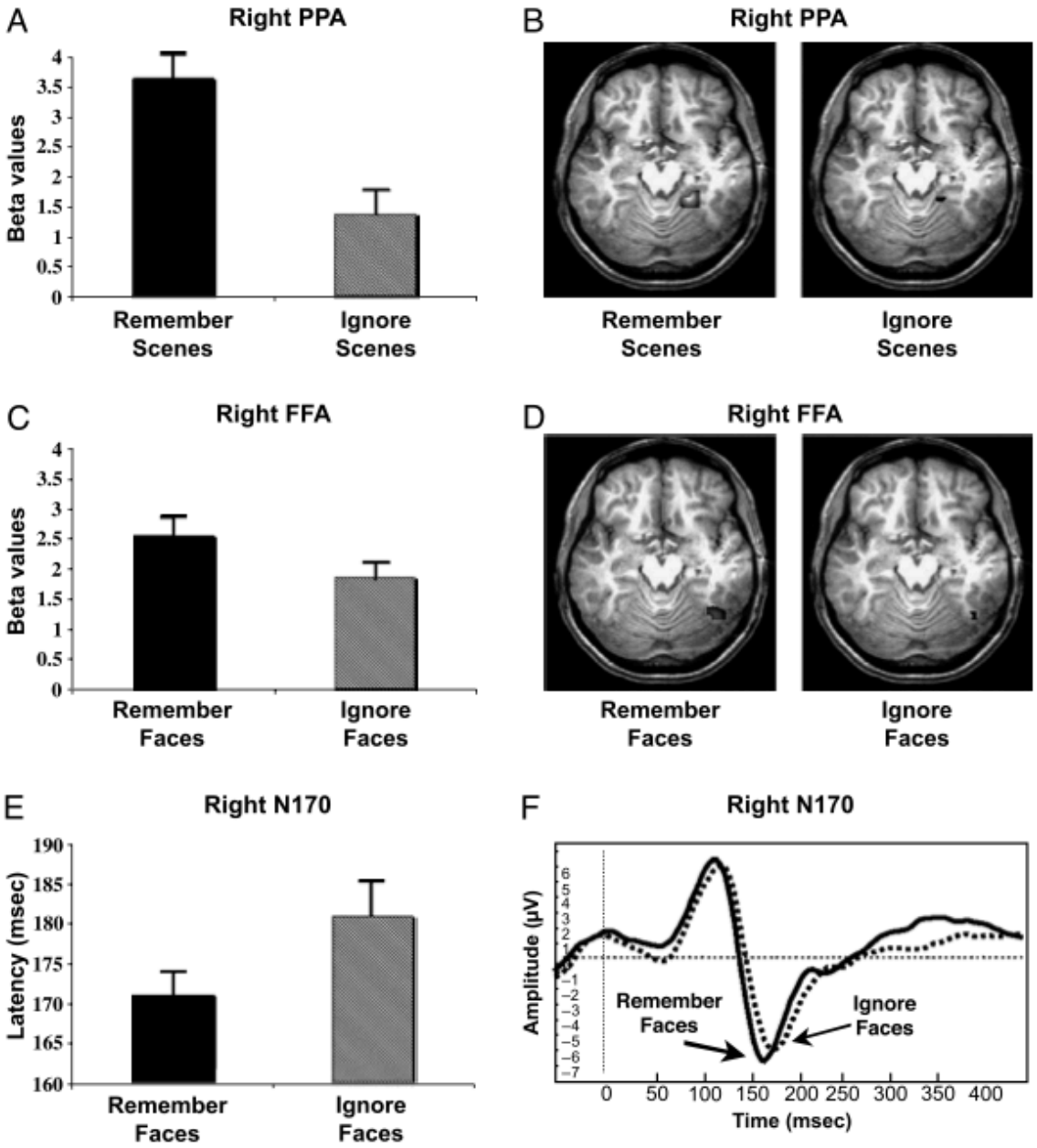

Figure 3.1 Enhancement and suppression effects in VAC. (A,C) fMRI BOLD beta values for the "Remember" and "Ignore" conditions show enhanced magnitude of activity in stimulus-relevant VAC regions (e.g. when subjects were asked to attend to scene stimuli, activity in the scene-selective PPA region

was enhanced; when subjects were instructed to ignore scenes, activity in this region was suppressed. **(B, D)** Example of BOLD signal in PPA and FFA between the Remember/Ignore conditions (single subject) **(E)** ERP data showing group-averaged peak latency of right N170 response is earlier for Remember Faces vs. Ignore Faces. **(F)** Time-locked ERP's to face stimuli, showing earlier latency during "Remember Faces" condition. (Figure modified from Gazzaley *et al*, 2005).

3.2 *Methods*

3.2.1 Experimental design

Procedures for subject recruitment, experimental design and test day mood and blood measures are identical to the procedures described in **2.3.1 - 2.3.7** (excluding 2.3.4). Twenty-four healthy young women performed a visual selective-attention task while undergoing fMRI scanning. Subjects were tested on two occasions, time-locked to their menstrual cycle (**Fig. 3.2**).

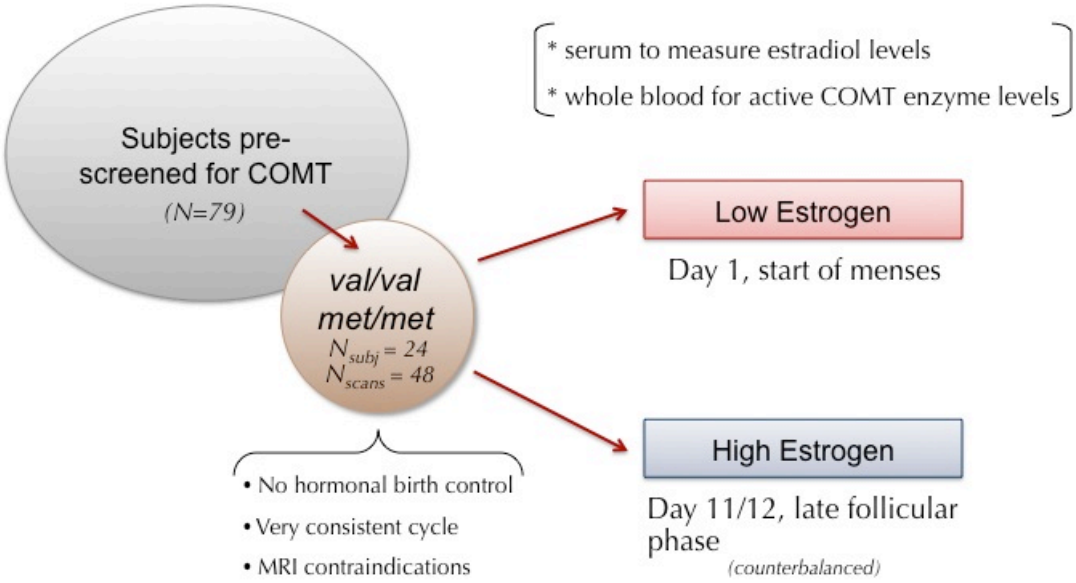

Figure 3.2 Experimental design. Individuals who met the exclusionary criteria and were homozygous for the COMT Val^{158}Met polymorphism underwent behavioral and fMRI testing on two occasions, time-locked to the low and high estrogen stage of their menstrual cycle. On each test day blood samples were acquired to assay estradiol and COMT enzyme activity.

3.2.2 fMRI task paradigm

In the scanner, participants performed a selective attention - working memory task comprised of four conditions: Passive View (Categorize), Attend Faces, Attend Scenes, and Attend Both (**Figure 3.3**). In each condition, participants observed a sequence of serially presented visual images from two object categories, faces and scenes. Stimuli were grayscale images of human faces and natural scenes, centrally presented. The "bottom-up" visual information was the same in all conditions, only the instructions differed. Instructions presented at the beginning of each run informed subjects which stimuli were relevant and which should be ignored. During the Attend Scenes condition, participants were instructed pay selective attention to scene stimuli (and to suppress/ignore face stimuli) and to respond to any scene stimulus that was identical to the previous scene stimulus (i.e. a 1-back match) by pressing Button 1. The participants were instructed to respond to all irrelevant face stimuli and non-matching scene stimuli by pressing Button 2. The instructions for the Attend Faces condition were identical to the Attend Scenes condition, with the exception that the relevant object category switched from scene to face stimuli. During the Passive View/Categorize condition participants were instructed to categorize each image (as 'face' or 'scene') with one of 2 button presses, with no attempt to remember them. During the Attend Both condition, participants were instructed to pay attention to both face and scene stimuli and to respond to any stimulus that was identical to the previous stimulus within the same object category (a 1-back match) by pressing Button 1. Participants were instructed to respond to all non-matching stimuli by pressing the Button 2. Data were acquired during sixteen scanner runs (4 runs of each of the 4 task conditions), with each run lasting approximately 2 minutes. Each run contained 20 trials, yielding a total of 80 trials per condition. Within a run, each trial was presented for 600 ms with a jittered inter-stimulus interval of 3, 5, or 7 seconds. To ensure constant motivation and effort across the task, feedback and point rewards were provided at the end of each task block.

Subjects performed a brief functional localizer prior to the main experiment to independently localize face- and scene-selective regions within the VAC. Subjects viewed alternating 16 sec blocks of face stimuli, scene stimuli and rest periods (7 blocks of each type). Subjects were instructed to attend to the stimuli and make a button press to any image that was a repeat of the image before it (a '1-back' match).

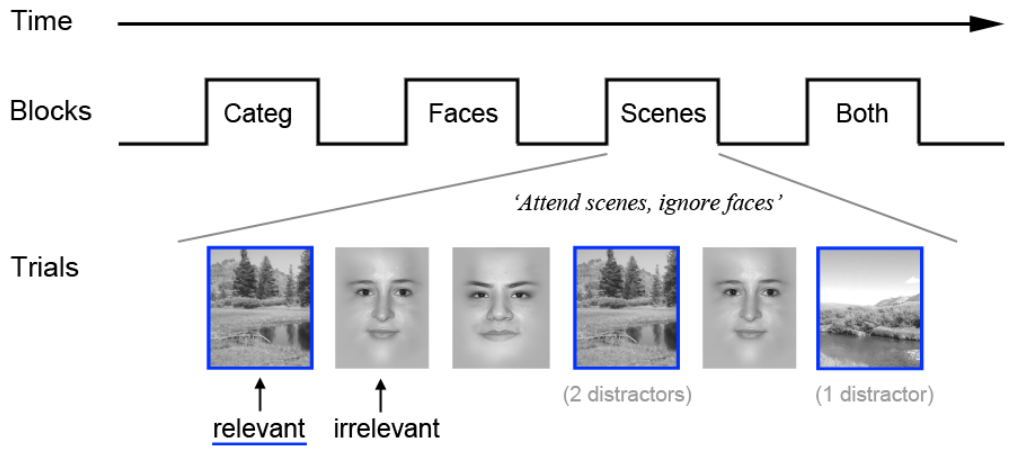

Figure 3.3 Selective attention task paradigm (hybrid block/event-related design). In all conditions, face and scene stimuli were serially presented, keeping 'bottom-up' visual information constant. Only the instructions differed: *attend to scenes* (and ignore faces; example shown); *attend to faces* (and ignore scenes); *attend to both* stimulus classes; *categorize* the images (a 'passive view'-like control condition). Stimuli were presented for 600 ms followed by a variable inter-stimulus interval of 3, 5, or 7 seconds.

A surprise post-scan recognition memory test was administered immediately after the fMRI scan. Subjects viewed novel and old items, with old items from each of the four task conditions (*Remember Faces, Remember Scenes, Remember Both and Categorize*). Forty images from each old category along with 40 novel stimuli were sequentially presented on a computer screen; subjects indicated on a 4-point scale whether the image was 1 = *Definitely New*, 2 = *Probably New,* 3 = *Probably Old,* 4 = *Definitely Old.*

3.2.3 fMRI acquisition and analysis

MRI data were acquired with a Siemens 3 Tesla Trio Magnetom scanner equipped with a transverse electromagnetic send-and-receive radio frequency head coil. Functional data were obtained using a 2-shot T2* weighted echo-planar imaging (EPI) sequence sensitive to BOLD contrast (TR = 1000 ms, TE = 27 ms, FOV = 225 mm, voxel size 3.5 x 3.5 x 5.0). Each functional volume consisted of eighteen 5-mm oblique axial slices separated by a 0.5 mm inter-slice gap. A high-resolution T1 (MPRAGE) anatomical scan was also acquired. Following acquisition MRI data were converted to Nifti format; pre-processing was performed using SPM5 software (http://www.fil.ion.ucl.ac.uk) and included correction for slice-timing and motion artifacts, correction for linear drift within runs, spatial normalization and spatial smoothing with a 3D Gaussian kernel (8 mm FWHM). Statistical parametric maps of BOLD activation were calculated in SPM5 using the general linear model approach (Worseley et al., 1995).

3.2.4 fMRI univariate and multivariate analyses

To assess activity within category-selective visual association cortex (VAC), ROI's were defined from an independent functional localizer obtained at the beginning of the scanning session. Bilateral face-selective FFA regions were defined from a Faces > Scenes contrast and bilateral scene-selective PPA regions were defined from a Scenes > Faces contrast (**Fig 3.2**).

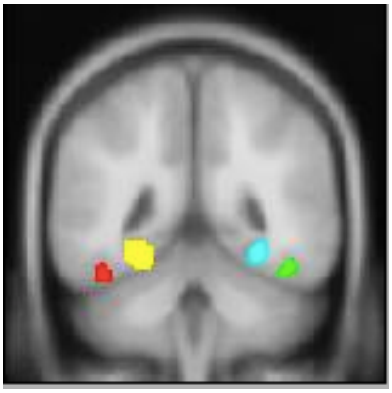

Figure 3.4 Face and scene-selective VAC regions. Left FFA (red – MNI coordinates of peak locus: -44, -48, -21), right FFA (green – 44, -48, -19), left PPA (yellow – -27, -49, -12), right PPA (blue – 29, -45, -13).

Univariate ROI analyses focused on the modulation of VAC activity across attend-ignore conditions. We predicted that activity in scene-selective ROI's would be enhanced during the *Remember Scenes* condition and suppressed during the *Ignore Scenes* condition relative to a passive-viewing baseline. We predicted that FFA would be similarly modulated (enhanced during *Remember Faces* and suppressed during *Ignore Faces*) although previous reports suggest that FFA modulation is less robust / more noisy than PPA modulation (Gazzaley et al, 2005). The encode period was modeled with a regressor spanning the duration of the encode period (600 sec) and the delay period was modeled with a 1 second regressor toggled around the mid-point of the delay (delay period data is not reported, our hypotheses are centered on the modulation of VAC activity when visual stimuli are being encoded). Comparisons were made between 'relevant' items (e.g. scenes presented in a *Remember Scenes* block) and 'irrelevant' items (e.g. scenes presented in an *Remember Faces* block).

After the VAC ROI's were identified, the statistical model was applied to the average signal within each ROI using MarsBar (www.mrc-cbu.cam.ac.uk/imaging/marsbar.html). Mean beta weights from the ROIs were extracted and entered into an ANOVA to examine condition x estrogen x genotype effects.

Functional connectivity analyses were performed at the block level to assess coherence between prefrontal and VAC regions (Sun et al, 2004). Coherence scores were derived between each ROI (including left, right PFC and left, right PPA). PFC ROI's were defined at the encoding stage, with a contrast of Relevant > Irrelevant items (relevant items include scene stimuli in the *Remember Scenes* condition blocks and face stimuli the *Remember Faces* condition; irrelevant items include scene stimuli in the *Remember Faces* condition and face stimuli in the *Remember Scenes* condition.) Coherence was computed between ROI's as a function of task condition. Coherence values were transformed via an arc-hyperbolic tangent function (so that the difference of coherence magnitudes approaches a zero-centered normal distribution (Rosenberg et al, 1989)) and submitted to an ANOVA to test for condition, group and condition x group interactions.

3.3 *Functional MRI Univariate Results*

3.3.1 Modulation of VAC activity – estrogen effects

The magnitude of activity in left and right PPA was significantly modulated by task condition (**Fig 3.5A**) (across all subjects and sessions, irrespective of genotype or estrogen status; N=40). PPA activity was enhanced during *Remember Scenes* and suppressed during *Ignore Scenes* compared to a passive viewing baseline ('Categorize'). Enhancement was also observed in the FFA, as the activity was significantly higher for *Remember Faces* than *Passive View* (left FFA $p < .005$; right FFA $p < .046$), but suppression effects were not observed. Thus, the focus of further analyses was restricted to bilateral PPA (the lack of a suppression effect in FFA has been noted elsewhere (Gazzaley et al, 2005; Gazzaley et al, 2007)).

Next, we evaluated whether the suppression and enhancement effect is modulated by estrogen status. Under high estrogen conditions (just prior to ovulation) subjects demonstrated a robust enhancement of activity in VAC regions in response to task-relevant representations, and a robust suppression of VAC activity in response to task-irrelevant stimuli (compared to a passive-viewing baseline). However, under 'low-estrogen' conditions subjects showed a marked deficit in their ability to suppress irrelevant representations. Their enhancement of task-relevant representations was preserved (**Fig 3.5B-C**). (The finding remained robust when *val/val* and *met/met* subjects were tested independently - **Appendix D**.) This failure to suppress neural processing of distracting information when estrogen levels are naturally diminished parallels suppression deficits observed in older adults (**Fig 3.6**).

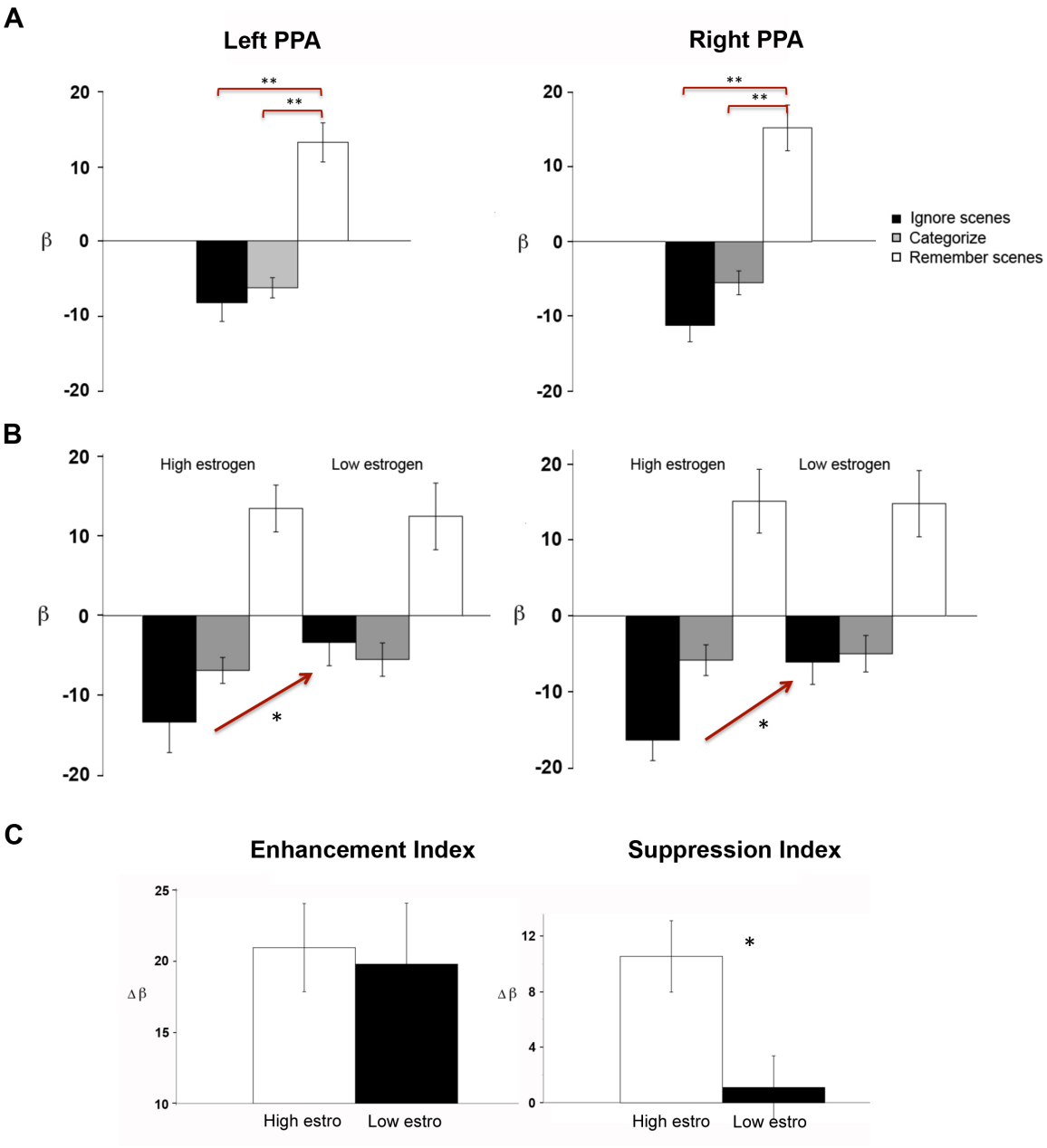

Figure 3.5 Enhancement and suppression effects in low vs. high estrogen conditions. **(A)** Across all subjects, enhancement and suppression of VAC activity (for *Remember Scenes* and *Ignore Scenes*, respectively) is evident within left and right PPA. (Remember vs Categorize: left PPA, $p < 0.005$; right PPA, $p < 0.005$; Remember vs. Ignore: left PPA, $p < 0.005$; right PPA, $p < 0.005$) **(B)** A comparison of high and low estrogen subjects reveals a suppression deficit (black bars) for low estrogen subjects (left PPA, $p = 0.043$; right PPA, $p = 0.015$). Enhancement of task relevant representations (white bars) was preserved. **(C)** An enhancement (*Remember scenes – Categorize*) and suppression (*Categorize – Ignore Scenes*) index (computed as a difference score of mean beta values between conditions) reveals this deficit more clearly: while both low and high estrogen subjects show a robust ability to enhance PPA activity in response to task-relevant scene stimuli, only high estrogen subjects show a suppression effect. Low estrogen (N = 21), high estrogen (N=19). Error bars represent s.e.m.

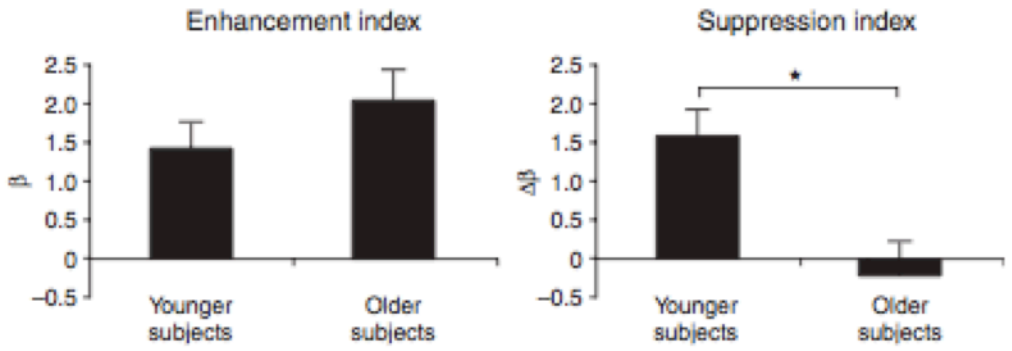

Figure 3.6 Enhancement and suppression index in young vs. older adults. While enhancement was robust for both groups, suppression indices reveal a striking suppression deficit in older subjects (mean age: 67) compared to younger subjects (mean age: 23.5). (Note that both samples scontained a near even distribution of men and women.) (Figure from Gazzaley *et al*, 2005).

3.3.2 Modulation of VAC activity when resources are taxed

When limited, top-down attentional resources are taxed VAC modulation is compromised. Gazzaley et al (2005) first noted this effect when comparing VAC activity during single versus dual-task conditions in their face/scene selective attention task. When subjects are asked to attend to scenes, PPA activity ramps up (as described in **3.3.1**). But what happens when subjects are asked to attend to both faces and scenes simultaneously? In this dual-task *Remember Both* condition, the PPA enhancement effect is diminished, with a magnitude of activity that is not significantly different than *Passive View* (**Fig. 3.7**).

A recent study by Rissman *et al* (2009) explicitly tested the effects of WM load on selective attention performance and VAC modulation. Subjects performed the attend/ignore face-scene task as before, but prior to each trial they were given a low or high load WM task (via auditory presentation) to complete while performing the visual selective attention task. Under high WM load conditions, subjects showed poor suppression of the distracting visual stimuli (as indexed by elevated PPA activity in response to stimuli that were meant to be ignored and higher incidental long-term memory of those items). High load conditions didn't deter from subjects ability to upregulate PPA activity when scenes were task-relevant. This inability to successfully filter distracting information when WM demands are taxed resembles the suppression deficit observed in older adults (**Fig. 3.6**) and in women when estrogen levels are low (**Fig. 3.5**).

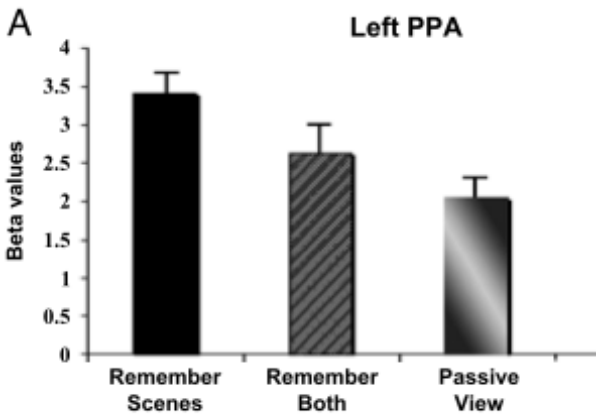

Figure 3.7 VAC modulation in young adults (men and women). Gazzaley et al (2005) showed that attentional load impacts VAC modulation: during the dual attention *Remember Both* condition, PPA activity was not significantly greater than *Passive View*. As expected, the *Remember Scenes* condition evoked greater PPA activity than *Passive View* (as well as *Remember Both)*. Their sample of young adults contained 10 men and 8 women. (Figure from Gazzaley et al, 2005).

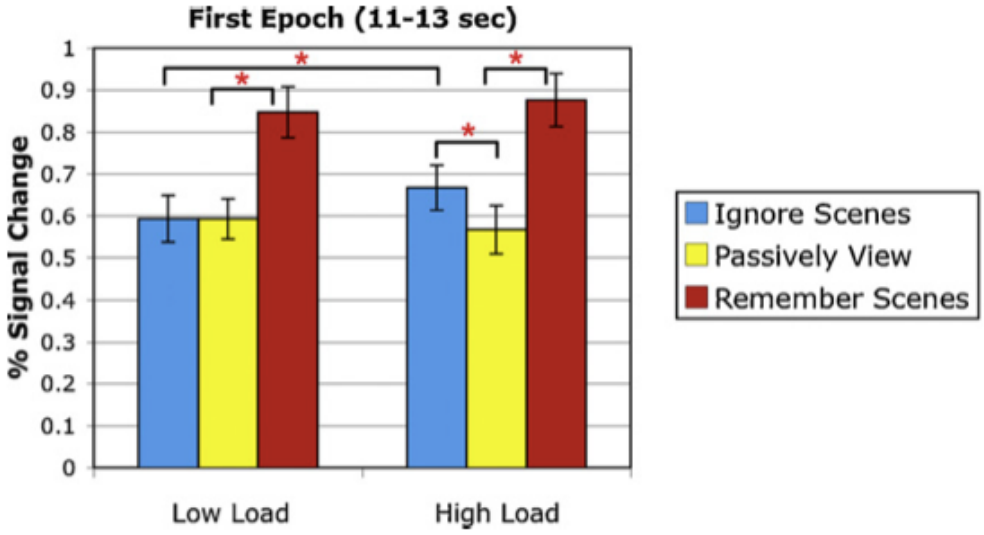

Figure 3.8 VAC modulation under low and high WM loads. Rissman et al (2009) demonstrated that when WM demands are high, distracting stimuli are not appropriately ignored. PPA activity during the *Ignore Scenes* condition (blue bars) is heightened when WM demands are high, but is appropriately diminished when WM demands are low. (Figure from Rissman et al, 2009)

How would low and high estrogen groups fare when faced with the more challenging dual-task condition, in which attentional resources had to be allocated across both stimulus categories? To probe estrogen's impact on VAC modulation when WM demands are taxed, we compared the magnitude of PPA activity during the dual-task *Remember Both* condition between low and high estrogen groups. During the *Remember Both* condition subjects were asked to keep two visual representations in mind (one face, on scene) over a delay and then indicate whether the item in mind matched or did not match the next image (from the same stimulus category) to appear. The paradigm followed a visual N-back-like working memory task, where the number of intervening distracters (irrelevant stimuli) ranged from 0-3.

In low estrogen subjects, the magnitude of PPA activity during the *Remember Both* condition was not significantly elevated above *Passive View,* indicating a lack of enhancement in the dual task above the perceptual baseline (**Fig. 3.9**), in keeping with the previous study of young adults (**Fig 3.7**). As expected, greater PPA BOLD activity was observed for *Remember Scenes* compared to *Passive View*. (An ANOVA assessing the effect of condition on PPA activity confirmed that the conditions differed ($p = 0.004$) for low estrogen subjects and post-hoc Bonferoni corrected comparisons indicated that activity was significantly greater for *Remember Scenes* versus *Passive View* ($p < .003$)).

In high estrogen subjects, the magnitude of activity in left PPA for *Remember Both was* significantly elevated above *Passive View*, reflecting an enhancement in the dual task above the perceptual baseline (**Fig. 3.9**). As expected greater PPA BOLD activity was observed for *Remember Scenes* compared to *Passive View*. (An ANOVA assessing the effect of condition on PPA activity confirmed that the conditions differed ($p < 0.005$) in high estrogen subjects and post-hoc Bonferoni corrected comparisons indicated that activity was significantly greater for *Remember Scenes* and *Remember Both* compared to *Passive View* (both $p < 0.005$)).

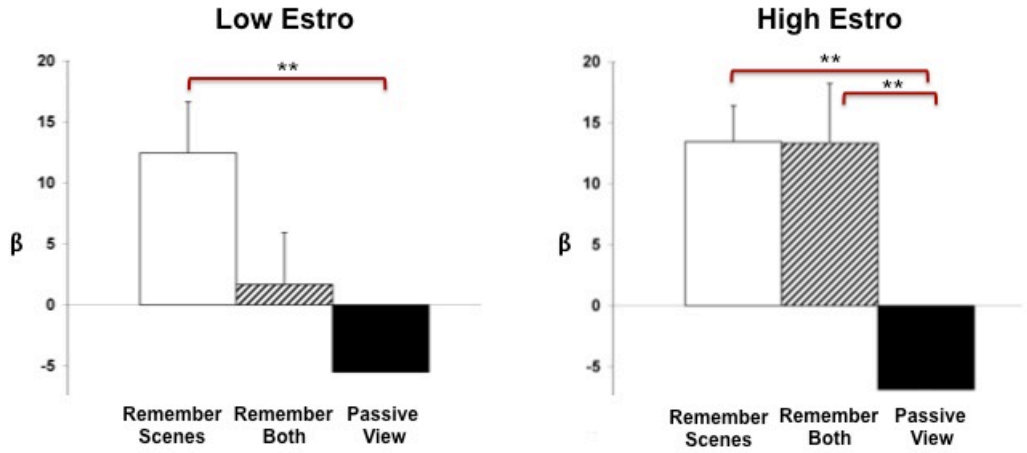

Figure 3.9 VAC modulation when selection demands are taxed. In a low estrogen state, subjects show weak activity in VAC regions during the *Remember Both* condition, when competition for attentional resources is high. These data parallel previous findings in young adults (Fig 3.7). However, in a high estrogen state, this dual-task deficit disappears: the magnitude of PPA enhancement is as robust in the dual-attention condition as it is in the *Remember Scenes* condition (data from left PPA shown). Error bars represent s.e.m.

3.3.2 Interim summary of univariate results

Thus, when asked to selectively attend to one image category (while ignoring the other) women in both the low and high-estrogen state showed a robust enhancement of VAC activity in response to task-relevant stimuli. However, low-estrogen women showed a suppression deficit (when scenes were meant to be ignored, bilateral PPA activity was not reduced below the passive viewing baseline), suggesting a difficultly in keeping out irrelevant, distracting information.

When attentional resources were challenged (by imposing stronger working memory demands in conjunction with the attentional-control task), low estrogen women succumbed to an additional 'enhancement deficit', in keeping with data from a previous sample of young adults (Gazzaley et al 2005). However, when these women were tested in the peak estrogen state (just before ovulation), they showed robust VAC activity during the dual-task condition, which may represent a neural index of their ability to upregulate goal-relevant activity in visual association cortices, in spite of taxed attentional resources.

3.4 *Functional MRI Multivariate Results*

3.4.1 PFC-VAC functional connectivity

Coherence between prefrontal and visual association cortices differed significantly as a function of task condition (irrespective of genotype and estrogen status; N=40) (**Fig. 3.10**). PFC-PPA coherence was enhanced during the *Attend Scenes* condition and suppressed during *Ignore Scenes*, relative to the *Passive View* condition. Coherence during the dual attention condition (*Attend Both)* was comparable to *Passive View*. These effects were evident in both ipsilateral and contralateral PFC-PPA ROI pairings.

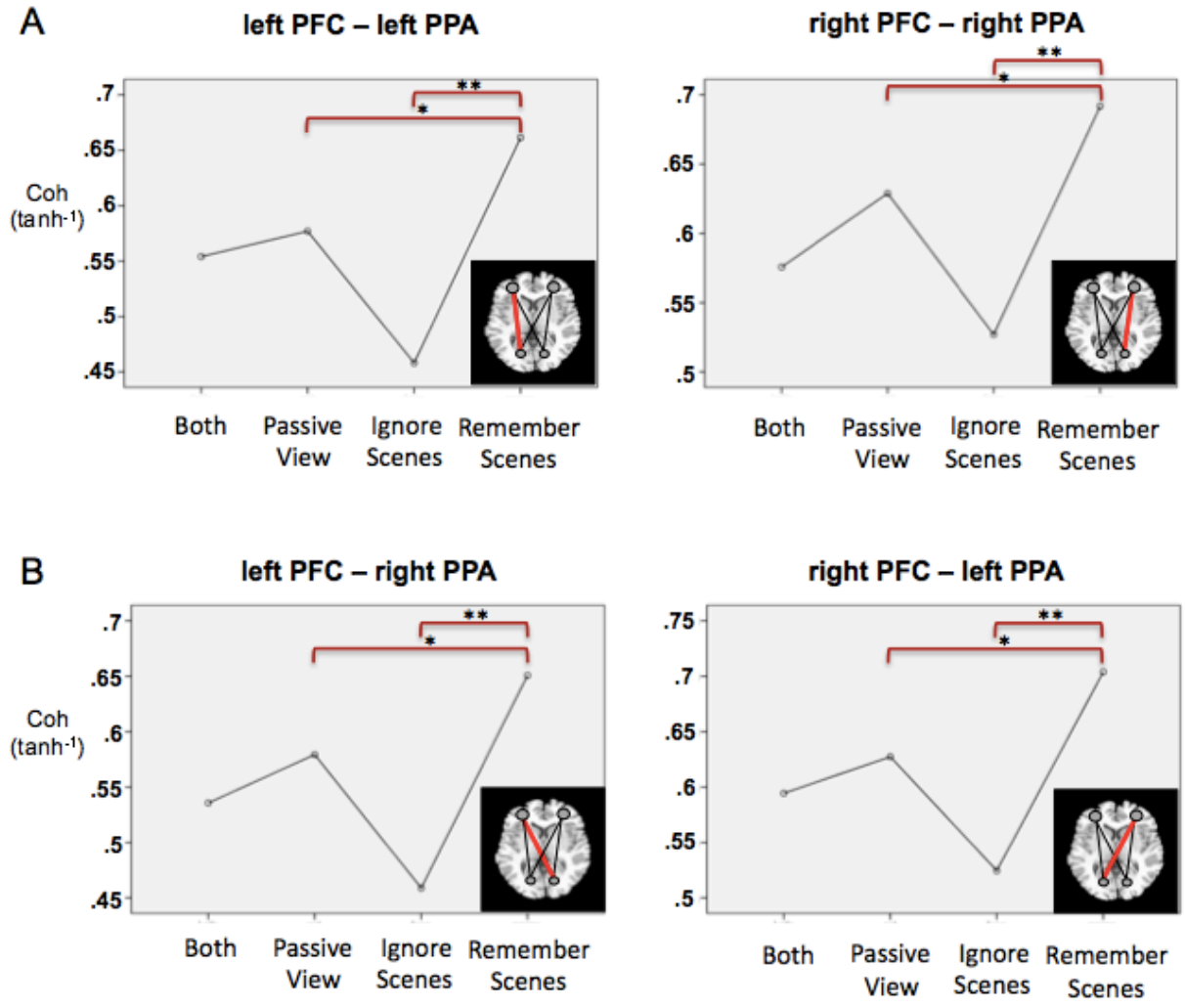

Figure 3.10 PFC-PPA functional connectivity is modulated by attentional goals. (A) Coherence between ipsilateral PFC-PPA ROIs as a function of task condition. Coherence is increased above a *Passive View* baseline during the *Remember Scenes* condition (Left: $p < 0.005$) and suppressed below a *Passive View* baseline during the *Ignore Scenes* condition (Left: $p = 0.02$). (*Attend Both* and *Remember Scenes* are also significantly different [Left: $p = 0.035$; Right: $p = 0.028$] as are *Attend Scenes* vs *Ignore Scenes* [Left: $p < 0.005$; Right: $p = 0.002$]. Condition ANOVA, $p = 0.001$). **(B)** Similar results were observed when assessing coherence between contralateral PFC-PPA ROIs. Inset brain schematics reflect the regions being examined in the companion line-graph.

Next, we assessed the impact of estrogen status (low, high) and COMT genotype (val, met) on PFC-PPA coherence. Based on the univariate results (in which low estrogen subjects showed a marked suppression deficit in PPA, irrespective of genotype) we predicted that low estrogen subjects would similarly show altered PFC-PPA connectivity

during the *Ignore Scenes* condition compared to high estrogen subjects, with negligible differences between genotypic groups.

A three-way Condition (*4*) x Estrogen status (*2*) x Genotype (*2*) ANOVA of PFC-PPA coherence revealed a significant main effect of condition, in addition to an estrogen * genotype interaction. For *val/val* subjects, PFC-PPA coherence was weakest in the low estrogen state and significantly stronger in the high estrogen state. The opposite effect was observed for *met/met* subjects, who had the strongest PFC-PPA coherence when estrogen levels were low and attenuated coherence when estrogen levels were high (**Fig. 3.11A**). Furthermore, at an individual level, circulating estradiol levels at the time of testing correlated with the degree of top-down coherence for *val/val* subjects (**Fig.3.11B**), demonstrating that beyond a straightforward 'low' and 'high' estrogen grouping (based on a median split of estradiol values) the relationship between estrogen and coherence extends to the individual subject level. In *met/met* subjects, a trend for a negative correlation between estradiol and PFC-PPA was observed, but it did not reach significance.

In summary, subjects with naturally reduced prefrontal DA (*val/val* genotype) showed greater functional connectivity between prefrontal control regions and visual association cortex when estrogen levels were elevated relative to low estrogen conditions, but subjects with naturally elevated prefrontal DA (*met/met* genotype) showed the opposite pattern, with the most robust coherence when estrogen levels were low. This interaction follows the theoretical inverted-U shaped DA curve, where an optimal DA level is necessary for maximal function and both insufficient and excessive levels lead to PFC dysfunction. These data parallel our finding in the N-back working memory task, in which behavioral and neural indices of PFC function showed an estrogen*genotype interaction. In both cases, naturally 'low dopamine' individuals received a neural and/or behavioral boost from high circulating estrogen, presumably because dopamine levels were elevated to a more optimal state. However, for subjects with already near-optimal DA, indices of PFC function were strongest when estrogen levels were at a minimum.

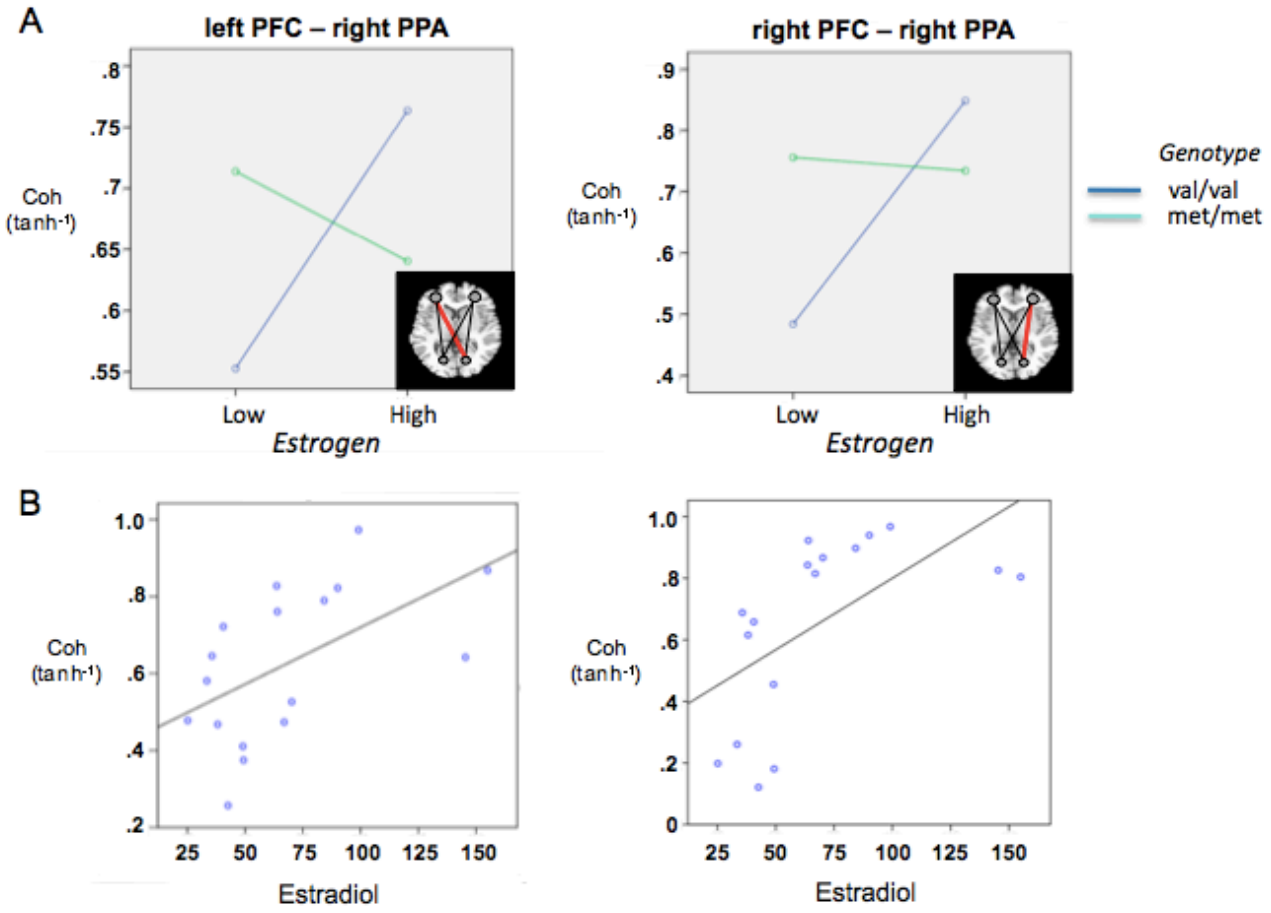

Figure 3.11 PFC-PPA functional connectivity is impacted by estrogen, genotype. (A) Coherence values (shown here for the *Remember Scenes* condition) differed between genotypic groups as a function of estrogen status. *Val/val* subjects (blue bars) showed robust coherence under high estrogen relative to low estrogen conditions, while *met/met* subjects (green bar) showed the opposite pattern. **(B)** At an individual level, circulating estradiol levels correlated with PFC-PPA coherence in *val/val* subjects (blue dots). Coherence is shown between two ROI pairings: left PFC/right PPA and right PFC/right PPA, although similar results were observed between most PFC-PPA pairings. Overall pattern of results (estro*genotype interaction) was similar for PFC-PPA coherence in the *Ignore Scenes* condition.

3.4 *Behavioral results*

Post-experiment recognition memory testing confirmed that recognition of previously viewed items varied significantly by task-condition. Passively viewed and irrelevant items (i.e. items meant to be ignored) were significantly less recognized than relevant images (i.e. items meant to be remembered) (**Fig 3.12**). Recognition ratings for passively viewed and irrelevant items were not significantly different than novel stimuli, indicating that subjects failed to recognize items they encountered during the *Ignore* conditions (i.e.

face stimuli during the *Remember Scenes* condition and scene stimuli during *Remember Faces* condition).

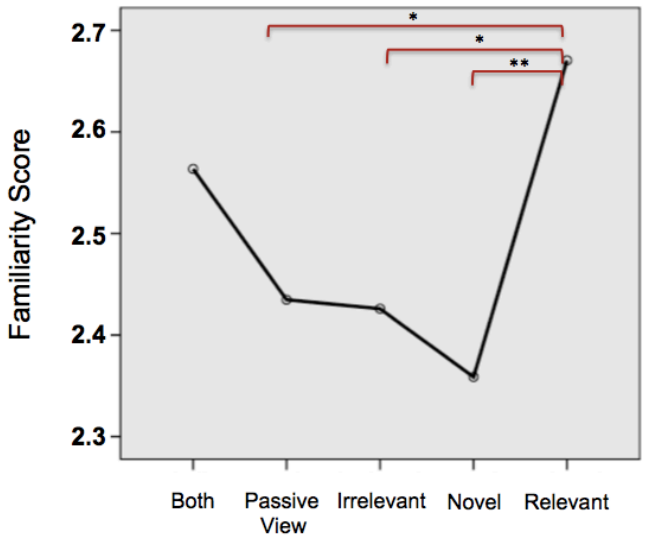

Figure 3.12 Long-term memory of novel and old items by task-condition. Familiarity ratings for face and scene stimuli indicate that items meant to be remembered (*Relevant*) were recognized more than *Novel*, *Irrelevant* and *Passively Viewed* items. (Three-way ANOVA for Conditon x Cycle x Genotype revealed a main effect of condition on familiarity ratings, $p = 0.002$, and post-hoc corrected comparisons showed a significant difference between Relevant and: Novel ($p < 0.005$), Irrelevant ($p = 0.015$) and Passive View ($p = 0.027$).

There was no main effect of estrogen on long-term incidental memory of irrelevant scene stimuli, which we predicted based on the univariate ROI data showing a PPA suppression deficit for low estrogen subjects (although the data suggests a trend toward low estrogen subjects having stronger recognition of irrelevant scenes relative to passively viewed stimuli – **Appendix E**). Instead, we observed an estrogen * genotype interaction on familiarity ratings, which mirrors the estrogen * genotype interaction found in the multivariate functional connectivity data (**Fig. 3.13**).

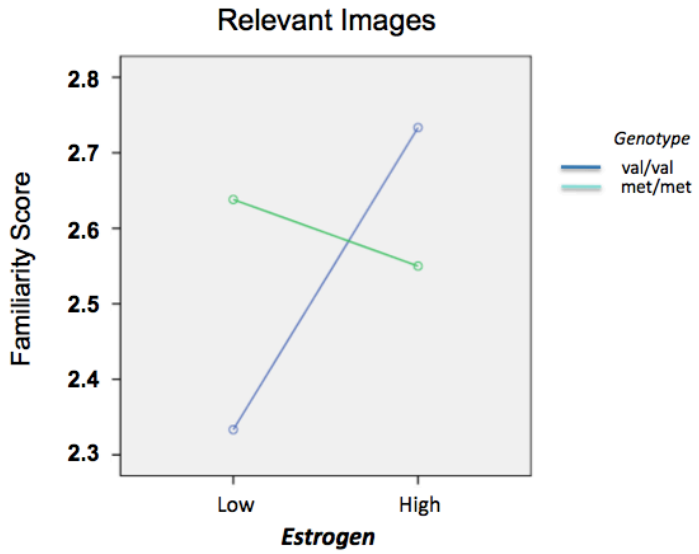

Figure 3.13 Recognition memory for relevant stimuli by estrogen status and COMT genotype. Familiarity ratings showed an estrogen * genotype interaction, with the strongest recognition ratings for met/low estrogen and val/high estrogen groups. The interaction was significant for face stimuli, but only a trend for scene stimuli. (Note that the coherence interaction was limited to PFC-PPA ROIs. PFC-FFA coherence analyses were not run, due to lack of a stable FFA suppression index at the univariate level).

3.5 *Interim summary*

In summary, univariate data from bilateral scene-selective regions within visual association cortices (PPA) indicate that when circulating levels of estrogen are high, women show a robust enhancement and suppression effect (i.e. VAC activity is modulated in synchrony with task demands, with an enhancement of PPA activity when scenes are relevant and a suppression of activity when scenes are irrelevant), which parallels previous work in young adults (Gazzaley et al, 2005). However, when estrogen levels are low (at the beginning of the menstrual cycle), female participants have a deficit suppressing PPA activity when scenes are irrelevant. (Enhancement of task-relevant PPA activity is preserved.)

Multivariate functional connectivity data assessing coherence between frontal control regions and visual association cortices revealed an estrogen*genotype interaction. "Optimal" DA groups (*val/val* + high estrogen and *met/met* + low estrogen) show stronger PFC-PPA coherence during multiple stages of the selective attention task (including both *Remember Scenes* and *Ignore Scenes*), while "non-optimal" DA groups (*val/val* + low estrogen and *met/met* + high estrogen) showed weak coherence.

Likewise, behaviorally, optimal dopamine groups had better long-term memory of relevant stimuli, while non-optimal DA groups had poorest long-term memory, in keeping with an inverted-U nature of the relationship between DA and PFC function (though, the behavioral effect was limited to familiarity for face stimuli).

4

CONCLUSIONS, IMPLICATIONS AND FUTURE DIRECTIONS

4.1 *Summary of novel experimental results*

In the series of studies presented herein we examined the impact of estrogen on dopamine- and PFC- dependent tasks while taking into consideration two indices of baseline dopaminergic activity: COMT Val^{158}Met genotype and COMT enzyme activity. By rooting the broad estrogen-cognition problem within a model of prefrontal DA function, we could ask with greater precision how estrogen impacts DA-dependent neural processes.

First, using a classic working memory paradigm (a verbal n-back task), we showed that taking baseline DA into account is pivotal to detecting the direction of estrogen's effect on WM. Specifically, we established that estrogen can be beneficial *or* detrimental to WM and, crucially, the direction of the effect depends on COMT genotype and, at a finer scale, COMT enzymatic activity. At the behavioral level, the effects of estrogen and COMT genotype emerged on trials that require a high demand of cognitive control. Neurally, hormonal and genotypic differences were apparent even in the absence of significant behavioral differences. We showed that 'optimal' cortical DA (as indexed by COMT genotype and estrogen status) was associated with a broadly efficient pattern of sustained activity (that which occurs across WM blocks), along with a selective, event-related enhancement of activity during episodes of high interference (e.g. lures), when the demand for cognitive control is greatest. Furthermore, the extent to which an individual enhanced PFC activation during the demanding lure trials was predictive of their performance.

Next, we turned to a visual selective attention paradigm that allowed us to assess the effects of estrogen and COMT genotype on top-down modulation of neural activity in visual association cortices. We used a recently established metric of goal-directed neural 'enhancement' and 'suppression' that is sensitive to identifying group differences in VAC modulation (e.g. between younger vs. older adults and under low vs. high cognitive load) (Gazzaley et al, 2005; Rissman et al, 2009). Scene-selective regions of interest showed robust suppression and enhancement effects at the group level, which were highly dependent on attentional goals, but further analyses revealed an important difference between low and high estrogen groups. While both groups successfully enhanced PPA activity during the *Remember Scenes* condition above a perceptual baseline, only the high estrogen subjects were able to appropriately attenuate the processing of task-irrelevant scenes in the *Ignore Scenes* condition. This effect of

estrogen on distracter filtering parallels the suppression deficit observed in older adults (Gazzaley et al, 2005), and in young adults when attentional resources are taxed (Rissman et al, 2009).

Furthermore, when attentional resources were imposed upon (during the dual-task *Remember Both* condition) low estrogen subjects succumbed to the 'enhancement deficit' that has been shown to occur in young adults when attentional resources are limited (Gazzaley et al 2005). For subjects in the low-estrogen state, like the previous sample of young adults, VAC enhancement is diminished in the dual-task condition compared to the single task of attending to one image category (note that in all conditions perceptual load is held constant, only task instructions differ). High estrogen subjects, however, were resilient to the working memory demands incurred by the high load condition. VAC enhancement during *Remember Both* was as robust as the enhancement observed during *Remember Scenes*. Thus, even when attentional/working memory resources were taxed, if estrogen levels were high women showed no evidence of strained top-down, goal-directed processing. When estrogen levels dropped (during the beginning of the cycle) the enhancement deficit emerged.

Multivariate functional connectivity data assessing coherence between frontal control regions and visual association cortices revealed an estrogen*genotype interaction. Subjects with naturally diminished prefrontal DA (*val/val* genotype) showed greater top-down coherence when estrogen levels were high versus low; but subjects with naturally elevated prefrontal DA (*met/met* genotype) showed the opposite pattern, with the most robust coherence when estrogen levels were low. This interaction follows the theoretical inverted-U shaped DA curve, where an optimal DA level is necessary for maximal function and both insufficient and excessive levels lead to PFC dysfunction. Behaviorally, subjects' long-term memory of attended items mirrored the functional connectivity data: optimal dopaminergic groups had robust connectivity between prefrontal control regions and visual association cortices as well as better long-term memory, while non-optimal groups had weaker coherence and poorer memory (though the stimulus categories for which the multivariate and behavioral effects were significant differed).

These data establish or advance three points. One, dopamine's influence on cognition cannot be fully understood without taking estrogen into account. Two, the functional impact of genetic variation can be explored via direct assays of brain function. Three, 'women's health' questions can be addressed within the central nervous system. What emerges from this work is fundamentally a very simple question: *what makes people differ?* How do people differ cognitively; how might they differ in response to stress; and how might they differ in response to drugs (including drugs of abuse and pharmacological treatments)? By taking an idiosyncratic approach to healthcare we can better identify those people who are most vulnerable to disorders rooted in dysfunction of frontal lobe and the dopamine system. That may translate into different approaches for men and women, and within women in different endocrine states.

4.2 *Implications, beyond 'proof of concept'*

Often in neuroscience sex is something to be controlled for. Or, scientists leapfrog over the matter altogether by studying only males of a species. To make that point clear, colleagues at Berkeley indexed all studies in mammals published in 2009, across 10 fields and over 40 journals. They found a widespread bias toward using only male animals, and the effect was especially striking in neuroscience, where the ratio of male-only to female-only studies is 5.5 to 1 (Hayden, *Nature News*, 2010). When males and females were used, the data were often not analyzed by sex. The bias was largest in neuroscience, pharmacology and physiology, fields for which basic science research has huge implications for human health (Wald and Wu, *Science News*, 2010). Yet, sex differences are evident: sex differences are repeatedly found for rates of depression, vulnerability to stress, pain, Parkinson's Disease, ADHD, and nearly all aspects of drug abuse (from acquisition to treatment).

In humans, the estrogen-cognition literature is famously inconsistent (e.g. Sherwin, 2005). According to the news, one day estrogen is good for you and the next day it's bad for you. Yes, it's a messy problem. (The good ones always are.) But the problem gets thrown into relief only after it's subjected to careful scientific study.

Studies on sex differences and neuroendocrine effects have implications for women at all stages of life. Enormous endocrine changes accompany menopause (circulating estradiol concentrations fluctuate widely during perimenopause before falling markedly in postmenopause) (Burger et al., 2002) yet we know relatively little from a human neuroscience perspective about how this normal aging process affects specific neurochemical and cognitive systems. One hundred years ago, this problem was irrelevant: the average age of menopause is 51.8 and in 1900 the average life expectancy for women was 52. Now average life expectancy is 82, meaning women live a third of their lives in an estrogen-depleted state. The conundrum is palpable in earlier stages of life too. Imagine you have a girl and boy who present with ADHD. The doctor prescribes 30 mg of methylphenidate (Ritalin) to both. It's possible that the effective dose differs between the two patients, especially if the girl is in the midst of puberty and estrogen levels are in flux. In short, a man and woman's milieu differ; until we understand how we cannot fully understand neural processes as they unfold in the healthy state, less still in the diseased state.

4.3 Future directions – pharmacological PET study of estrogen's impact on the dopamine system

4.3.1 Background and Purpose

Strong evidence indicates estrogen enhances activity in the DA system but a definitive study showing an estrogen-dopamine link in humans is lacking. Thus, we designed a pharmacological PET-MRI study to understand how estrogen impacts phasic dopamine release, and how both estrogen and dopamine are related to cognitive function and neuronal activation (fMRI BOLD). Positron Emission Tomography (PET) imaging is the only method available to assess DA release in vivo in humans. Currently available radioactive tracers can assess presynaptic dopamine function (e.g. by examining the dopamine transporter, DAT) as well as dopamine synthesis or synthesis capacity. More recently, transient dopamine release has been studied by combining PET imaging with pharmacological stimulation of the dopamine system.

Here, we combine pharmacological stimulation of dopamine using methylphenidate (compared to placebo) with raclopride PET to examine functional changes in dopamine in relation to circulating estrogen levels. We measure phasic dopamine release via [11C] raclopride (RAC) PET scanning under placebo conditions and after oral administration of 30 mg methylphenidate, which stimulates dopamine release. Young adult women with normal menstrual cycles are examined at two stages of their cycle: when circulating estrogen levels are at their peak (day 11-12) and trough (day 1-2). Brain activation is measured by the use of functional MRI during a working memory task. Specific hypotheses are: 1) higher levels of circulating estrogen will be associated with greater stimulated phasic dopamine release; 2) phasic dopamine release will be related to cognitive performance; 3) fMRI BOLD will be related to phasic dopamine release and cognitive performance.

The proposal is driven by a desire to understand dopamine's role in the cognitive and neural effects of estrogen. However, in addition to elucidating estrogen's role, the study will: 1) establish a link between dopamine-induced changes in BOLD & transient dopamine release (i.e. functional dopamine changes) 2) provide the necessary pilot work for setting up a displacement protocol at Berkeley, which will open up avenues to studying functional dopamine changes in other populations 3) allow us to assess the association between drug-related changes in task performance and transient changes in dopamine. The study will significantly extend previous dopaminergic-PET studies by relating indices of DA release to fMRI BOLD and estrogen, and it will open up the study of state rather than trait-related dopamine changes.

4.3.2 Methods

This is a cross-sectional study (semi-randomized, crossover design under single blind conditions) of 10 healthy women between the ages of 20 and 35 (inclusive). Before being enrolled in the PET-MRI portion of the study, subjects undergo two screenings to determine eligibility (a preliminary telephone screen and an in person consultation).

Subject menstrual cycles are tracked for 4+ months prior to scanning to determine cycle consistency and length.

Enrolled subjects are scanned on two occasions: when circulating levels of estrogen are low (the beginning of the menstrual cycle) and when estrogen levels peak (during the late follicular stage). During each scan day subjects undergo 1 PET scan and 1 fMRI scan under placebo conditions (oral administration of 30 mg lactose placebo), followed by 1 PET scan and 1 fMRI scan under drug conditions (oral administration of 30 mg methylphenidate). Drug or placebo administration cannot be randomized within the constraints of this study since the placebo condition must occur before the drug is administered. Dopamine release is indexed by the degree to which methylphenidate lowers the [11C] raclopride binding potential.

Scan schedule (session order counterbalanced across subjects):

Session 1 = High estrogen stage of cycle: PET, fMRI scan on placebo, followed by PET, fMRI scan on drug

Session 2 = Low estrogen stage of cycle: PET, fMRI scan on placebo, followed by PET, fMRI scan on drug

Two small blood samples (40 cc or 2-3 tablespoons) are collected immediately before the first RAC-PET scan on each day; one is used to assay catechol-O-methyltransferase (COMT) enzyme levels the other is used to assay estradiol levels. A small salivary sample (~ 1ml) is collected by passive drool for an additional measure of estradiol concentrations.

4.3.3 Predictions

Stimulated phasic DA release will be potentiated by estrogen, leading to greater displacement of the radioligand when circulated levels of estrogen are elevated.

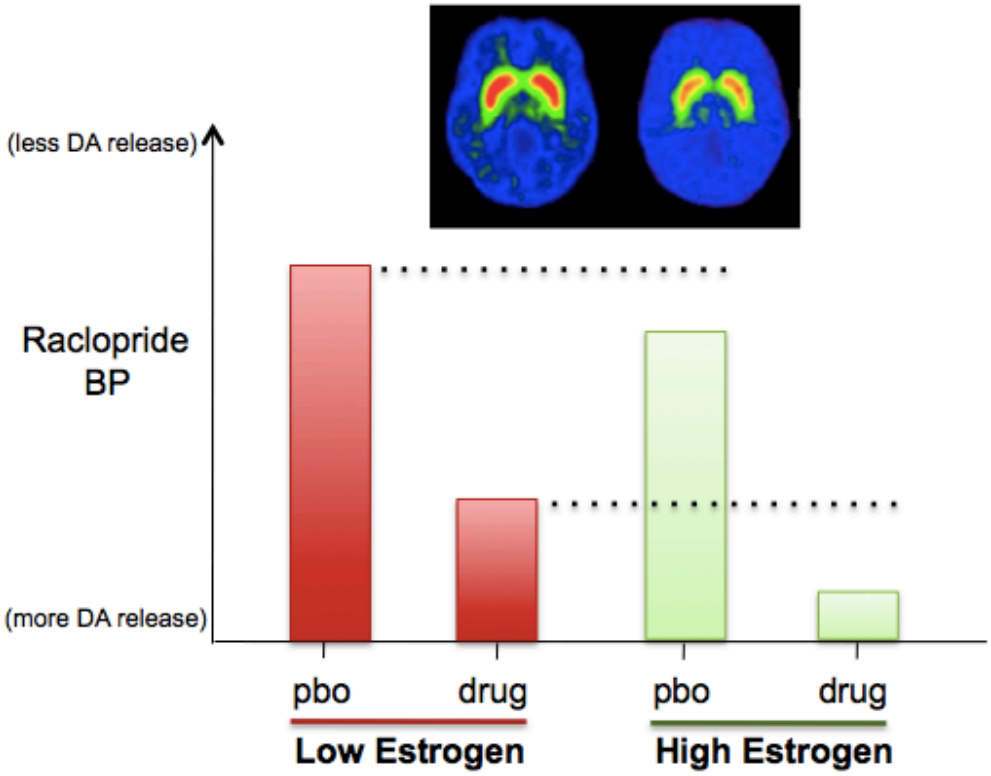

Figure 4.1 Raclopride-PET: predicted effects of estrogen on dopamine binding. Under low estrogen conditions (red bars) we expect to see a drug-induced decrease in raclopride binding potential (i.e. a drug-induced increase in dopamine release). Under high estrogen conditions (green bars) there may be an estrogen-related difference in baseline binding (on placebo - PBO), or a potentiation of the drug-related increase in dopamine release. Given the selectivity of the tracer to D2 receptors, we expect these results to be evident in dopamine-rich striatal regions (top image).

4.3 *Stress and development*

Stress is another factor that has a potent impact on catecholamines (Deutch and Roth, 1990; Arnsten and Goldman-Rakic, 1998; Arnsten, 2000). When a person mounts a stress response PFC dopamine increases dramatically. It is possible that like estrogen, stress may have a beneficial or detrimental effect on an individual depending on their genetic milieu: some individuals may show cognitive improvements under mild amounts of stress while others may be burdened by the same stressor. Similarly, circulating estrogen levels may alter subjects' sensitivity to stress. Future work can tease apart sex, hormone and genetic factors that alter individual vulnerabilities to stressors (both mild, controllable

stressors and more severe, uncontrollable stressors).

Finally, adolescence marks a unique time in brain development: substantial reorganization of neural pathways occurs within the frontal lobe, an area that subserves most of the "higher order" cognitive skills we rely on as functioning human beings. Adolescents have a heightened number of dopamine receptors expressed in the frontal lobe compared to adults. That, coupled with the fact that stress stimulates dopamine release in the prefrontal cortex, may make the risk for stress-induced prefrontal dysfunction especially high during puberty. It's a vulnerable group and even mild increases in dopamine (from stress, estrogen, lack of sleep etc.) could have crippling effects on cognition. This could be a contributing factor to a number of mental health disorders that emerge during adolescence, including schizophrenia and early-onset depression. It is a quirk of nature that at the very time adolescents are undergoing tremendous physiological changes that increase their vulnerability to stress, they enter a period of social stress that seems unparalleled in scope. An important overlying issue is how social factors, such as socioeconomic and perceived status, influence ongoing developmental changes. If we identify susceptible populations early enough, and understand the environmental conditions that contribute to that susceptibility, clinicians may be better equipped to minimize the manifestation of these disorders.

§

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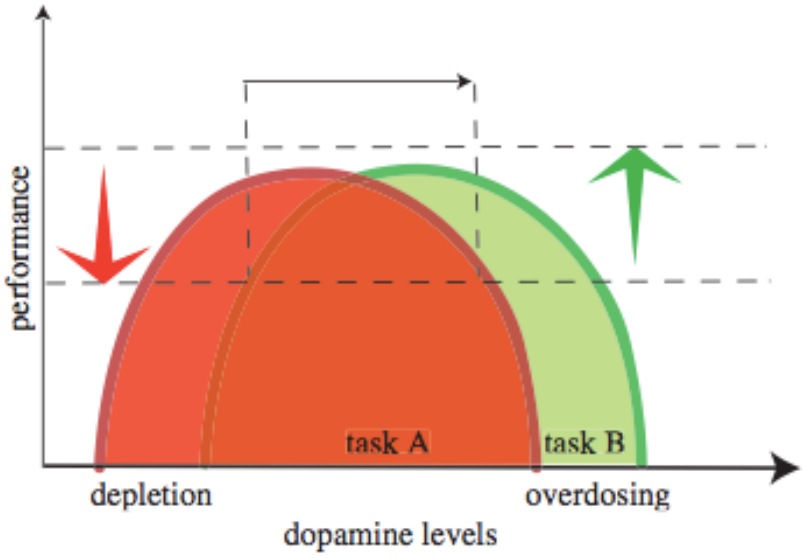
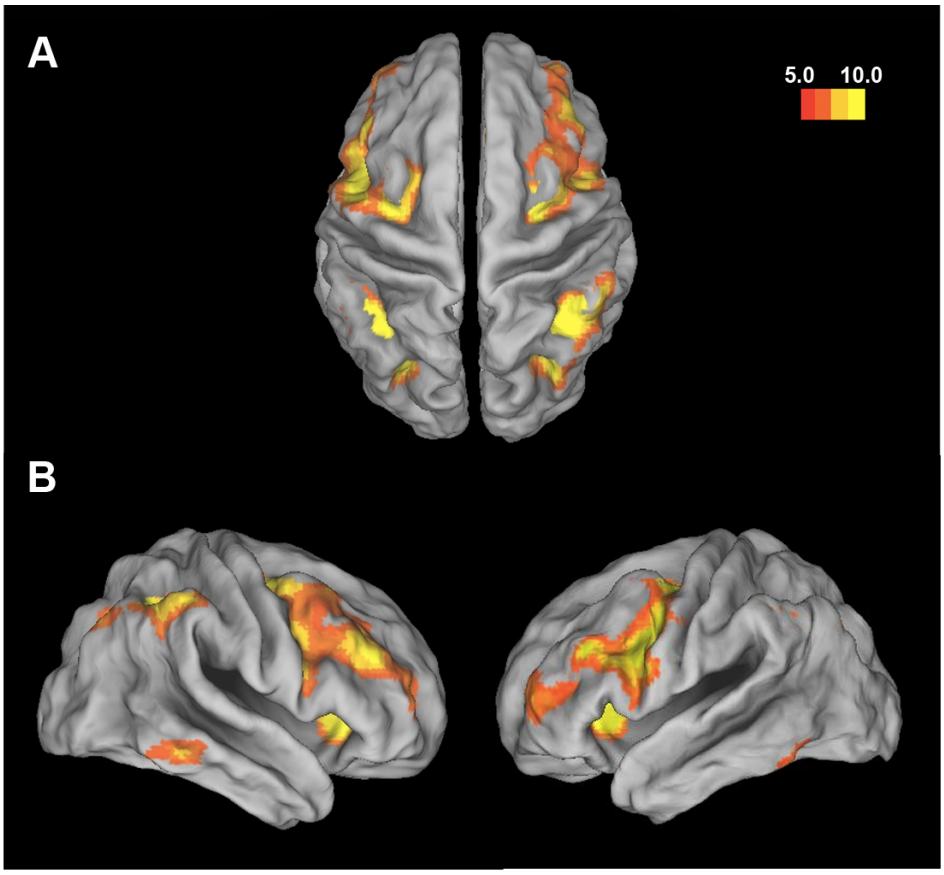
APPENDIX A.



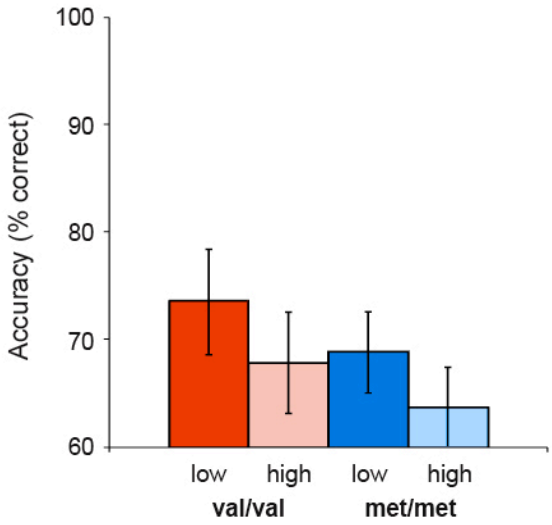
Inverted U shaped curve whereby insufficient and excessive levels of DA impairs performance. Optimal DA levels may vary from task to task, represented here by two dose- response curves. Depending on where along the curve a subject lies and what task is being probed, an increase in DA (e.g. from a DA agonist or during stress) may help or hinder performance. In this figure baseline levels of DA are represented by the first vertical dashed line. Performance is high on task A, low on task B. When a hypothetical increase in DA shifts levels (to the second vertical dashed line), performance on task A declines, performance on task B improves. *Figure from Cools et al, 2004.*

APPENDIX B.



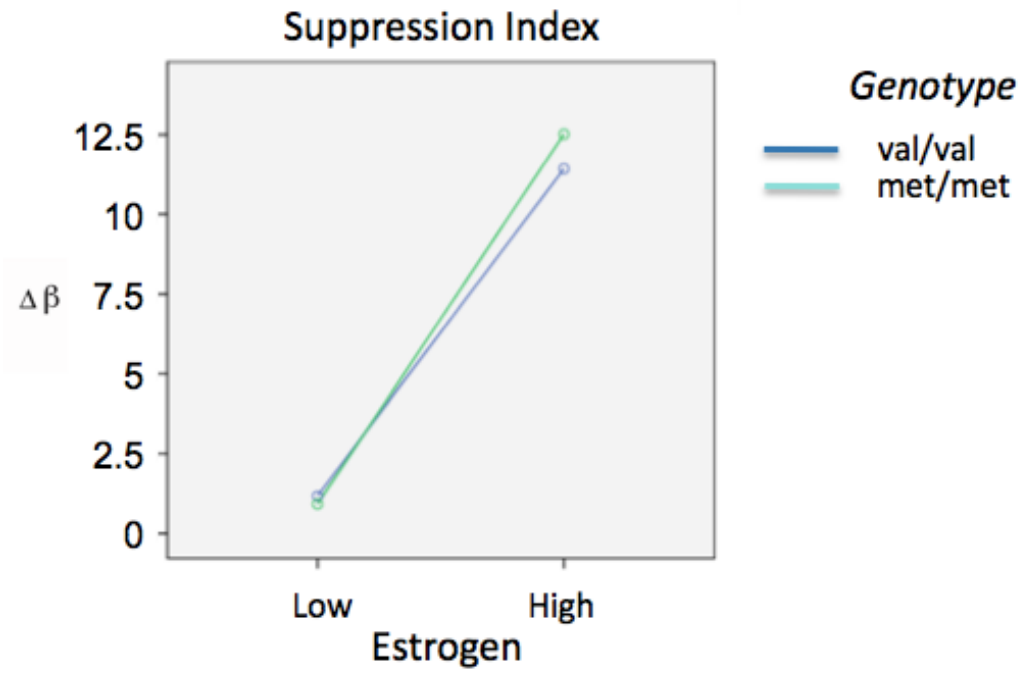
Working memory load-sensitive regions. **(A)** Dorsal and **(B)** lateral view shown at $P_{FWE} > 5e^{-06}$. These regions (including bilateral PFC, supplementary motor, bilateral superior parietal) exhibit a parametric increase in activity from 0-back -> 2-back -> 3-back blocks.

APPENDIX C.



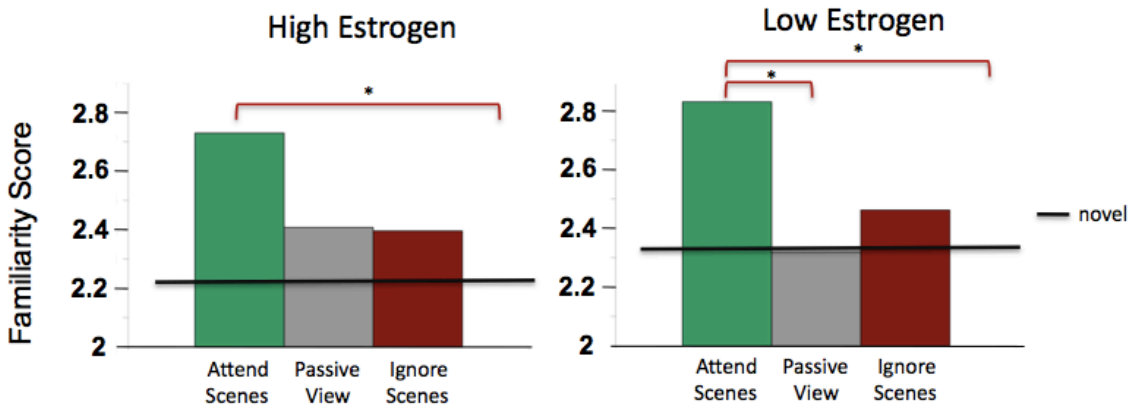
Three-back lure performance as a function of COMT genotype and estrogen state. As inferred DA status increases (left to right), performance decreases – in accord with a theoretical inverted-U model that accounts for load-dependent shifts in DA – but the differences were not significant.

APPENDIX D.



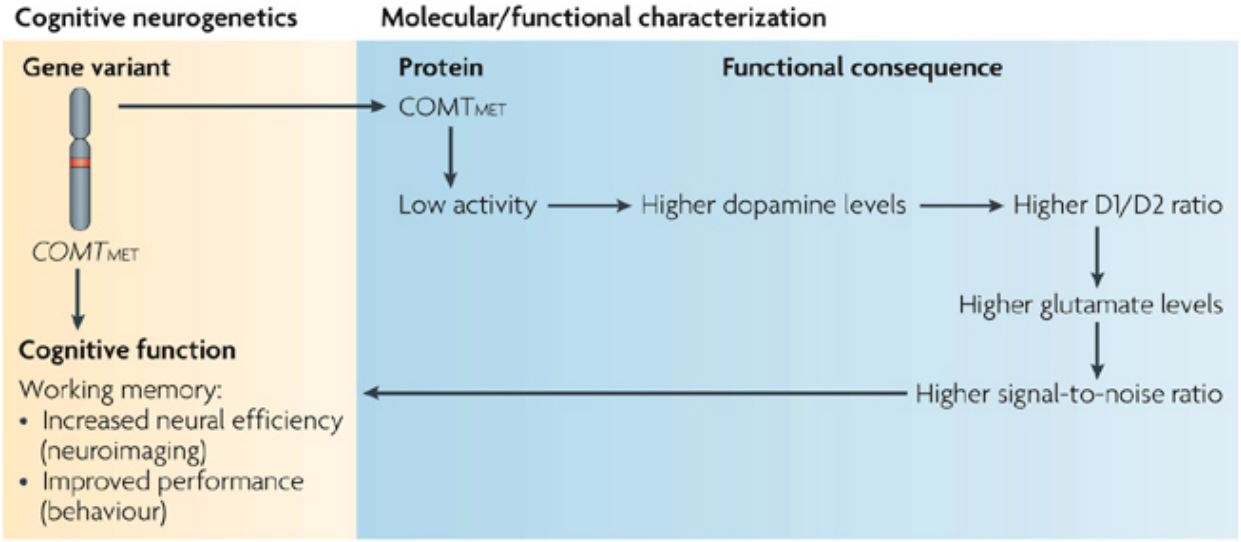
Suppression Index in PPA. The suppression deficit seen in low estrogen subjects (poor PPA suppression in response to distracting, task-irrelevant stimuli) did not vary as a function of COMT genotype. (The suppression index represents a subtraction of mean PPA beta values in the *Ignore Scenes* condition from mean beta values during *Passive View*.)

APPENDIX E.



Familiarity ratings by estrogen status. Low estrogen subjects show a trend toward having 'better' incidental long-term memory for scene items that were meant to be ignored (red bar) relative to passively viewed items (grey bar). Both low and high estrogen groups showed significantly better recognition memory for items in the Attend condition relative to novel stimuli.

APPENDIX F.



Current model of the relationship between COMT, DA, and 'cortical efficiency'. The *met/met* allele of the COMT Val[158]Met polymorphism is associated with reduced COMT enzyme activity, which leads to a greater accumulation of DA in the PFC (where COMT is highly expressed). Based on differences in the synaptic/extrasynaptic location of D1 and D2 receptor expression, increased PFC DA is thought to shift the ratio of D1 to D2 receptor stimulation in favor of D1. This in turn modulates the release of glutamate from pyramidal cells and increases the signal-to-noise ratio of cortical processing. It is hypothesized that this SNR boost is detected, using standard human cognitive neuroscience tools, as decreased BOLD in task-related regions and improved performance. *Figure from Green et al, 2008.*