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Impact Of Adverse Childhood Experiences On Behavioral And Neural Markers Of Executive Function In Menopausal Women

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Abstract
Many healthy women with no history of cognitive dysfunction experience subjective executive difficulties during menopause. Indicators of risk for executive function difficulties at menopause are lacking, as is a mechanistic understanding of how loss of estradiol unmasks this vulnerability. We hypothesized that adverse childhood experiences (ACE) increase the risk of executive dysfunction during menopause via alterations in monoaminergic neurotransmission. To test this hypothesis, we evaluated the effect of ACE on subjective and objective measures of executive function as well as executive activation, functional connectivity, and neurochemistry. We used tryptophan depletion (TD) and lisdexamfetamine (LDX) to probe serotonergic and catecholaminergic function, respectively. High ACE women endorsed greater symptoms of executive dysfunction and performed worse on tasks probing sustained attention and working memory. These negative ACE effects were partially mediated by anxiety and depressive symptoms. ACE moderated the impact of TD on DLPFC activation in hypogonadal women such that TD increased activation in high ACE participants but decreased activation in low ACE participants. Importantly, treatment with estradiol attenuated the effects of both ACE and TD. ACE similarly moderated the impact of TD on within-network connectivity. While ACE was associated with lower within-network connectivity regardless of depletion condition, TD increased connectivity in the high ACE group but had no effect on connectivity in the low ACE group. ACE also moderated response to LDX. In the high ACE group, LDX (vs placebo) increased activation in the insula and reduced symptoms related to difficulty with organization and activation for work. In contrast, response to LDX was not significantly different from placebo in the low ACE group.

These results have several clinical and mechanistic implications. First, they highlight that addressing concurrent mood changes is a critical step in treating menopause-induced executive difficulties. Second, this work suggests that early life adversity has latent impacts on serotonergic circuits underlying executive function that are unmasked by loss of estradiol during menopause. Third, they indicate that early adversity may have lasting effects on catecholaminergic neurotransmission and may moderate response to stimulant medications. Together, they emphasize the importance of considering ACE when treating executive difficulties with pharmacologic agents during menopause.

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ABSTRACT

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Many healthy women with no history of cognitive dysfunction experience subjective executive difficulties during menopause. Indicators of risk for executive function difficulties at menopause are lacking, as is a mechanistic understanding of how loss of estradiol unmasks this vulnerability. We hypothesized that adverse childhood experiences (ACE) increase the risk of executive dysfunction during menopause via alterations in monoaminergic neurotransmission. To test this hypothesis, we evaluated the effect of ACE on subjective and objective measures of executive function as well as executive activation, functional connectivity, and neurochemistry. We used tryptophan depletion (TD) and lisdexamfetamine (LDX) to probe serotonergic and catecholaminergic function, respectively. High ACE women endorsed greater symptoms of executive dysfunction and performed worse on tasks probing sustained attention and working memory. These negative ACE effects were partially mediated by anxiety and depressive symptoms. ACE moderated the impact of TD on DLPFC activation in hypogonadal women such that TD increased activation in high ACE participants but decreased activation in low ACE participants. Importantly, treatment with estradiol attenuated the effects of both ACE and TD. ACE similarly moderated the impact of TD on within-network connectivity. While ACE was associated with lower within-network connectivity regardless of depletion condition, TD increased connectivity in the high ACE group but

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General Introduction: Could Early Life Adversity Increase Risk for Executive Dysfunction during Menopause?

The Clinical Problem

The menopause transition is marked initially by fluctuating levels of ovarian hormones and ends with an overall dearth of estrogen in the postmenopausal period (Burger et al., 2007; Henderson and Popat, 2011; Shanmugan and Epperson, 2014). During this transition, many women report experiencing a decline in cognitive function (Greendale et al., 2009; Mitchell and Woods, 2011), specifically in areas of memory and attention (Halbreich et al., 1995; Weber and Mapstone, 2009; Schaafsma et al., 2010; Mitchell and Woods, 2011). Recent reports have suggested that healthy women with no history of cognitive dysfunction experience increased difficulty with everyday tasks requiring executive processes such as working memory, focus, attention, organization, and planning (Epperson et al., 2011; Shanmugan and Epperson, 2014; Epperson et al., 2015; Shanmugan et al., 2017). Evidence as to whether this midlife onset in cognitive decline can be attributed to the menopause transition is suggestive but not conclusive (Henderson and Popat, 2011). This variability in results across studies has traditionally been attributed to lack of consistency in the stages of menopause examined, covariates considered, and the use of cognitive measures with varying degrees of ecological validity (Luetters et al., 2007). A more recent hypothesis is that individual biological and environmental differences contribute to risk vs resilience for executive dysfunction with waning estradiol (Shanmugan and Epperson, 2014). In particular, there is preliminary evidence that adverse childhood experiences (ACE) including abuse, neglect, and household dysfunction may confer a vulnerability for executive difficulties that is revealed by loss of estradiol during menopause (Shanmugan and Epperson, 2014). This hypothesis is based on the negative impacts of ACE on executive functions and cognitive aging as well as the converging effects of estradiol and early life stress on monoaminergic function in the prefrontal cortex (PFC).

The Prefrontal Cortex: Location and Function

Before discussing the effects of estradiol and early life adversity on the PFC at the neurotransmitter level, it is important to review the anatomical and working nature of the PFC. The PFC is located at the anterior of the frontal lobes of the brain and includes Brodmann's areas 8, 9, 10, 11, 44, 45, 46, and 47 (Shanmugan and Epperson, 2014). The PFC is important for executive processes such as inhibiting distracting information and stimuli, planning, evaluating consequences when making decisions, and working memory. Working memory is often defined as the ability to keep desired or target information ''online'' and accessible as well as the ability to manipulate this information while being presented with new information that could serve as a distraction. Several clinical neuroimaging studies and preclinical studies in rodents and nonhuman primates have established the importance of the PFC in such behaviors. Rodents and monkeys with PFC lesions exhibit impaired performance on tasks requiring working memory (Butters and Pandya, 1969; Brito and Brito, 1990; Carlson et al., 1997; Chafee and Goldman-Rakic, 1998; Funahashi and Inoue, 2000; Lauwereyns et al., 2001; Chang et al., 2002; Baeg et al., 2003), reversal learning and flexibility (Rolls et al., 1996; Meunier et al., 1997; Dias and Aggleton, 2000; Chudasama and Robbins, 2003; Kim and Ragozzino, 2005; Floresco and Ghods-Sharifi, 2007; Rygula et al., 2010), and decision-making (Mobini et al., 2002; Floresco and Ghods-Sharifi, 2007; Kim et al., 2008; Churchwell et al., 2009; Hauber and Sommer, 2009; Endepols et al., 2010; O'Neill and Schultz, 2010). In healthy human subjects, performing tasks requiring these processes, particularly working memory, increases blood oxygen level dependent (BOLD) signal in the PFC (Barch et al., 1997; Braver et al., 1997; D'Esposito et al., 1998; Callicott et al., 1999; Clark et al., 2000; Diwadkar et al., 2000; Owen et al., 2005). The results of this research suggest that certain portions of the PFC are important for particular executive

functions. For example, the dorsal and lateral portions of the PFC are key in the regulation of attention, whereas the ventral and medial portions are important for emotional regulation (Arnsten, 2009). Optimal executive functioning and PFC regulation of subcortical brain structures are dependent upon prefrontal concentrations of monoamines, specifically dopamine (Arnsten, 2011), norepinephrine (Arnsten, 2011), and serotonin (5-HT) (Boulougouris and Tsaltas, 2008; Shanmugan and Epperson, 2014).

As suggested by Arnsten and colleagues, dopamine and norepinephrine exhibit an inverted U-shaped dose–response curve on PFC regulation of behavior (Arnsten, 2009, 2011). When present at ideal levels, norepinephrine maintains network connectivity through the stimulation of postsynaptic α2A-receptors and dopamine inhibits inappropriate attention to stimuli through stimulation of D1 receptors (Arnsten, 2009, 2011). Insufficient stimulation of these receptors at lower concentrations can lead to symptoms of executive dysfunction such as fatigue, distractibility, and impulsivity while the excessive D1 stimulation and inappropriate norepinephrine α1 and β1 stimulation that occur at higher neurotransmitter concentrations mimic symptoms of excessive stress such as mental inflexibility (Arnsten, 2009; Shanmugan and Epperson, 2014)

While serotonin also contributes to PFC modulation of executive function (Boulougouris and Tsaltas, 2008), its mechanisms of action are less well understood. Serotonin has a predominantly inhibitory effect on glutamatergic activity in the PFC (Puig and Gulledge, 2011). This inhibitory effect is driven by the 5-HT1A receptor, which reduces action potential generation at the cell body and axon of glutamatergic pyramidal neurons (Puig and Gulledge, 2011). The 5-TH1A receptor also acts as a presynaptic autoreceptor on projections from the dorsal raphe, inhibiting further release of serotonin (Li et al., 1999). Stimulation of the 5-TH1A receptor has been associated with improved

attentional control and decreased impulsivity (Carli et al., 2006; Puig and Gulledge, 2011). Serotonin also binds to 5-HT2A receptors on the apical dendrites of pyramidal neurons, which increases membrane excitability and excitatory post-synaptic potentials (Puig and Gulledge, 2011). Simulation of these 5-HT2A receptors has been associated with worse cognitive flexibility (Carli et al., 2006), reversal learning (Boulougouris et al., 2008; Boulougouris and Tsaltas, 2008), and impulsivity (Carli et al., 2006; Puig and Gulledge, 2011). These seemingly paradoxical effects result in serotonin inhibiting the activity of individual neurons while increasing the coherence of networks of neurons in the PFC (Puig and Gulledge, 2011).

Evidence supporting the withdrawal of estrogen modulation of these prefrontal monoaminergic systems as one mechanism leading to decline in executive functions will be discussed further below.

Estradiol's effect on executive function

Subjective cognitive complaints, particularly in areas of memory and attention, are commonly reported by women transitioning through menopause (Halbreich et al., 1995; Weber and Mapstone, 2009; Schaafsma et al., 2010; Epperson et al., 2011; Mitchell and Woods, 2011; Epperson et al., 2015; Shanmugan et al., 2017). Mitchell and Woods (Mitchell and Woods, 2011) found that 60% of their sample of menopausal women reported experiencing increasing memory problems while 79% of menopausal women in another sample reported experiencing memory loss (Weber and Mapstone, 2009). Rodent and non-human primate studies have suggested that this midlife cognitive decline can be attributed to the loss of estradiol experienced during the menopause transition. Estrogen, alone or in combination with progesterone, has been shown to be successful in correcting ovariectomy induced impairments in spatial working memory

and recognition memory consolidation, pre-clinical proxies for executive function (Rapp et al., 2003; Inagaki et al., 2010). In a delayed response test of spatial working memory, estrogen replacement reversed cognitive impairments associated with cognitive aging in intact aged female rhesus monkeys, while ovariectomy worsened the same symptoms (Rapp et al., 2003; Shanmugan and Epperson, 2014). Enhanced performance on working memory tasks in ovariectomized nonhuman primates receiving estradiol is thought to be in part due to estradiol-induced increases in dendritic spine density and the number of smaller, more motile dendritic spines thought to be important for synaptic plasticity (Rapp et al., 2003; Hao et al., 2007).

ACE effects on executive function

Unlike other cognitive domains, the executive system undergoes a protracted period of development that extends into young adulthood, making it particularly susceptible to the effects of early life adversity (Pechtel and Pizzagalli, 2011). The high density of glucocorticoid receptors and dopaminergic inputs make the PFC especially vulnerable (Brake et al., 2000; Pechtel and Pizzagalli, 2011). Inhibitory control and cognitive flexibility develop more slowly in preschool-aged children with less access to learning resources (Clark et al., 2013; Shanmugan and Satterthwaite, 2016). Impairments in inhibitory control (Navalta et al., 2006; Mueller et al., 2010) and PFC hyperactivation (Mueller et al., 2010) have also been observed in adolescents with high levels of early life stress (Mueller et al., 2010) as well as adult women with a history of sexual abuse (Navalta et al., 2006; Pechtel and Pizzagalli, 2011). A longitudinal neuroimaging study found an interactive effect of socioeconomic status and sex on both behavior and brain function, but no effect of socioeconomic status or sex alone (Spielberg et al., 2015; Shanmugan and Satterthwaite, 2016), highlighting the relevance

of considering interactions between early adversity and estradiol on neural function. This study suggested that inhibitory processing in the DLPFC is less efficient in females of lower socioeconomic status than those of higher socioeconomic status (Spielberg et al., 2015). The negative effects of early life stress on executive function last into adulthood. In healthy adults, early trauma has been associated with poorer working memory performance (Philip et al., 2016), executive system hyperactivation during a working memory task (Philip et al., 2016), and decreased functional connectivity within networks at rest (Philip et al., 2013). Findings from rodent studies have suggested that the impact of early life stress on later cognitive functions may become particularly acute during middle-age (Brunson et al., 2005). Significant adversity during childhood has similarly been associated with sub-optimal cognitive aging in human participants (Kremen et al., 2012; Zhang et al., 2016). Together, these studies suggest that early life adversity alters the developmental trajectory of executive functions, which may result in heightened vulnerability to executive dysfunction with loss of estradiol. Reviewing the mechanisms by which estradiol and ACE impact monoaminergic systems is necessary to understand why the effects of ACE may be particularly acute during the menopause transition.

Estradiol effects on prefrontal catecholamine systems

Estradiol modulates PFC concentrations of dopamine and norepinephrine, catecholamines crucial for executive functioning (Arnsten, 2011). Previous studies have demonstrated that treatment with 17b-estradiol both improves memory consolidation and regulates dopaminergic and noradrenergic neurotransmission in the rodent PFC. In rodents, estradiol treatment improves memory consolidation and raises concentrations of 3-Methoxy-4-hydroxyphenylglycol (metabolite of norepinephrine) and 3,4-Dihydroxyphenylacetic acid (metabolite of dopamine) in the PFC (Inagaki et al., 2010).

Estrogen benzoate or ERb agonist-induced elevations in prefrontal dopamine and norepinephrine metabolites are accompanied by improvements in recognition memory in ovariectomized rats (Jacome et al., 2010). However, treatment with significantly higher or lower doses of estradiol does not improve memory consolidation in ovariectomized rats despite increases in neurotransmitter levels observed at higher doses, indicating that estrogen's effects on cognition are dose dependent (Inagaki et al., 2010).

Estrogen also modulates these catecholaminergic systems through enzymatic regulation of transmitter metabolism. In female rats, OVX or treatment with tamoxifen, an estrogen antagonist, has been shown to elevate PFC protein expression and enzyme activity of catechol-o-methyl transferase (COMT), an enzyme involved in dopamine and norepinephrine metabolism (Schendzielorz et al., 2011). Jacobs and D'Esposito (2011) showed that the relationship between BOLD signal in the medial frontal gyrus (MFG) during performance of a working memory task (N-back task) and estradiol levels are dependent on catecholaminergic tone, as determined by COMT Val158Met genotype and enzyme activity. In this study, premenopausal women with the genotype Val/Val had greater COMT enzyme activity, and therefore metabolized dopamine more quickly, compared to women who were homozygous for the Met allele. Performance on the N-back task as well as MFG activation exhibited an inverted U-shaped curve dependent on both genotype and estradiol level. Women who were homozygous for the Val allele showed better working memory performance and increased MFG activation when estradiol levels were high compared with when estradiol levels were low, presumably because the increase in estradiol increased dopamine to optimum levels. The opposite was true of women with the genotype Met/Met. In their case, the increase in estradiol resulted in overly heightened PFC dopamine levels, pushing them to the right side of the inverted U-shaped curve and impairing executive functioning (Jacobs and D'Esposito,

2011). This literature suggests withdrawal of estrogen modulation of prefrontal catecholamine systems during menopause may be one mechanism leading to decline in executive functions (Shanmugan and Epperson, 2014).

ACE effects on prefrontal catecholamine systems

Maternal deprivation and separation paradigms have been used to model early life stress in rodents. In these rodent models of early life stress, adversity results in an increase in PFC dopamine turnover (Matthews et al., 2001; Rentesi et al., 2013) and may lower dopamine levels in comparison to controls (Rentesi et al., 2013). Rodents exposed to early life stress are more sensitive to the effects of d-amphetamine (Zimmerberg and Shartrand, 1992; Rentesi et al., 2013), a stimulant that increases concentrations of dopamine and norepinephrine, as well as apomorphine (Daskalakis et al., 2012; Rentesi et al., 2013), a dopamine agonist. Early stress also decreases dopamine D2 receptor expression in the PFC (Rentesi et al., 2013). Similarly, in nonhuman primates, early and life-long stress in the form of social subordination results in decreased D2 receptor function accompanying stressed behaviors in female monkeys (Shively, 1998).

Evidence from human participants suggests the consequences of these changes in neurotransmission manifest early in life. A large longitudinal study recently found that a child's executive functioning is determined by the interaction between genotype for COMT and history of early life adversity (Blair et al., 2015). This study found that between the ages of 3 and 5, the presence of early life adversity was associated with poorer executive function. Additionally, there was a COMT genotype x early life adversity interaction on the trajectory of executive functioning development during this time. In children with low levels of adversity, Val/Val children initially performed better on

executive functioning tasks, but children with a Met allele improved faster. In contrast, children with a Met allele and high levels of adversity demonstrated the slowest executive development, resulting in the lowest performance levels at 5 years of age among the four groups (Val/Val low adversity = Val-Met/Met-Met low adversity > Val/Val high adversity > Val-Met/Met-Met high adversity) (Blair et al., 2015).

These studies demonstrate that estradiol and early life adversity act on common molecular targets involved in catecholamine function and suggest that exposure to high levels of early life adversity may result in heightened vulnerability to loss of estradiol modulation of catecholamine function.

Estradiol effects on serotonin

Rodent and non-human primate studies have demonstrated that estradiol has profound impacts on serotonin function at the gene, enzyme, and receptor level. (Amin et al., 2005). Estradiol treatment increases serotonin concentration (Inagaki et al., 2010), synthesis (Cone et al., 1981), release (Cone et al., 1981) and turnover (Di Paolo et al., 1983), increases gene expression of tryptophan hydroxylase (the rate limiting enzyme in serotonin synthesis) (Pecins-Thompson et al., 1998), increases serotonin transporter binding (Fink et al., 1999), and increases serotonin receptor 2A mRNA and binding (Cyr et al., 1998; Fink et al., 1999; Amin et al., 2005). Using the tryptophan depletion (TD) paradigm, Epperson et al. (2012) provided novel evidence for estrogen by serotonin interactions during working memory in menopausal women. In this study, the TD paradigm was used to lower central serotonin levels in healthy menopausal women before and after treatment with transdermal estradiol. During the 2-back task, TD was associated with a decrease in BOLD signal in the right and left DLPFC and middle frontal/cingulate gyrus prior to estradiol treatment. This attenuation in BOLD was

prevented by estradiol treatment, suggesting that estradiol's effects on brain activation during working memory are in part via estradiol's effects on serotonergic function (Epperson et al., 2012; Shanmugan and Epperson, 2014).

ACE effects on serotonin

Preclinical studies have shown that early life adversity exerts lasting effects on the serotonin system (Comasco et al., 2013; van der Doelen et al., 2014a; van der Doelen et al., 2014b; van der Doelen et al., 2015), suggesting the interaction between these factors may further exacerbate executive difficulties during hypogonadism. In rodent models of early life stress, maternal deprivation results in a decrease in serotonin levels in the PFC (Matthews et al., 2001; Rentesi et al., 2013) as well as an increase in serotonin turnover (Masis-Calvo et al., 2013; Rentesi et al., 2013; Wong et al., 2015). Early life adversity also decreases tryptophan hydroxylase type 2, the rate-limiting enzyme in serotonin synthesis (Bethea et al., 2008) and increases monoamine oxidase-A (Wong et al., 2015), an enzyme involved in serotonin metabolism. Early life stress also attenuates 5-HT1A receptor function (Wong et al., 2015) and decreases 5-HT2A receptor expression (Rentesi et al., 2013) in the prefrontal cortex of adult rats. Furthermore, low serotonin levels have been shown to exacerbate the negative effects of early life stress on impulsivity in adult mice (Sachs et al., 2013). This literature indicates that estradiol and early life stress act on common serotonergic targets at the molecular level and suggests that one mechanism by which early life adversity increases risk for executive dysfunction during menopause may be via decreased serotonin concentration and receptor function.

Overview of original research chapters

The overarching purpose of this dissertation is to determine whether early life adversity is a risk factor for executive function difficulties during menopause and to examine the impact of ACE on monoaminergic function during this hormonal transition. To answer this question, we use behavioral and neuroimaging data from 3 separate samples of menopausal women with high and low levels of early life adversity.

First, we sought to determine whether ACE confers a risk for executive dysfunction during menopause. In chapter 2, we use behavioral data from a large sample of women who underwent surgical menopause to establish the impact of ACE on subjective and objective measures of executive function. We demonstrate that ACE is associated with greater symptoms of executive dysfunction as well as poorer task performance. Importantly, we show that these effects of ACE are partially mediated by anxiety and depression symptoms.

The strong associations between ACE and mood symptoms in chapter 2 suggested to us that ACE is likely associated with suboptimal serotonergic function. Given the previous work from our lab demonstrating the importance of intact serotonergic function to working memory function during menopause (Epperson et al., 2012) as well as the converging effects of estradiol and early life stress on common serotonergic targets at the molecular level, we hypothesized that one mechanism by which ACE exerts a negative impact on executive function during periods of low estradiol may be via effects on serotonin.

To test this hypothesis, we used functional magnetic resonance imaging and tryptophan depletion (TD), a paradigm used to lower central serotonin levels. We hypothesized that ACE would be associated with executive hyperactivation and that TD would exacerbate differences between ACE groups. However, given that previous studies have shown that TD may improve sustained attention and response inhibition

(Schmitt et al., 2000; Booij et al., 2005; Scholes et al., 2007), our competing hypothesis was that TD would attenuate differences in ACE groups. The common theme underlying both of these hypotheses is that, because early adversity negatively impacts multiple aspects of serotonergic function, the serotonin system of high ACE individuals would be more "vulnerable" to experimental manipulations affecting serotonin, resulting in differential response to TD.

In chapter 3, we present data consistent with our competing hypothesis. We show that in the absence of exogenous estradiol, TD increases DLPFC activation in high ACE subjects but decreases DLPFC activation in low ACE subjects. Importantly, treatment with estradiol attenuates the effects of both ACE and TD. Given that the TD x ACE interaction on BOLD signal, while consistent with our competing hypothesis, was not as initially predicated, we chose to further probe this finding by examining whether ACE and TD would show similar effects on a different neural marker of executive function: functional connectivity.

In chapter 4, we residualize task effects to obtain an approximate resting state timeseries. Using this pseudo-resting state, we examine the impact of ACE and TD on within-network connectivity, a measure shown to underlie executive functions across the lifespan (Betzel et al., 2014; Chan et al., 2014; Tsvetanov et al., 2016). We predicted that ACE would be associated with lower within-network connectivity. We also initially predicted that TD would further lower connectivity in women with high levels of early adversity but have no effect in in women with low levels of early adversity. However, given the results of chapter 3, we strongly considered the competing hypothesis that TD would attenuate differences between ACE groups by selectively increasing connectivity in the high ACE group. This was in fact what we found. We demonstrate that ACE is associated with lower within-network connectivity regardless of depletion condition. We

also show that TD increases connectivity in the high ACE group but has no effect on connectivity in the low ACE group.

We first examined whether serotonergic differences contribute to ACE effects on executive function because results of chapter 2 indicated that ACE likely alters serotonergic function and because previous work from our lab (Epperson et al., 2012) indicated that the serotonin system may be vulnerable to additional insults during periods of low estradiol. However, as reviewed earlier in the introduction, ACE likely affects executive function via multiple mechanisms involving monoamine neurotransmission.

Therefore, we next examined the impact of ACE on dopaminergic and noradrenergic function in menopausal women with subjective cognitive complaints. Because rodent studies have demonstrated that early life stress may increase sensitivity to amphetamine, we chose to use a stimulant medication as our pharmacological probe. We specifically selected lisdexamfetamine (LDX), because this medication successfully improves subjective measures of executive function in menopausal women with self-reported new-onset executive difficulties (Epperson et al., 2015).

Previous studies have shown that stimulant medications increase activation in executive regions, including the insula (Rubia et al., 2014) and DLPFC (Wong and Stevens, 2012) in subjects with attention deficit hyperactivity disorder (ADHD). Stimulants have also been shown to decrease glutamate levels in the PFC of children with ADHD (Wiguna et al., 2012). However, no brain imaging studies examining the effect of LDX on brain activation or neurochemistry have been performed. Therefore, before examining whether ACE alters response to this drug, we first characterize the impact of LDX on executive activation and neurochemistry in chapter 5. Results from chapter 5 demonstrate that LDX improves subjective symptoms of executive

dysfunction, increases insula activation, and decreases DLPFC glutamate. In chapter 6, we examine whether ACE moderates the impact of LDX on these outcomes and demonstrate that ACE is a moderator of both behavioral and brain response to LDX.

In summary, this body of work seeks to address why many healthy women with no history of cognitive dysfunction experience executive difficulties during the menopause transition. Indicators of risk for executive function difficulties at menopause are currently lacking, as is an understanding of the mechanisms contributing to this vulnerability with diminishing estradiol. Therefore, in the following chapters I will examine early life adversity as a risk factor for this decline and explore potential mechanisms by which early adversity confers this vulnerability to executive dysfunction with loss of estradiol.

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CHAPTER 2

Contribution of Mood Symptoms to Early Life Adversity Effects on Executive Function after Risk Reduction Salpingo-Oophorectomy

This chapter has been submitted for publication:

Shanmugan S, Sammel MD, Loughead J, Ruparel K, Gur R, Brown TE, Faust J, Domcheck S, Epperson CN. Contribution of Mood Symptoms to Early Life Adversity Effects on Executive Function after Risk Reduction Salpingo-Oophorectomy. (under review)

Abstract

Objective: Many women who undergo risk reduction salpingo-oophorectomy (RRSO) to reduce risk of breast and ovarian cancers experience onset of executive dysfunction. Adverse childhood experiences (ACE) may be one factor contributing to risk vs resilience for executive dysfunction after oophorectomy. Here the authors examined the effect of ACE on subjective and objective measures of executive function in women who underwent RRSO. The authors hypothesized that ACE would be associated with poorer executive function and that these effects would be mediated by mood symptoms.

Method: The authors studied 552 women who completed cognitive testing after RRSO at up to two time points (857 sessions total). Cognitive tasks included the Continuous Performance Task and letter n-back task to probe sustained attention and working memory, respectively. The ACE questionnaire was used to assess early life adversity. ACE effects on task performance and subjective symptoms of executive dysfunction were examined using generalized estimating equations. Mood (anxiety/depressive) symptoms were evaluated as a mediator of ACE effects on these outcomes.

Results: ACE was associated with more symptoms of executive dysfunction and worse performance on both cognitive tasks. Mood symptoms were a partial mediator of ACE effects on sustained attention and subjective report of executive dysfunction.

Conclusions: These data suggest that early life adversity is associated with increased risk of executive dysfunction after RRSO and that the negative effects of early adversity on executive function are in part mediated by mood symptoms, highlighting the importance of assessing anxiety and depressive symptoms in women who have undergone RRSO.

Introduction

Women with mutations in breast cancer type 1 or 2 susceptibility genes (BRCA1 and BRCA2) are at increased risk for developing breast and ovarian cancers (Finch et al., 2012). Risk reduction salpingo-oophorectomy (RRSO) by 35 – 40 years of age is the standard of care (Muto, 2015) and up to 90% of BRCA carriers undergo RRSO (Finch et al., 2012). While this procedure reduces the risk of ovarian cancer by 85-95% and the risk of breast cancers by 53-68% (Guidozzi, 2016), there are several consequences of being hypogonadal at such an early age.

Given that ovarian hormones such as estradiol and progesterone have numerous neuromodulatory and neuroprotective effects (Shanmugan and Epperson, 2014), recent studies have examined RRSO as a potential risk for central nervous system (CNS) impairment and overall decline in quality of life. For example, large cohort studies have shown that women who undergo oophorectomy before natural menopause are at increased risk of dementia (Rocca et al., 2007) and cognitive dysfunction during and after the seventh decade of life (Rocca et al., 2007; Ryan et al., 2014). More specifically, the largest declines in cognitive performance following oophorectomy occur in executive functioning domains (Kurita et al., 2016). This post-oophorectomy onset of executive difficulties may be a result of reduced estradiol modulation of brain regions such as the prefrontal cortex (PFC) that are critical to proper executive processes such as working memory, organization, focus, and sustained attention (Epperson et al., 2011; Shanmugan and Epperson, 2014; Epperson et al., 2015; Shanmugan et al., 2017).

Executive functioning capacity depends on the neurochemical environment in the PFC. Optimal executive function is maintained by a balance of factors in this region including dopamine, norepinephrine, and glutamate concentration and receptor function (Arnsten and Jin, 2014) as well as hormonal status (Jacobs and D'Esposito, 2011;

Shanmugan and Epperson, 2014). However, previous research has shown that significant adverse childhood experiences (ACE) not only alter the developmental trajectory of the executive system (Shanmugan and Satterthwaite, 2016) but also have lasting impacts on dopaminergic (Kasanova et al., 2016), noradrenergic (Zitnik et al., 2016), and glutamatergic (Llorente et al., 2012) function, which may leave the individual vulnerable to cognitive changes during periods of low estradiol such as after RRSO (Shanmugan and Epperson, 2014).

Together, these studies suggest that premature, abrupt loss of estradiol with RRSO induces executive function difficulties in many, but not all, hypogonadal women and that ACE may be one factor contributing to risk vs resilience for executive dysfunction after oophorectomy. However, no studies examining the effect of ACE on executive function after RRSO have been performed. Early life adversity has also been shown to increase risk for mood disorders during natural menopause (Epperson et al., In Press), and mood symptoms have been independently associated with executive dysfunction (Shanmugan et al., 2016). As such, it is not clear to what extent ACE effects on mood symptoms drive ACE effects on executive function in hypogonadal women.

Here we examined the effect of ACE on subjective and objective measures of executive function while rigorously controlling for multiple possible confounding variables in 552 women who underwent RRSO. We hypothesized that high levels of early life adversity would be associated with greater self-reported symptoms of executive dysfunction as well as poorer performance on neuropsychological tasks probing executive function. Furthermore, we hypothesized that these ACE effects on executive function would be partially mediated by ACE effects on mood symptoms.

Method

Participants

Subjects were recruited through mailings from the Cancer Risk Evaluation Program at the University of Pennsylvania, through local advertising, and through an advocacy group for women with genetic risk for breast and ovarian cancer (FORCE; Facing Our Risk of Cancer Empowered). Women over the age of 30 at high risk for breast or ovarian cancer who had undergone RRSO were eligible to participate. Exclusion criteria included inability to provide informed consent.

Subjects completed online surveys through Qualtrics. These surveys assessed mood, subjective and objective cognition, menopausal symptoms and related quality of life measures, early life adversity, and medication use. Subjects were asked to repeat the survey after 1 year. In this report, we consider the sample of subjects from this cohort that completed one or more outcomes of interest at least once.

Assessment of subjective executive function

Subjective symptoms of executive dysfunction were evaluated using a modified version of the 40-item Brown Attention Deficit Disorder Scale (BADDS), a validated subjective measure of executive function (Sandra Kooij et al., 2008) that assesses the frequency and severity of five domains of executive dysfunction: (1) organization and activating for work, (2) sustaining attention and concentration, (3) sustaining alertness, effort, and processing speed, (4) managing affective interference, and (5) using working memory and accessing recall. The original version of the BADDS was adapted for online use. Each item is rated on a scale from 0 to 3. Higher scores are indicative of more dysfunction. Total BADDS score served as the primary measure of subjective executive function, while BADDS subscales were secondary outcome measures.

Assessment of objective executive function

As part of a comprehensive computerized cognitive battery, (Gur et al., 2010) subjects completed two neuropsychological tasks probing executive domains. In this report, we specifically focused on measures related to executive functions given the converging evidence for the effects of estradiol and early life stress on this domain (Shanmugan and Epperson, 2014; Shanmugan and Satterthwaite, 2016). The Penn Continuous Performance Task (CPT) number and letter version (Kurtz et al., 2001) was used to assess sustained attention. During the task, subjects were instructed to respond when the image seen was a complete letter or number. The primary measure of sustained attention was *d'*, a signal detection metric that limits the influence of response bias.

A letter version of the n-back task (Ragland et al., 2002) was used to probe working memory function across four conditions that placed varying demands on working memory load: 0-back, 1-back, 2-back and 3-back. Stimuli were 4-letter non-words without vowels. In the 0-back condition, participants responded with a button press to a pre-specified target 4-letter stimulus, while subjects responded to a stimulus "n" number of stimuli before it in all other conditions. Because the 3-back condition places the greatest demands on working memory load, 3-back *d'* served as the primary measure of working memory.

Assessment of early life adversity

The Adverse Childhood Experiences (ACE) Questionnaire (Felitti et al., 1998) was used to assess history of child abuse, childhood neglect, and household dysfunction. The ACE questionnaire has been widely used to assess the association between early life stress and health-related outcomes in adult life (Felitti et al., 1998).

Using the ACE questionnaire allowed us to consider the effects of overall early life adversity as a predictor of cognitive dysfunction rather than examining the consequences of a particular trauma or stressor. The number of exposures was summed to create the ACE score (range: 0–10). Based on evidence from prior studies indicating increased risk of depressive disorders in later life in subjects with 2 or more ACEs (Chapman et al., 2004; Epperson et al., In Press), we considered subjects with an ACE score ≥ 2 “high ACE” and subjects with an ACE score of < 2 “low ACE”.

Assessment of mood symptoms

Presence of depressive and anxiety symptoms was assessed using the 14-item Hospital Anxiety and Depression Scale. This scale contains a 7-item depression subscale that assesses changes in mood, loss of interest, and psychomotor slowing and a 7-item anxiety subscale that assesses mental agitation and psychological distress. Each item is rated on a scale of 0 to 3. In line with prior studies of executive functions that have represented anxiety and depressive symptoms as a composite “mood” score (Shanmugan et al., 2016), total scores on the depression and anxiety subscales were summed to create a composite measure of mood symptoms.

Statistical analyses

Generalized estimating equations implemented using the *geepack* package (HÃ¸jsgaard et al., 2006) in R (R Core Team, 2015) were employed to evaluate the effect of ACE on executive outcomes. This method accommodates multiple survey assessments per woman, and adjusts for non-independence of these repeated measures. Age, time since oophorectomy, and age at oophorectomy were assessed as possible covariates. However, at baseline, age was highly correlated with both age at

oophorectomy ($r=0.85$, $p<0.0001$) and time since oophorectomy ($r=0.49$, $p<0.0001$). To fully account for the effects of age on cognitive outcomes, generalized estimating equations with cognitive measures as the outcomes and age as the predictor were used to obtain age-residualized scores for the BADDS, CPT, and n-back outcomes. Subsequent analyses were performed on these residuals rather than raw values. Time without hormones (a summary of time since oophorectomy and duration of hormone therapy use, if any), education level, and history of chemotherapy use were included as covariates in all models. Significance was defined as $p<=0.05$.

Mediation models were used to examine whether ACE confers a risk for executive dysfunction after RRSO via increased mood symptoms. The three primary outcome measures served as the dependent variable in 3 separate analyses. Total ACE served as the independent variable, while the composite mood score served as the mediator. The indirect effect was estimated using the Product of Coefficients approach followed by significance testing using a bootstrap resampling approach implemented with the *boot* package (Canty and Ripley, 2016) using ordinary nonparametric bootstrapping and 2,000 iterations.

Psychotropic medication use served as an additional covariate in supplemental analyses of primary outcome variables. Supplementary analyses also examined the effects of ACE in the subset of women who had undergone oophorectomy prior to age 47.4, 2 standard deviations (1 SD=2.26 years) below the average age (51.9 years) of post-menopause onset (Freeman et al., 2012). To evaluate whether timing of adversity was driving results, we also separately examined the effects of pre-pubertal ACE and post-pubertal ACE on primary outcome measures. Puberty was defined as 2 years prior to menstrual cycle onset.

Results

Participants

Eight hundred subjects completed cognitive testing at baseline and were eligible for inclusion in this report. Of these 800 subjects, 453 repeated cognitive testing after 1 year, yielding a total of 1,253 sessions that were eligible for inclusion. Sessions included in analyses must have completed at least one outcome of interest (n=1,058). Sessions missing information regarding age at testing (n=16), hormone therapy use (n=165), ACE (n=12), education level (n=8), and mood symptoms (n=30) were excluded. As part of data quality assurance, subjects who performed below chance (<50% true positives) on the CPT were excluded from analyses of the CPT (n=20). Similarly, subjects who performed below chance (<5 true positive responses) on the 0-back control condition were excluded from analyses of the n-back (n=13). The final sample included in analyses was 552 subjects (**Table 2-1**; **Figure 2-1**). Complete data for at least one outcome measure and all primary covariates were obtained on 494 subjects at baseline and 363 subjects at the 1-year follow-up session, yielding a total of 857 sessions included in analyses.

ACE effects on executive function

ACE was associated with higher total BADDS score (β= 7.1, p=0.0005) (**Figure 2-2a**) as well as each BADDS subscale (organization/activation for work, β=1.7, p= 0.002; attention/concentration, β=1.8, p=0.001; alertness/effort/processing speed, β=1.4, p=0.004; managing affective interference, β=1.3, p=0.0007; working memory/accessing recall, β=0.8 p=0.03) (**Figure 2-2b**). The high ace group also performed worse on both the n-back (β=-0.17, p=0.007) (**Figure 2-3a**) and CPT (β=-0.1, p=0.03) (**Figure 2-3b**) in comparison to the low ACE group. Chemotherapy use also had a negative impact on

sustained attention (β=-0.17, p=0.006). Time in a hypogonadal state was not significantly associated with any outcome measure.

Mood symptoms mediate ACE effects on executive function

We conducted mediation analyses to determine whether ACE effects on executive function are mediated by ACE effects on mood symptoms. Mood symptoms partially mediated the relationship between ACE and total BADDS score (**Figure 2-4a**). After accounting for the effects of previous chemotherapy use, education, and length of time hypogonadal, ACE was significantly associated with mood symptoms (β=2.1, p=0.0003) and mood symptoms were significantly associated with age-residualized total BADDS scores (β=2.1, p<0.0001). ACE continued to be associated with total BADDS scores when mood symptoms were included as a covariate (β=2.8, p=0.08). Mood symptoms mediated 62.8% (95% CI: 42.3% - 100%) of ACE effect on total BADDS score.

Mood symptoms partially mediated ACE effects on sustained attention (**Figure 2-4b**). ACE was significantly associated with higher mood symptoms (β=2.1, p=0.0002) and mood symptoms were significantly negatively associated with age-residualized CPT *d'* (β=-0.01, p=0.001). ACE continued to be associated with CPT *d'* when mood symptoms were included as a covariate (β=-0.09, p=0.08). Mood symptoms mediated 21.3% (95% CI: 9.3% - 100%) of ACE effects on CPT *d'*.

In contrast to the role of mood symptoms as a mediator of ACE effects on subjective executive function and sustained attention, mood symptoms did not mediate a significant proportion of the relationship between ACE and working memory performance (95% CI: -1.4% – 50%) (**Figure 2-4c**). However, ACE effects on n-back *d'* remained significant when mood symptoms were included as a covariate (β=-0.16, p=0.01).

Supplementary analyses

ACE effects on subjective executive function (β=6.1, p=0.002), sustained attention (β=-0.11, p=0.03), and working memory (β=-0.16, p=0.01) were similar when controlling for use of psychoactive medications. Results were also similar in the subset of women who underwent oophorectomy before age 47.4 (total BADDS, n=353, β =8.4, p=0.0008; CPT *d'*, n=350, β = -0.12, p=0.06; n-back *d'*, n=382, β = -0.16, p=0.06). While pre-pubertal ACE was associated with significantly more subjective executive difficulties (β=5.6, p=0.03), post-pubertal ace was not. In contrast, post-pubertal ACE was associated with worse sustained attention (β=-0.24, p=0.006), while pre-pubertal ACE was not. Pre-pubertal ACE (β=-0.13, p=0.08) and post-pubertal ACE (β=-0.22, p=0.07) were both associated with poorer working memory.

Discussion

In this large study of cognitive function in women who underwent RRSO, we examined associations between early life adversity and subjective and objective measures of executive function. To account for the fact that early adversity is associated with increased risk of mood disorders with loss of estradiol, we conducted mediation analyses to determine the extent to which ACE effects on executive function result from concurrent mood changes. Women with high levels of early life adversity reported more symptoms of dysfunction across executive domains. High ACE women also performed worse on executive tasks probing sustained attention and working memory. While mood symptoms partially mediated ACE effects on sustained attention and subjective report of executive dysfunction, the associations between ACE and poorer subjective and objective measures of executive function remained present after accounting for the

effects of anxiety and depression symptoms. Taken together, these data emphasize that early life adversity is associated with increased risk of executive dysfunction after RRSO.

Evidence for ACE effects on executive function

As predicted, we found that ACE was associated with greater subjective report of executive dysfunction as well as poorer performance on neuropsychological tasks of executive function. Several studies have demonstrated early life adversity is associated with poorer executive function during childhood and adolescence (Hostinar et al., 2012; Loman et al., 2013; Heyman and Hauser-Cram, 2015) and that this negative effect persists into adulthood (Philip et al., 2016). For example, a study of 90 young children found that early psychosocial deprivation was associated with lower composite scores of executive function after controlling for IQ (Hostinar et al., 2012). Such deprivation has been associated with slower neural responses and attentional processing in older children (Loman et al., 2013). Similarly, early life stress has been associated with poorer working memory performance and aberrant activation in associated brain regions in healthy adults (Philip et al., 2016). This literature suggests early life adversity likely alters the developmental trajectory of the executive system (Shanmugan and Satterthwaite, 2016).

Animal models suggest the mechanisms underlying the detrimental effects of early adversity on executive function development are likely multifold. For example, early life stress in mice has been shown to induce alterations in PFC dendritic architecture (Yang et al., 2015). Early adversity also alters dopaminergic (Kasanova et al., 2016), noradrenergic (Zitnik et al., 2016), serotonergic (Matsuzaki et al., 2009), and glutamatergic (Llorente et al., 2012) function in the PFC. Importantly, estradiol supports the normal development and maintenance of each of the above neurotransmitter

systems (Shanmugan and Epperson, 2014). Additionally, the effects of early adversity and estradiol converge on common molecular targets at the synaptic level (Shively et al., 2003; Amin et al., 2005; Matsuzaki et al., 2009; Michopoulos et al., 2014; Shanmugan and Epperson, 2014). Together, this literature suggests abrupt loss of estradiol regulation of these neurotransmitter systems after RRSO is likely a key mechanism underlying the observed differences in executive function between high and low ACE groups (Shanmugan and Epperson, 2014).

Evidence for mood as a mediator of ACE effects on executive function

ACE effects on subjective report of executive dysfunction and ACE effects on sustained attention performance after RRSO were partially mediated by anxiety and depressive symptoms. Many healthy women experience onset of executive function difficulties during menopause concurrent with loss of estradiol (Epperson et al., 2011; Epperson et al., 2015; Shanmugan et al., 2017). During this hormonal transition, women with no history of depression are also at increased risk of new onset depressive symptoms (Freeman et al., 2014). Early life adversity has been suggested as a risk factor for both executive function difficulties (Shanmugan and Epperson, 2014) as well as mood changes (Epperson et al., In Press) during periods of waning estradiol. Mood symptoms are also associated with executive system dysfunction (Shanmugan et al., 2016), highlighting the importance of jointly considering the role of early adversity and mood changes on executive functions after RRSO. Notably, the mediating effect of mood and timing of pre- vs post-pubertal adversity varied across domains, suggesting future studies of ACE effects on executive function would benefit from further dissociation between sustained attention, working memory, and subjective report of

executive dysfunction symptoms as well as timing of adversity onset in relation to puberty.

Our results highlight that addressing concurrent mood changes is a critical step in treating either natural or surgical menopause-induced executive difficulties. Importantly, we found that ACE effects were mediated by anxiety and depressive *symptoms*, which may or may not translate to a full diagnosis of generalized anxiety disorder, major depressive disorder, or other mood disorder. The mediating effect of mood was significant even though the average anxiety and depression subscale scores in this sample (Table 1) were below those corresponding to full categorical diagnoses of anxiety (8+) or depressive disorders (8+) (Bjelland et al., 2002), further emphasizing the importance of screening for sub-threshold mood symptoms in this population. However, mood was a partial, not full, mediator of ACE effects on BADDS and CPT performance. Additionally, mood did not mediate ACE effects on working memory. Furthermore, ACE effects on working memory remained significant when accounting for the effects of mood symptoms, age, chemotherapy use, time since oophorectomy, hormone therapy use, and education. Together, these data suggest that mood symptoms are only one component of the mechanism underlying new onset cognitive dysfunction with loss of estradiol and that at least a portion of ACE-associated risk for executive difficulties is independent of the association between ACE and anxiety and depressive symptoms. Further research is necessary to determine these mood-independent mechanisms, but dopaminergic (Shanmugan et al., 2017), glutamatergic (Shanmugan et al., 2017), and serotonergic (Shanmugan and Epperson, 2014) pathways likely play a role.

Limitations

While this study had a large sample size and controlled for multiple possible confounds, certain limitations should be acknowledged. First, subjects completed cognitive testing in an uncontrolled environment, potentially reducing validity of these neuropsychological tests. However, excluding subjects who performed below chance on control conditions allowed us to limit this effect. Additionally, such a study design enabled us to examine the effects of early adversity on cognition post-RRSO at a scale that would have been much less feasible with in-office visits. Second, while a previous study (Ryan et al., 2014) found effects of age at oophorectomy on cognitive outcomes, we did not find a similar effect of time without hormones. This difference may be due to differences in the predictive variable examined as well as the fact that that study was conducted in a much older population (age at baseline > 65). Third, all subjects in this study were surveyed after undergoing RRSO. As such, while results demonstrate that ACE is associated with worse executive function after RRSO, it is not clear whether ACE has similar effects prior to RRSO. Longitudinal studies testing subjects before and after RRSO would be necessary to determine whether the impacts of RRSO and ACE on executive function are synergistic or additive.

Conclusions

In summary, these data provide novel evidence regarding the impact of early life adversity on executive function after RRSO. These results emphasize that the negative effects of early adversity on executive function are at least in part mediated by ACE effects on mood, underscoring the importance of assessing anxiety and depressive symptoms in women who have undergone RRSO. These results suggest that the neurotransmitter systems underlying these processes, including serotonin, glutamate,

and dopamine, may be important targets of interventions that seek to improve executive function in this population. Further research utilizing longitudinal study designs and multi-modal neuroimaging would be helpful in elucidating ACE x RRSO effects on glutamatergic and monoaminergic function.

Table 2-1. Participant Characteristics

	Low ACE	High ACE
	Mean (SD) or number	
n	350	202
Age (years)	47.2 (7.2)	48.1 (7.9)
Age at oophorectomy (years)	43.4 (6.0)	43.9 (7.3)
Time since oophorectomy (months)	45.6 (46.2)	49.3 (48.3)
Time without hormones (months)	30.8 (41.3)	33.3 (43.0)
History of cancer	156	101
Previous hormone therapy use	150	80
Current hormone therapy use	98	52
Previous chemotherapy use	94	65
Education		
Graduate school	155	94
College	155	68
Some College	34	32
High school	3	8
Some high school	1	0
8th grade or less	2	0
Race		
Caucasian	326	181
African American	2	4
Asian	3	0
Hispanic	8	6
Other	6	3
Unknown or preferred not to disclose	10	8
Mood symptoms		
HADS total score	11.3 (6.7)	13.5 (7.1)
HADS depression subscale score	5.5 (3.8)	6.7 (4.0)
HADS anxiety subscale score	5.7 (3.6)	6.9 (3.4)
Current psychoactive medication indication or class		
Attention deficit disorder	4	4
Depression	98	62
Anxiety	9	12
Neurological conditions	13	7
Narcotics	3	2
SERM antagonist/Aromatase inhibitor	43	30
Tamoxifen/SERM	32	16
Mean ACE	0.32 (0.47)	3.4 (1.6)
Abuse		
Emotional abuse	6	119
Physical abuse	4	78
Sexual abuse	25	51
Neglect		
Emotional neglect	11	102
Physical neglect	1	25
Household dysfunction		
Parents divorced/separated	37	107
Witnessed domestic violence	2	26
Drug abuse in household	10	93
Mental illness in household	7	93
Incarceration of family member	2	13

ACE=Adverse Childhood Experiences; HADS=Hospital Anxiety and Depression Scale; SERM=Selective Estrogen Receptor Modulator

Figures

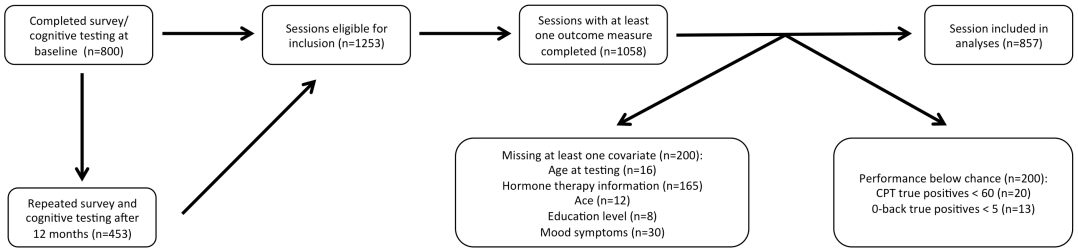


Figure 2-1. Consort diagram. Eight hundred subjects completed cognitive testing at baseline and 453 subjects repeated cognitive testing after 1 year, yielding a total of 1,253 sessions that were eligible for inclusion. Sessions included in analyses must have completed at least one outcome of interest (n=1,058). Sessions missing information regarding age at testing (n=16), hormone therapy use (n=165), ACE (n=12), education level (n=8), and mood symptoms (n=30) were excluded. Subjects who performed below chance (<50% true positives) on the CPT were excluded from analyses of the CPT (n=20). Similarly, subjects who performed below chance (<5 true positive responses) on the 0-back control condition were excluded from analyses of the n-back (n=13). Complete data for at least one outcome measure and all primary covariates were obtained on 494 subjects at baseline and 363 subjects at the 1-year follow-up session, yielding a total of 552 subjects and 857 sessions included in analyses. ACE=adverse childhood experiences

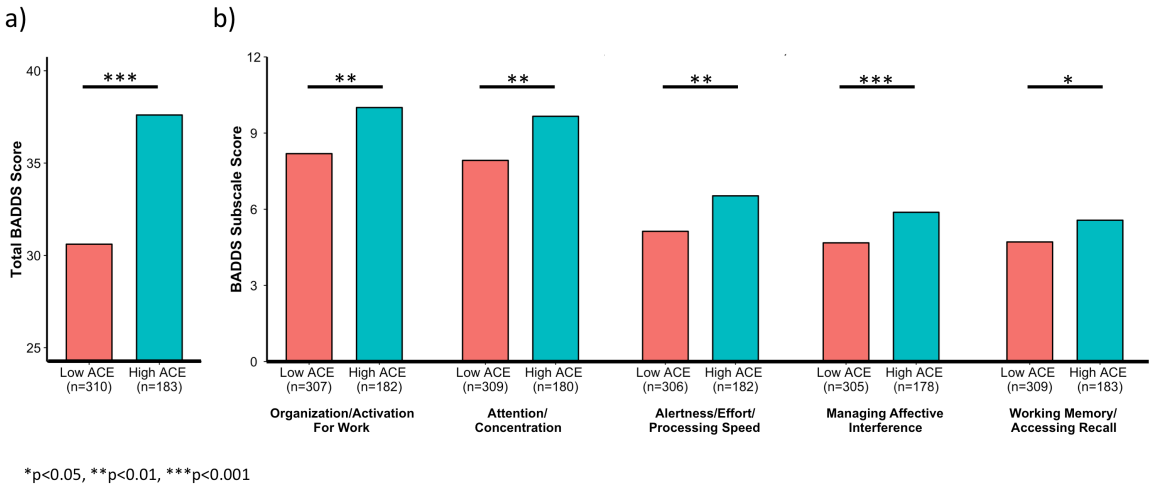


Figure 2-2. ACE effects on subjective symptoms of executive dysfunction. ACE was associated with higher total BADDS score (β= 7.1, p=0.0005) (**a**) as well as each BADDS subscale (**b**) (organization/activation for work, β=1.7, p= 0.002; attention/concentration, β=1.8, p=0.001; alertness/effort/processing speed, β=1.4, p=0.004; managing affective interference, β=1.3, p=0.0007; working memory/accessing recall, β=0.8 p=0.03). Bars represent means. BADDS=Brown Attention Deficit Disorder Scale. ACE=adverse childhood experiences

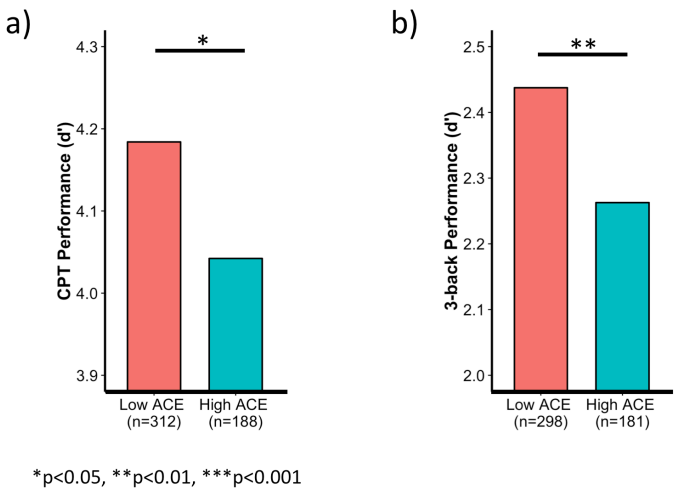
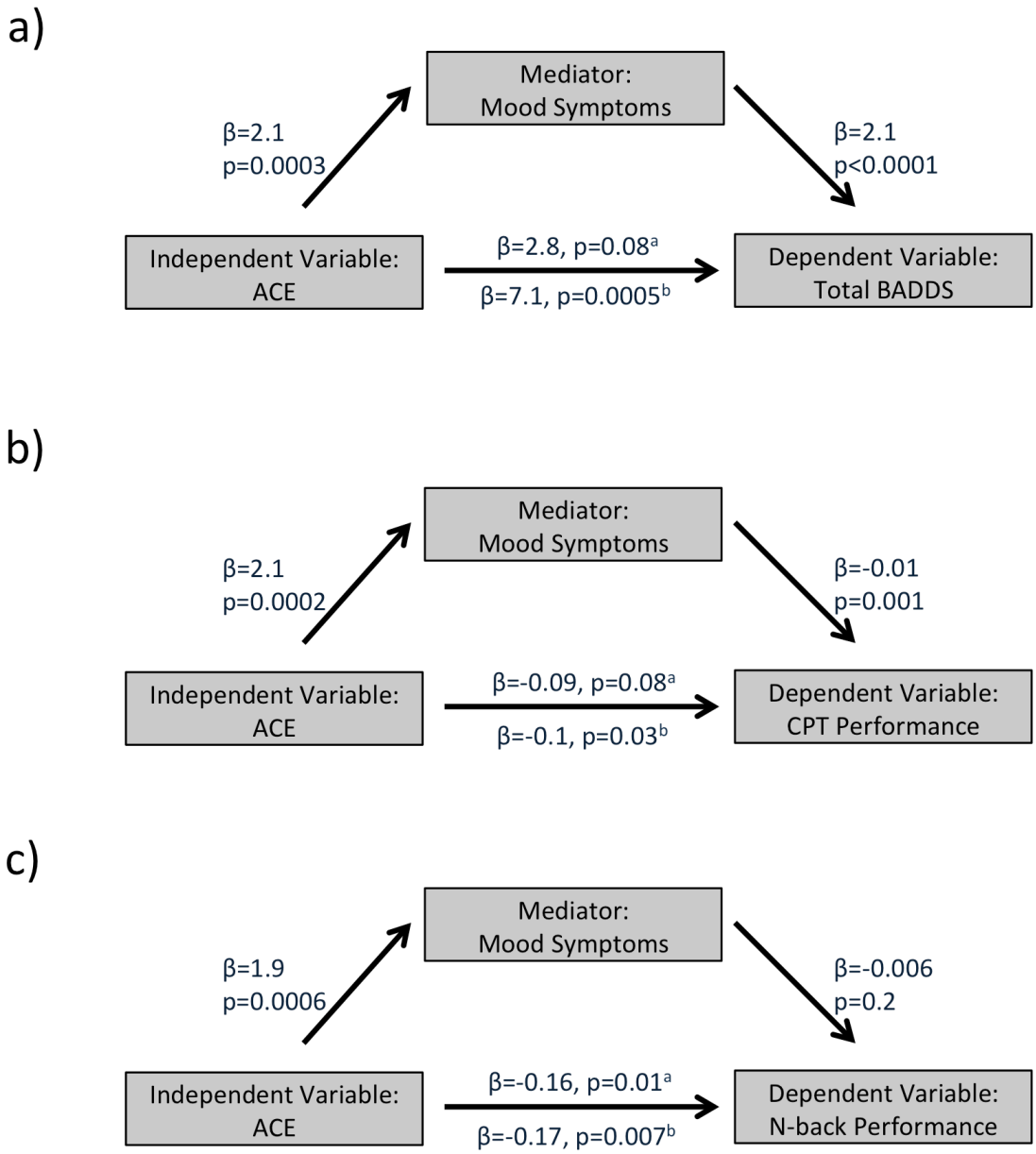


Figure 2-3. ACE effects on sustained attention and working memory. When controlling for age, time without hormones, chemotherapy use, and education level, ACE was associated with worse performance worse on both the CPT (β=-0.1, p=0.03) (**a**) and n-back (β=-0.17, p=0.007) (**b**). Bars represent means. CPT=continuous performance task. ACE=adverse childhood experiences.



[a] Direct effect of ACE on dependent variable
[b] Total effect of ACE on dependent variable

Figure 2-4. Mood symptoms mediate ACE effects on executive function. a) Mood symptoms partially mediated the relationship between ACE and total BADDS score. ACE was significantly associated with total BADDS scores (β=7.2, r^2=0.03, p=0.0005). ACE was significantly associated with mood symptoms (β=2.1, p=0.0003), mood

symptoms were significantly associated with age-residualized total BADDS scores (β=2.1, p<0.0001), and ACE was associated with BADDS scores when mood symptoms were included as a covariate (β=2.8, r^2=0.41, p=0.08). Mood symptoms mediated 62.8% (95% CI: 42.3% - 100%) of ACE effect on total BADDS score. **b**) Mood symptoms partially mediated ACE effects on sustained attention. ACE was significantly associated with CPT performance (β=-0.1, r^2=0.02, p=0.03). ACE was associated with greater mood symptoms (β=2.1, p=0.0002), mood symptoms were negatively associated with age-residualized CPT *d'* (β=-0.01, p=0.001), and ACE was associated with CPT *d'* when mood symptoms were included as a covariate (β=-0.09, r^2=0.04, p=0.08). Mood symptoms mediated 21.3% (95% CI: 9.3% - 100%) of ACE effects on CPT *d'*. **c**) ACE was significantly associated with working memory performance (β=-0.17, r^2=0.02, p=0.007). Mood symptoms did not mediate a significant proportion of the relationship between ACE and working memory performance (95% CI: -1.4% – 50%). However, ACE effects on n-back *d'* remained significant when mood symptoms were included as a covariate (β=-0.16, r^2=0.02, p=0.01). BADDS=Brown Attention Deficit Disorder Scale. CPT=continuous performance task. ACE=adverse childhood experiences

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CHAPTER 3

Impact of Tryptophan Depletion on Executive System Function during Menopause is Moderated by Childhood Adversity

This chapter has been accepted for publication:

Shanmugan S, Loughead J, Cao W, Sammel MD, Satterthwaite TD, Ruparel K, Gur R, Epperson CN (In Press). Impact of Tryptophan Depletion on Executive System Function during Menopause is Moderated by Childhood Adversity. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology

Abstract

Many healthy women with no history of cognitive dysfunction experience subjective executive difficulties during menopause. Preclinical literature suggests latent effects of early life adversity on serotonin function may play a role in this phenomenon. However, evidence in human participants regarding the mechanisms by which loss of estradiol contributes to this vulnerability is lacking. Here we examined the impact of tryptophan depletion (TD) and adverse childhood experiences (ACE) on brain activation during a working memory task in menopausal women. We hypothesized that an interactive effect between ACE and TD would be observed when women were hypogonadal, and that treatment with estradiol would attenuate this effect. Thirty-three women underwent functional imaging at four time points (123 total scans) in this double-blind, placebo controlled, crossover study. The effects of TD, ACE, and TD x ACE were evaluated using a voxelwise, mixed-effects, 2 x 2 ANOVA. In the absence of exogenous estradiol, a TD by ACE interaction was observed on BOLD signal in the right DLPFC such that TD increased activation in high ACE subjects but decreased activation in low ACE subjects. While a similar interaction was observed with placebo treatment, treatment with estradiol attenuated the effects of ACE and TD such that no between or within group differences were observed. Together, these results suggest that early life adversity may have a lasting impact on serotonergic circuits underlying executive function that are unmasked by loss of estradiol during menopause.

Introduction

During menopause, many healthy women experience new onset subjective difficulties with executive processes involving sustained attention, motivation for work, organization and working memory (Epperson et al., 2015; Shanmugan et al., 2017). Onset of these executive difficulties coincides with waning estradiol levels and may be a result of reduced estradiol modulation of the prefrontal cortex (PFC) (Shanmugan and Epperson, 2014). We previously showed that estradiol modulation of serotonergic function in the dorsolateral PFC (DLPFC) may be particularly important in maintaining intact working memory processes in menopausal women (Epperson et al., 2012). Early life experiences may also shape an individual's executive function during this hormonal transition (Shanmugan and Epperson, 2014). Significant adversity during childhood alters executive system development (Bruce et al., 2013; Shanmugan and Satterthwaite, 2016), resulting in poor working memory and executive system hyper-activation into adulthood (Majer et al., 2010; Bruce et al., 2013; Philip et al., 2016) as well as sub-optimal cognitive aging (Barnes et al., 2012). Moreover, adverse childhood experiences (ACE) have lasting impacts on serotonergic function (Comasco et al., 2013; van der Doelen et al., 2015), which may leave individuals vulnerable to cognitive changes during periods of low estradiol such as during menopause (Epperson et al., 2011; Shanmugan and Epperson, 2014; Epperson et al., 2015; Shanmugan et al., 2017).

Non-human primate studies indicate effects of early life stress and estradiol converge on key aspects of serotonergic function. Specifically, reduction in tryptophan hydroxylase type 2 (TPH2) gene expression resulting from social subordination stress in ovariectomized macaques is ameliorated by treatment with estradiol (Bethea et al., 2008). In non-stressed, ovariectomized rats and macaques, chronic estradiol treatment not only increases serotonin synthesis via TPH2, but also enhances serotonin release

and turnover, increases serotonin transporter binding, and increases serotonin receptor 2A mRNA and binding (Amin et al., 2005). Together, this preclinical literature suggests early life stress and estradiol act on common serotonergic targets at the molecular level. However, what roles early adversity and the interaction between early adversity and serotonin function play in conferring vulnerability to executive dysfunction during menopause is unknown. Human participants studies examining ACE effects on serotonergic function during conditions of low and high estradiol are needed to determine whether early adversity increases risk for executive dysfunction during menopause via effects on serotonin.

We used tryptophan depletion (TD) and a double-blind, placebo-controlled, cross-over design in 33 healthy menopausal women with high and low levels of early life adversity. We hypothesized that ACE would be associated with aberrant activation in the DLPFC. Additionally, we predicted TD would differentially impact DLPFC activation in high vs low ACE hypogonadal women. Moreover, we hypothesized that estradiol treatment would attenuate ACE and TD effects.

Methods and Materials

Participants were healthy women ages 48-60 with no psychiatric diagnoses and within 10 years of their last menstrual period. Participants were right-handed and had a FSH > 30 IU/ml, a normal mammogram within the last year, and a clear urine toxicology. Exclusion criteria included use of hormone therapy within the last year, contraindications to hormone therapy, IQ < 95, Mini Mental Status Exam Score < 25, lifetime history of psychotic or bipolar disorder, other psychiatric or substance use disorder in the last year, Hamilton depression score > 14, psychotropic medication use within the last month, metallic implant, and claustrophobia. This study was approved by the Institutional

Review Board at the University of Pennsylvania. All participants provided written informed consent.

Study design

Participants underwent 4 test days in this double-blind, placebo-controlled, cross-over study (**Figure 3-1a**). During test days 1 and 2 (phase 1), participants underwent 2 imaging sessions: active TD or sham TD. The order of condition was counterbalanced and double-blind. After phase 1, participants were randomized to 17β-estradiol (Vivelle Dot® 0.100 mg/d) or placebo patch treatment for 10 weeks. After completing ~8 weeks of estradiol or placebo, participants underwent active TD and sham TD (phase 2).

On test days, participants ingested capsules containing either amino acids without tryptophan (active TD) or microcellulose (sham TD). Blood was taken for free tryptophan analysis (**Figure 3-1b**) and mood ratings were completed prior to consumption of capsules and approximately 6 hours later, after which participants underwent neuroimaging. Subjects with percent change in tryptophan approximately >3 SD from the average for the assigned depletion status were excluded to ensure that tryptophan levels were consistent with the assigned condition.

Assessment of early life adversity

The Adverse Childhood Experiences Questionnaire (Felitti et al., 1998) was used to assess history of emotional, physical, or sexual abuse, childhood neglect, and household dysfunction. The ACE questionnaire assesses the number of exposures rather than severity (Teicher and Parigger, 2015) and has been used extensively to assess the association between early adversity and later life health outcomes (Felitti et al., 1998). Number of exposures was summed to create the ACE score (range: 0–10). Subjects with an ACE score ≥ 2 were considered “high ACE” while subjects with ACE

score of < 2 were considered “low ACE”. A threshold of 2 was used to define the high ACE group based on studies of depression prevalence in later life that demonstrate increased susceptibility at this level of exposure (Chapman et al., 2004; Epperson et al., In Press).

Assessment of mood

Mood was measured as a possible confound using the Profile of Mood States. Total score and “Depression-Dejection” sub-score assessed changes in overall mood and depressive symptoms, respectively. Difference in mood score (AM-PM) served as the outcome in regression models used to assess the effects of TD and ACE on mood.

Task paradigm

A letter n-back task was used to probe executive system function during fMRI. The task included four conditions: 0-back, 1-back, 2-back and 3-back. Stimuli were 4-letter non-words without vowels presented for 500 ms, followed by an interstimulus interval of 2500 ms. In the 0-back condition, participants responded to a target stimulus. In all other conditions, participants responded to a stimulus “n” number of stimuli before it. Each condition consisted of 3 20-trial blocks (60s; 1:2 target-foil ratio). Visual instructions (9s) preceded each block. Baseline rest periods occurred at the beginning (72s), middle (24s), and end (24s) of acquisition. Task duration was 924s. Equivalent n-back tasks with unique stimuli were used for each session and version order was counter-balanced. Performance measures of interest were overall true positive count, false positive count, and median true positive response time.

Image acquisition and processing

Imaging data were acquired on 3T Siemens Trio scanner. A magnetization-prepared, rapid acquisition gradient echo T1-weighted image (TR=1810 ms, TE=3.51 ms, FOV=180 x 241mm, matrix=192 x 256, 160 slices, effective voxel resolution of 0.94 x 0.94 x 1 mm) was acquired to aid spatial normalization to standard space. Functional images were acquired using a whole-brain, single-shot gradient-echo echoplanar sequence with the following parameters: TR/TE=3000/32 ms, FOV=192 × 192mm, matrix=64 x 64, slice thickness/gap=3/0 mm, 46 slices, effective voxel resolution of 3 x 3 x 3 mm.

BOLD time series data were skull stripped with BET (Smith, 2002), despiked with AFNI's 3dDespike (Cox, 1996), motion-corrected with MCFLIRT (Jenkinson et al., 2002), high pass filtered (120s), spatially smoothed (6mm FWHM), and mean-based intensity normalized. Subject-level timeseries analyses were carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al., 2001; Woolrich et al., 2009). The four condition blocks (0-back, 1-back, 2-back, and 3-back) and their temporal derivatives were modeled using a canonical (double-gamma) hemodynamic response function with standard plus extended motion parameters included as nuisance covariates. The rest condition (fixation point) served as the unmodeled baseline. Functional and anatomical volumes were co-registered using boundary-based registration (Greve and Fischl, 2009). The anatomical image was normalized to the MNI 152 T1 1mm template using diffeomorphic SyN registration in ANTs (Klein et al., 2009; Avants et al., 2011). Co-registration, normalization, and down-sampling to 3 mm^3 were concatenated so only one interpolation was performed. Images were assessed for excessive motion (mean relative displacement > 0.5 mm) and low temporal signal-to-noise ratio (tSNR < 2 SD).

Group-level analyses

Analyses of imaging data were completed using voxelwise linear mixed-effects models with FSL's FLAME1 procedure. A two-way, whole-brain, voxel-wise, mixed-effects ANOVA was used to determine the effects of TD, ACE, and TD x ACE on brain activation during phase 1. The primary analysis focused on the parametric contrast; 0-, 1-, 2-, and 3-back were weighted -3, -1, 1, and 3 to model a linear increase in activity across conditions. The parametric contrast is most sensitive to linear effects in signal associated with increasing working memory load and may not capture non-linear effects. Therefore, we also examined the 3-back > 0-back contrast. As prior imaging studies found effects of early life stress at 0-back > baseline (Philip et al., 2016), we also examined this contrast. To adequately control for Type I error (Eklund et al., 2016) and limit the probability of Type 2 error, we implemented stringent cluster correction procedures using 10,000 Monte-Carlo simulations executed with AFNI's 3dClustSim program (Cox, 1996). Parameters selected include voxel height threshold of $z>3.1$, cluster probability of $p<0.05$, non-Gaussian (long-tailed) filtering, padded simulated volumes to account for edge effect artifacts, 1-sided thresholding, and first-nearest neighbor clustering (clustering of above threshold voxels if faces touch). Smoothness was estimated with AFNI's 3dFWHMx program using residuals of the group level analysis and spatial autocorrelation computed as a function of the radius using a mixed Gaussian plus mono-exponential model (Eklund et al., 2016). Mean percent signal change from significant voxels was extracted for further statistical testing and visualization. Signal was also extracted from the 1-back>0-back and 2-back>0-back contrasts to investigate effects of TD and ACE at varying levels of working memory load (**Figure 3-S1**). To examine whether estradiol attenuated the impact of ACE and TD on BOLD, signal from significant phase 1 voxels was extracted from phase 2 scans.

Statistical analyses

Linear mixed-effects models implemented using the *nlme* package (Pinheiro et al., 2016) in R (R Core Team, 2015) were employed to evaluate the effect of TD, ACE and their interaction on mood, behavior, and BOLD signal during phase 1 as well as the 4-way interaction between ACE group x TD status x estradiol/placebo group x study phase. All models accounted for repeated measures and controlled for age, estradiol level, and time since last menstrual period. Hypotheses regarding the impact of TD and ACE status under estradiol and placebo conditions on significant phase 1 outcome measures were then tested using this modeling framework. Test day was included as an additional covariate in secondary analyses of significant behavioral findings to account for practice effects. Difference in percent errors between 3-back and 0-back was included as a covariate in supplementary analyses to account for differences in task performance between back levels that may be reflected in the 3-back > 0-back BOLD contrast. The square root of the coefficient of determination from a linear mixed-effects model was used to estimate the partial correlation between BOLD signal within the DLPFC and behavior while accounting for repeated measures. Spearman's rank correlation was used to examine the relationship between ACE as a continuous variable and BOLD signal during sham depletion. Statistical tests were two-sided with $p<=0.05$ considered significant.

Results

Participants

Sixty participants completed screening and were enrolled in this study. Two participants self-withdrew before the first test day and 3 were lost to follow-up. Six participants did not complete the first test day due to inability to complete the depletion

procedure (n=1), claustrophobia (n=4), and lack of space (n=1) inside the MRI scanner. All study procedures were otherwise well tolerated with no serious or unintended adverse events. Three participants self-withdrew prior to the second test day, and one subject was withdrawn due to artifacts produced by dental fillings. Per a priori exclusion criteria, participants were excluded from phase 1 analyses for excessive motion (n=0), having a percent change in tryptophan level approximately greater than 3 SD from the average for the assigned depletion status (n=3), or missing data regarding ACE (n=3), imaging (n=1), or tryptophan levels (n=4). The subject with the lowest tSNR (< 2 SD) was flagged for further inspection; independent component analysis confirmed lack of n-back network activation and the subject was excluded.

The final sample included in phase 1 analyses consisted of 19 low ACE and 14 high ACE participants (**Table 3-1**, **Table 3-S1**). One of these participants was lost to follow-up prior to randomization. Only subjects included in phase 1 analyses were considered for inclusion in analyses of phase 2. Sessions during phase 2 that had percent change in tryptophan level approximately 3 SD from the average for each condition (n=2), did not complete imaging (n=1), or had missing tryptophan levels (n=4) were excluded from analyses of phase 2. The final sample included in phase 2 analyses consisted of 10 low ACE participants (19 sessions) and 11 high ACE participants (19 sessions) randomized to estradiol and 8 low ACE participants (13 sessions) and 3 high ACE participants (6 sessions) randomized to placebo.

Behavioral results

Active TD significantly decreased free tryptophan concentration in comparison to sham TD ($p<0.0001$; **Figure 3-1b**). There was not a significant difference in percent change in tryptophan levels between ACE groups. As expected, increasing working

memory load was associated with fewer correct responses to targets and greater false positive responses to foils (**Figure 3-1c**). During phase 1 (pre-randomization), active TD increased true positive responses (p=0.03) in comparison to sham depletion, though had no effect on false positive responses or reaction time. This main effect of TD on true positive count remained significant (p=0.04) when accounting for non-significant practice effects, though was not present in the smaller subgroups randomized to estradiol or placebo. High ACE was associated with slower true positive reaction time in comparison to low ACE during phase 1 (p=0.1), but not in the smaller subgroups of phase 2. There was no effect of TD x ACE on behavior during either phase of the study. Similarly, there was no significant effect of ACE or TD on overall mood or depressive symptoms.

Imaging results: phase 1

As expected, the parametric (**Figure 3-S2**) and 3-back > 0-back (**Figure 3-1d**) contrasts robustly recruited the executive network (Owen et al., 2005; Loughead et al., 2009; Loughead et al., 2010; Satterthwaite et al., 2013; Shanmugan et al., 2016). Lower activation during 3-back relative to 0-back in regions associated with the default mode network were also robust. No effects of TD or ACE on the parametric or 0-back contrasts survived correction, and subsequent analyses focused on the 3-back > 0-back contrast. A whole-brain analysis demonstrated that TD differentially altered right DLPFC activation in high and low ACE groups (**Figure 3-2a**; MNI coordinates: x=45, y=21, z=48; volume=108 mm^3; peak z=3.94). BOLD signal in this region significantly correlated with true positive responses across all subjects (r=0.34, p=0.007) (**Figure 3-2b**). During sham depletion, BOLD signal in this region was significantly correlated with total ACE score (r=0.41, p=0.02; **Figure 3-2c**). When controlling for the effects of age, estradiol level, and time since last menstrual period, the interaction between ACE and TD

remained significant (p=0.0001). Post-hoc comparisons revealed the following effects: higher BOLD signal in low ACE participants on active TD compared to sham TD (p=0.03), lower BOLD signal in high ACE participants on active TD compared to sham TD (p=0.0003), and higher BOLD in high ACE participants compared to low ACE participants during sham TD (p=0.007). During active TD, there was no difference between ACE groups. This interactive effect of TD and ACE on DLPFC BOLD remained significant when true positive count was included as an additional covariate (p=0.0002). Similarly, this interaction remained significant (p=0.0002) when controlling for the difference in percent errors between 3-back and 0-back. Post-hoc comparisons revealed the following effects: higher BOLD signal in low ACE participants on active TD compared to sham TD (p=0.03), lower BOLD signal in high ACE participants on active TD compared to sham TD (p=0.0007), and higher BOLD in high ACE participants compared to low ACE participants during sham TD (p=0.005). There was no difference between ACE groups during active TD.

Imaging results: phase 2

Within the right DLPFC cluster from phase 1, a 4-way interaction between ACE group x TD status x estradiol/placebo group x study phase was detected (p=0.03). A comparable interactive effect to that observed in phase 1 between ACE and TD on BOLD was present in the participants randomized to placebo (**Figure 3-3b**; p=0.07). As in phase 1, lower BOLD signal was observed in high ACE participants on active TD compared to sham TD (p=0.08) and higher BOLD was observed in high ACE participants compared to low ACE participants during sham TD (p=0.04). However, in participants randomized to estradiol, there was no effect of ACE or TD on BOLD (**Figure**

3-3c). In these participants, estradiol attenuated differences between high and low ACE groups during sham TD as well as BOLD response to active TD in the high ACE group.

Results were similar when controlling for differences in percent errors between 3-back and 0-back. The 4-way interaction between ACE group x TD status x estradiol/placebo group x study phase remained significant (p=0.03). An ACE x TD interaction was observed in participants randomized to placebo (p=0.09); lower BOLD signal was observed in high ACE participants on active TD compared to sham TD (p=0.1) and higher BOLD was observed in high ACE participants compared to low ACE participants during sham TD (p=0.04). No effect of ACE or TD was observed in participants randomized to estradiol.

Discussion

This double-blind, placebo-controlled, cross-over study examined whether TD differentially impacted brain activation in high vs low ACE hypogonadal women in the presence and absence of exogenous estradiol. In the absence of exogenous estradiol, TD increased right DLPFC BOLD signal and improved working memory performance in women who experienced low levels of early life adversity. In contrast, TD decreased right DLPFC activation without worsening performance in high ACE women. However, treatment with estradiol attenuated the effects of ACE and TD such that no between or within group differences were observed. Together, these results suggest that ACE has lasting impacts on serotonergic circuits supporting executive function that are unmasked by loss of estradiol during menopause.

Evidence for TD x ACE effects on executive function

As expected, we found main effects of ACE and TD as well as an ACE by TD interaction on DLPFC activation in hypogonadal women. The DLPFC is part of the frontoparietal network responsible for goal-directed executive functions (Cortese et al., 2012) and dysfunction of this region contributes to executive deficits across multiple psychiatric disorders (Shanmugan et al., 2016). During sham depletion, the high ACE group exhibited significantly greater activation in this region and a trend for slower reaction time than the low ACE group This finding suggests early adversity is associated with inefficient hyperactivation of the DLPFC in hypogonadal menopausal women. Our findings are globally convergent with the only other study that used a whole-brain approach to examine early adversity effects on brain activation during the n-back in healthy adults, which found that early adversity was associated with hyperactivation of several brain regions (Philip et al., 2016). While that study found effects of early life adversity during 0-back, differences in results between our study and theirs are likely due to differences in correction methods employed and instruments used to assess early adversity.

While the high ACE group demonstrated inefficient hyperactivation of the DLPFC during sham TD, this ACE effect was not present during active TD. That TD ameliorates this ACE-associated inefficiency in DLPFC activation suggests that serotonergic differences likely contribute to baseline differences in BOLD between ACE groups observed during the sham condition. This finding is in agreement with two other n-back studies demonstrating TD-induced decreases in DLPFC activation in the absence of performance changes (Allen et al., 2006; Epperson et al., 2012). In contrast, TD increased activation and task performance in the low ACE group. Similar TD-induced improvements in performance (Schmitt et al., 2000; Booij et al., 2005; Scholes et al.,

2007) have been reported during tasks requiring focused attention and response inhibition (Mendelsohn et al., 2009). These studies may not be directly relevant to our results as they examined executive domains other than working memory. The combination of group differences observed during sham depletion and the differential direction of response to active depletion highlight that the effects of ACE should be accounted for in future neuroimaging studies utilizing neurotransmitter manipulations. Failure to account for this moderating effect is likely a major factor contributing to the inconclusive supporting literature regarding TD's effects on executive function.

In combination, these results suggest that ACE has lasting impacts on serotonin function and that hypogonadal middle-aged women with high and low levels of early adversity respond differently to experimental manipulations of the serotonergic system. As early adversity negatively impacts serotonin synthesis (Shively et al., 2003), metabolism (Wong et al., 2015), and signaling (Murrough et al., 2011), the mechanism contributing to differential TD response in low and high ACE participants is likely multifold. A human participants study found that low socioeconomic status is associated with lower serotonergic activity (Matthews et al, 2000), suggesting differences in baseline serotonin concentration may play a role. Animal studies demonstrating that early life stress lowers TPH2 expression (Shively et al., 2003) and increases monoamine oxidase-A (Wong et al., 2015) support this hypothesis. However, early adversity also reduces 5-HT1B expression (Murrough et al., 2011) and attenuates 5-HT1A function (Matsuzaki et al., 2009). This suggests alterations in feedback inhibition or receptor sensitivity may also contribute to differences in BOLD response to TD between high and low ACE individuals.

Evidence for estradiol modulation of TD x ACE effects on executive function

As predicted, treatment with estradiol attenuated ACE and TD effects on BOLD signal in the DLPFC. In contrast to the ACE by TD interaction seen in the absence of exogenous estradiol during phase 1 and participants randomized to placebo, no between or within group differences were observed in participants randomized to estradiol. This finding is in agreement with our previous study demonstrating that estradiol prevented TD-induced alterations in brain activation (Epperson et al., 2012). These data support the hypothesis that early adversity has long-term effects on serotonergic circuits involved in working memory that are revealed by loss of estradiol during menopause. Given that TD results in decreased serotonin synthesis, and the animal literature demonstrating effects of early adversity and estradiol on TPH2 gene expression (Shively et al., 2003), one likely mechanism by which ACE is exerting these latent effects on working memory may be via TPH2. However, the converging effects of early trauma (Matsuzaki et al., 2009) and estradiol (Michopoulos et al., 2014) on 5-HT1A make the presynaptic autoreceptor another promising alternative.

Limitations

While this study leveraged a unique study design with several levels of rigorous controls, certain limitations merit discussion. First, given the association between ACE and many adverse health-related outcomes (Felitti et al., 1998), the resilient sample of highly educated, physically and psychologically healthy hypogonadal women studied here reduces generalizability to the typical menopausal population, particularly those with substantial early life stress. However, using this sample allowed us to examine the effects of early life adversity on serotonin system function while limiting the potentially confounding impacts of comorbid psychiatric symptomatology and psychotropic

medication use. Second, no parametric effects of ACE or TD survived correction, and results are based on a post-hoc analysis of the 3-back > 0-back contrast. However, results using this contrast indicate ACE and TD effects are present when high demands are placed on working memory. Third, the sample size of 33 is relatively small. It is possible that lack of ACE and TD effects in the estradiol group could be driven by diminished power in the sub-sample of phase 1 participants. However, these findings are unlikely due to lack of power given the significant 4-way interaction as well as the ACE and ACE x TD effects observed in the smaller sub-sample randomized to placebo. Additionally, the double-blind, placebo-controlled, cross-over study design using repeated-measures allowed for decreased variance in estimates of treatment effects and increased power to detect between group differences using > 100 fMRI scans, a comparatively large data set for an imaging study employing a pharmacologic manipulation in a healthy population. Furthermore, the stringent Type 1 error correction method applied to our whole brain analysis highlights the robust nature of these findings.

Conclusions

In summary, these data imply that early life adversity exerts enduring effects on serotonergic circuits underlying executive processes that are unmasked by loss of estradiol during menopause. To confirm this effect of menopause, a longitudinal study of women pre- to postmenopause would be ideal. Our results emphasize serotonin's role in executive function and highlight that the moderating effects of early adversity must be accounted for when manipulating serotonin levels. Further, they provide preliminary mechanistic evidence as to why some, but not all, women experience executive difficulties during menopause and that brain responses to estradiol vary. Further research in a larger sample would be helpful in elucidating the impact of timing of early

life adversity on serotonin system function across brain networks, whether sex-differences exist with regard to TD x ACE interactions, and finally whether early life adversity alters the risk-benefit profile of peri- and postmenopausal estrogen treatment.

Table 3-1. Participant characteristics at admission

	Low ACE	**High ACE**
	Mean (SD) or number (%)	
n	19	14
Age (years)	55.4 (3.6)	54.8 (2.9)
Months since last menstrual period	52.9 (33.4)	62.5 (32.1)
Follicle-stimulating hormone (IU/L)	93.4 (35.6)	75.7 (42.8)
Estradiol (pg/mL)	26.5 (18.8)	24.1 (10.5)
Education		
Graduate	11 (58)	2 (14)
Some Graduate	1 (5)	3 (21)
College	4 (21)	4 (29)
Some College	2 (11)	5 (36)
High School	1 (5)	0 (0)
Race		
Caucasian	16 (84)	9 (64)
African American	2 (11)	4 (29)
Asian	1 (5)	1 (7)
Employment		
Full time	14 (64)	9 (64)
Part time	1 (12)	4 (29)
Retired	2 (11)	0 (0)
Unknown or preferred not to disclose	0 (0)	1 (7)

Figure Legends

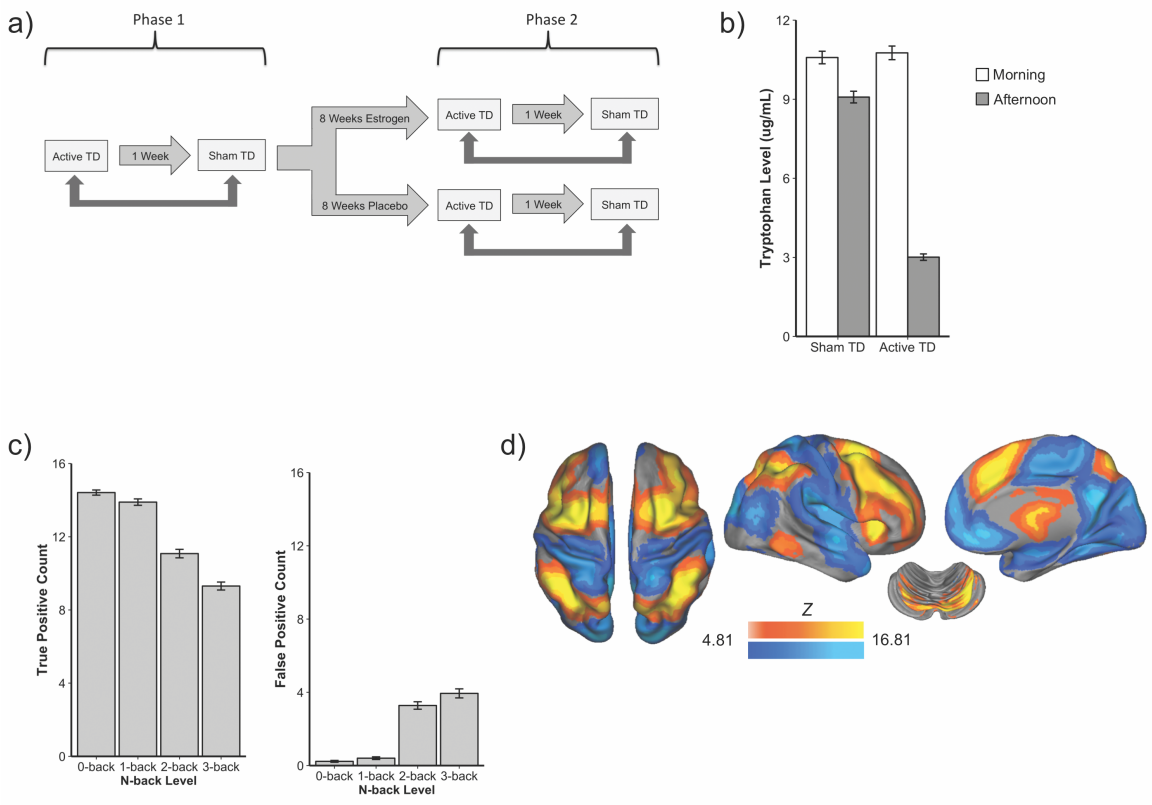

Figure 3-1. Study design, tryptophan levels, working memory performance, contrast of interest. a. This study used a double-blind, placebo-controlled, cross-over design. Each participant underwent 4 test days. During test days 1 and 2 (phase 1), each participant underwent 2 imaging sessions: active TD or sham TD. The order of condition (active TD vs sham TD) was counterbalanced and double-blind. After completing phase 1, participants were randomized to 17β-estradiol 0.100 mg/d or look-alike placebo patch treatment for a total of 10 weeks. Randomization was assigned by the research pharmacist and was blocked and stratified with a block size of 3 to maintain a 2:1 ratio of estrogen to placebo within each stratum. Randomization allocation sequence was generated using a random sequence generator in Excel. Lot and expiration information were withheld by investigational drug services to conceal

sequence and randomization status. After completion of approximately 8 weeks of estradiol treatment or placebo treatment, each participant again underwent active TD and sham TD on two separate test days (phase 2). On test days, participants presented to the Penn Center for Women's Behavioral Wellness (PCWBW) at 7:30 am after an overnight fast and ingested 70 capsules containing either 31.5g of amino acids without tryptophan (active TD) or 31.5g of microcellulose (sham TD). The active capsules consisted of L-isoleucine 4.2g, L-leucine 6.6g, L-lysine 4.8g, L-methionine 1.5g, L-phenylalanine 6.6g, L-threonine 3.0g and L-valine 4.8g. Enrollement and post-test day follow-up (24 hours for placebo, 3 weeks for estradiol) was conducted by research coordinators at PCWBW. **b.** Blood was taken for free tryptophan analysis prior to consumption of the study capsules (morning) and approximately 6 hours later (afternoon). The effect of active vs sham depletion was assessed using a linear mixed model with percent change in free tryptophan concentration as the outcome. Active TD resulted in a significant decrease in tryptophan level in comparison to sham TD ($p<0.0001$). There was not a significant difference in percent change in tryptophan levels between ACE groups. Bars represent means and error bars represent standard error. **c.** Increased working memory load was associated with fewer correct responses to targets and more false positive responses to foils **d.** The Bonferonni corrected group mean of the 3-back > 0- back contrast across the 123 scans included in analyses activated executive regions and de-activated default mode network regions. Images were displayed using caret. TD=tryptophan depletion.

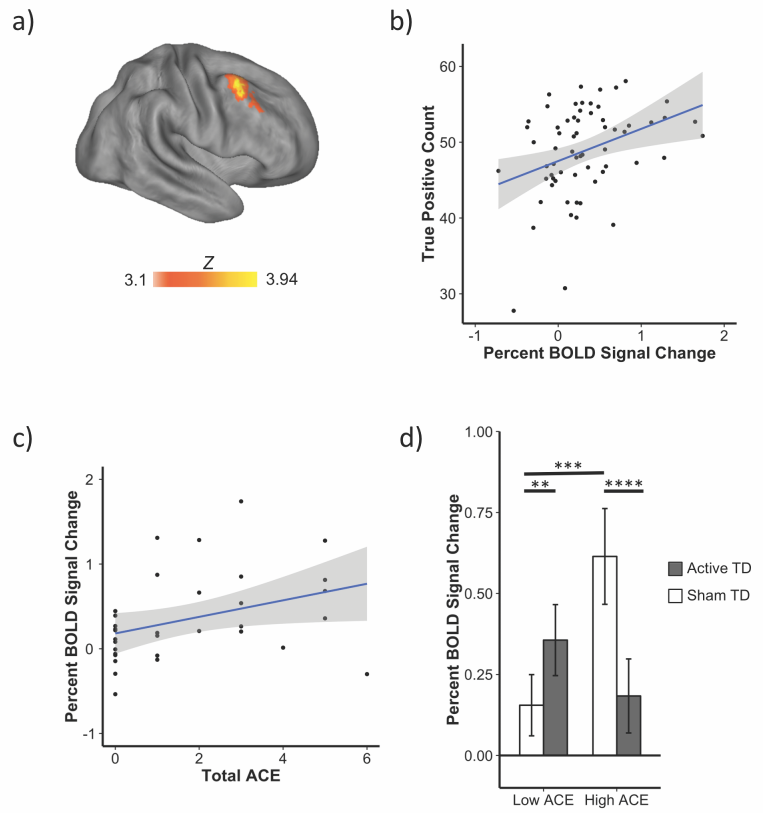

Figure 3-2. Phase 1 imaging results. a. A whole-brain mixed-effects 2 x 2 ANOVA demonstrated that TD differentially altered right DLPFC activation in high and low ACE groups (MNI coordinates: x=45, y=21, z=48; volume=108 mm^3; peak z=3.94). **b.** BOLD signal in this region significantly correlated with true positive responses across all subjects (r=0.34, p=0.007). This relationship remained significant when excluding the two sessions with the lowest BOLD signal (r=0.30, p=0.02). **c.** During sham depletion, BOLD signal in this region was significantly correlated with total ACE score (r=0.41, p=0.02) **d.** Signal from the region displayed in Figure 2a was extracted for visualization. In the low ACE group, active TD increased BOLD relative to sham TD (β=0.20, 95% CI: -0.16 – 0.21, p=0.03). In the high ACE group, active TD decreased activation relative to sham TD (β=-0.43, 95% CI: -0.65 – -0.22, p=0.0003). During sham depletion, high ACE was associated with greater activation relative to low ACE (β=0.49, 95% CI: 0.15 – 0.82,

p=0.007). During active TD, there was no difference between ACE groups. Bars represent means and error bars represent standard error. TD=tryptophan depletion. ACE=adverse childhood experiences. *p<0.1, **p<0.05, ***p<0.01, ****p<0.001

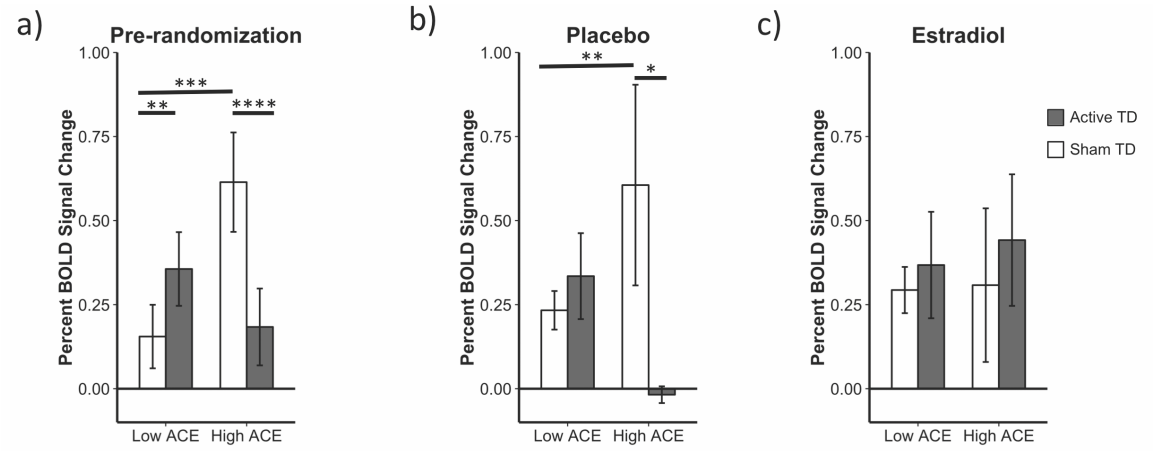

Figure 3-3. Phase 2 imaging results. a. TD and ACE exerted an interactive effect on right DLPFC BOLD during phase one. This panel is identical to Figure 2c. **b.** Within this cluster, a 4-way interaction between ACE group x TD status x estradiol/placebo group x study phase was detected (β=1.1, 95% CI: 0.12 – 2.1, p=0.03). A comparable interactive effect to that observed in phase 1 between ACE and TD on BOLD was present in the participants randomized to placebo. Active TD decreased activation in the high ACE group in comparison to sham TD (β=-0.62, 95% CI: -1.4 – 0.12, p=0.08) and high ACE was associated with increased activation in comparison to low ACE during sham TD (β=0.59, 95% CI: 0.12 – 1.1, p=0.04). **c.** Estradiol attenuated differences between high and low ACE groups during sham TD as well as BOLD response to active TD in the high ACE group. There was no effect of ACE or TD on BOLD after estradiol treatment. Bars represent means and error bars represent standard error. TD=tryptophan depletion. ACE=adverse childhood experiences. *p<0.1, **p<0.05, ***p<0.01, ****p<0.001

Table 3-S1. Occurrence of Individual ACEs in Study Sample

	Low ACE	High ACE
Mean ACE	0.32 ± 0.48	3.79 ± 1.25
Abuse		
Emotional abuse	0	8
Physical abuse	0	5
Sexual abuse	1	5
Neglect		
Emotional neglect	0	8
Physical neglect	0	0
Household dysfunction		
Parents divorced/separated	2	7
Witnessed domestic violence	0	8
Drug abuse in household	2	6
Mental illness in household	1	7
Incarceration of family member	0	0

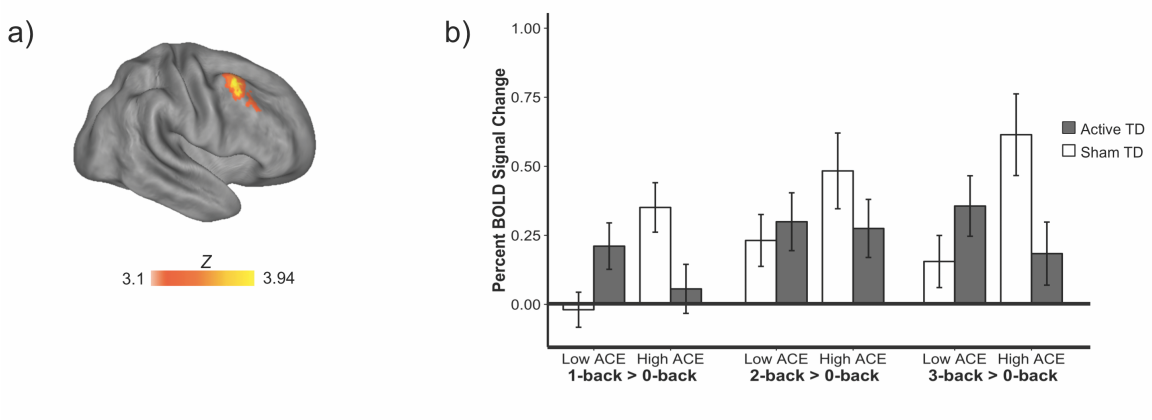

Figure 3-S1. TD and ACE effects on DLPFC activation across n-back levels. a. A whole-brain mixed-effects 2 x 2 ANOVA demonstrated that TD differentially altered right DLPFC activation in high and low ACE groups (MNI coordinates: x=45, y=21, z=48; volume=108 mm^3; peak z=3.94). This panel is identical to Figure 2a. **b.** Signal from the region depicted in part **a** was extracted from the 1-back > 0-back, 2-back > 0-back, and 3-back > 0-back contrasts to investigate the effects of TD and ACE across varying levels of working memory load**.**

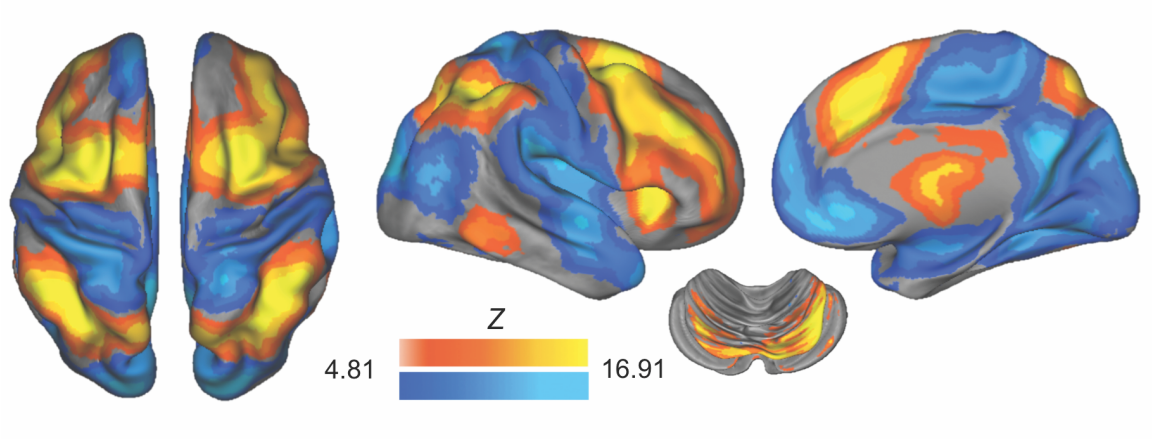

Figure 3-S2. Mean parametric contrast. The Bonferonni corrected group mean of the parametric contrast across the 123 scans included in analyses activated executive regions. Decreasing activation with increasing working memory load was observed in default mode network regions.

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CHAPTER 4

Impact of Early Life Adversity and Tryptophan Depletion on Functional Connectivity in Menopausal Women

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Shanmugan S, Satterthwaite TD, Sammel MD, Cao W, Ruparel K, Gur RC, Epperson CN, Loughead J. Impact of Early Life Adversity and Tryptophan Depletion on Functional Connectivity in Menopausal Women (submitted)

Abstract

During the menopause transition, women are at increased risk of subjective symptoms of executive dysfunction. Evidence from animal and human participant studies suggests adverse childhood experiences (ACE) may be a risk factor for executive complaints during this hormonal transition. Preclinical literature indicates early life adversity effects on serotonin function may play a role in this increased susceptibility. However, the mechanisms underlying this increase in vulnerability in human participants remain relatively unknown. Here we examined the impact of ACE and tryptophan depletion (TD) on functional network connectivity in discovery and replication datasets. We hypothesized that ACE would be associated with decreased within-network connectivity. We predicted that TD would further lower connectivity in women with high levels of early adversity, but have no effect in in women with low levels of early adversity. Forty women underwent two functional imaging sequences at two time points (141 total scans) in a double-blind, placebo controlled, crossover study. The effects of ACE and TD were evaluated using generalized estimating equations (GEE). As predicted, ACE was associated with lower within-network connectivity. While TD had no effect on connectivity in the low ACE group, TD increased connectivity in the high ACE group. The robust main effect of ACE remained significant in the replication dataset, though the ACE x TD interaction did not. Together, these results suggest that early life adversity has lasting impacts on large-scale functional networks underlying executive function. Alterations in functional network connectivity may be one mechanism by which early life adversity increases the risk of cognitive disorders during menopause.

1. Introduction

The menopause transition is associated with increased susceptibility to subjective symptoms of cognitive dysfunction (Shanmugan and Epperson, 2014). Several reports have suggested that healthy women with no history of cognitive dysfunction experience increased difficulty with everyday tasks requiring executive processes such as working memory, focus, attention, organization, and planning (Epperson et al., 2015; Shanmugan and Epperson, 2014; Shanmugan et al., 2017). There is preliminary evidence for adverse childhood experiences (ACE) such as abuse, neglect, and household dysfunction as a risk factor for executive dysfunction during this hormonal transition (Shanmugan and Epperson, 2014).

Significant adversity during childhood alters normal trajectories of brain maturation (Bruce et al., 2013; Hanson et al., 2015; Shanmugan and Satterthwaite, 2016) resulting in heightened vulnerability to executive dysfunction (Philip et al., 2016) in adult life. Neuroimaging studies have demonstrated that early adversity alters activation in brain regions subserving executive processes. In healthy adults, early trauma has been associated with executive system hyperactivation during working memory (Philip et al., 2016). In healthy menopausal women, estradiol treatment attenuates this ACE-associated executive hyperactivation (Shanmugan et al., 2016), suggesting the effects of ACE may be magnified when women are hypogonadal.

While these studies demonstrate that ACE has effects on activation in particular brain regions, the impact of ACE on functional network architecture is less clear. There is growing evidence that the functional connectivity between brain regions as part of larger cognitive systems is critical to maintaining intact executive function (reviewed by Vaidya and Gordon, 2013). A recent large neuroimaging study in healthy adults ages 18-88 demonstrated that the degree of connectivity between and within large-scale brain

networks becomes increasingly important to maintaining cognitive performance as we age (Tsvetanov et al., 2016). Additionally, altered neural connectivity has been suggested as a potential marker of menopausal women who may be at risk of late-life cognitive dysfunction (Vega et al., 2016). As such, understanding whether early adversity alters connectivity among these functional networks is particularly relevant in this middle-aged population.

During development, networks supporting cognitive processes segregate: brain regions become more connected with regions of the same network (increased within-network connectivity) and less connected with regions of other networks (decreased between-network connectivity) (Dosenbach et al., 2010; Fair et al., 2007a; Power et al., 2010; Satterthwaite and Baker, 2015; Shanmugan and Satterthwaite, 2016). Adversity-induced disruptions in this trajectory of network segregation have been observed as early as preschool (Demir-Lira et al., 2016) and last into adulthood (Philip et al., 2013). In healthy adults, early life trauma is associated with decreased connectivity between regions within the same network as well as increased connectivity between regions in different networks (Philip et al., 2013). Such patterns of dysconnectivity are associated with poorer working memory across the lifespan (Tsvetanov et al., 2016).

Together these studies suggest that early life adversity may confer a risk for executive dysfunction during menopause by altering connectivity between and within neural networks. However, the effects of ACE on functional network connectivity during this hormonal transition have not been studied. Converging evidence from animal and human studies suggests one mechanism by which ACE may alter brain function during periods of low estradiol may be via effects on serotonin. In non-human primates, both ovariectomy and social subordination stress are associated with reductions in tryptophan hydroxylase type 2 (TPH2) gene expression (Shively et al., 2003) and 5-HT1A binding

potential (Michopoulos et al., 2014). Similarly, lowering central serotonin levels differentially impacts prefrontal cortex activation in menopausal women with high and low levels of early life adversity (Shanmugan et al., 2016). However, whether ACE and sertononin exhibit a similar interactive effect on functional networks is not known.

Accordingly, we tested the hypothesis that ACE adversely impacts functional network connectivity during menopause via alterations in serotonin function. Forty healthy menopausal women with high and low levels of early life adversity underwent tryptophan depletion (TD) and performed two cognitive tasks during functional magnetic resonance imaging. Task effects were regressed to obtain discovery and replication timeseries approximate to the resting state (Fair et al., 2007b). The impact of ACE, TD, and the interaction between ACE and TD on within-network connectivity were examined in both datasets. We predicted that within-network connectivity would be lower in the high ACE group compared to the low ACE group during both active TD and sham TD. We also predicted that TD would further lower this measure in the high ACE group, but have no effect on connectivity in the low ACE group.

2. Methods

2.1. Participants

Participants were healthy menopausal women ages 48-60 with no current psychiatric diagnoses and within 10 years of their last menstrual period. Participants were right-handed and had a FSH > 30 IU/ml, a normal mammogram and pap smear within the last year, and a clear urine toxicology. Exclusion criteria included use of estrogen or hormone therapy within the last year, contraindications to hormone therapy, IQ less than 95, Mini Mental Status Exam Score less than 25, lifetime history of DSM-V psychotic or bipolar disorder, other psychiatric or substance use disorder in the last year,

Hamilton depression score > 14, psychotropic medication use within the last month, metallic implants, and claustrophobia. The sample considered here constitutes a superset of participants previously included in a report focusing on the impact of early adversity on prefrontal cortex activation during working memory (Shanmugan et al., 2016).

2.2 Study design

This study used a double-blind, placebo-controlled, cross-over design. Each participant underwent 2 imaging sessions: active TD or sham TD. The order of condition (active TD vs sham TD) was counterbalanced and double-blind. On each test day, participants presented after an overnight fast and ingested 70 capsules containing either 31.5g of amino acids without tryptophan (active TD) or 31.5g of microcellulose (sham TD). The active capsules consisted of L-isoleucine 4.2g, L-leucine 6.6g, L-lysine 4.8g, L-methionine 1.5g, L-phenylalanine 6.6g, L-threonine 3.0g and L-valine 4.8g. Blood was taken for free tryptophan analysis and mood ratings were completed prior to consumption of the capsules and approximately 6 hours later, after which participants underwent cognitive testing and neuroimaging.

2.3 Assessment of early life adversity

The Adverse Childhood Experiences (ACE) Questionnaire (Felitti et al., 1998) was used to assess history of emotional, physical, or sexual abuse, childhood neglect, and household dysfunction. The ACE questionnaire assesses the number of exposure categories and has been used extensively to assess the association between early life adversity and later life health-related outcomes. The number of exposures was summed to create the ACE score (range: 0–10). Participants with an ACE score greater than or

equal to 2 were considered “high ACE” while participants with ACE score of less than 2 were considered “low ACE”. A threshold of 2 was used to define the high ACE group based on studies of depression prevalence in later life that demonstrate increased susceptibility at this level of exposure (Chapman et al., 2004; Epperson et al., In Press). The continuous effects of ACE were explored in secondary analyses utilizing total ACE score.

2.4 Assessment of mood

Mood was measured as a possible confound. Participants completed the Profile of Mood States prior to TD procedure and again before fMRI. Total score and the “Depression-Dejection” sub-score were used to assess changes in overall mood and depressive symptoms, respectively. Difference in mood score (morning - afternoon) served as the outcome variable in regression models used to assess the effects of TD and ACE on mood.

2.5 Task paradigms

A letter version of the n-back task was used to probe working memory and executive system function during fMRI scans. The task consisted of four conditions: 0-back, 1-back, 2-back and 3-back. Stimuli were 4-letter non-words without vowels presented for 500 ms, followed by an interstimulus interval of 2500 ms. During the 0-back, participants responded to a target 4-letter stimulus with a button press. During other conditions, participants responded to a stimulus “n” number of stimuli before it. Each condition consisted of a 20-trial block (60s) and each class of blocks was repeated three times. A target-foil ratio of 1:2 was maintained in all blocks. Visual instructions (9s) preceded each block. The task began with a 48s baseline rest period. Additional 24s

baseline rest periods occurred at the beginning, middle, and end of the acquisition. Total task duration was 924s. Equivalent n-back tasks with unique stimuli were used for the four sessions and version order was counter-balanced.

An emotional identification task was used to probe emotional bias and affective processing. The task consisted of 60 faces displaying neutral, happy, sad, angry or fearful expressions presented in a fast event-related design. Each face was displayed for 5.5 seconds followed by a variable inter-stimulus interval (0.5–18.5 seconds) during which a complex crosshair (matched to the faces' perceptual qualities) was displayed. Participants were asked to select one of five labels (happy, sad, anger, fear, neutral) for each face presented using a scroll wheel. Equivalent versions of the task with unique stimuli were used for the four sessions and version order was counter-balanced. Total task duration was 630s.

2.6 Image acquisition

Imaging data were acquired on 3T Siemens Trio scanner. A magnetization-prepared, rapid acquisition gradient echo T1-weighted image (TR = 1810 ms, TE = 3.51 ms, FOV = 180 x 241 mm, matrix = 192 x 256, 160 slices, effective voxel resolution of 0.94 x 0.94 x 1 mm) was acquired to aid spatial normalization to standard space. Functional images were acquired using a whole-brain, single-shot gradient-echo (GE) echoplanar sequence with the following parameters: TR/TE = 3000/32 ms, FOV= 192 × 192 mm, matrix = 64 x 64, slice thickness/gap = 3/0 mm, 46 slices, effective voxel resolution of 3 x 3 x 3 mm.

2.7 Network construction and visualization

We examined the effects of ACE and TD on functional connectivity using a system of functional networks described by Power et al. (Power et al., 2011). In this system, networks are composed of spheres with a 5-mm radius (264 nodes) (**Figure 4-1**) and the connections between these nodes (34,716 unique edges). These nodes compose 14 functional network modules that correspond to brain networks that are present during both task performance and at rest (Power et al., 2011). This node system provides good coverage of the whole brain (Power et al., 2011) and has been used to examine functional connectivity between and within brain networks during cognitive tasks and at rest (Dosenbach et al., 2010; Satterthwaite et al., 2013b).

Image registration

Subject-level functional and anatomical T1 images were co-registered using boundary-based registration (Greve and Fischl, 2009). The anatomical image was normalized to the MNI 152 T1 1mm template using the top-performing diffeomorphic SyN registration in ANTs (Avants et al., 2011; Klein et al., 2009). Co-registration, normalization, and down-sampling of network nodes to subject space were concatenated so only one interpolation was performed.

2.8 Image Processing

BOLD time series data were skull stripped with BET (Smith, 2002), despiked with AFNI's 3dDespike (Cox, 1996), motion-corrected with MCFLIRT (Jenkinson et al., 2002), high pass filtered (120 s), spatially smoothed (6 mm FWHM), and mean-based intensity normalized. The event related emotion identification task time series were also slice-time corrected. Subject-level time series analyses were carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al., 2009).

Task condition (0-back, 1-back, 2-back, and 3-back blocks for the n-back or happy, sad, anger, fear, and neutral events for emotion identification) and their temporal derivatives were modeled using a canonical (double-gamma) hemodynamic response function with 24 motion parameters (Friston et al., 1996; Yan et al., 2013a) included as nuisance covariates. The rest condition (fixation point) served as the unmodeled baseline. Images were assessed for excessive motion (mean relative displacement > 0.3 mm) (Satterthwaite et al., 2013b). Time series were extracted from each of the 264 nodes in subject space using the residuals of this subject-level analysis that modeled task and motion. Task regression has been shown to aide test-retest reliability of connectivity measurements obtained during tasks (Cao et al., 2014).

2.9 Graph construction and analyses of network topology

For each participant, pair-wise Pearson correlations were calculated using the time series extracted from the 264 nodes. These values were then transformed using a Fisher-Z transformation to construct a 264 x 264 symmetric connectivity matrix for each participant. Our primary outcome of interest was within-network connectivity. Within-network connectivity is the mean strength of edges between nodes within a network, averaged across all networks. We also calculated total network strength to account for potential motion artifact (Saad et al., 2013; Yan et al., 2013b). Total network strength is the average strength of the connectivity matrix (Saad et al., 2013; Yan et al., 2013b).

2.9 Discovery and replication data sets

Prior studies have demonstrated that removing the effects of task from BOLD data acquired during cognitive tasks produces “resting-state” timeseries that are qualitatively similar to “true” resting-state data (Fair et al., 2007b). Several groups have

used these residual timeseries to examine the effect of pathology on “resting-state” functional connectivity (Fleisher et al., 2009; Weisberg et al., 2014). However, given that differences between task-regressed residuals and “true” resting-state timeseries have been noted (Fair et al., 2007b), we tested our hypotheses in both a discovery and replication dataset. We obtained these two approximate resting-state timeseries by regressing task effects during working memory and emotion identification tasks. Given the differences in both task design (block vs event-related) and task demands, consistency of results across timeseries would suggest differences due to intrinsic functional network architecture rather than task performance.

We defined the timeseries obtained during the n-back as the discovery timeseries for several reasons. First, the n-back task was longer and provided an additional 98 volumes for calculation of correlations between network nodes. Second, Cao et al. (2014) demonstrated that the test-retest reliability of graph-based connectivity measures calculated from residual timeseries was higher for the n-back than for an emotional face-processing task. Third, as described below in the results (section *3.1 Participants*), a greater number of timeseries were available for the n-back than the emotional identification task. Given these differences, we chose to first examine the impact of ACE and TD in the residuals of the n-back timeseries, which may be more consistent and a closer approximate to resting-state. The main effect of task on within-network connectivity was evaluated using generalized estimating equations to assess the validity of using residual timeseries from a separate task as the replication dataset.

2.9 Statistical analysis

Generalized estimating equations implemented using the *geepack* package (HÃ¸jsgaard et al., 2006) in R (R Core Team, 2015) were employed to evaluate the

effect of ACE, TD, and their interaction on within-network connectivity. This method accommodates multiple assessments per participant and adjusts for non-independence among these repeated measures. All models controlled for age, estradiol level, and time since last menstrual period. As previous studies have demonstrated that the impact of TD on brain function varies with estradiol level in menopausal women (Epperson et al., 2012), we also controlled for this interaction in our analyses. Both mean relative displacement (Power et al., 2015; Satterthwaite et al., 2013a) and mean network strength (Saad et al., 2013; Yan et al., 2013a; Yan et al., 2013b) were included as covariates to minimize the impact of motion. The effect of the number of ACEs (continuous) on functional connectivity was evaluated in supplementary analyses that examined interactions between TD and total ACE score. Statistical tests were two-sided with $p<=0.05$ considered significant.

3. Results

3.1 Participants

Sixty participants completed screening and were enrolled in the present study. Of these 60 participants, 2 withdrew from the study before the first test day, and 3 were lost to follow up. Six participants did not complete the first test day due to inability to complete the depletion procedure (n=1), claustrophobia (n=4), or lack of space (n=1) inside the MRI scanner. Three participants withdrew prior to the second test day, and one participant was excluded due to artifacts produced by dental fillings. Participants were excluded from analyses for excessive motion (n-back n=1; emotion identification n=2), having a percent change in tryptophan level approximately greater than 3 SD from the average for the assigned depletion status (n=3), or missing data regarding ACE (n=3), imaging (n-back n=1; emotion identification n=5), or tryptophan levels (n=4). The

final sample included in the discovery dataset consisted of 24 low ACE participants (43 sessions) and 16 high ACE participants (30 sessions; see **Table 4-1** and **Table 4-S1**). Four of these participants did not complete the emotional identification task; the final sample included in the replication dataset consisted of 24 low ACE participants (40 sessions) and 15 high ACE participants (28 sessions).

3.1 Tryptophan level and mood results

Blood was taken for free tryptophan analysis before consumption of the study capsules (morning) and approximately 6 hours later (afternoon). The effect of active depletion vs sham depletion was assessed using a generalized estimating equation with percent change in free tryptophan concentration as the outcome. Active TD resulted in a significant decrease in tryptophan level in comparison to sham TD ($p<0.0001$). There was no significant difference in percent change in tryptophan levels between ACE groups. There was no significant effect of ACE, TD, or ACE x TD on mood.

3.2 Validation of discovery and replication timeseries

As expected, nodes demonstrated greater connectivity with nodes of the same network than with nodes of different networks in both the discovery (**Figure 4-2a**) and replication (**Figure 4-2b**) data sets. The mean symmetric connectivity matricies from these two timeseries qualitatively appeared very similar. However, prior to testing our hypotheses regarding the impact of ACE and TD on functional connectivity, we evaluated the main effect of cognitive task performed on within-network connectivity to assess the validity of using residual timeseries from a separate task as the replication dataset. There was no significant effect of task (n-back vs emotional identification) on within-network connectivity. This effect remained non-significant when accounting for

total network strength as well as motion and was not evaluated further. The lack of significant task effect supported our use of these two sets of timeseries as discovery and replication datasets.

3.3 Discovery timeseries results

To test our hypotheses that ACE would be associated with lower within-network connectivity and that tryptophan depletion would selectively further lower connectivity only in the high ACE group, we used a model that evaluated the impact of ACE group, TD status, and ACE group x TD status on within-network connectivity. The effect of ACE in this model was robust; as predicted, connectivity was significantly lower in the high ACE group than in the low ACE group during sham depletion ($p=0.002$). In line with our hypothesis that TD would selectively impair the high ACE group, TD had no effect on connectivity in the low ACE group. Also as predicted, an interaction between ACE group and depletion status on within-network connectivity was observed at a trend level ($p=0.1$). However, contrary to our hypothesis that active TD would exacerbate the effect of ACE observed during sham TD, active depletion actually increased connectivity in the high ACE group ($p=0.02$; **Figure 4-3a**). This attenuated the effect of ACE during active TD. As in the sham condition, the high ACE group also displayed lower within-network connectivity during active TD, though this trend did not reach statistical significance ($p=0.1$).

Previous studies using the ACE questionnaire to evaluate the impact of early life adversity on mental health outcomes in adult life have used a threshold of ≥ 2 to define the high ACE group (Epperson et al., In Press). As such, our primary analysis utilized the same binary definitions of ACE. However, it is not clear whether such a threshold applies to ACE effects on functional network connectivity. Therefore, we also examined

the effect of the number of ACEs (continuous) using a model that evaluated the impact of total ACE score, TD status, and ACE score x TD status. As hypothesized and in agreement with our results using ACE group, there was a robust effect of ACE score as ACE score was significantly negatively associated with within-network connectivity during sham depletion (p=0.004; **Figure 4-3b**). Additionally, there was a significant ACE score x TD status interaction on within-network connectivity (p=0.04). Consistent with our TD x ACE group analysis, active TD diminished the impact of ACE score; there was no association between total ACE score and within-network connectivity during active TD (p=0.2).

3.3 Replication timeseries results

To probe the validity of our results, we tested our hypotheses regarding the impact of ACE and TD on functional connectivity using a separate set of task-regressed timeseries data acquired in a subsample of our participants. As in the discovery data set, we used a model that evaluated the impact of ACE group, TD status, and ACE group x TD status on within-network connectivity to test our hypotheses that ACE would be associated with lower connectivity and that tryptophan depletion would selectively lower connectivity only in the high ACE group. The effect of ACE group was robust and consistent with discovery results. Within-network connectivity was significantly lower in the high ACE group during both sham TD (p=0.002) and active TD (p=0.05; **Figure 4-4a**). The effect of TD in the low ACE group was also replicated and remained non-significant. However in contrast to discovery results, there was no significant ACE group x TD status interaction and no effect of TD in the high ACE group.

We also attempted to replicate our findings regarding the continuous effects of ACE using a model that evaluated the impact of total ACE score, TD status, and ACE

score x TD status. Consistent with our hypothesis and analyses using ACE group, total ACE score was significantly negatively associated with within-network connectivity during sham TD (p=0.008; **Figure 4-4b**). As in the discovery dataset, this association did not reach significance during active TD. However, it was present at a trend level (p=0.1), and the interaction between ACE score and TD status was not significant.

4. Discussion

In this double-blind, placebo-controlled, cross-over study, we examined the effects of ACE and TD on functional network connectivity in healthy menopausal women. In our discovery dataset, high ACE women displayed lower within-network connectivity than low ACE women regardless of depletion status. Similarly, total ACE score was negatively associated with within-network connectivity. Additionally, active TD increased within-network connectivity in women with high levels of early adversity but had no effect in women with low levels of early adversity. While the robust effects of both ACE group and ACE score were replicated, the more marginal ACE x TD interaction was not. These results indicate early life adversity has lasting impacts on intrinsic large-scale functional networks and provide preliminary evidence that serotonin's effect on these networks may be task-dependent.

As expected, we found a main effect of ACE on functional connectivity. High ACE participants displayed lower within-network connectivity in both the discovery and replication datasets. Connectivity within functional networks has been shown to underlie multiple cognitive domains including working memory (Dosenbach et al., 2007; Fair et al., 2007a; Power et al., 2010; Tsvetanov et al., 2016). Decrements in working memory are associated with lower connectivity within default and cingulo-opercular networks (Vaidya and Gordon, 2013). Decreases in within-network connectivity have been

observed in psychiatric disorders exhibiting dysfunction in executive domains (Satterthwaite and Baker, 2015; Shanmugan and Satterthwaite, 2016). While the impact of ACE on similar measures of within-network connectivity has not been examined, our findings are globally convergent with seed-based resting-state connectivity studies examining the impact of early life stress on functional connectivity in healthy adults (Philip et al., 2013) and adult women with posttraumatic stress disorder (Bluhm et al., 2009). Although seed regions selected and networks examined vary, these studies found that early adversity is associated with lower connectivity between regions within the same network (Bluhm et al., 2009; Philip et al., 2013).

The mechanisms by which ACE imparts this negative impact on connectivity are likely multifold. While we hypothesized that TD would selectively impair connectivity in the high ACE group, we found that TD instead selectively improved connectivity in the high ACE group. Though contrary to our hypothesis, this result is consistent with previous findings demonstrating that tryptophan depletion attenuates ACE effects on dorsolateral prefrontal cortex activation during the n-back in menopausal women (Shanmugan et al., 2016). That TD attenuated the negative effects of ACE on connectivity suggests that serotonergic differences may be contributing to baseline differences in functional connectivity between ACE groups. However, we were not able to replicate this ACE x TD interaction in our replication timeseries. This discordance may be due to differences in task duration, task design, or variations in non-linear effects of task remaining in the residual timeseries (Fair et al., 2007b). However, this lack of ACE x TD interaction in our replication timeseries as well as the consistent effects of ACE regardless of depletion status or cognitive task highlight the importance of considering other mechanisms by which early adversity exerts enduring effects on functional connectivity.

Given that functional connections may be reflective of underlying structural connections (Damoiseaux and Greicius, 2009), the effects of early adversity on neuronal development during childhood and adolescence likely also play a role. During development, within-network connectivity increases while between-network connectivity decreases (Satterthwaite and Baker, 2015; Shanmugan and Satterthwaite, 2016). Significant adversity during this developmental period may preclude this network segregation (Vaidya and Gordon, 2013), possibly via structural changes. Early life stress in mice has been shown to induce alterations in PFC dendritic architecture (Yang et al., 2015). Female mice that experience peripubertal stress also display increases in PFC myelin basic protein gene expression and proteolipid protein gene expression, both of which are indicators of myelination (Morrison et al., 2016). Importantly, estradiol supports the normal development and maintenance of serotonergic function (Epperson et al., 2012), dendritic morphology (Shanmugan and Epperson, 2014), and neuronal myelination (Luo et al., 2016).

4.1 Limitations

Certain limitations of the present study should be noted. First, we focused on middle-aged females who were within 10 years of their final menstrual period, as women are at risk of executive dysfunction during the menopause transition (Epperson et al., 2015; Shanmugan and Epperson, 2014; Shanmugan et al., 2017). Given the impact of estradiol on prefrontal cortex structure and function (Shanmugan and Epperson, 2014) as well as evidence for sex differences in the effects of early adversity (Shanmugan and Satterthwaite, 2016) on neural response, these data cannot be extrapolated to similar-aged males or pre-menopausal females. Similarly, while results demonstrate that ACE is associated with maladaptive connectivity patterns during menopause, it is not clear

whether ACE has comparable effects prior to menopause. Longitudinal studies testing women pre- and post-menopause would be necessary to determine whether the impacts of ACE and menopause on functional connectivity are synergistic or additive. Second, given the association between ACE and many adverse health-related outcomes (Felitti et al., 1998), the resilient sample of highly educated, healthy hypogonadal women without psychiatric disorders studied here reduces generalizability to the typical menopausal population, particularly those with substantial early life adversity. However, this sample allowed us to examine the impact of early adversity on functional network connectivity while limiting the confounding effects of psychiatric symptomatology and psychotropic medication use. Third, residuals from task-regressed timeseries, while similar, are not identical to true resting state data (Fair et al., 2007b). However, that ACE results were replicated in a separate set of residual timeseries lends confidence to our results. Third, the sample size of 40 is relatively small. However, the double-blind, placebo-controlled, cross-over study design using repeated-measures allowed for decreased variance in estimates of condition effects and increased power to detect between group differences.

4.2 Conclusions

In summary, these data suggest that early life adversity has lasting impacts on functional network connectivity in healthy menopausal women. These results provide preliminary evidence regarding the mechanisms by which early life adversity confers a vulnerability for executive dysfunction during menopause. Further research in a larger sample would be helpful in elucidating the impact of timing of early life adversity on brain networks and whether sex-differences exist with regard to TD and ACE effects on connectivity.

Table 4-1. Participant characteristics

	Low ACE	**High ACE**
	Mean (SD) or number (%)	
n	24	16
Age (years)	55.5 (3.3)	55.1 (3.1)
Months since last menstrual period	53.7 (33.0)	62.3 (30.9)
Follicle-stimulating hormone (IU/L)	93.5 (32.5)	74.0 (40.8)
Estradiol (pg/mL)	27.1 (17.7)	22.7 (11.3)
Education		
Graduate	14 (58)	2 (13)
Some Graduate	1 (4)	3 (29)
College	4 (17)	6 (38)
Some College	2 (8)	5 (31)
Associates	1 (4)	0 (0)
High School	2 (8)	0 (0)
Race		
Caucasian	19 (79)	11 (69)
African American	3 (13)	4 (25)
Asian	1 (4)	1 (6)
Other	1 (4)	
Employment		
Full time	16 (67)	10 (63)
Part time	2 (8)	5 (31)
Unemployed	1 (4)	0 (0)
Retired	2 (8)	0 (0)
Unknown or preferred not to disclose	0 (0)	1 (6)

Figures

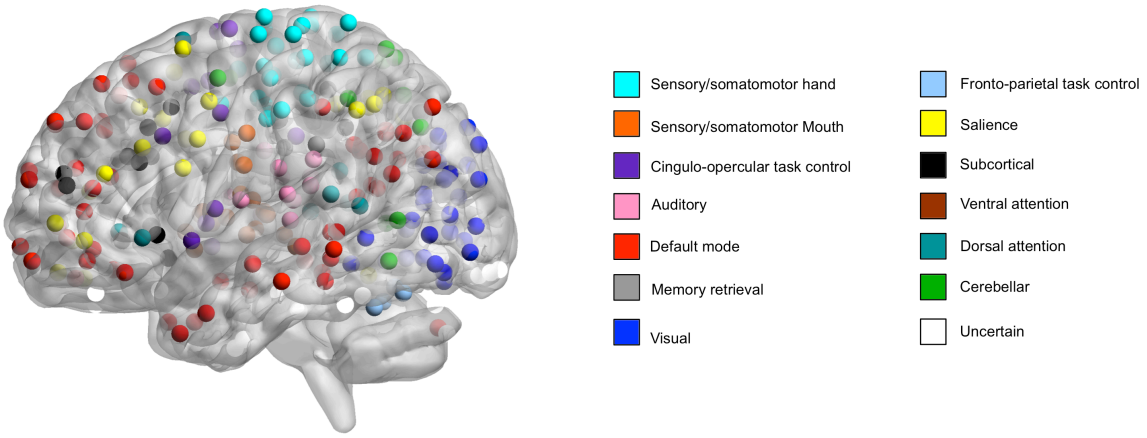

Figure 4-1. Power nodes. Nodes in the system of functional networks described by Power et al. (Power et al., 2011). In this system, networks are composed of spheres with a 5-mm radius (264 nodes) and the connections between these nodes (34,716 unique edges). These nodes compose 14 functional network modules that correspond to brain networks that are present during both task performance and at rest. This node system provides good coverage of the whole brain and has been used to examine functional connectivity between and within brain networks during cognitive tasks and at rest. Time series were extracted from each of the 264 nodes in subject space using the residuals of this subject-level analysis that modeled task and motion. Time series were extracted from each of these nodes in subject space. Image is displayed using BrainNet Viewer.

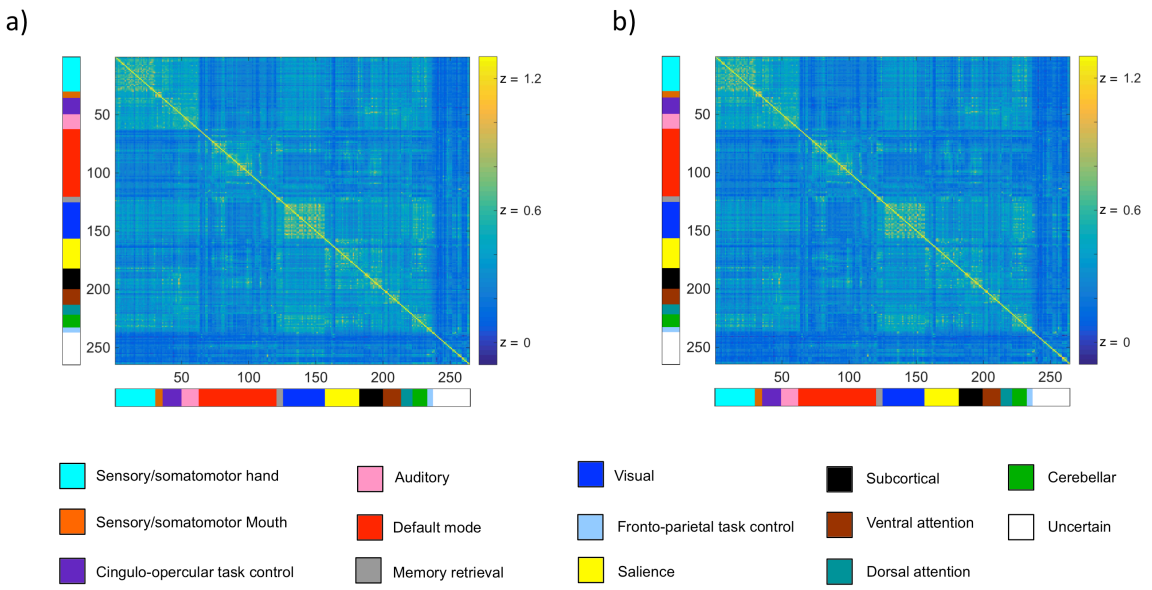

Figure 4-2. Mean symmetric connectivity matrices. The mean symmetric connectivity matrix of discovery (**a**) and replication (**b**) timeseries confirmed nodes demonstrated greater connectivity with nodes of the same network than with nodes of different networks. The mean symmetric connectivity matrices from these two timeseries qualitatively appeared very similar. We evaluated the main effect of cognitive task performed on within-network connectivity to assess the validity of using residual timeseries from a separate task as the replication dataset. There was no significant effect of task (n-back vs emotional identification) on within-network connectivity. This effect remained non-significant when accounting for total network strength as well as motion. Values displayed are Fisher Z transformed pair-wise Pearson's correlations. Color bars to the left and bottom of connectivity matrices indicate community membership.

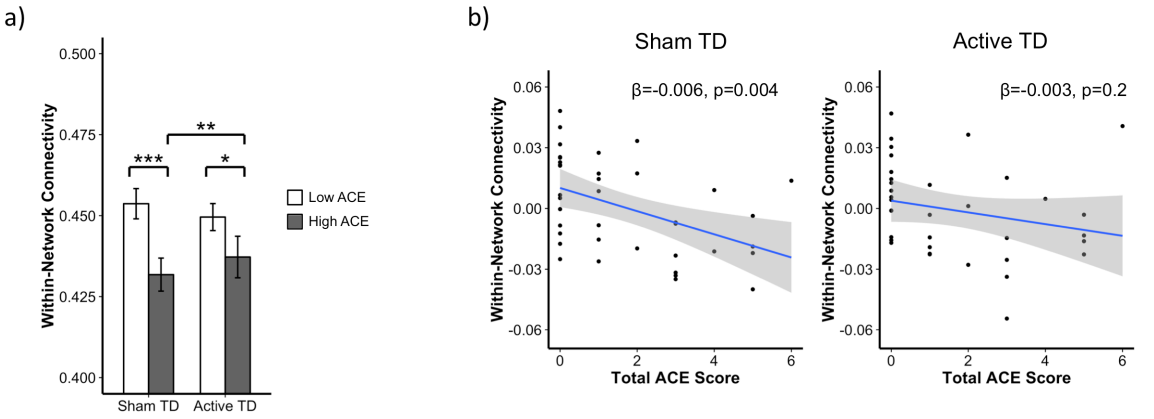

Figure 4-3. ACE and TD effects on within-network connectivity in discovery timeseries. a. To test our hypotheses that ACE would be associated with lower within-network connectivity and that tryptophan depletion would selectively further lower connectivity only in the high ACE group, we used a model that evaluated the impact of ACE group, TD status, and ACE group x TD status on within-network connectivity. The effect of ACE in this model was robust; as predicted, connectivity was significantly lower in the high ACE group than in the low ACE group during sham depletion (p=0.002). In line with out hypothesis that TD would selectively impair the high ACE group, TD had no effect on connectivity in the low ACE group. Also as predicted, an interaction between ACE group and depletion status on within-network connectivity was observed at a trend level (p=0.1). However, contrary to our hypothesis that TD would exacerbate the effect of ACE observed during sham TD, active depletion actually increased connectivity in the high ACE group (p=0.02). This attenuated the effect of ACE during active TD. As in the sham condition, the high ACE group also displayed lower within-network connectivity during active TD, though this trend did not reach statistical significance (p=0.1). Bars represent least-square means adjusted for age, time since last menstrual period, estradiol level, total network strength, and motion. Error bars represent standard error. **b.**

We also examined the effect of the number of ACEs (continuous) using a model that evaluated the impact of total ACE score, TD status, and ACE score x TD status. As hypothesized and in agreement with our results using ACE group, there was a robust effect of ACE score as ACE score was significantly negatively associated with within-network connectivity during sham depletion ($p=0.004$). Additionally, there was a significant ACE score x TD status interaction on within-network connectivity ($p=0.04$). Consistent with our TD x ACE group analysis, active TD diminished the impact of ACE score; there was no association between total ACE score and within-network connectivity during active TD ($p=0.2$). The effect of all covariates included in analyses have been regressed from the connectivity values depicted. ACE= adverse childhood experiences; TD = tryptophan depletion; *$p \leq 0.1$, **$p \leq 0.5$, ***$p \leq 0.01$

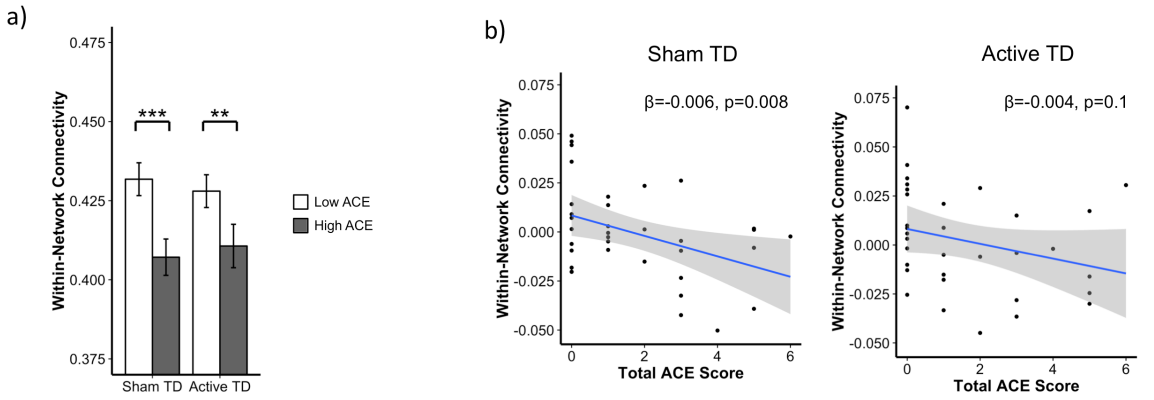

Figure 4-4. ACE and TD effects on within-network connectivity in replication timeseries. a. To probe the validity of our results, we tested our hypotheses regarding the impact of ACE and TD on functional connectivity using a separate set of task-regressed timeseries data acquired in a subsample of our participants. As in the discovery dataset, we used a model that evaluated the impact of ACE group, TD status, and ACE group x TD status on within-network connectivity to test our hypotheses that ACE would be associated with lower connectivity and that tryptophan depletion would selectively lower connectivity only in the high ACE group. The effect of ACE group was robust and consistent with discovery results. Within-network connectivity was significantly lower in the high ACE group during both sham TD (p=0.002) and active TD (p=0.05). The effect of TD in the low ACE group was also replicated and remained non-significant. However in contrast to discovery results, there was no significant ACE group x TD status interaction and no effect of TD in the high ACE group. Bars represent least-square means adjusted for age, time since last menstrual period, estradiol level, global connectivity, and motion. Error bars represent standard error. **b**. We also attempted to replicate our findings regarding the continuous effects of ACE using a model that evaluated the impact of total ACE score, TD status, and ACE score x TD status. Consistent with our hypothesis and analyses using ACE group, total ACE score was

significantly negatively associated with within-network connectivity during sham TD ($p=0.008$). As in the discovery dataset, this association did not reach significance during active TD. However, it was present at a trend level ($p=0.1$), and the interaction between ACE score and TD status was not significant. The effect of all covariates included in analyses have been regressed from the connectivity values depicted. ACE= adverse childhood experiences; TD = tryptophan depletion; *$p \leq 0.1$, **$p \leq 0.5$, ***$p \leq 0.01$

Table 4-S1. Occurrence of Individual ACEs in Study Sample

	Low ACE	**High ACE**
Mean ACE	0.29 ± 0.46	3.75 ± 1.18
Abuse		
Emotional abuse	0	10
Physical abuse	0	5
Sexual abuse	1	5
Neglect		
Emotional neglect	0	9
Physical neglect	0	1
Household dysfunction		
Parents divorced/separated	2	8
Witnessed domestic violence	0	8
Drug abuse in household	3	6
Mental illness in household	1	9
Incarceration of family member	0	0

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CHAPTER 5

Lisdexamfetamine Effects on Executive Activation and Neurochemistry in Menopausal Women with Executive Difficulties

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Shanmugan S, Loughead J, Nanga RP, Elliott M, Hariharan H, Appleby D, Kim D, Ruparel K, Reddy R, Brown TE, Epperson CN (2017) Lisdexamfetamine Effects on Executive Activation and Neurochemistry in Menopausal Women with Executive Function Difficulties. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 42:437-445.

Abstract

Many women with no history of executive dysfunction report difficulties in this domain during the menopause transition. Lisdexamfetamine (LDX) has been suggested to be a safe and effective treatment option for these women. However, the mechanisms by which LDX improves executive functioning in these women is not known. Here we investigated the effects of LDX on brain activation and neurochemistry, hypothesizing that LDX would be associated with increased activation and decreased glutamate in executive regions. Fourteen women underwent multi-modal neuroimaging at 7T at three time points in this baseline-corrected, double-blind, placebo controlled, crossover study. Effects of LDX on symptom severity, BOLD signal, and DLPFC glutamate + glutamine (Glx) were measured using a clinician-administered questionnaire, fMRI during performance of a fractal n-back task, and ^{1}H-MRS, respectively. The effect of treatment (LDX minus baseline vs. placebo minus baseline) on these behavioral and neural markers of executive function was examined using repeated measures mixed-effects models. LDX treatment was associated with decreased symptom severity, increased activation in the insula and DLPFC, and decreased DLPFC Glx. Additionally, magnitude of LDX-induced improvement in symptom severity predicted both direction and magnitude of LDX-induced change in insular and DLPFC activation. Moreover, symptom severity was positively correlated with Glx concentration in the left DLPFC at baseline. These findings provide novel evidence that the neural mechanisms by which LDX acts to improve self-reported executive functioning in healthy menopausal women with midlife onset of executive difficulties include modulation of insular and DLPFC recruitment as well as decrease in DLPFC Glx concentration.

Introduction

During the menopause transition, many women with no history of executive functioning deficits report cognitive difficulties in domains including working memory, organization, focus, and attention (Epperson et al., 2011). This midlife onset of executive difficulties may be a result of reduced estradiol modulation of the executive system (Shanmugan and Epperson, 2014). Proper executive functioning depends on the neurochemical environment of executive regions including the dorsolateral prefrontal cortex (DLPFC), the region of the prefrontal cortex (PFC) critical for attention and working memory (Arnsten and Jin, 2014). PFC function has been characterized as an inverted U-shaped curve such that optimal executive function (EF) depends on a balance of factors including baseline monoaminergic and glutamatergic function (Arnsten and Jin, 2014) as well as hormonal status (Jacobs and D'Esposito, 2011). In such a model, a decrease in estradiol levels during menopause could cause executive difficulties in women whose genetics or previous life experiences make them susceptible to such a cognitive decline (Kudielka et al., 2009; Shanmugan and Epperson, 2014; Culpepper, 2015) (**Figure 5-1**).

Estradiol affects working memory through modulation of PFC dopamine activity. In women who metabolize dopamine more quickly, low estradiol levels result in improper PFC activation during working memory tasks (Jacobs and D'Esposito, 2011). Preclinical models have suggested estradiol modulation of glutamatergic function may be an additional mechanism by which estradiol loss induces changes in cognition. Estradiol loss reduces the density of healthy dendritic spines in the DLPFC (Bailey et al., 2011) that are necessary for communication between delay cells (Wang and Arnsten, 2015), neurons capable of maintaining representations across a delay in the absence of sensory input. Loss of this regulation could lead to disinhibition of neurons that fire in

response to a stimulus, potentially resulting in higher DLPFC glutamate levels and worse EF (Wang and Arnsten, 2015).

As such, interventions that increase dopamine and decrease glutamate may improve executive difficulties induced by loss of estradiol input to the PFC during menopause. We have shown lisdexamfetamine (LDX), a stimulant medication, successfully improves subjective measures of EF in menopausal women with self-reported new onset executive difficulties (Epperson et al., 2015). In addition to increasing extracellular dopamine and norepinephrine, stimulants also have direct and indirect effects on striatal and cortical glutamate critical to optimal PFC function (Paspalas and Goldman-Rakic, 2005).

Previous studies have shown that stimulant medications increase blood oxygen level dependent (BOLD) signal in executive regions, including the insula (Rubia et al., 2014) and DLPFC (Wong and Stevens, 2012) in subjects with ADHD. Additionally, stimulants also decrease glutamate levels in the PFC of children with ADHD (Wiguna et al., 2012). However, no neuroimaging studies examining the effect of LDX on brain activation or neurochemistry have been performed. Additionally, whether stimulants improve executive deficits in populations other than ADHD with similar cognitive difficulties by analogous mechanisms is not known. Furthermore, while preclinical studies suggest glutamatergic tone may mediate executive capabilities (Wang and Arnsten, 2015), information regarding the role that DLPFC glutamate levels play in EF in human subjects is limited. Thus, human subject studies evaluating the effect of stimulants on neural markers of executive dysfunction in populations other than ADHD are necessary to determine the mechanisms by which such medications improve executive deficits in these populations.

Accordingly, here we used multi-modal neuroimaging and a baseline corrected, double-blind, placebo controlled, crossover design to examine the effects of LDX on executive system recruitment and neurochemistry in 14 healthy menopausal women with midlife onset executive dysfunction. We hypothesized LDX would increase executive activation during a working memory task and decrease glutamate + glutamine (Glx) levels in the DLPFC at rest. Furthermore, we predicted baseline DLPFC Glx levels would be reflective of the degree of executive dysfunction.

Methods

Participants

Subjects in this study represent a subset of women who participated in a clinical trial investigating the effects of LDX on EF (Epperson et al., 2015) and were recruited from the parent trial to participate in this neuroimaging adjunct study. Women ages 45 to 60 with EF difficulty onset during menopause and were within 5 years of their last menstrual period (LMP) were eligible to participate. Perimenopausal women had irregular menstrual cycles for ≥12 months, no period for ≥ 3 months, and serum follicle stimulating hormone (FSH) level of ≥ 20 IU/L. LMP of ≥12 months and serum FSH levels ≥35 IU/L indicated postmenopausal status. Subjective EF symptom severity was assessed using the Brown Attention Deficit Disorder Scale (BADDS) (Brown, 1996). BADDS score of ≥ 20 and onset of symptoms coinciding with the initiation of menstrual cycle irregularity were required.

Women with a lifetime history of a DSM-IV psychotic disorder or psychostimulant abuse, substance abuse disorder in the previous year, or present Axis I psychiatric disorder were excluded. Psychotropic medication use, ET use within the previous 6 months, positive pregnancy test, Mini-mental Status Examination score < 26, IQ ≤ 90,

history of seizures, cardiac disease, active hypertension, claustrophobia, abnormal electrocardiogram at screening, left-handedness, and metallic implants were all exclusionary. Subjects were excluded from ROI analyses for poor co-registration (n=2). Excessive motion (mean relative displacement > 0.5 mm; *n*=1) was an additional exclusionary criterion in whole-brain analyses.

Study Design

This study was a double-blind, placebo controlled, crossover study. After screening, subjects underwent neuroimaging and cognitive assessment at three time points. After baseline, subjects were randomized to one pill of study medication (LDX 20 mg or placebo) daily for the first week, two pills daily for the second week, and, if tolerated, three pills daily for the final 2 weeks of each trial. Participants were allowed to remain in the study if they could tolerate at least 1 pill per day throughout the study. Upon completion of the first 4-week trial, participants underwent a 2-week washout and were crossed over to the other treatment condition. Testing and imaging were conducted approximately 2 – 6 hours after last LDX dose.

Assessment of Executive Function

Assessment of subjective EF was as previously reported (Epperson et al., 2015). Briefly, subjects completed the BADDS, a validated subjective measure of EFs (Sandra Kooij et al., 2008). The BADDS is a clinician-administered questionnaire that assesses the frequency and severity of five clusters of executive dysfunction: (1) organization and activating for work, (2) sustaining attention and concentration, (3) sustaining alertness, effort, and processing speed, (4) managing affective interference, and (5) using working memory and accessing recall. Subjects rate symptoms on a scale from 0 to 3, with 0

meaning the problem described does not relate to them and 3 indicating the problem occurs almost daily. Subjects also completed an out-of-scanner cognitive battery consisting of a letter n-back task, NYU paragraph recall task, and Penn Continuous Performance Task to assess working memory, verbal memory, and sustained attention, respectively (See Epperson et al, 2015 for further detail).

fMRI Task paradigm

Subjects completed a fractal n-back task (Ragland et al., 2002) with 3 conditions (0-, 2-, and 3-back) during each scan to probe working memory. Subject performance was monitored to ensure adequate task engagement. See Supplementary Methods for further detail.

Image Processing

Imaging data were acquired on 7T Siemens Trio scanner (Supplementary Methods). In comparison to 3T, the higher signal to noise ratio and greater sensitivity to BOLD signal at 7T allows for increased ability to distinguish differences between conditions, though 7T is more sensitive to distortion and artifacts.

Timeseries were analyzed with FEAT (fMRI Expert Analysis Tool) (Supplementary Methods). To determine the effect of treatment (active-baseline vs. placebo-baseline), mean signal change for the 3-back contrast was extracted from functionally defined ROIs in the right and left DLPFC, medial frontal/cingulate gyrus (MF/CG), right and left insula, right and left parietal cortex, and posterior cingulate cortex. ROI masks for these regions were functionally defined using the parametric contrast of the group mean of all sessions (baseline, active, and placebo). The activation map of the group mean of the parametric contrast was cluster corrected at a voxel

threshold of z>4.5 and cluster probability of p <0.001 (Woolrich et al., 2009). ROI masks were transformed into subject space using FLIRT. An exploratory whole brain analysis using a two sample paired t-test in FSL was also performed on the 3-back contrast to characterize the effect of treatment (active vs. placebo). Type I error control was provided by cluster correction using Gaussian Random Field Theory (voxel height of z >1.6; cluster probability of p <0.05).

MRS and Quantification of Glutamate

Water reference single voxel spectroscopy (SVS) and water suppressed SVS scans were obtained on a voxel placed in the left DLPFC (15x30x20 mm^3) (**Supplementary Figure 5-1a**). Voxels were placed approximately 1 cm from the skull to avoid lipid contamination. Automated shimming of the B_0 field was performed on the voxel to obtain localized water line width of ~20 Hz or less using FASTMAP. Variable power RF pulses with optimized relaxation delays (Tkac et al., 1999) were used to obtain water suppression spectra (Cai et al., 2012). SVS for Glx were obtained using short TE SVS with modified Point-Resolved Spectroscopy sequence (PRESS) having the following parameters: spectral width=4kHz, number of points=2048, averages=16 (water reference) or 64 (water suppressed), TE=20 ms, TR=3000 ms. Chemical shift artifact for Glx was minimized by setting water acquisition spectrum excitation and refocusing pulses in resonance with the water peak at 4.7ppm, and also setting water suppressed spectrum excitation and refocusing pulses in resonance with the Glx peak at 2.35ppm.

Water reference data were used to obtain channel wise time dependent phase shifts due to eddy current and amplitude scale factors. Using a fitting method validated by Cai et al. (Cai et al., 2012) metabolite peaks from water suppressed spectrum were fitted as Lorentzian functions with non-linear least squares fitting using *lsqcurvefit* in

MATLAB. This procedure accounted for amplitudes, line widths and peak positions for 8 macromolecular and 14 metabolite peaks. Normalization by water reference signal allowed for quantification of Glx (**Supplementary Figure 5-1b**). See Supplementary Information for goodness of fit assessment.

Statistical Analysis

Mixed effects models were employed to compare the BADDS scores, cognitive task performance, BOLD in ROIs, and glutamate levels measured at the end of the active trial minus baseline vs. those measured at the end of the placebo minus baseline for each participant to account for repeated measures. While the parametric activation map used to identify ROIs was corrected for multiple comparisons using Gaussian Random Field Theory (Woolrich et al., 2009), we did not further correct for the number of ROIs examined and p-values reported are uncorrected. BMI was included as a covariate in models examining the effect of LDX on BOLD and glutamate to ensure LDX-induced change in BMI did not affect results. Age and the proportion of grey matter in the spectroscopy voxel were included as covariates in a model examining the drug effect on glutamate (Supplementary Results).

Spearman rank-order correlations were used to compare 1) BADDS scores to glutamate levels at baseline and 2) change in BADDS scores between active and baseline to change in BOLD between active and baseline. Association between glutamate and BOLD was evaluated with linear regression. Measures of anxiety, depression, sleep, and overlap between BOLD and spectroscopy voxels were controlled for in supplementary analyses (Supplementary Methods). The effect of LDX on n-back performance was evaluated using mixed effects models with condition as a 3-level predictor variable to minimize the effect of missing data.

Results

Participants

Eighteen subjects from the parent clinical trial were enrolled in this neuroimaging adjunct study. Of these 18 subjects, 14 subjects completed all 3 imaging session. One subject was unable to see the screen during the fMRI task and did not complete the baseline session, two subjects withdrew after the baseline session, and one subject was unable to tolerate being in the scanner during her final scan due to nasal congestion. Baseline characteristics for the 14 women who completed both active LDX and placebo conditions are depicted in **Table 5-1**. The order of active and placebo visits across these 14 women was counterbalanced: 7 received active first and 7 received placebo first. The 14 subjects who completed imaging had higher total BADDS scores at baseline than subjects from the parent clinical trial who did not participate in this imaging adjunct study (imaging: 44.7 ± 17.8; other: 28.6 ± 12.3; t=-3.01, DF=30, p=0.005) but were similar in terms of age, race, education (See Supplementary Information for Methods). All but two participants finished each trial taking three pills per day. One participant experienced jitteriness and increased heart rate after increasing from one to two pills and remained on one pill for the remainder of the trial (Active LDX). The other experienced an increase in blood pressure upon increasing from two to three pills and remained on two pills for the remainder of that trial (Placebo).

Behavioral Results

LDX significantly decreased total BADDS (F=26.6, DF=13, p<0.0002) (**Figure 5-2a**, **Supplementary Figure 5-2a**) and each BADDS subscale (organization/activation for work, F=14.3, DF=13, p=0.002; attention/concentration, F=31.7, DF=13, p<0.0001; alertness/effort/processing speed, F=12.3, DF=13, p=0.004; managing affective

interference, F=4.8, DF=13, p=0.05; working memory/accessing recall, F=14.4, DF=13, p=0.002) (**Figure 5-2b**, **Supplementary Figure 5-2b**). While there was a trend towards an effect of LDX in decreasing out-of-scanner 2-back true positive reaction time (F=3.2, DF=10, p=0.1), no significant effect of LDX was observed on delayed paragraph recall, continuous performance task, or in-scanner n-back performance (p>0.05) (**Supplementary Figure 5-3**).

BOLD Results

As expected, the n-back task robustly activated executive network regions and deactivated non-executive regions (**Figure 5-3a**). ROI analyses demonstrated LDX increased activation in the right Insula/IFG (F=20.7, DF=11, p=0.0008) and left DLPFC (F=2.9, DF=11, p=0.12) (**Figure 5-3b**, **Supplementary Table 5-1**) during the 3-back. This robust insular finding remained significant in an exploratory whole-brain analysis (MNI coordinates: x=48, y=52, z=18; *k*=1416) (**Figure 5-3c**).

LDX-induced change in right insula activation was positively associated with LDX-induced decrease in total BADDS score (r=0.95, p=<0.0001, t=9.88, DF=11), BADDS subscale 1 (r=0.86, p=0.0004, t=5.22, DF=11), and BADDS subscale 3 (r=0.83, p=0.0008, t=4.70, DF=11). Similar results were obtained for correlation between LDX-induced change in activation in the left DLPFC and total BADDS score (r=0.78, p=0.003, t=3.90, DF=11), BADDS subscale 1 (r=0.78, p=0.0028, t=3.93, DF=11), and BADDS subscale 3 (r=0.81, p=0.0014, t=4.38, DF=11) (**Figure 5-3d**). Correlations remained significant when controlling for measures of anxiety, depression, and sleep (Supplementary Results).

Spectroscopy Results

LDX was associated with decreased Glx concentration in left DLPFC (F=4.6, DF=13, p=0.052) (**Figure 5-4a**, **Supplementary Figure 5-4**). Glx values (mean mM ± SD) at baseline, active, and placebo were 9.14 ± 0.81, 8.4 ± 1.03, and 8.91 ± 0.86, respectively. At baseline, left DLPFC Glx concentration was positively correlated with total BADDS (r=0.64, p=0.01, t=2.90, DF=13), BADDS subscale 1 (r=0.70, p=0.005, t=3.43, DF=13), and BADDS subscale 3 (r=0.58, p=0.03, t=2.47, DF=13) (**Figure 5-4b**). There were no other significant correlations between Glx and BADDS or between Glx and DLPFC BOLD (p>0.05).

Discussion

In this double-blind, placebo controlled, cross-over study, we examined the effect of LDX on behavioral and neural markers of EF at 7T. To account for the inherent variability in the BOLD signal, a repeated measures, baseline corrected study design was used. LDX treatment was associated with improved self-reported EF in comparison to placebo, as shown by a significant reduction in BADDS scores. Furthermore, LDX treatment significantly increased recruitment of executive regions during performance of a working memory task. Additionally, LDX treatment decreased Glx concentrations in the left DLPFC at rest. Taken together, these data provide novel evidence for the neural mechanisms by which this drug acts to improve self-reported EF in previously healthy menopausal women with midlife onset of executive difficulties.

Evidence for psychostimulant effects on EF

As in the parent clinical trial (Epperson et al., 2015), we found that LDX improved EF in this subset of women as measured by self-reported symptom severity. We did not, however, find an effect of LDX on working memory task performance. While

the task used in this study was validated in a large sample of healthy volunteers (Gur et al., 2010), education level has been shown to be correlated with performance on executive tasks, particularly the n-back (Gur et al., 2010). Given the large portion of our sample that had at least a college degree (79%) and graduate degree (35%), ceiling effects from the high performance at baseline likely rendered these tests unable to detect any objective improvements in EF.

Evidence for psychostimulant effects on executive activation

The most robust finding in the present study is evidence of LDX's effect of increasing activation in the insula/IFG region. This was observed in both an ROI analysis at all levels of the n-back task performed as well as in a whole-brain analysis. These results accord with a copious literature of neuroimaging studies of stimulant effects in individuals with ADHD (Rubia et al., 2014), and moreover highlight the mechanism of this drug's action in a population that exhibits similar executive deficits but who do not meet criteria for ADHD.

A meta-analysis of 14 fMRI data sets found stimulants relative to placebo/off-medication most consistently increase activation in the insula/IFC (Rubia et al., 2014). This finding is present during tasks probing executive domains of interference inhibition (Rubia et al., 2014), time discrimination (Rubia et al., 2014) and working memory (Spencer et al., 2013). The insula is part of the cinglo-opercular control network critical for cognitive control, and is particularly important in the maintenance of task sets and error monitoring (Power et al., 2011).

While the DLPFC is a part of the frontoparietal network supporting goal-directed executive processes (Cortese et al., 2012) and is an area implicated in the pathogenesis of executive deficits seen in ADHD (Cortese et al., 2012), the effect of stimulants on the

DLPFC is inconsistent. Studies have found stimulants increase (Wong and Stevens, 2012), decrease (Cubillo et al., 2014), or have no effect (Kobel et al., 2009; Rubia et al., 2014) on DLPFC activation. Such inconsistency may be due in part to the type or dosage of medication used and level of working memory load probed (Cubillo et al., 2014).

We also found the magnitude of LDX-induced improvement in symptom severity predicted both the direction and magnitude of LDX-induced change in both insular and DLPFC activation. While subjects with the greatest improvements in BADDS scores demonstrated less task-induced activation, subjects with the least improvements in BADDS scores demonstrated greater task-induced activation, suggesting that other factors such as genetics, adverse childhood experiences, baseline activation and symptom severity, and time since last menstrual period impact the relationship between LDX treatment and brain and behavioral response. Our observation that LDX treatment resulted in an overall increase in DLPFC and insular cortex BOLD (**Figures 5-3b – 5-3c**) was likely driven by the greater proportion of individuals who experienced an increase rather than a decrease in activation from baseline (**Figure 5-3d**). One study found no correlation between activation during a stop-signal task and symptom severity after controlling for psychostimulant use in adults with ADHD (Congdon et al., 2014). However, no studies have looked at the correlation between stimulant-induced changes in brain activation during working memory and stimulant-induced changes in symptom severity in adults. Thus, our findings make an important step towards understanding factors that impact stimulant effects on brain activation and may in part explain the contradictory findings (Kobel et al., 2009; Wong and Stevens, 2012; Cubillo et al., 2014; Rubia et al., 2014) of stimulants on brain activation in the literature.

Evidence for psychostimulant effects on neurochemistry

A third important finding in this study was that LDX significantly decreases Glx concentrations in the DLPFC from baseline in comparison to placebo. Results of the few studies that have examined the effect of stimulants on left PFC glutamate levels are inconsistent and have shown that stimulants either decrease (Wiguna et al., 2012) or have no effect (Carrey et al., 2002; Husarova et al., 2014b) on glutamate concentrations. Differential effects of stimulants on glutamate levels have been attributed to differences in medication formulation, treatment duration, or a combination of both (Husarova et al., 2014b).

Importantly, we found that left DLPFC Glx levels at baseline positively correlated with self-reported executive function symptom severity. Only one other study has examined the relationship between DLPFC glutamate concentration and subjective executive dysfunction symptom severity (Husarova et al., 2014a). While this study found no correlation between left DLPFC glutamate and parent-reported symptom severity, associations between executive symptom severity and other neurometabolites including N-acetylaspartate/creatine, glutamate + glutamine/creatine, and choline/creatine in the DLPFC or white matter behind the DLPFC were observed. However, it is important to note that subjects in that study were children who met criteria for ADHD combined subtype and almost one-third of subjects also had comorbid oppositional defiant disorder.

Interestingly, we did not find an association between treatment-induced change in Glx and treatment-induced change in symptom severity. This would seem to indicate that LDX disrupts the relationship between Glx and symptom severity seen at baseline. The implications of the lack of association between these variables with placebo treatment is less clear, but could indicate that either placebo also causes a change in

neurochemistry, prior LDX treatment alters the relationship between Glx and symptom severity, or that the washout period was not of sufficient duration to return the relationship between these variables to baseline. Glutamate antagonists have been shown to improve symptoms of anxiety and depression in preclinical models (Gerhard et al., 2016), suggesting the moderating effect of glutamate on mood symptoms could be a confounding factor when evaluating the relationship between glutamate and executive symptom severity. However, we found that correlations between Glx and total BADDS score remained significant even when controlling for measures of depression and sleep, indicating that this relationship is not simply a reflection of the effect of LDX on mood. No other studies have directly examined the correlation between stimulant-induced changes in left DLPFC Glx and stimulant induced changes in symptom severity.

We did not find an association between Glx concentration and BOLD signal in the left DLPFC. Interestingly, both of these measures were correlated with the same measures of symptom severity: total BADDS, Subscale 1, and Subscale 3. There was not, however, an association between neural outcome measures and other BADDS subscales even though LDX significantly improved symptoms in all domains, suggesting certain subscales may be more sensitive to neural markers of EF in this population. The relationship between glutamate concentration and BOLD signal is not well established. One study found a positive correlation between dorsal anterior cingulate cortex (ACC) glutamate and BOLD during a cognitive control task in several regions including the retrosplenial cortex, orbitofrontal cortex, inferior parietal lobule, and basal ganglia (Falkenberg et al., 2012). Another study found a positive correlation between dorsomedial PFC glutamate and pregenual ACC BOLD during emotional processing (Stan et al., 2014). In contrast, another study found no correlation between glutamate and BOLD in the ACC or inferior frontal gyrus before, during, or after performance of an

interference inhibition task (Kuhn et al., 2015) even though voxel placement was guided by individual's BOLD activity. However, no studies have examined the relationship between DLPFC glutamate concentration and activation during a working memory task at baseline or with psychostimulant treatment.

Limitations

Certain limitations of the present study should be noted. First, the sample size is relatively small. However, the repeated-measures design allowed for decreased variance in estimates of treatment effects and increased power to detect between group differences. Additionally, while the BOLD signal is inherently variable, this study included a baseline session allowing comparisons between active and placebo to be baseline corrected. Doing so contrasted the minimal differences in behavior, executive activation, and Glx concentrations between placebo and baseline with the significant differences between active and baseline. Furthermore, the high signal to noise ratio at 7T allows for increased ability to distinguish differences between conditions.

A second limitation of this study is that ^{1}H-MRS does not distinguish between intra- and extracellular glutamate levels. Preclinical studies show the extracellular pool of glutamate is between 1-4 micromoles (Lerma et al., 1986). Glutamate measured using ^{1}H-MRS is between 8-12 millimoles, suggesting our findings are based primarily on the intracellular pool of glutamate. Another limitation is the inability to resolve glutamate from glutamine, even at 7T, due to spin-spin coupling. However, the small values in Glx fit uncertainty (<0.1) and strong intraclass correlation (0.6) between Glx values at baseline and placebo suggest robust goodness of fit and test-retest reliability, respectively.

Third, current substance abuse could affect both behavioral and imaging outcomes and subjects were not required to undergo a drug screen on each test day.

However, a clear urine toxicology during screening was required for inclusion in the study. Additionally, because these subjects did not have any history of substance abuse, substance abuse during the study was unlikely. Fourth, we did not formally assess whether participants could accurately guess when they were on active LDX or placebo. However, we did examine carry-over effects in the larger clinical trial and did not find any order effects on behavioral outcome measures (Epperson et al, 2015) suggesting that if participants accurately identified the active LDX from placebo trials, this knowledge did not unduly impact our outcomes.

Conclusions

In summary, these data provide novel evidence regarding the neural mechanisms by which stimulants act to improve executive functioning in healthy menopausal women with midlife onset of executive difficulties. Further research is needed to determine if other factors such as genetics or adverse childhood experiences modulate the effect of LDX on glutamate and executive system recruitment. Such information could serve as markers of women who may experience cognitive difficulties upon entering menopause and of women who would benefit from early intervention. Furthermore, the mechanisms by which loss of estradiol and healthy dendritic spines as well as higher cortical glutamate concentrations contribute to worse executive functioning in human subjects are yet to be elucidated, and future research should consider the role of such factors in promoting neuroinflammation and oxidative stress (Kitamura et al., 2009). Additional research in a larger sample would be helpful in ascertaining whether there is a three-way interaction between symptom severity, BOLD, and Glx. A larger sample may also reveal an effect of menopause stage on these measures. Executive function has been shown to decline between early and late post-

menopause (Elsabagh et al., 2007), though declines observed in other cognitive domains such as verbal memory (Epperson et al., 2013) suggest executive dysfunction may begin earlier. Moreover, it would be important to confirm whether LDX acts by similar mechanisms after surgery- or chemotherapy-induced menopause.

Table 5-1. Participant Characteristics

	Mean (SD) or number (%)
Age (years)	53.1 (3.0)
Time since last menstrual period (months)	31.8 (18.1)
IQ	115.9 (11.3)
Mini-mental status exam	28.8 (1.1)
Marital status	
Single	2 (14.3)
Married	9 (64.3)
Divorced/separated	2 (14.3)
Widowed	0 (0)
Did not disclose	1 (7.1)
Menopause status	
Perimenopause	2 (14.3)
Postmenopause	12 (85.7)
Race	
Caucasian	9 (64.3)
African American	3 (21.4)
American Indian/Alaska native	1 (7.1)
Others	1 (7.1)
Ethnicity	
Hispanic	0 (0)
Non-Hispanic	14 (100)
Education	
High school	1 (7.1)
Some college/vocational	2 (14.3)
College graduate/some graduate school	6 (42.9)
Graduate/professional degree	5 (35.7)
Household income	
Unknown or did not disclose	4 (28.6)
< $50,000	2 (14.3)
$50,000 to $100,000	3 (21.4)
$100,000 to $200,000	4 (28.6)
> $200,000	0 (0)
Employed	
Full time	14 (100)

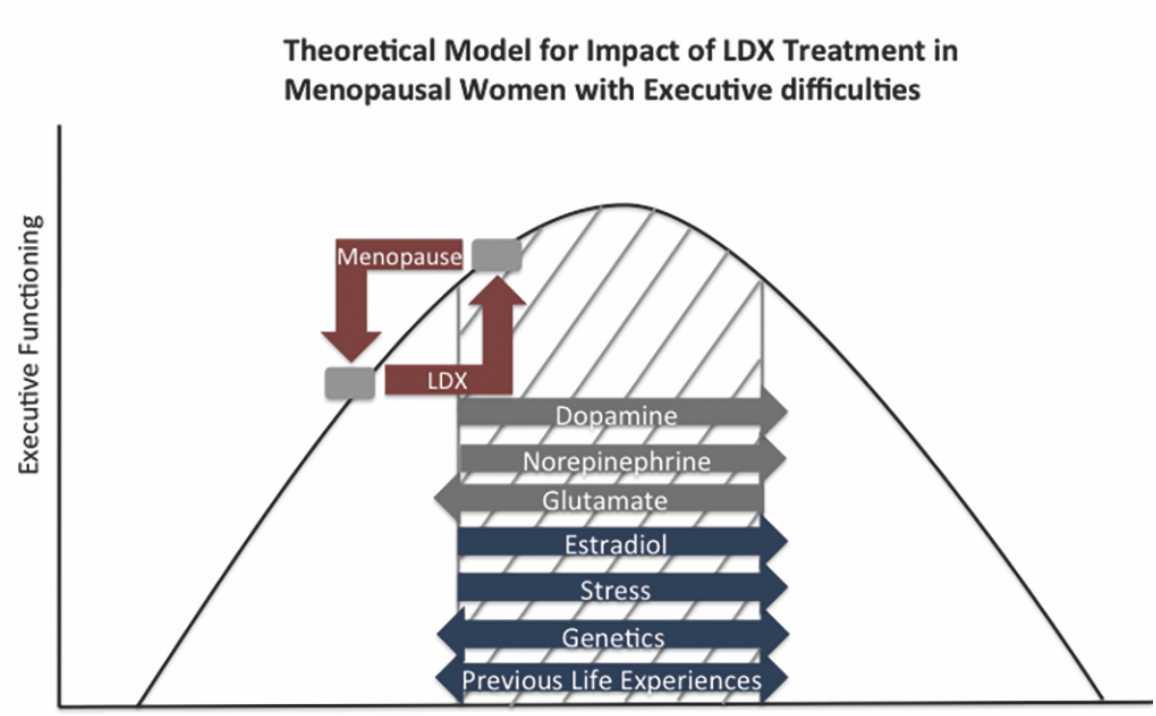

Figure 5-1. Model for effects of estradiol and LDX on executive function. Executive functioning capacity depends on the neurochemical environment in the PFC. Within the optimum range of neurotransmitter concentrations, represented by the grey crosshatching in the figure, normal executive functioning is maintained by a balance of factors including dopamine, norepinephrine, and glutamate concentrations as well as estradiol level. Where a woman falls on this curve depends on her genetics for catecholaminergic and glutamatergic metabolism and signaling as well as hormonal status and stress level. Response to stress is modified by numerous factors such as age, hormonal and reproductive status, current or previous use of medications such as glucocorticoids or psychoactive drugs, smoking, coffee and alcohol consumption, caloric intake, genetics, exposure to prenatal stress, birth weight, gestational age, level of early family adversity, and position in social hierarchy indicating individual lifestyle choices and adverse childhood experiences may impact an individual's executive functioning capacity before and during menopause [reviewed by Kudielka et al., 2009]. Additionally,

factors such as depressive symptoms (Culpepper, 2015), substance use, and medication side effects (Kudielka et al, 2009) may also contribute to an individual's executive functioning capacity. During menopause, women whose combination of such factors place them toward the left of the optimal range may experience executive functioning difficulties due to loss of estradiol modulation of prefrontal systems. LDX may improve executive functioning in these women by increasing dopamine and norepinephrine as well as decreasing glutamate in the PFC, though response to LDX should be considered in the context of underlying genetic variability in catecholaminergic and glutamatergic neurotransmission.

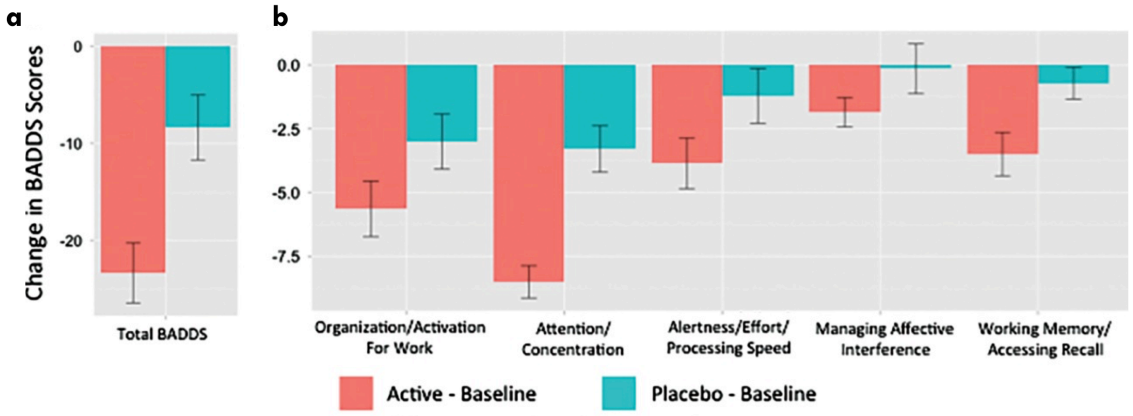

Figure 5-2. LDX improves subjective measures of executive function. The decrease in BADDS scores from baseline was significantly greater with LDX versus placebo for both **(a)** total BADDS (F=26.6, DF=13, p<0.0002) as well as **(b)** each BADDS subscale (organization/activation for work, F=14.3, DF=13, p=0.002; attention/concentration, F=31.7, DF=13, p<0.0001; alertness/effort/processing speed, =12.3, DF=13, p=0.004; managing affective interference, F=4.8, DF=13, p=0.05; working memory/accessing recall, F=14.4, DF=13, p=0.002). Error bars represent standard error.

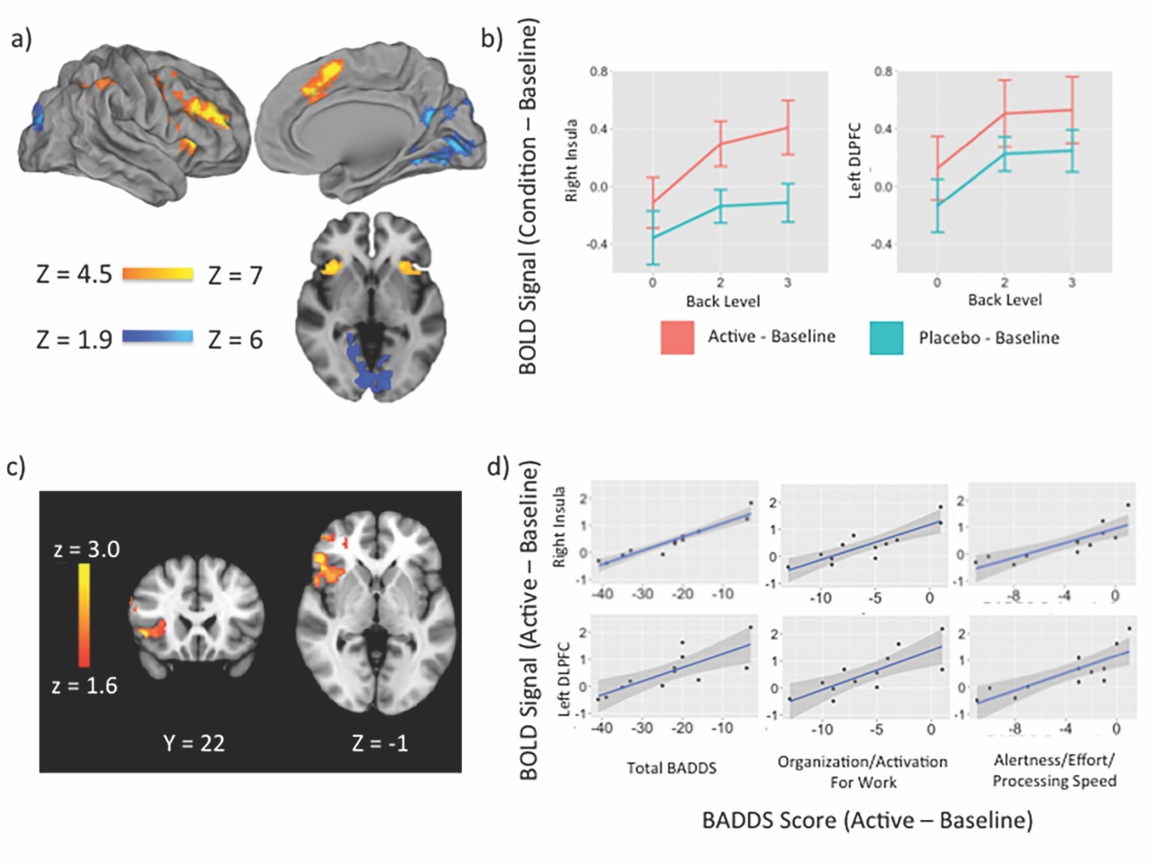

Figure 5-3. LDX increases executive activation during working memory. (a) The n-back task robustly activated executive network regions and deactivated non-executive regions, as depicted by the parametric contrast (z>4.5, p<0.001). **(b)** Recruitment of the right insula, and left DLPFC increased with increasing levels of working memory load. Values plotted represent mean difference in activation from baseline at each task level. Error bars represent standard deviations. **(c)** Whole-brain analysis using a paired t-test (z>1.6, p <0.05) of the 3-back contrast demonstrated that activation in the right insula (MNI coordinates: x=48, y=52, z=18; *k*=1416) was significantly greater than placebo. **(d)** LDX-induced increase in both right insula and left DLPFC BOLD signal from baseline during the 3-back was positively correlated with LDX-induced decrease in total BADDS Scores as well as BADDS subscales measuring organization/activation for work and

alertness/effort/processing speed. Subjects with the greatest improvements in BADDS scores demonstrated less task-induced activation with LDX while subjects with the least improvements in BADDS scores demonstrated greater task-induced activation with LDX. Right insula: total BADDS ($r=0.95$, $p=<0.0001$, $t=9.88$, $DF=11$); organization/activation for work ($r=0.86$, $p=0.0004$, $t=5.22$, $DF=11$); alertness/effort/processing speed ($r=0.83$, $p=0.0008$, $t=4.70$, $DF=11$). Left DLPFC: total BADDS ($r=0.78$, $p=0.003$, $t=3.90$, $DF=11$); organization/activation for work ($r=0.78$, $p=0.0028$, $t=3.93$, $DF=11$); alertness/effort/processing speed ($r=0.81$, $p=0.0014$, $t=4.38$, $DF=11$).

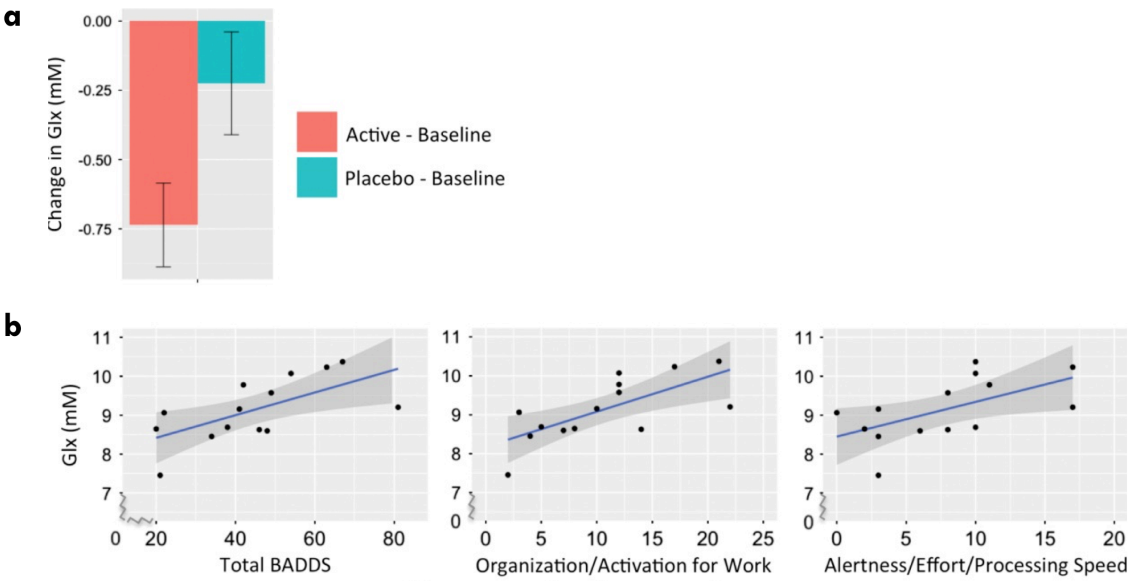

Figure 5-4. Left DLPFC Glx concentrations. (a) Decrease in left DLPFC Glx concentration from baseline was greater with LDX vs placebo (F=4.5, DF=12, p=0.056). Error bars represent standard error. **(b)** At baseline, left DLPFC Glx concentration was positively correlated with total BADDS (r=0.64, p=0.01, t=2.90, DF=13) as well as BADDS subscales measuring organization/activation for work (r=0.70, p=0.005, t=3.43, DF=13) and alertness/effort/processing speed (r=0.58, p=0.03, t=2.47, DF=13).

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Supplementary Information

Supplementary Methods

fMRI Task Paradigm

Subjects completed a fractal version of the n-back task during each fMRI scan. During the task, a fractal was presented for 500 ms followed by a 2500 ms interstimulus interval. This task was used to probe working memory and had 3 conditions: 0-, 2-, and 3-back. Each condition consisted of three 20-trial blocks, each preceded by a 9s instruction period, with a target to foil ratio of 1:3. The task included a total of 45 targets and 135 foils, as well as three 24 s blocks of rest during which a fixation crosshair was displayed. Analyses on in-scanner n-back performance focused on true positives, false positives, and reaction time.

Image Acquisition

Structural and functional images were acquired on 7T Siemens Trio scanner. Structural image acquisition sequence consisted of a gradient echo localizer, a 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) whole brain acquisition (TR, 2110 ms; TE, 3.17 ms; TI, 1500 ms; FOV, 240×180 mm; matrix, 256×192; 160 slices; slice thickness, 1 mm; flip angle, 10°; effective voxel resolution, 0.9×0.9×1 mm), and 3D reformatting of the MPRAGE data. Functional images were obtained using a whole-brain, single-shot, multislice, gradient-echo echoplanar sequence (239 volumes; TR, 3000; TE, 27 ms; flip angle, 70°; FOV, 220×220 mm; matrix 110×110; 37 slices; slice thickness 2.2 mm; effective voxel resolution, 2×2×2.2 mm).

Image Processing

BOLD timeseries data were analyzed with FEAT (fMRI Expert Analysis Tool) in FSL, using skull removal with BET (Smith, 2002), motion-correction with MCFLIRT (Jenkinson et al., 2002), high pass filtering, spatial smoothing (6 mm FWHM), and mean-based intensity normalization. Subject-level timeseries analyses were carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al., 2009). The three condition blocks (0-back, 2-back, and 3-back) were modeled using a canonical (double-gamma) hemodynamic response function with standard plus extended motion parameters included as nuisance covariates. The rest condition (crosshair) served as the unmodeled baseline. The median functional and anatomical volumes were co-registered using boundary-based registration (Greve and Fischl, 2009). The anatomical image was normalized to the MNI 152 T1 2 mm template using linear registration with 12 degrees of freedom. Statistical maps for the contrasts of interest (3-back) were used in group-level analyses.

MRS and Quantification of Glutamate

Goodness of fit for the fitting method used to quantify Glx was evaluated on all scans for all subjects. Using residuals and the Jacobian matrix (dYi/dPj) where Yi represents the fitted result at point i and Pj represents the parameter j, 90% confidence limits were determined for all parameters. Upper and lower integral limits and concentrations for Glx were calculated using the amplitude and width limits for these two metabolites. Goodness of fit was estimated from these integral limits. Fit uncertainty was conservatively represented by the higher of these values. Additionally, test-retest reliability of acquisition and fitting methods were evaluated using intraclass correlation, which assessed agreement between Glx concentrations at baseline and placebo. An F-test was used to test whether intraclass correlation is different from zero.

Statistical Analysis

We examined how representative subjects who completed this neuroimaging adjunct study were of subjects who participated in the parent clinical trial in terms of age, race, education, and baseline BADDS scores. Age and BADDS were assessed using pooled t-tests while race and education were assessed using Fisher's Exact Test.

Modulation of glutamate has been shown to effectively reduce symptoms of depression and anxiety (Gerhard et al., 2016). Primary analyses also included Spearman rank-order correlations to compare 1) BADDS scores to Glx levels at baseline and 2) change in BADDS scores between active and baseline to change in BOLD signal between active and baseline. Therefore, we also included measures of anxiety, depression, and sleep in models examining the change in BADDS scores between active and baseline to change in BOLD signal between active and baseline in subsets of subjects for whom data were available. Anxiety, depression, and sleep were assessed using the Beck Anxiety Inventory, Beck Depression Inventory, and Pittsburgh Sleep Quality Index, respectively. Baseline scores for these indices were used as covariates in the model examining correlations between BADDS scores to Glx levels at baseline.

BMI was included as a covariate in models examining the effect of LDX on BOLD and Glx to ensure LDX-induced change in BMI did not affect results.

Separate linear regression models were used to test for association between Glx and BOLD signal at each condition (baseline, active, placebo). These models were repeated with distance between peak of the BOLD signal and the center of the spectroscopy voxel was included as a covariate to account for variations in overlap between the functionally defined DLPFC ROI and DLPFC spectroscopy voxel. The functionally defined left DLPFC ROI is superficial with respect to cortical tissue making voxel placement for spectroscopy somewhat of a challenge, as any overlap of the voxel

with skull would introduce lipid contamination and partial voluming, obscuring results. While we measured Glx before individuals performed the n-back in order to avoid any potential impact of task activation effecting Glx levels, we could not ensure the voxel used for MRS of Glx always completely overlapped with the functionally defined ROI used for BOLD analyses. However, distance between peak of the BOLD signal and the center of the spectroscopy voxel was included as a covariate to account for variations in overlap between the functionally defined DLPFC ROI and DLPFC spectroscopy voxel. This metric was calculated as follows. The group level DLPFC ROI was transformed into subject space using FLIRT and binarized with *fslmaths*. This mask was multiplied by the subject's zstat image to isolate the subject's BOLD signal within the DLPFC voxel. This image was then transformed to the same T1 space as the subject's spectroscopy voxel. *Fslstats* was then used to obtain the coordinates of the peak of the bold signal and the center of the spectroscopy voxel.

The volume of grey matter in the spectroscopy voxel relative to the total volume of grey matter, white matter, and CSF was determined as follows. FAST was used to segment each skull-stripped T1 image into grey matter, white matter, and CSF. Each segmented image was then multiplied by a mask of the subject's spectroscopy voxel to obtain the number of volumes of grey matter, white matter, and CSF in this voxel. Age and proportion of grey matter volume in the spectroscopy voxel were included as covariates in a model examining the effect of LDX on Glx.

Supplementary Results

BOLD Results

In the right insula, results were similar to the unadjusted model (F=20.7, DF=11, p=0.0008) when controlling for the effects of BMI (F=17.6, DF=9, p=0.0023), anxiety

(F=9.1, DF=7, p=0.019), depression (F=24.0, DF=9, p=0.0009), and sleep (F=14.1, DF=9, p=0.0046). In the left DLPFC, results were similar to the unadjusted model (F=2.9, DF=11, p=0.12) when controlling for the effects of BMI (F=2.2, DF=9, p=0.17), anxiety (F=2.1, DF=7, p=0.19), depression (F=4.8, DF=9, p=0.06), and sleep (F=2.5, DF=9, p=0.15).

As stated in the primary manuscript, significant correlations were observed between LDX-induced change in activation in the right insula and total BADDS score, BADDS subscale 1, and BADDS subscale 3. The correlation between LDX-induced change in activation and LDX-induced change in total BADDS score remained significant when controlling for measures of anxiety ($p<0.0004$, $t=7.07$, $DF=8$), depression ($p<0.0001$, $t=7.04$, $DF=10$), and sleep ($p<0.0001$, $t=9.05$, $DF=10$). Correlations also remained significant for BADDS subscale 1 (anxiety $p<0.008$, $t=3.99$, $DF=8$; depression $p<0.005$, $t=3.09$, $DF=10$; sleep $p<0.002$, $t=4.71$, $DF=10$) and BADDS subscale 3 (anxiety $p<0.02$, $t=3.58$, $DF=8$; depression $p<0.02$, $t=3.13$, $DF=10$; sleep $p<0.003$, $t=4.41$, $DF=10$). Likewise, the correlation between LDX-induced change in left DLPFC activation and LDX-induced change in total BADDS score remained significant when controlling for measures of anxiety ($p=0.0007$, $t=6.42$, $DF=8$), depression ($p<0.03$, $t=2.64$, $DF=10$), and sleep ($p<0.007$, $t=3.65$ $DF=10$). Results were similar for BADDS subscale 1 (anxiety $p<0.005$, $t=3.99$, $DF=8$; depression $p<0.03$, $t=2.81$, $DF=10$; sleep $p<0.006$, $t=3.72$, $DF=10$) and BADDS subscale 3 (anxiety $p<0.002$, $t=5.31$, $DF=8$; depression $p<0.007$, $t=3.66$, $DF=10$; sleep $p<0.003$, $t=4.22$, $DF=10$).

Spectroscopy Results

The SNR for NAA, Glx, Cho, and Cr is ~110, ~20, 60, and 90, respectively. fWHM for NAA and Cr are ~18 Hz (We used line broadening of 6Hz when processing raw data). Glx fit uncertainty (mean ± SD) was 0.096 ± 0.082, 0.095 ± 0.068, and 0.093 ± 0.087 at baseline, placebo and active conditions, respectively. The intraclass correlation between baseline and placebo was 0.63 ($F(6,7)=4.35$, $p=0.038$).

As stated in the main manuscript, significant correlations were observed between baseline Glx concentration in the left DLPFC and total BADDS score, BADDS subscale 1, and BADDS subscale 3. The correlation between Glx and total BADDS score remained significant when controlling for measures of sleep ($p<0.04$, $t=2.49$, $DF=10$) but not measures of anxiety or depression ($p>0.05$). Correlations also remained significant for BADDS subscale 1 when measures of sleep ($p<0.01$, $t=3.05$, $DF=13$) and depression ($p<0.03$, $t=2.60$, $DF=13$) were included, though correlations between Glx and BADDS subscale 3 were no longer significant.

To examine whether variations in voxel placement in relation to the BOLD peak could be affecting results, we calculated a distance metric that was a measure of the distance between the peak of the BOLD signal and the center of the spectroscopy voxel. However, including this distance metric as a covariate did not alter results and correlations between Glx and BOLD did not reach significance.

To ensure LDX-induced decreases in Glx were not due to changes in the amount of grey matter in the spectroscopy voxel, we examined the proportion of grey matter in the voxel at each time point. A mixed model was used to determine whether the proportion of grey matter within this voxel differed by condition. There was no significant difference in this ratio across conditions ($F=0.27$, Numerator $DF=2$, Denom $DF = 26$; $p=0.77$) (Supplementary Figure 5). This proportion (mean ± SD) was 0.17 ± 0.06, 0.17 ± 0.07, and 0.16 ± 0.05 at baseline, active, and placebo, respectively. Results were

consistent with the original model examining the effect of LDX on Glx concentration when the change in this proportion between baseline and condition was included as a covariate (F=4.72, Num DF=1, Denom Df=12, p=0.051).

Effects of LDX on Glx concentration were similar when BMI (p=0.058, t=-2.10, DF=12) and age (p=0.056, t=-2.10, DF=13) were included as covariates.

Supplementary Tables

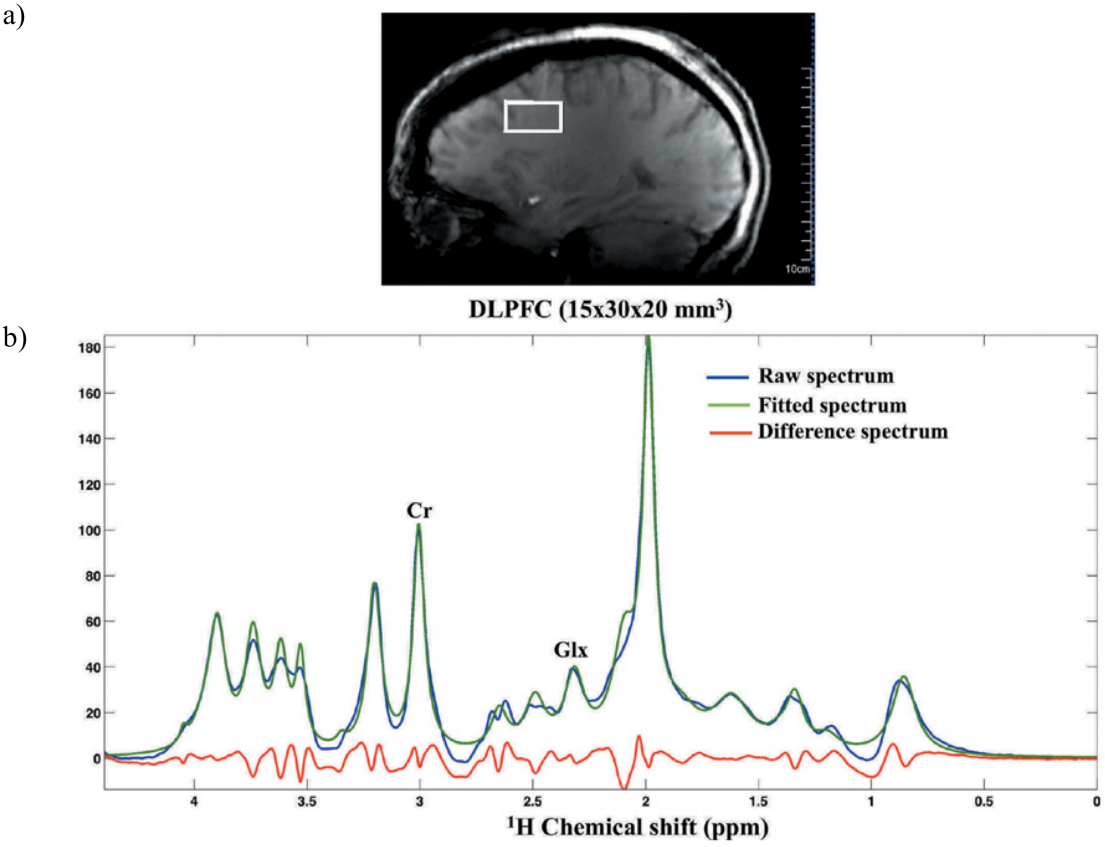
Region	k	Peak *z*	x	y	z
Activation					
Frontal Pole, Right DLPFC	1935	6.55	36	40	18
Precentral Gyrus, Left DLPFC	1360	6.03	-34	-6	42
MF/CG	897	6.14	2	8	54
Right Parietal lobe	473	5.89	42	-48	46
Right Insular Cortex	379	6.49	30	22	6
Left Insular Cortex	356	5.96	-28	22	4
Left Parietal lobe	320	6.31	-50	-48	40
Deactivation					
Occipital Pole, PCC	5243	5.6	-18	-94	26

Supplementary Table 5-1. Effect of n-back task on executive and non-executive regions. Performance of the n-back task robustly activated executive regions ($z>4.5$, $p<0.001$) and deactivated non-executive regions ($z>1.9$, $p<0.05$). Values displayed for mean of parametric contrast for all sessions.

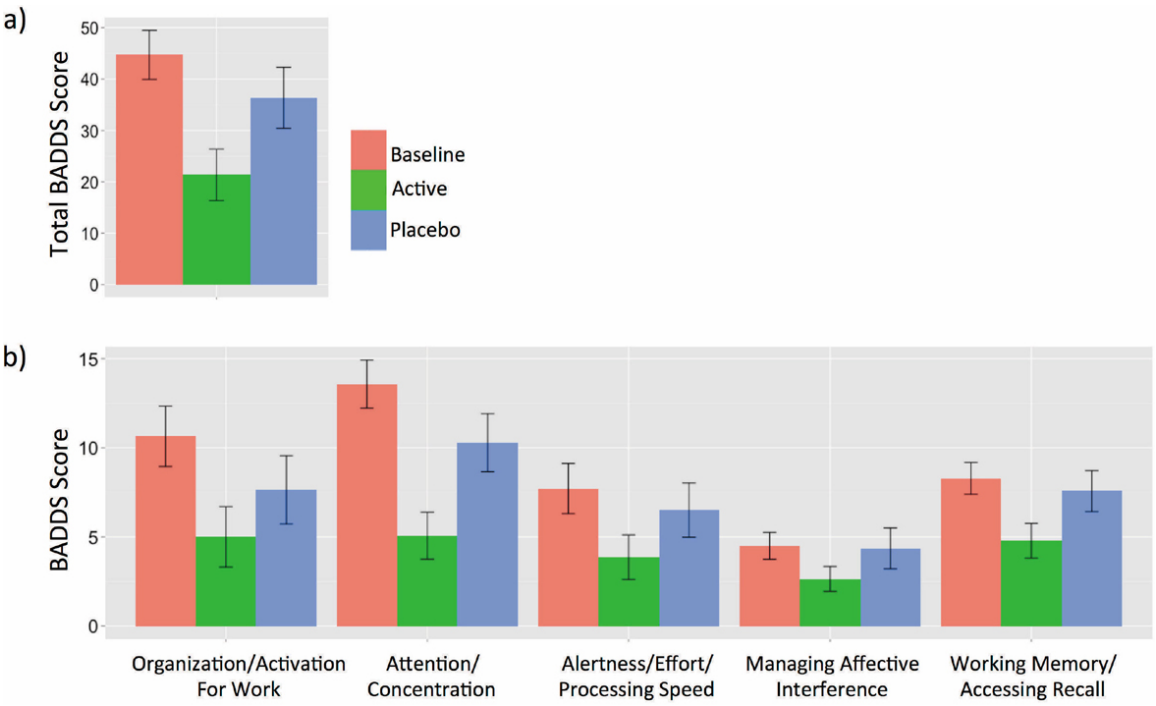
Supplementary Figures

a)

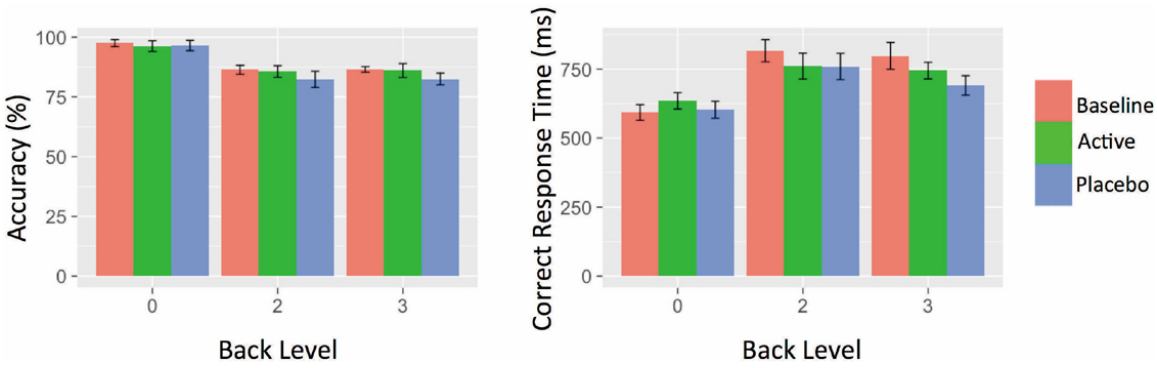
b)



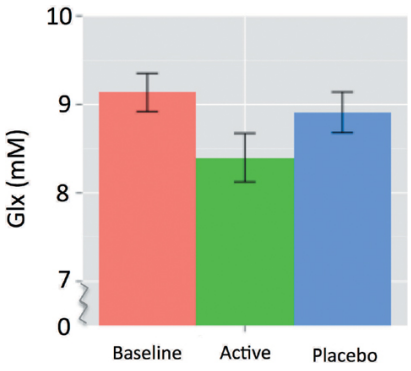
Supplementary Figure 5-1. ^{1}H-MRS of left DLPFC voxel. (a) Localization of spectroscopy voxel in the left DLPFC. (**b)** Representative water suppressed raw spectrum, fitted spectrum, and difference spectrum from one scan session.



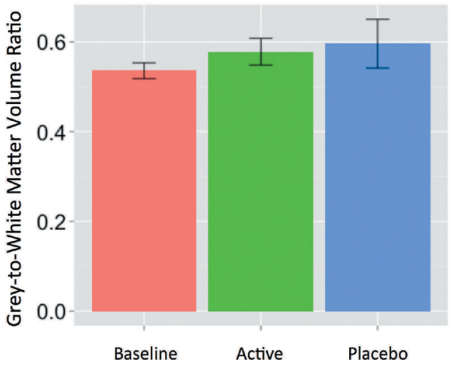
Supplementary Figure 5-2. BADDS scores across the study. BADDS scores measured across the study for total BADDS **(a)** and BADDS subscales **(b)** Error bars represent standard error.



Supplementary Figure 5-3. In-scanner n-back performance across the study. Accuracy (% correct) **(a)** and true positive reaction time **(b)** measured across the study.



Supplementary Figure 5-4. Glx measurements across the study. Left DLPFC Glx concentrations measured across the study. Error bars represent standard error.



Supplementary Figure 5-5. Proportion of grey matter volume in Left DLPFC spectroscopy voxel across the study. The proportion of grey matter in the spectroscopy voxel did not significantly differ at any time point. This value (mean ± SD) was 0.17 ± 0.06, 0.17 ± 0.07, and 0.16 ± 0.05 at baseline, active, and placebo, respectively. Error bars represent standard error.

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CHAPTER 6

Early Adversity as a Moderator of Lisdexamfetamine's Effects on Executive Function in Menopausal Women: An Addendum to Chapter 5

Rodent studies have indicated that early life stress may increase sensitivity to amphetamine (Zimmerberg and Shartrand, 1992; Rentesi et al., 2013). Therefore, in this addendum to chapter 5, we examined whether adverse childhood experiences (ACE) altered brain and behavioral response to lisdexamfetamine (LDX) in menopausal women with subjective cognitive complaints. We hypothesized that women with high levels of early life adversity would demonstrate greater response to LDX vs placebo in comparison to women with low levels of early life adversity. Specifically, we predicted that high ACE women would report greater symptom improvement with LDX than low ACE women. We also predicted that high ACE women would show a greater increase in insula activation and a greater decrease in dorsolateral prefrontal cortex (DLPFC) glutamate concentration from baseline with LDX than low ACE women.

Methods

Inclusion and exclusion criteria, study design, assessment of executive function, image acquisition and processing, and quantification of glutamate were as described in chapter 5 (Shanmugan et al., 2017) but are reproduced below for completeness.

Participants

Subjects in this addendum represent a subset of women who participated in a clinical trial investigating the effects of LDX on executive function (Epperson et al., 2015). Women ages 45 to 60 with EF difficulty onset during menopause and were within 5 years of their last menstrual period (LMP) were eligible to participate. Perimenopausal women had irregular menstrual cycles for ≥12 months, no period for ≥ 3 months, and serum follicle stimulating hormone (FSH) level of ≥ 20 IU/L. LMP of ≥12 months and serum FSH levels ≥35 IU/L indicated postmenopausal status. Subjective EF symptom

severity was assessed using the Brown Attention Deficit Disorder Scale (BADDS) (Brown, 1996). BADDS score of ≥ 20 and onset of symptoms coinciding with the initiation of menstrual cycle irregularity were required.

Women with a lifetime history of a DSM-IV psychotic disorder or psychostimulant abuse, substance abuse disorder in the previous year, or present Axis I psychiatric disorder were excluded. Psychotropic medication use, ET use within the previous 6 months, positive pregnancy test, Mini-mental Status Examination score < 26, IQ ≤ 90, history of seizures, cardiac disease, active hypertension, and abnormal electrocardiogram at screening were all exclusionary. Left-handedness, metallic implants, and claustrophobia were additional exclusion criteria for subjects who underwent neuroimaging. Subjects were excluded from ROI analyses for poor coregistration (n = 2).

Study Design

This study was a double-blind, placebo-controlled, crossover study. After screening, subjects underwent neuroimaging and cognitive assessment at three time points. After baseline, subjects were randomized to one pill of study medication (LDX 20 mg or placebo) daily for the first week, two pills daily for the second week, and, if tolerated, three pills daily for the final 2 weeks of each trial. Participants were allowed to remain in the study if they could tolerate at least 1 pill per day throughout the study. Upon completion of the first 4-week trial, participants underwent a 2-week washout and were crossed over to the other treatment condition. Testing and imaging were conducted ~ 2–6 h after last LDX dose.

Assessment of Executive Function

Assessment of subjective EF was as previously reported (Epperson et al., 2015). Briefly, subjects completed the BADDS, a validated subjective measure of EFs (Sandra Kooij et al., 2008). The BADDS is a clinician-administered questionnaire that assesses the frequency and severity of five clusters of executive dysfunction: (1) organization and activating for work, (2) sustaining attention and concentration, (3) sustaining alertness, effort, and processing speed, (4) managing affective interference, and (5) using working memory and accessing recall. Subjects rate symptoms on a scale from 0 to 3, with 0 meaning the problem described does not relate to them and 3 indicating the problem occurs almost daily.

Assessment of early life adversity

The Adverse Childhood Experiences Questionnaire (Felitti et al., 1998) was used to assess history of emotional, physical, or sexual abuse, childhood neglect, and household dysfunction. Number of exposures was summed to create the ACE score (range: 0–10). Subjects with an ACE score ≥ 2 were considered “high ACE” while subjects with ACE score of < 2 were considered “low ACE”. A threshold of 2 was used to define the high ACE group based on studies of depression prevalence in later life that demonstrate increased susceptibility at this level of exposure (Chapman et al., 2004; Epperson et al., In Press).

fMRI Task Paradigm

Subjects completed a fractal version of the n-back task during each fMRI scan. During the task, a fractal was presented for 500 ms followed by a 2500 ms interstimulus interval. This task was used to probe working memory and had 3 conditions: 0-, 2-, and

3-back. Each condition consisted of three 20-trial blocks, each preceded by a 9s instruction period, with a target to foil ratio of 1:3. The task included a total of 45 targets and 135 foils, as well as three 24 s blocks of rest during which a fixation crosshair was displayed. Analyses on in-scanner n-back performance focused on true positives, false positives, and reaction time.

Image Acquisition

Structural and functional images were acquired on 7T Siemens Trio scanner. Structural image acquisition sequence consisted of a gradient echo localizer, a 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) whole brain acquisition (TR, 2110 ms; TE, 3.17 ms; TI, 1500 ms; FOV, 240×180 mm; matrix, 256×192; 160 slices; slice thickness, 1 mm; flip angle, 10°; effective voxel resolution, 0.9×0.9×1 mm), and 3D reformatting of the MPRAGE data. Functional images were obtained using a whole-brain, single-shot, multislice, gradient-echo echoplanar sequence (239 volumes; TR, 3000; TE, 27 ms; flip angle, 70°; FOV, 220×220 mm; matrix 110×110; 37 slices; slice thickness 2.2 mm; effective voxel resolution, 2×2×2.2 mm).

Image Processing

BOLD timeseries data were analyzed with FEAT (fMRI Expert Analysis Tool) in FSL, using skull removal with BET (Smith, 2002), motion-correction with MCFLIRT (Jenkinson et al., 2002), high pass filtering, spatial smoothing (6 mm FWHM), and mean-based intensity normalization. Subject-level timeseries analyses were carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich et al., 2009). The three condition blocks (0-back, 2-back, and 3-back) were modeled using a canonical (double-gamma) hemodynamic response function with standard plus

extended motion parameters included as nuisance covariates. The rest condition (crosshair) served as the unmodeled baseline. The median functional and anatomical volumes were co-registered using boundary-based registration (Greve and Fischl, 2009). The anatomical image was normalized to the MNI 152 T1 2 mm template using linear registration with 12 degrees of freedom.

Functional ROI analysis

Functional ROIs were defined using the parametric contrast of the group mean of all sessions (baseline, active, and placebo) for subjects who completed the neuroimaging adjunct study (Shanmugan et al., 2017). The activation map of the group mean of the parametric contrast was cluster corrected at a voxel threshold of $z > 4.5$ and cluster probability of $p<0.001$ (Woolrich et al., 2009). While this analysis identified 8 functionally defined regions, we limited our analysis of ACE x LDX effects specifically to outcomes where the effect of LDX was significant in chapter 5. However, in chapter 5, we examined the effect of LDX on BOLD in 8 regions and therefore restricted statistical tests on BOLD to the 3-back condition to limit multiple comparisons. Because we focus only on the right insula in this chapter, we evaluated the effect of ACE x LDX at each condition of working memory load. Therefore, mean signal change for the 0-back, 2-back, and 3-back contrast was extracted from this functionally defined right insula ROI. The ROI mask was transformed into subject space using FLIRT.

MRS and Quantification of Glutamate

Water-reference single voxel spectroscopy (SVS) and water- suppressed SVS scans were obtained on a voxel placed in the left DLPFC (15×30×20mm3). Voxels were placed ~1cm from the skull to avoid lipid contamination. Automated shimming of the B0

field was performed on the voxel to obtain localized water line width of ~ 20 Hz or less using FASTMAP. Variable power RF pulses with optimized relaxation delays (Tkac et al., 1999) were used to obtain water suppression spectra (Cai et al., 2012). SVS for glutamate + glutamine (Glx) was obtained using short TE SVS with modified point-resolved spectroscopy sequence (PRESS) having the following parameters: spectral width = 4 kHz, number of points = 2048, averages = 16 (water reference) or 64 (water suppressed), TE = 20 ms, TR = 3000 ms. Chemical shift artifact for Glx was minimized by setting water acquisition spectrum excitation and refocusing pulses in resonance with the water peak at 4.7 p.p.m., and also setting water-suppressed spectrum excitation and refocusing pulses in resonance with the Glx peak at 2.35 p.p.m.

Water-reference data were used to obtain channel-wise time-dependent phase shifts due to eddy current and amplitude scale factors. Using a fitting method validated by Cai et al (2012), metabolite peaks from water-suppressed spectrum were fitted as Lorentzian functions with nonlinear least squares fitting using *lsqcurvefit* in MATLAB. This procedure accounted for amplitudes, line widths, and peak positions for 8 macromolecular and 14 metabolite peaks. Normalization by water-reference signal allowed for quantification of Glx.

Statistical analysis

In chapter 5, we demonstrated that, relative to baseline, LDX (vs placebo) decreased scores on the Brown Attention Deficit Disorder Scale (BADDS), increased activation in the insula, and decreased glutamate in the DLPFC. Here we examined the impact of ACE as a moderator of the effect of LDX specifically on these outcomes where the effect of LDX was significant. Linear mixed effects models implemented using the *nlme* (Pinheiro *et al*, 2016) in R (R Core Team, 2015) were employed to test the effect of

ACE, LDX, and ACE x LDX on BADDS scores, insula BOLD, and DLPFC glutamate measured at the end of the active trial minus baseline vs those measured at the end of the placebo trial minus baseline for each participant to account for repeated measures. Spearman's rank-order correlations were used to compare (1) change in BOLD between active and baseline to ACE scores, (2) glutamate concentration at baseline to ACE score, and (3) change in glutamate concentration between active and baseline to ACE score. Statistical tests were two-sided with $p<=0.05$ considered significant.

Results

Participants

The effect of ACE on BADDS scores was evaluated using the sample of participants who completed the clinical trial examining the effect of LDX on executive function (Epperson et al., 2015). Of the 32 participants who completed this clinical trial, 3 subjects did not complete the ACE questionnaire and were excluded from analyses. The final sample included in analyses of ACE x LDX effects on BADDS (n=29) consisted of 18 low ACE participants and 11 high ACE participants. The mean +/- SD ACE score in the high ACE group was 3.8 +/- 1.6. The effect of ACE on BOLD and glutamate was evaluated using the sample of participants who completed the neuroimaging adjunct study described in chapter 5 (Shanmugan et al., 2017). One subject in that sample did not complete the ACE questionnaire and was excluded from analyses. The final sample included in analyses of ACE x LDX effect on BOLD (n=12) consisted of 8 low ACE subjects and 4 high ACE subjects. The mean +/- SD ACE score in the high ACE group was 3.3 +/- 1.3. The final sample included in analyses of ACE x LDX effect on glutamate (n=13) consisted of 8 low ACE subjects and 5 high ACE subjects. The mean +/- SD ACE score in the high ACE group was 3.8 +/- 1.6.

Behavioral results

Linear mixed-effects models were used to test the hypothesis that the high ACE group would show a greater reduction in symptom severity with LDX vs placebo than the low ACE group. A significant ACE x LDX interaction was detected on BADDS scores relating to organization and activation for work (p=0.01). Post-hoc comparisons revealed that low ACE subjects demonstrated a greater response to placebo than high ACE subjects (p=0.03). While there was no significant difference between LDX vs placebo treatment in the low ACE group, the high ACE group showed a significantly greater reduction in symptoms with LDX treatment in comparison to placebo (p=0.0002; **Figure 6-1**). There was no significant effect of ACE or ACE x LDX on total BADDS or other BADDS subscales. There was an effect of LDX vs placebo in decreasing total BADDS score (low ACE p=0.004; high ACE p=0.02) as well as BADDS subscales assessing attention and concentration (low ACE p=0.01; high ACE p=0.09) and alertness, effort, and processing speed (low ACE p=0.008; high ACE p=0.07). At a trend level, LDX decreased symptoms related to managing affective interference in the high ACE group (p=0.09), but had no effect in the low ACE group (p=0.16). In contrast, LDX decrease symptoms related to working memory/accessing recall in the low ACE group (p=0.002) but had no effect in the high ACE group (p=0.15). These differences in LDX effects between ACE groups where the interaction was not significant are likely due to insufficient power to detect the drug effect in the small sub-samples.

BOLD results

In chapter 5, we saw that LDX resulted in an increase in activation in the right insula during the 3-back condition (Shanmugan et al., 2017). Given that previous literature has indicated that early life stress may increase sensitivity to amphetamine

(Zimmerberg and Shartrand, 1992; Rentesi et al., 2013), we tested the hypothesis that higher ACE would be associated with a greater increase in BOLD in this ROI with LDX. As predicted, an ACE x LDX interaction was present at a trend level in the 0-back condition (p=0.06). In the high ACE group, the difference in BOLD between LDX and baseline was significantly higher than the difference in BOLD between placebo and baseline (p=0.01). In contrast, in the low ACE group, LDX did not significantly change BOLD from baseline in comparison to placebo. There was no significant ACE x LDX interaction at either 2-back or 3-back. The increase in BOLD signal from baseline was significantly greater with LDX than with placebo during both 2-back (low ACE p= 0.006, high ACE=0.02) and 3-back (low ACE p=0.009, high ACE p=0.01). The high ACE group exhibited greater change in BOLD from baseline with LDX in comparison to the low ACE group during the 2-back (p=0.08) and 3-back (p=0.09). While a similar ACE effect was present with LDX during the 3-back (p=0.09), there was no difference between ACE groups during placebo (p=0.02) (**Figure 6-2**).

The above analyses demonstrated that LDX-induced change in BOLD from baseline was higher in the high ACE group than in the low ACE group at the higher levels of working memory load. To further probe this finding, we examined the association between the number of ACEs (continuous) and LDX-induced change in BOLD signal. At each level of working memory load, change in BOLD signal was correlated with total ACE score, though this correlation only reached significance during the 2-back (0-back r=0.47, p=0.1; 2-back r=0.58, p=0.05; 3-back r=0.51, p=0.09; **Figure 6-3**).

Spectroscopy results

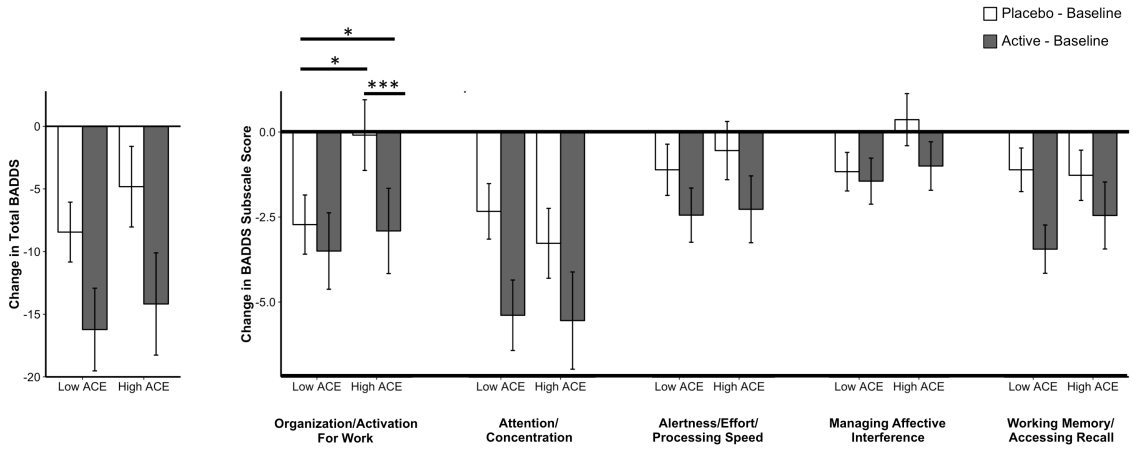
There is preliminary evidence that individuals with a history of early adversity may have higher PFC glutamate levels (Duncan et al., 2015), though it is unclear whether adversity would moderate the effect of stimulant medications on prefrontal glutamate concentration. Based on evidence from rodent studies suggesting increased sensitivity to amphetamines with early life stress (Zimmerberg and Shartrand, 1992; Rentesi et al., 2013), we hypothesized the difference in LDX-induced change from baseline vs placebo-associated change from baseline would be greater in the high ACE group compared to the low ACE group. Contrary to this hypothesis, we did not find an effect of ACE or an ACE x LDX interaction on DLPFC glutamate concentration. The effect of LDX was present at a trend level in this subsample of subjects who completed the ACE questionnaire ($p=0.1$). There was also no significant correlation between total ACE score and either baseline glutamate concentration or change in glutamate between baseline and active.

Conclusions

In this double-blind, placebo-controlled, crossover study, we examined whether ACE moderates the effect of LDX on behavioral and neural markers of executive function. In the high ACE group, LDX (vs placebo) reduced symptoms related to difficulty with organization and activation for work. However, response to LDX was not significantly different from placebo in the low ACE group. Additionally, the extent to which LDX increased activation in the insula increased linearly with total ACE score. These findings are in agreement with animal studies indicating that early adversity may increase sensitivity to amphetamine. In contrast, there was no effect of ACE or ACE x LDX on glutamate concentration in the left DLPFC. Given the literature suggesting that early adversity has multiple, complex effects on PFC glutamate systems including

increases in glutamate concentration (Duncan et al., 2015), alterations in NMDA receptor subunit composition (Kinnunen et al., 2003), and decreases in glutamatergic dendritic spine density in the PFC (Michelsen et al., 2007), this finding is likely due to insufficient power in this small sample. Behavioral and BOLD results provide preliminary evidence that early adversity may also have lasting effects on catecholaminergic neurotransmission. Furthermore, they emphasize the importance of considering ACE when treating executive difficulties with stimulant medications during menopause.

Figures



*p≤0.05, **p≤0.01, ***p≤0.001

Figure 6-1. ACE x LDX effect on BADDS. Linear mixed-effects models were used to test the hypothesis that the high ACE group would show a greater reduction in symptom severity with LDX vs placebo than the low ACE group. A significant ACE x LDX interaction was detected on BADDS scores relating to organization and activation for work (p=0.01). Post-hoc comparisons revealed that low ACE subjects demonstrated a greater response to placebo than high ACE subjects (p=0.03). While there was no significant difference between LDX vs placebo treatment in the low ACE group, the high ACE group showed a significantly greater reduction in symptoms with LDX treatment in comparison to placebo (p=0.0002). There was no significant effect of ACE or ACE x LDX on total BADDS or other BADDS subscales. There was an effect of LDX vs placebo in decreasing total BADDS score (low ACE p=0.004; high ACE p=0.02) as well as BADDS subscales assessing attention and concentration (low ACE p=0.01; high ACE p=0.09) and alertness, effort, and processing speed (low ACE p=0.008; high ACE p=0.07). At a trend level, LDX decreased symptoms related to managing affective interference in the

high ACE group (p=0.09), but had no effect in the low ACE group (p=0.16). In contrast, LDX decrease symptoms related to working memory/accessing recall in the low ACE group (p=0.002) but had no effect in the high ACE group (p=0.15). These differences in LDX effects between ACE groups where the interaction was not significant are likely due to insufficient power to detect the drug effect in the small sub-samples.

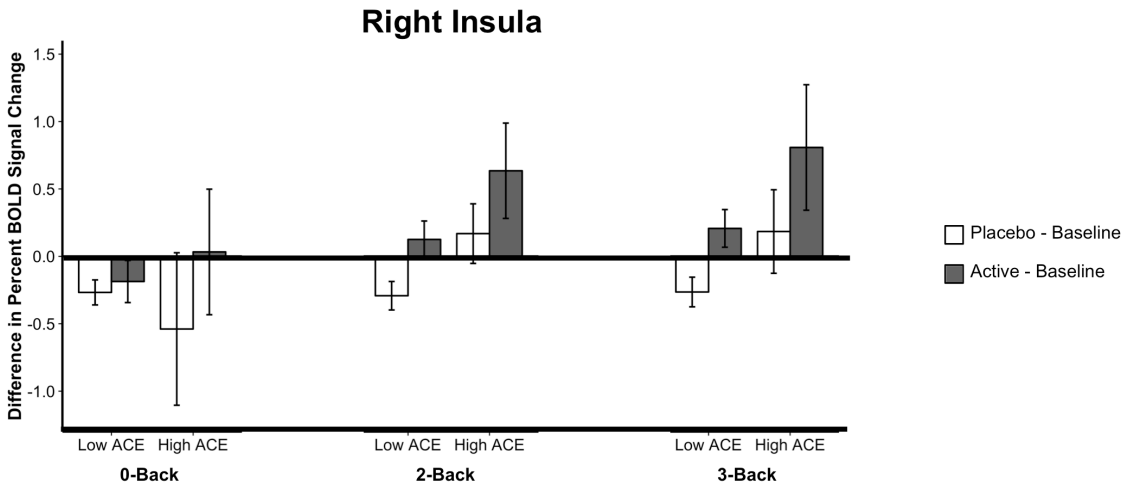


Figure 6-2. ACE x LDX effect on insular activation. We tested the hypothesis that higher ACE would be associated with a greater increase in BOLD in the right insula with LDX. As predicted, an ACE x LDX interaction was present at a trend level in the 0-back condition (p=0.06). In the high ACE group, the difference in BOLD between LDX and baseline was significantly higher than the difference in BOLD between placebo and baseline (p=0.01). In contrast, in the low ACE group, LDX did not significantly change BOLD from baseline in comparison to placebo. There was no significant ACE x LDX interaction at either 2-back or 3-back. The increase in BOLD signal from baseline was significantly greater with LDX than with placebo during both 2-back (low ACE p= 0.006, high ACE=0.02) and 3-back (low ACE p=0.009, high ACE p=0.01). The high ACE group exhibited greater change in BOLD from baseline with LDX in comparison to the low ACE group during the 2-back (p=0.08). While a similar ACE effect was present with LDX during the 3-back (p=0.09), there was no difference between ACE groups during placebo (p=0.2).

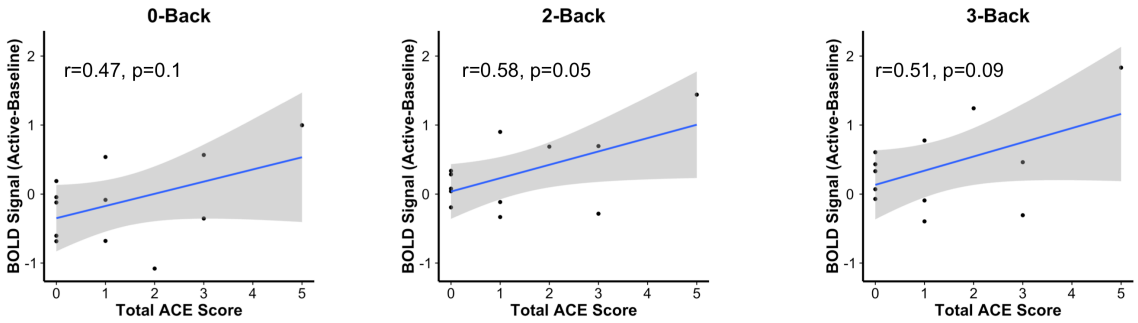


Figure 6-3. LDX-induced change in activation correlates with ACE score. Figure 2 demonstrated that LDX-induced change in BOLD from baseline was higher in the high ACE group than in the low ACE group at the higher levels of working memory load. To further probe this finding, we examined the association between the number of ACEs (continuous) and LDX-induced change in BOLD signal. At each level of working memory load, change in BOLD signal was correlated with total ACE score, though this correlation only reached significance during the 2-back (0-back r=0.47, p=0.1; 2-back r=0.58, p=0.05; 3-back r=0.51, p=0.09).

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CHAPTER 7

Overall Discussion and Future Directions

Synthesis of results and overall discussion

During the menopause transition, many healthy women with no history of cognitive dysfunction experience subjective difficulties with sustained attention, motivation for work, organization and working memory (Epperson et al., 2011; Shanmugan and Epperson, 2014; Epperson et al., 2015; Shanmugan et al., 2017). Indicators of risk for cognitive decline during menopause are lacking, as is an understanding of the mechanisms contributing to this vulnerability with waning estradiol. Results of this dissertation support the hypothesis that adverse childhood experiences (ACE) is a risk factor for this cognitive decline and that ACE increases the risk of executive dysfunction during menopause via alterations in monoaminergic neurotransmission.

In chapter 2, we examined the impact of ACE on subjective and objective measures of executive function in a large sample of women who underwent surgical menopause. We found that high ACE women reported more subjective symptoms of executive dysfunction and performed worse on neuropsychological tasks probing working memory and sustained attention. To account for the fact that early adversity is associated with increased risk of mood disorders with loss of estradiol (Epperson et al., In Press), we conducted mediation analyses to determine the extent to which ACE effects on executive function result from concurrent mood (anxiety and depressive symptoms) changes. We determined that mood symptoms were a partial mediator of ACE effects on subjective symptoms of executive dysfunction and sustained attention. However, mood did not mediate ACE effects on working memory. Moreover, ACE effects on working memory remained significant when accounting for the effects of mood symptoms, age, chemotherapy use, time since oophorectomy, hormone therapy use, and education. Together, results of chapter 2 suggest that mood symptoms are one

component of the mechanism underlying new onset cognitive dysfunction with loss of estradiol but that at least a portion of ACE-associated risk for executive difficulties is independent of the association between ACE and anxiety and depressive symptoms. We next pursued these mood-independent mechanisms in chapters 3 through 6.

Given the strong associations between ACE and mood symptoms in chapter 2, we hypothesized that an additional mechanism by which ACE exerts a negative impact on executive function during periods of low estradiol may be via effects on serotonin. In chapter 3, the effects of tryptophan depletion (TD), ACE, and TD x ACE were evaluated using a voxelwise, whole-brain analysis. In the absence of exogenous estradiol, a significant TD by ACE interaction was observed on BOLD signal in the right DLPFC such that TD increased activation in high ACE subjects but decreased activation in low ACE subjects. While a similar interaction was observed with placebo treatment, treatment with estradiol attenuated the effects of ACE and TD such that no between or within group differences were observed. In chapter 4, we observed a similar ACE x TD interaction on within-network connectivity in the absence of exogenous estradiol. While ACE was robustly associated with lower within-network connectivity regardless of depletion condition, TD increased connectivity in the high ACE group but had no effect on connectivity in the low ACE group.

Overall, results from chapters 3 and 4 suggest that early life adversity has lasting impacts on serotonergic circuits underlying executive function that are unmasked by loss of estradiol during menopause. These results also emphasize serotonin's role in executive function and highlight that the moderating effects of early adversity must be accounted for when manipulating serotonin levels. Further research targeting specific components of the serotonin system would be necessary to pinpoint the molecular targets of ACE. In animal models, early life stress is associated with lower tryptophan

hydroxylase type 2 gene expression (Shively et al., 2003), higher monoamine oxidase-A expression (Wong et al., 2015), and attenuated 5-HT1A function (Matsuzaki et al., 2009), indicating that future research in human participants should focus on understanding how ACE affects serotonin synthesis, metabolism, and signaling during menopause.

While the TD x ACE interactions on DLPFC activation and functional connectivity seen in chapters 3 and 4 provided evidence for ACE effects on serotonergic function, the robust effects of ACE on functional connectivity that were significant regardless of depletion status highlight that ACE effects on serotonin are only part of the story. Given the preclinical literature suggesting that ACE negatively impacts other monoaminergic systems (Shively, 1998; Matthews et al., 2001; Rentesi et al., 2013), we next examined the impact of ACE on dopaminergic and noradrenergic function in menopausal women with subjective cognitive complaints.

We probed catecholaminergic function in women with high and low levels of early adversity using lisdexamfetamine (LDX), a stimulant medication that has been shown to improve subjective measures of executive function in menopausal women with self-reported new-onset executive difficulties (Epperson et al., 2015). Because no previous imaging studies had utilized LDX, before examining whether ACE altered response to this drug, we first characterized the impact of LDX on executive activation and neurochemistry in chapter 5. We found that relative to baseline, LDX (vs placebo) decreased scores on the Brown Attention Deficit Disorder Scale (BADDS), increased activation in the insula, and decreased glutamate in the DLPFC. In chapter 6, we examined whether ACE moderated the effect of LDX on these outcomes. A significant ACE x LDX interaction was observed on subjective report of executive difficulties related to organization and activation for work. In the high ACE group, LDX (vs placebo)

reduced these symptoms from baseline while response to LDX was not significantly different from placebo in the low ACE group. Additionally, LDX-induced change in insular activation correlated with ACE score across varying levels of working memory load. These findings are in agreement with animal studies indicating that early adversity may increase sensitivity to amphetamine (Zimmerberg and Shartrand, 1992; Rentesi et al., 2013). Given that stimulants increase dopamine and norepinephrine levels in the PFC (Arnsten, 2009, 2011), lower baseline concentrations of catecholamines in the high ACE group may play a role in the differential treatment response to LDX. Animal studies demonstrating that early adversity is associated with increased dopamine transporter (DAT) expression (Novick et al., 2015), increased catechol-o-methyl transferase (COMT) expression (Grissom et al., 2015), and increased D2 autoreceptor expression (Lovic et al., 2013) and activity (Watt et al., 2014) support this hypothesis.

In sum, chapters 5 and 6 suggest that early adversity may have lasting effects on catecholaminergic neurotransmission. Furthermore, they emphasize the importance of considering ACE when treating executive difficulties with stimulant medications during menopause.

In contrast to our findings regarding ACE x LDX effects on subjective symptoms of executive dysfunction and brain activation in the insula, there was no effect of ACE x LDX effect on glutamate concentration in the left DLPFC. The lack of ACE x LDX interaction was surprising given that prior studies have demonstrated that individuals with high levels of early adversity have higher PFC glutamate concentrations (Duncan et al., 2015). Evidence from rodent studies indicates that early life stress alters the composition of N-methyl-D-aspartic acid (NMDA) receptor subunits in the prefrontal cortex (Kinnunen et al., 2003), which are important for the persistent firing of glutamatergic neurons during working memory and sustained attention (Arnsten and

Wang, 2016). Early stress in mice has also been shown to decrease mGluR2 and mGluR3 mRNA and protein levels in the frontal cortex (Matrisciano et al., 2012), which are important for anxiolytic and anti-psychotic drug response (Marek, 2010). Additionally, chronic postnatal stress has been shown to decrease dendritic spine density in the PFC of rats (Michelsen et al., 2007). This negative impact of early life stress on dendritic spine density is particularly intriguing given that executive functioning is dependent on the number of prefrontal small spines (Hara et al., 2012) and the substantial literature demonstrating negative effects of ovariectomy and positive effects of estradiol on dendritic spine morphology (Shanmugan and Epperson, 2014). Estrogen treatment has been shown to reverse both the decreases in PFC dendritic spine density and number as well as the impairments in PFC dependent functions caused by ovariectomy in both rodents and nonhuman primates (Rapp et al., 2003; Tang et al., 2004; Hao et al., 2007; Chen et al., 2009). In particular, long-term 17b-estradiol administration following ovariectomy increases apical and basal dendritic spine density and the number of smaller, more motile spines thought to be important for synaptic plasticity (Hao et al., 2007). Enhanced performance on working memory tasks in ovariectomized nonhuman primates receiving estradiol is thought to be at least in part due to these changes in spine density (Rapp et al., 2003; Hao et al., 2007). This literature suggests that our lack of ACE x LDX effect on glutamate levels may be due to insufficient power in the small sample of n=13. It is also possible that ACE predominantly impacts other aspects of glutamate system function (per above) which cannot be assessed with magnetic resonance spectroscopy. Clearly, the impact of ACE and the interaction between ACE and LDX on glutamatergic function in hypogondal women warrant further study in a larger sample.

Future directions

In the preceding chapters, we provided evidence that ACE confers a vulnerability for executive dysfunction during menopause via alterations in monoaminergic neurotransmission. However, there are likely several other mechanisms by which ACE impacts executive function that merit further investigation.

In chapter 4, we found that ACE was robustly associated with lower within-network connectivity. Given that functional connectivity may be reflective of underlying structural connections, impairments in neuronal development during childhood and adolescence may be one mechanism by which ACE predisposes individuals to executive dysfunction. As discussed above, early life stress in mice has been shown to induce alterations in PFC dendritic architecture (Yang et al., 2015). Female mice that experience peripubertal stress also display increases in PFC myelin basic protein gene expression and proteolipid protein gene expression, both of which are indicators of myelination (Morrison et al., 2016). Importantly, estradiol supports the normal development and maintenance dendritic morphology (Shanmugan and Epperson, 2014) and neuronal myelination (Luo et al., 2016). Future neuroimaging studies utilizing diffusion tensor imaging would be helpful in determining whether ACE effects on white matter integrity mediate the relationship between ACE and executive function during menopause.

Immune markers may also mediate the effects of early adversity on later life cognitive function. In healthy adults, early life stress has been associated with increases in pro-inflammatory cytokines such as IL-6, IL1-beta, and TNF-alpha (Hartwell et al., 2013). Elevated inflammatory markers have been implicated in the associations between early adversity and increased risk of major depressive disorder (Miller and Cole, 2012; Grosse et al., 2016), schizophrenia (Khandaker and Dantzer, 2016), and posttraumatic

stress disorder (Eraly et al., 2014). There is preliminary evidence that such chronic inflammation and oxidative stress may play a role in the pathogenesis of executive dysfunction seen in ADHD (Buske-Kirschbaum et al., 2013; Verlaet et al., 2014). As such, whether immune dysregulation contributes to the increased vulnerability for executive dysfunction seen with ACE should be investigated.

This work has also generated several hypotheses with implications for clinical practice. In chapter 2, we have provided evidence that ACE is a risk factor for executive dysfunction after surgical menopause. However, the majority of our sample completed cognitive testing shortly after oophorectomy. This limited our ability to separate the effects of current age from those of age at oophorectomy. Future studies should examine whether the impact of ACE is moderated by age at oophorectomy. Given that estradiol attenuates the effects of ACE on working memory (as seen in chapter 3) and greater duration of endogenous estrogen exposure may be beneficial for PFC dependent cognitive functions (Shanmugan and Epperson, 2014), one could hypothesize that the negative effects of ACE on executive function may be magnified by earlier age at oophorectomy. If future research in a sample with greater variability in the relationship between age and age at oophorectomy supported this hypothesis, physicians may be more informed when counseling patients who are high ACE as to the risks and benefits of undergoing vs postponing oophorectomy as a way to mitigate the negative effects of ACE on cognitive function.

In chapter 3, we demonstrated that neural response to estradiol treatment varies depending on levels of early adversity. This finding supports the hypothesis that individual biological and environmental differences contribute to risk vs resilience for executive dysfunction with waning estradiol. Failure to account for this moderating effect of ACE is likely a major factor contributing to the inconclusive literature regarding

estradiol's effects on executive function in human participants. Future studies examining the efficacy of estradiol in alleviating menopause-associated cognitive dysfunction should account for history of early adversity. Validation of ACE as a moderator of estradiol's impact on executive functions would support the use of ACE as a potential indicator of a patient's response to estrogen therapy.

However, many perimenopausal and postmenopausal women are not candidates for estrogen therapy due to medical contraindications or family history (Shifren and Schiff, 2010). Our results regarding ACE x monomanine interactions provide preliminary evidence for the use of serotonergic and catecholaminergic medications to treat menopause-associated executive dysfunction. In addition to psychotherapy, selective serotonin reuptake inhibitors (SSRIs) may be an appropriate treatment for the ACE-associated anxiety and depressive symptoms contributing to executive dysfunction. Because decreases in serotonin concentration may be one mechanism by which ACE confers a vulnerability for executive difficulties in healthy women without associated mood symptoms, future clinical trials should examine whether SSRIs could also ameliorate cognitive dysfunction in this population. Previous studies have indicated that serotonin-norepinephrine reuptake inhibitors (Epperson et al., 2011) and stimulants (Epperson et al., 2015; Shanmugan et al., 2017) are successful in reducing subjective symptoms of executive dysfunction in women who have undergone natural menopause. Here we provided preliminary evidence that ACE moderates treatment response to LDX. Given that this research into catecholaminergic agents as an alternative to hormone therapy for the alleviation of executive dysfunction is particularly relevant to women who are BRCA1/BRCA2 positive, future studies in a larger sample would be helpful in determining whether ACE moderates both brain and behavioral response to catecholaminergic medications after surgery- or chemotherapy-induced menopause.

Conclusions

In summary, this body of work supports the hypothesis that ACE is a risk factor for executive function difficulties during menopause and that ACE increases the risk of executive dysfunction with loss of estradiol via alterations in monoaminergic neurotransmission. Our results highlight that addressing concurrent mood changes is a critical step in treating menopause-induced executive difficulties. They also suggest that early life adversity has latent effects on serotonergic circuits underlying executive function that are unmasked by loss of estradiol during menopause. Additionally, our findings indicate that early adversity may have lasting effects on catecholaminergic neurotransmission and may alter treatment response to stimulant medications. Together, they emphasize the importance of considering ACE in neuroimaging studies utilizing neurotransmitter manipulations and when treating executive function difficulties during menopause.

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Appendix: Additional Published Manuscripts

Common and Dissociable Mechanisms of Executive System Dysfunction Across Psychiatric Disorders in Youth

This section has been published:

Shanmugan S, Wolf DH, Calkins ME, Moore TM, Ruparel K, Hopson RD, Vandekar SN, Roalf DR, Elliott MA, Jackson C, Gennatas ED, Leibenluft E, Pine DS, Shinohara RT, Hakonarson H, Gur RC, Gur RE, Satterthwaite TD (2016) Common and Dissociable Mechanisms of Executive System Dysfunction Across Psychiatric Disorders in Youth. The American journal of psychiatry 173:517-526.

ABSTRACT

Objective: Disruption of executive function is present in many neuropsychiatric disorders. However, determining the specificity of executive dysfunction within these disorders is challenging given high comorbidity of conditions. Here we investigated executive system deficits in association with dimensions of psychiatric symptoms in youth using a working memory paradigm, hypothesizing that common and dissociable patterns of dysfunction would be present.

Methods: We studied 1,129 youths who completed a fractal n-back task during fMRI at 3T as part of the Philadelphia Neurodevelopmental Cohort. Factor scores of clinical psychopathology were calculated using an itemwise confirmatory bifactor model, describing overall psychopathology as well as four orthogonal dimensions of symptoms including anxious-misery (mood / anxiety), behavioral disturbance (ADHD / conduct), psychosis-spectrum symptoms, and fear (phobias). The impact of psychopathology dimensions on behavioral performance and executive system recruitment (2-back > 0-back) were examined using both multivariate (matrix regression) and mass-univariate (linear regression) analyses.

Results: Overall psychopathology was associated with both abnormal multivariate patterns of activation and a failure to activate executive regions within the cingulo-

opercular control network including the frontal pole, cingulate cortex, and anterior insula. Additionally, psychosis-spectrum symptoms were associated with hypo-activation of left dorsolateral prefrontal cortex, whereas behavioral symptoms were associated with hypo-activation of fronto-parietal cortex and cerebellum. In contrast, anxious-misery symptoms were associated with widespread hyper-activation of the executive network.

Conclusions: These findings provide novel evidence that common and dissociable deficits within the brain's executive system are present in association with dimensions of psychopathology in youth.

INTRODUCTION

Deficits of executive function are present in a wide range of psychiatric disorders including attention deficit hyperactivity disorder (ADHD) (1), conduct disorder (2), and psychotic disorders such as schizophrenia (3,4). Executive deficits negatively impact everyday functioning (5), and contribute to diminished quality of life in many clinical populations (6,7). Consequences of executive deficits may be particularly acute in childhood and adolescence, and include increased interpersonal conflict, decreased academic achievement, and risk-taking behavior (7,8).

Many studies have investigated the neural basis of executive impairments in individual psychiatric disorders. Working memory is one of the most commonly studied components of executive function. For example, meta-analyses in patients with ADHD demonstrate hypoactivation within a network of regions including the dorsolateral prefrontal cortex, anterior cingulate cortex, thalamus, superior parietal lobule, and precuneus (9,10). Patients with schizophrenia also fail to recruit portions of the executive network including the dorsolateral prefrontal cortex (11-13). Likewise, conduct disorder is associated with a failure to activate a similar network of regions (14). Taken together,

these studies suggest there are likely overlapping effects across disorders, with hypoactivation of the executive system being a common underlying brain phenotype. However, evidence exists for dissociable abnormalities among psychiatric disorders. For example, hyperactivation of executive regions have been reported in major depressive disorder and anxiety disorders (15-17). Nonetheless, some heterogeneity in results exists, and such hyperactivation has also frequently been reported in schizophrenia (18).

A case-control design in a single disorder is the predominant approach in the studies reviewed above, and thus most studies are unable to directly evaluate whether common executive deficits exist across disorders. Furthermore, while the incidence of psychiatric co-morbidity is quite high, studies typically do not explicitly evaluate its impact. In epidemiological studies, approximately 40% of individuals meeting criteria for one diagnosis met criteria for at least one additional disorder in a different class (19). Thus, studies that use subjects with “pure” single diagnoses may not be representative. More recently, the neural basis of executive deficits across disorders have been investigated in an attempt to isolate regions of dysfunction specific to each diagnosis (20). However, studies comparing multiple clinical groups are typically hampered by small sample size, and few prior studies compared more than two disorders at a time. Furthermore, as psychiatric symptomatology exists on a continuum of normal to abnormal, dimensional analyses that cut across categorical clinical diagnoses may both enhance power and improve biological interpretability (21). Thus, studies in large samples evaluating the impact of multiple dimensions of psychopathology on executive functioning are necessary.

Accordingly, here we used a dimensional approach to examine executive dysfunction with a working memory fMRI task in a sample of 1,129 youth imaged as part

of the Philadelphia Neurodevelopmental Cohort (22,23). Notably, the ascertainment strategy used in the Philadelphia Neurodevelopmental Cohort differs substantially from studies using clinical help-seeking samples, instead employing a population-based approach that examines symptoms in non-help seeking youth. While such a design will likely have lower symptom severity than clinical samples, a population-based approach may nonetheless be valuable for investigating dimensions of psychopathology. We hypothesized that there would be both common and dissociable deficits in executive recruitment associated with different dimensions of psychopathology. Specifically, we predicted that general psychopathology would be associated with executive hypo-activation regardless of clinical diagnosis. Furthermore, we predicted that specific dimensions of psychopathology would be linked to dissociable regional patterns of impairment within the executive network.

METHODS

Participants

As previously described, the Philadelphia Neurodevelopmental Cohort is a collaboration between the Center for Applied Genomics at the Children's Hospital of Philadelphia and the Brain Behavior Laboratory at the University of Pennsylvania (22,23). The current report considers the entire cross-sectional sample of 1,601 subjects imaged as part of the Philadelphia Neurodevelopmental Cohort; of these, 1,462 completed the *n*-back task described below. Following subject exclusions including medical co-morbidity, task non-performance, and image quality assurance (see Supplementary Methods), the final sample included in analyses was *n*=1,129 (mean age = 15.5 years; SD = 3.4 years; 520 males). This sample thus constitutes a super-set of

subjects previously included in reports that focused on effects of working memory performance (24) and psychosis-spectrum symptoms (25).

Clinical assessment

As previously detailed (26,27), psychopathology symptoms were evaluated using a structured screening interview (GOASSESS). Computerized algorithms used endorsement of symptoms, their frequency and duration, and the presence of distress or impairment to determine whether DSM-IV criteria were met (see Supplementary Methods). The frequency and relevant demographic data for each screening diagnosis considered are detailed in **Table 1**. As in large-scale epidemiologic datasets (19), co-morbidity was quite common, with more subjects meeting criteria for more than one category (n=529) than a single category (n=249).

Psychopathology factor analysis

In order to parse this co-morbidity into orthogonal dimensions of psychopathology, we performed factor analyses of item-level data from GOASSESS. To produce stable factor scores, analyses utilized all subjects for whom clinical data were available (n=9,498) rather than only the subset who completed neuroimaging (26,27). As initial exploratory factor analyses indicated correlated traits of psychopathology, we used a bifactor confirmatory model, which can produce orthogonal scores from correlated traits (28).

Methods regarding estimation of the bifactor model will be presented in detail elsewhere (also see Supplementary Methods). Briefly, the confirmatory bifactor model (**Figure 1A**) was estimated using a Bayesian estimator in Mplus. As predicted by theory and supported by initial exploratory models, the four factors primarily represent anxious-

misery (mood & anxiety) symptoms, psychosis-spectrum symptoms, behavioral symptoms (conduct and ADHD), and fear symptoms (phobias). Additionally, the bifactor model estimated a general psychopathology factor, representing the overall burden of psychopathology while controlling for the presence of specific symptom dimensions. Importantly, all five factors (including general psychopathology) from the bifactor model are orthogonal and can be considered jointly in analysis of imaging data. Factors scores within each of the categorical screening categories were as expected given item-level loading (**Figure 1B**).

Task paradigm, image acquisition and image processing

Task paradigm, image acquisition, and pre-processing methods were as previously reported (24). Briefly, a fractal version of the n-back task (29) was used to probe executive system function across three levels of working memory load (**Figure 2A**). The primary behavioral measure was *d'*, a signal detection metric that limits the influence of response bias. Task performance (*d'*) across all levels of working memory load was related to categorical diagnosis from GOASSESS and dimensional factor scores using linear models while controlling for age and sex. Testing five dimensions of psychopathology were accounted for using Bonferroni correction.

fMRI, T1, and B0 images were acquired on the same scanner (Siemens 3T Tim Trio) for all subjects (see Supplementary Methods). Timeseries analysis of subject-level imaging data used FSL (30) to model three condition blocks (0-back, 1-back, and 2-back); the primary contrast was 2-back > 0-back, which robustly recruits the executive network (24). Subject-level statistical maps were distortion corrected, co-registered to the T1 image using boundary-based registration, and normalized to the MNI 152 1mm

template using ANTs (31) and then downsampled to 2mm. All transformations were concatenated so only one interpolation was performed.

Multivariate group-level analysis: Global associations with psychopathology

As a first step, we evaluated the degree to which dimensions of psychopathology from the factor analysis impacted overall multivariate patterns of activation (see **Figure S1**). To do this, we used multivariate distance-based matrix regression, a statistical technique developed originally for large-scale ecologic datasets that has recently been used in image analysis (32); this was implemented using the *vegan* package in R (33). Subject-level activation maps are compared on a pairwise basis (Euclidean distance) to yield a distance matrix. Matrix regression is then used to test whether each phenotypic variable explains the distances among each participant's activation patterns. In contrast to other multivariate methods, this approach allowed us to examine the influence of multiple dimensions of psychopathology simultaneously while also controlling for covariates (age, sex, and in-scanner motion). Multiple tests were accounted for using Bonferroni correction as above.

Mass-univariate group-level analysis: Regional associations with psychopathology

While the multivariate analysis detailed above provided an estimate of the degree to which dimensions of psychopathology impacted the global pattern of activation, this analysis does not evaluate regional effects. Accordingly, we next conducted a standard mass-univariate analysis using a general linear model implemented in FSL (30). We evaluated the effect of each dimension of psychopathology within this linear model, with covariates as above. Additionally, we investigated whether dimensions of psychopathology that showed a significant effect were significantly different from each

other using an *F*-test across dimensions. Type I error control was provided by cluster correction using 10,000 Monte-Carlo simulations (voxel height of $z > 3.09$; cluster probability of $p < 0.001$; minimum cluster size of $k=67$) (34). All analyses described below used unmasked, whole-brain voxelwise data. Images were displayed using Caret.

Supplementary analyses

In order to evaluate whether potentially confounding factors influenced observed results, we conducted a series of supplementary analyses. This included a) removing the minority (11.4%) of subjects who were taking psychoactive medication; b) including task performance (*d'*) as a covariate; c) excluding subjects with poor performance (>7 non-responses) on the 2-back condition; and d) including mean accuracy on an out-of-scanner computerized neuropsychological battery, race, assessment-scan interval, maternal education, and in-scanner performance (*d'*) together as covariates in one model.

RESULTS

Multiple dimensions of psychopathology impact task performance

As expected, increasing working memory load was associated with fewer correct responses to targets and increased false positive responses to foils (**Figure 2B & C**). These measures were integrated using the signal detection measure *d'*. As expected, *d'* varied considerably by screening categorical diagnosis (see **Table 1**). When summarized as psychopathology dimensions, several factors significantly impacted working memory task performance (**Figure 2D**). Higher levels of both overall psychopathology ($t[1124]=2.58$, $p=0.001$) and behavioral symptoms ($t[1124]=3.82$,

p=0.0001) were associated with lower working memory performance. In contrast, higher levels of anxious-misery symptoms were associated with a trend towards better working memory performance (*t*[1124]=2.45, p=0.015). There was not a significant relationship between fear or psychosis and working memory performance.

Overall psychopathology alters global patterns of executive system recruitment

As expected, the 2-back > 0-back contrast robustly recruited the entire executive network, and resulted in de-activation of non-executive regions (**Figure 2E**). We next evaluated whether dimensional psychopathology was associated with changes in this multivariate pattern of activation and de-activation using multivariate distance-based matrix regression. This procedure revealed that overall psychopathology was associated with a significant disturbance in the global pattern of executive system recruitment (p=0.009). Other symptom dimensions did not have a significant relationship; non-significant trends were observed for behavioral (p=0.044) and anxious-misery (p=0.092) symptoms.

Dimensions of psychopathology differentially impact regional executive activation

Multivariate distance-based matrix regression evaluates the presence of global multivariate effects, but is not sensitive to regional changes. Accordingly, we used mass-univariate generalized linear models to examine the relationship between dimensions of psychopathology and executive system activation (**Figure 3**, **Table S1**). As suggested by multivariate results, overall psychopathology had the most robust impact on executive activation. Higher levels of overall psychopathology were associated with diminished activation of bilateral frontal pole, anterior cingulate cortex, anterior insula, thalamus, and precuneus. Behavioral symptoms were associated with diminished activation of

frontoparietal cortex as well as thalamus and cerebellum. Psychosis-spectrum symptoms were associated with diminished activation of the left dorsolateral prefrontal cortex. Fear (phobia symptoms) was associated with diminished activation of a marginally significant cluster of medial frontal cortex, but this effect did not survive supplementary analyses (see below) and was not evaluated further. Finally, in contrast to the *hypo*-activation described above, anxious-misery symptoms were associated with a marked *hyper*-activation of multiple executive regions including anterior cingulate cortex, dorsolateral prefrontal cortex, parietal cortex, and thalamus. Notably, a small area of overlapping significant effects across dimensions was seen in the left dorsolateral prefrontal cortex (MNI coordinates: x=-28, y=-6, z=58; *k*=10). Significant differential effects of each dimension were present in multiple executive regions (**Figure 4**, **Table S2**). This was driven by the divergent impact of global psychopathology (*hypo*-activation) and anxious misery (*hyper*-activation). We did not find any differences in default mode regions where task-induced deactivation was present.

Supplementary analyses

Overall, convergent results were obtained from supplementary analyses where participants taking psychoactive medications were excluded (**Table S3**), when working memory performance was included as a covariate (**Table S4**), and when multiple additional covariates were included (**Table S5**). When participants with poor performance on the 2-back condition were excluded, psychosis-spectrum symptoms were associated with a marginally significant cluster of hyperactivation in the cingulate gyrus; otherwise results were similar (**Table S6**). As noted above, fear did not have a significant association with activation in any of the supplementary analyses.

DISCUSSION

In this large study of psychopathology in youth, we examined associations between diverse psychopathology and activation of the brain's executive system during a working memory task. To account for the fact that psychiatric disorders are highly co-morbid, factor analysis of the psychopathology data using a bifactor model allowed us to examine both general psychopathology and orthogonal dimensions of psychopathology. Overall psychopathology was associated with a significant alteration of the global multivariate pattern of activation. Furthermore, dimensions of psychopathology significantly influenced regional patterns of activation in different ways. In contrast to the hypo-activation seen in association with overall psychopathology, anxious misery symptoms were associated with hyper-activation of the same network. Taken together, these data emphasize that dimensions of psychiatric symptomatology are associated with both common and distinct deficits within the executive system.

Evidence for common executive deficits across psychiatric syndromes in youth

The most robust finding in the present study is evidence of common executive deficits attributable to overall psychopathology present across categorical clinical diagnoses. This was observed on both a global and local scale: overall psychopathology was associated with an alteration of the global multivariate pattern of activation, driven by regional hypoactivation in a network of executive regions including the frontal pole, anterior cingulate, anterior insula, and precuneus. These results accord with a copious literature of case-control studies from multiple individual disorders, and moreover highlight the centrality of executive dysfunction across major psychiatric syndromes.

We were able to estimate the influence of overall psychopathology across disorders through the use of a bifactor analysis of the item-level responses from the psychopathology screening interview. This approach obviates several major obstacles to estimating common deficits across traditional categorical diagnoses. First, when co-morbidity is represented in standard models as shared variance, it is controlled for but cannot be estimated. Estimating overall psychopathology may be particularly important in youth where the pattern of psychopathology may not fit standard diagnostic criteria in context of ongoing development. Second, strongly non-random patterns of comorbidity exist between different disorders (19). For example, mood and anxiety disorders have a high rate of comorbidity, as do ADHD and conduct disorders. Third, the frequency of each diagnosis varies considerably; thus the statistical power to estimate the impact of each individual diagnosis is not equal. Fourth, categorical diagnoses are by definition dichotomous, and important dimensional effects cannot be examined.

Crucially, each of these problems can be overcome through modeling of latent dimensions of psychopathology. Here we used confirmatory factor analysis to estimate a bifactor model, which provides orthogonal scores for every subject in each psychopathology domain, as well as a score for general psychopathology. These orthogonal scores can thus be included in a single model that can estimate dissociable dimensions of psychopathology with equal power, as well as the impact of overall psychopathology through the general score. The regions implicated by overall psychopathology include the frontal pole, anterior cingulate cortex, and anterior insula. Notably, all these regions are part of the cingulo-opercular control network (also called the salience or ventral attention network), which is consistently identified as among the most reproducible large-scale functional brain networks both at rest and during task performance (35). This network is critical for cognitive control, and is particularly

implicated in maintenance of task sets and error monitoring (36). These regions are among the most commonly impaired in case-control studies of schizophrenia, ADHD, and conduct disorder, suggesting that dysfunction of this network is not associated with one specific domain of psychopathology (4,10,37). Further support is provided from a large-scale meta-analysis by Goodkind et al., who found that gray matter loss in these cingulo-opercular regions was present across psychiatric disorders and associated with impaired executive function (38).

Orthogonal dimensions of psychopathology are associated with dissociable deficits

In addition to robust effects of overall psychopathology, we also identified both regionally and directionally dissociable effects of specific dimensions of psychopathology. Behavioral symptoms were associated with diminished activation of a network of regions including fronto-parietal cortex, thalamus, and cerebellum, whereas psychosis-spectrum symptoms were associated with hypo-activation of the left dorsolateral prefrontal cortex. The behavioral dimension loaded most prominently onto items assessing externalizing disorders such as ADHD, conduct disorder, and oppositional defiant disorder. Results are consistent with case-control studies in each of these disorders, which have demonstrated hypo-function of executive regions including most commonly fronto-parietal regions (10,20). Similarly, dysfunction of the dorsolateral prefrontal cortex is considered one of the cardinal impairments of psychosis (39). However, it should be noted that the effects of the psychosis spectrum observed here are more circumscribed than those typically reported; this may be due to the use of a population rather than a clinical sampling strategy, a focus on sub-threshold psychosis symptoms, and accounting for overall psychopathology through the bifactor model. However, consistent with the current results, in our previous report on psychosis-

spectrum symptoms in this sample we observed that hypo-activation of executive regions was linked to cognitive performance but not to severity of positive psychotic symptoms, suggesting a substantial effect of overall impairment rather than psychosis *per se* (25).

In contrast to the diminished recruitment seen in other symptom domains, anxious-misery symptoms were associated with widespread *hyper*-activation of the executive network. Indeed, after accounting for overall psychopathology, anxious-misery symptoms were associated with a trend towards better working memory performance. However, executive hyper-activation is unlikely to be simply an epiphenomenon associated with working memory performance, as hyper-activation was still seen in sensitivity analyses that controlled for both in-scanner and out-of-scanner cognitive performance. This result is consistent with several studies of executive function in major depression and anxiety disorders that have found over-activation of executive regions (15-17). In contrast to the *deficient* ability to recruit the executive network seen in association with other symptom dimensions, these data suggest that mood and anxiety symptoms are associated with an *inefficient* executive system, where higher levels of executive network activation occur. It should be noted that participants in our sample who screened positive for mood and anxiety disorders frequently also had high levels of overall psychopathology. As these dimensions have directionally opposite associations with executive recruitment, the relative presence of co-morbidity in a given sample could conceivably result in divergent results.

Limitations

Certain limitations of the present work should be noted. First, the young community-based sample studied here had diminished symptom severity compared to

that found in typically older help-seeking samples drawn from clinical practices. Drawing from a different distribution of symptoms may result in reduced generalizability to clinical populations. However, the approach used here allowed us to accrue a larger, mainly unmedicated sample at a single site and scanner, and is well suited to investigating broad dimensions of psychopathology. A second important limitation is that this cross-sectional report does not include longitudinal data. As our prior work in this dataset has demonstrated that between-subject cross-sectional age effects are relatively subtle (24), within-subject longitudinal data will be particularly informative regarding whether specific symptom domains are associated with development of executive deficits over time. Third, certain important classes of psychopathology such as substance use that have been shown to impact executive function were not considered in the current analysis (40). Fourth, despite prior research showing abnormalities of the default mode network in multiple psychiatric disorders, we did not find significant effects in the default mode network; this may be due to the task paradigm employed. Finally, as previously noted (26,27), the highly-structured screening format of GOASSESS may have resulted in high sensitivity but relatively diminished specificity, leading to over-estimation of the frequency of several disorders. Alternatively, high rates of endorsed symptoms may accurately reflect this particular sample. Although factor-analytic results of the GOASSESS appear consistent with prior findings and lend support to the validity of the measure, ongoing longitudinal follow-up with semi-structured assessments applying DSM disorder criteria will allow further evaluation of the clinical relevance and stability of the current findings.

Conclusions

These data provide novel evidence regarding common and dissociable executive system deficits across multiple dimensions of psychopathology in young people. Results emphasize that executive dysfunction is present in association with overall psychopathology across traditional categorical psychiatric diagnoses, underscoring this system's central relevance for circuit-based conceptualizations of neuropsychiatric disorders such as the NIMH Research Domain Criteria (RDoC) (21). These results may suggest that interventions seeking to enhance executive function may not fit well within the existing categorical diagnostic framework, and may be beneficial to individuals across diverse clinical syndromes where executive deficits are present. Future research employing longitudinal designs may motivate targeted early interventions that seek to mitigate executive dysfunction in youth before negative outcomes accrue.

TABLES

Screening Diagnostic Category	N	Female (%)	Caucasian (%)	Age (Years)		Maternal Education (Years)		*N*-Back Performance (d')	
				Mean	SD	Mean	SD	Mean	SD
Typically developing	351	50.7	58.7	15.34	3.88	14.81	2.57	2.96	0.65
ADHD	174	40.8	42.0	14.70	3.16	14.12	2.56	2.69	0.64
Agoraphobia	66	77.3	28.8	16.13	2.62	13.55	2.08	2.69	0.57
Anorexia	13	69.2	53.8	16.12	1.98	13.62	2.26	2.78	0.81
Bulimia	4	100.0	100.0	18.31	1.04	15.50	2.52	3.41	0.38
Conduct disorder	95	43.2	18.9	16.20	2.77	12.93	2.20	2.60	0.61
Major depression	166	63.9	45.8	17.47	2.36	13.93	2.38	2.96	0.61
Generalized anxiety disorder	21	52.4	57.1	16.46	3.02	14.76	3.03	3.04	0.89
Mania	13	61.5	15.4	16.84	2.94	13.75	1.82	2.85	0.55
Obsessive-compulsive disorder	34	70.6	41.2	16.82	3.07	13.85	2.58	2.98	0.84
Oppositional defiant disorder	368	51.1	35.3	15.59	2.90	13.74	2.38	2.74	0.65
Panic	8	75.0	25.0	15.68	2.63	12.75	1.04	2.75	0.37
Phobias	351	66.1	42.2	15.24	3.33	14.15	2.41	2.80	0.67
Psychosis-spectrum	321	51.4	32.7	16.14	2.97	13.79	2.19	2.76	0.64
PTSD	146	65.8	35.6	16.80	2.90	13.66	2.35	2.85	0.61
Separation anxiety	55	58.2	54.5	15.15	3.50	14.25	2.28	2.92	0.48
Social phobia	281	58.7	35.9	15.88	3.02	13.91	2.41	2.84	0.66

Table 1. Demographic information by screening diagnostic category. Individual subjects may be present in multiple categories due to co-morbidity.

FIGURE LEGENDS

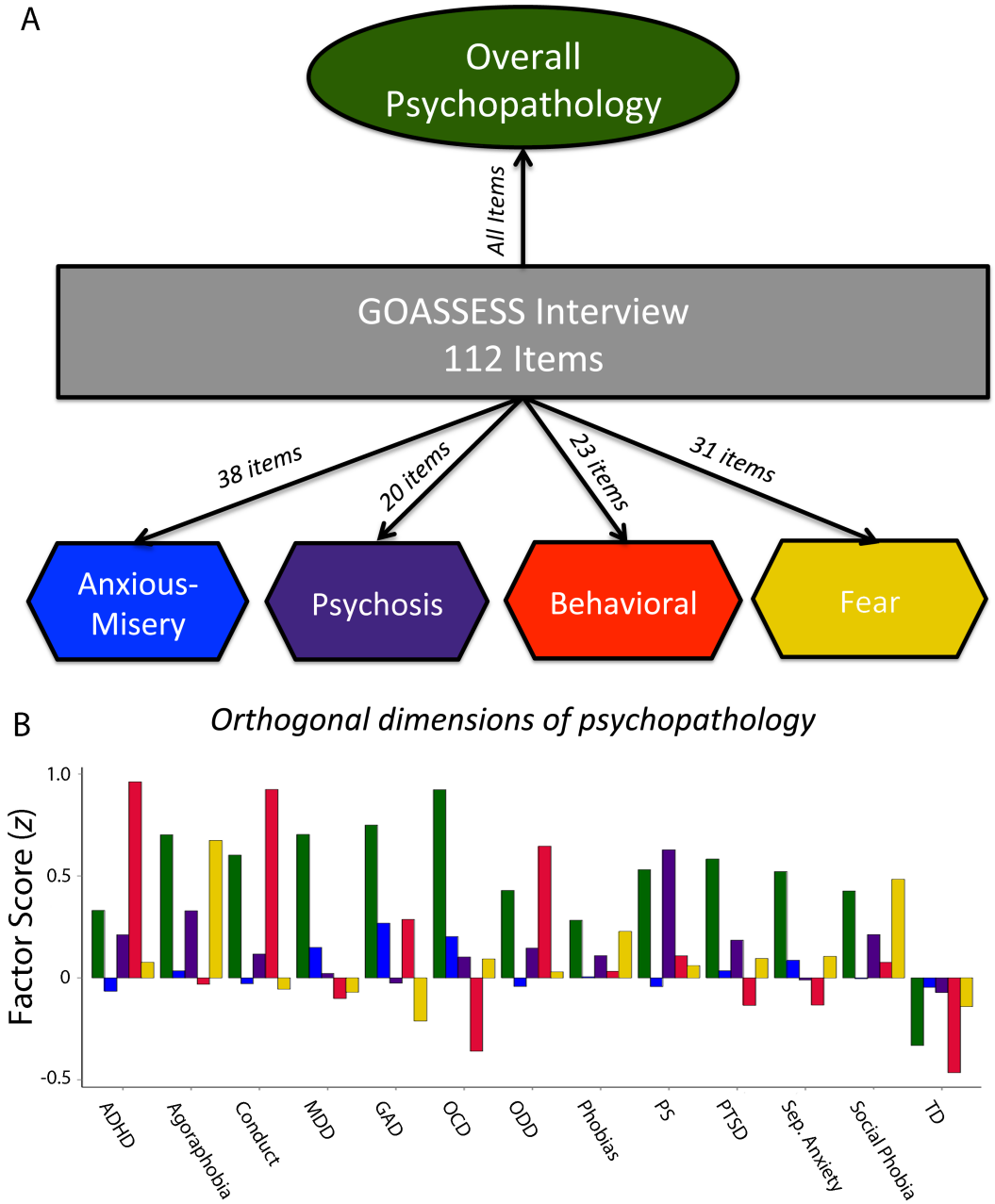

Figure 1. Bifactor model of common and divergent dimensions of psychopathology across categorical screening diagnoses. The input data to the bifactor model were 112 items from the GOASSESS screening diagnostic interview, resulting in orthogonal dimensions of psychopathology (**A**). Mean (SD) factor scores of each dimension for each screening diagnosis (with at least *n*=20) is displayed in (**B**).

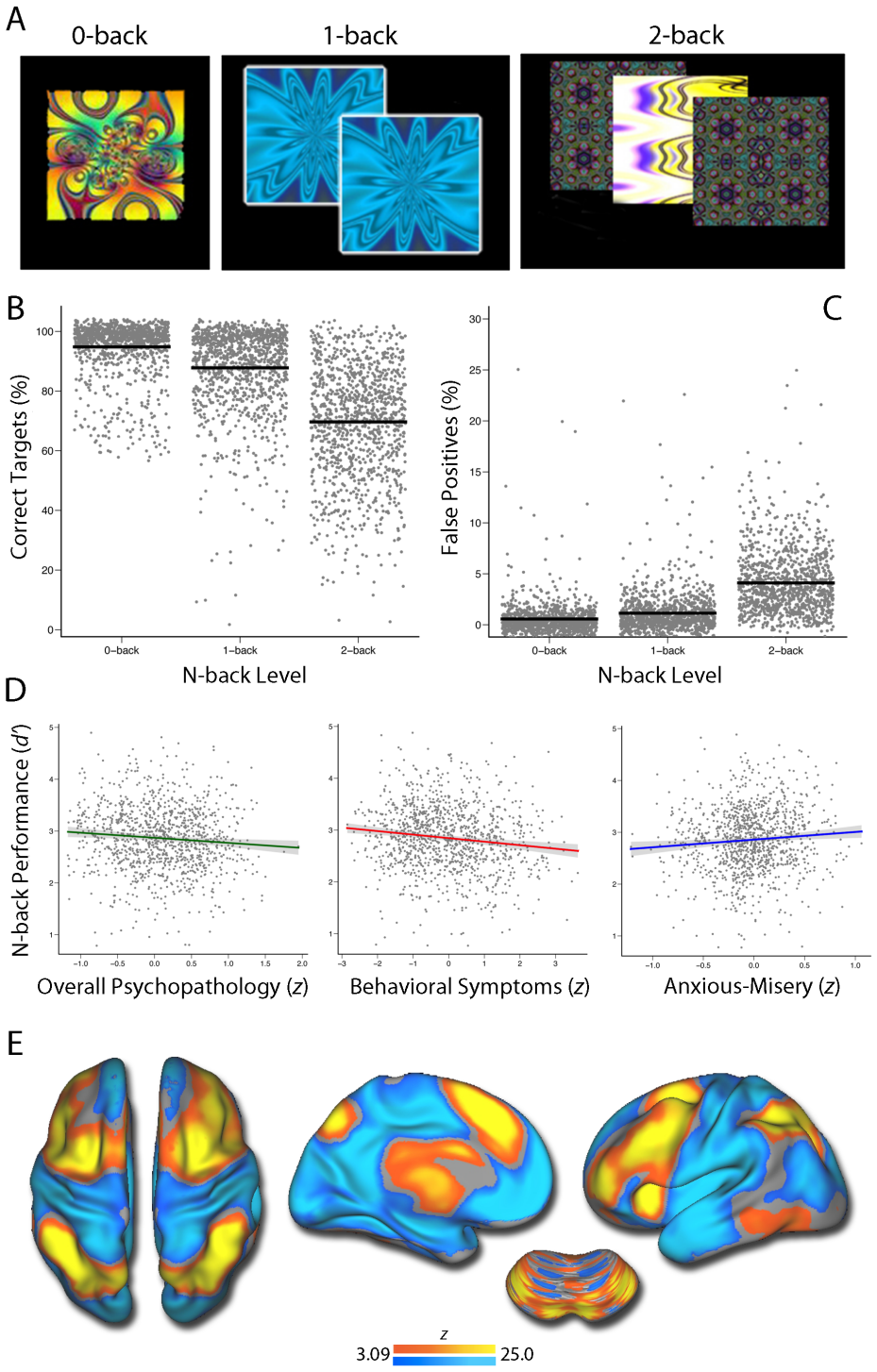

Figure 2. Working memory task paradigm, behavioral performance and contrast of interest. A fractal version of the *n*-back task was used to probe working memory

function where working memory load was varied parametrically across three levels (**A**). Higher working memory load was associated with fewer correct responses to targets (**B**) and more false positive responses to foils (**C**). Correct responses and false positives were summarized across all levels of working memory load using the signal detection metric *d'* to provide an integrated measure of working memory performance. Task performance was reduced in association with both overall psychopathology and behavioral symptoms; anxious-misery was conversely associated with a trend towards improved task performance (**D**). The contrast of interest (2-back > 0-back) robustly activated the entire executive system and de-activated non-executive regions (**E**).

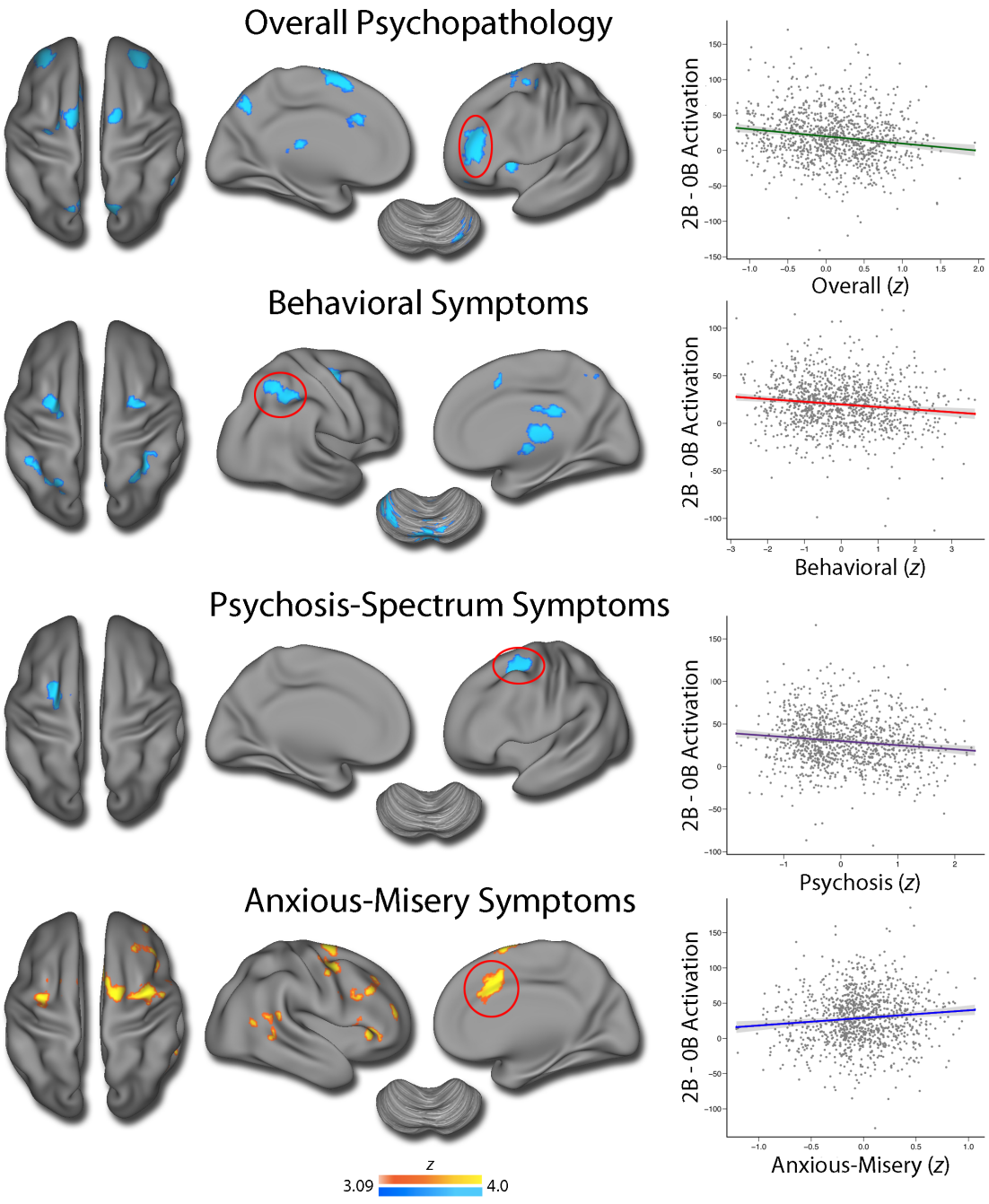

Figure 3. Relationship of orthogonal dimensions of psychopathology with executive network recruitment. The association of each dimension of psychopathology with executive network activation (2-back > 0-back) was examined

using linear regression. The most robust effects were seen for overall psychopathology, where greater symptom severity was associated with diminished activation of the frontal pole, anterior cingulate, anterior insula, thalamus, and precuneus. Behavioral symptoms were associated with fronto-parietal, thalamic, and cerebellar hypoactivation; psychosis-spectrum symptoms were associated with diminished activation of the left dorsolateral prefrontal cortex. In contrast to the observed hypo-activation for all other dimensions of psychopathology, anxious misery was associated with *hyper*-activation of regions within the executive network, including the dorsolateral prefrontal cortex and anterior cingulate cortex. All analyses included age, sex, and in-scanner motion as covariates; images thresholded at $z>3.09$, corrected $p<0.001$. Scatter plots were drawn from significant clusters identified by red circles. Activation is plotted in arbitrary BOLD units. For detailed results see Tables S1.

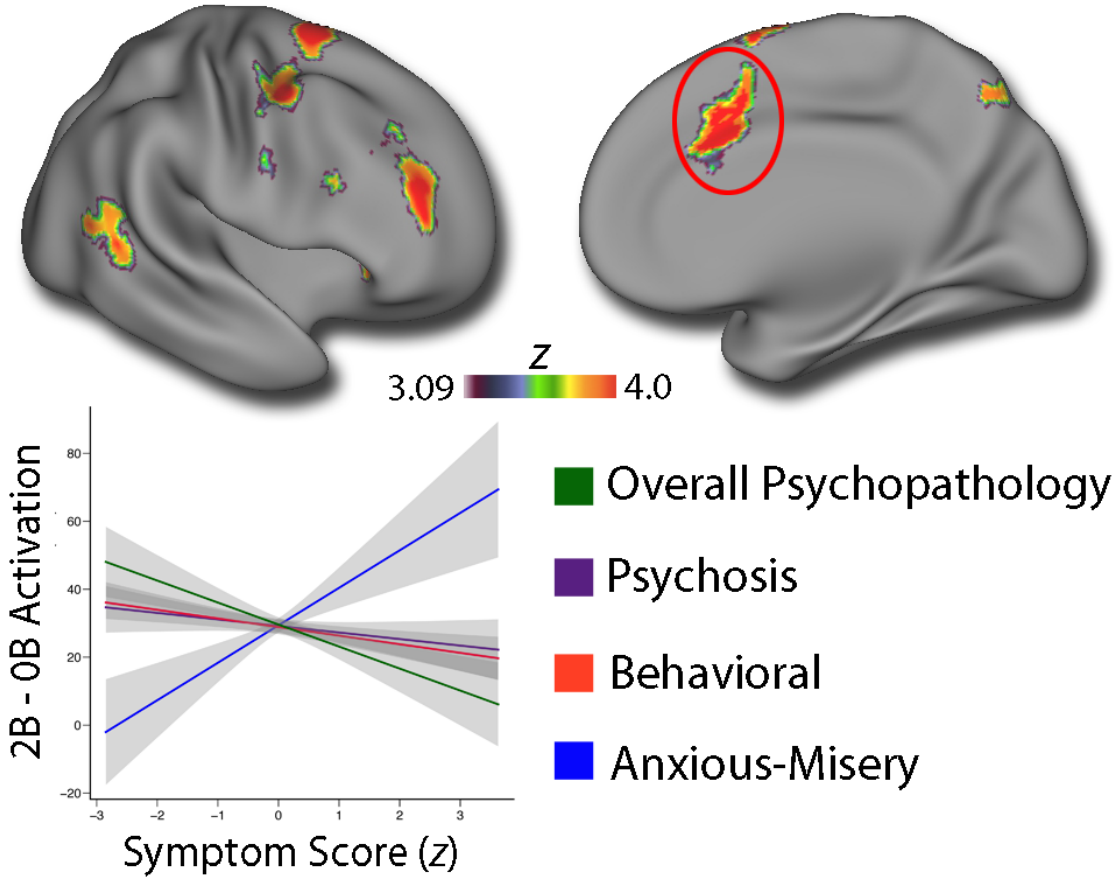

Figure 4. Differential effects of psychopathology on executive system activation. A linear model comparing the differential impact of each dimension of psychopathology revealed significant differences in multiple executive regions including the anterior cingulate cortex, frontal pole, and dorsolateral prefrontal cortex. Differential effects were driven by the divergent impact of anxious misery (*hyper*-activation) and overall psychopathology (*hypo*-activation). Activation is plotted in arbitrary BOLD units. Shaded area represents 95% confidence intervals. For detailed results see Table S2.

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SUPPLEMENTARY METHODS

Participants: Exclusion Criteria

The Philadelphia Neurodevelopmental Cohort is a collaboration between the Center for Applied Genomics at CHOP and the Brain Behavior Laboratory at the University of Pennsylvania (Penn). Children, adolescents, and young adults between the ages of 8 and 21 who presented to the Children's Hospital of Philadelphia (CHOP) or a CHOP-affiliated clinic for a pediatric visit and volunteered to participate in genomic studies of complex pediatric disorders were eligible for inclusion in the Philadelphia Neurodevelopmental Cohort (1-3). Participants and/or their parents signed an informed consent form approved by the Institutional Review Boards at CHOP and Penn. After stratification by age and sex, a subsample of 1,601 randomly selected participants underwent multi-modal neuroimaging. Of these participants, 1,462 completed the n-back task described below. Subject exclusion criteria included medical conditions that might impact brain function (*n*=151), incidentally encountered structural brain abnormalities (*n*=20), and inadequate task performance (>7 nonresponses on the 0-back condition; *n*=65). As part of image quality assurance, subjects were also excluded for excessive motion (mean relative displacement > 0.5mm or maximum displacement > 6 mm; *n*=94) or poor image coverage (*n*=73). Many of the subjects excluded met multiple exclusion criteria. The final sample included in analyses was 1,129. This sample thus constitutes a super-set of subjects previously included in reports which focused on normative development (4) and psychosis-spectrum symptoms (5).

Clinical Assessment

Psychopathology was assessed using a structured screening instrument (GOASSESS) administered by trained assessors (1). Participants age 11-21 were

interviewed individually; collateral information was obtained independently from a caregiver for children age 8-17. To allow rapid training and standardization across a large number of assessors, GOASSESS was designed to be highly structured, with screen-level symptom and episode information. The instrument is abbreviated and modified from the epidemiologic version of the NIMH Genetic Epidemiology Research Branch Kiddie-SADS (6). Assessors underwent a common training protocol developed and implemented by MEC that included didactic sessions, assigned readings, and supervised pair-wise practice. They were certified for independent assessments through a standardized procedure requiring observation by a certified clinical observer who rated the proficiency of the assessor on a 60-item checklist of interview procedures.

The psychopathology screen in GOASSESS assessed psychiatric and psychological treatment history, as well as lifetime occurrence of major domains of psychopathology including mood (major depressive episode, mania), anxiety (agoraphobia, generalized anxiety, panic, specific phobia, social phobia, separation anxiety), behavioral (oppositional defiant, attention deficit/hyperactivity, conduct), eating disorders (anorexia, bulimia), and suicidal thinking and behavior. Substance use disorders were assessed with a different instrument for a subset of participants and are not evaluated here. Each section included a screen for relevant symptoms and additional DSM-IV criteria such as symptom frequency, duration, onset, and offset. Associated distress and impairment were each rated on 11-point scales ranging from 0 to 10. To establish screening diagnostic criteria for each domain of psychopathology, a computerized algorithm integrated information regarding symptom frequency and duration approximating each DSM-IV disorder or episode criteria, accompanied by significant distress or impairment rated>=5 on the 11-point scale.

Assessment of Psychosis Spectrum Symptoms

As described in detail in our prior articles focused on psychosis-spectrum symptoms (5,7,8), GOASSESS provided three measures of psychosis-spectrum symptoms. First, sub-threshold positive symptoms in the past year were assessed with the 12-item assessor administered PRIME Screen-Revised (PS-R)(9). Items were read aloud by the assessor and self-rated on a 7-point scale (0 = Definitely Disagree; 6 = Definitely Agree). Second, lifetime threshold hallucinations and delusions were evaluated with the K-SADS (6) psychosis questions and structured follow-up probes. Third, negative and disorganized symptoms were evaluated using six assessor rated items from the Scale of Prodromal Symptoms (SOPS) in the Structured Interview for Prodromal Syndromes (SIPS)(10), including: N2 Avolition; N3 Expression of Emotion; N4 Experience of Emotions and Self; N6 Occupational Functioning; D3 Trouble with Focus and Attention; and P5 Disorganized Communication. We identified subjects as psychosis-spectrum if they: 1) had an age-deviant PRIME total score >= 2SD above age matched peers or had >= one PRIME item rated 6 or >= three items rated 5 (Somewhat Agree); 2) endorsed definite or possible hallucinations or delusions on the K-SADS psychosis screen; or 3) had an age deviant total negative/disorganized SOPS score >=2SD above age-matched peers.

Factor Analysis Methods

Factor analysis methods and results have been previously presented (11) and will be detailed in a separate manuscript (Calkins et al., In Preparation). Briefly, factor analyses proceeded in several phases. First, we performed exploratory factor analyses in order to determine which items loaded on which factors, with the ultimate goal of estimating a confirmatory factor analysis model from which to calculate scores. Because

there are multiple exploratory factor analyses extraction methods (and an even greater number of factor rotations), we decided to estimate several exploratory factor analyses for thoroughness. Methods provided convergent results, supporting a four-factor model.

Because all exploratory factor analyses indicated *correlated* traits of psychopathology, and because a goal of the item-wise confirmatory analysis was to generate *orthogonal* scores, we opted to use the only type of confirmatory model capable of accommodating such a combination, which is the bifactor model (12-14). Bifactor modeling is a way to estimate the contribution of an item to an overall dimension (psychopathology in this case) after controlling for its specific factor, and vice versa. Bifactor models are similar to higher-order models, except in a bifactor model there are direct effects of the general factor on the individual items.

The confirmatory bifactor model was estimated using the Bayesian estimator in Mplus (15). The Bayesian estimator comes with the drawback of not producing conventional fit indices, but was chosen because the computation time of other estimators (e.g. wlsmv) was anticipated to be much longer. The model was later estimated using the other estimators (wlsmv and MLR), and the minimum correlation between scores calculated using the different methods was 0.93 (mean = 0.98). The fit indices provided by the wlsmv-estimated model suggest acceptable fit, with a Comparative Fit Index (CFI) of 0.91 and Root Mean-Square Error of Approximation (RMSEA) of 0.027 ± 0.0005.

Task Paradigm

Subjects completed a fractal version of the n-back task (16) during their fMRI scan. During the task, a fractal was presented for 500 ms followed by a 2500 ms interstimulus interval. This task was used to probe working memory and had 3

conditions: 0-, 1-, and 2-back. During the 0-back, subjects responded by pressing a button when the fractal presented matched a predefined fractal. During the 1-back, subjects responded when the fractal presented was the same as the one preceding it. During the 2-back, subjects responded when the fractal was identical to the one two before it. Each condition consisted of three 20-trial blocks, each preceded by a 9s instruction period, with a target to foil ratio of 1:3. The task included a total of 45 targets and 135 foils, as well as three 24 s blocks of rest during which a fixation crosshair was displayed.

Image Acquisition

Imaging data were acquired on 3T Siemens TIM Trio whole-body scanner using a 32-channel head coil. A magnetization-prepared rapid acquisition gradient echo T1-weighted (MPRAGE) image (TR, 1810 ms; TE, 3.51 ms; TI, 1100 ms; FOV, 180 × 240 mm; matrix, 192 × 256; 160 slices; slice thickness/gap, 1/0 mm; flip angle, 9°; effective voxel resolution, 0.9 × 0.9 × 1 mm) and B0 field map (TR, 1000 ms; TE1, 2.69 ms; TE2, 5.27 ms; 44 slices; slice thickness/gap, 4/0 mm; FOV, 240 mm; effective voxel resolution, 3.8 × 3.8 × 4 mm) were acquired to aid spatial normalization to standard space and application of distortion correction procedures, respectively. Functional images were then obtained using a whole-brain, single-shot, multislice, gradient-echo echoplanar sequence (231 volumes; TR, 3000; TE, 32 ms; flip angle, 90°; FOV, 192 × 192 mm; matrix 64 × 64; 46 slices; slice thickness/gap 3/0 mm; effective voxel resolution, 3.0 × 3.0 × 3.0 mm).

Image Processing

As previously described (4), fMRI data was pre-processed with FSL (17), including skull removal with BET (18), slice time correction, motion-correction with MCFLIRT (19), spatial smoothing (6 mm FWHM), and mean-based intensity normalization. Subject-level timeseries analyses were carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (20). The three condition blocks (0-back, 1-back, and 2-back) were modeled using a canonical (double-gamma) hemodynamic response function with six motion parameters and the instruction period included as nuisance covariates. The rest condition served as the unmodeled baseline. The median functional and anatomical volumes were co-registered using boundary-based registration (21) with integrated distortion correction using FUGUE. The anatomical image was normalized to the Montreal Neurologic Institute 152 1 mm template using the top-performing diffeomorphic SyN registration of ANTS (22,23). All transformations (distortion correction, co-registration, normalization, and down-sampling to $2mm^3$) were concatenated so only one interpolation was performed. The statistical maps for the contrast of interest (2-back > 0-back) were then used in the group-level analyses. Statistical maps were downsampled further to $4mm^3$ for the multivariate distance-based matrix regression analysis (see below) for computational feasibility; generalized linear model analyses used $2mm^3$ resolution data.

Matrix Regression

Multivariate distance-based matrix regression operates in two steps. In the first step, the overall multivariate pattern of activation and de-activation in down-sampled data (4mm isotropic voxels) was compared among subjects using a distance metric (Euclidean distance). This produces a matrix (size 1,129 x 1,129) representing how similar the overall pattern of activation is between each combination of subject pairs.

Second, matrix regression is used to test how well each phenotypic variable explains the distances between each participant's pattern of activation created in step 1. This provides a measure of how the overall pattern of activation is impacted by each group level variable entered into the design matrix in standard regression format. Our group-level design matrix included symptom dimensions (from the factor analysis), age, sex, and in-scanner motion. As in our prior work, motion was summarized for each subject as the mean relative displacement of realignment parameters across the time series (24-26). This multivariate distance-based matrix regression procedure yields a pseudo-F statistic, the significance of which was tested using 1,000 permutations.

Figure S1

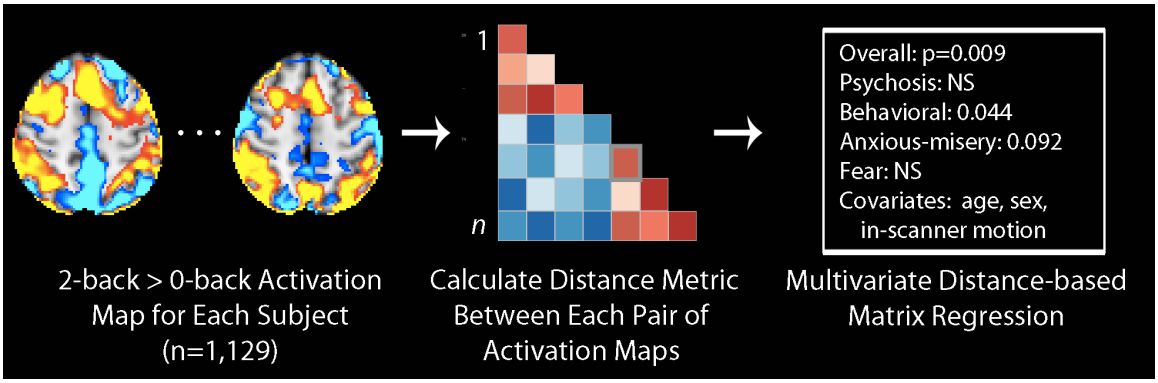
1
n
Overall: p=0.009
Psychosis: NS
Behavioral: 0.044
Anxious-misery: 0.092
Fear: NS
Covariates: age, sex, in-scanner motion
2-back > 0-back Activation Map for Each Subject (n=1,129)
Calculate Distance Metric Between Each Pair of Activation Maps
Multivariate Distance-based Matrix Regression

Table S1. Main effect of psychopathology dimensions in complete sample (n=1129)

Region	k	Peak *z*	x	y	z
HYPO-ACTIVATION					
Overall Psychopathology					
Frontal pole	993	4.96	-34	48	16
Precuneus cortex	752	4.54	10	-70	56
Frontal pole	698	5.07	30	44	20
Superior frontal gyrus	600	4.65	-2	10	56
Central operculum	283	4.9	-42	12	4
Precentral gyrus	172	4.26	-28	-8	60
Cerebellar crus I	167	4.56	34	-76	-22
Middle temporal gyrus	159	3.93	54	-44	4
Superior frontal gyrus	131	3.93	16	4	62
Anterior insula	131	3.94	22	30	4
Anterior cingulate gyrus	123	4.08	-8	14	28
Thalamus	104	3.92	-10	-20	16
Anterior cingulate gyrus	84	3.92	8	8	32
Psychosis					
Superior frontal gyrus	293	4.09	-22	-4	58
Fear					
Juxtapositional lobule cortex	118	3.91	-12	6	56
Behavioral					
Superior parietal lobule	641	4.49	38	-46	50
Supramarginal gyrus	568	4.88	-28	-46	38
Cerebellar crus I	510	3.88	-30	-52	-36
Cerebellar vermis crus II	477	3.92	0	-80	-28
Thalamus	440	4.45	6	-20	10
Middle frontal gyrus	319	4.58	-26	-4	56
Posterior cingulate gyrus	256	3.95	6	-28	26
Superior frontal gyrus	150	4.1	24	4	46
Juxtapositional lobule cortex	97	3.58	-6	8	58
Caudate	77	3.76	-8	4	2
Cerebellar V	77	3.99	-2	-58	-24
Anterior insula	69	3.85	-44	14	-8

Table S1 continued.

Region	k	Peak *z*	x	y	z
HYPER-ACTIVATION					
Anxious Misery					
Superior frontal gyrus	863	5.12	0	10	68
Frontal pole	535	4.27	32	36	42
Paracingulate gyrus	454	4.51	8	12	42
Anterior insula	355	4.14	36	34	-2
Precentral gyrus	334	4.4	-32	-6	56
Angular gyrus	182	3.62	48	-46	18
Frontal pole	91	4.02	38	52	16
Angular gyrus	84	3.6	62	-60	24

Table S2. Differential effects of psychopathology dimensions in complete sample (n=1129)

Region	k	Peak *z*	x	y	z
Superior frontal gyrus	1459	5.51	18	-2	70
Frontal pole	575	4.72	32	44	20
Precentral gyrus	502	5.06	-32	-6	58
Middle temporal gyrus	355	4.16	54	-44	4
Frontal pole	320	4.4	-32	46	14
Inferior frontal gyrus	211	3.83	56	18	30
Superior temporal gyrus	115	4.68	-52	-8	-6
Anterior insula	95	3.89	34	32	-2
Precuneus	80	3.68	8	-72	52
Temporal occipital fusiform cortex	74	4.05	-38	-48	-20

Table S3. Main effect of psychopathology dimensions in sub-sample of participants not taking psychotropic medication (n=1007)

Region	k	Peak *z*	x	y	z
HYPO-ACTIVATION					
Overall Psychopathology					
Frontal pole	1686	5.43	-34	48	16
Superior frontal gyrus	1587	5.25	-2	10	56
Precuneus cortex	1455	4.71	10	-70	54
Frontal pole	823	5.09	34	44	26
Cerebellar crus VI	397	4.47	30	-60	-30
Middle temporal gyrus	249	4.11	54	-48	12
Lateral occipital cortex	150	3.85	-44	-58	58
Thalamus	126	4.05	-8	-20	16
Anterior insula	104	3.6	22	22	0
Middle frontal gyrus	86	3.89	-42	14	38
Occipital fusiform cortex	84	4.31	-42	-74	-24
Psychosis					
Superior frontal gyrus	97	3.62	-22	6	52
Superior parietal lobule	69	4.2	-30	-52	60
Superior parietal lobule	69	3.99	-18	-64	58
Fear	none				
Behavioral					
Superior parietal lobule	810	4.16	-38	-48	-18
Supramarginal gyrus	337	4.44	-28	-46	38
Superior parietal lobule	178	4.02	38	-46	50
Middle frontal gyrus	174	3.96	-26	-4	56
Precuneus cortex	128	3.75	14	-58	54
HYPER-ACTIVATION					
Anxious Misery					
Superior frontal gyrus	491	4.59	-2	12	68
Frontal pole	125	4.11	38	34	40
Paracingulate gyrus	124	3.91	6	12	44
Middle frontal gyrus	112	4.06	50	16	32

Table S4. Main effect of psychopathology dimensions with working memory performance (*d'*) as a covariate (n=1129)

Region	k	Peak *z*	x	y	z
HYPO-ACTIVATION					
Overall Psychopathology					
Frontal pole	330	4.41	28	44	20
Frontal pole	208	4.3	-30	48	26
Central operculum	139	4.29	-42	12	4
Psychosis					
Superior frontal gyrus	158	3.87	-24	6	52
Fear	none				
Behavioral					
Supramarginal gyrus	221	4.02	38	-46	52
Superior parietal lobule	124	4.18	-28	-46	38
Thalamus	69	3.63	6	-28	26
HYPER-ACTIVATION					
Anxious Misery					
Temporal pole	233	4.09	52	12	-22
Inferior frontal gyrus	232	3.97	52	30	-4
Middle frontal gyrus	219	4.13	36	0	46
Superior frontal gyrus	169	4.67	-2	12	70
Cingulate gyrus	152	4	8	12	40
Inferior frontal gyrus	131	3.93	56	16	32
Superior temporal gyrus	125	4.06	-52	-8	-6
Temporal pole	95	3.87	-44	8	-20
Supramarginal gyrus	78	3.52	62	-40	14

Table S5. Main effect of psychopathology dimensions while including additional covariates (n=1112 due to missing covariate data in 17 subjects). Covariates included: age, sex, race, in-scanner motion, maternal education, time between assessment and imaging, out-of-scanner cognitive performance, and in-scanner working memory performance.

Region	k	Peak *z*	x	y	z
HYPO-ACTIVATION					
Overall Psychopathology					
Frontal pole	448	4.57	30	42	18
Frontal pole	353	4.57	-32	48	16
Central operculum	153	4.32	-42	12	4
Middle temporal gyrus	102	3.92	54	-44	4
Precuneus cortex	87	3.71	10	-70	56
Psychosis					
Superior frontal gyrus	145	3.87	-22	-4	60
Fear	none				
Behavioral					
Supramarginal gyrus	73	3.98	-28	-46	38
HYPER-ACTIVATION					
Anxious Misery					
Anterior insula	224	3.98	50	32	-2
Middle frontal gyrus	198	4.02	36	0	46
Superior frontal gyrus	137	4.57	-2	12	70
Superior temporal gyrus	133	3.87	58	-4	-10
Inferior frontal gyrus	114	3.83	56	16	32
Middle temporal gyrus	110	3.61	62	-42	2
Frontal pole	107	3.86	8	12	40
Superior temporal gyrus	79	3.86	-52	-8	-6

Table S6. Main effect of psychopathology dimensions after excluding for poor 2-back performance (n=963)

Region	k	Peak *z*	x	y	z
HYPO-ACTIVATION					
Overall Psychopathology					
Frontal pole	376	4.38	28	44	20
Frontal pole	232	4.47	-34	48	16
Frontal operculum	226	4.68	-42	12	4
Precuneous cortex	206	4.46	10	-70	54
Supramarginal gyrus	138	4.28	56	-40	46
Thalamus	125	4.14	-4	-32	8
Superior frontal gyrus	120	4.1	-2	10	56
Fear	none				
Behavioral					
Thalamus	321	4.32	8	-20	6
Superior parietal lobule	244	4.45	-28	-46	38
Superior parietal lobule	215	4.11	38	-46	52
Cingulate gyrus	213	4.04	6	-30	26
Cerebellar VI	169	3.87	-18	-60	-22
Cerebellar Vermis VI	109	3.54	2	-72	-16
Caudate	97	3.93	-8	4	2
Superior frontal gyrus	83	4.01	-26	-2	58
HYPER-ACTIVATION					
Anxious Misery					
Cingulate gyrus	309	4	8	14	38
Middle frontal gyrus	152	3.99	36	0	46
Frontal pole	102	4.06	32	36	42
Superior frontal gyrus	75	4.38	0	10	68
Inferior frontal gyrus	73	3.65	56	16	32
Psychosis					
Cingulate gyrus	67	4.21	2	-44	14

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Neural Markers of the Development of Executive Function: Relevance for Education

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ABSTRACT

Executive functions are involved in the development of academic skills and are critical for functioning in school settings. The relevance of executive functions to education begins early and continues throughout development, with clear impact on achievement. Diverse efforts increasingly suggest ways in which facilitating development of executive function may be used to improve academic performance. Such interventions seek to alter the trajectory of executive development, which exhibits a protracted course of maturation that stretches into young adulthood. As such, it may be useful to understand how the executive system develops normally and abnormally in order to tailor interventions within educational settings. Here we review recent work investigating the neural basis for executive development during childhood and adolescence.

Introduction

Executive function (EF) is a broad category of cognition, including cognitive control, sustained attention, inhibition, error monitoring, and working memory. Unlike other cognitive domains, the executive system undergoes a protracted period of development that extends into young adulthood. Behavioral studies have suggested that different domains of EF may develop at different rates during this time [1]. These processes are necessary for the normal development of nearly all academic areas

including math [2,3] and language [3] skills. The importance of early EF capacity to academic achievement begins as early as preschool [4] and continues throughout adolescence [5]. As such, there has been much interest in methods to facilitate the development of EFs in school settings in an effort to improve academic performance. Accordingly, an understanding of the neural basis of the executive system's development could help target interventions to facilitate the development of executive function.

Here we review the existing literature regarding brain systems implicated in the development of the executive system and its significance for academic achievement. We first describe recent behavioral and neuroimaging studies regarding the maturation of the executive system from childhood through adolescence. Second, we describe moderating factors that have been shown to affect the course of executive development. Third, we review studies documenting executive dysfunction in neuropsychiatric disorders, which often begin in adolescence and are associated with poor educational outcomes [6]. Finally, we present examples of educational interventions that have been proposed as ways of facilitating EF development.

Normative development of executive function: behavioral studies

Childhood and adolescence are periods of rapid development of EF. Dajani and Uddin [7] note that different domains of EF develop at various times during development: inhibition and attentional control emerge and develop in early childhood, information processing abilities improve in mid childhood, while cognitive flexibility and goal setting continue to develop into adolescence. For instance, a longitudinal study of young children charting trajectories of inhibitory control and cognitive flexibility found that these processes developed most rapidly during 3-4 years of age [1]. Though cognitive

flexibility develops more slowly than inhibitory control [1], age-related improvements in inhibition have been observed through mid-childhood [8]. Attention and working memory (WM) are additional domains of EF that continue to mature throughout adolescence, while other cognitive domains such as spatial memory and verbal memory do not [9].

Normative development of executive function: neuroimaging studies

Though it is clear from behavioral studies that EF matures during childhood and adolescence, neuroimaging studies provide information regarding how neurodevelopmental processes may underlie the improvement in performance. Studies of brain structure, function, and connectivity provide complementary evidence regarding maturation of the executive system. Structural brain imaging studies indicate that grey matter volume increases during childhood and subsequently declines during adolescence, whereas white matter volumes increase throughout development [10]. However, the trajectory of development may be more important in predicting intelligence and EF than cross-sectional measurements [11]. For example, the correlation between prefrontal cortical thickness and IQ is negative in early childhood but becomes positive in late childhood through early adulthood. Additionally, the trajectory of these changes in cortical thickness are more reflective of IQ than cortical thickness itself [11].

More recent studies have examined how changes in cortical thickness of specific brain regions correlate with EF. For example, in healthy older children, cortical grey matter thinning in the inferior frontal gyrus and anterior cingulate cortex is associated with age-related improvements in cognitive control, while thinning in the superior parietal cortex is associated with improvements in working memory [12]. In contrast, a longitudinal study showed that increased rate of cortical thinning in medial cortical regions during development is associated with more executive deficits in adulthood [13].

While age is strongly related to changes in brain structure, this relationship is less robust for functional measures of executive function. In young children, age was found to be correlated with increased lateral PFC recruitment during a working memory task [14]. In addition, cognitive control studies have shown that children with good task performance recruit different prefrontal regions than adults [15]. However, age effects on task-induced activation of executive regions during sustained attention and inhibition are not completely consistent [16-18]. Cognitive control studies have shown that there is a shift from diffuse to focal patterns of activation during development, with increased activation in regions necessary for effective task performance [19,20].

More recently, a large study of 951 youths showed that increased activation of executive regions and reciprocal deactivation of default mode network (DMN) regions underlie the improvements in WM seen during adolescence (see **Figure 1**). Executive activation and DMN de-activation was more strongly correlated with WM performance than chronological age [21], suggesting that executive development may be understood as a process of functional (rather than chronologic) maturation. Notably, multivariate pattern regression demonstrated that while both executive activation and DMN de-activation could be used to predict performance, maximum accuracy required integration of features from both networks.

Studies of resting-state functional connectivity provide evidence regarding the development of interacting functional networks, which support EF. Both cross-sectional [22] and longitudinal studies [23] demonstrate that correlations among regions within the DMN are weak in childhood but become stronger with age. Furthermore, a recent study in a large cohort of youth found that the DMN's role as a "cohesive connector" system within the functional connectome increased with age, and was correlated with cognitive performance [24]. This finding contrasts with a longitudinal study in young adolescents,

which found weaker between network correlations between the central executive network and the DMN with age [23]. The divergence of these findings may be due to the different edge measures used for the network analyses of the two studies: wavelet coherence was used as an edge measure in Gu et al., 2015, which quantifies highly related, but out-of-phase (anti-correlated) signals as strongly connected, and all values range from 0-1. This contrasts with the commonly-used Pearson's correlation (as in [23]), where out-of-phase signals will be represented with negative values.

Such developmental changes in resting state connectivity have been associated with EF capacity. Hyper-connectivity within the DMN and cingulo-opercular network are seen in children with executive deficits [25]. Greater anti-correlation between the DMN and lateral frontal cortex is associated with better inhibitory performance in children [25], but the relationship between inhibition and strength of anti-correlation between the task-positive executive system and DMN is also stronger in adults than in children [26]. These studies support the idea that the development of functional network topology contributes to the maturation of EF during this critical period.

Moderating factors

Development of EF is influenced by several factors including sex and socioeconomic status (SES). Females demonstrate better working memory and attention from childhood through adolescence [9], though males show more improvement in these domains during adolescence [27]. In contrast, males show better processing speed at the beginning of adolescence but improve less [27]. SES also affects multiple domains of EF, including sustained attention and inhibition [27]. Inhibitory control and cognitive flexibility develop more slowly in preschool-aged children with less access to learning resources [1]. In contrast to findings from behavioral studies, a longitudinal neuroimaging

study found an interactive effect of SES and sex on both behavior and brain function, but no effect of SES or sex alone [28]. Sex differences in EF that are present before puberty have been attributed to organizational effects of exposure to gonadal hormones in early development on emerging neural circuits, whereas sex differences that first appear during puberty may be due to activational effects of differences in gonadal steroid levels associated with puberty [29].

Relevance for psychopathology

Executive deficits are present in a wide range psychiatric disorders affecting children and adolescents including attention deficit hyperactivity disorder (ADHD) [30], conduct disorder [31], substance use disorders [32], and psychotic disorders [33,34]. Consequences of executive deficits during childhood and adolescence include decreased academic achievement, social isolation, low self-esteem, and risk-taking behavior [35]. Individuals with these disorders are thus most likely to benefit from educational interventions aimed at improving EF. Therefore, understanding how psychopathology is related to failures of executive development is critical.

Recent behavioral studies in neuropsychiatric disorders have examined how executive dysfunction impacts academic and social aspects of school performance, with most evidence accumulating in ADHD and substance use disorders. Developmental trajectories in adolescents with ADHD compared to healthy individuals are delayed for inhibition and shifting but similar for WM and planning [36]. In youths with ADHD, those with worse EF have more problems with academic performance and peer interactions [37]. Additionally, poor EF in adolescence is associated with early and greater use of alcohol and other substances [32].

Many neuroimaging studies have examined the relationship between executive system dysfunction and neuropsychiatric disorders during development. Case-control studies often attribute dysfunction of executive regions such as the dorsolateral prefrontal cortex, anterior cingulate, and anterior insula to disorders such as ADHD [38], schizophrenia [39], and conduct disorder [40]. However, a recent study of 1,129 youths with diverse psychopathology found that overall psychopathology (as measured using a bi-factor model, **Figure 2A**) is associated with reduced recruitment of the executive network (**Figure 2B**) [41]. Such functional data is convergent with recent evidence demonstrating grey matter loss in similar regions across several disorders that exhibit executive deficits [42]. In contrast, anxious-misery symptoms are associated with widespread *hyper*-activation of the executive network (**Figure 2C**) [41], suggesting that there are both common and dissociable deficits in executive system recruitment among neuropsychiatric disorders. Taken together, these studies emphasize the degree to which failures of executive function are a central feature of diverse psychopathology that often emerge in school-age children and adolescents, and frequently present as difficulties within educational environments.

Educational interventions

Understanding executive development and the factors that moderate it are important for developing interventions to improve it. Cognitive remediation has been shown to significantly improve multiple executive domains in patients with schizophrenia [43] and ADHD [44]. These improvements in EF are correlated with changes in brain activation such that cognitive remediation diminishes the degree of hypoactivation observed in psychosis [45]. However, the majority of recent studies proposing educational interventions that would improve EF, such as music and physical education

classes, do not include a neuroimaging component, and thus brain-based data are limited. Two recent exceptions include one study that demonstrated improved EF and increased task-induced prefrontal activation in healthy young adults following exercise [46]. Another recent study suggested that musically trained children exhibit better verbal fluency and processing speed as well as increased activation of executive regions [47]. While cognitive training [48], neurofeedback training [49], and mindfulness training [50] are additional methods that have shown early positive results in improving executive deficits, the impact of these interventions on brain function has not yet been evaluated. Given the paucity of studies in this area, it is clear that more work is needed to understand how proposed educational interventions may impact EF at a neural level.

Limitations of current research

While much progress has been made regarding our understanding of executive system development, there are several limitations that should be noted. First, many of the studies investigating EF in development are cross-sectional, and thus are unable to delineate the longitudinal developmental trajectory of EF. While cross-sectional studies are often the basis of longitudinal inferences regarding development, factors such as the selection of time points, trajectory of developmental process, and test-retest reliability of methods employed affect the extent to which cross-sectional findings accurately represent longitudinal processes [51]. Many of the few longitudinal neuroimaging studies suffer from relatively small sample sizes or relatively brief longitudinal follow-up. Furthermore, existing work has used a variety of tasks to probe EF, and results may not be generalizable across tasks or executive domains. Additionally, task difficulty likely decreases with age, and the strategies used to perform such tasks evolve during development [7], limiting the ability to similarly probe EF across age groups.

Similarly, few studies evaluate the impact of educational interventions on brain measures of EF. As such, it is not known how such interventions lead to improvements in EF or whether they produce lasting neural changes that affect the course of executive system development. Additionally, methods of assessing EF and task paradigms vary across studies, limiting replication of existing results.

Future directions

Studies reviewed here emphasize that EF undergoes an extended maturational period throughout childhood and young adulthood. It is clear from both behavioral and neuroimaging studies that different domains of EF exhibit different developmental trajectories. However, charting these trajectories remains incomplete. Large longitudinal neuroimaging studies that consistently account for factors that would affect the course of development are necessary in determining how the executive system develops normally and abnormally in association with performance deficits. Determining the normal developmental trajectory of the executive system is a necessary step in identifying both abnormal development in youth at risk for poor outcomes as well as critical periods where interventions might be most effective. Notably, the Adolescent Brain Cognitive Development (ABCD) initiative will provide detailed cognitive and clinical phenotyping in concert with longitudinal multi-modal neuroimaging on a large cohort of 10,000 youth followed over 10 years. This landmark study has the potential to provide data that may transform our understanding regarding how the executive system develops during youth.

Additionally, it is evident that there are several factors that moderate the development of EFs. Preliminary studies indicate cognitive remediation [43,44], cognitive training [48], music classes [47], and physical education [46] may be ways to improve EF during development. However, more information regarding their impact and mode of

action are needed before these factors can be used to inform the implementation of educational strategies that can facilitate EF development. For example, while several studies have identified factors that correlate with better or worse EF, the optimal time periods for implementation remain unclear. Similarly, the mechanisms by which educational interventions exert their effects are yet to be determined. Incorporating brain-based measurements within such studies may be an important step in understanding the mechanisms of interventions that have the potential to produce long-term benefits for academic achievement.

FIGURE LEGENDS

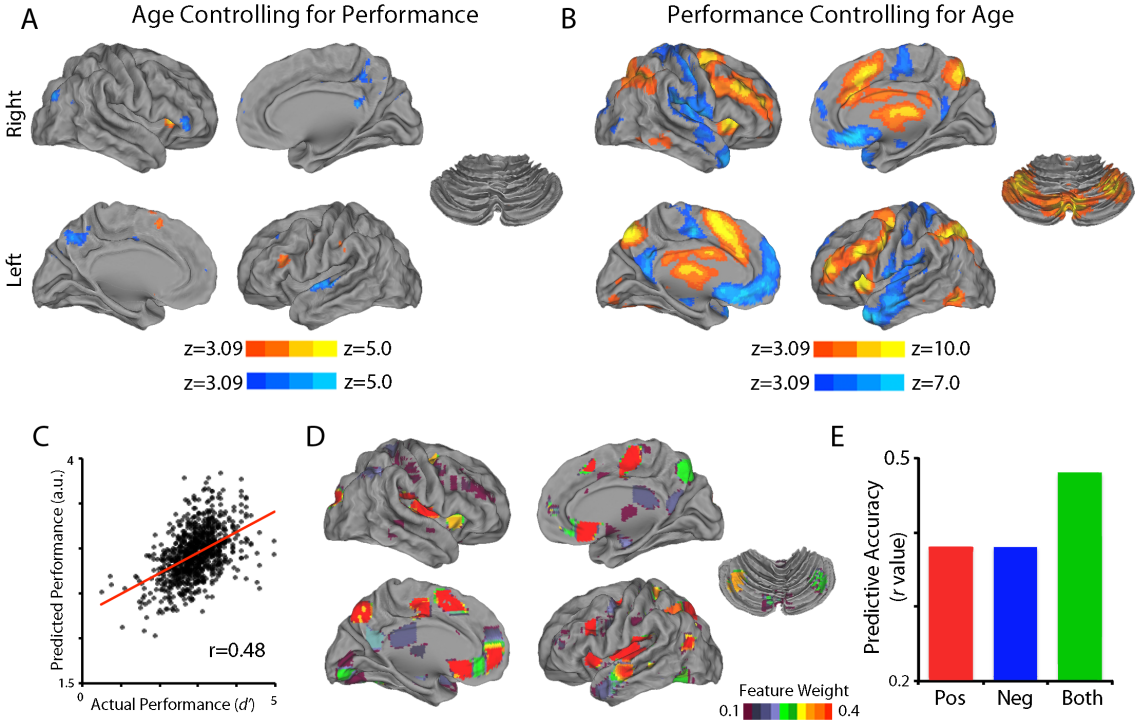

Figure 1. Brain response to WM load is more significantly related to WM performance than subject age. In a sample of 951 youths ages 8-22 imaged as part of the Philadelphia Neurodevelopmental Cohort using a fractal version of the n-back working memory task, working memory load was associated with executive activation and reciprocal de-activation of non-executive regions including the default mode network (DMN). This pattern of activation was weakly associated with chronological age (**A**), but strongly associated with WM performance (**B**). The complex multivariate pattern of brain response could be used to predict WM performance with a relatively high degree of accuracy using a cross-validated multivariate pattern regression (**C**), which included features from both the executive and default mode system (**D**). Notably, while both executive and non-executive regions could predict WM performance, maximally accurate predictions required the combined feature set which spanned both networks (**E**). All data from Satterthwaite et al., 2013.

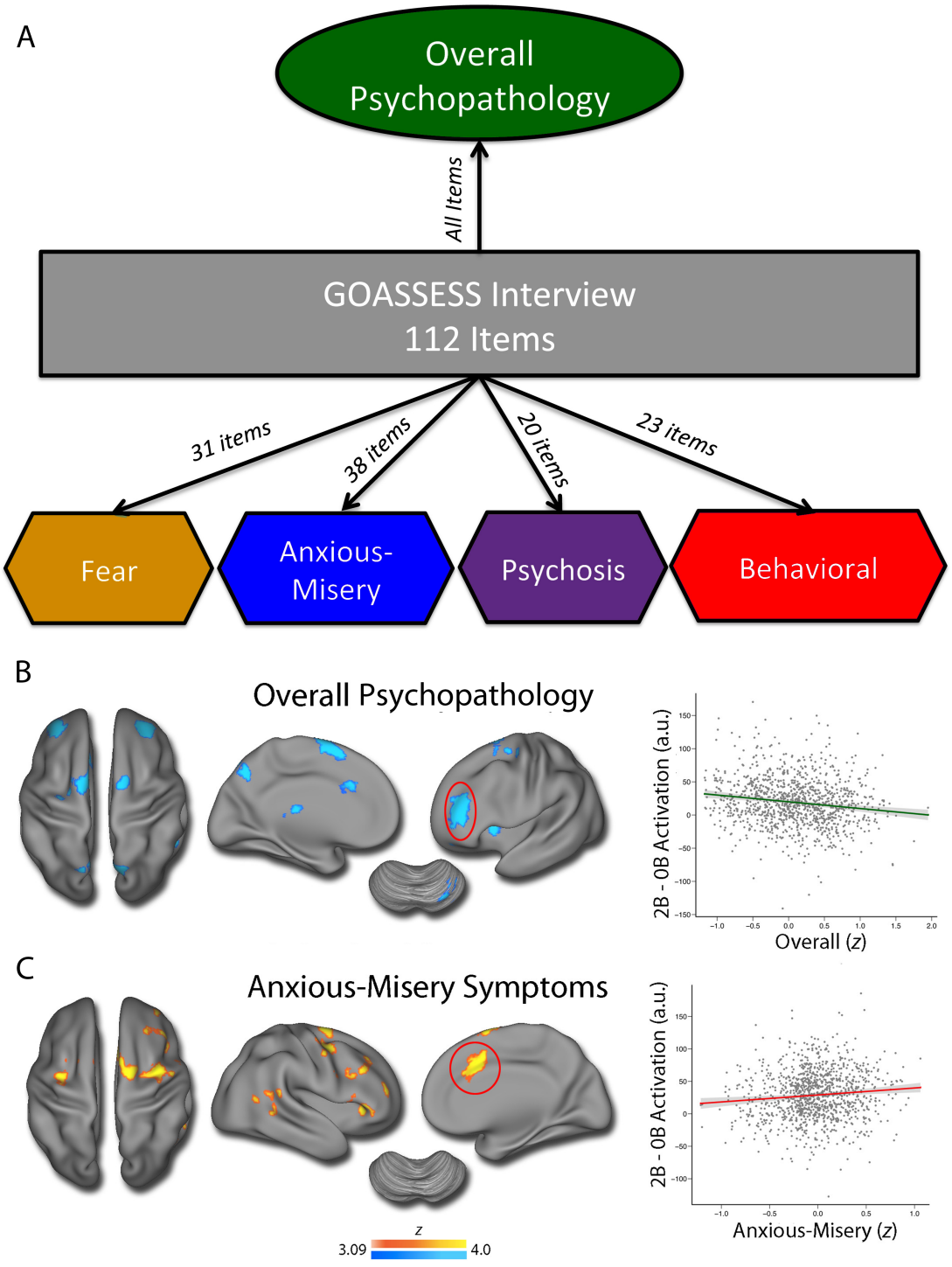

Figure 2. Psychopathology is associated with common and dissociable patterns of executive dysfunction. In a sample of 1,129 youths imaged as part of the Philadelphia Neurodevelopmental Cohort, a bifactor factor analysis identified common and divergent

dimensions of psychopathology across categorical screening diagnoses (**A**). When these dimensions of psychopathology were related to activation during a fractal version of the n-back working memory task, overall psychopathology across categorical screening diagnoses was associated with hypo-activation of the executive system (**B**), whereas anxious-misery symptoms (depression, anxiety) were associated with elevated activation of the executive system (**C**). All data from Shanmugan et al., 2016.

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