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Chronic Stress Effects on Prefrontal Cortical Structure and Function

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CHRONIC STRESS EFFECTS ON PREFRONTAL CORTICAL STRUCTURE AND FUNCTION

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Stressful life events have been implicated clinically in the pathogenesis of major depression, but the neural substrates that may account for this observation remain poorly understood. Attentional impairments symptomatic of depression are associated with structural and functional abnormalities in the prefrontal cortex. In three parallel rodent and human neuroimaging studies, this project assessed the effects of chronic stress on prefrontal cortical structure and function and the behavioral correlates of these changes.

The first study used fMRI to elucidate the precise computational contributions of frontoparietal circuitry to attentional control in human subjects, using a task that could be adapted for rats. The results confirmed that the contributions of dorsolateral frontoparietal areas to visual attentional shifts could be dissociated from the regulatory influences of more ventrolateral areas on stimulus/response mappings, in a manner consistent with studies in animal models. They also indicated that anterior cingulate and posterior parietal cortex may act in concert to detect dissociable forms of information processing conflicts and signal to dorsolateral prefrontal cortex the need for increased attentional control. Stress-induced alterations

in these regions and in the connections between them may therefore contribute to attentional impairments.

The second study tested this hypothesis in rats by examining whether chronic stress effects on medial prefrontal (mPFC) and orbitofrontal (OFC) dendritic morphology underlie impairments in the behaviors that they subserve. Chronic stress induced a selective impairment in attentional control and a corresponding retraction of apical dendritic arbors in mPFC. By contrast, stress did not adversely affect reversal learning or OFC dendritic arborization. These results suggest that prefrontal dendritic remodeling may underlie the attentional deficits that are symptomatic of stress-related mental illness.

The third study was designed to extend these findings to human subjects, using the techniques developed in Study 1. Accordingly, chronic stress predicted selective attentional impairments and alterations in prefrontal functional coupling that were reversible after four weeks. Together, these studies outline in broad strokes a mechanistic model by which chronic stress may predispose susceptible persons to the attentional impairments that are characteristic of major depression. Future studies will assess the roles of serotonin and neurotrophins in mediating these changes.

For A.L. and B.L.

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CHAPTER 1:

Introduction

As early as the fourth century BC, Hippocrates described a syndrome characterized by "aversion to food, despondency, sleeplessness, irritability, and restlessness". Hippocrates believed that *melancholia*—unipolar major depression in modern parlance—was attributable to a pathologic excess of "black bile", one of the four bodily humours that determined health and disease in Hippocratic medicine (Adams, 1891). Accordingly, therapy comprised a strict regimen of dietary changes, blood-letting, purging, and emesis, all aimed at restoring humoural balance. Perhaps not surprisingly, efficacious treatments for major depression are not to be counted among the formidable accomplishments of history's most famous physician.

Of course, this fact probably mattered little to Hippocrates and his contemporaries; ancient Greek doctors had more pressing concerns. Average life expectancy for someone born in Greece in the time of Hippocrates was 28 years, a number that is thought to have increased only marginally over most of the past 100,000 years of human existence (Caspari and Lee, 2004). Not until the early 19th century—when public sanitation, antibiotics, and access to the most rudimentary medical care vastly diminished the death tolls of infectious disease, malnutrition, and infant mortality—did average life expectancies begin to increase appreciably. As life expectancies evolved, so too did the causes of morbidity and mortality.

Today, few Westerners will die from tuberculosis or dysentery or malnutrition. Instead, they must cope with the stresses of more chronic cardiovascular, metabolic, rheumatic, and psychiatric illnesses (Murray and Lopez, 1997b; 1997c; 1997d). Ironically, mankind through its ingenuity has eliminated the scourges that have plagued it for millenia, only to see them replaced with slower, more insidious forms of suffering. Mother Nature, it seems, has a morbid sense of humor.

The major causes of morbidity and mortality in developed countries today are diseases that develop slowly but steadily over time, diseases that are thought to be the products, in part, of a lifetime's accumulation of environmental insults. In this view, the autonomic nervous system and hypothalamic-pituitary-adrenal axis work together to achieve allostasis—stability through carefully tuned changes aimed at coping with a stressor. In the short term, these compensations are adaptive. In states of chronic stress, however, allostatic regulatory mechanisms may have deleterious consequences for health in nearly every organ system, a phenomenon known as allostatic load (McEwen and Stellar, 1993; McEwen, 1998). A growing body of work indicates that chronic stress and allostatic load contribute to seven of the ten leading causes of disability in developed countries (Murray and Lopez, 1997a; 1997b; 1997d; McEwen, 1998).

Propelled by an ever-improving understanding of their pathology and pathophysiology, medical advances over the past half-century have revolutionized the treatment of chronic cardiovascular, metabolic, and rheumatic conditions, yielding more effective drugs targeted specifically at relevant pathologies, thereby generating minimal side effects. Likewise, a growing awareness of the role of stress as a risk factor has facilitated efforts at prevention. Regrettably, a few noteworthy exceptions notwithstanding, efforts to treat stress-related psychiatric diseases medically have progressed slowly (Nestler et al., 2002; Berton and Nestler, 2006). This failure is all the more disconcerting in light of the fact that unipolar major depression is expected to become the second leading cause of disability worldwide by 2020, with a disease burden exceeding those of tuberculosis, cholera, and dysentery combined (Murray and Lopez, 1997a; Murray and Lopez, 1997c).

While reasonable people might disagree on the relative merits of a sudden death at 33 versus a prolonged, disease-addled decline, these statistics point to at least one inescapable conclusion: society will be well served by efforts to ameliorate the impact of chronic diseases in general, and of depression in particular. Initiatives to devise new treatments for depression and other stress-related psychiatric conditions have relied largely on trial-and-error, as our cursory understanding of their neural substrates has

hindered more theory-driven efforts (Nestler et al., 2002; Berton and Nestler, 2006). By comparison, the cellular and molecular mechanisms by which the brain mediates allostasis acutely and adapts to states of chronic stress have been explicated in considerable detail. Associations between stress and psychiatric illnesses—particularly disorders of affect and anxiety—might therefore be exploited to facilitate an understanding of the pathology of these diseases at a level of analysis not readily amenable to other approaches.

To this end, this project was an effort to explore the association between chronic stress and the neural substrates of stress-related affective and anxiety disorders in a series of parallel rodent and human neuroimaging studies. By necessity, it was limited in scope. Building on work delineating the brain's response to stress, particularly in the hippocampus and amygdala, this project examined the effects of chronic stress on particular structural and functional properties of the prefrontal cortex, a region of the brain believed to play a critical role in depression and anxiety.

The remainder of this chapter is devoted to a brief review of selected studies that motivate the work reported here. This includes a discussion of 1) the brain's role in initiating the stress response; 2) structural and functional changes associated with states of chronic stress, particularly in the hippocampus, amygdala, and prefrontal cortex; 3) the behavioral and clinical

correlates of these changes; and 4) the mechanisms by which they are mediated. More detailed reviews are available elsewhere and are noted when relevant.

Neurocircuitry of the Stress Response

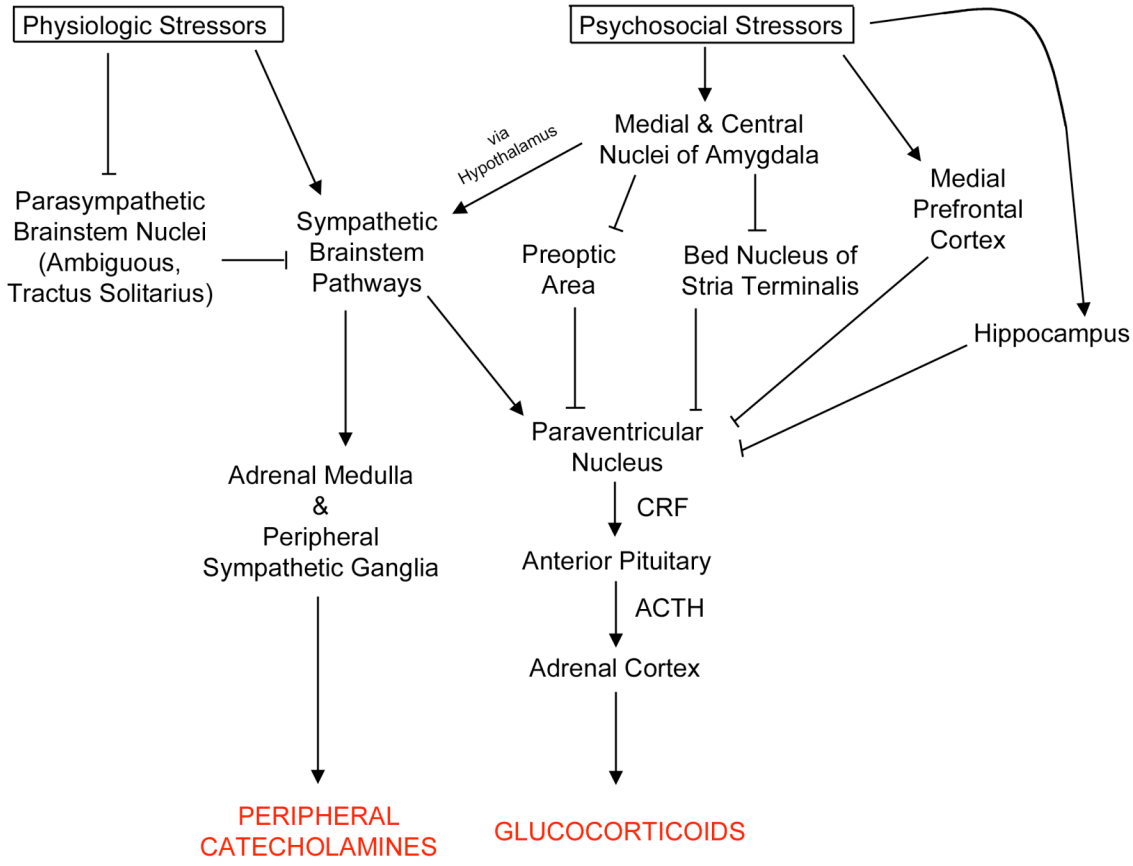
Hans Selye, who pioneered the study of the stress response, recognized the importance of resolving semantic ambiguities inherent in the study of a concept like stress. "Everybody knows what stress is," Selye wrote, "and nobody knows what it is" (Selye, 1973). Following the publication of Selye's landmark 1936 study of the physiological consequences of acute stress (Selye, 1936), biologists have adopted his definition of a stressor as any condition that threatens homeostasis in the organism. Selye and others have identified two unique classes of stressors. Systemic or physiologic stressors, like hemorrhage, infection, or anoxia, are those that pose a direct challenge to homeostasis (Selye, 1936; Sapolsky, 2005). Processive or psychosocial stressors are defined principally by cognitive or affective components and reflect an anticipation of a looming challenge to homeostasis (Selye, 1936; Sapolsky, 2005).

Stressors of both classes elicit a stereotyped set of neural and hormonal responses—the stress response—aimed at maintaining

homeostasis via two distinct pathways (Figure 1.1). Diverse physiologic stressors, including hemorrhage, hypotension, respiratory distress, and immune challenge, stimulate catecholaminergic brainstem pathways directly (Herman and Cullinan, 1997). Systemic sympathetic hyperactivity is enhanced by a relative inhibition of parasympathetic outputs, particularly from the nucleus ambiguous and the nucleus of the tractus solitarius, among others (Thayer and Brosschot, 2005). Additionally, sympathetic brainstem pathways project to cell bodies in the hypothalamic paraventricular nucleus (PVN), stimulating release of corticotropin-releasing factor (CRF) into the pituitary portal circulation. CRF, in turn, induces the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary, leading finally to glucocorticoid synthesis and secretion from the adrenal cortex (Herman and Cullinan, 1997).

Psychosocial stressors, by contrast, require cortical processing, which is mediated by inhibitory and excitatory limbic forebrain circuits. Negative feedback is mediated by the hippocampus, via direct projections to the PVN (Herman and Cullinan, 1997), and by the medial prefrontal cortex (mPFC) via direct inputs to the PVN and via GABAergic interneurons, suggesting a role for this structure in the "unlearning" of stress-related associative memories as they lose their predictive value (Diorio et al., 1993; Quirk et al.,

Figure 1.1: Neurocircuitry of the Stress Response. Both physiologic and psychosocial stressors act to stimulate peripheral catecholamine release and glucocorticoid synthesis. The former do so through direct stimulation of sympathetic brain stem pathways. The latter act via positive feedback loops originating in the amygdala and negative feedback loops originating in the hippocampus and medial prefrontal cortex. See text for details.



2000; Milad and Quirk, 2002; Quirk et al., 2003). Stress-provoking stimuli are processed by cells of the medial and central nuclei of the amygdala, which stimulate CRF release by inhibiting GABAergic projections from the preoptic area and the bed nucleus of stria terminalis to the PVN. Amygdaloid neurons also stimulate brainstem catecholamine release via direct projections to the hypothalamus. Thus, both pathways terminate in sympathetic nervous system excitation and activation of the hypothalamic-pituitary-adrenal (HPA) axis (Herman and Cullinan, 1997; McEwen, 1998; Joels et al., 2006).

Together, the autonomic nervous system, operating on a timescale of seconds to minutes, and the HPA axis, operating on a timescale of minutes to hours, engender a multisystem allostatic response aimed at mobilizing and conserving limited resources to cope with imminent threats to survival. Glucocorticoids mobilize blood sugar, for example, by stimulating hepatic gluconeogenesis, releasing free fatty acids from adipose tissue, and accelerating protein catabolism (McEwen, 1998; Sapolsky et al., 2000). They also inhibit costly anabolic processes such as lymphocyte proliferation, tumor surveillance, inflammation, and gametogenesis (McEwen, 1998; Sapolsky et al., 2000).

In the short term, these responses are adaptive. However, in states of chronic stress, especially psychosocial stress, the neuroendocrine response can have deleterious health consequences that outweigh short-term benefits and that collectively constitute allostatic load. Accordingly, chronic stress is associated with a variety of long-term immunosuppressive effects and an increased risk for coronary artery disease, hypertension, atherosclerosis, and diabetes (Schnall et al., 1992; Bosma et al., 1997; Everson et al., 1997; Seeman et al., 1997; McEwen, 1998).

Some evidence suggests that this association may be mediated by autonomic imbalance, as heart rate variability, a measure of autonomic balance, appears to be a marker for stress-related disease. Resting heart rate is a factor of both sympathetic inputs, which tend to increase heart rate, and parasympathetic inputs, which tend to reduce it, and it has been shown that variability in the heart rate time series (HRV) depends critically on parasympathetic vagal inputs acting on a millisecond timescale, because the sympathetic influence on heart rate acts too slowly to produce beat-to-beat changes (Saul, 1990; Thayer and Brosschot, 2005). Accordingly, HRV tends to decrease in states of autonomic imbalance that are characterized by hypersympathetic activity, as observed in chronic stress. Decreased HRV, in turn, predicts increased risk of all-cause mortality (Thayer and Brosschot,

2005) and has been associated with several markers of stress-related disease, including increased fasting glucose, increased hemoglobin A1c, and excess proinflammatory cytokines (Ershler and Keller, 2000; Kiecolt-Glaser et al., 2002; Thayer and Brosschot, 2005).

Stressful life events have also been implicated clinically not only in metabolic dysregulation but also in several psychiatric conditions, including major depression, post-traumatic stress disorder, anxiety disorders, and schizophrenia (Sapolsky, 1996; Heim et al., 1997a; b). Links to psychiatric disease may be related in part to the set of cortical and subcortical structural and functional adaptations that are associated with states of chronic stress. These are discussed in the section that follows, with an emphasis on changes in the limbic forebrain circuits known to regulate the HPA response to the psychosocial stressors that are the focus of this project.

Chronic Stress in the Frontolimbic Forebrain: Structural and Functional Alterations

Chronic stress in rodents induces a well-defined set of structural and functional alterations in the hippocampus, amygdala, and medial prefrontal cortex, three key regulators of the HPA response to psychosocial stressors. In the rodent hippocampus, which provides negative feedback to the HPA

via inputs to the PVN (Herman and Cullinan, 1997), repeated restraint stress induces a selective reduction in apical dendritic material and apical dendritic debranching in CA3 pyramidal cells, while sparing basal dendrites (Watanabe et al., 1992). Remodeling of apical dendritic arbors is associated with impaired long-term potentiation (Pavlides et al., 1993) and a variety of learning deficits, discussed in the following section.

Other forms of stress induce hippocampal atrophy as well. Chronic psychosocial stress, for example, causes apical dendritic atrophy in hippocampal CA3 pyramidal cells (Magarinos et al., 1996) and decreased neurogenesis in the dentate gyrus in subordinate tree shrews (Gould et al., 1997) and marmoset monkeys (Gould et al., 1998). Magnetic resonance imaging studies have extended these findings to human populations, demonstrating that normal age-related increases in glucocorticoids predict decreased hippocampal volumes (Lupien et al., 1998) and that chronic stress-related psychiatric conditions such as post-traumatic stress disorder (PTSD) and recurrent major depression are associated with hippocampal atrophy (Bremner et al., 1995; Sheline et al., 1996; Bremner et al., 1997; Sheline et al., 1999). If the stressor is short-lived, stress-induced impairments in dendritic arborization and neurogenesis are reversible and may serve a neuroprotective function by limiting glutamatergic

excitotoxicity (McEwen, 1999; 2003). However, chronic stress over a period of months or years can induce irreversible dendritic atrophy and eventually cell death (Uno et al., 1989).

In the amygdala, which provides positive feedback to the HPA by diminishing GABAergic tone in the PVN (Herman and Cullinan, 1997), repeated restraint stress has a proliferative effect that contrasts with changes in the hippocampus. In rats, this includes increased apical dendritic material and enhanced apical branching in the basolateral amygdala (BLA) and the related bed nucleus of the stria terminalis (Vyas et al., 2002; Vyas et al., 2003). Stress also increases apical spine density in BLA pyramidal-like cells, an effect that precedes changes in dendritic arborization (Mitra et al., 2005).

Although less thoroughly studied, several recent reports have examined the effects of stress on the structure and function of the medial prefrontal cortex (mPFC), which provides negative feedback to the HPA axis (Diorio et al., 1993). These reports show that the mPFC responds to stress in a manner comparable to the hippocampus, consistent with their similar roles in HPA axis regulation. In the rodent dorsal mPFC, including the anterior cingulate and prelimbic cortices, repeated restraint stress induces apical dendritic atrophy and debranching (Cook and Wellman, 2004; Radley

et al., 2004), effects that are reversible after four weeks of rest (Radley et al., 2005). Similar morphologic changes have been reported in the ventral infralimbic region of mPFC (Izquierdo et al., 2006), coupled with impaired long-term potentiation in amygdaloid projections to this area (Maroun and Richter-Levin, 2003). Interestingly, the mPFC may be more sensitive to mild stressors than the hippocampus, as exposure to just two hours of immobilization stress is sufficient to induce dendritic remodeling in the mPFC that persists for at least ten days (Miller et al., 2005; Izquierdo et al., 2006). The behavioral correlates of this heightened plasticity and their relevance for stress-related psychiatric disease are discussed below.

Chronic Stress in the Frontolimbic Forebrain: Behavioral and Clinical Correlates

A growing body of literature demonstrates that chronic stress induces a pattern of behavioral effects consistent with the atrophy of dendritic arbors observed in the hippocampus and mPFC and the proliferation observed in the amygdala, thereby confirming the functional significance of these morphologic alterations. Early work in this field focused on the hippocampus, whose contributions to spatial learning and memory in rodents and declarative memory in human subjects are well established. Using a

variety of stress paradigms and behavioral assays, these studies demonstrated that stress impairs hippocampus-dependent memory. In rats, for example, 21 days of repeated restraint stress impairs acquisition learning in an eight-arm radial maze (Luine et al., 1994) and short-term retention of spatial recognition memory in a Y-maze task (Conrad et al., 1996). Repeated corticosterone treatment induces analogous spatial learning deficits as measured by the Morris water maze (Bodnoff et al., 1995), as does chronic psychosocial stress in tree shrews (Ohl and Fuchs, 1999).

The link between stress and impaired memory is strengthened further by studies of aged rats, in which sympathetic and HPA activity fail to return efficiently to baseline after a stressor. Prolonged glucocorticoid exposure leads to hippocampal atrophy and impaired spatial memory with age but not in a subset of aged rats in which basal glucocorticoids were unelevated relative to younger rats (Issa et al., 1990). This effect may occur independently of impairments in neurogenesis (Rapp and Gallagher, 1996), lending tentative support to the hypothesis that dendritic atrophy alone may be sufficient to impair cognitive function.

Stress does not impair learning universally, however. On the contrary, repeated stress enhances cue-based fear conditioning (Shors et al., 1992), in which the acquisition and retention of conditioning depends on the

integrity of the amygdala but not the hippocampus (Phillips and Ledoux, 1992; LeDoux, 2000). This double dissociation, whereby stress impairs hippocampus-dependent learning while enhancing amygdala-dependent learning, extends to other aspects of amygdaloid function. In addition to promoting fear conditioning, stress potentiates the expression of anxiety-related behaviors that have been linked to amygdala hyperactivity. Stress-induced increases in dendritic arborization in the basolateral amygdala, for example, have been linked to reduced exploratory activity in the open arm of an elevated plus maze (Vyas et al., 2002). Strikingly, gradual increases in dendritic spine density in the BLA following a single acute immobilization stress are associated with gradual increases in anxiety behavior (Mitra et al., 2005), further underscoring the sensitivity of limbic forebrain structures to stress and the behavioral significance of the changes it invokes.

Together, these studies indicate that a variety of stressors induce a common set of morphologic alterations in the hippocampus and amygdala, which in turn are associated with predictable behavioral effects, across a range of species. A complementary body of literature suggests that comparable effects may occur in healthy human subjects, and these effects may have some relevance for stress-related psychiatric diseases. One source of evidence comes from neuroimaging studies of normal aging, analogous to

those described in animal models above. Like aged rats, aged humans exhibit prolonged elevations in cortisol, which may be attributable to HPA axis dysregulation following a stressor; deficits on an array of hippocampus-dependent memory tasks relative to young adults; and reductions in hippocampal volume that correlate with estimates of cumulative cortisol exposure (Lupien et al., 1998).

Intriguingly, this model of normal aging captures at least two features of major depression and several other stress-related psychiatric conditions. These disorders often feature prominent increases in basal cortisol levels and circadian cortisol dysregulation, accompanied by reductions in hippocampal volume. This has been observed in both major depression (Sheline et al., 1996), where the duration of disease predicts the degree of hippocampal volume loss independent of age (Sheline et al., 1999), and post-traumatic stress disorder (Bremner et al., 1995; Bremner et al., 1997). Both conditions also feature enhanced amygdala reactivity (Drevets et al., 1992; Rauch et al., 2000), a finding consistent with the animal models of stress discussed above.

Of course, these studies do not demonstrate a causative link between depression or PTSD and stress-related structural changes in the hippocampus and amygdala. It remains unclear whether hippocampal volume reductions precede the onset of disease or are simply symptomatic of HPA axis

dysregulation secondary to more fundamental pathologies. However, at least one report provides evidence to support the former hypothesis (Gilbertson et al., 2002). This study compared hippocampal volumes in a cohort of healthy combat veterans with those of combat veterans who developed PTSD and their identical twins, who never went to war. In accord with previous reports, the PTSD patients showed significant reductions in left hippocampal volume. Importantly, they found that the PTSD victims' identical twins also showed reduced hippocampal volumes relative to the healthy controls, even though the twins had no history of PTSD. Important issues beyond the scope of this discussion remain unresolved,[1] but this report provides evidence to suggest that decreased hippocampal volume, whatever the cause, may predispose vulnerable individuals to stress-related psychiatric conditions.

Although early work focused on the role of the hippocampus and amygdala in the pathogenesis of these diseases, more recent work suggests

[1] Sapolsky (2002) notes that this association is probably more complicated than might be inferred from the report by Gilbertson and colleagues (2002). Their results suggest that genetic factors may predispose some people to PTSD by reducing the inhibitory influences of the hippocampus on the HPA axis, but this interpretation is not easily reconciled with conflicting reports of higher than normal serum cortisol in some PTSD patients versus lower than normal levels in others. It is likely that diverse factors including the type of trauma and the timecourse of the disease interact with hippocampal structural differences to modulate glucocorticoid profiles. See Sapolsky (2002) for further discussion.

that stress-induced alterations in prefrontal cortical structure and function may be equally important, particularly in the case of mood disorders. Indeed, the principal symptomatic features of mood disorders are not fear, anxiety, and heightened stress reactivity, but rather deficits in affective and attentional regulation (APA, 1994), which in turn are well-established functions of the prefrontal cortex (Desimone and Duncan, 1995; Davidson, 1998; Casey et al., 2000; Davidson et al., 2000; Miller and Cohen, 2001; Casey et al., 2002; Ochsner and Gross, 2005). Unsurprisingly, then, mood disorders feature functional alterations not just in the hippocampus and amygdala, but also in a frontoparietal network of structures that includes lateral prefrontal, anterior cingulate, and posterior parietal regions (Davidson et al., 2002).

Findings of altered activity in subgenual cingulate cortex have received special attention by virtue of this region's contributions to affective and autonomic regulation (Drevets et al., 1997; Mayberg et al., 1999). Accordingly, a recent clinical trial demonstrated that electrical stimulation of subgenual cingulate white matter effectively cured four of six patients with unipolar depression refractory to all other therapeutic modalities (Mayberg et al., 2005). The mechanisms by which deep brain stimulation may ameliorate the symptoms of depression remain unclear, however, and the

risks inherent in intracranial neurological surgery mandate that this intervention be reserved for only the most recalcitrant cases. Current medical treatments are also unsatisfactory, due to their unreliable efficacies and their frequently unfavorable side effect profiles (Nestler et al., 2002; Berton and Nestler, 2006), which in turn are attributable in large part to our rudimentary understanding of the underlying pathology. A more mechanistic understanding of the links between stress, structural and functional alterations in prefrontal cortex, and the cognitive and affective symptoms of mood disorders might therefore inform some of these pressing questions.

Regrettably, relatively few studies have examined how chronic stress-related changes at the level of the cell might modulate PFC-dependent behaviors. (Note that the issue of how stressors affect prefrontal cortical function acutely is a separate one, discussed in more detail in Chapter 4.) Mizoguchi and colleagues (2000) demonstrated that chronic unpredictable stress impairs performance on an mPFC-dependent spatial working memory task in rats (Vanhaaren et al., 1985), and that this impairment is reversed by administration of a dopamine D1 receptor-specific agonist. It is unclear whether this effect reflects changes in dopaminergic projections to mPFC or local alterations in dopamine receptor availability as might be predicted by

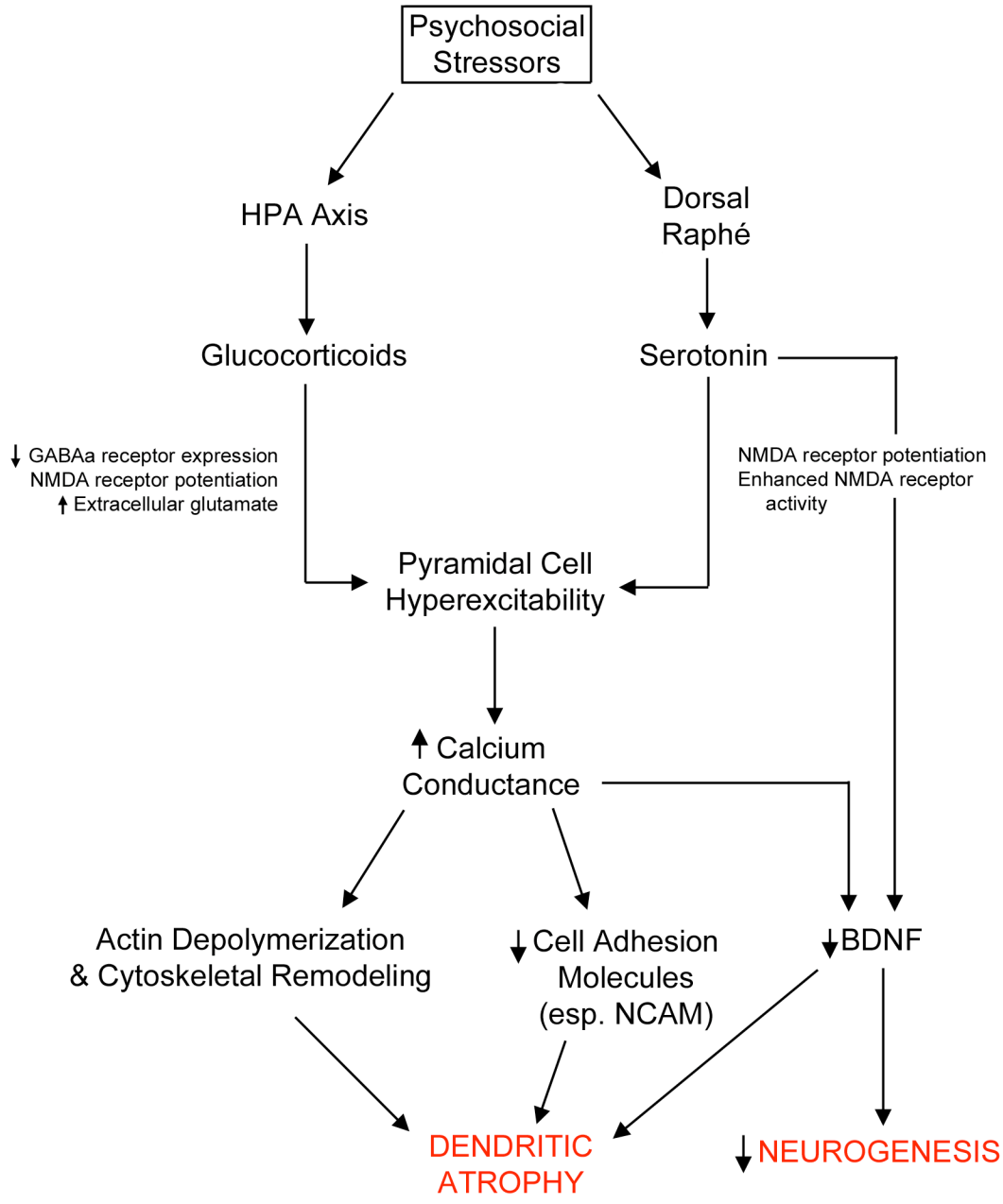
previous reports of dendritic atrophy and spine loss in this region (Radley et al., 2004; Radley et al., 2006). In either case, this finding highlights the contributions of hypodopaminergic tone to stress-related working memory impairments. Other groups have reported that repeated restraint stress modulates the acquisition and retention of conditioned fear extinction (Izquierdo et al., 2006; Miracle et al., 2006), which may be related to stress-induced changes in medial prefrontal structural and electrophysiologic properties (Milad and Quirk, 2002; Quirk et al., 2003; Lebron et al., 2004; Santini et al., 2004; Liston et al., 2006). While these reports represent important contributions to our understanding of stress-related fear and anxiety, their relevance for mood disorders is less clear.

Thus, the goal of this project was to examine the effects of stress on prefrontal cortical structure and function in an effort to contribute to a mechanistic understanding of the association between stress and one of the core features of mood disorders, namely impaired attentional control. With this in mind, this introduction concludes with a brief review of the mechanisms by which stress mediates morphologic changes in the limbic forebrain, followed by an explication of the project's specific aims.

Chronic Stress in the Frontolimbic Forebrain: Mechanisms of Action

The mechanisms by which repeated stress mediates dendritic remodeling in the hippocampus, amygdala, and prefrontal cortex are the subject of a rapidly growing area of research. A variety of studies, most of which focus on the hippocampus, point collectively to a complex interaction between glucocorticoids, excitatory neurotransmission, the serotonergic neuromodulatory system, and ultimately altered expression of neurotrophins and cell adhesions molecules (Figure 1.2). As described above, psychosocial stressors—particularly uncontrollable psychosocial stressors—induce the release of glucocorticoids, via activation of the HPA axis, and serotonin, via direct stimulation of the midbrain dorsal raphé nucleus (Herman and Cullinan, 1997; McEwen, 1998; Amat et al., 2005). Glucocorticoids, in turn, promote excitatory neurotransmission in the CA3 region of the hippocampus by reducing GABAa receptor mRNA expression, potentiating NMDA receptor binding, and increasing the availability of extracellular glutamate (McEwen, 1999). Excitatory activity in the CA3 is a prerequisite for dendritic remodeling, as administration of a selective

Figure 1.2: Mechanisms of Hippocampal Structural Remodeling. Chronic psychosocial stress mediates dendritic remodeling and impairs hippocampal neurogenesis via a complex series of interactions initiated by glucocorticoids and serotonin and terminating in cytoskeletal remodeling and altered expression of cell adhesion molecules (e.g. NCAM) and neurotrophins (e.g. BDNF). See text for details.



NMDA receptor antagonist, the antiepileptic drug phenytoin, or the benzodiazepine adinazolam is sufficient to block stress-induced remodeling (Magarinos et al., 1999; McEwen, 1999; 2000).

Serotonin is believed to play an essential role as well, since dendritic remodeling and spatial learning deficits are prevented by tianepine, an atypical antidepressant that promotes presynaptic serotonin reuptake, but not by selective serotonin reuptake inhibitors like fluoxetine and fluvoxamine (Luine et al., 1994; Conrad et al., 1996; Magarinos et al., 1999). Serotonin may contribute to remodeling in part by potentiating NMDA receptor binding by glutamate and by enhancing the activity of NMDA receptors via a $5HT_2$-mediated pathway (Mennini and Miari, 1991; Rahmann and Neumann, 1993).

Thus, glucocorticoids and serotonin are thought to act in concert to enhance excitatory neurotransmission and voltage-gated calcium conductances, which in turn may trigger dendritic atrophy directly, by promoting actin depolymerization and cytoskeletal remodeling, or indirectly, via downstream transcriptional regulation and post-translational modification of cell adhesion molecules (McEwen and Sapolsky, 1995; McEwen, 2000; Cambon et al., 2004; Sandi, 2004). Downstream effects on brain-derived neurotrophic factor (BDNF) may also play a role, as both

repeated immobilization stress (Smith et al., 1995) and chronic corticosterone treatment (Ueyama et al., 1997) have been shown to decrease BDNF expression in the hippocampus, though this effect may be more relevant for impairments in neurogenesis in the dentate gyrus than for dendritic remodeling in the CA3, per se (Kuroda and McEwen, 1998).

These mechanistic details are particularly informative in light of a series of recent studies that implicate common genetic polymorphisms in the association between stress and mood disorders. Lesch and colleagues (1996) identified a simple repeat sequence in the promoter for the serotonin reuptake transporter (5HTT) gene (SLC6A4), located upstream of the coding sequence on chromosome 17q12, and reported that expression of the 5HT transporter and 5HT reuptake in lymphoblasts varied with the number of repeats in the promoter: the short variant of the promoter reduced transcriptional efficiency. In a study of 505 individuals, they demonstrated that carriers of the short allele were more likely to express anxiety-related personality traits. In their study, this polymorphism was found to account for 3-4% of total variance and 7-9% of inherited variance in these personality traits—a small but statistically significant effect, suggesting that 5HTT polymorphism status may modulate individuals' risk for developing anxiety disorders and implicating 5HT neurotransmission in anxiety in

human subjects, though it should be noted that efforts to replicate this finding have yielded inconsistent results (Ohara et al., 1998; Deary et al., 1999; Flory et al., 1999).

Subsequently, Caspi and colleagues (2003) demonstrated that 5HTT polymorphism status may predispose susceptible individuals to psychiatric conditions by modulating the maladaptive effects of chronic stress, suggesting that inconsistent replications may be attributable in part to a previously unidentified interaction between genotype and environment. In a prospective longitudinal study of 1037 individuals, they found that accumulation of stressful life events predicted an increased probability of developing symptoms characteristic of depression, and that this effect was much more pronounced in carriers of the short allele: whereas there was essentially no relation between stress and depression in individuals homozygous for the long allele, carriers of the short allele were much more likely to suffer from depression following major life stressors.

A single nucleotide polymorphism in the BDNF gene, common in human populations, may also modulate stress responsivity in the hippocampus. This polymorphism leads to a valine to methionine substitution at codon 66 (Val66Met) in the prodomain of the BDNF peptide. The BDNF gene encodes a precursor peptide, which is proteolytically

cleaved and packaged into secretory vesicles in a process that appears to involve interactions with sortilin, a sorting protein (Chen et al., 2005). The Met substitution is thought to hinder interactions with sortilin; accordingly, the Met allele is associated with reduced depolarization-induced BDNF secretion, decreased localization to secretory granules and synapses, altered hippocampal activity patterns, and episodic memory deficits (Egan et al., 2003; Chen et al., 2004). The Met allele has also been linked to an increased risk for depression and anxiety disorders (Jiang et al., 2005; Kaufman et al., 2006). Some studies have failed to replicate these results (Sen et al., 2003; Lang et al., 2005), though none have examined how stress may interact with these effects, so this confound may account in part for these inconsistencies.

A few caveats should be noted here. As described above, efforts to replicate some of these results have been inconsistent. Furthermore, it remains unclear whether these findings are germane to structures outside the hippocampus. However, at least two studies suggest that they are. Using functional MRI, Hariri and colleagues (2002) demonstrated that 5HTT polymorphism status modulates the responsiveness of anxiety-related neural circuits to fearful stimuli. Subjects viewed images of angry and fearful faces and were required to make judgments about the affective content of the images, a paradigm known to engage the amygdala reliably. Carriers of the

short allele exhibited greater amygdala activity in response to the fearful faces, as indexed by fMRI, relative to individuals homozygous for the long allele. Likewise, Pezawas and colleagues (2005) have reported that carriers of the short allele exhibit a functional decoupling of amygdala and ventromedial prefrontal activity as indexed by fMRI while viewing a similar set of angry and fearful faces. Although neither study assessed whether stress modulated the observed effects, these results, in conjunction with findings from animal models described above, suggest that stress-induced 5HT release may exacerbate dendritic remodeling and the cognitive and affective impairments that are characteristic of chronic stress.

Summary and Specific Aims

Collectively, the studies reviewed here show that stress induces a characteristic set of morphologic and electrophysiologic changes in the frontolimbic forebrain in animal models; that comparable morphologic changes are observed in normal aging and in a variety of stress-related psychiatric conditions in human subjects; that these changes are associated with cognitive and affective impairments linked to hippocampus and mPFC dysfunction and enhanced expression of amygdala-dependent behaviors; and that common polymorphisms in the 5HTT and BDNF genes may warrant

particular interest for human populations, by virtue of their involvement in regulating these changes. They also point to the special relevance of prefrontal cortical alterations for mood disorders and highlight the need for further study in this area.

Thus, the goal of this project was to examine the effects of chronic stress on structural and functional properties of the prefrontal cortex and the behavioral correlates of these changes, with a special focus on the cognitive control of attention. Parallel rodent and human neuroimaging studies, employing cell morphometric techniques, behavioral assays, and a variety of functional neuroimaging analytic tools, were designed to address three specific aims:

1. ***To characterize in greater detail the frontoparietal circuitry that mediates the cognitive control of attention.*** Flexible attentional modulation is mediated in humans and non-human primates chiefly by lateral prefrontal cortex, anterior cingulate cortex, and posterior parietal cortex (Desimone and Duncan, 1995; Miller and Cohen, 2001; Casey et al., 2002) and in rodents by an analogous network of structures (Birrell and Brown, 2000; Fox et al., 2003; McAlonan and Brown, 2003). The computational contributions of individual components of this network were characterized in a functional neuroimaging study of healthy human

subjects, using a cognitive task designed to capture the principal features of a comparable task suitable for use in animal models (Dias et al., 1996; Birrell and Brown, 2000). The results of this work, in turn, informed subsequent efforts to understand the association between stress-related attentional effects and the structural and functional alterations observed in this circuit.

2. ***To investigate in a rodent model how stress modulates structural and functional properties of the frontoparietal circuit characterized in Aim 1.*** Building on earlier investigations (Radley et al., 2004; Radley et al., 2006), this study examined how chronic stress affects dendritic profiles in the medial (mPFC) and lateral orbitofrontal (OFC) regions of the prefrontal cortex in rats. The same rats were then tested on a perceptual attentional set-shifting task that yields dissociable measures of reversal learning and attentional control. These behavioral capacities are known to depend on the integrity of the lateral OFC and mPFC, respectively.
3. ***To extend the results of Aim 2 to a healthy human population.*** Healthy human subjects were scanned and tested using the behavioral and neuroimaging techniques developed in Aim 1. Building on the results of the cell morphometry analyses described in Aim 2, functional connectivity analysis was used to assess whether stress modulates

functional coupling and patterns of activity in lateral prefrontal cortex and whether these changes are associated with predictable attentional effects. All subjects were requested to return for a second scanning session four weeks later, thereby facilitating within-subjects, pairwise comparisons to assess the reversibility of stress effects and to control for intersubject variability unrelated to stress.

The results of these experiments are reported in the three chapters that follow. The final chapter is devoted to a general discussion of the results, their caveats and limitations, their relevance for clinical populations, and directions for further research, including a brief discussion of preliminary results from an on-going effort to assess whether BDNF and 5HTT polymorphism status may modulate the effects of chronic stress on functional connectivity and attention.

CHAPTER 2:

Functional Characterization of Attentional Circuitry

Attentional impairments, broadly defined, feature prominently in the symptomatology of mood disorders and other stress-related psychiatric conditions. They are often accompanied by structural and functional abnormalities in a network of structures, including anterior cingulate and lateral prefrontal cortices (Cohen & Servan-Schreiber, 1992; APA, 1994; Drevets et al., 1997; Mayberg et al., 1999; Davidson, 1998; Casey et al., 2002; Davidson et al., 2002; Bishop et al., 2004). A more concrete understanding of the contribution of subcomponents of this circuitry to the cognitive control of attention is crucial for understanding the relationship between clinical symptomatology and stress-induced neuropathology in these conditions.

Converging evidence from rodent, primate, and human imaging studies implicate a network of structures including lateral prefrontal cortex, anterior cingulate cortex, and posterior parietal cortex in dissociable aspects of attentional regulation (Dias et al., 1996; Birrell & Brown, 2000; O'Reilly et al., 2002; McAlonan & Brown, 2003; Fox et al., 2003). There is a growing consensus that the primate lateral prefrontal cortex acts to support task relevant representations of stimulus information and stimulus-response mappings, thus favoring them in competitions with task-inappropriate representations in posterior cortex (Desimone & Duncan, 1995; Miller &

Cohen, 2001). Distinct regions of lateral prefrontal cortex are thought to regulate representations at different levels of abstraction (Dias et al., 1996; O'Reilly et al., 2002; Koechlin et al., 2003) and contribute differentially to enhancement versus inhibition (Casey et al., 2000).

Lesion studies lend support to this hypothesis. Dias and colleagues (1996) identified a double dissociation of attention shifting and response reversals within primate prefrontal cortex. Marmosets were trained to discriminate between visual stimuli based on different features of the stimuli. Excitotoxic lesions of lateral prefrontal cortex impaired attentional shifts, in which discrimination learning required the monkey to shift its attention to a different dimension of the stimulus. Response reversals, in which discrimination learning required the monkey to override a well-learned stimulus/response association, were unaffected. Conversely, orbitofrontal lesions impaired response reversals but not attentional shifts. Other groups extended these findings to rats, demonstrating that medial prefrontal (Birrell and Brown, 2000) and orbitofrontal (McAlonan and Brown, 2003) lesions impaired attention shifts and response reversals, respectively, but not vice versa. Subsequent work demonstrated that posterior parietal lesions in rats selectively impair attentional shifts as well (Fox et al., 2003), a finding consistent with studies in primates implicating

this region in the generation of motor plans via transformations of sensory inputs from multiple modalities (Anderson & Buneo, 2002). Posterior parietal cortex may also play a role in integrating representations of reward information in the service of perceptual decision-making (Platt & Glimcher, 1999; Gold & Shadlen, 2001).

Together, these studies demonstrate that distinct regions of prefrontal and parietal cortex play critical and dissociable roles in attentional regulation in rats and non-human primates. The goal of the study reported in this chapter was to elucidate in greater detail the precise computational contributions of these structures to attentional control in an effort to inform interpretations of stress effects on attention in subsequent chapters. One influential theory in this field, known as the conflict-monitoring hypothesis, posits that the anterior cingulate region of prefrontal cortex monitors conflicts in information processing and recruits lateral prefrontal cortex to resolve competition as needed (Botvinick et al., 2001). The conflict-monitoring hypothesis makes a variety of testable predictions, several of which have been confirmed in a series of recent experiments. These studies demonstrated that ACC activity is higher on trials associated with multiple competing responses; that DLPFC activity is increased across blocks of trials expected to require greater control; and that increased ACC activity on

a given trial predicts increased DLPFC activity and more effective behavioral regulation on the subsequent trial (Carter et al., 1998; Carter et al., 2000; MacDonald et al., 2000; Kerns et al., 2004).

The conflict-monitoring hypothesis provides a plausible account of how anterior cingulate and dorsolateral prefrontal cortices act in concert to detect conflict and implement control to resolve it, while producing testable, mechanistically specific predictions, many of which have been verified. However, recent findings complicate this model. Using variations of the Stroop task, Milham and colleagues showed that with practice, DLPFC is engaged independently of ACC, a finding at odds with the assertion that ACC acts to recruit DLPFC in this paradigm (Milham et al., 2003). They also showed that while DLPFC, posterior parietal cortex, and ACC respond to manipulations of conflict, the role of ACC is limited to conflict at the level of the response and not at the level of the stimulus representation (Milham et al., 2005), leading some to speculate that ACC may act with DLPFC to resolve conflicts, not detect them (Paus, 2001).

We reasoned that conflict should be particularly robust, and therefore more amenable to measurement, in a task-switching paradigm, in which subjects responded to either the color or the motion of a visual stimulus in a manner analogous to the attentional shifts tested in the animal models

described above (Dias et al., 1996; Birrell and Brown, 2000; Fox et al., 2003; McAlonan and Brown, 2003). The principal aims of this study were 1) to examine the role of these structures in the detection and resolution of conflicts in information processing; and 2) to dissociate the contributions of response conflict and stimulus conflict to cognitive control demands, using task switching as a tool to probe and accentuate these effects. Other studies have implicated ACC in responding to conflict by comparing trials associated with multiple incongruent stimulus-response mappings to congruent trials. Conflict in these studies was defined in cognitive rather than physiologic terms (MacDonald et al., 2000; Kerns et al., 2004). Here, we adopted a complementary approach: in accord with computational formulations (Botvinick et al., 2001), we defined conflict in terms of the product of activities in areas specialized for color or motion processing and examined how the BOLD signal in lateral prefrontal, anterior cingulate, and posterior parietal cortices varied with this measure on a trial-by-trial basis.

Experimental Procedures

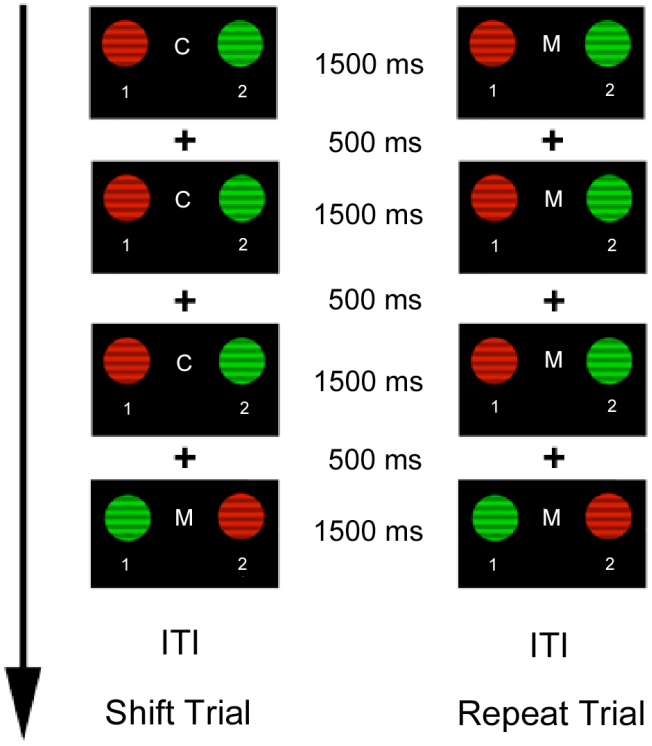
Subjects. 19 right-handed, healthy young adults (10 males) were scanned. All subjects were screened for contraindications for MRI and a history of any psychiatric or neurological conditions. The experimental procedure was

approved by the Weill Medical College of Cornell University IRB, and written informed consent was obtained from all subjects prior to scanning.

Attentional Control Paradigm. On each trial, subjects were presented with two circular square-wave gratings, one red and one green, each subtending 4.6^{O} of visual space at an eccentricity of 4.6^{O} from fixation, for 1500 ms. Each grating moved either up or down. A centrally located cue ("M" or "C") instructed the subject to attend to either the motion or the color of the stimuli. If the cue was an "M", the subject responded by choosing the side with the upward moving grating, regardless of color. If the cue was a "C", the subject responded by choosing the side with the red grating, regardless of motion (Figure 2.1). Repeat trials were defined as those preceded by 2-5 trials of the same dimension. Shift trials were those preceded by 2-5 trials of the opposite dimension. Trials also varied with manipulations of response conflict and stimulus conflict. In a low response conflict trial, the red grating was also the upward moving grating so the correct response was the same in both dimensions. In a high response conflict trial, the red grating was downward moving, and the green grating was upward moving so the correct response depended on the cue (Figure 2.2B). Stimulus conflict was parametrically manipulated by adjusting the color saturation on motion trials

Figure 2.1: Attentional Control Paradigm.

The task cue and square-wave stimuli were presented simultaneously for 1500 ms followed by a 500-ms fixation cross. Repeat trials were preceded by 2-5 trials of the same dimension. Shift trials were preceded by 2-5 trials of the opposing dimension. Shift trials and repeat trials were followed by a variable intertrial interval of 0.5-12.5 seconds (mean 5.0 seconds).



and the square-wave contrast on color trials, yielding three levels of conflict (low, medium, high; see Figure 2.2C) that varied with the salience of the irrelevant dimension, consistent with psychophysical studies of motion detection and color discrimination (Campbell & Maffei, 1980). Each trial ended with a centrally located white fixation cross, subtending 1.2^{O} of visual space, with a variable duration (500-12,500 ms). Reaction times and accuracies were recorded for all trials using the E-Prime and IFIS software packages (Psychology Software Tools, Pittsburgh PA).

Prior to scanning, subjects were trained on 3 blocks of 36 trials consisting of color discriminations, motion discriminations, and alternating color/motion discriminations, respectively. In the scanner, subjects completed 6 blocks of 72 trials, which were presented in a jittered task design. Counterbalancing procedures and other details of the task design are described in Appendix 1.

Behavioral Data Analysis. Reaction time and accuracy were recorded for all trials. Effects of attention shifting, response conflict, and stimulus conflict were assessed using a 2 (repeat/shift) x 2 (low/high response conflict) x 3 (low/medium/high stimulus conflict) factorial within-subjects ANOVA. Only correct trials were included in reaction time analyses.

MRI Procedure. Functional and 3D high-resolution anatomical images were acquired on a GE 3-Tesla MRI scanner using a quadrature head coil. Functional MR images were preprocessed and coregistered to the anatomical volume using the BrainVoyager QX software package (Brain Innovations, Maastricht, The Netherlands). MRI parameters and preprocessing procedures are described in Appendix 2.

Imaging Data Analysis: Planned Contrasts and Correlations. After preprocessing, the 114 (6 runs x 19 subjects) z-normalized functional timecourses were analyzed based on the least mean squares solution to each of two general linear models. The first GLM used level of response conflict (low or high) for each trial type (shift or repeat) as the primary predictors. The second GLM used level of stimulus conflict (low, medium, high) for each trial type (shift or repeat) as the primary predictors. Only correct trials were included in these predictors. Each contrast analysis was performed based on wholebrain voxelwise t-tests of the difference between the beta weights of the relevant predictors using a random effects analysis. Interactions were assessed using a multifactorial within-subjects ANOVA based on the beta weights of the relevant predictors for each ROI, as noted in the text. The shift versus repeat contrast was thresholded at $p < 0.005$ with a

minimum cluster size of 8 contiguous voxels (~320 transformed voxels) to minimize the likelihood of a Type I error. Because the contrasts for response conflict and stimulus conflict included only 50% and 33% as many trials, respectively, these contrasts were thresholded at $p < 0.01$ with a minimum cluster size of 11 contiguous voxels, yielding an equivalent correction for multiple comparisons and increased power for detecting relatively large volumes of activation. Monte Carlo simulation confirmed that the probability of a Type I error was less than 0.05 based on these criteria (Forman et al, 1995).

Correlations between the conflict index and activity (BOLD signal, % change from run-average baseline) in DLPFC, ACC, and PPC were assessed by performing linear regressions for each subject, followed by a one-sample t-test of the resultant beta values versus zero, to account for inter-subject variance. The relative strengths of these associations were assessed by comparison of the Fisher Z-transformed correlation coefficients for each subject as described in Meng et al. (1992). Z-values reported in the text represent the group mean, with corresponding significance levels. Partial correlations controlling for shared variance with other structures in the circuit (see text) were also performed separately for each subject, followed by a one-sample t-test versus zero. Correlation coefficients reported in the

text represent the group mean, while significance levels reflect the results of each one-sample t-test.

Imaging Data Analysis: Post-hoc Analyses. To assess the role of PPC in regulating DLPFC activity and executing appropriate behavioral adjustments, we performed two post-hoc analyses based on the methods described in Kerns et al. (2004). The motivation for these analyses is described below. First, we examined how activity in ACC and dorsal PPC on high response conflict and high stimulus conflict trials, respectively, correlated with activity in DLPFC on the subsequent trial, in accord with the analysis described in Kerns et al. (2004). For this purpose, activity for a given trial type was defined as the mean of activity (BOLD signal, % change from run-average baseline) recorded on the second, third, and fourth scan post stimulus-onset, accounting for the lag in the hemodynamic response. To control for the effects of task-associated brain activity, we calculated the partial correlation between activity in either ACC or dorsal PPC on a given trial and DLPFC activity on the subsequent trial, while controlling for shared variance with a task-relevant region in the right temporal lobe, again following the approach described in Kerns et al. (2004). We then performed a one-sample t-test versus zero to account for inter-subject variance, as

described above. Significance levels reported in the text represent the results of these t-tests. Second, we examined whether activity in ACC and dorsal PPC predicted subsequent behavioral adjustments. Again, we adopted the methods of Kerns et al. (2004): trials immediately following a shift trial were sorted by reaction time relative to the mean repeat-trial RT. Trials in the fastest quintile were classified as "high adjustment", and those in the slowest quintile were classified as "low adjustment." We then tested the prediction that activity in ACC and dorsal PPC should be higher for shift trials followed by high adjustment trials than for those followed by low-adjustment trials.

Results

Behavioral Results. Analysis of behavioral data confirmed the validity of the attention shifting and conflict manipulations (Figure 2.2). All behavioral effects were observed for both color and motion trials, so these results are collapsed across dimension. Shift trials were slower ($F(1,18) = 388.03$, $p < 0.001$) and less accurate ($F(1,18) = 6.50$, $p = 0.02$) than dimension-matched repeat trials. As predicted, both conflict manipulations were also associated with significant behavioral impairments. Response conflict was associated with impairments in reaction time ($F(1,18) = 50.1$, $p < 0.001$) and accuracy

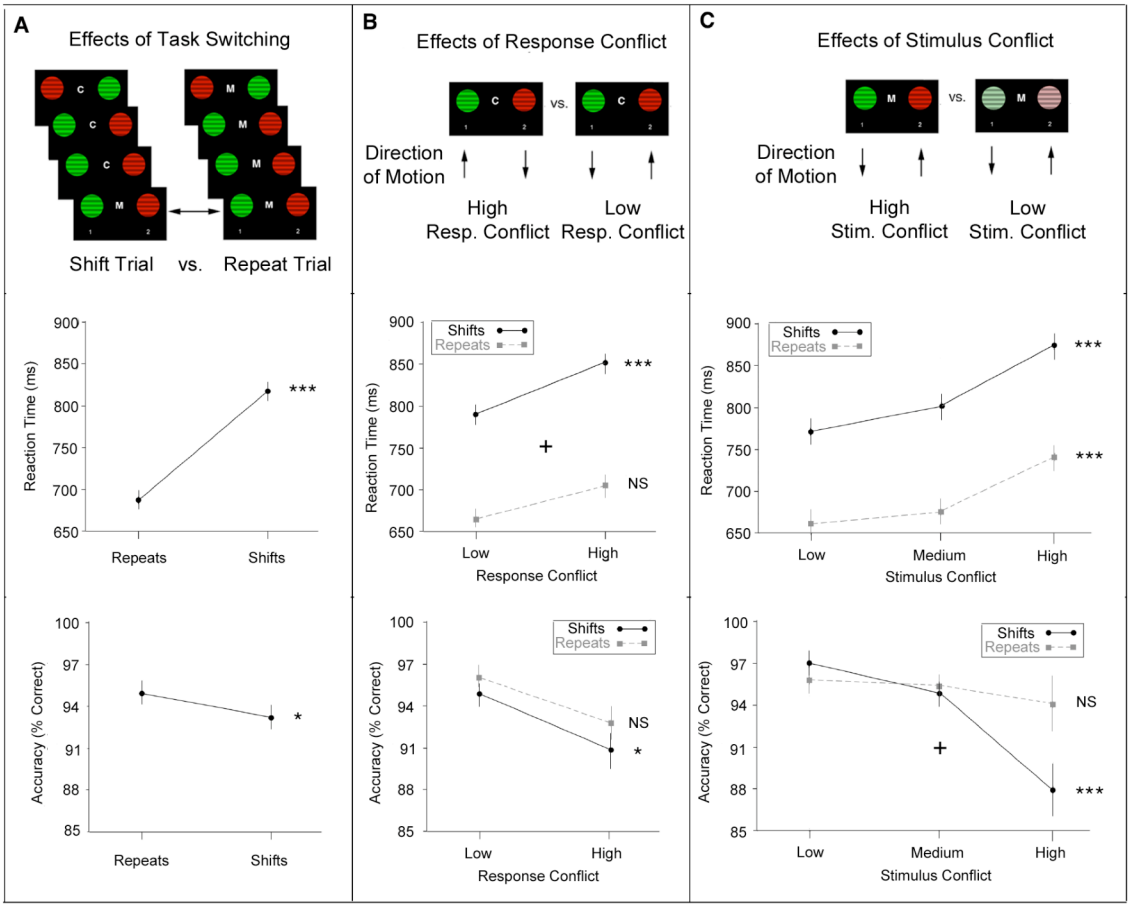
Figure 2.2 (see following page): Behavioral Effects of Conflict. High conflict trial types were associated with significant costs in task performance.

A) Shift trials were significantly slower (middle panel: $F(1,18) = 388.03$, $p < 0.001$) and less accurate than repeat trials (bottom panel: $F(1,18) = 6.50$, $p = 0.02$).

B) Response conflict was associated with impairments in reaction time (middle panel: $F(1,18) = 50.1$, $p < 0.001$) and accuracy (bottom panel: $F(1,18) = 4.37$, $p = 0.05$), but these effects were driven by shift trials. Whereas high conflict shifts were slower ($t = 3.81$, $p < 0.001$) and less accurate ($t = 1.95$, $p = 0.05$) than low conflict shifts, the effects of response conflict on repeat trial RT ($t = 1.81$, $p = 0.07$) and accuracy ($t = 1.27$, $p = 0.21$) did not reach significance. This was reflected in an interaction between response conflict and task switching for reaction time ($F(1,18) = 14.79$, $p < 0.001$).

C) Stimulus conflict was associated with impairments in reaction time ($F(2,36) = 36.14$, $p < 0.001$). There was also an effect of stimulus conflict on accuracy that was specific to shifts but not repeats, as reflected in an interaction between stimulus conflict and task switching ($F(2,36) = 9.35$, $p < 0.001$). Error bars = SEM. Main effects: * = $p < 0.05$, *** = $p < 0.001$. Interactions: + = $p < 0.001$.

Figure 2.2: Behavioral Effects of Conflict. See caption on preceding page.



($F(1,18) = 4.37$, $p = 0.05$). Post-hoc contrasts indicated that these effects were driven by shift trials: whereas high conflict shifts were slower ($t = 3.81$, $p < 0.001$) and less accurate ($t = 1.95$, $p = 0.05$) than low conflict shifts, the effects of response conflict on repeat trial RT ($t = 1.81$, $p = 0.07$) and accuracy ($t = 1.27$, $p = 0.21$) did not reach significance. This was reflected in an interaction between response conflict and attention shifting for reaction time ($F(1,18) = 14.79$, $p < 0.001$).

The effects of stimulus conflict were confined primarily to reaction time ($F(2,36) = 36.14$, $p < 0.001$): for both shift ($F(2,36) = 10.60$, $p < 0.001$) and repeat trials ($F(2,36) = 7.74$, $p = 0.001$), a main effect of stimulus conflict was observed such that increasing interference from the irrelevant dimension was associated with slower reaction times. There was also an effect of stimulus conflict on accuracy ($F(2,36) = 4.77$, $p = 0.01$) that was specific to shifts ($F(2,36) = 7.27$, $p = 0.001$) but not repeats ($F(2,36) = 0.47$, $p = 0.63$), as reflected in an interaction between stimulus conflict and attention shifting ($F(2,36) = 9.35$, $p < 0.001$). However, this effect was confounded by an interaction between stimulus conflict and response conflict ($F(2,36) = 17.66$, $p < 0.001$). That is, the main effect of stimulus conflict on accuracy was limited to high response conflict shift trials

($F(2,36) = 27.05$, $p < 0.001$) and not low response conflict shifts ($F(2,36) = 1.47$, $p = 0.24$). No other main effects or interactions were observed.

To summarize, stimulus conflict was associated with an impairment in reaction time but not accuracy per se, but this effect persisted even for repeat trials. Response conflict was associated with impairments in accuracy as well as reaction time that were compounded when response conflict and stimulus conflict were both high, but these effects diminished rapidly over the course of two to five repetitions.

This behavioral paradigm was designed to address three questions in the imaging data. First, we identified the principal regions involved in shifting attentional set and reconfiguring task rules by contrasting shift trials with dimension-matched repeat trials. Next, we sought to dissociate the contributions of conflict at the level of the stimulus representation and at the level of the response to these patterns of activity. Finally, we examined how activity in these regions varied with a physiologic index of conflict.

Effects of Attention Shifting. Shift trials relative to dimension-matched repeat trials engaged a network of prefrontal and parietal structures (Figure 2.3), including bilateral dorsolateral prefrontal cortex (BA 8/9), bilateral anterior cingulate cortex (BA 24/32), and bilateral posterior parietal cortex

(BA 40/7). These regions were also engaged when color trials and motion trials were examined separately so the results depicted in Figure 2.3 are collapsed across dimension. The precise locations of these regions, as well as the locations of regions engaged for color trials but not motion trials or vice versa, are detailed in Table 2.1.

Effects of Response Conflict and Stimulus Conflict. Next, we examined the effects of response conflict and stimulus conflict on activity in DLPFC, ACC, and PPC. This served both to dissociate the contributions of each type of conflict and to identify regions of interest that were particularly sensitive to conflict within the relatively large areas of DLPFC, ACC, and PPC activated in the task switching contrast. High response conflict shifts relative to low response conflict shifts (Figure 2.4A) engaged a network of structures that included rostral anterior cingulate cortex (BA 24/32, $p < 0.05$) but not the region of dorsal PPC (BA 7, $p > 0.62$) illustrated in Figure 2.3, which was reflected in an interaction between region (ACC, PPC) and response conflict ($F(1,18) = 5.22$, $p = 0.035$). Other areas that were sensitive to response conflict included ventrolateral prefrontal cortex (inferior frontal gyrus: BA 44/45), orbitofrontal cortex (BA 11), and inferior aspects of posterior parietal cortex (BA 40; Figure 2.4C: $p < 0.05$; see Table 2.2 for

Figure 2.3: Attention Shifting Engaged a Frontoparietal Network of Structures.

Shift trials contrasted with repeat trials engaged a network of prefrontal and parietal structures**.** 3D renderings of areas engaged by the shift-repeat contrast (left) are paired with coronal sections (right). These include bilateral DLPFC (orange), bilateral ACC (yellow), and bilateral posterior parietal cortex (BA 7/40; violet).

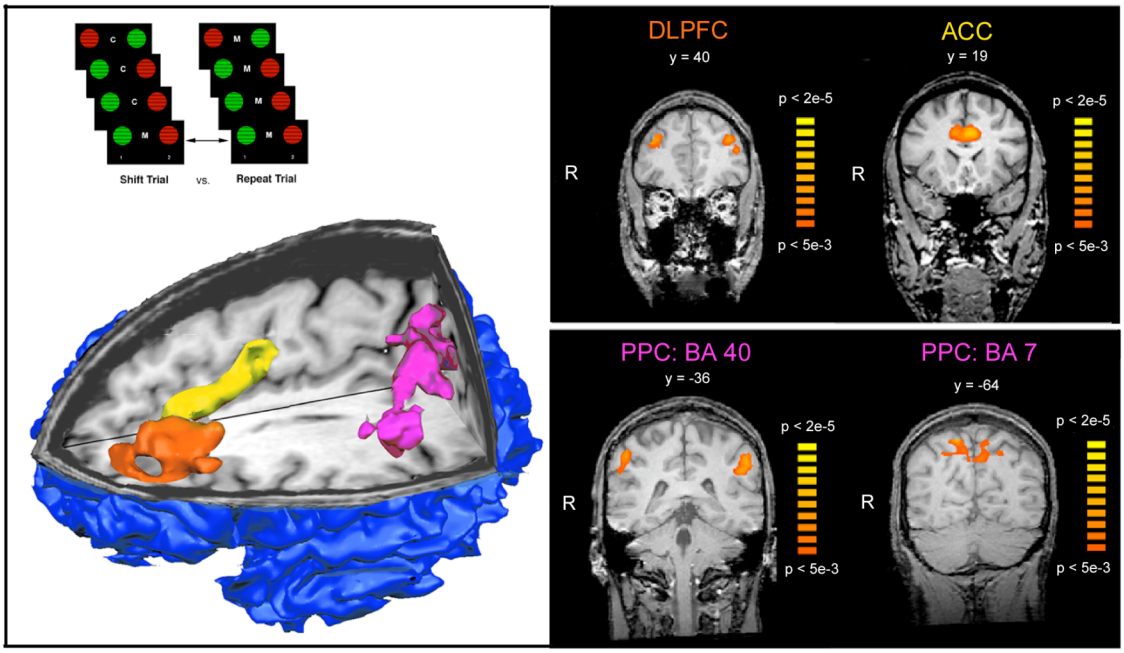


Table 2.1: Main Effects of Attention Shifting. DLPFC = dorsolateral prefrontal cortex; ACC = anterior cingulate cortex; PPC = posterior parietal cortex; BA = Brodmann Area.

Contrast	Region	BA	Talairach Coord. (x, y, z)	Peak Z Value
Shift > Repeat				
Color & Motion	R. DLPFC	8/9	36, 34, 36	4.61
	L. DLPFC	8/9	-33, 41, 35	4.57
	Bilateral ACC	24/32	1, 15, 34	4.78
	R. PPC	40	53, -38, 40	4.39
		7	17, -62, 48	4.07
	L. PPC	40	-54, -36, 32	4.13
		7	-32, -54, 50	4.14
Color Only	L. Premotor	6	-39, 0, 51	4.72
	Middle Temporal Gyrus	21	51, -39, -6	4.70
Motion Only	Left Medial Frontal Gyrus	6	-3, -1, 50	5.45
	Right Premotor Cortex	6	19, -9, 65	4.24

Figure 2.4 (see following page): Response Conflict and Stimulus Conflict Engaged Dissociable Frontoparietal Networks.

A) High response conflict shift trials relative to low conflict shift trials (green regions in (C)) engaged a network of structures including orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), ventrolateral prefrontal cortex (vlPFC) and inferior aspects of posterior parietal cortex (PPC). No significant differences were observed in these areas for high versus low conflict repeat trials.

B) High stimulus conflict shifts relative to low conflict shifts (red regions in (C)) engaged a distinct network of structures including anterior prefrontal cortex, dorsolateral prefrontal cortex (DLPFC), and dorsal aspects of posterior parietal cortex (PPC). A similar pattern was observed for repeat trials.

C) 3D rendering of regions engaged in the high versus low response conflict contrast (green) and the high versus low stimulus conflict contrast (red). In general, stimulus conflict was associated with increased activity in a network of structures located dorsal to those sensitive to response conflict.

Figure 2.4: Response Conflict and Stimulus Conflict Engaged Dissociable Frontoparietal Networks. See caption on preceding page.

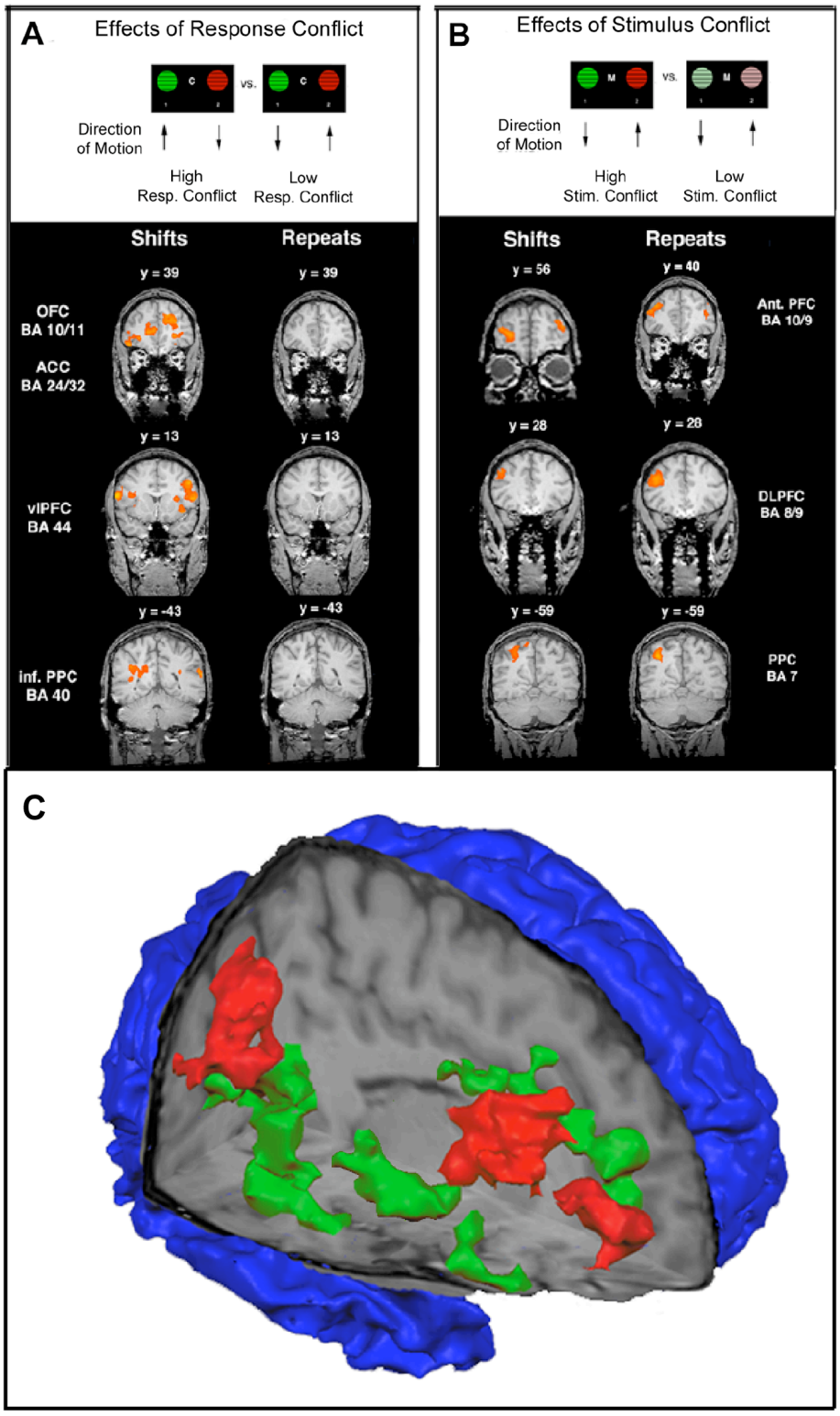


Table 2.2: Main Effects of Response Conflict. OFC = orbitofrontal cortex; ACC = anterior cingulate cortex; IFG = inferior frontal gyrus; MdFG = medial frontal gyrus; IPL = inferior parietal lobule; BA = Brodmann Area.

Contrast	Region	BA	Talairach Coord. (x, y, z)	Peak Z Value
High Resp. Conf. > Low Resp. Conf.				
Shifts Only:	Bilateral Medial OFC	11	1, 59, -5	5.02
	R. Lateral OFC	47/11	43, 35, -8	4.25
	R. ACC	24/32	12, 40, 15	4.08
	L. ACC	32	-13, 36, 28	4.49
	R. IFG	44/45	56, 15, 17	4.51
	L. IFG	44/45	-52, 16, 17	4.11
	Bilateral MdFG	6	1, 5, 61	4.41
	R. IPL	40	64, -20, 26	5.80
	L. IPL	40	-61, -46, 25	4.41

n.b. No regions showed a main effect of response conflict for repeat trials.

precise locations). These effects were limited to shift trials; no effects of response conflict on repeat trials were observed, which was confirmed by an interaction between attention shifting (shift vs. repeat) and response conflict ($F(1,18) = 13.46$, $p = 0.002$).

High stimulus conflict shifts relative to low stimulus conflict shifts (Figure 2.4B) engaged a network of structures located dorsal to the areas sensitive to response conflict (compare red and green regions, respectively, in Figure 2.4C). This included a region of right dorsal posterior parietal cortex (BA 7, $p < 0.05$) that converged with the region depicted in Figure 2.3, but not anterior cingulate cortex ($p > 0.85$). This dissociation was reflected in an interaction between region (ACC, PPC) and stimulus conflict ($F(2,36) = 3.19$, $p = 0.05$). Other areas that were sensitive to stimulus conflict included right DLPFC (BA 8/9), which converged with the ROI depicted in Figure 2.3, and right anterior prefrontal cortex (BA 9/10; Figure 5C: $p < 0.05$; for precise locations, see Table 2.3). A similar pattern was observed when shifts and repeats were analyzed together and for repeat trials alone, though activations were more robust in this contrast. Although high and low stimulus conflict shifts included equal numbers of high and low response conflict trial types, we further controlled for the confounding effects of response conflict by performing this contrast on low response

Table 2.3: Main Effects of Stimulus Conflict. PFC = prefrontal cortex; DLPFC = dorsolateral prefrontal cortex; PPC = posterior parietal cortex; BA = Brodmann Area.

Contrast	Region	BA	Talairach Coord. (x, y, z)	Peak Z Value
High Stim. Conf. > Low Stim. Conf.				
Shifts Only:	R. anterior PFC	9/10	26, 54, 15	3.59
	R. DLPFC	8/9	39, 29, 36	3.07
	R. PPC	7	29, -60, 45	2.92
Repeats Only:	R. anterior PFC	9/10	36, 48, 25	3.56
	R. DLPFC	8/9	37, 26, 33	3.75
	R. PPC	7	33, -59, 42	3.07

conflict shift trials exclusively. Activities in all three areas remained significant. Thus, ACC but not DLPFC or dorsal PPC were sensitive to conflict at the level of the response, while DLPFC and dorsal PPC but not ACC were sensitive to conflict at the level of the stimulus representation.

Conflict Sensitivity in Frontoparietal Cortex. Finally, we examined how activity in these three structures varied with a physiologic index of conflict. The conflict monitoring hypothesis states that anterior cingulate cortex acts to detect conflicts in information processing in posterior cortex. According to this view, when activity in two competing neural units is high, activity in anterior cingulate cortex should also be elevated (Botvinick et al., 2001). To test this prediction, we identified six occipitotemporal regions that were primarily motion-sensitive or primarily color-sensitive by contrasting color shift trials with motion shift trials. The three most significant color-sensitive areas were located in the middle and superior temporal gyri (BA 20, 21; Talairach coord: -43, 7, -21; -42, 2, -5; 58, -34, 3). Primarily motion-sensitive regions were located in two areas of extrastriate occipital cortex and in the middle temporal gyrus (BA 18, 19, 21; Talairach coord: 36, -65, 1; 26, -79, 18; -44, -52, -9). In accord with Botvinick and colleagues' (2001) computational formulation, conflict was indexed as a normalized

product of the activities (% change in BOLD signal from the run-average baseline) in these three color-sensitive regions and three motion-sensitive regions, summed across all nine combinations:

$$\text{Conflict} = \text{sqrt} \sum_{i,j} C_i \times M_j$$

or

$$\text{Conflict} = \text{sqrt}[(C1 + C2 + C3)(M1 + M2 + M3)]$$

Importantly, this formulation differs from that adopted in Botvinick and colleagues' (2001) computational model in that they ensured that competing units were connected by negative weights, whereas we could not reliably assess the association between the color- and motion-sensitive regions examined in our study. Instead, our index was intended to serve as a measure of conflict not between two competing perceptual areas per se, but rather between two stimulus-response processing streams, which are assumed to compete with each other. This was most easily measured in components of these processing streams that are anatomically distinct, i.e. in perceptual regions. Activities in the color- and motion-sensitive regions were assumed to be proxies for activity in their respective processing streams. Justifications for these assumptions and further discussion of the

limitations of this analysis are included in Appendix 3. To confirm the validity of the construct, we examined how the conflict index varied by trial type. As predicted, conflict was higher for shift trials than for repeat trials (Figure 2.5A: $t = 1.85$, $p = 0.033$, one-tailed).

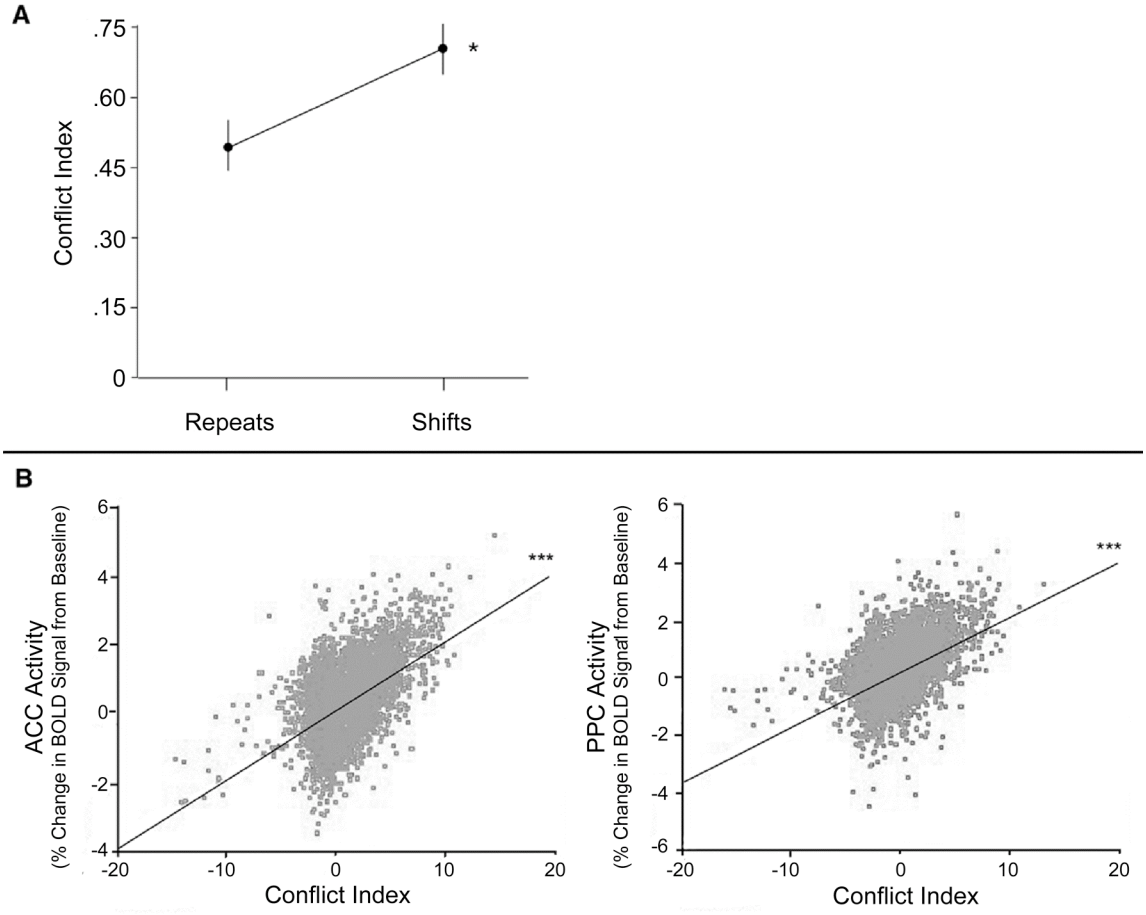
We then examined how this physiologic index of conflict predicted activity in DLPFC, ACC, and dorsal PPC on a trial-by-trial basis, excluding trials when color- and motion-sensitive regions were both below baseline. Within the relatively large areas of DLPFC, ACC, and PPC activated in the task switching contrast, we selected regions that overlapped with either conflict contrast to maximize conflict sensitivity. These included right DLPFC (36, 32, 36) and right dorsal PPC (24, -60, 45), which were active in both the attention shifting and stimulus conflict contrasts, and an area of right rostral ACC (Talairach coord: 5, 37, 17), which was active in both the attention shifting and response conflict contrasts. (See Appendix 3 for further discussion of ROI selection.)

The conflict index was significantly correlated with the BOLD signal (% change from baseline) in all three regions of interest, but the strength of this correlation varied from region to region. Correlations with anterior cingulate cortex (BA 24/32, $r = 0.49$, $p < 0.001$; Figure 2.5B) and posterior parietal cortex (BA 7, $r = 0.48$, $p < 0.001$; Figure 2.5C) were significantly

Figure 2.5: Activity in ACC and PPC Increased with Conflict.

A) As predicted, the conflict index was significantly higher for shift trials than repeat trials ($t = 1.85$, $p = 0.033$, one-tailed). Error bars = SEM.

B) Activity (% change in BOLD signal from run-average baseline) in anterior cingulate cortex (left) and posterior parietal cortex (right) is plotted against the conflict index. Activity in these regions increased with increasing conflict as indexed by the product of activities in color- and motion-sensitive regions ($r = 0.49$ [ACC], $r = 0.48$ [PPC], $p < 0.001$). * = $p < 0.05$, *** = $p < 0.001$.



stronger (ACC: $Z = 3.08$, $p < 0.001$; PPC: $Z = 2.49$, $p < 0.007$) than the correlation with dorsolateral prefrontal cortex (BA 8/9, $r = 0.38$, $p < 0.001$). Interestingly, the conflict index predicted activity in ACC independent of PPC ($r = 0.31$, $p < 0.001$) and vice versa ($r = 0.26$, $p < 0.001$). In contrast, it accounted for only 1% of the variance in DLPFC activity ($R^2 = 0.012$) independent of activity in these two structures.

Conflict-monitoring in Posterior Parietal Cortex. Thus, dorsal PPC as well as ACC were uniquely sensitive to conflict. Moreover, ACC and PPC were sensitive to dissociable forms of conflict at the level of the response or at the level of the stimulus representation, respectively, which suggests that the central tenet of the conflict-monitoring hypothesis may apply to PPC as well as ACC. Previous work has confirmed several additional predictions of the conflict-monitoring hypothesis concerning the role of ACC in regulating DLPFC activity and executing appropriate behavioral adjustments. These investigations have shown that increased ACC activity precedes increased DLPFC activity and is associated with enhanced behavioral performance on subsequent trials (Kerns et al., 2004). We attempted to replicate the findings reported in Kerns et al. (2004) and then tested whether these predictions also applied to posterior parietal cortex.

First, we tested whether increased activity in ACC and dorsal PPC on high response conflict and high stimulus conflict trials, respectively, preceded increased activity in DLPFC. As predicted, increased ACC activity on the current trial preceded increased DLPFC activity on the subsequent trial ($r = 0.45$, $p < 0.001$). This was also observed for dorsal PPC ($r = 0.48$, $p < 0.001$), consistent with an analogous role for this structure in recruiting DLPFC (Figure 2.6A). Importantly, dorsal PPC predicted subsequent DLPFC activity independent of shared variance with ACC ($r = 0.19$, $p < 0.001$), and vice versa ($r = 0.20$, $p < 0.001$), which is suggestive of independent roles for these structures in DLPFC regulation.

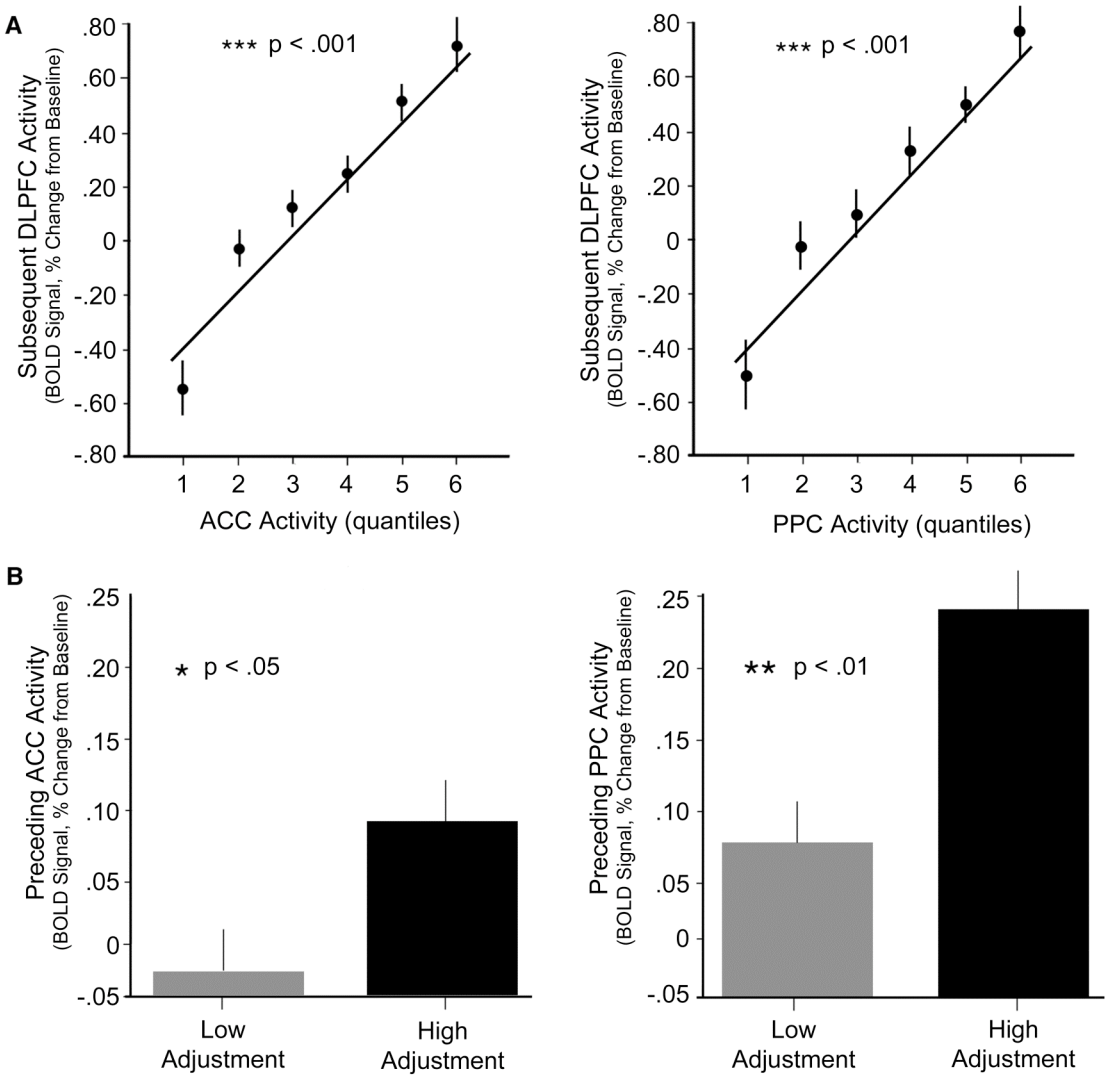
Next, we tested whether ACC and dorsal PPC activity predicted subsequent behavioral adjustments. In accord with Kerns et al. (2004), we classified each trial following a shift trial as "high adjustment" (fastest quintile relative to average repeat trial RT) or "low adjustment" (slowest quintile relative to average repeat trial RT). As predicted, ACC activity on the preceding shift trial was significantly higher for high adjustment (fast) trials than for low adjustment (slow) trials ($t = 2.21$, $p = 0.027$). Again, this was also observed for dorsal PPC ($t = 3.48$, $p = 0.001$), indicating that increased PPC activity on a given trial was associated with enhanced performance on the subsequent trial (Figure 2.6B).

Figure 2.6 (see following page): Activity in ACC and Dorsal PPC Predicted Increased Activity in DLPFC and Enhanced Performance on Subsequent Trials.

A) Activity in ACC (left: $p < 0.001$) and dorsal PPC (right: $p < 0.001$) on high conflict trials predicted increased DLPFC activity on the subsequent trial. ACC and dorsal PPC activities are plotted in six quantiles against the means for DLPFC activity (% change in BOLD signal from run-average baseline).

B) Activity in ACC (left: $t = 2.00$, $p = 0.047$) and dorsal PPC (right: $t = 2.70$, $p < 0.007$) on the preceding shift trial was significantly higher for high adjustment (fast) trials than for low adjustment (slow) trials. See text for details. Error bars = SEM. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Figure 2.6: Activity in ACC and Dorsal PPC Predicted Increased Activity in DLPFC and Enhanced Behavioral Performance on Subsequent Trials. See caption on preceding page.



Discussion

In accord with other work (Sohn et al., 2000; Rushworth et al., 2002; Barber & Carter, 2005), the implementation of attentional control associated with task switching engaged a network of structures including dorsolateral prefrontal cortex (BA 8/9), anterior cingulate cortex (BA 24/32), and posterior parietal cortex (BA 7/40). Activity in all three structures was sensitive to the conflict index but to varying degrees. The strongest associations were observed in ACC and superior aspects of the posterior parietal cortex, which were independently sensitive to dissociable forms of conflict at the level of the response and the stimulus representation, respectively.

Although activities in all three structures were positively correlated with each other, this finding cannot fully account for the relation between the conflict index and activity in PPC and ACC. The conflict index predicted activity in ACC and PPC independent of activity in DLPFC: increased conflict predicted increased activity in these structures above and beyond that associated with whole-circuit increases in activity associated with attention, for example. In contrast, the conflict index predicted only about 1% of the variance in DLPFC activity independent of ACC and PPC, suggesting a relation specific to these structures.

Activity in both ACC and PPC increased with the conflict index, but these structures were sensitive to different forms of conflict. Response conflict varied with the congruency of the stimulus-response mapping in each dimension, similar to the approach adopted in previous studies of response conflict (e.g. MacDonald et al., 2000; Rushworth et al., 2002; Weissman et al., 2003; Kerns et al., 2004). Stimulus conflict varied with the salience of the irrelevant dimension, which was manipulated in accord with psychophysics findings (Campbell & Maffei, 1980). It is important to note that factors such as the salience of the *relevant* stimulus dimension, the location of the target stimulus, the level of response conflict, and the preceding context were all controlled. Thus, the stimulus conflict manipulation was not confounded by conflict at the level of the response or by other (e.g. spatial) attentional demands, independent of competing stimulus information from the irrelevant dimension.

These manipulations revealed a double dissociation for conflict sensitivity in ACC and PPC. ACC, but not dorsal PPC, was sensitive to conflicts at the level of the response: ACC activity was elevated on high response conflict shifts relative to low response conflict shifts (Figure 2.4B), but this effect was not observed in dorsal PPC (BA 7). In contrast, posterior parietal cortex, but not ACC, was sensitive to conflict at the level of the

stimulus representation, as activity in PPC increased with the salience of stimulus information from the irrelevant dimension. Interestingly, the conflict index also predicted activity in PPC independently of ACC and vice versa, which lends further support to the interpretation that these structures are sensitive to distinct forms of conflict. The conflict index predicted approximately 25% of the variance in ACC and PPC, nearly half of which was independent of activity in the other structure.

The selective sensitivity of ACC activity to response conflict but not stimulus conflict is consistent with at least two other reports (Van Veen et al., 2001; Van Veen & Carter, 2002), which used the Eriksen flanker task. Although others have observed ACC activity in association with non-response conflict (e.g. Weissman et al., 2003; Badre & Wagner, 2004; Van Veen & Carter, 2005), non-response conflict in these studies occurred at a level intermediate between the stimulus input and the response. In contrast, non-response conflict in our task and in the Eriksen flanker task occurred at the level of the stimulus representation, which may account for this discrepancy, as described in Van Veen and Carter (2005). The precise locus of ACC activity may also be important. For example, Rushworth and colleagues (2003) reported that lesions to ACC in monkeys caused task-switching deficits only if the lesions were extensive and included the

cingulate sulcus. In two of the reports cited above (Weissman et al., 2003; Van Veen & Carter, 2005), ACC activity sensitive to non-response conflict was observed in more caudal aspects of ACC, while Van Veen and Carter's (2005) report indicates that rostral ACC may be selectively sensitive to response conflict, consistent with the locations described in other studies that emphasize interference at the level of the response (Casey et al., 2000; Van Veen et al., 2001).

In other respects, though, patterns of activity in ACC and PPC were similar. Increased activity in both ACC and PPC predicted increased DLPFC activity and enhanced behavioral performance on subsequent trials. Importantly, the correlation between PPC activity and subsequent DLPFC activity was independent of shared variance with ACC (and vice versa), lending further support to the hypothesis that PPC may act to regulate DLPFC activity independently of ACC. These results are consistent with a role for both of these structures in regulating DLPFC activity by signaling the need for greater control. Indeed, just as ACC is anatomically well situated to detect conflicts at the level of the motor response and signal these to lateral prefrontal cortex (Barbas & Pandya, 1989; Bates & Goldman-Rakic, 1993), several studies suggest that posterior parietal cortex is anatomically well suited to detect stimulus conflict and signal this to

prefrontal cortex: primate posterior parietal cortex receives ample, direct input from extrastriate visual cortex and sends direct projections to lateral prefrontal cortex (Wise et al., 1997). Previous studies have emphasized a role for posterior parietal cortex in detecting unexpected or behaviorally relevant stimuli and facilitating goal-directed attention to task-relevant aspects of a visual stimulus (Corbetta et al., 2000; Corbetta & Shulman, 2002). Our results suggest one mechanism by which these processes may be mediated: detection of conflicts in information processing at the level of the stimulus representation may signal to lateral prefrontal cortex the need for enhanced top-down control, with distinct subregions regulating representations at various levels of abstraction (Desimone & Duncan, 1995; Dias et al., 1996; Casey et al., 2000; O'Reilly et al., 2002; Koechlin et al., 2003). Further experimentation is necessary to assess the importance of conflict detection as a mechanism by which PPC mediates selective attention, especially in the context of other task paradigms.

These findings may also inform efforts to integrate the conflict-monitoring hypothesis with a growing body of research exploring the role of posterior parietal cortex in generating categorical perceptual decisions about sensory stimuli. Electrophysiological studies in non-human primates suggest that PPC plays a critical role in generating plans for movement

through coordinate transformations of sensory inputs from multiple modalities into a common frame of reference (Anderson & Buneo, 2002), and stimulus-related inputs to PPC can evoke neuronal activity associated with more than one potential motor plan (Snyder et al., 1996). Recent experiments have demonstrated that these responses are modulated by decision-theoretic variables such as expected gain and outcome probability (Platt & Glimcher, 1999), suggesting that cells in PPC may function to accumulate over time stimulus information favoring one decision over another; perceptual decisions could be made by calculating the difference between activity in cells favoring decision A and in those favoring decision B (Gold & Shadlen, 2001). Cells with these electrophysiological properties would be ideally suited for detecting and signaling conflicts at the level of the stimulus representation: an activity difference that fails to exceed the required threshold could serve as a signal for the recruitment of prefrontally mediated control mechanisms, which would facilitate the representation of task-appropriate stimulus information.

It is also interesting to note that response conflict and stimulus conflict played different roles in shift trials relative to repeat trials, which may help to reconcile the conflict monitoring hypothesis with a recent report by Milham and colleagues (2003). They scanned subjects while performing

a variant of the Stroop task in which subjects attained rapid practice-related improvements in performance. ACC and DLPFC activity were observed initially on incongruent relative to congruent trials, but with practice, ACC activity decreased to baseline while DLPFC activity remained elevated. Several groups have noted that this finding is inconsistent with the assertion that ACC plays a necessary role in recruiting DLFPC (Paus, 2001; Milham & Banich, 2005).

Our results suggest an alternative interpretation. They confirm that with repetition, the role of ACC in regulating control mechanisms diminishes: ACC was not engaged on high conflict repeat trials, and the behavioral costs associated with response conflict diminished commensurately. Instead, posterior parietal cortex may substitute for ACC in regulating the activity of DLPFC: high stimulus conflict repeat trials engaged both PPC and DLPFC robustly, and the behavioral effects of stimulus conflict persisted. With repeated exposure, PPC may suffice to detect conflicts at the level of the stimulus representation and recruit DLPFC to resolve them before they affect response selection. Alternatively, response selection may with repetition become tonically regulated by the more ventral regions of lateral prefrontal cortex depicted in Figure 2.4B, in accord with other studies of behavioral inhibition and practice-related

changes in executive function (Casey et al., 1997; Raichle et al., 1994; Petersen et al., 1998; Durston et al., 2002). Further experimentation would be required to test these hypotheses.

Although we focus here on the role of DLPFC, PPC, and ACC in detecting and resolving conflicts in information processing in the service of attentional control, this focus should not obscure the fact that these structures also serve additional functions that are essential to task switching as required in this paradigm. Accordingly, Rogers and Monsell (1995) have demonstrated that switch costs persist even in the absence of conflict at the level of the response or the stimulus representation. Indeed, our results replicate this finding: when both response and stimulus conflict were minimized, shift trials were still significantly slower than repeat trials ($p < 0.007$). Other studies have examined the various contributions of these structures to task switching in detail, and our results are generally in accord with this work. Reports of activity in DLPFC and PPC, for example, are common in these investigations (Sohn et al., 2000; Dreher & Berman, 2002; Luks et al, 2002; Barber et al., 2004; Dreher & Grafman, 2004), which ascribe to DLPFC a role in selecting and maintaining task-relevant representations and selecting task-appropriate responses. Studies that emphasize response inhibition (e.g. Sohn et al., 2000, Barber et al., 2004)

highlight more inferior aspects of lateral PFC (BA 46/45), also in accord with our results. Posterior parietal cortex is believed to play a role in reconfiguring stimulus-response mappings (Barber et al., 2004) and in executing stimulus-driven task-set adjustments (Sohn et al., 2000). It is likely that the behavioral costs associated with attentional shifts, independent of manipulations of response and stimulus conflict, can be attributed in part to these adjustments and reconfigurations. By focusing on the detection and resolution of conflict, our results complement this body of work.

In contrast to DLPFC and PPC, reports of ACC activity in studies of task switching and conflict detection are somewhat inconsistent, and it is important to understand the source of these discrepancies. Several studies have reported ACC activity in association with task switching (Burgess et al., 2000; Dreher & Berman, 2002; Swainson et al., 2003; Rushworth et al., 2002). However, several other studies describe results that question the importance of ACC for task switching, per se (Sohn et al., 2000; Luks et al., 2002; Dreher & Grafman, 2003). Important variations in task design, especially in the timing and predictability of the switch, may account for these differences. ACC activity may play a critical role only in conditions that yield high response conflict on switch trials. If the task structure

provides more time for subjects to prepare for a switch or if the switch itself is more predictable, response conflict may diminish (e.g. Sohn et al., 2000; Luks et al., 2002). Alternatively, if task switching occurs rapidly (e.g. Dreher & Grafman, 2003), response conflict may persist even on repeat trials, in which case switches and repeats might engage ACC equivalently. Our task was designed to maximize switch-related response conflict, so ACC activity was to be expected.

Collectively, our findings suggest that the basic tenets of the conflict-monitoring hypothesis (Botvinick et al., 2001) may apply to dorsal posterior parietal cortex as well as anterior cingulate cortex. Anterior cingulate and posterior parietal cortices were components of two distinctly dissociable networks, sensitive to conflict at the level of the response or the stimulus representation, respectively. Activity in these structures varied uniquely and independently with a physiologic index of conflict in competing processing streams and predicted increased DLPFC activity and enhanced behavioral adjustments. Together, ACC and PPC may act to detect dissociable forms of conflict, signaling to prefrontal cortex the need for increased control. Structural and functional abnormalities in DLPFC and ACC are commonly reported in schizophrenia, major depression, and anxiety disorders, among other psychiatric conditions, all of which feature prominent deficits in

attentional control. Our results confirm the importance of posterior parietal cortex in this circuitry (Fox et al., 2003; Sohn et al., 2000, Barber et al., 2005) and highlight a new potential role for this structure. A more thorough understanding of the functional significance of each component of this circuit may facilitate future efforts to link clinical symptomatology with neuropathology and more effective treatments.

With this goal in mind, these results laid the groundwork for studies reported in subsequent chapters. Importantly, the task developed here captures the principal features of the attentional set-shifting paradigms suitable for work in animal models. The attention shifting manipulation, in which subjects redirect attention from motion to color information or vice versa, was designed to mimic the extradimensional attentional shifts tested in rats and monkeys. Likewise, the response conflict manipulation, which taps circuitry important for overriding a stimulus/response association independent of attentional shifts, mimics the response reversal phase in animal models. In accord with these hypotheses, attention shifting and response reversals engaged dissociable networks of structures in a manner consistent with the dissociations observed in rodent and primate lesion studies (Dias et al., 1996; Birrell and Brown, 2000; Fox et al., 2003; McAlonan and Brown, 2003). Exploiting these analogies, the studies

reported in the following two chapters examined the effects of chronic stress on attentional regulation and the circuitry that subserves it in rats and in healthy human subjects.

CHAPTER 3:

Chronic Stress Effects on Attentional Control and Prefrontal Dendritic Morphology in Rats

As reviewed in Chapter 1, stressful life events may predispose susceptible individuals to a variety of psychiatric conditions, including depression, post-traumatic stress disorder (PTSD), and other anxiety disorders (Sapolsky, 1996; Heim et al, 1997; McEwen, 1998; Caspi et al., 2003). Increasing evidence suggests that the prefrontal pathologies may contribute to the attentional impairments that are symptomatic of these conditions (Cohen and Servan-Schreiber, 1992; Drevets et al., 1997; Casey et al., 2002; Rauch et al., 2003). Results reported in Chapter 2 highlight the dissociable contributions of components of this circuit to behavioral regulation. Whereas ventral regions play a role in overriding well-learned stimulus/response associations, dorsal regions mediate attentional shifts. Experiments reported in this chapter examined the effects of chronic stress on the integrity of these networks in rats.

Some progress has been made in elucidating the cellular morphologic changes in mPFC following chronic stress in rats. Repeated restraint stress induces retraction and debranching of apical dendrites (Radley et al., 2004; 2006; Cook and Wellman, 2004). Subsequently, our group showed that apical dendritic atrophy is accompanied by axospinous synapse loss in medial prefrontal pyramidal cells (Figure 3.1: adapted from Radley et al., 2006).

Figure 3.1: Effects of Stress on mPFC Dendritic Spine Density. In a previous report, our group demonstrated that apical dendritic atrophy in medial prefrontal cortex is accompanied by spine loss. **A)** A typical anterior cingulate layer II/III pyramidal cell with the apical dendrite (arrow) extending to the right toward the pial surface and the axon (arrowheads) projecting to deeper layers of cortex. **B)** Confocal images of dendritic segments, which were sampled at random in 50-µm increments from the cell body. The number in the lower right corner of each image denotes the spine density. Images (i) and (ii) were collected from the control group, while (iii) and (iv) were collected from the stressed group. Adapted from Radley et al. (2006).

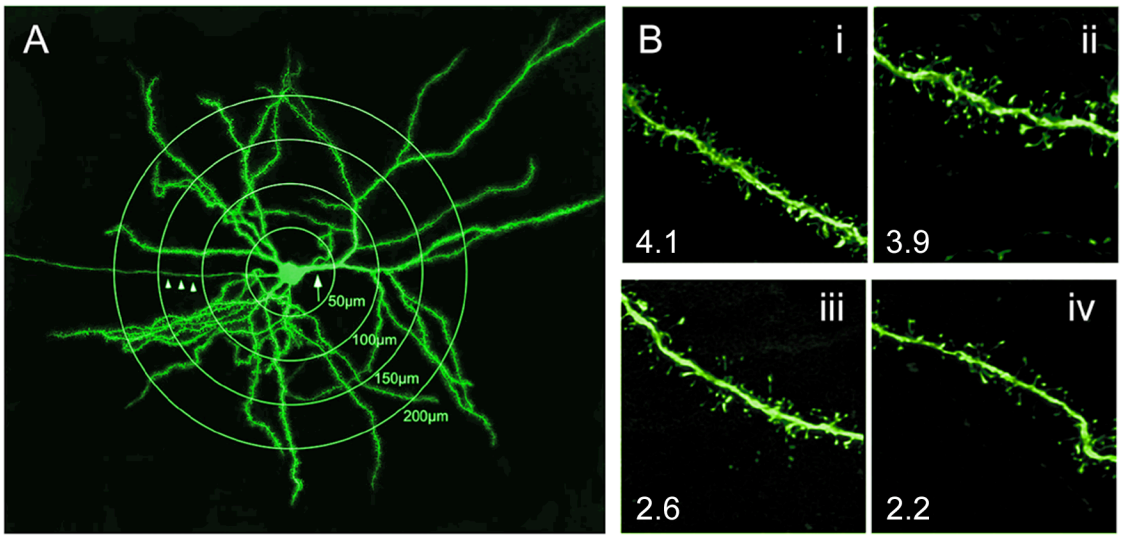
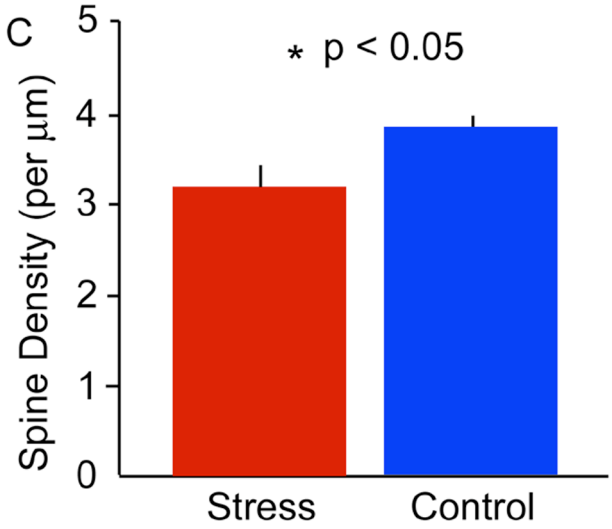


Figure 3.1 (cont.): C) Spine densities were significantly reduced in chronically stressed rats relative to controls ($p < 0.05$). Error bars = SEM. Adapted from Radley et al. (2006).



The functional consequences of these morphological alterations remain unclear. The rodent mPFC is believed to play a critical role in modulating attention. As reviewed in Chapter 2, excitotoxic lesions to mPFC selectively impair attention shifts but not simple discrimination learning or reversal learning (Birrell and Brown, 2000). In contrast, orbitofrontal lesions impair reversal learning but not attentional set-shifting (McAlonan and Brown, 2003). Collectively, these studies raise the possibility that stress-induced morphologic alterations may impair attentional control and that the functional consequences of these effects in mPFC and OFC could be dissociated based upon their differential roles in attentional processing.

Thus, the aim of the present study was to examine the relationship between stress-related effects on prefrontal cortical dendritic morphology and attentional control. After 21 days of repeated restraint stress, 24 rats (12 stressed, 12 controls) were tested on a perceptual attentional set-shifting task. We then performed intracellular iontophoretic injections of Lucifer Yellow in a subset of these rats to examine dendritic morphology in layer II/III pyramidal cells of lateral OFC and the anterior cingulate (ACg) region of mPFC.

Experimental Procedures

Animals. 24 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 250-280g at the start of the experiment were pair-housed and maintained on a 12-hour light/dark schedule (lights on 07:00 to 19:00). All rats were handled daily for seven days before beginning the experiment. 12 of these rats were restrained in wire-mesh restrainers for six hours daily (10:00 to 16:00) for 21 days. Control rats (N=12) and stressed rats (N=12) were housed in separate rooms. During the first 11 days of the experiment, all rats had *ad libitum* access to food and water, except during restraint sessions. Thereafter, all rats were maintained on a restricted diet of 15-20 grams of food per day, with 85% of *ad libitum* body weight serving as a guideline. Water remained available *ad libitum* throughout the experiment. All procedures were approved by the Rockefeller University Institutional Animal Care and Use Committee (IACUC).

Behavioral Paradigm: Apparatus. Rats can be trained to dig in bowls filled with sawdust to retrieve a food reward (Wood et al., 1999). The testing apparatus used in this experiment was adapted from those described in Birrell and Brown (2000) and Fox et al. (2003). We used plastic bowls with an internal diameter of 12 cm and a depth of 6 cm. The bowls were filled

with a digging medium, which could be scented. Thus, the bowls varied along two dimensions: odor and texture of the digging medium. One half of a Honey Nut Cheerio (General Mills, Minneapolis, MN) served as a reward.

The testing apparatus was a plexiglas box measuring 50 x 37.5 x 25 cm with a removable opaque divider separating one third of the box from the rest. On each trial, two bowls were placed at the opposite end, separated from each other by a permanent central divider running one third of the length of the box. The opaque divider was then removed, giving the rat access to the bowls. The divider was replaced once the trial had begun. The purpose of the dividers was to block access to the bowls between trials and after an error.

Behavioral Paradigm: Habituation. On day 22 of the experiment, rats were placed in the testing apparatus and given access to the two bowls, each filled with corn cob bedding from their home cages and baited with several cheerios. The bowls were continuously rebaited, approximately every five minutes, until the rats were digging reliably to retrieve the food rewards. This took approximately 45 minutes. Next, the rats were trained to a criterion of six consecutive correct trials on two simple discriminations: sage versus parsley and shredded latex versus crumpled tissue paper. These

stimuli were not used again. As described in Fox et al. (2003), the reward was buried deep within the bowl, and digging was defined as vigorous displacement of the digging medium. Thus, the rats could sample the digging medium with paws or snout without executing a digging response so they could rely on either tactile or visual properties of the stimulus to make a decision in this dimension. The purpose of this phase of the experiment was to habituate the rats to the apparatus, to acquaint them to the discrimination learning procedure, and to draw their attention to the task-relevant dimensions of the stimuli (odor and texture).

Behavioral Paradigm: Testing Procedure. The testing procedure was identical to that described in Fox et al. (2003), except for the stimulus pairs as described below. Briefly, a trial began by raising the barrier, giving the rat access to the bowls, only one of which was baited. An error was recorded if the rat dug first in the unbaited bowl. The first four trials of each discrimination constituted a discovery period: the rat was permitted to dig in both bowls until it retrieved the reward, regardless of where it dug first. On subsequent trials, if the rat dug first in the unbaited bowl, an error was recorded and the trial was terminated. This procedure was repeated until the rat reached a criterion of six consecutive correct trials.

Rats were tested on a series of five discriminations (Figure 3.2) in a single session on the day following habituation. Testing started with a simple discrimination (SD), in which the rat discriminated between either two odors or two digging media. Next, in a compound discrimination (CD), a new dimension was introduced, but the positive stimulus was the same as in the SD. This was followed by an intradimensional attentional shift (IDS), in which two new exemplars from each dimension were introduced, but the task-relevant dimension was the same as in the SD and CD. Next, the IDS was reversed (Rev), such that the formerly negative stimulus became the positive stimulus. Finally, an extradimensional attentional shift (EDS) occurred. Here, two new exemplars from each dimension were introduced, and the formerly task-irrelevant dimension became the relevant one. One possible combination of discriminations is provided in Table 3.1.

Half of the rats in each group started with discriminations based on medium and shifted to odor (see example in Table 3.2), and half started with odor and shifted to medium. Each rat in the stressed group was paired with a control rat that was tested on an identical sequence of stimuli. The order of stimuli within a dimension was also counterbalanced across subjects. Because there were too many possible stimulus pairings and orderings to permit full counterbalancing, the stimuli were assigned to pairs that were

Figure 3.2 (see following page): Perceptual attentional set-shifting task. Rats were trained to dig in bowls to retrieve a food reward and tested on five discriminations. In the simple discrimination (SD) depicted below, the bowls varied by medium only, and one exemplar (plastic beads) predicted the reward (denoted in red). In a compound discrimination (CD), the bowls varied independently by both medium and odor, but the task was the same. In an intradimensional attentional shift (IDS), two new exemplars from each dimension were introduced. A new exemplar from the medium dimension (shredded paper towel) predicted the reward. In a reversal shift (REV), the stimulus/reward association was reversed; shredded newspaper predicted the reward, not shredded paper towel. Finally, in an extradimensional attentional shift (EDS), two new exemplars from each dimension were introduced. A new exemplar from the odor dimension (cinnamon) predicted the reward. The digging medium was irrelevant for task performance, so the rat was required to shift attention to a new dimension of the stimulus to obtain the reward. This figure depicts just one permutation of stimuli. See text and Tables 3.1 and 3.2 for further details.

Figure 3.2: Perceptual attentional set-shifting task. See caption on preceding page.

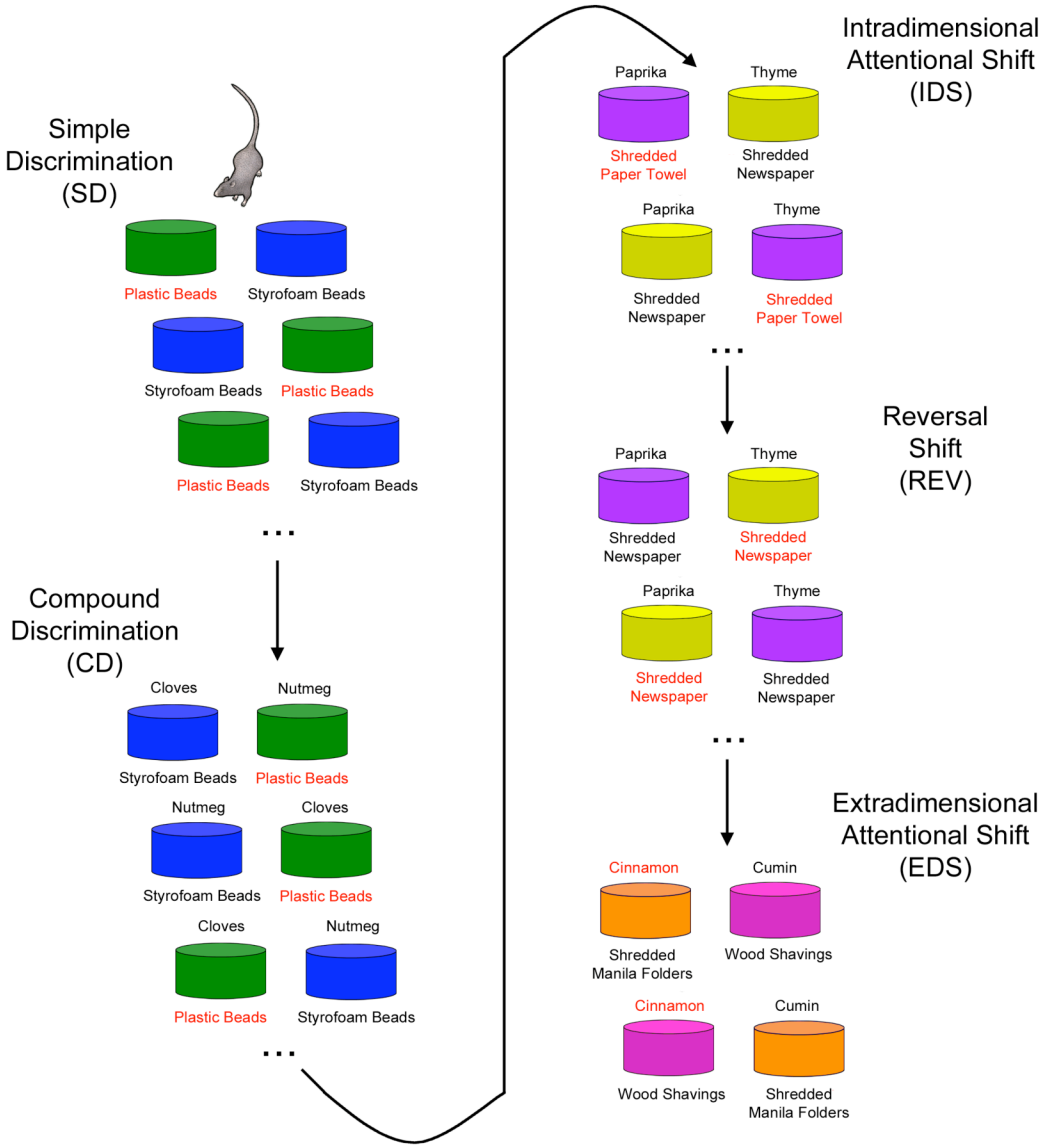


Table 3.1: Task Sequence. A typical sequence of discriminations, including the task-relevant dimension and all possible exemplar combinations. The correct exemplar is depicted in bold for each discrimination.

	Dimensions		**Exemplars**	
Discrimination	**Relevant**	**Irrelevant**	**+**	**-**
Simple (SD)	Medium		**M1**	M2
Compound (CD)	Medium	Odor	**M1**/O1 **M1**/O2	M2/O2 M2/O1
Intradimensional Shift (IDS)	Medium	Odor	**M3**/O3 **M3**/O4	M4/O4 M4/O3
Reversal (Rev)	Medium	Odor	**M4**/O3 **M4**/O4	M3/O4 M3/O3
Extradimensional Shift (EDS)	Odor	Medium	**O5**/M5 **O5**/M6	O6/M6 O6/M5

Table 3.2: Stimulus Pairs. Because there were too many possible stimulus pairings and orderings to permit full counterbalancing, the stimuli were assigned to pairs (listed below) that were maintained across subjects.

Odor:	**Medium:**
Cloves (O1) vs. Nutmeg (O2)	Plastic (M1) vs. Styrofoam Beads (M2)
Thyme (O3) vs. Paprika (O4)	Shredded Paper Towel (M3) vs. Shredded Newspaper (M4)
Cinnamon (O5) vs. Cumin (O6)	Wood Shavings (M5) vs. Shredded Manila Folders (M6)

maintained across subjects (see Table 3.2), and the order of presentation was counterbalanced to the extent possible. In particular, each exemplar was the positive stimulus of the EDS for one pair of rats and the negative stimulus for another. Except for pair-matched controls, no two rats were tested on the same sequence of stimuli.

Analysis of Prefrontal Dendritic Morphology. On the day after behavioral testing, 12 rats (6 stressed, 6 controls) were given a euthanizing dose of Nembutal and perfused transcardially with cold 4% paraformaldehyde in phosphate buffered saline (PBS; pH 7.4), followed by fixation in cold 4% paraformaldehyde with 0.125% glutaraldehyde in PBS. Brains were dissected and post-fixed for 2-3 hours in the same fixative. All procedures were approved by the Rockefeller University IACUC. To minimize bias in morphometric analyses, each brain was coded prior to the perfusion, and the code was not broken until the analyses were completed.

The iontophoretic cell loading procedure was identical to that described in Radley et al. (2006). Coronal sections (250-μm thick) were prepared on a Vibratome and exposed to a fluorescent nucleic acid stain (4,6-diamidino-2-phenylindole; Sigma, St. Louis, MO) for 1-2 minutes for visualization of cortical lamination patterns. They were then mounted on

nitrocellulose filter paper. Neurons in layer II/III of the anterior cingulate region of mPFC and lateral OFC were loaded with intracellular iontophoretic injections of 5% Lucifer Yellow (Molecular Probes, Eugene, OR) under a DC current of 1-8 nA for 7-10 minutes. The anterior cingulate cortex (Cg1-3 in Paxinos and Watson, 1998) was delineated based on criteria described in Radley et al. (2006). The lateral orbitofrontal cortex (LO in Paxinos and Watson, 1998) was delineated by loading cells on the orbital aspect of frontal cortex, dorsal to the olfactory bulb, in coronal sections located ~2-3 mm anterior to the rostral aspect of the genu of the corpus callosum, where LO occupies the lateral ~60% of orbital cortex. Loaded cells that were later determined not to lie clearly within the limits of LO based on comparison with Paxinos and Watson (1998) schematics (i.e. those adjacent to AI dorsolaterally or VO medially) were not included in the analysis. These regions were selected to coincide with the locations of the mPFC and OFC lesions in two studies demonstrating a double dissociation for these structures in mediating attention shifting and reversal learning, respectively (Birrell and Brown, 2000; McAlonan and Brown, 2003).

After cell loading, sections were coverslipped under PermaFluor and reconstructed in 3D at 400x using a Zeiss Axiophot 2 Microscope and Neurolucida software (MicroBrightField, Williston, VT). Dendrograms and

3D Sholl analyses were generated for each neuron using NeuroExplorer software (MicroBrightField). Dependent measures included total basal dendritic material, total apical dendritic material, total apical branch number, and quantity of apical dendritic material and number of intersections per radial distance from the cell body, in 30-μm increments. Inclusion in the analysis required that neurons lie within layer II/III of anterior cingulate or lateral orbitofrontal cortex; exhibit complete filling of dendritic arbors as evidenced by well-defined endings; and display pyramidal cell morphology (Radley et al., 2004; 2006).

Results

After 21 days of repeated restraint, stressed rats appeared well groomed and healthy such that they were indistinguishable from controls. However, stressed rats weighed significantly less than controls ($t = 2.96$, $p = 0.007$), consistent with previous reports (Watanabe et al., 1992; Radley et al., 2004; 2006). Earlier studies have confirmed that the 21-day repeated restraint stress model induces increased plasma corticosterone and increased adrenal weights (Watanabe et al., 1992; Magarinos and McEwen, 1995) so these assays were not performed here.

Effects of stress on dendritic morphology in ACg and OFC. Figure 3.3 depicts the results of our cell loading procedure, including a typical layer II/III orbitofrontal pyramidal cell and Neurolucida tracings of typical ACg and OFC pyramidal cells from stressed rats and controls. 72 lateral OFC cells (36 stressed, 36 controls) and 54 ACg cells (16 controls, 38 stressed) met the criteria for inclusion in the morphometric analysis.

Repeated restraint stress induced contrasting effects on apical dendrites in ACg and lateral OFC. Consistent with previous reports (Radley et al., 2004), medial frontal apical dendritic material ($t = 2.83$, $p = 0.007$) and branching ($t = 1.99$, $p = 0.05$) were reduced by 20% and 11%, respectively, in stressed animals relative to controls (Figure 3.4A), whereas basal dendritic material was unaffected ($t = 0.41$, $p = 0.69$). A Sholl analysis (Figure 3.4C) revealed main effects of stress ($F_{(1,52)} = 8.17$, $p = 0.006$) and radial distance from cell body ($F_{(9,468)} = 84.69$, $p<0.001$) on apical dendritic material. Post-hoc contrasts indicated that the effect of stress was most pronounced at distances of 90 ($t = 2.04$, $p = 0.05$), 150 ($t = 2.31$, $p = 0.03$), and 180 ($t = 2.32$, $p = 0.05$) microns.

By contrast, stress induced a proliferative effect in lateral OFC that extended to more distal aspects of the apical dendrite. Total apical dendritic material ($t = 4.64$, $p < 0.001$) and branching ($t = 3.64$, $p = 0.001$) increased

Figure 3.3: Neurolucida Tracings of mPFC and OFC Pyramidal Cells. **A)** Coronal hemisection of the prefrontal cortex (Bregma +3.20 μm, adapted from Swanson, 1992) depicting anterior cingulate (ACg) and lateral orbitofrontal (OFC) regions of interest. **B)** and **C)** Dendritic reconstructions of neurons from ACg (B) and lateral OFC (C), with apical dendrites highlighted in blue (controls) and red (stressed). **D)** A typical pyramidal neuron from lateral OFC, with the apical dendrite (arrow) extending from the soma toward the pial surface at right and the axon (arrowheads) extending to the left. Scale bar = 50 μm.

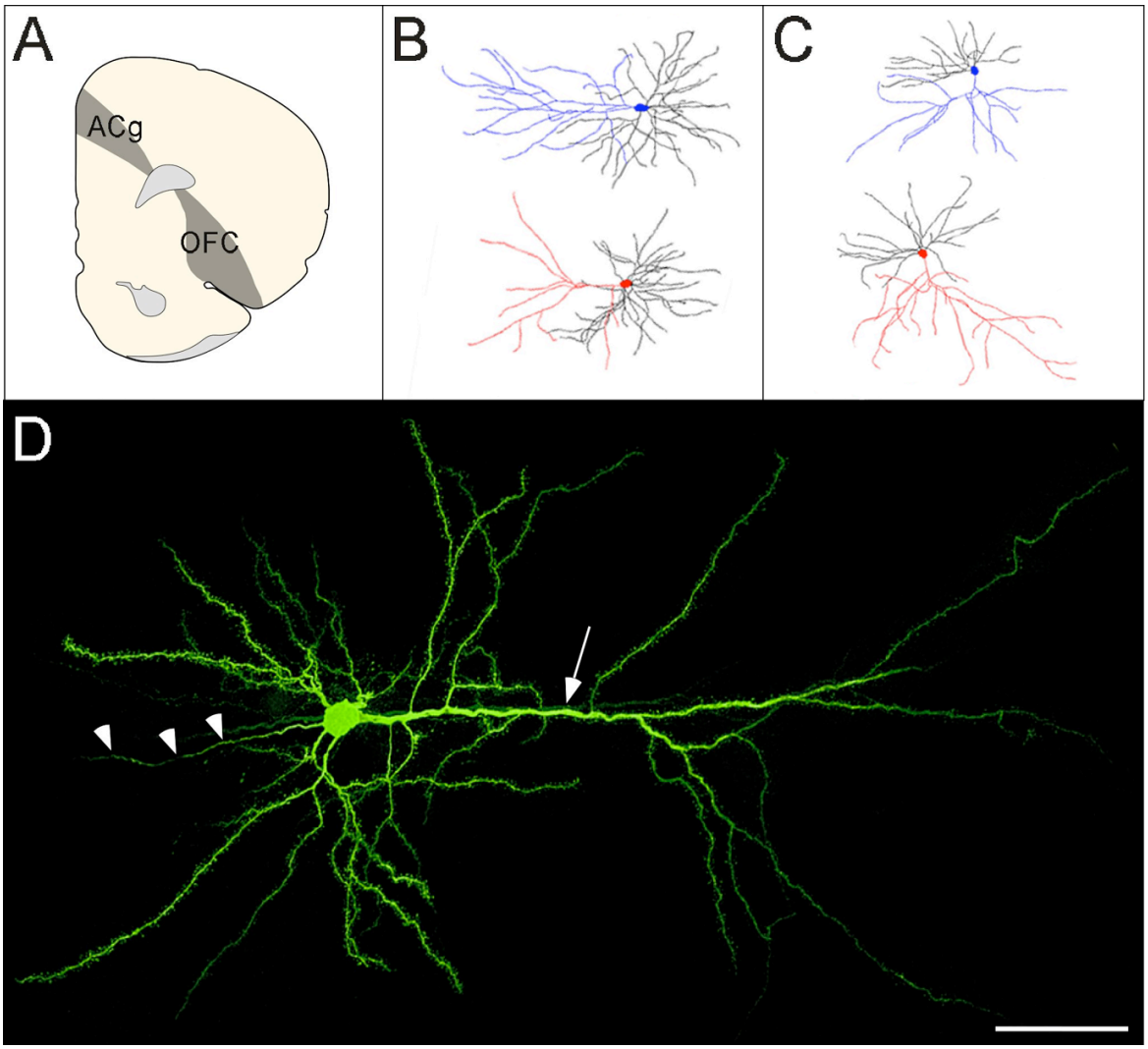
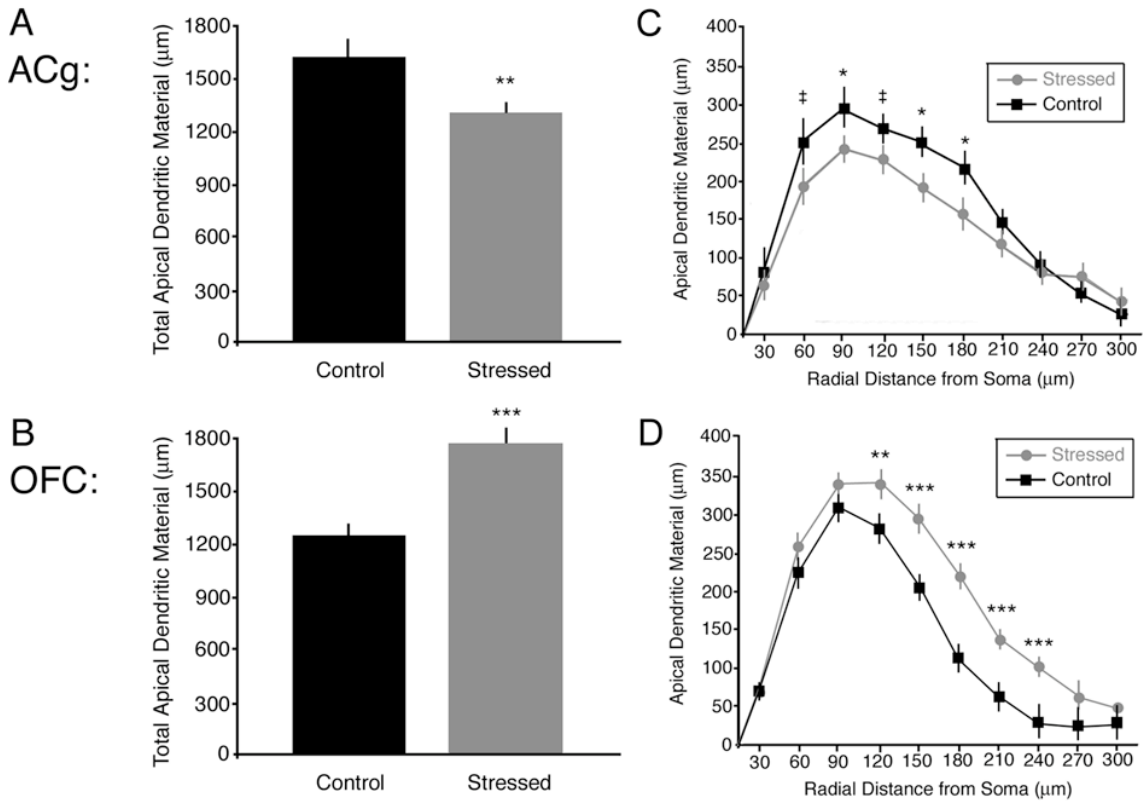


Figure 3.4: Effects of Stress on Dendritic Morphology. Chronic stress induced contrasting effects on apical dendritic arborization in ACg and OFC. **A)** and **B)** In ACg (A), total apical dendritic material decreased with stress, whereas a stress-related increase in apical dendritic material was observed in OFC (B). **C)** and **D)** Sholl analyses. Stress effects on apical dendritic arborization were most pronounced at distances of 90-180 μm from the soma in ACg (C). In OFC (D), stress affected more distal aspects of the dendrite, with significant increases at 120-240 μm. Error bars = SEM. ‡ $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.



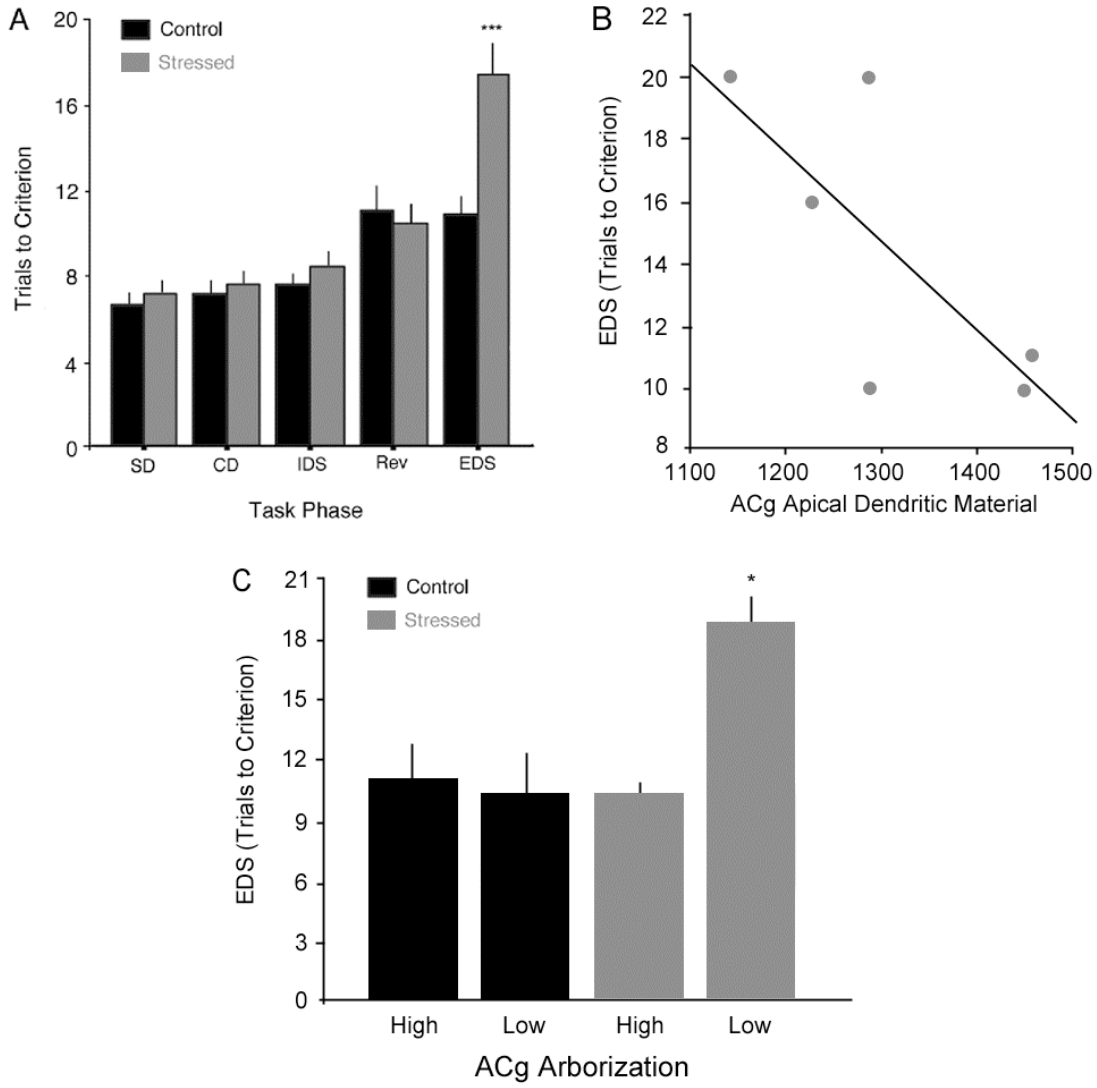
by 43% and 36%, respectively, in stressed animals relative to controls (Figure 3.4B). As in ACg, no significant differences were observed for basal dendritic material ($t = 0.94$, $p = 0.35$). A Sholl analysis (Figure 3.4D) revealed main effects of stress ($F_{(1,70)} = 18.50$, $p < 0.001$) and radial distance ($F_{(9,630)} = 213.88$, $p < 0.001$) on apical dendritic material. Post-hoc contrasts revealed stress-related increases in apical dendritic material at distances of 120 to 240 microns from the cell body ($t > 2.71$, $p = 0.009$). A significant region by group by distance interaction ($F_{(9,1098)} = 4.96$, $p < 0.001$) confirmed that stress affected more distal aspects of the apical dendrite in OFC relative to ACg.

Effects of stress on attentional set shifting. Repeated restraint stress induced a selective impairment in extradimensional attentional set shifting, but not discrimination or reversal learning (Figure 3.5A). An overall ANOVA with task phase as a within-subjects factor and group (stressed or control) and initial relevant dimension (medium or odor) as between-subjects factors revealed main effects of task phase ($F_{(4,80)} = 26.6$, $p < 0.001$) and group ($F_{(1,20)} = 7.63$, $p = 0.01$) and a task phase by group interaction ($F_{(4,80)} = 5.85$, $p < 0.001$). *Post hoc* analyses demonstrated that this interaction was driven by the EDS phase, with stressed rats significantly impaired relative to

Figure 3.5 (see following page): Effects of Stress on Task Performance.
A) Chronic stress selectively impaired extradimensional attention shifting (EDS) leaving discrimination learning (SD, CD) and reversal learning (Rev) unaffected.
B) Stress-related decreases in ACg apical dendritic material predicted attentional impairments in the EDS phase ($r = -.74$, $p = .09$). No association was observed in controls (data not shown).
C) Stressed rats and controls were divided into four groups based on median splits of their respective ACg apical dendritic lengths. Stressed rats (gray) with the largest ACg morphologic effects ("low ACg arborization") showed significant attention shifting impairments, while stressed rats with minimal morphologic changes ("high arborization") performed comparably to controls. No association between ACg arborization and attention shifting was observed in controls. Error bars = SEM. * $p < 0.05$; *** $p < 0.005$.

Figure 3.5 (see following page): Effects of Stress on Task Performance.

See caption on preceding page.



controls ($t = 3.51$, $p = 0.002$) and no significant between-group differences on any other task phase ($t < 1.33$, $p > 0.20$). Importantly, stressed rats were not impaired on reversal learning ($t = 0.43$, $p = 0.67$), which in control rats was equivalent in difficulty to the EDS. This indicates that the effect of stress was specific to attention shifting and did not reflect a generalized learning impairment. No other main effects or interactions were observed in this analysis ($p > 0.60$).

The validity of the task design was confirmed with a separate within-group ANOVA examining control rats only, with task phase as a within-subjects factor and initial relevant dimension (medium or odor) as a between-subjects factor. As expected, there was a main effect of task phase ($F_{(4,40)} = 8.73$, $p < 0.001$). The EDS was significantly more difficult than the IDS ($t = 3.65$, $p = 0.001$), confirming the validity of the extradimensional attention shifting manipulation. There was no main effect of initial relevant dimension or interaction of this effect with other factors ($p > 0.40$), indicating that the odor-to-medium and medium-to-odor shifts were equivalent in difficulty.

Analysis of individual differences in morphology and attention shifting. To assess whether intersubject morphologic variation predicted behavioral

performance, we computed mean apical dendritic lengths for each region in each subject. Rats with the largest stress-related morphologic alterations showed the greatest impairments in attention shifting. In the stressed group, rats with the greatest stress-related retractions of ACg apical dendrites tended to show larger attention-shifting impairments (Figure 3.5B: $r = -0.74$, $p = 0.09$). No association between ACg arborization and attention was detectable in controls ($r = -0.54$, $p = 0.35$). To confirm these results, we classified stressed and control rats into four groups based on median splits of their respective ACg arbors. ANOVA confirmed a main effect of this grouping on EDS performance ($F_{(3,8)} = 7.35$, $p = 0.01$). Stressed rats with the largest ACg morphologic alterations were significantly impaired on the EDS phase relative to rats in the other three groups (Figure 3.5C: $t > 3.43$, $p < 0.026$), while stressed rats with lesser morphologic alterations performed equivalently to controls on the EDS phase ($t < 0.38$, $p > 0.72$). By contrast, ACg arborization in control rats had no effect on EDS performance ($t = 0.25$, $p = 0.82$), in accord with the correlations reported above.

Discussion

Our results indicate that chronic stress induces contrasting morphologic effects in medial and lateral orbitofrontal cortices. In accord with previous

reports (Radley et al., 2004; 2006), chronic stress was associated with a 20% decrease in apical dendritic material in the anterior cingulate region of mPFC. By contrast, stress induced a 43% increase in apical dendritic material in layer II/III pyramidal cells of lateral OFC. Accordingly, stress selectively impaired extradimensional attention shifting, which depends on mPFC function, but not reversal learning, an OFC-dependent function.

Our morphologic results from ACg are in agreement with several previous studies using the Golgi impregnation and iontophoretic cell loading techniques (Radley et al, 2004; 2006; Cook and Wellman, 2004). In separate studies using iontophoretic cell loading, Radley and colleagues (2004; 2006) observed decreases in medial prefrontal apical dendritic material of 20-22%, in close accord with the 20% reduction reported here. The design of these studies was identical to that employed here, except that rats in our study were maintained on a restricted diet for the last ten days of the experiment. The fact that our results are in close agreement suggests that dietary restriction did not confound our findings. Indeed, numerous reports indicate that mild dietary restriction can prolong life span, ameliorate age-related declines in physiologic functions, and reduce the incidence of autoimmune disease (e.g. Weindruch and Walford, 1988; Kubo et al., 1992). The fact that these results were highly replicable also highlights the utility of

iontophoretic cell loading, which facilitates more precise morphometry relative to the Golgi technique by ensuring that cells are completely filled and by eliminating overlapping dendritic fields.

Although several studies have reported decreased apical dendritic arborization in ACg with stress, our OFC morphology findings are, to our knowledge, the first report of stress-related increases in arborization in any region of frontal cortex. This finding is interesting, given the well-established role of stress as a risk-factor for drug abuse and addiction, which in turn has been linked to altered orbitofrontal cortical function in both humans and animal models (Schultz, 2000; Volkow and Fowler, 2000). There is considerable evidence to suggest that atypical stress responses may play a causative role in the development of addictive states (Kreek, 1996; Koob and LeMoal, 1997; Koob and Le Moal, 2001; Kreek et al., 2005). The results reported here raise the possibility that stress-induced alterations in dendritic morphology in the orbitofrontal cortex may contribute to these effects.

Two previous studies reported contrasting effects of chronic stress on hippocampal and amygdaloid pyramidal cells, with cells of the basolateral amygdala undergoing marked increases in apical dendritic arborization and spine density (Vyas et al., 2002). The mechanisms by which chronic stress

induces contrasting morphologic effects in these two regions remain unclear. As described in Chapter 1, previous studies indicate that glucocorticoids and excitatory neurotransmitters may act in concert in the hippocampus to induce dendritic atrophy (Magarinos and McEwen, 1995). Glucocorticoids also act to enhance calcium currents in hippocampal pyramidal cells, which can induce dendritic remodeling, and it has been suggested that the contrasting effects of stress on hippocampal and amygdaloid plasticity may be attributable to differences in the spatiotemporal dynamics of intracellular calcium concentrations (Kerr et al, 1992; McEwen, 2000). Neuronal cell adhesion molecules (Sandi, 2004) and serotonergic neuromodulatory influences (Conrad et al., 1996; Stutzmann et al, 1998) may also play a prominent role. Similar pharmacologic manipulations may shed light on the mechanisms by which stress induces contrasting effects in ACg and lateral OFC.

Given these contrasting morphologic effects, the selective impairment in attention shifting can be easily understood in the context of previous lesion studies. In separate studies, Brown and colleagues demonstrated a double dissociation by which mPFC lesions impair attention shifting but not reversal learning (Birrell and Brown, 2000), while OFC lesions impair reversal learning but not attention shifting (McAlonan and Brown, 2003).

Our results confirm that chronic stress reduces apical dendritic material by ~20%, which, in combination with a 16% reduction in spine density (Radley et al., 2006), may lead to a 33% reduction in axospinous input to layer II/III apical dendrites in ACg. Layer II/III pyramidal cells are both the origin and target of long-range corticocortical connections and are likely to play an important computational role in cognitive (e.g. attentional) functions mediated by a distributed network of structures (Dehaene et al., 1998). As such, it is likely that a stress-related reduction in axospinous input to these cells contributed to the selective impairment in attention shifting (Figure 3.5A).

This hypothesis is in accord with a recent report demonstrating an association between corticosteroid-induced atrophy of mPFC layer II and impairments in behavioral flexibility (Cerqueiras et al., 2005). It is also supported by our observation that the magnitude of ACg morphologic alterations predicted the degree of attentional impairment (Figure 3.5B-C). Interestingly, this correlation was specific to stressed rats: ACg arborization was not associated with task performance in controls, and only rats with the largest stress-induced retractions of ACg arbors showed significant attentional impairments, suggesting that the circuitry may be resilient to smaller variations in axospinous input.

Although earlier lesion studies provide a framework for understanding the effects reported here, a few anatomical distinctions are worth noting. In particular, the lesions in the Birrell and Brown (2000) study extended into regions of both dorsal mPFC—including ACg and prelimbic cortex—and ventral mPFC (infralimbic cortex) in all animals, so the mPFC contribution to attention shifting cannot be localized to ACg based on these results. In a series of similar studies, Ragozzino and colleagues (1998; 1999) examined the effects of dorsal versus ventral mPFC lesions on two measures of behavioral flexibility and found that only the latter impaired performance. However, like Birrell and Brown's work, their ventral mPFC lesions encompassed an extensive swath of cortex, including IL, PL, and ventral ACg, whereas their dorsal mPFC lesions were relatively limited, including only the medial precentral area (Fr1 in the parlance of Paxinos and Watson) and the dorsal tip of ACg (so-called Cg1). Thus, dysfunction in PL and ventral ACg may have contributed to the behavioral deficits in the ventral group, while the limited involvement of ACg in the dorsal group may have spared performance.

Indeed, converging findings from a variety of studies (reviewed in Heidbreder and Groenewegen, 2003) indicate that ventral mPFC, including infralimbic cortex, functions primarily in the top-down regulation of

autonomic responses (e.g. HPA axis regulation as discussed in Chapter 1) and in conditioned fear learning paradigms (for a review, see Gabbott et al., 2005). By contrast, dorsal mPFC—including ACg and dorsal PL—is thought to play a critical role in purely cognitive functions such as decision making, response selection, spatial learning, attention, and working memory (Stuesse and Newman, 1990; Harrison and Mair, 1996; Kesner and Ragozzino, 2003; Dalley et al., 2004). These functions are consistent with region-specific patterns of afferent and efferent connections. Whereas dorsal mPFC has reciprocal connections with the dorsal striatum and sensorimotor and neocortical association areas, ventral mPFC has more extensive connections with the ventral striatum, amygdala, and limbic association cortex (Heidbreder and Groenewegen, 2003; Gabbott et al., 2005). Collectively, these results support the idea that rodent dorsal mPFC, rather than infralimbic cortex, may play a critical role in attentional control and other functions ascribed to lateral PFC in primates. They are also consistent with our hypothesis that stress-related reductions in axospinous input to ACg may contribute to impairments in attentional control.

By contrast, enhanced OFC arborization was not associated with a significant enhancement in reversal learning. This may be due in part to a ceiling effect, whereby the speed of reversal learning observed in control

rats cannot be substantially improved upon by enhanced OFC inputs. Alternatively, it is likely that attention shifting and reversal learning are both mediated by multiple structures acting in concert (Fox et al., 2003; O'Reilly et al., 2002; Liston et al., 2006), and our observations may reflect alterations in other regions not examined here. Future work will target additional regions of association cortex and examine how they interact to mediate these behaviors. Likewise, our results point to an association between ACg arborization and attentional control, but they do not rule out contributions from other stress-dependent factors. For example, some reports suggest that mPFC-dependent cognitive functions may be modulated by stress-related alterations in noradrenergic (Roozendaal et al., 2004) and dopaminergic (Mizoguchi et al., 2000) inputs to mPFC, which in turn may reflect local post-synaptic structural changes consistent with our results or pre-synaptic changes specific to the cells that are the source of these projections. In either case, our findings highlight the need for more detailed anatomical and pharmacological studies that could distinguish between these possibilities.

Collectively, these results show that chronic stress induces contrasting morphologic effects in lateral OFC and ACg, which in turn predict the severity of impairments in attention shifting. They provide direct evidence that prefrontal dendritic remodeling may contribute to the attentional

impairments that are symptomatic of depression and other stress-related psychiatric disorders. Experiments reported in the following chapter examine the extent to which comparable effects are detectible in human subjects.

CHAPTER 4:

Chronic Stress Effects on Attentional Control and Prefrontal Functional Connectivity in Humans

In the experiments reported in Chapter 3, chronic stress induced contrasting morphologic alterations in mPFC and lateral OFC, coupled with a predictable pattern of behavioral effects. In mPFC, stress induced a 20% retraction of apical dendritic arbors and a corresponding impairment in attentional set-shifting that correlated with the magnitude of the morphologic effect across subjects. In contrast, stress was not found to adversely affect reversal learning or dendritic morphology in lateral OFC, where arborization increased by 43%.

The goal of the experiments reported in this chapter was to assess whether similar effects are detectible in chronically stressed human subjects using functional MRI measurements of regional brain activity and an analogous behavioral assay, characterized in detail in Chapter 2. As described in Chapter 1, this question is an important one by virtue of its implications for an improved understanding of the association between stress, mood disorders, and the prefrontal cortical anomalies that characterize them, but it has not been addressed elsewhere, perhaps because chronic stress effects on the prefrontal cortex have only recently been identified (Cook and Wellman, 2004; Radley et al., 2004; Izquierdo et al., 2006; Radley et al., 2006). A complementary body of literature delineates the effects of stress on prefrontal function acutely, highlighting the

significance of short-term stress-related changes in monoaminergic neuromodulatory inputs to the PFC for working memory and attention (Arnsten, 1998); these findings are discussed in greater detail at the end of this chapter.

The approach adopted here complements this body of work. Effects of chronic stress on attentional control and the functional integrity of the frontoparietal network that subserves it were assessed in a cohort of healthy human subjects. In Chapter 3, chronic stress induced a selective impairment of attention shifting and a corresponding retraction of the apical dendrites of layer II/III pyramidal cells in the rat mPFC. Layer II/III pyramidal cells are both the origin and target of long-range corticocortical connections and are likely to play an important computational role in cognitive (e.g. attentional) functions mediated by a distributed network of structures (Dehaene et al., 1998). As such, it was predicted that chronic stress in human subjects would be associated with reduced functional coupling within the dorsolateral frontoparietal attentional network and a selective impairment of attentional shifts, while sparing response reversals.

Subjects also returned for a second scanning session, approximately four weeks after the first. Half of these subjects were chronically stressed medical students who had spent 4-5 weeks prior to the first session preparing

for the United States Medical Licensing Exam, which was followed by 4-5 weeks of rest, relatively free of major academic responsibilities. Thus, the second scanning session, which facilitated within-subjects, pairwise comparisons, served two purposes: 1) to assess whether stress effects observed in session one were reversible; and 2) to control for the confounding influences of intersubject variability unrelated to stress. Based on previous work showing that alterations in dendritic morphology in mPFC are reversible after four weeks in rats (Radley et al., 2005), it was hypothesized that task performance and functional coupling would improve in session two in medical students but not in a group of controls matched for age, gender, and IQ.

Experimental Procedures

Subjects. 46 right-handed, healthy young adults (22 males; mean age = 25.9 years) participated in this study. 24 subjects (12 males; mean age = 25.0 years) were second-year medical students preparing for the United States Medical Licensing Exam. They were scanned initially after 3-4 weeks of intensive exam preparation, approximately 7-10 days prior to the examination, which in turn was followed by a month of rest, free of any major academic responsibilities. 21 of these subjects (11 males; mean age =

25.0 years) returned for a second scanning session at the end of this period, approximately five weeks after the first session. Of the remaining 22 subjects, 21 (10 males; mean age = 27.0 years) returned for a second session approximately five weeks after the first. The procedure for the second session was identical to the first.

All subjects were screened for contraindications for MRI and a history of any psychiatric or neurological conditions. The experimental procedure was approved by the Weill Medical College of Cornell University IRB, and written informed consent was obtained from all subjects prior to scanning.

Perceived Stress Quantification. Stress was quantified by self-report at the start of each session using the Perceived Stress Scale (PSS) Questionnaire, a standardized and reliable measure of an individual's perception of chronic stress that yields a numerical score on a scale of zero to forty. A PSS score exceeding one standard deviation above the established population mean for this age group served as an exclusion criterion for the control group. The PSS questionnaire is described in greater detail in Appendix 4.

Behavioral Paradigm. All subjects were tested on the attentional control paradigm developed in Chapter 2. On each trial, subjects were presented

with two circular square-wave gratings, one red and one green, each subtending 4.6^{O} of visual space at an eccentricity of 4.6^{O} from fixation, for 1500 ms. Each grating moved either up or down. A centrally located cue ("M" or "C") instructed the subject to attend to either the motion or the color of the stimuli. If the cue was an "M", the subject responded by choosing the side with the upward moving grating, regardless of color. If the cue was a "C", the subject responded by choosing the side with the red grating, regardless of motion (see Figure 2.1). Repeat trials were defined as those preceded by 2-5 trials of the same dimension. Shift trials were those preceded by 2-5 trials of the opposite dimension. Trials also varied with the congruency of the correct stimulus-response mapping.[1] In a congruent trial, the red grating was also the upward moving grating so the correct response was the same in both dimensions. In an incongruent trial, the red grating was downward moving, and the green grating was upward moving so the correct response depended on the task cue (see Figure 2.2B).

Each trial ended with a centrally located white fixation cross, subtending 1.2^{O} of visual space, with a variable duration (500-12,500 ms). Reaction times and accuracies were recorded for all trials using the E-Prime and IFIS software packages (Psychology Software Tools, Pittsburgh, PA).

[1] Note that these manipulations are identical to those described in Chapter 2, though the stimulus conflict manipulation is not considered here.

The task was designed to capture the principal features of the attentional set-shifting task used in Chapter 3, namely dissociable measurements of attention shifting and response reversals. Attention shifts were assessed by contrasting shift trials with dimension-matched repeat trials. Response reversals were assessed by contrasting incongruent shift trials with congruent shift trials. On incongruent shift trials, the subject was required not only to shift his attention to the newly relevant dimension but also to override the response that would have been correct in the previous dimension.

Prior to each scanning session, subjects were trained on 3 blocks of 36 trials consisting of color discriminations, motion discriminations, and alternating color/motion discriminations, respectively. In the scanner, subjects completed 6 blocks of 72 trials, which were presented in a jittered task design. Counterbalancing procedures and other details of the task design are described in Appendix 1.

MRI Procedure. Functional and 3D high-resolution anatomical images were acquired on a GE 3-Tesla MRI scanner using a quadrature head coil. Functional MR images were preprocessed and coregistered to the anatomical volume using the BrainVoyager QX software package (Brain Innovations,

Maastricht, The Netherlands). MRI parameters and preprocessing procedures are described in Appendix 2.

Behavioral Data Analysis: Effects of Stress on Attentional Control. Reaction time (RT) and accuracy were recorded for all trials, and only correct trials were included in reaction time analyses. Main effects of attention shifts and response reversals and interactions between these factors were explored in detail in Chapter 2 and are not considered here. The goal of the present study was to examine how chronic stress modulates these functions. To this end, subjects were classified into four groups based on quartile splits of their perceived stress scores. These groups were matched for age and gender (see Table 4.1 for details). Four-level (PSS grouping) one-way ANOVA was used to detect main effects of stress on behavioral measures (described below), and post-hoc t-tests were used to identify significant between-group differences. For each analysis, boxplots were visually inspected for outliers, which were defined as data points that differed from the group mean by more than two standard deviations in either direction on either dimension. Outliers were excluded from analysis. No more than two outliers were excluded in any analysis unless otherwise stated in the text.

Table 4.1: Demographic Details by Group. All groups were matched as closely as possible for age and gender.

			Gender	
Group	**N**	**Mean Age (years)**	**Male**	**Female**
Overall	46	25.9	22	24
PSS ANOVA:				
1st Quartile (PSS < 10)	12	25.8	5	7
2nd Quartile (10 < PSS < 14)	12	25.6	7	5
3rd Quartile (14 < PSS < 18)	12	25.8	6	6
4th Quartile (PSS > 18)	10	26.5	4	6
Reversibility:				
Medical Students	21	25.0	11	10
Non Med Students	21	27.0	10	11

To minimize the need for statistical corrections for multiple comparisons, dependent measures for these analyses comprised a selected set of variables identified *a priori* based on findings reported in Chapters 2 and 3. Attention shifts were quantified for each subject in terms of the reaction time cost associated with a shift trial corrected for repeat trial reaction times (i.e. mean shift RT – mean repeat RT). Response reversals were quantified in terms of the cost associated with an incongruent shift relative to a congruent one (i.e. mean incongruent shift RT – mean congruent shift RT). These two variables were the primary measures of interest, based on the results described in Chapter 3. Accuracy costs were not considered in this analysis for two reasons: 1) they were found to be irrelevant for attention shifts after controlling for the effect of response reversals in Chapter 3, and 2) RT costs were found to be more sensitive measures of both functions (see Figure 2.2). However, in a secondary analysis, shift and repeat accuracy were examined to rule out confounding influences of a speed/accuracy trade-off.

MRI Data Analysis: Stress Effects on PFC Function. Analysis of functional imaging data occurred in two steps. The goal of the first step was to identify salient areas that were engaged during attention shifts and response reversals

for use as regions of interest in the two subsequent analyses, thus confirming the validity of the results reported in Chapter 2 in this larger sample of subjects. After preprocessing, Z-normalized functional timecourses for all subjects were analyzed together based on the least mean squares solution to a general linear model in which trial type (shift or repeat) and response congruency (incongruent or congruent) were the primary predictors. Only correct trials were included in these predictors. As described above, attention shifting circuitry was identified by contrasting shift trials with repeat trials, and response reversal circuitry was identified by contrasting incongruent shifts with congruent shifts. Each contrast analysis was performed based on wholebrain voxelwise t-tests of the difference between the beta weights of the relevant predictors using a random effects analysis. Both contrasts were thresholded at $p < 0.005$ with a minimum cluster size of eight contiguous voxels (~320 transformed voxels) to minimize the likelihood of a Type I error. Monte Carlo simulation confirmed that the probability of a Type I error was less than 0.05 using these criteria (Forman et al., 1995).

The goal of the second step was to investigate the effects of chronic stress on functional coupling within the two networks identified above by adapting analytical tools developed elsewhere (Pezawas et al., 2005).

Functional coupling between a given pair of regions was assessed in each subject by regressing the functional timecourse for the first region (i.e. the mean signal in that cluster) on the second region's timecourse. The resultant subject beta values then served as dependent measures in a four-level (PSS grouping) one-way ANOVA based on quartile splits of perceived stress scores, using post-hoc t-tests to identify significant between-group differences, as described above. Projections to the lateral prefrontal areas that may be homologous to the regions examined in Chapter 3 were of primary interest here.

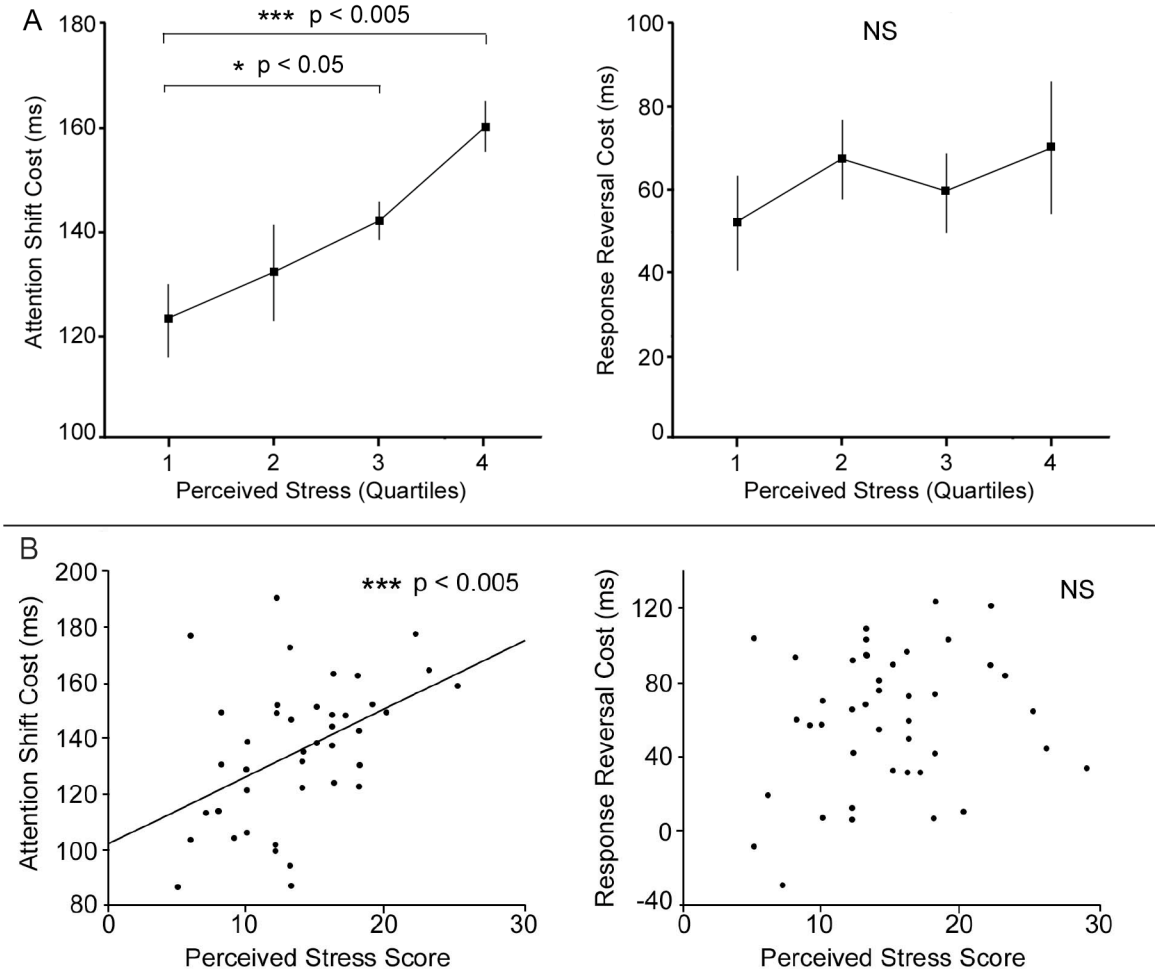
Reversibility of Stress Effects. All subjects were requested to return for a second scanning session approximately one month after the pre-exam session. Reversibility of stress effects was assessed in terms of within-subject, pair-wise t-tests of pre- and post-test PSS scores, attention shifting costs, and measures of functional coupling in medical students. The remaining subjects served as a control for between-session changes in performance attributable to task experience or other factors not related to stress. The within-subject design also provided an additional control for the confounding influences of between-subject variations unrelated to chronic stress (e.g. sample biases).

Results

Effects of stress on behavioral measures. Stress selectively impaired attention shifts but not response reversals (Figure 4.1A). Attention shifting costs tended to increase with stress ($F(3,38) = 3.55$, $p = 0.02$), while response reversal costs were unaffected ($F(3,41) = 0.49$, $p = 0.69$).[2] Accordingly, increased PSS scores predicted larger attention shifting costs across subjects (Figure 4.1B: $r = 0.45$, $p = 0.003$). By contrast, response reversal costs were not associated with PSS scores (Figure 4.1B: $r = 0.02$, $p = 0.91$). This effect was driven chiefly by subjects who reported supra-median PSS scores (i.e. greater than 14 out of 40). Subjects in the second quartile did not differ significantly from subjects in the lowest quartile, whereas subjects in both the third ($t = 2.50$, $p = 0.02$) and fourth ($t = 3.29$, $p = 0.005$) quartiles showed significantly elevated attention shifting costs. Among these subjects, PSS scores were strongly predictive of increased attention shifting costs ($r = 0.61$, $p = 0.005$), but not among subjects with lesser PSS scores ($r = 0.14$, $p = 0.53$).

[2] It should be noted that the shift cost analysis excluded three outliers at the high end of the perceived stress scale. Shift costs for these subjects were more than two standard deviations below the group mean. This effect appeared to reflect not more efficient task performance, but rather exceptionally slow repeat trial reaction times, which in turn yielded deceptively small shift costs in this subtraction. A post-hoc analysis seeks to explore the significance of this observation in greater detail and is described in Chapter 5.

Figure 4.1: Effects of Stress on Behavioral Measures. A) Chronic stress impaired attention shifting costs selectively ($F(3,38) = 3.55$, $p = 0.02$), while response reversal costs were unaffected ($F(3,41) = 0.49$, $p = 0.69$). **B)** Across subjects, perceived stress scores were positively correlated with attention shifting costs ($r = 0.45$, $p = 0.003$) but not with response reversal costs ($r = 0.02$, $p = 0.91$).



Stress effects on attention shifting did not reflect a speed/accuracy trade-off. Stress effects on accuracy were undetectable ($F(3,41) < 2.03$, $p > 0.12$), and PSS scores were not significantly correlated with shift or repeat accuracy ($r < 0.20$, $p > 0.20$).

Effects of stress on functional connectivity. Attention shifts and response reversals engaged dissociable frontoparietal networks, consistent with the results reported in Chapter 2. Attention shifts (Figure 4.2) engaged a largely dorsolateral network including bilateral dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex, right insular cortex, left premotor cortex, and bilateral posterior parietal cortex (see Table 4.2 for details). Response reversals (Figure 4.3) engaged a predominantly ventrolateral and right lateralized network including right ventrolateral prefrontal cortex (VLPFC), anterior cingulate cortex, right ventral premotor cortex, ventral aspects of posterior parietal cortex bilaterally, and the head of the right caudate nucleus (see Table 4.3 for details).

Functional connectivity analysis (described above) was used to examine how stress modulates functional coupling between 1) DLPFC and other structures in the attention shifting network and 2) VLPFC and other structures in the response reversal network. In left DLPFC, stress modulated

Figure 4.2: Attention Shifting Network. Attention shifting engaged a predominatly dorsolateral network that included bilateral dorsolateral prefrontal cortex (DLPFC), right insular cortex, anterior cingulate cortex (ACC), left premotor cortex, and bilateral posterior parietal cortex (PPC, including BA 40 and BA 7).

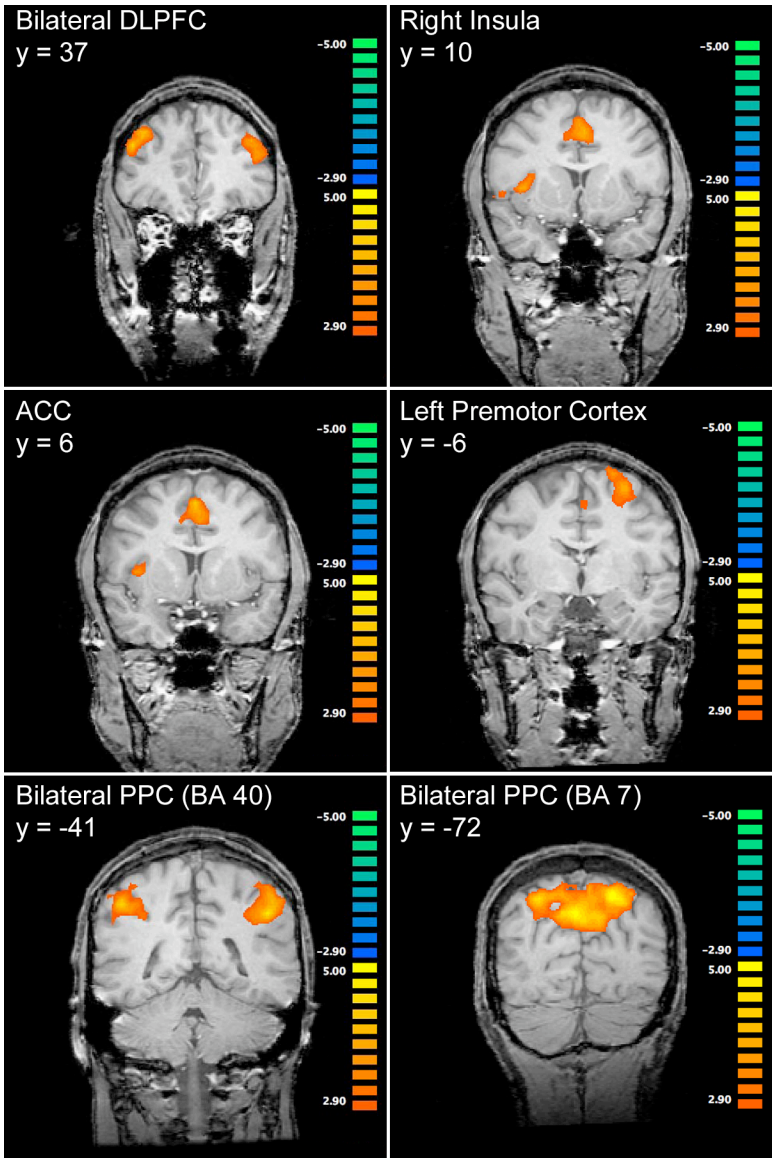


Table 4.2: Attention shifting engaged a predominantly dorsolateral frontoparietal network.

Region	BA	Talairach Coord. (x, y, z)	Peak Z Value
right dorsolateral prefrontal	8/9	36, 37, 41	4.41
left dorsolateral prefrontal	8/9	-35, 33, 36	4.45
anterior cingulate cortex	32/2	0, 6, 38	4.57
right insular cortex	44	36, 10, 8	4.00
left premotor cortex	6	-27, -6, 61	4.27
right posterior parietal cortex	40	48, -41, 43	4.69
	7	27, -72, 46	4.91
left posterior parietal cortex	40	-47, -41, 39	5.09
	7	-21, -74, 49	5.89

Figure 4.3: Response Reversal Network. Response reversals engaged a predominantly ventrolateral network that included right ventrolateral PFC (IFG), anterior cingulate cortex (ACC), right ventral premotor cortex, the right caudate nucleus, the right thalamus, and bilateral ventral posterior parietal cortex (PPC).

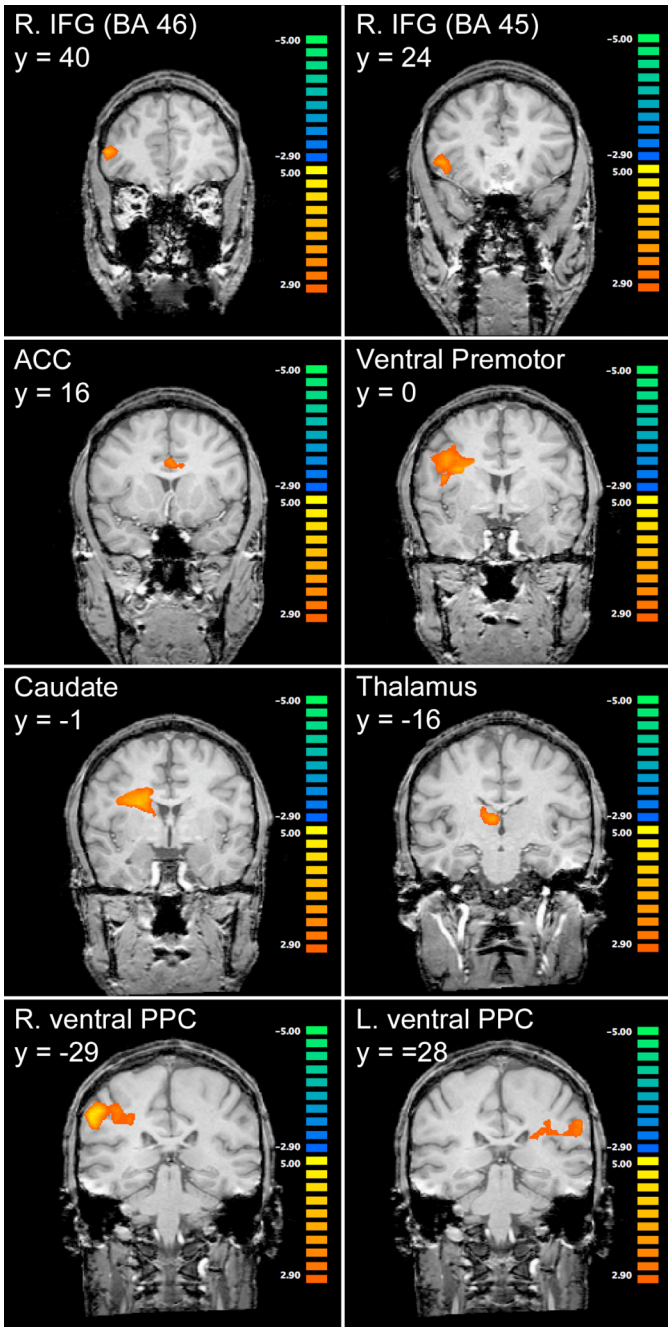


Table 4.3: Response reversals engaged a predominantly right lateralized ventrolateral frontoparietal network.

Region	BA	Talairach Coord. (x, y, z)	Peak Z Value
right ventrolateral prefrontal cortex (IFG)	46	46, 40, 16	4.31
	45	45, 24, 5	4.10
anterior cingulate cortex	24	-2, 16, 28	3.75
right ventral premotor cortex	6	41, 0, 32	4.08
right caudate nucleus	n/a	17, -1, 21	4.33
right thalamus (nucleus dorsomedialis)	n/a	9, -16, 10	4.07
right ventral posterior parietal cortex	40	59, -29, 36	5.14
left ventral posterior parietal cortex	40	-58, -28, 25	3.68

functional coupling with left premotor cortex (BA 6) and posterior parietal cortex (right BA 40 & bilateral BA 7; Figure 4.4). In all four cases, stress effects on functional coupling appeared nonlinear. Functional coupling increased with stress among subjects with sub-median PSS scores but decreased thereafter, such that coupling was maximal in the second quartile and reached a minimum in the fourth quartile at a value that was ~20% lower than the peak value (see Table 4.4B for statistics). Similar trends were observed for functional coupling in right DLPFC, but they did not approach significance. A post-hoc analysis examining all possible permutations of couplings in the attentional network indicated that this effect was specific to DLPFC. Among these (eight per region, 36 unique permutations in total), only one—coupling between left and right posterior parietal cortices—showed an effect comparable to those observed for left DLPFC, and this effect was not significant after Bonferroni correction for multiple comparisons.

Functional coupling within the ventrolateral response reversal network also varied with stress but in a manner distinct from the pattern observed in DLPFC (Figure 4.5). In right VLPFC (BA 45 & BA 46), coupling with ventral posterior parietal cortex (BA 40) and the caudate nucleus increased with stress among subjects in the first three quartiles by as

Figure 4.4: Functional Connectivity Analysis: DLPFC. A) The first functional connectivity analysis assessed whether functional coupling between DLPFC and other structures in the attention shifting network varied with stress. In left DLPFC, stress modulated functional coupling with left premotor cortex and bilateral posterior parietal cortex. Affected couplings are depicted in red. DLPFC = dorsolateral prefrontal cortex; ACC = anterior cingulate cortex; PPC = posterior parietal cortex.

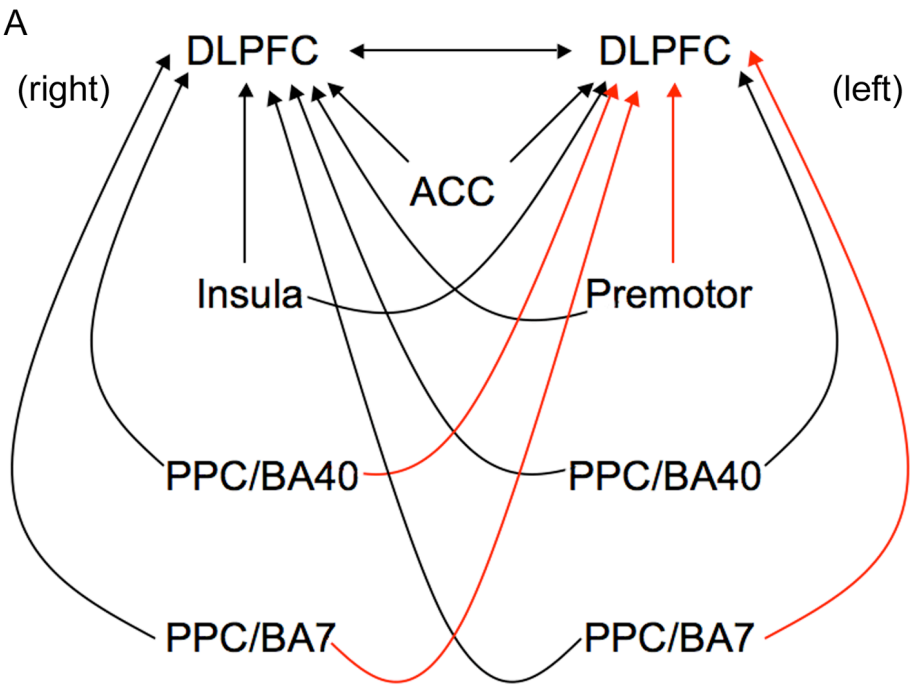


Figure 4.4B: Mean beta weights for each stress quartile are depicted for functional coupling between left DLPFC and i) left posterior parietal cortex (BA 7); ii) left premotor cortex (BA 6); and iii) & iv) right posterior parietal cortex (BA 40 and BA 7, respectively). In general, functional coupling tended to increase with stress from the first to the second quartile, peak in the second quartile, and decrease with stress thereafter. Absolute minima occurred in the fourth quartile, though these values did not differ significantly from those in the first quartile. Post-hoc t-test statistics are depicted in Table 4.4.

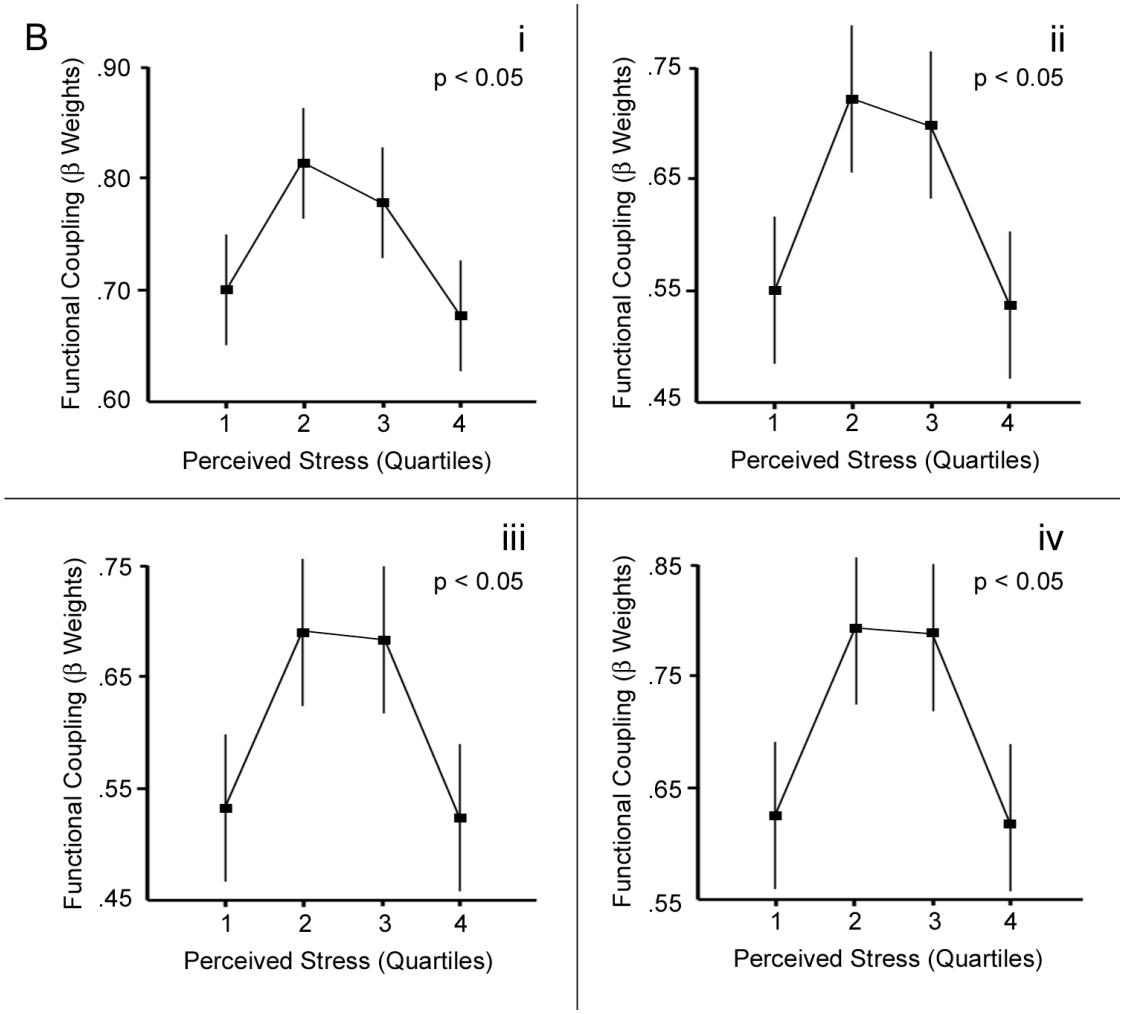


Table 4.4 (see following page): Functional Connectivity Analysis: DLPFC. In left DLPFC, stress modulated functional coupling with bilateral posterior parietal cortex (right BA 40, bilateral BA 7) and left premotor cortex. ANOVA statistics and post-hoc t-test results are presented for each effect. P values for post-hoc t-tests are corrected for multiple comparisons using Fisher's Least Significant Difference procedure. d.f. = degrees of freedom; PPC = posterior parietal cortex. * = $p < 0.05$; italics = $p < 0.10$.

Table 4.4: Functional Connectivity Analysis: DLPFC. See preceding page for caption.

Functional Coupling Between Left DLPFC and:		**F/t**	**d.f.**	**p**
Right PPC (BA 40)		3.57	3,42	**0.02***
	Post-hoc t-tests:			
	PSS1 vs. PSS2	2.06	22	**0.05***
	PSS1 vs. PSS3	2.92	22	**0.02***
	PSS1 vs. PSS4	0.08	22	0.93
	PSS2 vs. PSS3	0.64	22	0.62
	PSS2 vs. PSS4	1.82	22	**0.05***
	PSS3 vs. PSS4	2.49	22	**0.02***
Right PPC (BA 7)		3.03	3,42	**0.04***
	Post-hoc t-tests:			
	PSS1 vs. PSS2	2.46	22	**0.05***
	PSS1 vs. PSS3	3.05	22	**0.02***
	PSS1 vs. PSS4	0.17	22	0.84
	PSS2 vs. PSS3	0.59	22	0.65
	PSS2 vs. PSS4	1.46	22	*0.09*
	PSS3 vs. PSS4	1.85	22	**0.04***
Left PPC (BA 7)		3.32	3,42	**0.03***
	Post-hoc t-tests:			
	PSS1 vs. PSS2	2.55	22	**0.02***
	PSS1 vs. PSS3	2.43	22	**0.04***
	PSS1 vs. PSS4	0.09	22	0.92
	PSS2 vs. PSS3	0.29	22	0.80
	PSS2 vs. PSS4	2.07	22	**0.02***
	PSS3 vs. PSS4	1.95	22	**0.04***
Left Premotor Cortex (BA 6)		2.88	3,42	**0.05***
	Post-hoc t-tests:			
	PSS1 vs. PSS2	2.27	22	**0.03***
	PSS1 vs. PSS3	1.85	22	0.12
	PSS1 vs. PSS4	0.31	22	0.72
	PSS2 vs. PSS3	0.75	22	0.55
	PSS2 vs. PSS4	2.16	22	**0.02***
	PSS3 vs. PSS4	1.81	22	*0.07*

Figure 4.5: Functional Connectivity Analysis: VLPFC. A) The second functional connectivity analysis assessed whether functional coupling between right ventrolateral prefrontal cortex (inferior frontal gyrus) and other structures in the response reversal network varied with stress. The analysis included two distinct regions of activity in the inferior frontal gryus (BA 46 and BA 45). In BA 45, stress modulated functional coupling with the right caudate nucleus and right ventral posterior parietal cortex. Similar trends approached significance in BA 46. Significantly affected couplings ($p < 0.05$) are depicted in red. Trends ($p < 0.10$) are depicted in orange. IFG= inferior frontal gyrus; ACC = anterior cingulate cortex; PPC = posterior parietal cortex.

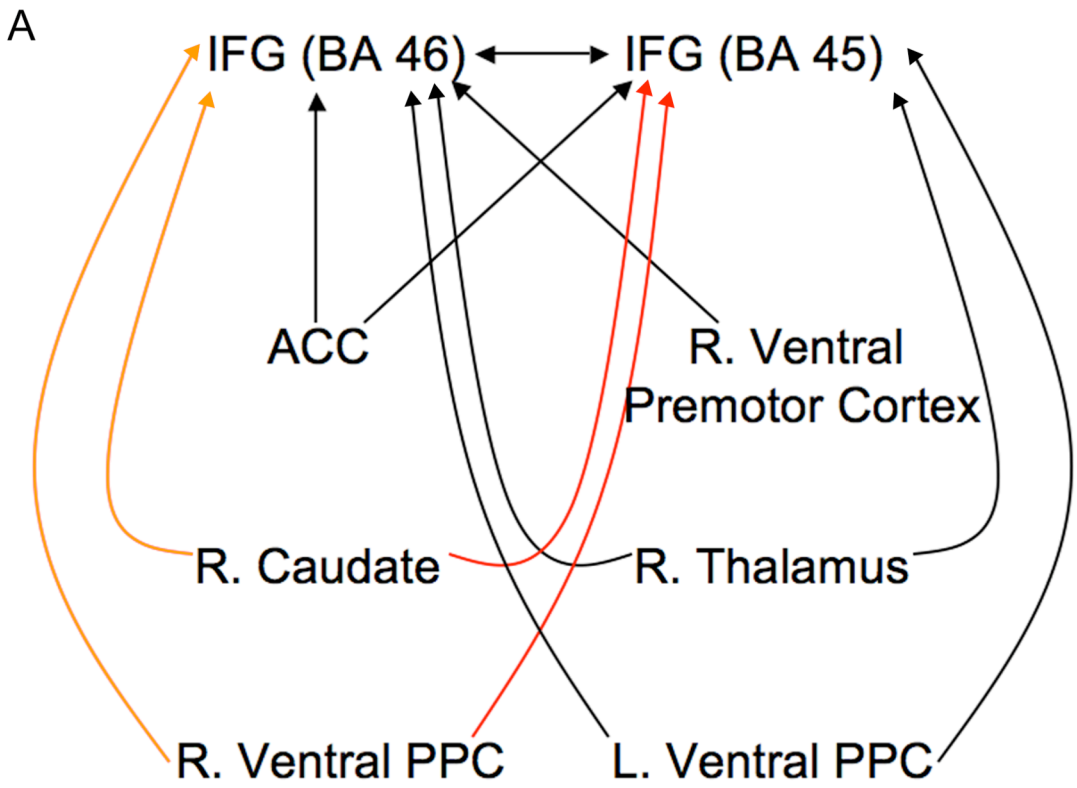
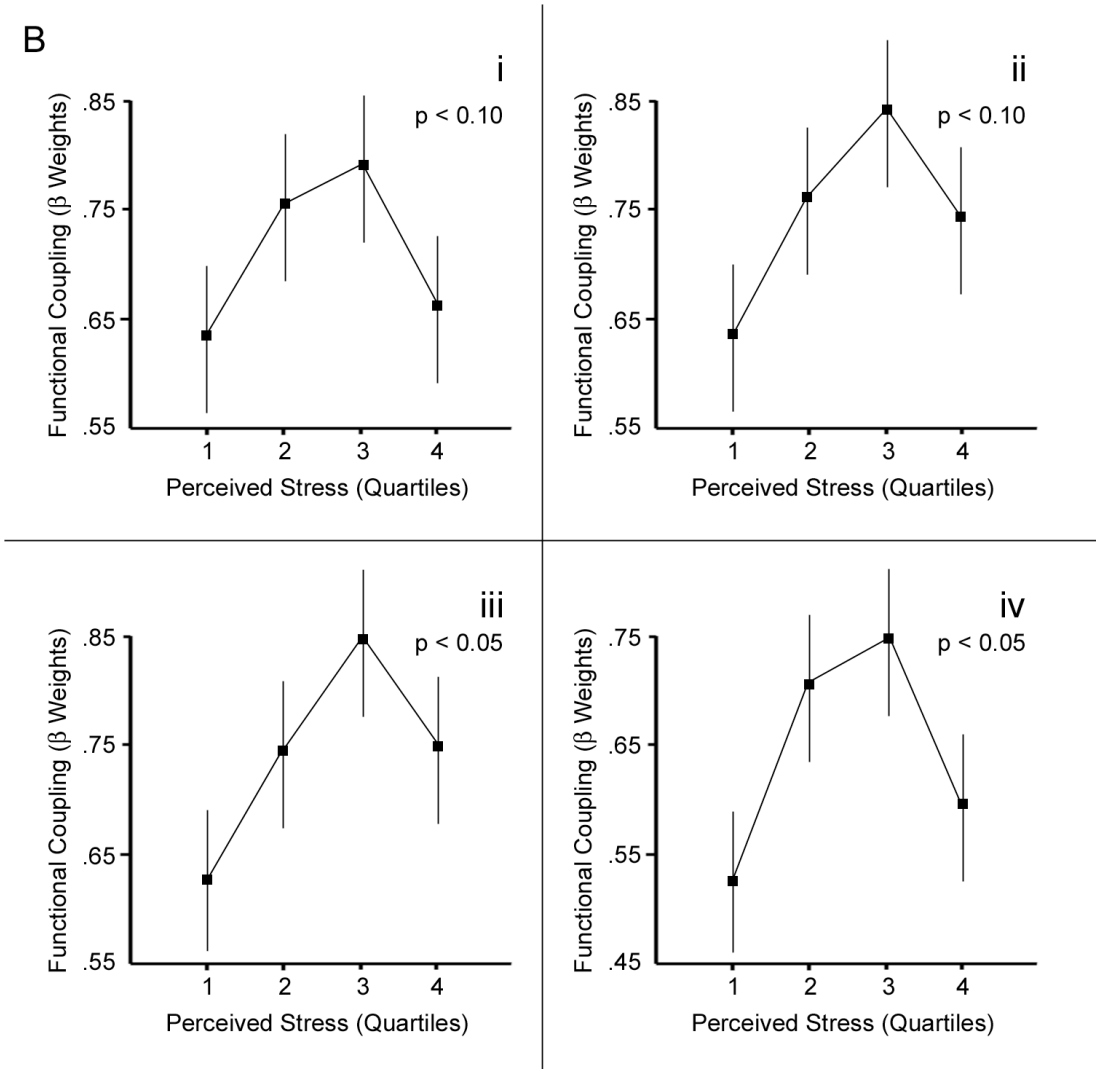


Figure 4.5B: Mean beta weights for each stress quartile are depicted for functional coupling between the ventrolateral BA 46 and i) right caudate and ii) right ventral PPC (BA 40). Panels iii) and iv) depict comparable effects of stress on coupling between BA 45 and iii) right caudate and iv) right ventral PPC (BA 40). In general, functional coupling tended to increase with stress across the first three quartiles, peak in the third quartile, and then decrease slightly thereafter. This decrease did not reach significance in all cases, and minimal values occurred in the first quartile. Post-hoc t-test statistics are depicted in Table 4.5.



much as 35%, before decreasing slightly, but in most cases significantly, in the fourth quartile, such that functional coupling was minimal in the first quartile and maximal in the third (see Table 4.5 for statistics). Again, these effects were specific. In a post-hoc analysis of all other functional coupling permutations in the response reversal network (seven per region, 28 unique permutations in total), none varied significantly with stress.

Thus, the predominant effect of stress in the ventrolateral response reversal network was to enhance functional coupling, though these gains were offset by a smaller stress-related decrease among subjects in the highest quartile. Conversely, functional coupling in the dorsolateral attentional network peaked at a lower level of stress and decreased thereafter.

Reversibility of Stress Effects. In the analyses reported above, chronic stress was associated with a selective impairment of attention shifting but not response reversals and corresponding effects on functional coupling in the prefrontal networks that subserve these functions. In order to assess the reversibility of these effects, subjects returned for a second scanning session approximately one month after the first one, thereby permitting pair-wise within-subjects comparisons.

Table 4.5 (see following page): Functional Connectivity Analysis: VLPFC. In ventrolateral prefrontal cortex (BA 45 and 46), stress modulated functional coupling with the right caudate nucleus and right ventral PPC. ANOVA statistics and post-hoc t-test results are presented for each effect. P values for post-hoc t-tests are corrected for multiple comparisons using Fisher's Least Significant Difference procedure. d.f. = degrees of freedom; PPC = posterior parietal cortex. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.005$; italics = $p < 0.10$.

Table 4.5: Functional Connectivity Analysis: VLPFC. See caption on preceding page.

Functional Coupling btw/ BA 46 and:		**F/t**	**d.f.**	**p**
Right caudate		2.68	3,42	*0.06*
	Post-hoc t-tests:			
	PSS1 vs. PSS2	1.48	22	0.10
	PSS1 vs. PSS3	2.68	22	**0.01****
	PSS1 vs. PSS4	1.23	22	0.18
	PSS2 vs. PSS3	1.20	22	0.30
	PSS2 vs. PSS4	0.27	22	0.80
	PSS3 vs. PSS4	1.54	22	0.21
Right ventral PPC (BA 40)		2.39	3,42	*0.08*
	Post-hoc t-tests:			
	PSS1 vs. PSS2	1.55	22	0.10
	PSS1 vs. PSS3	2.24	22	**0.03***
	PSS1 vs. PSS4	0.14	22	0.88
	PSS2 vs. PSS3	0.60	22	0.60
	PSS2 vs. PSS4	1.44	22	0.16
	PSS3 vs. PSS4	2.18	22	*0.06*
Functional Coupling btw/ BA 45 and:				
Right caudate		3.57	3,42	**0.02***
	Post-hoc t-tests:			
	PSS1 vs. PSS2	1.38	22	*0.10*
	PSS1 vs. PSS3	3.19	22	**0.002*****
	PSS1 vs. PSS4	1.56	22	*0.09*
	PSS2 vs. PSS3	1.67	22	0.15
	PSS2 vs. PSS4	0.09	22	0.93
	PSS3 vs. PSS4	2.05	22	**0.05***
Right ventral PPC (BA 40)		4.06	3,42	**0.01****
	Post-hoc t-tests:			
	PSS1 vs. PSS2	2.24	22	**0.02***
	PSS1 vs. PSS3	2.89	22	**0.003*****
	PSS1 vs. PSS4	0.78	22	0.33
	PSS2 vs. PSS3	0.94	22	0.55
	PSS2 vs. PSS4	1.72	22	0.17
	PSS3 vs. PSS4	2.68	22	**0.05***

Medical students (see Table 4.1 for demographic details), who had been preparing for their licensing exams during session one, reported significantly lower perceived stress in session two (Figure 4.6A: $t = 4.44$, $p < 0.001$), and attention shifting costs decreased accordingly (Figure 4.6B: $t = 2.50$, $p = 0.02$). In contrast, neither perceived stress scores ($t = 0.78$, $p = 0.45$) nor attention shifting costs ($t = 0.77$, $p = 0.46$) differed significantly between sessions for age- and gender-matched controls, indicating that enhancements in attention shifting among medical students were not attributable to experience-dependent learning, practice effects, or other between-session differences unrelated to stress.

The reversibility of stress effects on functional connectivity in DLPFC and VLPFC was more difficult to ascertain, as these effects appeared to be nonlinear, so the *direction* of the between-session difference could vary as a factor of a subject's particular perceived stress scores in sessions one and two.[3] In an effort to address this problem, reversibility in DLPFC was assessed in a subset of subjects whose PSS scores exceeded the first quartile

[3] To illustrate, consider functional coupling between left DLPFC and left PPC (BA 7) as an example (see Figure 4.4B, panel iii). In a subject whose PSS score placed him in the top quartile in session one and the second quartile in session two, functional coupling would be expected to increase between sessions. In a subject whose PSS score placed him in the third quartile in session one and the first quartile in session two, functional coupling would be expected to decrease between sessions, despite comparable between-session differences in perceived stress scores.

in both sessions (i.e. those who drove the DLPFC decoupling effect) and decreased between sessions by at least one standard deviation. In these subjects, stress effects on functional coupling in left DLPFC reversed in the second session (Figure 4.6C: $t > 2.16$, $p < 0.03$, one-tailed). Conversely, reversibility in VLPFC was assessed in a subset of subjects whose PSS scores were below the fourth quartile in both sessions (i.e. those who drove the VLPFC enhanced coupling effect) and decreased by at least one standard deviation in session two. Again, stress-related enhancements in functional coupling in right VLPFC reversed in the second session (Figure 4.6D: $t > 1.78$, $p < 0.05$, one-tailed). No significant between-session differences were observed for any of these measures in a group of controls in which PSS scores differed by less than one standard deviation between sessions ($t < 1.29$, $p > 0.23$), indicating again that the reversal effect was not attributable to practice or other experience-dependent changes.

Figure 4.6: Reversibility of Stress Effects. A) Perceived stress scores for medical students were significantly lower in session two. **B)** Accordingly, stress effects on attention shift costs reversed in the second session, but not in a group of controls for whom perceived stress remained constant across sessions (data not shown).

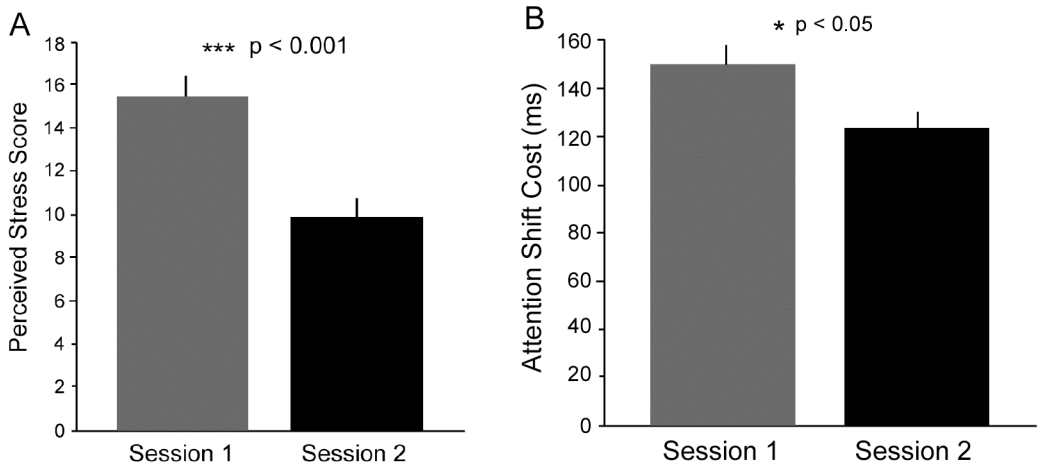
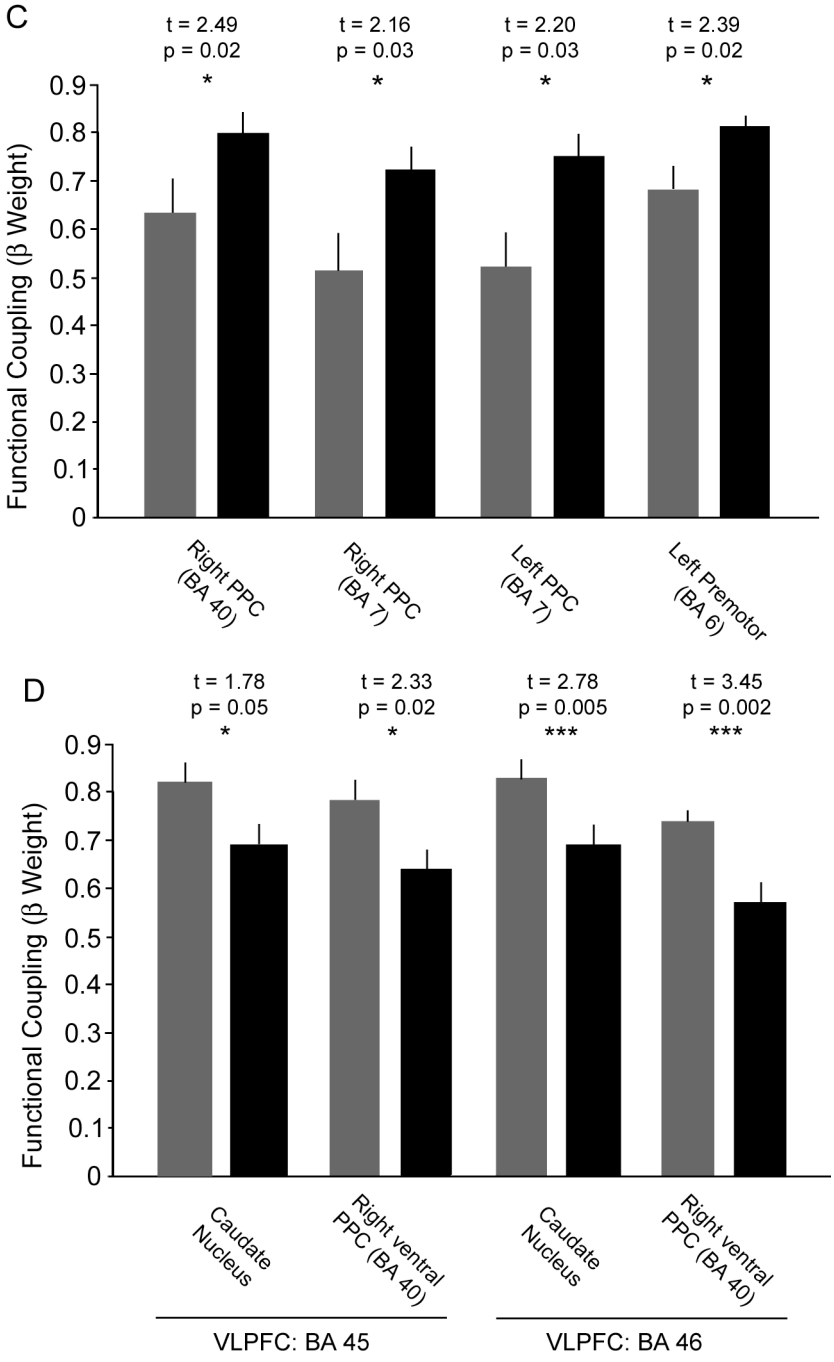


Figure 4.6 (cont.): Stress effects on functional coupling in **C)** left DLPFC and **D)** right ventrolateral prefrontal cortex in session one (grey bars) also reversed as stress decreased in session two (black bars). Again, functional coupling in both networks did not change appreciably in a group of controls for whom perceived stress remained constant. See text for details.



Discussion

In accord with findings in rats reported in Chapter 3, the results described here indicate that chronic stress alters prefrontal cortical function selectively, adversely affecting some functions while sparing others. Across a range of stress scores that included a majority of subjects, functional coupling in the dorsolateral prefrontal attentional network decreased with stress, particularly at higher levels of perceived stress, and increased in the ventrolateral prefrontal response reversal network, except in the highest quartile. Accordingly, stress impaired attention shifts but not response reversals, and attentional impairments were most significant in those subjects who showed stress-related functional decoupling in DLPFC (quartiles three and four).

These findings are in general agreement with the rodent model, though several unexpected differences should not be discounted and are considered in more detail below. In rats, as in the human subjects that were the focus of this chapter, chronic stress selectively impaired attentional shifts, while sparing response reversals. Stress also modulated dendritic morphology in a manner consistent with the functional coupling effects here. Stress reduced apical dendritic arborization in the dorsal mPFC, a putative homolog of primate lateral prefrontal cortex (Brown and Bowman, 2002),

just as functional coupling in DLPFC decreased with stress after peaking in the second quartile. Likewise, stress enhanced arborization in the rodent orbitofrontal cortex, which mediates response reversals (McAlonan and Brown, 2003), just as functional coupling in VLPFC increased with stress, reaching a peak in the third quartile. The magnitudes of these effects were also comparable: functional coupling decreased by ~20% from its peak in the attentional network, in accord with the 20% reduction in mPFC arborization observed in rats in Chapter 3, and increased by as much as 35% from its minimum in the response reversal network, which closely approximates the 42% increase observed in the rodent OFC.

Together, these results raise the possibility that chronic stress-induced changes in dendritic arborization may manifest themselves at the systems level as alterations in functional connectivity, which in turn affect network function and ultimately behavior. Whether changes in functional coupling in the left DLPFC bear any relevance for stress-related psychiatric diseases is a separate question beyond the scope of this work, though it is interesting to note that they mirror several findings from neuroimaging studies of major depression, including selective deficits in left but not right dorsolateral prefrontal cortical function (Henriques and Davidson, 1991; Davidson et al., 1999; Davidson et al., 2002).

A working model like this one might have some clinical utility, particularly in light of the fact that all of these effects appear to be reversible. After four weeks of rest, behavioral measures of attentional control improved. Likewise, functional coupling increased within the dorsolateral frontoparietal network that subserves this function in subjects who had shown stress-related impairments a month earlier (Figure 4.6). The enhancements were not attributable to experience-dependent learning, since they were not observed in control subjects, in whom perceived stress remained approximately constant across session. These findings are consistent with previous studies that have shown that apical dendritic atrophy in the medial prefrontal cortex and hippocampus is reversible after cessation of a repeated stressor (Conrad et al., 1999; Radley et al., 2005). Thus, the effects of stress on attention and the integrity of distributed frontoparietal networks may not be permanent, at least not initially.

In that sense, these findings, building on other recent studies, are promising. That said, they also raise several perplexing issues and leave others unresolved, thereby highlighting the need for further research. Several interpretive caveats deserve special consideration. First, it bears repeating that analogies between rodent and primate prefrontal cortex should be considered with caution. Indeed, whether rodents possess a region of

cortex homologous to primate lateral prefrontal cortex remains controversial (Preuss, 1995; Brown and Bowman, 2002). Evidence in support of this hypothesis stems from the seminal work of Rose and Woolsey (1948) and Akert (1964) who argued that homologs to primate lateral prefrontal cortex could be identified in rodents and other mammals on the basis of projections from the mediodorsal thalamic nucleus (MD). In the rat, these areas include the lateral orbitofrontal cortex and anterior cingulate, prelimbic, and infralimbic regions of mPFC (Preuss, 1995). Later studies identified rich dopaminergic innervation as a second distinctive characteristic of the primate lateral prefrontal cortex (Bjorklund et al., 1978; Brown and Goldman, 1977) and showed that the distribution of dopaminergic projections coincide with MD projections in both primates and rodents (Divac et al., 1978; Glowinski et al., 1984) and include the mPFC in rats. Behavioral studies represent a third source of evidence. They have shown that lesions to mPFC produce a pattern of behavioral impairments comparable to lateral prefrontal lesions in primates on spatial delayed alternation, delayed response, and attentional set-shifting tasks (Berger 1992; Kolb, 1984; Passingham et al., 1988; Birrell and Brown, 2000; McAlonan and Brown, 2003).

However, others have argued that primate lateral prefrontal cortex possesses histological characteristics that are sufficiently distinctive to cast doubt on the notion of a true homolog in nonprimates. Brodmann (1909) argued that dorsolateral prefrontal cortex is a region unique to primates in that it includes a well-developed internal granular layer (layer IV), lacking in nonprimates, a finding that has been delineated in much greater detail with modern methods (Preuss and Goldman-Rakic, 1991a; 1991b). Critics also note that MD and dopaminergic projections are not restricted to the lateral prefrontal cortex in primates and that on histologic and cytoarchitectonic grounds, the rodent mPFC resembles primate premotor and anterior cingulate cortex more closely than lateral PFC (Preuss, 1995).

A full discussion of the nuances of this debate is beyond the scope of this work (for an excellent review, see Preuss, 1995). Instead, this discussion serves to emphasize the point that the region-specific structural and functional changes in rodent mPFC and human DLPFC observed here may reflect analogous adaptations to stress mediated by comparable but potentially distinct mechanisms rather than truly homologous changes per se. Further work will be required to assess these possibilities.

A second limitation of this study concerns the difficulties inherent in quantifying stress in human subjects. Different people may respond in

different ways to the same stressor, and their physiologic profiles may reflect this variability. In this respect, the Perceived Stress Scale used here seems well suited to measuring stress, since it emphasizes the individual's *perception* of stressors and their impact on daily living and has been shown to predict stress-related physiologic changes more accurately than questionnaires that merely quantify exposure to psychosocial stressors (Cohen et al., 1983). Still, other unknown factors may interact with perceived stress to influence HPA responsivity, and PSS scores may be biased in favor of recent events in the subject's life at the expense of older stressors that may nevertheless affect measurements of stress-related changes in prefrontal function. It is likely that each of these factors contributed to the noise inherent in the data depicted above. Future work will complement PSS scores with measures of salivary cortisol and heart rate variability, which may be a marker of autonomic imbalance (Thayer and Brosschot, 2005).

Gender may also influence intersubject variability. Several studies indicate that estrogens may modulate stress reactivity, both acutely, where estrogens appear to heighten HPA reactivity and lower the threshold for stress-related PFC dysfunction (Shansky et al., 2004; Arnsten and Shansky, 2004), and chronically, where intact females may be *less* vulnerable to

stress-induced hippocampal atrophy than males and ovariectomized females females (McEwen, 2000b). Although the cause of this discrepancy remains unclear, these studies certainly highlight the importance of counterbalancing for gender in group-wise comparisons. Efforts to this end probably minimized the impact of this confound.

It is also worth emphasizing once again that the correlations reported here, while certainly intriguing, should not be mistaken for a causative association. Other factors not evaluated in this study may contribute to attentional impairments. For example, excess dopaminergic and noradrenergic tone may also contribute to the attentional deficits associated with chronic stress. Stress has been shown to alter monoaminergic neuromodulatory inputs to the prefrontal cortex acutely (Thierry et al., 1976; Goldstein et al., 1996), and acute stress-related working memory impairments can be reversed by pretreatment with D1 and α1 receptor blockers like haloperidol and clozapine or a selective D1 receptor antagonist (Murphy et al., 1996a; Murphy et al., 1996b). Future experiments must examine how chronic stress and dendritic atrophy are associated with alterations in monoaminergic tone acutely, if at all. Such studies would facilitate efforts to determine whether changes in dendritic profiles and functional coupling might interact with acute changes in neuromodulatory

inputs and other factors to generate the attentional deficits that are characteristic of major depression and other stress-related psychiatric conditions.

Finally, and perhaps most importantly, it is not clear how changes in dorsolateral prefrontal functional coupling may contribute to attentional impairments. In both DLPFC and VLPFC, the association between perceived stress and functional coupling appeared to be nonlinear, as coupling increased with stress initially before ultimately decreasing at higher levels (Figures 4.4 and 4.5). By contrast, stress effects on attention shifting were more uniform, increasing linearly with stress (Figure 4.1). One possibility is that attention shifting capacities may be resilient to small changes in functional coupling such that changes below a certain threshold do not affect performance. In accord with this hypothesis, stress effects on attention were driven strongly by subjects in the third and fourth stress quartiles—subjects who also drove the functional decoupling effect in DLPFC. This is also consistent with work in animal models. In the experiments described in Chapter 3, not all rats subjected to repeated restraint stress showed the same levels of dendritic atrophy, and attentional impairments were driven by those with the most severe morphologic changes. Similar effects have been reported in experiments examining

anxiety behavior following a single immobilization stress. Again, not all animals showed mPFC arborization effects, and only those that did showed increased anxiety (Miller et al., 2005).

Yet this hypothesis cannot account fully for the observed pattern of results either: although functional coupling in DLPFC was lowest in the fourth stress quartile, where attention shifting was most impaired, it was still comparable to coupling in the first quartile, where attention shifting was most efficient. Thus, it seems likely that other factors are contributing to stress-related attentional impairments. Another possibility is that enhanced functional coupling in the ventrolateral network may modulate behavioral measures of attentional capacities indirectly. Computational modeling work in an analogous task suggests that functional alterations (e.g. lesions) in the response reversal circuit, though not strictly required for attention shifting, may nevertheless modulate attentional capacities and vice versa (O'Reilly et al., 2002). Their results suggest that attention shifting costs may be a function of the integrity and efficiency of both the shift network, which tends to reduce them by promoting behavioral flexibility, and of the response reversal network, which may hinder attention shifting by enhancing performance in one dimension at the expense of the other.

Further experimentation would be required to test these hypotheses explicitly. In any case, the results indicate that chronic stress effects on dendritic arborization, functional coupling, and behavior are probably nonlinear. This result is not inconsistent with a large body of literature demonstrating an "inverted U"-shaped dose-response curve for the effects of acute stress-related variables on electrophysiological (Diamond et al., 1992; Sandi et al., 1997) and cognitive properties (Murphy et al., 1996; Arnsten, 1998; Arnsten and Goldman-Rakic, 1998; Lidow et al., 2003). These issues are discussed in greater detail in Chapter 5.

CHAPTER 5:

Conclusions and Avenues for Future Research

The goal of this work was to evaluate how chronic stress affects attentional control and the frontoparietal network that subserves this function in parallel rodent and human neuroimaging studies. The experiments described in Chapter 2 were designed to characterize the computational roles of components of this network in an attentional control task that could be adapted for use in rats. They confirmed that the contributions of dorsolateral frontoparietal areas to visual attentional shifts can be dissociated from the regulatory influences of more ventrolateral areas on stimulus/response mappings, in a manner consistent with studies in animal models (Birrell and Brown, 2000; Fox et al., 2003; McAlonan and Brown, 2003).

Exploiting this analogy, the experiments reported in Chapter 3 examined the effects of chronic stress on the integrity of this network at the level of dendritic morphology. Chronic stress induced a retraction of apical dendritic arbors in the medial prefrontal cortex and corresponding impairments of attentional shifts that correlated with the magnitude of dendritic atrophy. By contrast, stress did not adversely affect reversal learning or dendritic morphology in lateral orbitofrontal cortex, where apical arborization actually increased by 43%. The studies described in Chapter 4 extended these findings to healthy human subjects using the task developed in Chapter 2. Perceived stress scores predicted selective impairments in

attentional shifts and altered functional coupling in the dorsolateral attention network.

Together, these results outline in broad strokes a mechanistic model by which chronic stress may predispose susceptible persons to the attentional impairments that are characteristic of mood disorders and other stress-related psychiatric conditions. Repeated stressors induce a remodeling of apical dendrites via a complex interaction between glucocorticoids, excitatory amino acids, neurotrophins, and serotonergic neuromodulatory influences. Dendritic atrophy, in turn, may impair functional connectivity in salient neocortical networks. Impaired connectivity may disrupt network function and ultimately, attentional control mechanisms.

Of course, like most decent science, these findings raise more questions than they answer. What function, if any, does stress-related dendritic remodeling serve? What are the precise cellular and molecular mechanisms by which this remodeling is mediated? And most importantly from a medical perspective: are these findings relevant for stress-related neuropsychiatric conditions? These questions will be the focus of future experiments, some of which are already under way. Each is considered in

turn in the pages that follow, along with preliminary results from two on-going studies that may shed light on these issues.

Functional Significance of Stress-Induced Dendritic Remodeling

As reviewed in Chapter 1, early work in the field focused on stress effects on cell morphology in the hippocampus, a key regulator of the HPA axis. More recent studies indicate that stress modulates dendritic morphology in a much broader distribution of cortical and subcortical structures that includes the amygdala (Vyas et al., 2002; Vyas et al., 2003), several subregions of the medial prefrontal cortex (Cook and Wellman, 2004; Radley et al., 2004; Izquierdo et al., 2006; Liston et al., 2006b; Radley et al., 2006), and the lateral orbitofrontal cortex (Liston et al., 2006b). This remodeling process is thought to involve actin depolymerization and other ATP-dependent cytoskeletal changes (McEwen, 1999; 2000a). What benefits, then, might the organism derive from these energy expenditures?

It has been suggested that dendritic atrophy in the CA3 region of the hippocampus may serve a neuroprotective function (McEwen, 2000b; a). The hippocampus provides negative feedback to the HPA axis (Herman and Cullinan, 1997), and in states of chronic stress, overstimulated hippocampal pyramidal cells may be subject to excitatory neurotoxicity. In accord with

this hypothesis, repeated restraint stress induces a reorganization of mossy fiber terminals in the CA3 region, including increased synaptic vesicle density and increased numbers of presynaptic mitochondria (Magarinos et al., 1997), which may reflect an enhanced capacity for excitotoxic input (de Kloet et al., 2005). Likewise, glucocorticoids have been shown to potentiate cell death secondary to seizure activity (Sapolsky, 1985; Sapolsky et al., 1988), and this effect may be ameliorated by increasing glucose availability, thereby reducing excitotoxicity (Sapolsky, 1986; Ozawa et al., 2000). In light of these reports, it stands to reason that hippocampal dendritic atrophy may benefit the organism by attenuating the impact of stress-related excitotoxic inputs.

However, this model cannot easily account for the proliferative changes found in the amygdala, which also provides feedback to the HPA axis (Herman and Cullinan, 1997), nor for changes in the lateral orbitofrontal cortex, which does not play any known role in HPA regulation. An alternative (and not exclusive) possibility is that stress-induced dendritic remodeling and its cognitive correlates may serve an adaptive purpose. This idea has been applied successfully in diverse areas of medicine to explain how manifestations of disease like chronic pain, inflammation, fever, and iron sequestration may in fact reflect adaptations shaped by natural selection

to cope with infection, chemical injury, and mechanical wear-and-tear (Williams and Nesse, 1991; Nesse and Berridge, 1997). In this view, some manifestations of disease may arise directly from physiological defects whereas others may be by-products of the body's normal defenses or of dysregulated defensive mechanisms gone awry (Nesse, 2000). It has been suggested that several cognitive and affective symptoms of major depression may fall into one of the latter two categories (Nesse, 2000). Similarly, stress-related changes in dendritic morphology might serve an adaptive purpose by altering network properties in a manner that enhances some cognitive functions at the expense of others. For example, whereas chronic stress impairs hippocampus-dependent learning and memory (Luine et al., 1994; Conrad et al., 1996), it promotes the acquisition and retention of fear conditioning (Shors et al., 1992; Conrad et al., 1999). Thus, dendritic remodeling in the amygdala may serve to protect the organism from real dangers in the environment by enhancing the processing of threatening stimuli.

The experiments reported in Chapters 3 and 4 suggest that stress-related enhancements in network function may extend beyond the amygdala. In Chapter 3, repeated restraint stress induced a 43% increase in dendritic arborization in the rodent lateral orbitofrontal cortex (Figure 3.4A).

Likewise, functional coupling in the ventrolateral PFC increased in human subjects across a wide range of stress scores, peaking in the third stress quartile and then decreasing slightly (Figure 4.5). Conversely, functional coupling in the dorsolateral attentional network increased with stress, but only in subjects who reported below-average stress scores, and decreased in the second and third quartiles (Figure 4.4). These trends suggest that chronic stress may modulate connectivity and cognitive capacities in a nonlinear manner, yielding enhancements at lower levels of stress that may be offset by complementary impairments at higher levels.

To assess this hypothesis, a post-hoc analysis of the behavioral and functional coupling data presented in Chapter 4 was conducted to evaluate whether the effects of stress on these measures could be captured more accurately by a nonlinear model versus a linear one. A curve-fitting algorithm (SPSS, SPSS Inc., Chicago, IL) was used to generate curve estimation regression statistics, which in turn were used to compare the goodness-of-fit of a linear model versus a quadratic (U-shaped) one. Nonlinear, quadratic models provided a more accurate fit for associations between perceived stress and functional coupling in dorsolateral and ventrolateral prefrontal cortices in all cases (Figure 5.1; Table 5.1). The stress effect on attention shifting costs was captured more accurately with a

Figure 5.1: Association Between Stress and Functional Connectivity is Nonlinear. A) Functional coupling in dorsolateral prefrontal cortex and tended to increase with stress initially and then decrease at higher levels of stress in all eight pairs where stress effects were observed in Chapter 4. This U-shaped association was modeled accurately in quadratic terms.

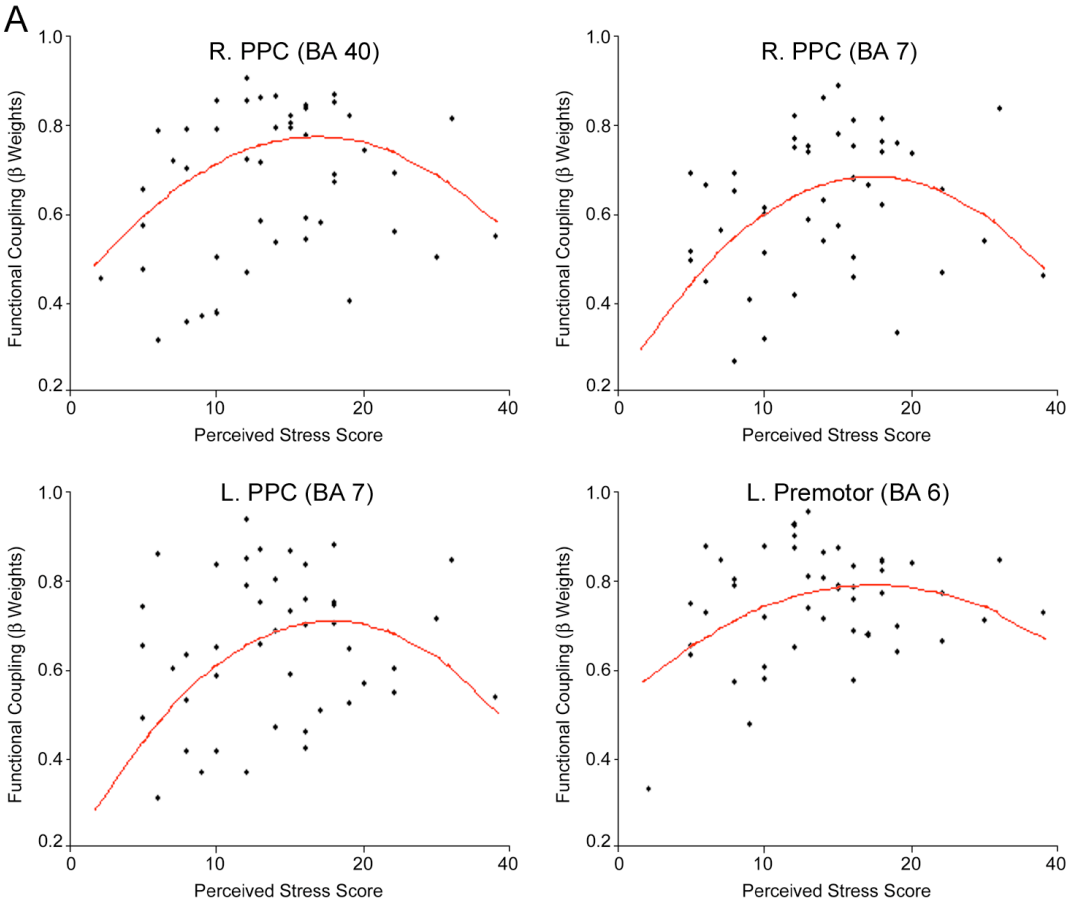


Figure 5.1 (cont.): Association Between Stress and Functional Connectivity is Nonlinear. B) Similar effects were observed in ventrolateral prefrontal cortex (top = BA 45; bottom = BA 46).

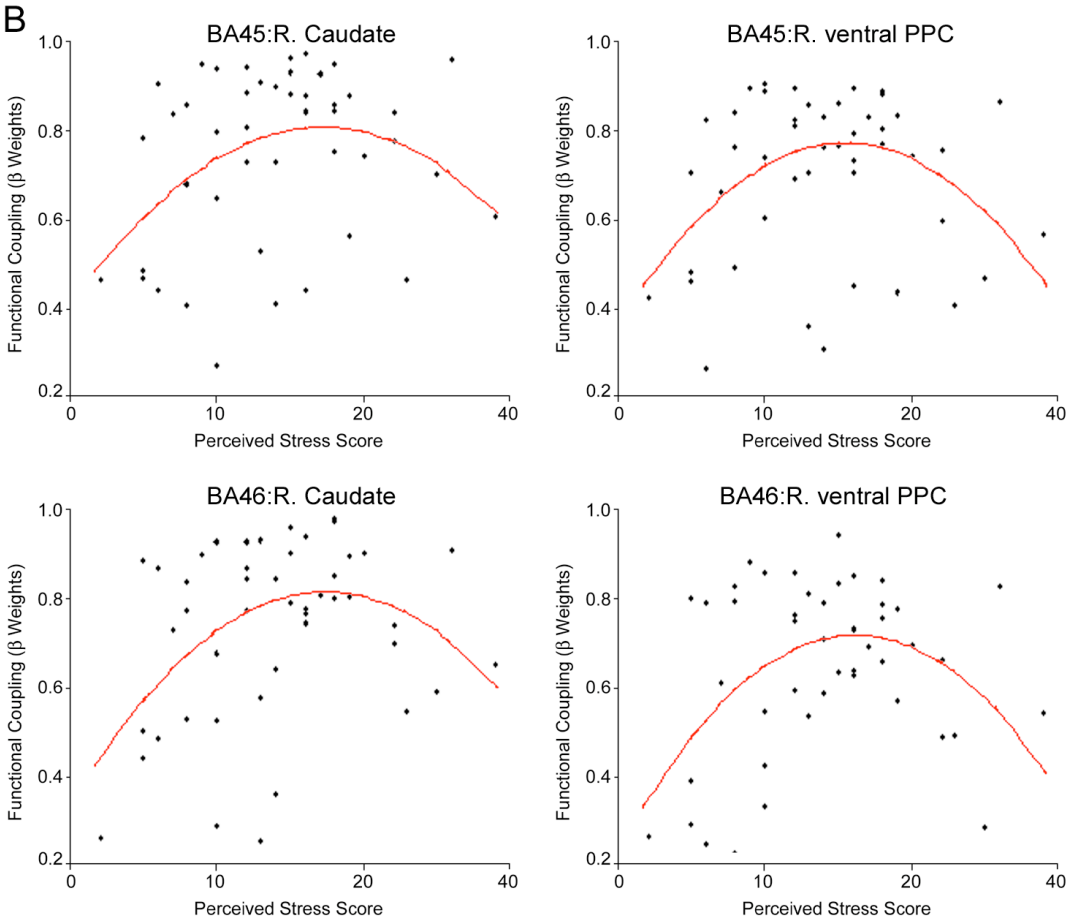


Table 5.1: Stress and Connectivity: Curve-fitting Statistics. Quadratic models provided a more accurate fit for associations between perceived stress and functional coupling in dorsolateral and ventrolateral prefrontal cortices in all eight pairs where significant effects were observed in Chapter 4. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.005$.

	Linear Model:			**Quadratic Model:**		
Dependent Measure:	**F**	**p**	**R^2**	**F**	**p**	**R^2**
L. DLPFC:R. PPC (BA40)	0.14	0.71	0.003	3.20	0.05*	0.13
L. DLPFC:R. PPC (BA7)	1.03	0.32	0.02	5.12	0.01**	0.19
L. DLPFC:L. PPC (BA7)	1.19	0.28	0.03	4.32	0.02*	0.16
L. DLPFC:L. Premotor	0.32	0.58	0.007	2.98	0.06	0.12
R. BA46:R. Caudate	2.10	0.15	0.05	3.75	0.03*	0.15
R. BA46:R. PPC	0.30	0.58	0.007	5.10	0.01*	0.19
R. BA45:R. Caudate	3.68	0.06	0.08	6.31	0.004***	0.23
R. BA45:R. PPC	1.31	0.26	0.03	6.18	0.004***	0.22

linear model (Table 5.2), a finding consistent with the seemingly linear association depicted in Figure 4.1B. However, stress effects on other behavioral measures were more clearly quadratic (U-shaped). Reaction times and accuracies for both shifts and repeats tended to improve with stress initially and then decline at higher levels of stress (Figure 5.2; Table 5.2).

Thus, whereas attention shifting costs appear to increase linearly with stress, stress effects on speed, accuracy, and functional coupling may be more nuanced. These findings are consistent with reports in monkeys, rats, and mice, demonstrating an "inverted U"-shaped dose-response curve for the effects of acute stress-related variables on hippocampal and prefrontal cortical function. Arnsten, Goldman-Rakic, and colleagues have demonstrated that prefrontal cortical cognitive function may be impaired by either too little (Sawaguchi and Goldman-Rakic, 1991; Kozlov et al., 2001; Lidow et al., 2003) or too much dopamine (Zahrt et al., 1997; Arnsten and Goldman Rakic, 1998; Lidow et al., 2003), whose release is stimulated by stress acutely. Corticosterone also enhanced hippocampal primed burst potentiation and learning in an inverted U-shaped, dose-dependent manner (Diamond et al., 1992; Sandi et al.; 1997). Our results suggest that chronic stress-related changes in dendritic morphology and functional coupling may

Figure 5.2: Association Between Stress and Speed or Accuracy is Nonlinear. Speed and accuracy tended to improve with stress initially for both shifts and repeats, whereas stress-related impairments showed up at higher levels of stress. This U-shaped association was modeled accurately in quadratic terms.

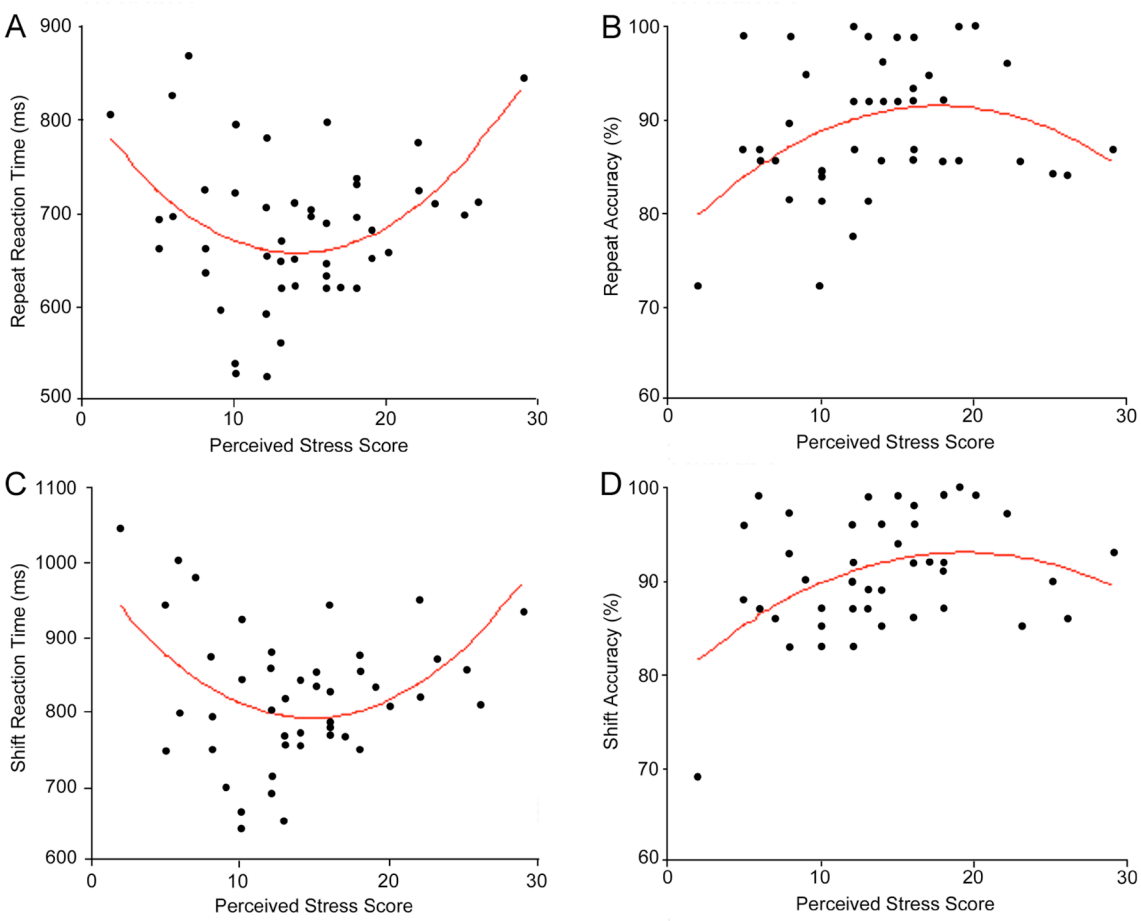


Table 5.2: Stress and Behavior: Curve-fitting Statistics. The stress effect on attention shifting costs was captured accurately with a linear model, but stress effects on other behavioral measures (speed and accuracy) were modeled more accurately in quadratic terms.

	Linear Model:			**Quadratic Model:**		
Dependent Measure:	**F**	**p**	**R^2**	**F**	**p**	**R^2**
Attention Shift Cost (RT)	9.90	0.003***	0.20	4.92	0.01*	0.20
Shift RT	0.006	0.94	<0.001	5.62	0.007**	0.21
Repeat RT	0.34	0.56	0.008	6.09	0.005***	0.22
Shift Accuracy	1.58	0.22	0.04	4.64	0.02*	0.18
Repeat Accuracy	0.56	0.46	0.01	3.29	0.05*	0.13

follow a similar pattern and may interact with acute changes to yield the observed pattern of behavioral effects.

These hypotheses need to be tested explicitly in future work and grounded in the functional characterization of the circuitry reported in Chapter 2, an effort in turn that will require considerably more subjects. If confirmed, they may inform future studies in animal models by helping to resolve frequently conflicting reports wherein the same behavioral and morphologic measures are impaired by stress in some paradigms (Izquierdo et al., 2006) but enhanced in others (Liston et al., 2006a).

Cellular and Molecular Mechanisms of Stress-Induced Remodeling

The findings discussed above are interesting from an academic perspective, but they must be supplemented with a more detailed understanding of the cellular and molecular mechanisms that mediate stress-induced remodeling if they are to inform efforts to develop more effective medical and surgical treatments for psychiatric disease. Work in animal models has been particularly fruitful in this regard. These studies, reviewed in Chapter 1, indicate that glucocorticoids and serotonin are released in response to repeated stressors and may act together to promote dendritic remodeling by enhancing calcium conductances and regulating transcription and translation

of BDNF, cell adhesion molecules, and other factors (McEwen, 1999; 2003; Sandi, 2004).

Human neuroimaging studies that exploit common genetic polymorphisms typify an alternate strategy that may provide information complementary to findings in animal models. Genes encoding BDNF and the serotonin reuptake transporter (5HTT) have received considerable attention (again, see Chapter 1 for a brief review). Common polymorphisms in these genes are thought to modulate extracellular BDNF and 5HT availability by decreasing activity-dependent secretion and impairing presynaptic reuptake, respectively. The 5HTT variant (the "short" allele), in turn, has been associated with amygdala hyperreactivity (Hariri et al., 2002) and decreased functional coupling between the amygdala and subgenual PFC (Pezawas et al., 2005), while the BDNF variant (the "Met" allele) has been linked to hippocampal LTP impairments, decreased hippocampal volume, and episodic memory deficits (Egan et al., 2003; Chen et al., 2004). Such studies are important in that they confirm that these polymorphisms have functionally meaningful consequences for network function and cognition. Their relevance for the pathology of stress-related mood disorders is less clear, however, since the behavioral deficits that they

highlight are not critical features of depressive symptomatology, and they do not address interactions with stress.

With this in mind, future work will assess whether BDNF and 5HTT polymorphism status modulate stress effects on attention and prefrontal functional coupling like those reported in Chapter 4. Again, these questions are best addressed in considerably larger subject cohorts, but preliminary findings are promising. 40 subjects who participated in the study reported in Chapter 4 were genotyped and classified as "high stress" or "low stress" based on a median split of perceived stress scores, as described previously. For each polymorphism, a 2 (genotype: Met/short allele carrier versus homozygous Val/long) x 2 (stress: high vs. low) factorial ANOVA assessed whether attention shifting costs varied with genotype and stress and identified interactions between these factors. There was a significant main effect of stress on attention shifting as expected ($F(1,39) = 6.67$, $p = 0.01$), and both polymorphisms interacted with stress to modulate attention shifting, but they did so in different ways (Figure 5.3). The BDNF Val allele appeared to play a protective role. Attention shifting costs were unaffected by stress in Val allele homozygotes ($t < 0.20$, $p > 0.65$), whose performance at high stress was comparable to low stress subjects of both genotypes. Accordingly, the main effect of stress on attention shifting was

Figure 5.3: Modulation of Stress Effects by BDNF and 5HTT Polymorphisms. A) BDNF polymorphism status modulated the effect of stress on attention shifting. Whereas attention shifting costs did not vary with stress in Val/Val homozygotes, they increased significantly in the high stress group in carriers of the Met allele.

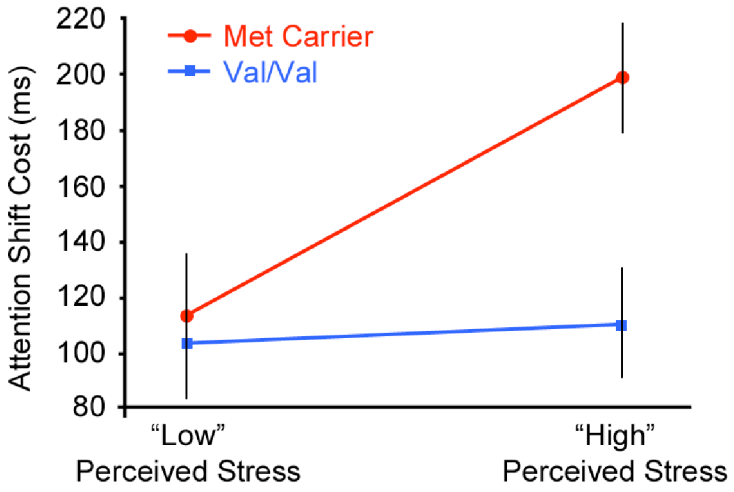
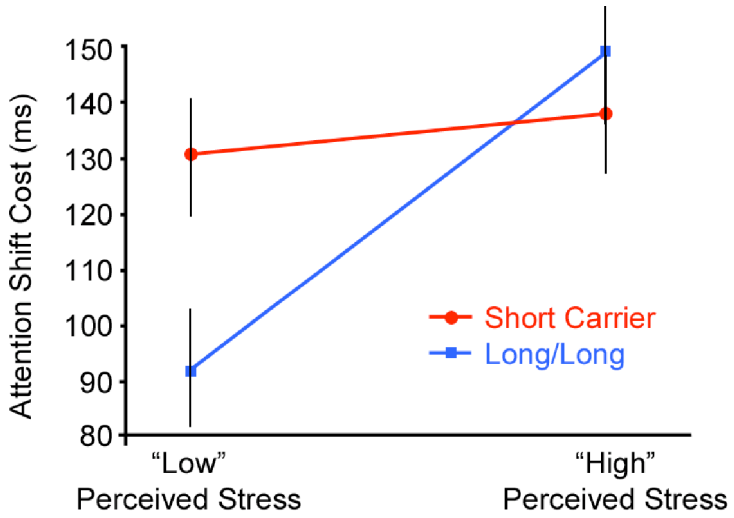


Figure 5.3B) 5HTT polymorphism status also modulated stress effects on attention shifting. Attention shifting costs increased with stress in Long/Long homozygotes. Stress did not modulate attention shifting costs in carriers of the short allele. However, short allele carriers showed attention shifting costs at low levels of stress that were comparable in magnitude to subjects of either genotype at high levels of stress and were significantly larger than Long/Long homozygotes at low stress.



driven by Met allele carriers, which was reflected in an interaction between BDNF genotype and stress ($F(1,39) = 7.26$, $p = 0.007$).

5HTT polymorphism status also modulated attention shifting costs via an interaction with stress ($F(1,39) = 4.26$, $p = 0.04$), but it was the long allele homozygotes who drove the stress effect in this case. Attention shifting costs did not increase significantly with stress in carriers of the short allele ($t < 0.20$, $p > 0.65$). Instead, these subjects performed at low stress at a level comparable to high stress subjects of both genotypes. This unexpected result suggests that carriers of the short allele may develop stress-related attentional impairments at lower levels of stress. More subjects would be required to test this hypothesis explicitly by stratifying subjects into more than two stress groups. Future work will confirm these findings in a larger sample and examine how 5HTT and BDNF polymorphism status may interact with stress to modulate functional coupling within these networks.

Relevance for Mood Disorders

As outlined in Chapter 1, this project was motivated by a need for a greater understanding of the pathology and pathophysiology of depression and of the role of stress as a risk factor. In considering the clinical relevance of the results, it is important to distinguish between pathophysiology and

pathogenesis. Chronic stress was found to alter dendritic morphology, functional coupling, and attentional control in rats and in human subjects. Attentional deficits in major depression are by definition symptomatic of the pathophysiology, which in turn might reasonably be expected to share critical features with the neural substrates of stress-related attentional impairments, since chronic stress appears to be both a cause and a consequence of the disease in many cases. What is less clear is whether these findings reflect changes that contribute meaningfully to pathogenesis and disease progression—changes that represent potential targets for therapy. Put another way, these issues can be framed simplistically as a chicken-and-egg dilemma: Which came first? The disease or the attentional deficits?

This question, like the two that preceded it, is beyond the scope of this project and will not be resolved here. Instead, I shall conclude with a brief consideration of one line of evidence that supports the latter proposition—that these findings may inform efforts to understand pathogenesis. In a recent series of studies, Anderson and colleagues examined the neural substrates of controlling attention to undesirable thoughts and memories (Anderson and Green, 2001; Anderson et al., 2004). They trained subjects on a task that tested their memory for a list of novel word pairs. On each

trial, subjects were presented with a cue word, and on some trials, they were instructed to recall the word that was paired with it in the list they had memorized. Critically, on other trials, they were instructed not only to refrain from responding but also to avoid even attending to the correct response word, silently or otherwise. Suppressing attention to unwanted memories was associated with increased activity in DLPFC and impaired post-test retention of those memories in a manner predicted by the degree of DLPFC activity (Anderson et al., 2004).

This finding suggests that DLPFC plays a central role in regulating attention to unwanted thoughts and memories, which have figured prominently in psychodynamic models of depression since the time of Freud (Freud, 1966; Anderson et al., 2004) and remain a critical feature of the symptomatology in modern definitions (APA, 1994). Likewise, efforts to enhance a patient's capacity for cognitive regulation of emotionally salient thoughts are among the core components of the only talk therapy whose efficacy in treating mood disorders has been reliably and consistently established (Beck, 1970; Kovacs et al., 1981). Stress-induced impairments in DLPFC function may therefore prove clinically relevant if they act to exacerbate the patient's tendency to ruminate uncontrollably on negative

thoughts, a finding that would place these changes squarely in Nesse's category of adaptations gone awry (Nesse, 2000).

Ironically, Hippocrates seems to have anticipated this possibility. Reflecting on the links between major life stressors, rumination, and the symptoms that characterize depression, he noted that "Grief and fear, *when lingering* [emphasis added], provoke melancholia." As was frequently the case with this paragon of medical wisdom, his remarkably astute clinical observations were often more insightful than the treatments he devised to remedy them. Future work will assess these hypotheses more directly.

APPENDICES

Appendix 1:

Attentional Control Task: Design Details

This appendix describes the details of the task design for the attentional control paradigm used in the experiments reported in Chapters 2 and 3. In this task, subjects performed visual discriminations concerning either the color or the motion of a series of visual stimuli. On each trial, subjects viewed a pair of circular square-wave gratings, one red and one green. Each grating moved either up or down. The gratings flanked a simultaneously presented, centrally located task cue ("M" or "C"). If the cue was an "M", the subject responded by choosing the side with the upward moving grating, regardless of color. If the cue was a "C", the subject responded by choosing the side with the red grating, regardless of motion (Figure 2.1). Repeat trials were defined as those preceded by 2-5 trials of the same dimension (e.g. MMMM). Shift trials were those preceded by 2-5 trials of the opposite dimension (e.g. CCCM).

Trials also varied with manipulations of conflict at the level of the response and the stimulus representation. In a low response conflict trial, the red grating was also the upward moving grating so the correct response was the same in both dimensions. In a high response conflict trial, the red

grating was downward moving, and the green grating was upward moving so the correct response depended on the cue. Stimulus conflict was parametrically manipulated by adjusting the color saturation on motion trials and the square-wave contrast on color trials, yielding three levels of conflict (low, medium, high; see Figure 2.2C) that varied with the salience of the irrelevant dimension (Campbell & Maffei, 1980). For each trial of a given dimension, the salience of the relevant dimension was held constant; only the salience of the irrelevant dimension was varied. Thus, the difficulty of the relevant visual discrimination did not vary from trial to trial independently of competing stimulus information from the irrelevant dimension.

Color and motion trials were presented in a pseudorandomized order such that the task cue could not be predicted, and each contrast performed in the analyses described in Chapters 2 and 4 was counterbalanced for dimension, side of target presentation, response conflict, and stimulus conflict. Importantly, this counterbalancing ensured that the stimulus conflict manipulation was not confounded by conflict at the level of the response (and vice versa) or by other attentional demands, independent of those associated with interference from the irrelevant dimension.

Notes on Response Conflict Manipulation. Response conflict varied with the congruency of stimulus-response mappings in each dimension, in accord with previous studies (e.g. MacDonald et al., 2000; Rushworth et al., 2002; Weissman et al., 2003; Kerns et al., 2004).

Notes on Stimulus Conflict Manipulation. Stimulus conflict varied parametrically with the salience of the irrelevant dimension, as described above. All trial types were counterbalanced for dimension, side of target presentation, response conflict, and level of stimulus interference. This counterbalancing ensured that the stimulus-related conflict manipulation was not confounded by other factors. Three additional points are worth noting:

1. Even when controlling for the location of the target stimulus, the effects of the stimulus conflict manipulation on reaction time, accuracy, and activity in PPC and DLPFC remained significant. Thus, the conflict was not spatial and was not solely associated with the to-be-attended location.
2. Even when holding the level of response conflict constant, these effects remained significant, indicating that the effect was related to the stimulus conflict manipulation and not confounded by an interaction with response conflict.

3. Most importantly, it should be noted that for every trial of a given dimension, the salience of the relevant dimension was held constant; only the salience of the irrelevant dimension was varied. Thus, the difficulty of the relevant visual discrimination did not vary from trial to trial independently of the competing stimulus information from the irrelevant dimension. It was predicted that while holding color salience constant, increasing salience of the motion dimension would be associated with increased activity in the stimulus-response processing stream that mediates motion-based responses and is assumed to compete with the processing stream mediating color-based responses. (Justifications for this assumption are provided in Appendix 3.) It was predicted that this competition, which depends on the representation of the stimulus independent of the target location and motor response and which we define as stimulus conflict, would be associated with impaired behavioral performance. As described in the text, this was exactly what was observed.

Appendix 2:

MRI Parameters and Preprocessing Procedures

Appendix 2 describes the MRI parameters and data preprocessing procedures used to collect and analyze the imaging data described in Chapters 2 and 4.

MRI Parameters. Images were acquired on a GE 3T MRI scanner using a quadrature head coil. Functional scans were acquired using a spiral in-and-out sequence (Glover & Thomason, 2004) with the following parameters: TR=2000, TE=30, FOV=200mm, 64x64 matrix, 29 5-mm axial slices. Anatomical data sets included 3D high-resolution SPGR images (TR=25, TE=5, 124 1.5-mm coronal slices) and a T1-weighted in-plane scan (TR=500, TE=min, FOV=200mm, 256x256 matrix, 29 5-mm axial slices).

Preprocessing Procedures. MR images were preprocessed and analyzed using the BrainVoyager QX software package (Brain Innovations, Maastricht, The Netherlands). Preprocessing of fMRI data included slice scan time correction, temporal filtering, linear trend removal, spatial smoothing using a 4-mm full-width half-maximum Gaussian kernel, and 3D motion correction. Functional data sets were manually co-registered to the

3D SPGR anatomical volume. Both functional and anatomical data sets were then transformed into Talairach space. Finally, z-normalized functional timecourses were analyzed based on the least mean squares solution to a general linear model. These techniques are described in detail in the Experimental Procedures sections of Chapters 2 and 4.

Appendix 3:

Methdological Notes on MRI Analytic Techniques

This appendix describes additional details of the statistical and analytic methodologies applied in Chapter 2. This includes a discussion of the approach we adopted for operationalizing conflicts in information processing; alternative formulations that were also considered; a more detailed description of the statistical approach for regressions on the conflict index; and notes on our methods for selecting regions of interest. These are not essential for understanding the results reported in Chapter 2, but they may be of interest to some readers.

Notes on Conflict Index Formulation. The conflict monitoring hypothesis states that anterior cingulate cortex acts to detect conflict in information processing in posterior cortex. According to this view, when activity in two competing neural units is high, activity in anterior cingulate cortex should also be elevated (Botvinick et al., 2001). To test this prediction, we identified six occipitotemporal regions that were primarily motion-sensitive or primarily color-sensitive by contrasting color shift trials with motion shift trials. Conflict was indexed as a normalized product of the activities (% change in BOLD signal from the run-average baseline) in these three color-

sensitive regions and three motion-sensitive regions, summed across all nine combinations:

$$\text{Conflict} = \text{sqrt}\ \Sigma_{i,j}\ C_i \times M_j$$

or

$$\text{Conflict} = \text{sqrt}[(C1 + C2 + C3)(M1 + M2 + M3)]$$

Importantly, this formulation differs from the formulation of conflict adopted in Botvinick and colleagues' (2001) computational model in that they ensured that competing units were connected by negative weights, whereas we could not reliably assess the association between the color- and motion-sensitive regions examined in our study. Instead, our conflict index was intended to serve as a measure of conflict not between two competing perceptual areas per se, but rather between two competing stimulus-response processing streams, which seems consistent in principle with the formulation of conflict outlined in Botvinick et al. (2001). This form of conflict was most easily measured using functional MRI in components of these processing streams that are anatomically distinct, i.e. in perceptual regions. Activities in the color- and motion-sensitive regions were assumed to be proxies for activity in their respective processing streams. Accordingly, the conflict index was higher during shift trials than during repeat trials (t =

1.85, p = .03, one-tailed). Three additional sources of evidence also support this assumption:

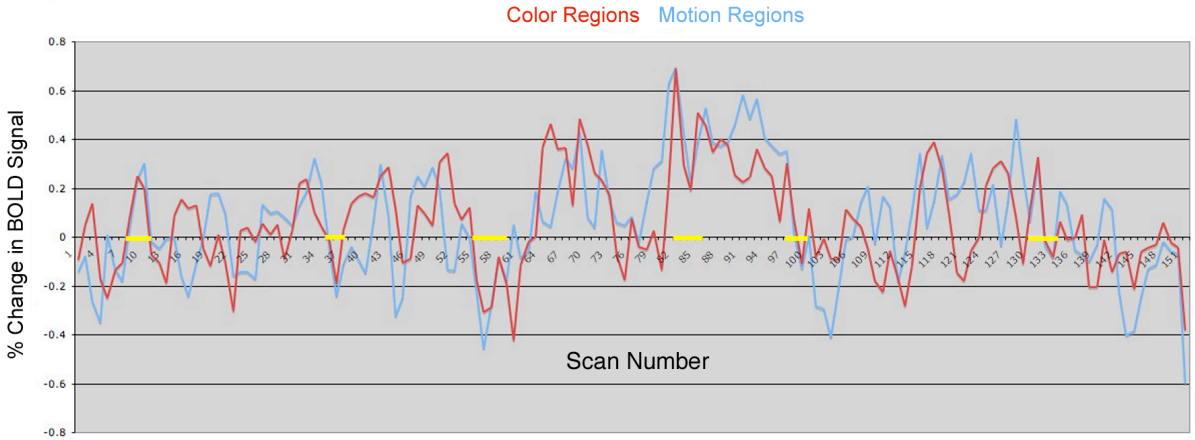
1. It is well established that the nature of a visual stimulus itself is but one of several factors that modulate visual processing even in lower-level perceptual regions. In particular, top-down attentional processes and behavioral context can modulate activity in perceptual regions extending as far down the processing chain as primary visual cortex (Ito & Gilbert, 1999; Li et al, 2004; Sigman et al., 2005). Attention directed at one aspect of a visual stimulus (e.g. color) can also enhance lower-level perceptual processing relative to other aspects of the stimulus (e.g. motion) despite the constancy of the physical attributes of the stimulus itself (for review, see Desimone & Duncan, 1995). Thus, activity in color- and motion-sensitive perceptual regions would be expected to vary not only with the nature of the visual stimulus being represented but also with other attentional demands, such as those induced by processing conflicts at the level of the response. This also applies to manipulations of stimulus-related conflict, where it should not be assumed that the sole determinant of processing in a given perceptual region is the salience of the visual stimulus.

2. Behavioral measures of task performance in our study varied with activity in color- and motion-sensitive regions, even when controlling for the physical characteristics of the visual stimulus. That is, when activity in color-sensitive regions was high, color discriminations tended to be faster and more accurate than motion discriminations, and the converse held for activity in motion sensitive regions. Although these are inherently noisy measures, these trends reached significance ($p < 0.05$) in two contrasts. Thus, activity in these perceptual regions was related to the final output of the processing stream as a whole, even when controlling for the nature of the visual stimulus.
3. We also examined how activity in color-sensitive regions was associated with activity in motion-sensitive regions. Overall, a weak positive correlation was observed ($r = 0.12$, $p < 0.001$). However, this correlation appeared to reflect the average of two distinct patterns of association, modulated by the difference in overall activation between color- and motion-sensitive regions: during blocks of scans when the difference was small (lowest quintile), color- and motion-activities were highly correlated ($r = .97$, $p < .001$), and activity in all regions of the circuit was significantly elevated ($p < .01$), perhaps reflecting an

attention-related increase in activation throughout the network, which would be associated with a high correlation among the perceptual regions. No readily identifiable association with experimental condition was observed. When this quintile of trials was excluded from analysis, activities in color- and motion-sensitive regions were anticorrelated ($r = -.21$, $p < .001$), consistent with a competitive relationship between these two processing streams. These two patterns of association are illustrated in Figure A1, which depicts the BOLD signal timecourses for color- and motion-sensitive regions from a single run, averaged across the 19 subjects. Although a uniformly competitive association could not be verified as in computational models, the predominant association was an inverse one, as predicted. Importantly, even when these trials were excluded, the conflict index still independently predicted activity in PPC and ACC but not DLPFC ($p < .001$), suggesting that this limitation did not significantly confound our results.

Figure A1: Color- and Motion-sensitive BOLD Signal Timecourses

Mean activity (% change in BOLD signal) for the three color-sensitive regions (red) and three motion-sensitive regions (blue), averaged across all 19 subjects, are plotted as a timecourse for a typical run. Overall, a weak positive correlation was observed for activities in these regions ($r = .12$, $p < .001$). However, this correlation appeared to reflect the average of two distinct patterns of association, modulated by the difference in overall activation between color- and motion-sensitive regions: across blocks of scans when the difference was small (highlighted in yellow), color- and motion-activities were highly correlated ($r = .97$, $p < .001$). When this quintile of trials was excluded from analysis, activities in color- and motion-sensitive regions were anticorrelated ($r = -.21$, $p < .001$), consistent with a competitive relationship between these two processing streams.



It should also be noted that two other formulations of conflict were considered.

1. We examined how activation of the irrelevant perceptual region predicted activity in ACC and PPC. Activity in the irrelevant perceptual region predicted activity in ACC and PPC independently of DLPFC ($p < .001$), but this measure was not as strong a predictor as the conflict index described in the text, perhaps because it failed to account for variance in the relevant processing stream.
2. We also considered the difference in overall activation between color- and motion-sensitive areas as an alternative to the formulation described in the text. We found that this difference measure did not reliably differentiate between shifts and repeats, high and low response conflict trials, or high and low stimulus conflict trials; nor did it account for any significant portion of the variance in activity in PPC, ACC, or DLPFC.

Thus, the formulation adopted by Botvinick et al. (2001) seemed to be the most reliable measure for our task paradigm.

Correlations between the conflict index and activity (BOLD signal, % change from run-average baseline) in DLPFC, ACC, and PPC were assessed by performing simple linear regressions for each subject, followed by a one-

sample t-test of the resultant beta values versus zero, to account for inter-subject variance. We also assessed whether the conflict index predicted activity in ACC independent of PPC, and vice versa, using within-subject partial correlations controlling for shared variance with the relevant structure, followed by a one-sample t-test accounting for inter-subject variance. When performing the conflict index correlation analyses, trials were excluded if the sums of the activities in color- and motion-sensitive regions were both negative, as noted in the text. This was because the computed conflict index would be distorted on these trials: neither color- nor motion-sensitive regions were significantly active, but the conflict index would increase paradoxically for trials with the least activity. This excluded approximately 30% of data points. There was no association with the experimental condition.

Notes on Region-of-Interest Selection. Within the relatively large areas of DLPFC, ACC, and PPC activated in the attention shifting contrast, we selected regions that overlapped with either conflict contrast for the analyses depicted in Figures 2.5 and 2.6. That is, we selected regions of DLPFC, ACC, and PPC that were engaged in the attention shifting contrast and *either* the response conflict contrast *or* the stimulus conflict contrast. This served

to highlight areas of DLPFC, ACC, and PPC that were particularly sensitive to conflict. These regions of interest are reasonable based on previous work. Our DLPFC ROI (Talairach coordinates: 36, 32, 36) is nearly identical in location to that defined in Kerns et al. (2004: 30, 34, 37). Kerns et al. (2004) highlights a region of ACC (3, 14, 41) similar to that observed in our attention shifting contrast (1, 15, 34). We selected a region at the rostral end of this area of activation that overlapped with the response conflict contrast to ensure maximal conflict sensitivity. Although our ACC ROI (5, 37, 17) is located somewhat rostral to the one defined in Kerns et al. (2004), reports of more rostral ACC activity in conflict detection studies are not uncommon (Swainson et al., 2003; Badre & Wagner, 2004; Van Veen & Carter, 2005)

Importantly, ROI selection did not bias the results. An analysis of all areas engaged by the shift versus repeat contrast (see Table A1) yielded essentially the same results as those described in Figure 2.5. Thus, different criteria could have been used for selecting ROIs, but our conclusions would have been the same: the conflict index predicted activity in ACC independent of PPC, and vice versa, but it accounted for only ~1% of the variance in DLPFC independent of activity in these structures.

Table A1 (see following page): Correlations with the Conflict Index. Within the relatively large areas of DLPFC, ACC, and PPC activated in the task switching contrast, we selected regions that overlapped with either conflict contrast. Statistics for these regions of interest are presented in the first three rows of the table below. The next three rows contain statistics for the partial correlations derived from these regions of interest. Importantly, ROI selection did not bias the results. Statistics for other regions of DLPFC, PPC, and ACC engaged in the task switching contrast are presented in the last five rows. These demonstrate that different criteria could have been used for selecting ROIs, but our conclusions would have been the same: the conflict index predicted activity in ACC independent of PPC, and vice versa, but it accounted for only ~1% of the variance in DLPFC independent of activity in these structures.

Table A1: Correlations with the Conflict Index. See caption on preceding page.

ROI	Talairach Coord. (x, y, z)	r (group mean)	t	p
Right DLPFC	36, 32, 36	.38	8.97	<.001
Right PPC (BA 7)	24, -60, 45	.48	16.41	<.001
Right rostral ACC	8, 36, 18	.49	11.37	<.001
Right PPC (controlling for shared variance with ACC)	24, -60, 45	.26	10.56	<.001
Right rostral ACC (controlling for shared variance with PPC)	8, 36, 18	.31	7.97	<.001
Right DLPFC (controlling for shared variance with PPC and ACC)	36, 32, 36	.11	5.56	<.001
Left DLPFC	-33, 41, 35	.41	9.99	<.001
Left PPC (BA 7)	-32, -54, 50	.46	14.49	<.001
Left rostral ACC	-13, 36, 28	.46	11.23	<.001
Right PPC (BA 40)	53, -38, 40	.44	13.86	<.001
Left PPC (BA 40)	-54, -36, 32	.45	11.69	<.001

Appendix 4

Perceived Stress Scale

Stress was quantified in human subjects in Chapter 4 using the Cohen Perceived Stress Scale. This widely used self-report measures the degree to which situations in a subject's life are perceived as stressful. Subjects are asked to respond to the following ten items by describing how often they felt or thought a certain way during the last month using a five-point scale (0 = never, 4 = very often):

1. In the last month, how often have you been upset because of something that happened unexpectedly?
2. In the last month, how often have you felt that you were unable to control the important things in your life?
3. In the last month, how often have you felt nervous and "stressed"?
4. In the last month, how often have you felt confident about your ability to handle your personal problems?
5. In the last month, how often have you felt that things were going your way?
6. In the last month, how often have you found that you could not cope with all the things that you had to do?

7. In the last month, how often have you been able to control irritations in your life?
8. In the last month, how often have you felt that you were on top of things?
9. In the last month, how often have you been angered because of things that were outside of your control?
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

The questionnaire is scored by reversing responses (i.e. 0 = 4, 1= 3, 2 = 2, 3 = 1, 4 = 0) to items 4, 5, 7, and 8 and then summing across all items, yielding a total score on a scale of zero to forty. The scale has been extensively validated, and higher PSS scores are reliably associated with a variety of stress-related health status measures (for further details, see Cohen et al., 1988). In a sample of 2,387 respondents, the mean score for subjects aged 18-29 years was 14.2, in accord with the median score of 14 among subjects who participated in the experiments reported in Chapter 4.

REFERENCES

Adams F (1891) The Genuine Works of Hippocrates. New York: William Wood and Company.

Akert K (1964) Comparative anatomy of frontal cortex and thalamofrontal connections. In JM Warren & K Akert (Eds), The Frontal Granular Cortex and Behavior. New York: McGraw-Hill.

Amat J, Baratta MV, Paul E, Bland ST, Watkins LR, Maier SF (2005) Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. Nature Neuroscience 8:365-371.

Anderson, R.A., and Buneo, C.A. (2002). Intentional maps in posterior parietal cortex. Ann Rev Neurosci *25*,189-220.

Anderson MC, Green C (2001) Suppressing unwanted memories by executive control. Nature 410:366-369.

Anderson MC, Ochsner KN, Kuhl B, Cooper J, Robertson E, Gabrieli SW, Glover GH, Gabrieli JDE (2004) Neural systems underlying the suppression of unwanted memories. Science 303:232-235.

APA (1994) Diagnostic and Statistical Manual of Mental Disorders, 4 Edition. Washington, DC: APA.

Arnsten AFT (1998) Catecholamine modulation of prefrontal cortical cognitive function. Trends in Cognitive Sciences 2:436-447.

Arnsten AFT, Goldman-Rakic PS (1998) Noise stress impairs prefrontal cortical cognitive function in monkeys - Evidence for a hyperdopaminergic mechanism. Archives of General Psychiatry 55:362-368.

Arnsten AFT, Shansky RM (2004) Adolescence: Vulnerable period for stress-induced prefrontal cortical function? - Introduction to Part IV. In: Adolescent Brain Development: Vulnerabilities and Opportunities, pp 143-147.

Badre, D., and Wagner, A.D. (2004). Selection, integration, and conflict monitoring: Assessing the Nature and Generality of Prefrontal Cognitive Control Mechanisms. Neuron *41,* 473-487.

Barbas H., and Pandya, D.N. (1989). Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. J Comp Neurol *286*, 353-375.

Barber, A.D., and Carter, C.S. (2005). Cognitive control involved in overcoming prepotent response tendencies and switching between tasks. Cereb Cortex *15*, 899-912.

Bates, J.F., and Goldman-Rakic, P.S. (1993). Prefrontal connections of medial motor areas in the rhesus monkey. J Comp Neurol *336*, 211-228.

Beck AT (1970) Cognitive Therapy - Nature and Relation to Behavior Therapy. Behavior Therapy 1:184-200.

Berger B (1992) Dopaminergic innervation of the frontal cerebral cortex: Evolutionary trends and functional implications. In P Chauvel, AV Delgado-Escuerta, E Halgren, J Bancaud (Eds) Frontal Lobe Seizures and Epilepsies. New York: Raven.

Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nature Reviews Neuroscience 7:137-151.

Bjorklund A, Divac I, Lindvall O (1978) Regional distribution of catecholamines in monkey cerebral cortex: Evidence for a dopaminergic innervation of primate prefrontal cortex. Neuroscience Letters 7:115-119.

Bishop, S., Duncan, J., Brett, M., and Lawrence, A.D. (2004). Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli. Nat Neurosci *7*, 184-188.

Birrell JM, Brown VJ (2000) Medial frontal cortex mediates perceptual attentional set shifting in the rat. Journal of Neuroscience 20:4320-4324.

Bodnoff SR, Humphreys AG, Lehman JC, Diamond DM, Rose GM, Meaney MJ (1995) Enduring Effects of Chronic Corticosterone Treatment on Spatial-Learning, Synaptic Plasticity, and Hippocampal Neuropathology in Young and Mid-Aged Rats. Journal of Neuroscience 15:61-69.

Botvinick, M.M., Braver, T.S., Barch, D.M., Carter C.S., and Cohen J.D. (2001). Conflict monitoring and cognitive control. Psychol Rev *108*, 624-652.

Bosma H, Marmot MG, Hemingway H, Nicholson AC, Brunner E, Stansfeld SA (1997) Low job control and risk of coronary heart disease in Whitehall II (prospective cohort) study. British Medical Journal 314:558-565.

Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, Delaney RC, McCarthy G, Charney DS, Innis RB (1995) Mri-Based Measurement of Hippocampal Volume in Patients with Combat-Related Posttraumatic-Stress-Disorder. American Journal of Psychiatry 152:973-981.

Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure C, Capelli S, McCarthy G, Innis RB, Charney DS (1997) Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse - A preliminary report. Biological Psychiatry 41:23-32.

Brodmann K (1909) Vergleichende Lokalisationslehre der Grosshirrhinde. Leipzig: Barth.

Brown RM, Goldman PS (1977) Catecholamines in neocortex of rhesus monkeys: Regional distribution and ontogenetic development. Brain Research 124:576-580.

Burgess, P.W., Veitch, E., Costello, A.D., and Shallice, T. (2000). The cognitive and neuroanatomical correlates of multitasking. Neuropsychologia *38,* 848-863.

Brown VJ, Bowman EM (2002) Rodent models of prefrontal cortical function. Trends in Neurosciences 25:340-343.

Cambon K, Hansen SM, Venero C, Herrero AI, Skibo G, Berezin V, Bock E, Sandi C (2004) A synthetic neural cell adhesion molecule mimetic peptide promotes synaptogenesis, enhances presynaptic function, and facilitates memory consolidation. Journal of Neuroscience 24:4197-4204.

Campbell, F.W., and Maffei, L. (1980). The influence of spatial frequency and contrast on the perception of moving patterns. Vision Res *21*, 713-721.

Carter, C.S., Braver, T.S., Barch, D.M., Botvinick, M.M., Noll, D., and Cohen, J.D. (1998). Anterior cingulate cortex, error detection, and the online monitoring of performance. Science *280*, 747-749.

Carter, C.S., MacDonald, A.M., Botvinick M., Ross, L.L., Stenger, V.A., Noll, D., and Cohen, J.D. (2000). Parsing executive processes: strategic vs. evaluative functions of the anterior cingulate cortex. Proc Nat Acad Sci U.S.A., *97*, 1944-1948.

Casey, B.J., Thomas, K.M., Welsh, T.F., et al (2000). Dissociation of response conflict, attentional selection, and expectancy with functional magnetic resonance imaging. Proc Natl Acad Sci U S A *97*, 8728-8733.

Casey BJ, Tottenham N, Fossella J (2002) Clinical, imaging, lesion, and genetic approaches toward a model of cognitive control. Developmental Psychobiology 40:237-254.

Casey BJ, Thomas KM, Welsh TF, Badgaiyan RD, Eccard CH, Jennings JR, Crone EA (2000) Dissociation of response conflict, attentional selection, and expectancy with functional magnetic resonance imaging. Proceedings of the National Academy of Sciences of the United States of America 97:8728-8733.

Caspari R, Lee SH (2004) Older age becomes common late in human evolution. Proceedings of the National Academy of Sciences of the United States of America 101:10895-10900.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science 301:386-389.

Cerqueiras JJ, Pego JM, Taipa R, Bessa JM, Almeida OFX, Sousa N (2005) Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. J Neurosci 25:7792-7800.

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. Journal of Neuroscience 24:4401-4411.

Chen ZY, Ieraci A, Teng H, Dall H, Meng CX, Herrera DG, Nykjaer A, Hempstead BL, Lee FS (2005) Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. Journal of Neuroscience 25:6156-6166.

Cohen, J.D., and Servan-Schreiber, D. (1992). Context, cortex and dopamine: a Connectionist approach to behavior and biology in schizophrenia. Psychol Rev *99*, 47.

Cohen S, Kamarck T, Mermelstein R (1983) A global measure of perceived stress. Journal of Health and Social Behavior 24:385-396.

Conrad CD, Galea LAM, Kuroda Y, McEwen BS (1996) Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. Behavioral Neuroscience 110:1321-1334.

Conrad CD, Magarinos AM, LeDoux JE, McEwen BS (1999) Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. Behavioral Neuroscience 113:902-913.

Cook SC, Wellman CL (2004) Chronic stress alters dendritic morphology in rat medial prefrontal cortex. Journal of Neurobiology 60:236-248.

Corbetta, M., Kincade, J.M., Ollinger, J.M., McAvoy, M.P., Shulman, G.L. (2000). Voluntary orienting is dissociated from target detection in human posterior parietal cortex. Nat Neurosci *3*, 292-297.

Corbetta, M., and Shulman, G.L. (2002). Control of goal-directed and stimulus-driven attention in the brain. Nat Rev Neurosci *3*, 201-215.

Dalley JW, Cardinal RN, Robbins TW (2004) Prefrontal executive and cognitive functions in rodents: Neural and neurochemical substrates. Neuroscience and Biobehavioral Reviews 28:771-784.

Davidson RJ (1998) Affective style and affective disorders: Perspectives from affective neuroscience. Cognition & Emotion 12:307-330.

Davidson RJ, Jackson DC, Kalin NH (2000) Emotion, plasticity, context, and regulation: Perspectives from affective neuroscience. Psychological Bulletin 126:890-909.

Davidson RJ, Abercrombie H, Nitschke JB, Putnam K (1999) Regional brain function, emotion and disorders of emotion. Current Opinion in Neurobiology 9:228-234.

Davidson RJ, Pizzagalli D, Nitschke JB, Putnam K (2002) Depression: Perspectives from affective neuroscience. Annual Review of Psychology 53:545-574.

de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adapatation to disease. Nature Reviews Neuroscience 6:463-475.

Deary IJ, Battersby S, Whiteman MC, Connor JM, Fowkes FGR, Harmar A (1999) Neuroticism and polymorphisms in the serotonin transporter gene. Psychological Medicine 29:735-739.

Dehaene S, Kerszberg M, Changeux JP (1998) A neuronal model of a global workspace in effortful cognitive tasks. Proceedings of the National Academy of Sciences of the United States of America 95:14529-14534.

Desimone R, Duncan J (1995) Neural Mechanisms of Selective Visual-Attention. Annual Review of Neuroscience 18:193-222.

Diamond DM, Bennett MC, Fleshner M, Rose GM (1992) Inverted-U Relationship between the Level of Peripheral Corticosterone and the Magnitude of Hippocampal Primed Burst Potentiation. Hippocampus 2:421-430.

Dias R, Robbins TW, Roberts AC (1996) Dissociation in prefrontal cortex of affective and attentional shifts. Nature 380:69-72.

Divac I, Bjorklund A, Lindvall O, Passingham RE (1978) Converging projections from the mediodorsal thalamic nucleus and mesencephalic dopaminergic neurons to the neocortex in three species. Journal of Comparative Neurology 180:59-72.

Diorio D, Viau V, Meaney MJ (1993) The Role of the Medial Prefrontal Cortex (Cingulate Gyrus) in the Regulation of Hypothalamic-Pituitary-Adrenal Responses to Stress. Journal of Neuroscience 13:3839-3847.

Dreher, J.C., and Berman, K.F. (2002). Fractionating the neural substrate of cognitive control processes. Proc Nat Acad Sci USA *99,* 14595-14600.

Dreher, J.C., and Grafman J. (2003). Dissociating the roles of the rostral anterior cingulate and the lateral prefrontal cortices in performing two tasks simultaneously or successively. Cereb Cortex *13,* 329-339.

Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME (1992) A Functional Anatomical Study of Unipolar Depression. Journal of Neuroscience 12:3628-3641.

Drevets WC, Price JL, Simpson JR, Todd RD, Reich T, Vannier M, Raichle ME (1997) Subgenual prefrontal cortex abnormalities in mood disorders. Nature 386:824-827.

Durston, S., Thomas, K.M., Worden, M.S., Yang, Y., and Casey, B.J. (2002). The effect of preceding contex on inhibition. Neuroimage *16*, 449-453.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257-269.

Ershler WB, Keller ET (2000) Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annual Review of Medicine 51:245-270.

Everson SA, Lynch JW, Chesney MA, Kaplan GA, Goldberg DE, Shade SB, Cohen RD, Salonen R, Salonen JT (1997) Interaction of workplace demands and cardiovascular reactivity in progression of carotid atherosclerosis: Population based study. British Medical Journal 314:553-558.

Flory JD, Manuck SB, Ferrell RE, Dent KM, Peters DG, Muldoon MF (1999) Neuroticism is not associated with the serotonin transporter (5-HTTLPR) polymorphism. Molecular Psychiatry 4:93-96.

Forman SD, Cohen JD, Fitzgerald M, Eddy WF, Mintun MA, Noll DC (1995) Improved Assessment of Significant Activation in Functional Magnetic-Resonance-Imaging (Fmri) - Use of a Cluster-Size Threshold. Magnetic Resonance in Medicine 33:636-647.

Fox MT, Barense MD, Baxter MG (2003) Perceptual attentional set-shifting is impaired in rats with neurotoxic lesions of posterior parietal cortex. Journal of Neuroscience 23:676-681.

Freud S (1966) The Standard Edition of the Complete Psychological Works of Sigmund Freud. London: Hogarth.

Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby S (2005) Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. Journal of Comparative Neurology 492:145-177.

Gilbertson MW, Shenton ME, Ciszewski A, Kasai K, Lasko NB, Orr SP, Pitman RK (2002) Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. Nature Neuroscience 5:1242-1247.

Glowinski J, Tassin JP, Thierry AM (1984) The mesocortico-prefrontal dopaminergic neurons. Trends in Neurosciences 7:415-418.

Glover, G.H., and Thomason, M.E. (2004). Improved combination of spiral-in/out images for BOLD fMRI. Magn Reson Med *51*, 863-868.

Gold, J.I., and Shadlen, M.N. (2001). Neural computations that underlie decisions about sensory stimuli. Trends Cog Sci *5*, 10-16.

Goldstein LE, Rasmusson AM, Bunney BS, Roth RH (1996) Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. Journal of Neuroscience 16:4787-4798.

Gould E, McEwen BS, Tanapat P, Galea LAM, Fuchs E (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated

by psychosocial stress and NMDA receptor activation. Journal of Neuroscience 17:2492-2498.

Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E (1998) Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proceedings of the National Academy of Sciences of the United States of America 95:3168-3171.

Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, Egan MF, Weinberger DR (2002) Serotonin transporter genetic variation and the response of the human amygdala. Science 297:400-403.

Harrison LM, Mair RG (1996) A comparison of the effects of frontal cortical and thalamic lesions on measures of spatial learning and memory in the rat. Behavioral Brain Research 75:195-206.

Heidbreder CA, Groenewegen HJ (2003) The medial prefrontal cortex in the rat: Evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neurosci Biobehav Rev 27:555-579.

Heim C, Owens MJ, Plotsky PM, Nemeroff CB (1997a) Persistent changes in corticotropin-releasing factor systems due to early life stress: Relationship to the pathophysiology of major depression and post-traumatic stress disorder. Psychopharmacology Bulletin 33:185-192.

Heim C, Owens MJ, Plotsky PM, Nemeroff CB (1997b) The role of early adverse life events in the etiology of depression and posttraumatic stress disorder - Focus on corticotropin-releasing factor. In: Psychobiology of Posttraumatic Stress Disorder, pp 194-207.

Henik, A., Bibi, U., Yanai, M., and Tzelgov, J. (1997) The stroop effect is largest during first trials. Abstracts Psychonomic Soc *2*, 57.

Henriques JB, Davidson RJ (1991) Left Frontal Hypoactivation in Depression. Journal of Abnormal Psychology 100:535-545.

Herman JP, Cullinan WE (1997) Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. Trends in Neurosciences 20:78-84.

Ito M, Gilbert CD (1999) Attention modulates contextual influences in the primary visual cortex of alert monkeys. Neuron 22:593-604.

Issa AM, Rowe W, Gauthier S, Meaney MJ (1990) Hypothalamic-Pituitary-Adrenal Activity in Aged, Cognitively Impaired and Cognitively Unimpaired Rats. Journal of Neuroscience 10:3247-3254.

Izquierdo A, Wellman CL, Holmes A (2006) Brief uncontrollable stress causes dendritic retraction in infralimbic cortex and resistance to fear extinction in mice. Journal of Neuroscience 26:5733-5738.

Jiang XY, Xu K, Hoberman J, Tian F, Marko AJ, Waheed JF, Harris CR, Marini AM, Enoch MA, Lipsky RH (2005) BDNF variation and mood disorders: A novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. Neuropsychopharmacology 30:1353-1361.

Joels M, Pu ZW, Wiegert O, Oitzl MS, Krugers HJ (2006) Learning under stress: How does it work? Trends in Cognitive Sciences 10:152-158.

Kaufman J, Yang BZ, Douglas-Palumberi H, Grasso D, Lipschitz D, Houshyar S, Krystal JH, Gelernter J (2006) Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. Biological Psychiatry 59:673-680.

Kerns, J.G., Cohen, J.D., MacDonald, A.W., Cho, R.Y., Stenger, V.A., and Carter, C.S. (2004). Anterior cingulate conflict monitoring and adjustments in control. Science *303*, 1023-1026.

Kerr DS, Campbell LW, Thibault O, Landfield PW (1992) Hippocampal glucocorticoid receptor activation enhances voltage-dependent Ca2+ conductances: Relevance to brain aging. Proc Natl Acad Sci USA 89:8527-8531.

Kesner RP, Ragozzino ME (2003) The role of the prefrontal cortex in object-place learning: A test of the attribute specificity model. Behavioral Brain Research 146:159-165.

Kiecolt-Glaser JK, McGuire L, Robles TF, Glaser R (2002) Emotions, morbidity, and mortality: New perspectives from psychoneuroimmunology. Annual Review of Psychology 53:83-107.

Koechlin, E., Ody, C., and Kouneiher F (2003). The architecture of cognitive control in the human prefrontal cortex. Science *302*, 1181-1185.

Kolb B (1984) Functions of the frontal cortex of the rat: A comparative review. Brain Research Reviews 8:65-98.

Koob GF, LeMoal M (1997) Drug abuse: Hedonic homeostatic dysregulation. Science 278:52-58.

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97-129.

Kovacs M, Rush AJ, Beck AT, Hollon SD (1981) Depressed Outpatients Treated with Cognitive Therapy or Pharmacotherapy - a One-Year Follow-Up. Archives of General Psychiatry 38:33-39.

Kozlov AP, Druzin MY, Kurzina NP, Malinina EP (2001) The role of D1-dependent dopaminergic mechanisms of the frontal cortex in delayed responding rats. Neurosci Behav Physiol 31:405-411.

Kreek MJ (1996) Opiates, opioids and addiction. Molecular Psychiatry 1:232-254.

Kreek MJ, Nielsen DA, Butelman ER, LaForge KS (2005) Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nature Neuroscience 8:1450-1457.

Kubo C, Gajjar A, Johnson BC, Good RA (1992) The effects of dietary restriction on immune function and development of autoimmune disease in BXSB mice. Proc Natl Acad Sci USA 89:3145-3149.

Kuroda Y, McEwen BS (1998) Effect of chronic restraint stress and tianeptine on growth factors, growth-associated protein-43 and microtubule-associated protein 2 mRNA expression in the rat hippocampus. Molecular Brain Research 59:35-39.

Lang UE, Hellweg R, Kalus P, Bajbouj M, Lenzen KP, Sander T, Kunz D, Gallinat J (2005) Association of a functional BDNF polymorphism and anxiety-related personality traits. Psychopharmacology 180:95-99.

Lebron K, Milad MR, Quirk GJ (2004) Delayed recall of fear extinction in rats with lesions of ventral medial prefrontal cortex. Learning & Memory 11:544-548.

LeDoux JE (2000) Emotion circuits in the brain. Annual Review of Neuroscience 23:155-184.

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274:1527-1531.

Li W, Piech V, Gilbert CD (2004) Perceptual learning and top-down influences in primary visual cortex. Nature Neuroscience 7:651-657.

Lidow MS, Koh P, Arnsten AFT (2003) D1 Dopamine Receptors in the Mouse Prefrontal Cortex: Immunocytochemical and Cognitive Neuropharmacological Analyses. Synapse 47:101-108.

Liston C, Sepulveda-Orengo M, Miller MM, Morrison JH, Hof PR, McEwen BS, Quirk GJ (2006a) The effects of chronic and acute restraint stress on extinction of conditioned fear. Society for Neuroscience Annual Meeting Abstract.

Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, Morrison JH, McEwen BS (2006b) Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. Journal of Neuroscience 26:7870-7874.

Liston C, Matalon S, Hare TA, Davidson MC, Casey BJ (2006c) Anterior cingulate and posterior parietal cortices are sensitive to dissociable forms of conflict in a task-switching paradigm. Neuron 50:643-653.

Luine V, Villegas M, Martinez C, McEwen BS (1994) Repeated Stress Causes Reversible Impairments of Spatial Memory Performance. Brain Research 639:167-170.

Luks, T.L., Simpson, G.V., Feiwell, R.J., Miller, W.J. (2002). Evidence for anterior cingulate cortex involvement in monitoring preparatory attentional set. Neuroimage *17,* 792-802.

Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Thakur M, McEwen BS, Hauger RL, Meaney MJ (1998) Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nature Neuroscience 1:69-73.

MacDonald, A.W., Cohen, J.D., Stenger, V.A., and Carter, C.S. (2000). Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. Science *288*, 1835-1838.

Magarinos AM, McEwen BS (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Involvement of glucocorticoid secretion and excitatory amino acid receptors. Neurosci 69:89-98.

Magarinos AM, Verdugo JMG, McEwen BS (1997) Chronic stress alters synaptic terminal structure in hippocampus. Proceedings of the National Academy of Sciences of the United States of America 94:14002-14008.

Magarinos AM, Deslandes A, McEwen BS (1999) Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. European Journal of Pharmacology 371:113-122.

Magarinos AM, McEwen BS, Flugge G, Fuchs E (1996) Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. Journal of Neuroscience 16:3534-3540.

Maroun M, Richter-Levin G (2003) Exposure to acute stress blocks the induction of long-term potentiation of the amygdala-prefrontal cortex pathway in vivo. Journal of Neuroscience 23:4406-4409.

Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, Schwalb JM, Kennedy SH (2005) Deep brain stimulation for treatment-resistant depression. Neuron 45:651-660.

Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA, Silva JA, Tekell JL, Martin CC, Lancaster JL, Fox PT (1999) Reciprocal limbic-cortical function and negative mood: Converging PET findings in depression and normal sadness. American Journal of Psychiatry 156:675-682.

McAlonan K, Brown VJ (2003) Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. Behavioural Brain Research 146:97-103.

McEwen BS (1998) Protective and damaging effects of stress mediators. New England Journal of Medicine 338:171-179.

McEwen BS (1999) Stress and hippocampal plasticity. Annual Review of Neuroscience 22:105-122.

McEwen BS (2000a) Effects of adverse experiences for brain structure and function. Biological Psychiatry 48:721-731.

McEwen BS (2000b) The neurobiology of stress: from serendipity to clinical relevance. Brain Research 886:172-189.

McEwen BS (2003) Mood disorders and allostatic load. Biological Psychiatry 54:200-207.

McEwen BS, Stellar E (1993) Stress and the Individual - Mechanisms Leading to Disease. Archives of Internal Medicine 153:2093-2101.

McEwen BS, Sapolsky RM (1995) Stress and Cognitive Function. Current Opinion in Neurobiology 5:205-216.

Meng, X.L., Rosenthal, R., Rubin, D.B. (1992). Comparing correlated correlation-coefficients. Psychol Bull *111,* 172-175.

Mennini T, Miari A (1991) Modulation of [H-3] Glutamate Binding by Serotonin in the Rat Hippocampus - an Autoradiographic Study. Life Sciences 49:283-292.

Milad MR, Quirk GJ (2002) Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420:70-74.

Milham, M.P., Banich, M.T., Claus, E.D., and Cohen, N.J. (2003). Practice-related effects demonstrate complementary roles of anterior cingulate and dorsolateral prefrontal cortices in attentional control. Neuroimage *18*, 483-493.

Milham, M.P., and Banich, M.T. (2005). Anterior cingulate cortex: an fMRI analysis of conflict specificity and functional differentiation. Hum Brain Map *25*, 328-335.

Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annual Review of Neuroscience 24:167-202.

Miller MM, Liston C, McEwen BS (2005) Prolonged effects of acute stress on anxiety behaivor and morphology in regions of the amygdala and frontal cortex. Society for Neuroscience Annual Meeting Abstract.

Miracle AD, Brace MF, Huyck KD, Singler SA, Wellman CL (2006) Chronic stress impairs recall of extinction of conditioned fear. Neurobiology of Learning and Memory 85:213-218.

Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S (2005) Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. Proceedings of the National Academy of Sciences of the United States of America 102:9371-9376.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T (2000) Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. Journal of Neuroscience 20:1568-1574.

Murphy BL, Arnsten AFT, GoldmanRakic PS, Roth RH (1996a) Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. Proceedings of the National Academy of Sciences of the United States of America 93:1325-1329.

Murphy BL, Arnsten AFT, Jentsch JD, Roth RH (1996b) Dopamine and spatial working memory in rats and monkeys: Pharmacological reversal of stress-induced impairment. Journal of Neuroscience 16:7768-7775.

Murray CJL, Lopez AD (1997a) Regional patterns of disability-free life expectancy and disability adjusted life expectancy: Global Burden of Disease Study. Lancet 349:1347-1352.

Murray CJL, Lopez AD (1997b) Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. Lancet 349:1436-1442.

Murray CJL, Lopez AD (1997c) Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet 349:1269-1276.

Murray CJL, Lopez AD (1997d) Alternative projections of mortality and disability by cause 1990-2020: Global burden of disease study. Lancet 349:1498-1504.

Nesse RM (2000) Is depression an adaptation? Archives of General Psychiatry 57:14-20.

Nesse RM, Berridge KC (1997) Psychoactive drug use in evolutionary perspective. Science 278:63-66.

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM (2002) Neurobiology of depression. Neuron 34:13-25.

Ochsner KN, Gross JJ (2005) The cognitive control of emotion. Trends in Cognitive Sciences 9:242-249.

Ohara K, Nagai M, Tsukamoto T, Tani K, Suzuki Y, Ohara K (1998) Functional polymorphism in the serotonin transporter promoter at the SLC6A4 locus and mood disorders. Biological Psychiatry 44:550-554.

Ohl F, Fuchs E (1999) Differential effects of chronic stress on memory processes in the tree shrew. Cognitive Brain Research 7:379-387.

O'Reilly, R.C., Noelle, D.C., Braver, T.S., and Cohen J.D. (2002). Prefrontal cortex and dynamic categorization tasks: representational organization and neuromodulatory control. Cereb Cortex *12*, 246-257.

Ozawa CR, Ho JJ, Tsai DJ, Ho DY, Sapolsky RM (2000) Neuroprotective potential of a viral vector system induced by a neurological insult. Proceedings of the National Academy of Sciences of the United States of America 97:9270-9275.

Passingham RE, Myers C, Rawlins N, Lightfoot V, Fearns S (1988) Premotor cortex in the rat. Behavioral Neuroscience 102:101-109.

Paus, T. (2001). Primate anterior cingulate cortex: where motor control, drive, and cognition interface. Nat Rev Neurosci *2*, 417-424.

Pavlides C, Watanabe Y, McEwen BS (1993) Effects of Glucocorticoids on Hippocampal Long-Term Potentiation. Hippocampus 3:183-192.

Petersen, S.E., van Mier H., Fiez, J.A., and Raichle, M.E. (1998). The effects of practice on the functional anatomy of task performance. Proc Natl Acad Sci USA *95*, 853-860.

Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BA, Munoz KE, Kolachana BS, Egan MF, Mattay VS, Hariri AR, Weinberger DR (2005) 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. Nature Neuroscience 8:828-834.

Phillips RG, Ledoux JE (1992) Differential Contribution of Amygdala and Hippocampus to Cued and Contextual Fear Conditioning. Behavioral Neuroscience 106:274-285.

Platt, M.L., and Glimcher, P.W. (1999). Neural correlates of decision variables in parietal cortex. Nature 400, 233-238.

Preuss TM, Goldman-Rakic PS (1991a) Myelo- and cytoarchitecture of the granular frontal cortex and surrounding regions in the strepsirhine primate Galago and the anthropoid primate Macaca. Journal of Comparative Neurology 310: 429-474.

Preuss TM, Goldman-Rakic PS (1991b) Ipsilateral cortical connections of granular frontal cortex in the strepsirhine primate Galago, with comparative comments on anthropoid primates. Journal of Comparative Neurology 310:507-549.

Preuss TM (1995) Do rats have prefrontal cortex? The Rose-Woolsey-Akert Program Reconsidered. Journal of Cognitive Neuroscience 7:1-24.

Quirk GJ, Russo GK, Barron JL, Lebron K (2000) The role of ventromedial prefrontal cortex in the recovery of extinguished fear. Journal of Neuroscience 20:6225-6231.

Quirk GJ, Likhtik E, Pelletier JG, Pare D (2003) Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. Journal of Neuroscience 23:8800-8807.

Radley JJ, Rocher AB, Janssen WGM, Hof PR, McEwen BS, Morrison JH (2005) Reversibility of apical dendritic retraction in the rat medial prefrontal cortex following repeated stress. Experimental Neurology 196:199-203.

Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, McEwen BS, Morrison JH (2004) Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. Neuroscience 125:1-6.

Radley JJ, Rocher AB, Miller M, Janssen WGM, Liston C, Hof PR, McEwen BS, Morrison JH (2006) Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. Cerebral Cortex 16:313-320.

Ragozzino ME, Adams S, Kesner RP (1998) Differential involvement of the dorsal anterior cingulate and prelimbic-infralimbic areas of the rodent prefrontal cortex in spatial working memory. Behavioral Neuroscience 112:293-303.

Ragozzino ME, Detrick S, Kesner RP (1999) Involvement of the prelimbic/infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. Journal of Neuroscience 19:4585-4594.

Rahmann S, Neumann RS (1993) Activation of 5-HT2 receptors facilitates depolarization of neocortical neurons by N-methyl-D-aspartate. European Journal of Pharmacology 231:347-354.

Raichle, M.E., Fiez, J.A., Videen, T.O., Macleod, A.M.K., Pardo, J.V., Fox, P.T., and Petersen, S.E. (1994). Practice-related changes in human brain functional-anatomy during nonmotor learning. Cereb Cortex *4*, 8-26.

Rapp PR, Gallagher M (1996) Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. Proceedings of the National Academy of Sciences of the United States of America 93:9926-9930.

Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, Orr SP, Pitman RK (2000) Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: A functional MRI study. Biological Psychiatry 47:769-776.

Rogers, R.D., and Monsell, S. (1995) Costs of a predictable switch between simple cognitive tasks. J Exp Psychol Gen *124,* 207-231.

Roozendaal B, McReynolds JR, McGaugh JL (2004) The basolateral amygdala interacts with the medial prefrontal cortex in regulating glucocorticoid effects on working memory impairment. J Neurosci 24:1385-1392.

Rose JE, Woolsey CN (1948) The orbitofrontal cortex and its connections with the mediodorsal nucleus in rabbit, sheep, and cat. Research Publications Association for Research in Nervous and Mental Disease 27:210-232.

Rushworth, M.F.S., Hadland, K.A., Paus, T., and Sipila, P.K. (2002). The role of the medial frontal cortex in task switching: a combined fMRI and TMS study. J Neurophysiol *87*, 2577-2592.

Rushworth, M.F.S., Hadland, K.A., Gaffan, D., Passingham, R.E. (2003). The effect of cingulate cortex lesions on task switching and working memory. J Cogn Neurosci *15,* 338-353.

Sandi C, Loscertales M, Guaza C (1997) Experience-dependent facilitating effect of corticosterone on spatial memory formation in the water maze. European Journal of Neuroscience 9:637-642.

Sandi C (2004) Stress, cognitive impairment and cell adhesion molecules. Nature Reviews Neuroscience 5:917-930.

Santini E, Ge H, Ren KQ, de Ortiz SP, Quirk GJ (2004) Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. Journal of Neuroscience 24:5704-5710.

Sapolsky RM (1985) A Mechanism for Glucocorticoid Toxicity in the Hippocampus - Increased Neuronal Vulnerability to Metabolic Insults. Journal of Neuroscience 5:1228-1232.

Sapolsky RM (1986) Glucocorticoid Toxicity in the Hippocampus - Reversal by Supplementation with Brain Fuels. Journal of Neuroscience 6:2240-2244.

Sapolsky RM (1996) Why stress is bad for your brain. Science 273:749-750.

Sapolsky RM (2005) The influence of social hierarchy on primate health. Science 308:648-652.

Sapolsky RM, Packan DR, Vale WW (1988) Glucocorticoid Toxicity in the Hippocampus - Invitro Demonstration. Brain Research 453:367-371.

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21:55-89.

Saul JP (1990) Beat-to-Beat Variations of Heart-Rate Reflect Modulation of Cardiac Autonomic Outflow. News in Physiological Sciences 5:32-37.

Sawaguchi T, Goldman-Rakic PS (1991) D1 dopamine receptors in prefrontal cortex: Involvement in working memory. Science 251:947-950.

Schnall PL, Schwartz JE, Landsbergis PA, Warren K, Pickering TG (1992) Relation between Job Strain, Alcohol, and Ambulatory Blood-Pressure. Hypertension 19:488-494.

Schultz W (2000) Multiple reward signals in the brain. Nature Reviews Neuroscience 1:199-207.

Seeman TE, McEwen BS, Singer BH, Albert MS, Rowe JW (1997) Increase in urinary cortisol excretion and memory declines: MacArthur studies of successful aging. Journal of Clinical Endocrinology and Metabolism 82:2458-2465.

Selye H (1936) A syndrome produced by diverse nocuous agents. Nature 138:32-32.

Selye H (1973) The evolution of the stress concept. American Psychologist 61:692-699.

Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burmeister M (2003) A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology 28:397-401.

Shansky RM, Glavis-Bloom C, Lerman D, McRae P, Benson C, Miller K, Cosand L, Horvath TL, Arnsten AFT (2004) Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. Molecular Psychiatry 9:531-538.

Sheline YI, Sanghavi M, Mintun MA, Gado MH (1999) Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. Journal of Neuroscience 19:5034-5043.

Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996) Hippocampal atrophy in recurrent major depression. Proceedings of the National Academy of Sciences of the United States of America 93:3908-3913.

Shors TJ, Weiss C, Thompson RF (1992) Stress-induced facilitation of classical conditioning. Science 257:537-539.

Sigman M, Pan H, Yang YH, Stern E, Silbersweig D, Gilbert CD (2005) Top-down reorganization of activity in the visual pathway after learning a shape identification task. Neuron 46:823-835.

Smith MA, Makino S, Kvetnansky R, Post RM (1995) Stress and Glucocorticoids Affect the Expression of Brain-Derived Neurotrophic Factor and Neurotrophin-3 Messenger-Rnas in the Hippocampus. Journal of Neuroscience 15:1768-1777.

Snyder, L.H., Batista, A.P., Andersen, R.A. (1997). Coding of intention in the posterior parietal cortex. Nature *386*, 167-170.

Sohn, M.H., Ursu, S., Anderson, J.R., Stenger, V.A., and Carter, C.S. (2000). The role of the prefrontal cortex and posterior parietal cortex in task switching. Proc Natl Acad Sci USA *97*, 13448-13453.

Stuesse SL, Newman DB (1990) Projections from the medial agranular cortex to brain steam visuomotor centers in rats. Experimental Brain Research 80:532-544.

Stutzmann GE, McEwen BS, LeDoux JE (1998) Serotonin modulation of sensory inputs to the lateral amygdala: Dependency on corticosterone. J Neurosci 18: 9529-9538.

Swainson, R., Cunnington, R., Jackson, G.M., Rorden, C., Peters, A.M., Morris, P.G., and Jackson, S.R. (2003). Cognitive control mechanisms revealed by ERP and fMRI: Evidence from repeated task-switching. J Cogn Neurosci *15,* 785-799.

Swanson (1992) Brain maps: Structure of the rat brain. New York: Elsevier.

Thayer JF, Brosschot JF (2005) Psychosomatics and psychopathology: looking up and down from the brain. Psychoneuroendocrinology 30:1050-1058.

Thierry AM, Tassin JP, Blanc G, Glowinski J (1976) Selective Activation of Mesocortical Da System by Stress. Nature 263:242-244.

Ueyama T, Kawai Y, Nemoto K, Sekimoto M, Tone S, Senba E (1997) Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. Neuroscience Research 28:103-110.

Uno H, Tarara R, Else JG, Suleman MA, Sapolsky RM (1989) Hippocampal Damage Associated with Prolonged and Fatal Stress in Primates. Journal of Neuroscience 9:1705-1711.

Vanhaaren F, Debruin JPC, Heinsbroek RPW, Vandepoll NE (1985) Delayed Spatial Response Alternation - Effects of Delay-Interval Duration and Lesions of the Medial Prefrontal Cortex on Response Accuracy of Male and Female Wistar Rats. Behavioural Brain Research 18:41-49.

Van Veen, V., Cohen, J.D., Botvinick, M.M., Stenger, V.A., and Carter C.S. (2001). Anterior cingulate cortex, conflict monitoring, and levels of processing. Neuroimage *14,* 1302-1308.

Van Veen, V., and Carter, C.S. (2002). The timing of action-monitoring processes in the anterior cingulate cortex. J Cogn Neurosci *14,* 593-602.

Van Veen, V., and Carter, C.S. (2005). Separating semantic conflict and response conflict in the Stroop task: A functional MRI study. Neuroimage *27,* 497-504.

Volkow ND, Fowler JS (2000) Addiction, a disease of compulsion and drive: Involvement of the orbitofrontal cortex. Cerebral Cortex 10:318-325.

Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S (2002) Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neurosci 22:6810-6818.

Vyas A, Bernal S, Chattarji S (2003) Effects of chronic stress on dendritic arborization in the central and extended amygdala. Brain Research 965:290-294.

Vyas A, Mitra R, Rao BSS, Chattarji S (2002) Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. Journal of Neuroscience 22:6810-6818.

Watanabe Y, Gould E, McEwen BS (1992) Stress Induces Atrophy of Apical Dendrites of Hippocampal Ca3 Pyramidal Neurons. Brain Research 588:341-345.

Weindruch R, Walford RL (1988) The Retardation of Aging and Disease by Dietary Restriction. Springfield, IL: Charles C. Thomas.

Weismann, D.H., Giesbrecht, B., Song, A.W., Mangun, G.R., and Woldorff, M.G. (2003). Conflict monitoring in the human anterior cingulate cortex during selective attention to global and local object features. Neuroimage, *19*, 1361-1368.

Williams GC, Nesse RM (1991) The Dawn of Darwinian Medicine. Quarterly Review of Biology 66:1-22.

Wise, S.P., Boussaoud, D., Johnson, P.B., and Caminiti, R. (1997). Premotor and parietal cortex: corticocortical connectivity and combinatorial computations. Ann Rev Neurosci *20*, 25-42.

Wood ER, Dudchenko PA, Eichenbaum H (1999) The global record of memory in hippocampal neuronal activity. Nature 397:613-616.

Zahrt J, Taylor A, Mathew RG, Arnsten AFT (1997) Supranormal stimulation of dopamine D1 receptors in the rodent prefrontal cortex impairs spatial working memory performance. Journal of Neuroscience 17:8258-8535.