NORADRENERGIC MODULATION OF THE ALERTING SYSTEM: CUE-RELATED BOLD SIGNAL CHANGES IN A DOUBLE-BLIND, PLACEBO CONTROLLED, GUANFACINE CHALLENGE

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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ABSTRACT

NORADRENERGIC MODULATION OF THE ALERTING SYSTEM: CUE-RELATED BOLD SIGNAL CHANGES IN A DOUBLE-BLIND, PLACEBO CONTROLLED, GUANFACINE CHALLENGE

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Suzanne M. Clerkin

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Background: Bottom-up regulation of alerting, or the capacity to phasically increase readiness to detect and respond to an impending stimulus, is thought to be mediated by the locus coeruleus noradrenergic (NA) system. Prior research has focused on the role of presynaptic α_{2a} autoreceptor inhibition of locus coeruleus (LC) firing and resultant down-regulation of NA release in alerting. However, α_{2a} adrenoceptors also act as heteroceptors to regulate neuronal excitability in select terminal regions, including prefrontal cortex (PFC). PFC regions provide top-down control of LC phasic activity. We tested bottom-up and top-down NA modulation of the alerting network by utilizing a 1.0mg oral dose of guanfacine in a double-blind, placebo-controlled challenge. **Method:** Sixteen healthy young adult volunteers performed a simple cued reaction time task while being scanned with functional magnetic resonance imaging (fMRI). Repeated Measures analysis of variance was used to test the effect of guanfacine treatment versus placebo on reaction time (RT) to cued and uncued targets. **Results:** There was a strong alerting effect for both treatment conditions, with decreased RT to cued targets versus uncued targets. However, there was no main effect of Treatment, nor was there a significant Treatment X Cue

interaction for RT. Fairly similar patterns of cue-related BOLD signal changes were observed for both guanfacine and placebo in regions of the alerting network, including dorsolateral PFC (DLPFC), cingulate and frontal motor areas, temporoparietal junction (TPJ), thalamus, and striatum. However, comparison of treatment effects on cue-related BOLD signal changes revealed significantly greater extent and/or magnitude of cue-related activation following guanfacine in bilateral DLPFC (BA 46), left ventromedial PFC (BA 9), right orbitofrontal cortex (BA 47), and posterior rostral cingulate zone. **Conclusions:** Guanfacine might increase signal to noise ratio and delay-related firing in response to relevant cues, thereby priming appropriate regions to respond to stimuli. The lack of behavioral effects suggests that cue-related BOLD signal increases were induced by guanfacine, rather than by a change in behavior.

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Specific Aims

Attention has been defined as focused consciousness, or the allocation of processing resources to relevant stimuli. One of the most influential models of attention was presented by Posner and Petersen (1991), who distinguished between three aspects of attention: alerting, orienting, and executive attention, with alerting being an underlying aspect of the other two subsystems. Alerting is defined as the capacity to phasically increase readiness to detect and respond to an impending stimulus (Coull, 1998). This capacity is separate from but dependent on more tonic regulation of arousal levels (Aston-Jones, 2005), and is critical for organisms to identify and respond rapidly to salient stimuli in an ever-changing environment (Aston-Jones, Rajkowski, & Cohen, 2000).

The pontine nucleus locus coeruleus (LC) plays a crucial role in the regulation of alerting and arousal states. The LC, which contains most of the noradrenergic (NA) neurons in the brain, receives multimodal sensory afferents from select nuclei, and in turn, sends diffuse efferents throughout the neuraxis, with particular innervation of areas associated with attention, including the thalamus, parietal cortex, and prefrontal cortex (PFC) (Foote, Bloom, & Aston-Jones, 1983). Behaviorally salient stimuli evoke large phasic increases in LC firing (Rajkowski, Kubiak, Aston-Jones, 1994), and the resultant rise in synaptic NA increases the signal-to-noise ratio of neurons in terminal regions, including the thalamus, inferior (LPi) and superior parietal lobules (LPs), supplementary motor area (SMA), and PFC (Berridge & Waterhouse, 2003; Hurley, Devilbliss, & Waterhouse, 2004). Many of these effects are mediated by the α_2-adrenoceptor, particularly the α_{2a} subtype (MacDonald, Kobilka, & Schenin), which serves as both an

autoreceptor to inhibit LC firing (Arima, Kubo, Ischibashi, & Akaike, 1998) and reduce NA release (Boehm & Huck, 1996), and as a heteroceptor to regulate neuronal excitability in select terminal regions (Timmons, Geisert, Stewart, Lorenzon & Foehring, 2004), such as the PFC (Arnsten, Steere, & Hunt, 1996). α_2-adrenoceptor agonists such as clonidine and guanfacine might attenuate bottom-up arousal mechanisms (Coull, Middleton, Robbins, & Sahakian, 1995; Coull, Sahakian, Middletone, Young, Park, McShane, Cohen, & Robbins, 1995), likely through reduced activation of thalamic and parietal regions secondary to presynaptic down regulation of LC and NA release (Coull, Nombre, & Frith, 2001). In contrast, α_2-adrenoceptor agonists might improve cognitive function, via heterosynaptic α_2-adrenoceptor activation of the PFC (Avery, Franowicz, Studholme, van Dyck, & Arnsten). Taken together, these data indicate that α_2-adrenoceptors may have important regulatory functions in alerting.

The aim of the current study was to characterize the role of α_2-adrenoceptors in the regulation of the attentional process of alerting. Sixteen healthy young adult volunteers performed a simple cued reaction time task (i.e., Stay Alert) while being scanned twice with functional magnetic resonance imaging (fMRI) in a double-blind, placebo-controlled challenge with the α_2-adrenoceptor agonist guanfacine (1.0mg oral). Three competing hypotheses were posited:

1. Decreases in alerting via α_2-autoreceptor inhibition of LC. This hypothesis predicts that guanfacine stimulation of α_2-autoreceptors in LC will attenuate evoked responses to salient stimuli, resulting in less NA release in terminal regions, which will be reflected in: i) reduced signal-to-noise ratio or activation of thalamus, LPi, LPs,

cerebellum, CMA, SMA, and PFC and ii) a selective increase in RT to cued targets but not uncued targets;

2. Increases in alerting via α_2-heteroceptor facilitation of PFC This hypothesis predicts that guanfacine stimulation of postsynaptic α_2-heteroceptors will increase the signal-to-noise ratio and attentional functions of the PFC, which will be reflected in: i) increased activation of PFC and ii) a selective decrease in RT to cued targets but not uncued targets;

3. A general sedative effect. This hypothesis predicts that guanfacine stimulation of α_2-autoreceptors in LC will inhibit both basal and evoked firing, resulting in less synaptic NA, as reflected in: i) global decreases in neural activation ii) a nonselective increase in RT to both cued and uncued targets.

Introduction

Attention has been a topic of study since the advent of psychological science, and its importance to psychology is demonstrated by Tichener's (1908) statement: "the doctrine of attention is the nerve of the whole psychological system, and that as men judge of it, so shall they be judged before the general tribunal of psychology" (p. 404). Numerous researchers have sought understanding of the relationship of attention to inhibition, sensory perception, memory, consciousness, and behavioral reactions. In addition, researchers have pondered the role of the central and peripheral nervous systems in producing and maintaining attention states, and the role of arousal and motivation in attention. However, over time, the focus of the study of attention was also largely influenced by culture, the prominent psychological milieu, and, of course, available technologies and advancements in physiology and medicine.

Theories of Attention

Cognitive Theories of Attention

Psychologists of the late 1800s and early 1900s laid the foundation for modern theories of attention. According to William James (1890), attention is a process that allows individuals to perceive, conceive, distinguish, remember, and react in the most efficient manner. William James described various types of attention that differed in target and source. For example, attention could be directed toward an actual object or directed toward an internal representation of an object. James referred to attention as immediate when the object of attention was interesting itself, and referred to attention as derived when the target was only interesting in association with another target that elicited immediate attention. Finally, James distinguished between passive or effortless

attention and active attention. Maturation was thought to bring about a change from predominantly passive attention to more selective and active attention.

The parcellation of attention into specific processes continued during the first half of the 20th century. An understanding of vigilance, or the capability for detecting changes in stimulus events over long periods of time (Frankman & Adams, 1962), and selective attention, which is the capacity to focus or concentrate on specific information to the exclusion of other information (Coull, 1998), was of particular importance at the time. The World Wars brought about a need to determine the limits of attention for proper selection of military personnel, and to determine the impact of limited attention on signal detection and maintaining vigilance in military operations. During this period, there were three important cognitive theories of the underlying mechanisms of vigilance: i) inhibition theory; ii) expectancy theory; and iii) attention theory; and at least two important cognitive theories of selective attention: i) filter theory; and ii) attenuation theory.

Macworth (1950) proposed the inhibition hypothesis, which interpreted vigilance behavior in terms of Pavlonian conditioning. He applied the principles of conditioning to explain well established performance decrements that occurred as a function of time on task. Macworth proposed that task-related responses served as unconditioned stimuli, and instructions from the experimenter regarding when to respond served as a reward. The experimental condition was viewed as an extinction period, during which the unconditioned stimulus was not paired with the reward, and as result of which detection declined, purportedly due to internal inhibition. However, participant responding never approximated complete extinction, with responding usually stabilizing around 70-75%,

and performance decrements could be avoided if participants were given continuous feedback. Macworth attributed the continued high rate of responding to the replacement of external reinforcement with internal speech and self-instructions. While this interpretation was consistent with the popular literature of the time, it was not viewed as an adequate explanation of attention.

In direct contrast to Macworth's description of an inhibitory process in vigilance, Deese (1955) characterized vigilance as an excitatory process and proposed the expectancy hypothesis. This hypothesis states that previous experience determines a participant's level of expectancy, which in turn, determines the level of vigilance and probability of accurate target detection. For example, if the frequency of targets is maintained at a high or low rate, then expectancy is maintained at a high or low rate, respectively. Other factors such as motivation also contributed to individual differences in expectancy. Specifically, the expectancy for a target signal is determined by the average inter-target interval of prior target signals during a search task and therefore, should be the same for all signals, as long as the inter-target interval is random. The rate of stimulus presentation does impact performance in the predicted direction, but no other significant evidence supporting expectancy theory was developed.

Broadbent (1953) proposed one of the first theories of vigilance that considered the central nervous system (CNS), claiming that when faced with a continuous array of stimuli, an individual will selectively focus on subsets of stimuli. Selection was due to the limited ability of the nervous system to handle large amounts of information at one time, and the inability to adequately respond to more than one stimulus at a time. Increased physical intensity, biological importance, and novelty increase the probability of

selection. Accordingly, individual performance will decrease over time because the repeated presentation of the same stimulus decreases the novelty of that stimulus, therefore decreasing the probability of selection and allowing for selection of competing stimuli. A period of rest can facilitate reinstatement of novelty among test stimuli.

Expanding his theory further, Broadbent proposed the Filter Theory (Broadbent, 1958) to explain selective attention. Broadbent's theory characterized attention as an active process, allowing an organism to selectively attend to important environmental stimuli. Filter theory was an early selection model of attention, whereby there are differences between the processing of attended and unattended events prior to complex processing. The model consisted of two stages of perceptual processing, and assumed that the CNS had a limited capacity to process information. Simple, physical properties would be extracted from stimuli entering parallel sensory channels. Since multi-sensory information often bombards the system, exceeding system capacity of a central processing channel, a filter is present that allows only part of the information from one or more channels to enter the second stage of processing. During the second stage of processing, more complex features are analyzed (e.g., word meaning), probably in a serial manner. Novel or intense stimuli are more likely to make it to the central processor and elaborate processing does not occur unless the stimulus enters the filter and the second stage of processing. The filter is not fixed on a single channel for a long period of time, providing the possibility of distraction or switching the focus of attention. Broadbent's theory placed an emphasis on the limited capacity of attention. Many researchers continued to debate whether processing began before or after initial filtering, proposing contrasting early- and late-selection models of attention (Deutsch & Deutsch,

1963; Treisman, 1966, 1969), the boundaries of input channels, and whether processing by the filter was an all-or-none phenomenon (Treisman, 1966, 1969).

Classic experiments exploring these questions of capacity and filtering utilized two methodologies: the split-span method (Broadbent, 1954) and the shadowing method (Cherry, 1953). Split-span experiments involved the presentation of stimuli (e.g., digits read aloud) at a certain rate simultaneously to two sensory channels (i.e., digits read to opposite ears; digits read to one ear and shown to one eye). Participants were required to write down the stimuli presented to both channels. Shadowing experiments involved the simultaneous presentation of stimuli (e.g., stream of speech) to both ears, requiring the participant to follow and repeat one stream of stimuli, while ignoring the other stream (Cherry, 1953). Split-span experiments indicated that in the auditory modality, when the same stimuli are presented to both ears, there is a high rate of correct responses (Broadbent, 1954). However, the rate of correct response decreases when different stimuli are presented simultaneously to opposite ears and the participant is required to list all of the presented stimuli, suggesting that each ear is a separate channel (Broadbent, 1954). However, performance is enhanced when there is a lag in presentation of stimuli to opposite ears (Moray, 1960). During auditory shadowing experiments, individuals usually notice the gender or pitch of the voice in the ignored stream changes; however, the identity and meaning of the words are not processed (Cherry, 1953). According to an early selection model, lag time allows for switching of attention between two separate channels, and processing of an unattended stream of information is successful only when there are clear physical differences between the streams of information, allowing for preattentive, early, parallel processing of physical characteristics of the stimuli. In

contrast, late selectionists (Deutsch and Deustch, 1963) suggested that the inability of participants to report ignored information may have less to do with depth of processing and more to do with the exclusion of the ignored stream in memory formation, or an inability to base responses on this information.

One of the most influential modifications to filter theory, known as attenuation theory, was based on many of the findings cited above (Treisman, 1966, 1969). Treisman defined attention as "the selective aspect of perception and response" (Treisman, 1969, pg 282), and concluded that Broadbent's filter could not be an all-or-none block. It was evident that words in a rejected message sometimes overcame the block during shadowing and because words from the rejected ear sometimes intrude when shadowing ears are switched. Therefore, filtering must occur during stimulus recognition, and filtering attenuated, but did not completely block competing information. All stimuli are partially analyzed, but only important stimuli activate internal representations. These representations can be ordered serially, in parallel or hierarchically, and encode independent features of stimuli. The model assumes that there are limits to the amount of information that can be handled by the system, and that different representations and response outputs compete for precedence.

During the 20th century many cognitive theories of attention were posited. These theories attempted to account for limitations as well as flexibility of attentional capacity. These theories were mainly concerned with mental processes, but had little to say about the specific brain mechanisms underlying these processes. The exclusion of the brain was not always a denial of its importance. Broadbent realized the importance of the central nervous system, but also stated that it would be dangerous to make premature

assertions about the biological mechanisms of attention and psychology in general. Over the course of the twentieth century, there was great progress in neurobiological techniques in animal research and in humans that gradually allowed for hypotheses about how the brain impacted attention. Specifically, theorists were beginning to apply knowledge about the central nervous system to explicate the neural and peripheral mechanisms of cognition.

Neuropsychological Theories of Attention

Human neurological disorders provided clues about the function of the brain by disclosing cognitive and behavioral deficits associated with brain lesions. A particularly influential neurological disorder was hemispatial neglect. Hemispatial neglect, also known as contralateral attention hemianopia, results from unilateral injury to different aspects of cortex, including cingulate cortex and human parietal cortex. The disorder is characterized by neglect for extrapersonal space contralateral to the lesion, such that patients may ignore stimuli such as food and written material, as well as fail to attend to personal hygiene and grooming on one side of the body. Patients have been describes as "behaving almost as if that half of the universe has abruptly ceases to exist (Mesulam, 1981, p. 309)." In addition to contralateral attention hemianopia, unilateral extinction to simultaneous stimulation, such that patients are unable to detect skin touch contralateral to the lesion, is a defining feature, and one that is present in the most subtle cases.

Understanding of unilateral neglect was facilitated by work in the macaque monkey. The ability to record activity in single-cells of awake and behaving animals greatly facilitated the translation of basic science research findings to complex human behavior. Recordings in brain regions associated with attention, such as the parietal lobe,

suggested that processing of auditory and visual stimuli depended on the direction and maintenance of attention to such stimuli. In addition, these single-cell physiology experiments were followed by experiments utilizing tracer substances to delineate the neural connections of PG, allowing scientists to identify possible neural networks associated with attention.

Ablation of the posterior parietal lobe was sufficient to elicit contralateral attention hemianopia and sensory extinction in macaque. Single cell recordings in awake and behaving macaque indicated that cells in the inferior parietal lobule, or area PG (von Bonin and Bailey, 1947), increased activity in response to attention to motivationally significant stimuli that was either in the animals gaze (Lynch, Mountcastle, Talbot, & Yin, 1977), or required a manual grasp to obtain the object (Hyvarinene & Poranen, 1974). Random physical movements did not result in increased cellular activity and the increase in activity ceased abruptly once the motivationally significant object was obtained, even without a shift in eye gaze (Lynch et al., 1977). These results suggest that PG is important in processing motivational salience, and has a role in maintaining attention on such objects. Therefore, unilateral damage to this area of the brain, or its human equivalent, was expected to illicit hemispatial neglect through an inability to process the motivational significance of a stimulus.

The dorsolateral PG seemed particularly important to hemispatial neglect. This area receives efferents from the reticular core (including the intralaminar thalamus, the brainstem raphe, and the locus coeruleus), which convey arousal mechanisms to produce and maintain alert states necessary for attention (Mesulam, Van Hoesen, Pandya, & Geschwind, 1978); sensory association cortices, which convey highly processed sensory

information from polymodal sensory processing areas; and limbic areas of the cingulate cortex and basal forebrain (Mesulam & Geschwind, 1978), which convey motivational significance. In addition, PG sends efferent projections to the frontal eye fields and the superior colliculus (Barbas & Mesulam, 1981; Pandya & Kuypers, 1969; Petras, 1971), which are important in establishing and maintaining gaze (through eye and head movements), and interacting with additional regions in establishing motor programs for responding to stimuli.

The combination of findings from single-cell physiology and neural tracing studies pointed to the importance of the parietal lobe in attention. Marcel Mesulam (1980) used these findings to present a comprehensive modern theory of attention that combined a century of knowledge from the fields of neurology, neurophysiology, and psychology. According to this theory, inputs from sensory association areas (i.e., medial pulvinar and polymodal association cortex), limbic areas (i.e., cingulate cortex and the basal forebrain), and reticular areas, (i.e., intralaminar thalamus, brainstem raphe, and the locus coeruleus) were respectively proposed to provide the sensory information, motivational salience, and underlying arousal necessary to guide visual attention in space. Gaze was controlled by the frontal eye fields and superior colliculi. The posterior parietal cortex was responsible for integrating information, and producing elaborate representations of extrapersonal space.

Hemispatial neglect and the plethora of physiological studies in animals conducted to explain the disorder also influenced the thinking and studies of Posner. Possibly the most important contemporary theory of attention was posited by Posner and Peterson (Posner & Petersen, 1990; Posner, Petersen, Fox, & Raichle, 1988), who

eloquently provided evidence for three aspects of attention, each of which is governed by separable but somewhat overlapping neural systems. They subdivided attention into mechanisms of alerting, orienting, and executive function. Alerting is defined as the achievement and maintenance of an arousal state, which increases the capacity to detect and respond to an impending stimulus. Orienting can be overt (i.e., foveation of a stimulus), or covert (i.e., directing attention without moving the head or eyes), and has the effect of improving efficiency of information processing. Executive attention is involved in conflict resolution. The orienting system involves a network comprised of the superior parietal cortex, temporal parietal junction, frontal eye field and superior colliculus (Corbetta, Kincade, Ollinger, McAvoy, & Shulman, 2000). Acetylcholine is a neurochemical modulator of the orienting system (Stewart, Burke, & Marrocco, 2001; Thiel, Zilles, & Fink, 2005; Witte, Davidson, & Marrocco, 1997). The executive attention system involves the anterior cingulate, lateral ventral prefrontal cortex, and basal ganglia, with dopamine acting as a neuromodulator (Marrocco & Davidson, 1998). Finally, the alerting system is comprised of the locus coeruleus, right frontal and parietal cortex, with norepinephrine acting as a neuromodulator (Witte & Marrocco, 1997).

The Attention Network Test (ANT) (Fan, McClandiss, Sommer, Raz, & Posner, 2002) was developed as a test of the efficiency and independence of the three attention networks. It was designed as a combination of the Cued Reaction Time task (Posner, 1980) and the Flanker Task (Eriksen & Eriksen, 1974). Participants are presented with a central fixation point, above or below which a series of arrows will appear. A center arrow is flanked by two arrows on either side or two neutral lines on either side. The center arrow is the target, and participants must respond with a button press to the

direction of the center arrow. The flankers can point in the same (congruent trials) or opposite (incongruent trials) direction as the center arrow. Cues are presented that appear either in the same location as the fixation (neutral cues) above or below the central fixation in the same location (congruent spatial cue) or opposite location (incongruent spatial cue) as the subsequent target, or asterisks appear simultaneously above and below the central fixation (double-cue). The dependent measure is reaction time to various targets, such that executive attention is measured as the difference in mean reaction time across all conditions to congruent versus incongruent flanker trials; orienting is measured by the difference between reaction time to targets following a spatial cue versus a neutral cue, and alerting is measured as the difference in reaction time to targets following a double-cue versus no cue.

Reaction times to targets with congruent flankers, congruent spatial cueing, or neutral cueing are shorter than reaction times to targets, which are among incongruent flankers, following neutral spatial cues, or following no cues, respectively (Fan et al., 2002). Early tests of the independence of these three networks based on correlational analyses found that these networks function orthogonally, however, when interactions between the networks were explored using analysis of variance, it was found that the alerting cues decreased the cost of incongruent flanker interference (Fan et al, 2002). Activation of the alerting system is thought to inhibit higher order thought within the executive system, leading to faster responses to salient stimuli; and alerting is thought to accelerate the response of the orienting system (Callejas, Lupianes, & Tudela, 2004; Posner & Petersen, 1990). Therefore, although each attentional network is comprised of different anatomical substrates, these systems work in concert to produce efficient

sensory processing and behavioral responding, and the alerting system is of particular importance to the functioning of the orienting and executive attention systems (Fan, Byrne, Worden, Guise, McCandliss, Fossella, & Posner (2007); Fan, Kolster, Ghajar, Suh, Knight, Sarkar, & McClandliss, 2007).

Mesulam (1981) and Posner and Petersen (1991) postulated two of the most influential neuropsychological theories of attention. Both theories are based on data collected from human lesion studies and animal electrophysiological studies. Both emphasize the importance right-lateralized attention networks centered on frontal and parietal cortices, and the particular importance of the cingulate cortex in determining motivational salience of stimuli. One of the most important similarities between the two theories is the acknowledgement of an underlying arousal mechanism that prepares organisms to evaluate and respond to stimuli. This acknowledgement was based on almost a century of research linking general arousal levels and performance. Given the importance of the arousal system to Mesulam's (1981) theory and particularly Posner and Peterson's (1991) alerting system, an understanding of how this system works to create and maintain arousal states is imperative to our understanding of attention mechanisms.

The Biological Basis of Attention

Long before the advent of modern imaging techniques, cognitive processes, including allocation of attention, were assumed to be accompanied by physical manifestations, including changes in blood flow through the central nervous system (Roy and Sherrington, 1890) and periphery (James, 1890), and changes in muscle tone (Fechner, 1860) in the respiratory organs, facial muscles, and sensory organs were thought to be indispensable to the allocation of attention. General arousal states, defined

by autonomic measures of galvanic skin response (GSR) and heart rate (Malmo, 1959) were hypothesized to impact attention and other performance measures. Early work with Electroencephalogram (EEG) allowed for localization of arousal centers in the brain (Moruzzi & Magoun, 1949), leading to general hypotheses of how arousal centers in the brainstem could influence could cognition. The ability to record cellular activity from alive, awake animals, in addition to the data accumulated from lesion studies in animals and humans, researchers were in a position to begin bridging basic neuroscience with psychology (Mesulam, 1980; Posner & Petersen, 1991). The following section will detail this progression in the field.

Arousal and Attention

Intuitively, arousal refers to a general state of physiological readiness to respond to stimuli that is driven by the central nervous system. However, the construct has been notoriously difficult to operationally define, and therefore definitions of arousal and its usefulness in psychological research have been subject to much debate. For example, in addition to 'arousal" different terms have been used to describe such states, including activation (Pibram & McGuinness, 1975), and more recently, alerting (Posner & Petersen, 1991). Not all investigators have used these terms in the same way. However, advances in physiological recording techniques have allowed for refinement of the arousal construct. It is now generally accepted that arousal is not a unitary construct, and that arousal interacts with subject variables (e.g., motivation) and task variables (e.g., cognitive load) to influence performance (Pibram & McGuinness, 1975; Sanders, 1983).

Early researchers studying "energetics" relied on peripheral measures, such as muscle tension (Duffy, 1932), and skin conductance (Duffy & Lacey, 1946). These gross

measures indicated that there was some consistent relationship between what they termed arousal, energy mobilization, and activation, on performance. Physiological recordings from electroencephalogram indicated that arousal states were characterized by a distinct wave pattern (Malmo, 1958). Deep sleep was characterized by low frequency waves. Relaxed but awake individuals demonstrate alpha waves, and moderate alertness is characterized by beta waves. Alertness not only brings about increased frequency of waves, but also a desynchronization, characterized by reduced amplitude of waveforms. Activation Theory (Lindsley, 1957) posited that desynchronization was the most important EEG measure. Lindsey believed that the brain mediated behavior through alert states, however, the mechanisms by which desynchronization were achieved were not yet known.

Moruzzi and Magoun (1949) identified a diffuse network of neurons, called the reticular activating system (RAS), which was thought to mediate arousal states. They performed a series of experiments in cats to observe changes in cortical and thalamic electroencephalogram (EEG) activity as a result of reticular formation lesions. Direct stimulation of the RAS was found to desynchronize the EEG, replacing high-voltage slow waves with low-voltage fast waves. This change in electrical activity was caused by stimulation of the medial bulbar reticular formation, pontine and mid-brain tegmentum, dorsal hypothalamus and subthalamus, with the effect observed in the cortex and mediated by the thalamus. However, a certain level of background synchrony of EEG activity was necessary to elicit the response, which was posited to be important for maintenance of wakefulness. These findings began influencing attention theory and provided evidence of a clear mechanism for maintenance of alert states.

Hebb (1955) provided a detailed description of the role of the RAS in performance. The direct route of sensory information is through the sensory nerves, to the sensory tracts, through the sensory nuclei in the thalamus, and finally to the sensory projection areas of the cortex. The RAS was viewed as a second major sensory pathway, which was slow and inefficient for the transfer of specific sensory information. This system was said to provide a background of supporting activity that was necessary for proper transmission of sensory information through the primary route, without which information would not spread efficiently through the cortex allowing for proper behavioral responses. Stimuli elicit a general level of arousal and provide information to guide behavior. Hebb (1955) described an inverted "U" relationship between general arousal and the ability to utilize cues. Under a low level of general arousal, further stimulation will serve to enhance signal transmission through the cortex and facilitation of behavior. However, if there is a high level of general arousal, further information entering the system might overwhelm the system, hindering the ability to utilize specific cue information to guide appropriate behavior.

The notion of an inverted U relationship between arousal and performance was not new. Yerkes and Dodson (1908), performed an experiment with rats involving a discrimination task, during which an electric shock was used to produce physiological arousal and brightness of the stimuli was varied to produce differences in the difficulty. The results of this experiment led to the proposal of what is now known as the Yerkes-Dodson Law: there is an inverted-U relationship between arousal and performance, such that performance decrements are seen and low and high levels of arousal, and optimal performance is seen at moderate levels of arousal. In addition, there is an effect of task

difficulty, such that more demanding tasks require less arousal for optimal performance, and less demanding tasks require more arousal for optimal performance. Hebb's work identified attention as one mechanism behind the performance decrements and enhancements due to arousal.

The works of Hebb (1955) and Yerkes and Dodson (1908) began to perforate studies of energetics and attention theory. Scott (1957) concluded that performance decrements during vigilance tasks were related to a reduction in stimulus variation, which resulted in sensory habituation, a loss of general arousal, and a loss of vigilance. When targets are infrequent and background stimuli are minimal performance decrements can be expected to occur rapidly. Strategies to overcome such decrements include rest periods and increasing the number of extraneous stimuli to increase stimulus variation, and thereby increase general arousal. Arousal level was also found to impact cue utilization, defined as the number of environmental cues that one orients to, attends, or responds to in a given situation (Easterbrook, 1959). Arousal was found to lead to a progressive reduction in the number of environmental cues an individual utilizes. As arousal increases, the number of irrelevant cues from the environment decreases until cue utilization reached an optimal level, beyond which, utilization of relevant cues disrupts performance. Further, Deutsch and Deutsch (1963) connected arousal to selective attention in their modification to attenuation theory. Their late selection theory posits that every stimulus evokes an internal representation, but only the most important internal representations are chosen as the target of attention, memory, and motor output. They proposed that a structure with widespread connections to the rest of the central nervous

system, such as the RAS would be responsible for the selection of the most important inputs for attention.

The work of Moruzzi and Magoun (1949), Yerkes and Dodson (1908), and Hebb (1955) solidified the role of the RAS in arousal and the relationship between arousal and attention. In addition, it became clear the reticular activating system was comprised of distinct nuclei that projected to rather specific areas of the lower brain and cortex. These groups of nuclei were subdivided according to their neurochemical cell bodies (Dahlstrom & Fuxe, 1964). Of particular importance to modern neuropsychological theories of arousal and performance, are the noradrenergic cell groups (Posner and Peterson, 1991).

The Noradrenergic System

The following section will detail the anatomy, physiology, and neurochemistry of the noradrenergic system. The summary is based on over a century of research performed in animals (i.e., rat, monkey, and cat, among others), as well as humans. With few exceptions, the noradrenergic system retains much of its anatomical characteristics across species. Through advances in neurophysiological and neurochemical techniques, there has been considerable refinement in our understanding of NA as not just an inhibitory neurotransmitter, but a neuromodulator that impacts cognitive processing. Finally, advances in neuroimaging have brought the field closer to understanding the importance of the noradrenergic system in normal and disordered human cognition.

Noradrenergic Cell Groups

The original designation of the noradrenergic cell groups by Dahlstrom and Fuxe (1964) included seven cell groups, numbered A1 – A7. These cell groups have been

further categorized into three major groups by Lindavall and Bjorklund (1983); the locus coeruleus complex (A6) and its caudal extension (A4); the lateral tegmental group, which is further subdivided into the pontine subcoerulues (A5 and A7) and a medullary group (A1); and the dorsal medullary group (A2). The original A3 cell group is now considered part of the A1 cell group.

The A1 cell group is located at the level of the area postrema in the ventral lateral reticular formation of the caudal medulla, and the A2 cell group is located in the nucleus of the solitary tract and the nucleus of the vagus. The A5 cell group is located ventral to the locus coeruleus, within the lateral reticular nucleus, and the A7 cell group, is located in and around the ventral nucleus of the lateral lemniscus. The A6 and A4 cell groups are located in the lateral periventricular gray, in the dorsal rostral pons. The A6 cell group, which is the largest noradrenergic cell group, was first described in the early 19th century (Reil, 1809). Shortly thereafter A6 was given the name Locus Coerulues (LC), or "blue spot" due to a natural pigment, neuromelanin, that gives the area its color (Simpson & Lin, 2007), and due to its size and massive projections it has been the most widely studies NA cell group. Therefore, the description that follows will focus on the locus coeruleus cell group.

Anatomical Organization of Locus Coeruleus Noradrenergic System

The locus coeruleus (LC) has been studied in a wide array of species. The LC can be found at a similar location and demonstrates similar structural organization across species. However, there are notable exceptions, for example, cat LC consists of non-NA containing cells (Jones & Moore, 1974), and is characterized by a much more diffuse arrangement of cells than is common among other species (Chu & Bloom, 1974; Jones &

Moore, 1974), such as rabbit. Further, non-human primates (i.e., pygmy marmosets, rhesus, macaque, and squirrel monkeys) share more features in common with rat and human LC than with cat, in terms of cellular arrangement (Jacobowitz & MacLean, 1978), and expression of catecholamine by all cells (Garver & Sladek, 1975). The presence of an LC analogue that projects to the forebrain does not occur until avian (Tohyama, 1976; Tohyama et al., 1975) and reptilian (Parent & Poitras, 1974) classes. Tohyama et al (1974, 1975) proposed that the development of the LC parallels the elaboration of its cortical target areas.

The LC contains a relatively small number of cells across species. Estimations in rat LC are approximately 1500 – 1600 cells per nucleus, while rhesus monkey LC contains approximately 7500 cells, and human LC contains 10,000 – 15,000 cells (Berridge & Waterhouse, 2003). Golgi impregnation (Swanson, 1976) and DBH immunohistochemical (Grzanna & Molliver, 1980) techniques have been used to characterize the morphological characteristics of LC neurons. Different cell types are found in dorsal and ventral portions of rat LC. Small fusiform cells are found mainly in the dorsal portion of the LC. These cells are 20-25µm in diameter, and have long, thin dendrites. Medium-sized multipolar neurons are found in the ventral portion of the nucleus, range from 25-30µm in diameter, and have large dendrites that extend long distances in all directions. In the rostral extent, a few large multipolar neurons (35 to 45µm in diameter) have also been demonstrated. Cells within human LC are also heterogeneous, with large multipolar, small multipolar, large elliptical, bipolar, and small, ovoid, bipolar neurons all identified by DBH or TH immunostaining (Baker, Tork,

Hornung, & Halasz, 1989; Chan-Palay & Asan, 1989; Pearson, Goldstein, Markey, & Brandeis, 1983).

The Locus Coeruleus Noradrenergic Projections: Although small in size, the importance of the LC became strikingly evident when it was identified as the largest group of NA-containing neurons in the brain (Dahlstrom & Fuxe, 1964), which send immensely ramified axons throughout the neuraxis (Anden et al 1966; Fuxe et al 1968; Olson & Fuxe, 1971; Ungerstedt, 1971). Glyoxylic acid (Lindvall & Bjorklund, 1974a, 1974b) and autoradiographic tracing (Jones & Moore, 1977) methodologies in rat have detailed LC-NA projections rather extensively. Three major ascending pathways from LC have been described. The dorsal pathway, or dorsal catecholamine bundle (Ungerstedt, 1971), is the largest ascending projection and extends into the mesencephalic tegmentum. The second projection ascends as a component of the dorsal longitudinal fasciculus. The third ascending component travels through the central tegmental tract and then through the ventral tegmental area into the medial forebrain bundle (MFB).

The dorsal pathway is comprised of fibers arising from the LC that course rostrally through the subcoeruleus to the caudal midbrain tegmentum, where they are located lateral and ventral to the periaqueductal gray. This pathway turns ventrally at the level of the fasciculus retroflexus to traverse the prerubral field at the medial portion of the zona incerta. At this level some fibers are given off which terminate in the parafascicular nucleus, while others course rostrally or dorsally to innervate the thalamus. Other fibers continue coursing through the MFB to innervate telencephalon.

The first source of telencephalic NA innervation is through the fibers entering the internal capsule, which continue along the lateral border of the external capsule. Then,

the fibers course laterally to enter the cortex. Fibers within the ventral amygdaloid bundle and the ansa peduncularis are distributed to the piriform cortex, amygdaloid complex, entorhinal complex, and to areas of the lateral and caudal neocortex. The second source of telencephalic innervation arises from ascending MFB fibers that turn medially into the diagonal band to innervate the septum. Most of these fibers terminate in the nucleus of the diagonal band though some terminate in the medial and lateral septal nuclei. Many fibers continue through the medial septal nucleus in Zuckerland's bundle to course around the rostrum of the corpus callosum to the cingulum. Fibers leave the cingulum and enter the cingulate and retrospenial cortex, and dorsal neocortex. Some also continue around the genu of the corpus collosum and enter the subiculum and hippocampal formation. The third group of fibers arising from the MFB projection continues rostrally as it enters the basal telencephalon. A portion of these fibers continue rostrally into the external capsule to innervate the frontal cortex. Other projections go to deep layers of the olfactory tubercle and the anterior olfactory nucleus.

Smaller projections from the rostral medial forebrain bundle ascend in the medial septum to the dorsal fornix and the fimbria to the hippocampus; pass through the interstitial nucleus into the stria terminalis, and terminate in the amygdaloid complex; or enter the stria medullaris and give off fibers to the paraventricular thalamus and terminate in the habenular complex.

The LC system has five commisures that give rise to contralateral projections. The dorsal pathway fibers cross ventrally to the medial longitudinal fasciculus, in the posterior commisure, at the ventral lateral surface of the internal capsule adjacent to the

optic tract; in the anterior commisure; and lastly, a small group of fibers cross at the genu of the corpus callosum.

Terminal Projections of the Locus Coeruleus: Dramatic decreases in cortical fluorescence (Fuxe et al, 1968), NA levels (Kobayashi, Palkovita, Kopin & Jacobowitz, 1974), and NA metabolite (Arbuthnot, Christie, Crow, Eccleston, & Walter, 1973; Korf, Roth & Aghajanian, 1973) are observed following lesions to the LC. Limbic regions, including cingulate cortex (Browstein, Saavedra, & Palkovits, 1974; Kobayashi et al, 1974; Pickel, Segal, & Bloom, 1974), the hippocampus and dentate gyrus (Browstein et al, 1974; Kobayashi et al, 1974; Koda and Bloom, 1977; Pickel et al, 1974; Segal and Landis, 1974), and piriform cortex (Pickel et al, 1974) receive particularly dense innervation. Although LC efferent projections are immense, they demonstrate a high degree of specificity in topographic organization of cells projecting to specific targets, preferential innervation of cortical layers, and dense innervation of regions known to subserve somatosensory processing, attention, temporal prediction of events, and motor response preparation. These areas include the spinal cord (Kuypers & Maisky, 1975; Nygren & Olson, 1977), cerebellum (Bloom, Hoffer, & Siggins, 1971; Mugnaini & Dahl, 1975; Olson & Fuxe, 1971), thalamus (Lindavall, Bjorklund, Nobin, Stenevi, 1974), and the entire neocortex (Fuxe, Hamburger, & Hokfelt, 1968; Levitt & Moore, 1978; Ungerstedt, 1971).

Fibers arising from the ventral and dorsal LC heavily innervate the spinal cord and cerebellum. Multipolar cells from the ventral LC and fusiform cells from dorsal LC innervate all sections of the spinal cord, including the ventral and dorsal horns, the intermediate gray, and at all levels. LC-NA innervation to cerebellum has been

extensively surveyed. The cerebellum receives all of its NA from the LC. LC-NA fibers project to the molecular layer of the cerebellar cortex. Some fibers run along the Purkinje cell layer and others course vertically toward the surface of the folium, along the Purkinje cell dendrites. The fibers bifurcate in the outer molecular layer and run parallel to the pial surface.

Fibers arising from the rostral pole of the LC innervate the hypothalamus (Mason & Fibiger, 1979). Fibers that course through the periaqueductal gray form a plexus that innervates the periventricular nucleus of the hypothalamus. The dorsomedial, paraventricular, and supraoptic nuclei are innervated by fibers coursing the periventricular system. In addition, all areas of the telencephalon, except the basal ganglia, olfactory tubercle, and nucleus accumbens, have demonstrated LC-NA innervation. Innervation to the central nucleus of the amygdala, the septal nuclei, nucleus of the diagonal band, and the interstitial nucleus of the stria terminalis do not originate only from the LC. However, there are many areas that receive innervation exclusively from the LC, including the hippocampus. NA axons projecting to the hippocampus largely originate in the dorsal LC. The ventral hippocampal formation receives LC-NA from the ansa peduncularis-ventral amygdaloid bundle system, the dorsal part of Ammon's horn receives innervation from the cingulum, and the stratum radiatum of CA3 and the area dentate receive innervation through the fornix.

The thalamus is a major terminal projection of the LC-NA system. The anterior nuclei receive the densest innervation. At the level of the fasciculus retroflexus, fibers from the caudal LC exit the MFB to innervate the anterior, ventral, and lateral nuclear complexes, and the medial and lateral geniculate bodies. Innervation of the medial

geniculate body is very dense. The dorsal lateral geniculate nucleus demonstrated dense plexiform innervation that appears to be predominantly axodendritic.

Within neorcortex, there is regional and laminar variability of fiber density. Cortical layers III and IV receive densest innervation, and Layer I receives sparse innervation (Foote & Morrison, 1987). Further evidence of specificity comes from retrograde tracer studies that suggest a rough topographic organization of LC efferents. Cortical targets receive primarily ipsilateral innervation from more caudal area of the LC (Waterhouse, Lin, Burne, & Woodward, 1983).

Afferent Projections to LC. In contrast to the diffuse efferent projections, the LC proper receives a discrete set of afferents. Among these are the nucleus paragigantocellularis, which responds to the sympathetic nervous system, and the nucleus prepositus hypoglosus, which is related to control of eye movements (Aston-Jones, Ennis, Pieribone, Nickell, & Shipley, 1986). Information from these targets to the LC might facilitate orienting of attention, or relay of arousal signals to the forebrain. The pericoerulear region, which consists of LC dendrites, receives input from the central nucleus of the amygdala (Van Bockstaele, Colago, & Valentino, 1996), the lateral hypothalamus, the bed nucleus of the stria terminalis (Van Bockstaele, Peoples, Telegan, 1999) and the dorsal raphe. In addition, the LC receives top-down cortical projections from the orbitofrontal cortex and anterior cingulate cortex (Arnsten & Goldman-Rakic, 1984; Jodo, Chiang, & Aston-Jones, 1998). Retrograde and anterograde tracer studies in monkey LC have confirmed these projections arising from the LC and pericoerulear dendritic field. Retrogradely labeled neurons also extend caudally from the OFC to the

anterior insular cortex. These projections are stronger in monkey LC than they are in rat LC, where they terminate in the peri-LC dendritic zone, not the LC proper.

These findings indicate that the LC provides NA innervation of cortical and subcortical regions that mediate the effects of alerting. Of particular note, the prefrontal cortex has been demonstrated to be a major source of afferent drive to the LC (Arnsten and Goldman-Rakic, 1984; Jodo et al, 1998), directly linking the LC with input from areas responsible for higher cognition. The PFC serves a "top-down" role in modulating the activity of the LC. Thus, the firing cycles of LC neurons and the efficacy of PFC regulation of the LC are both controlled by central levels of NA (Aston-Jones et al, 2000; Arnsten & Li, 2005).

Physiology of the Locus Coeruleus

Tonic activity in LC neurons is characterized by a low-frequency, sustained, and highly irregular discharge pattern. NA-LC neurons display a slow spontaneous discharge rates (0-5 Hz), broad action potential waveforms (1-2 ms) and burst discharges that are followed by a prolonged period of decreased firing. Electrotonically coupled dendrites allow for mass discharge of LC neurons. LC axons are thin and unmyelinated, with slow conduction velocities (0.2 – 0.86 m/s) (Aston-Jones, Segal, & Bloom, 1980). The physiology of LC neurons contributes to fluctuations in general arousal states and transitions between focused attention and unfocused scanning of the environment, or vigilance (Aston-Jones, Rajkowski, & Cohen, 2000; Foote et al, 1993; Rajkowski, Kubiak, & Aston-Jones, 1994). Arousal, or tonic alertness, refers to a general state of physiological reactivity that varies on a continuum from sleep to panic (Coull, 1998). Indeed, LC neurons display fluctuations in tonic firing modes that vary with the stages of

the sleep-wake cycle, firing most rapidly during waking, and become almost completely silent during paradoxical sleep (Aston-Jones & Bloom, 1981). Changes in LC firing anticipate changes in behavioral state and learning (Aston-Jones & Bloom, 1981; Aston-Jones, Rajkowski, Kubial, 1997).

Behaviorally salient stimuli evoke large phasic increases in LC firing (Rajkowski et al, 1994), which are of relatively short latency and are characterized by a burst of action potentials, followed by prolonged decrease in activity. Latency of phasic activation following targets is short (~100 ms onset) and precedes lever-release by about 200 ms (Aston-Jones et al., 1985). Phasic discharge is dependent on tonic discharge. There is generally a classic Yerkes-Dodson relationship between tonic activity of the LC and performance on tests of attention (Aston-Jones et al, 2000). Animals tend to perform optimally (i.e., respond to targets and not to distracters) when they are alert and demonstrate a moderate level of tonic activity, but perform poorly when they are drowsy with low levels of tonic activity or are hyperaroused with high levels of tonic activity (Aston-Jones et al, 2000). Alternately, when animals are engaged in maintenance behaviors such as feeding or grooming, tonic activity of the LC is low, and only behaviorally salient environmental stimuli can evoke a phasic response (Aston-Jones & Bloom, 1981; Aston-Jones et al, 2000; Grant, Aston-Jones, & Redmond, 1998; Rajkowski et al, 1994). Such a phasic response results in disruption of vegetative behavior, narrowing of attentional focus, and orienting toward the stimulus. Phasic responses habituate following repeated presentation of some stimuli.

The phasic LC response has been linked to the meaning and significance of a stimulus, rather than physical sensory attributes. Aston-Jones et al (1997) obtained

extracellular recordings from noradrenergic neurons in the locus coeruleus of three cynomolgus monkeys performing a vigilance task. The subjects were required to release a lever rapidly in response to improbable target stimuli (20% of trials) that appeared randomly intermixed with non-target stimuli. Stimuli were presented on a video display, and the subjects received a juice reward for each correct response. The LC neurons examined were all activated phasically and selectively by the target stimuli. No consistent responses from LC neurons were elicited by the juice reward, lever release, fixation spot, or non-target stimuli. Further, with a reversal of the task contingency, LC neurons no longer responded to the old target stimuli, but began responding to the new target stimuli. The reversal in LC responding to stimuli after contingency reversal occurred rapidly, and before reversal was expressed behaviorally. This study emphasizes many important aspects of LC phasic responses. First, conditioned responses reflect stimulus meaning and not physical stimulus attributes. Second, conditioned responses of LC neurons are plastic and easily altered by changes in stimulus meaning. Lastly, these results suggest a role of the LC in learning the significance of behaviorally important stimuli.

Early studies of iontophoretic application of NA to target neurons of cat (Freedman, Hoffer, & Woodward, 1975) and squirrel monkey (Nelson, Hoffer, Chu, & Bloom, 1973) demonstrated that NA led to a decrease in spontaneous activity in cortical neurons, leading to the suggestion that NA acted as an inhibitory neurotransmitter. However, several researchers disputed this interpretation. The work of Woodward, Moises, and Waterhouse (1979) demonstrated that NA acted to enhance evoked inhibitory and excitatory inputs. Their work in cerebellar purkinje cells demonstrated that concurrent application of glutamate or GABA with NA enhanced the effects of both

neurotransmitters. Synaptically evoked excitation was also enhanced by NA. Later work demonstrated NA enhancement of signal to noise ratio in a variety of species and in several brain regions associated with somatosensory processing, attention, and learning. Concurrent application of NA with ACh increased signal to noise ratio in somatosensory neurons of layers I-IV of the cortex, and GABA-induced inhibition of spontaneous cortical discharge was also augmented. Moreover, to demonstrate specificity of NA effect, dopamine was also tested, and failed to demonstrate an impact on excitability of cells at doses high enough to reduce background firing. More naturalistic studies of single unit responses generated by afferent synaptic input, such as foot tap (Waterhouse & Woodward, 1980) or species specific vocalizations (Foote, Freedman, & Oliver, 1975) indicated that administration low dose of NA differentially suppressed background discharge more than stimulus-bound excitation, and in one case doubled the signal to noise ratio. Yet another important finding reported by Madar and Segal (1980) suggested that NA enhanced selectivity of important stimuli and perhaps decreased attention to less important stimuli. They tested the impact of NA on orientation detectors in the visual system. NA facilitated responses to lines that were presented in the optimal orientation, and depressed reactivity to lines that were in suboptimal orientations.

Based on these important findings, NA was no longer thought of as an inhibitory neurotransmitter, rather, researchers were approaching norepinephrine as a neuromodulator that impacted neuronal firing patterns to increase signal and reduce noise in target areas, increasing efficiency of processing and facilitating learning and adaptive behavior. Electrotonic coupling allows LC neurons to fire synchronously, producing phasic increases in synaptic NA, which enhances the excitability, afferent inputs, signal-

to-noise ratio, receptive field, and temporal dynamics of neurons in terminal regions, the net effect of which is to reduce spontaneous activity and enhance evoked responses (Berridge & Waterhouse, 2003; Hurley et al, 2004).

Behavioral Functions of Locus Coeruleus Noradrenergic System

Pharmacologically induced lesions studies have also proven useful in delineating the function of the LC-NA system in learning and attention, and highlight the importance of arousal in LC-NA effects. Infusions of the neurotoxin, 6-hydroxydopamine (6-OHDA) into the DNAB of rat brain results in profound depletion of cortical norepinephrine to less than 10% of control values. Lesions do not result in any gross behavioral deficits. For example, DNAB-lesioned rats can eat and drink normally, and do not demonstrate any changes in locomotor activity. However, DNAB-lesioned animals have deficits in the acquisition phase of conditional discrimination tasks (Cole and Robbins, 1987; Everitt, Robbins, Gaskin, & Fray, 1983), suggesting a greater role of NA in learning than performance. Even though these deficits in acquisition of learning are consistent, there is evidence that DNAB-lesioned rats demonstrate an enhanced ability to be conditioned to context. The lesions seem to broaden the attention span of the animal, resulting in preference of distal rather than proximal cues. DNAB lesions enhance performance on the Morris Water Maze under certain circumstances (Selden, Cole, Everitt, & Robbins, 1990). Deficits were present only when cold water was used, not when warmer water was used. The cold water was more physiologically arousing, reducing core body temperature and increasing plasma cortisol. Therefore, the effects of DNAB lesions were particularly apparent under stressful conditions, and the cortical projections of the LC-NA system may function to overcome the detrimental effects of high arousal on attention.

Further evidence for the impact of arousal on the dependence of NA in learning and attention has been demonstrated in 6-OHDA lesioned rats using a continuous performance task. The rats were trained to detect brief visual stimuli presented unpredictably at one of five locations. Rats are impaired under three conditions: when brief bursts of white noise are presented just prior to the presentation of each stimulus (Carli, Robbins, Evenden & Everitt, 1983); when stimuli are presented unpredictably in time (Cole and Robbins, 1992), and when the rats receive treatment with d-amphetamine, injected peripherally or directly into the nucleus accumbens, where it causes a large increase in locomotor activity and also impulsive and premature responding (Cole and Robbins, 1987).

Thus, the LC seems to be the nexus of a neural system that enables organisms to ignore irrelevant environmental stimuli during vegetative behaviors, and yet remain responsive to behaviorally salient stimuli. In addition, it is evident that coeruleocortical NA depletion impairs performance of controlled processes under conditions of high arousal. Therefore, the LC-NA system might serve to preserve attentional focus, allowing for proper responding when conditions are arousing. Identification of adrenoceptors and increased understanding of their functional roles in the CNS have provided mechanisms by which NA exerts its impact on signal to noise ratio, arousal, and attention.

Noradrenergic Receptors and Mechanisms of Locus Coeruleus Noradrenergic Modulation of Attention: Five adrenergic receptors have been cloned: α_1, α_2, β_1, β_2, β_3. The α_1 and α_2 receptors are each further differentiated into three subtypes: α_{1a}, α_{1b}, α_{1d} and α_{2A}, α_{2B}, and α_{2C} (MacDonald, Kobilka, Scheinin, 1997). Subtype differentiation has been based on a variety of factors, such as location (e.g., pre versus post-synaptic),

affinity for different ligands, and activation of second messenger systems. Despite these differences, all adrenergic receptors are composed of seven transmembrane domains, each consisting of a single polypeptide chain with seven hydrophobic regions that are thought to form α helical structures and span or transverse the membrane. In general, adrenergic receptors are coupled to guanine nucleotide regulatory binding proteins (G proteins). Agonist binding to a receptor terminal leads to a conformational change that allows the receptor to activate a G protein. The conformational change is followed by the exchange of guanine diphosphate (GDP) for guanine triphosphate (GTP), leading to the dissociation of the α and βγ subunits of the G proteins. Activation of G proteins leads to dissociation of the α and β subunits, and alters activity of the enzyme adenylate cyclase and various second messenger systems.

Considerable evidence suggests that the α_2 adrenoceptor is of particular importance to cognition, and attention. The α_{2A} adrenoceptor is the predominant subtype in the brain, with large numbers found in the LC, and its terminal regions in cerebellum, striatum, hypothalamus, thalamus, amygdala, hippocampus, septum, and cerebral cortex, especially the PFC (Aoki, Go, Venkatesan, & Kurose, 1994). The majority of α_{2A} adrenoceptors serve as somatodendritic autoreceptors to suppress the excitability of LC neurons (Arima, Kubo, Ishibashi, Akaike, 1998; Feuerstein, Huber, Vetter, Aranda, Van Velthoven & Limberger, 2000) or presynaptic autoreceptors that inhibit NA release (Boehm & Huck, 1996). The effect of these α_{2A} adrenoceptor actions is to reduce NA tone throughout the brain and may be of particular importance to alerting, given the effects of NA on terminal regions (Berridge & Waterhouse, 2003; Hurley, Devilbliss, & Waterhouse, 2004). α_{2A} adrenoceptors are also found pre- and postsynaptically on non-

NA terminals (i.e., heteroreceptors) (Aoki, Venkatesan, & Kurose, 1994). Prejunctional α_{2A} receptors inhibit the release of such transmitters as dopamine (Hertel, Nomikos, Syensson, 1999) and glutamate (Boehm, 1999), and are involved in such processes as of corticothalamic gating (Castro-Alamancos & Calcagnotto, 2001). Post-synaptic α_{2A} adrenoceptors mediate many of the aforementioned effects of NA on neurons in select terminal regions (Berridge & Waterhouse, 2003; Hurley et al., 2004), such as the PFC (Sawaguchi, 1998). These α_{2A} receptor effects play an important role in regulating attention and reducing distractibility (Arnsten & Li, 2005; Arnsten, Steere & Hunt, 1996).

The synaptic NA released from LC terminals in response to salient stimuli primes target neurons to detect, process, and respond (Berridge & Waterhouse, 2003; Hurley et al, 2004). This priming effect is instrumental in the facilitation of sensory signaling (Waterhouse et al, 1981), regulation of kindling in the amygdalohippocampal area (Boehm,1999), gating of corticothalamic synapses (Castro-Alamancos & Calcanotto, 2001), and control of sympathetic outflow in the rostral ventrolateral medulla (Milner et al, 1999), all of which are involved in the readiness to detect and respond to salient stimuli. Evoked increases in synaptic NA also enhance delay-related firing of the PFC during working memory, which provides a cellular basis for the maintenance of attention on salient stimuli in the presence of interference (Arnsten et al, 1996; Arnsten & Li, 2005).

Human Studies of the Locus Coeruleus-Norepinephrine System

The small size of deep brain nuclei, the limited resolution of current neuroimaging techniques, and lack of approved ligands for the study of norepinephrine have made it difficult to directly study the LC-NA system in humans. Many

methodologies have been attempted, each differing in temporal and spatial resolution. Electroencephalogram (EEG) technology provided one of the first methods of studying human brain function *in vivo*. More recent advances in functional neuroimaging, including Positron Emission Tomography (PET) and Functional Magnetic Resonance Imaging (fMRI) have provided much greater spatial resolution in the study of human brain function. The following section will review EEG, PET, and fMRI studies, including their strengths, weaknesses, and an increasing trend toward the use of multiple techniques and pharmacological challenges to better understand human brain function, particularly human attention.

Electroencephalography and Functional Neuroimaging

EEG studies of the sleep/wake cycle indicated that activity in alpha and beta bands (high frequency bands) decreases with sleep onset. At the same time, activity in low frequency bands, delta and theta, increase. These changes have also been observed as a function of time on task, with decreasing levels of arousal occurring as time on task increases. Electrophysiological measures have also been used to characterize the role of arousal in cognitive processes. Event-related potentials (ERPs) are a measure of changes in electrical voltage across many sites over the surface of the scalp. ERPs reflect neuronal activity, and produce a waveform, the components of which can be used to differentiate between cognitive processes.

The P300 Waveform and the Locus Coeruleus-Noradrenergic System. One of the most studied components of the ERP waveform, and perhaps the most relevant to the influence of the LC-NA system on attention, is the P300 (P3). The P3 is a broad, large amplitude, positive potential. The peak latency is between 300 and 400 ms following

presentation of a stimulus. Many factors influence the amplitude of the P3, including the probability of the presentation of the stimulus, the motivational significance of the stimulus, and the amount of attention paid to the stimulus.

The oddball paradigm is the most common way of studying the P3. During this task, participants are asked to respond to low frequency target stimuli (oddballs), which are embedded in a stream of nontarget stimuli. P3 amplitude is inversely related to the probability of the targets (Duncan-Johnson & Donchin, 1977). Emotional significance of targets is also associated with larger amplitude P3 components (Johnston, Miller, & Burleson, 1986; Keil et al., 2002). Stimuli that would normally elicit a P3 response do not when they are intentionally ignored, or when participants' attention is occupied by another task (Donchin & Cohen, 1967; Duncan-Johnson & Donchin, 1977; Hillyard, Hink, Schwent, & Picton, 1973).

There are two components of the P3; the P3b is distributed over parietal electrodes and the P3a, which is more frontally distributed. The P3b is usually associated with familiar, but infrequent task-relevant stimuli (Courchesne, Hillyard, & Galambos, 1975; Squires, Squires, & Hillard, 1975; Yamaguchi & Knight, 1975; Friedman, Cyowicz & Gaeta, 2001), while the P3a is associated with novelty, such as within the oddball paradigm. The P3a peaks 60-80 ms earlier than the P3b, and rapidly habituates as novelty decreases (Courchesne et al., 1975; Yamaguchi & Knight, 1991). Nieuwenhuis, Aston-Jones, and Cohen (2005) proposed the LC-NA hypothesis, which posits that phasic activity of the LC and release of NA at terminal regions is critical in generating the P3.

In support of the LC-P3 hypothesis, the LC-NA system demonstrates regional specificity that closely resembles the specificity of P3 activity. Nieunhuis et al (2005)

offer anatomical and physiological evidence in support of the LC-P3 hypothesis. Anatomical evidence from primate literature suggests that NA innervation seems particularly high in inferior parietal and somatosensory cortex, and is least dense in visual cortical areas. In addition, there is great innervation throughout the frontal pole. There is also greater innervation of the superior temporal gyrus, in the temporo-parietal junction region, than of inferior temporal gyrus. NA fibers also preferentially innervate Layer V, which contains the large, radially oriented pyramidal cells, which are thought to generate EEG activity. Physiologically, the latency of LC phasic responses, the slow conduction velocity of NA fibers, and the time course of impact of LC responses on cortical processing is on the order of magnitude of the typical P3 latency.

ERPs provide excellent temporal resolution; however, they lack spatial resolution. Signals are measured on the surface of the scalp; therefore, definitive conclusions cannot be made about the anatomical sources of ERP waveforms. Advances in functional neuroimaging have provided a good balance between temporal and spatial resolution, and thus new methods for exploring the relationship between arousal systems and attention.

Functional Magnetic Resonance Imaging Studies. One of the first fMRI studies exploring the effect of autonomic arousal on attentional focus utilized a simple letter discrimination task (Tracy, Mohamen, Faro, Tiver, Pinus, Bloomer, Pyrros, & Harvan, 2000). The task consisted of sets of capitalized letters presented bilaterally, with each trial alternating between wide and narrow eccentricities. Participants were asked to judge whether the two letters were the same or different by pointing their hand to the left for similar judgments and to the right for difference judgments. Arousal was manipulated with the use of an audiotape, which consisted of randomized segments of unpleasant

noises such as jackhammers, traffic jams, etc. Skin conductance was measured by attaching electrodes to the two largest toes of the dominant foot. They proposed that excessive LC phasic activity associated with heightened arousal would lead to a constrained selection process, with the thalamus acting as the site for interaction between arousal and attention. In addition, they proposed that the parietal cortex would be involved in spatial localization, and through inputs from the dorsolateral prefrontal cortex would exert top-down control.

While response accuracy did not differ significantly between arousal and nonarousal conditions, accuracy was significantly lower for wide eccentricity same judgments compared to wide eccentricity difference judgments. Skin conductance measures confirmed greater autonomic arousal under the arousal condition. fMRI analyses revealed that compared to rest, arousal activated posterior lateral thalamus, inferior temporal lobe (BA 20), and cingulate cortex (BA 23), all left lateralized. Only under the high arousal condition did wide eccentricity activate posterior lateral thalamus. This suggests that the thalamus may act to filter stimuli during conditions of high arousal. In addition, skin conductance was associated with activation in the anterior medial thalamus, brainstem, and middle frontal gyrus (BA 10). The investigators suggest that lateral inhibition within the reticular nucleus reduces neural activity of neighboring thalamic columns and heightens signal in central columns, thus increasing signal to noise ratio. The increase in signal to noise ratio is thought to be initiated by LC phasic activity. In addition, this study provides neuroanatomical evidence for a relationship between brainstem arousal and thalamocortical gating.

Simultaneous EEG and fMRI Measurement. While there is evidence for a relationship between general arousal and performance, there have also been attempts to further specify the relationship between exogenous and endogenous arousal and attention. Endogenous, or top-down attention, is a voluntary orienting process, allowing for focus on relevant stimuli. This controlled process is dependent on activity within the dorsolateral prefrontal cortex, and the parietal cortex. In contrast, exogenous, or bottom-up attention, is an automatic process that is mostly right lateralized within the inferior frontal gyrus and temporo-parietal junction. The exogenous attention system allows for quick orienting to salient events, such as oddballs. While most studies have defined arousal as a general constant, some have proposed that low arousal can best be described as greater fluctuations rather than an average decrease in arousal. Foucher, Otzenberger, and Gounot (2004) performed a study of arousal and attention, simultaneously measuring fMRI signal and EEG to measure changes in cortical arousal associated with BOLD signal variations. Specifically, they investigated whether arousal modulates top-down processes, bottom-up processes, or both.

The investigators utilized a rare target detection task that was sensitive to bottom-up and top-down processing. Participants viewed stimuli on a screen that included frequent distracters and never-repeated oddballs (half letters, half pictures). Participants were required to either detect target stimuli (either letters or pictures depending on the block), and respond with a button press, or passively view stimuli. Higher arousal, as measured by the power of the 5- to 9.5 Hz frequency band, was associated with faster responding. The top-down network was associated with activation in dorsolateral prefrontal cortex, anterior medial frontal cortex, and the parietal cortices, mostly right

lateralized. The bottom-up network, associated with rare events (letters and pictures) was correlated with activation in the inferior frontal gyrus and the temporoparietal junction, especially on the right side.

Recent ERP and fMRI studies add support to the role of the LC-NA system and thalamo-fronto-parietal networks in arousal, alerting, and performance. The thalamus arises as an important structure in thalamo-cortico gating, increasing signal to noise ratio, especially under conditions of high arousal. However, ERP and fMRI methodologies do not provide direct evidence of neurotransmitter function.

Pharmacological Challenge Studies

The Effect of α_2-Adrenoceptor Agonists on Alerting. As heretofore mentioned, EEG and functional neuroimaging studies have focused on the cortical and subcortical targets of the LC-NA system. The use of pharmacological challenges brings functional neuroimaging one step closer to linking functional activation to actual neurotransmitter action. Researchers have begun to apply α_2 adrenoceptor agonists and antagonists to behavioral and functional neuroimaging studies of learning and attention. Quite a few agents have been used, each differing slightly in their pharmacological profiles. For example, although the adrenoceptor antagonists Idazoxan and Yohimbine are rather selective for the α_2 adrenoceptor subtypes, they only block α_1 and β receptors at higher concentrations. Clonidine and guanfacine are two commonly prescribed α_2 adrenoceptor agonists that differ slightly in their pharmacological profiles. Guanfacine has greater affinity for the α_{2A} receptor and much lower affinity for the α_{2B} and α_{2C} receptors than clonidine, and does not bind to α_1 adrenoceptors and imidazoline sites like clonidine (Uhlen, Muceniecce, Rangalm Tiger, & Wikberg, 1995). Guanfacine is also more rapidly

absorbed, has a longer plasma half-life, and has longer-lasting behavioral effects than clonidine (Physician's Desk Reference, 2005). The following section will focus on the impact of these agents on behavioral performance as well as on functional networks associated with learning and attention.

Pharmacological studies in rodents and monkeys have directed attention toward the role of NA actions at postsynaptic α_2 adrenoceptors in the PFC. Systemically administered clonidine and guanfacine have both been shown to improve working memory impairment caused by induced catecholamine depletion in young monkeys (Arnsten & Goldman-Rakic, 1985; Cai et al., 1993) as well as naturally occurring PFC catecholamine depletion in aged monkeys (Arnsten & Goldman Rakic, 1985; Arnsten et al., 1988; Rama et al., 1996), aged rats (Carlson et al., 1992; Ramos et al., 2006), and at higher doses improves WM performance in intact animals (Franowicz &Arnsten, 1998). Supporting specific action at α_2 receptors, the benefits of these agonists can be reversed with administration of α_2 but not α_1 antagonists, and α_2 antagonists impair PFC function. The importance of α_2 receptors in attention is also highlighted in the WM literature. WM enhancements are particularly pronounced during distracting conditions (Jackson & Buccafusco, 1991; Arnsten & Contant, 1992).

Human Behavioral Studies of Clonidine and Guanfacine. Clonidine and guanfacine exert differential effects on cognitive function in humans. Two research groups (Coull, Nobre, Frith, 2001; Jakala, Riekkinen, Sirvio, Koivisto, Kejonen, Vanhanen, & Reikkinen, 1999a, 1999b, 1999c) each published a series of behavioral studies of the impact of clonidine and/or guanfacine on human cognition. Both groups utilized a neuropsychological testing battery comprised of different combinations of

CANTAB (Owen et al, 1990) tasks to test the impact of different doses of these treatments on choice reaction time, planning, spatial working memory, delayed match to sample, and/or paired associates learning. In the Jakala series, each study participant was assigned to either 0.5 μg/kg (N=6) clonidine; 2.0μg/kg clonidine (N = 8), or 5.0μg/kg clonidine (N = 8); or 7μg/kg guanfacine (N = 9) or 29μg/kg Guanfacine (N = 12). Coull et al utilized intravenous administration of either 1.5 μg/kg clonidine or 2.5 μg/kg clonidine in a placebo-controlled cross over design.

During the choice RT task, participants were asked to hold down a touch pad until a dot appeared on a computer screen at one location (simple RT) or several locations (Choice RT). Simple and choice RT was measured as the latency to touch the dots on the screen. The possible sedating motor effects of treatment were accounted for with a motor screening test. Planning was assessed with the Tower of London task, with number of moves to completion and various measures of selection latency (initial and subsequent selection of moves) being used and indices of performance. The spatial working memory task required participants to search for tokens that had been hidden in boxes. Two types of errors were assessed: within search errors were defined as returning to a previously searched box within a trial; and between search errors were defined as searching a box that actually contained the token on a previous trial. For paired associates learning, participants were required to match a color patterns to a display. Visual working memory was assessed by asking participants to search for a token in boxes that displayed different color patterns.

While no dose of any treatment impacted performance on the motor screening test, and guanfacine did not impact simple or choice RT, the lowest dose of clonidine

decreased accuracy and increased RT during a choice RT task. The two groups found disparate results for the impact of clonidine on working memory performance. Jakalla et al reported increased between search errors at the 0.5 μg/kg and 5.0 μg/kg doses of clonidine, while Coull et al reported an improvement with the 2.5 μg/kg dose or clonidine. Coull et al's effect was limited to participants' performance during the second session when clonidine was administered after placebo. While this result suggests a practice effect, Coull et al. present evidence that healthy controls do not significantly improve their performance on this task of working memory across testing sessions. Therefore, the authors interpret this result as evidence that 2.5 μg/kg dose of clonidine facilitated performance. In contrast to some of the clonidine results, a decrease in between search errors was reported for the group who received 29 μg/kg guanfacine. On the tower of London task, individuals in the 29 μg/kg guanfacine group demonstrated poorer planning by utilizing more moves to complete the task. For paired associates learning, there was no effect of 1.5 μg/kg clonidine, however 2.0 μg/kg and 2.5 μg/kg clonidine, and 29 μg/kg guanfacine improved performance decreasing the number of trials to criterion.

Human behavioral studies have suggested an interaction between the impact of clonidine on behavioral and arousal level. Smith and Nutt (1996) reported in healthy volunteers that the impact of clonidine on performance of a selective attention task depended on arousal level, which was manipulated with exposure to white noise. Following clonidine administration, performance was significantly more impaired in the white noise condition than in the control condition. Coull et al (1995) reported participants who received clonidine and were already familiar with a RVIP task

demonstrated greater decrements in performance than those who were unfamiliar and presumably in a state of higher arousal.

These studies offer preliminary reports of the impact of guanfacine and clonidine on different cognitive processes. Both treatments improve paired associates learning (Jakala et al, 1999a), but only guanfacine improved planning and working memory (Jakala et al, 1999b), and only clonidine impaired choice RT performance (Jakala et al, 1999c). However, the small sample sizes and a lack of a measure of physiological or neural effects are weaknesses of these studies. These studies do suggest that clonidine and guanfacine have important post-synaptic effects in motor planning and execution areas and prefrontal working memory areas, consistent with the work done by Arnsten et al (1996) in animals. In addition, there seems to be an interaction between levels of arousal and the impact of clonidine on performance. However, these behavioral results do not speak to the changes in functional brain networks that mediate cognition.

Functional Neuroimaging Studies of Clonidine and Guanfacine challenges. Early PET studies of the impact of clonidine on attention, learning, and memory indicated that the impact of clonidine was greater during rest than during performance of cognitive tasks. Specifically, in a study of healthy volunteers, Coull et al (1997) reported attenuation of right thalamic regional cerebral blood flow during the control conditions of both a rapid visual information processing task which places demands on both sustained attention and working memory, and a paired associates learning task. This is suggestive of a greater effect of clonidine on neural functioning during period of low arousal irrespective of specific task demands. Of note, these neural effects were reported in the absence of behavioral effects.

Utilizing fMRI in conjunction with a Dexmethotomidine (DEX) noradrenergic challenge, Coull et al (2004) again reported an interaction between arousal level and the impact of NA agents on neural functioning and performance. While DEX impaired performance on a target detection task, this impairment was attenuated by the pseudorandom presentation of white noise throughout the trials. Attenuation of impairment was associated with a selective increase in activation of the left medial pulvinar nucleus, suggesting an increase in phasic arousal allowed participants to overcome the deleterious effects of DEX on performance.

The role of the thalamus in the interaction between arousal and performance during pharmacological challenge studies that are designed target α_{2A} receptor function are somewhat inconsistent with the lack of α_{2A} receptors in human thalamus. One explanation is that the effect of clonidine might occur through α_{2B} receptors, which are abundant in human thalamus. However, a plausible alternative is that the impact of clonidine, and perhaps other treatments that target α_{2A} receptors, occurs at other brain areas that exert influence over the thalamus, or that these pharmacological agents are impacting functional connectivity between other areas of the brain and thalamus. Coull et al (1999) used PET to explore the impact of clonidine on functional connectivity during a rest condition and during performance of a RVIP. During rest, clonidine reduced functional integration between frontal cortex and thalamus and between visual cortex and its targets. However, during performance, clonidine increased functional integration between frontal and parietal cortices and between parietal cortex and thalamus. There were also task-specific effects of clonidine on connections from the LC to neocortical areas. There were decreases in connectivity between the LC and visual cortex during rest,

but increased connectivity between the LC and intraparietal sulcus during task performance. These results indicate that clonidine does impact neuronal functioning in a task-dependent manner, and without looking at the functional connectivity between regions these relationships would have been missed.

Coull et al. (2001) went on to explore the specific impact of clonidine and guanfacine on a task of attention, which tested spatial orienting and alerting functions. Participants performed the task, which provided spatially informative, temporally informative, or non-informative cues prior to targets while being scanned with fMRI. The alerting component was measured by comparing neural responses of the temporally informative cue and the non-informative cue, while spatial orienting was measured by comparing the spatially informative cue and the neutral cue. With placebo, there was increased activation in the left prefrontal, premotor, and parietal cortices, for the temporally informative cue versus the non-informative cue. Notably, they did not find increased activation in thalamus. Clonidine, but not guanfacine, impaired behavioral measures of alerting and attenuated activity in the left temporal-parietal junction, left PFC, and insula during temporal orienting, and attenuated right superior parietal activation during spatial orienting.

Several methodological shortcomings may explain this discrepancy. First, these studies were not expressly designed to assess alerting, but rather used tests of learning (Coull et al, 1997), target detection (Coull et al., 1999), spatial attention (Coull et al., 2001), and working memory (Coull et al, 1997). Thus, guanfacine and clonidine effects on alerting have never been directly tested. Second, most studies of alerting to date (Fan et al., 2005; Sturm et al, 1999; Weis et al., 2000; Achten et al., 1999; Sturm & Wilmes,

2000), including the one study of guanfacine (Coull et al., 2001), have contrasted cued and uncued target stimuli, which introduced the confounding effect of the presence versus absence of a stimulus. Either a warning cue is presented prior to the target stimulus or nothing appears. Finally, and most tentatively, it is possible that the warning cues used in these studies (Coull et al, 2001; Coull et al, 2004; Coull et al, 1999; Coull et al, 1997) were not sufficiently arousing to elicit strong enough neural responses to discern treatment-induced attenuations in activation by fMRI (i.e., floor effects).

The current study was designed as an attempt to rectify the methodological shortcomings of prior studies of human alerting by imaging performance during the newly developed Stay Alert task. This task directly tests alerting using a simple RT paradigm in which a stimulus (warning cue or noncue) is always presented prior to the target stimulus (i.e., "X"). Thus, the contrast for alerting is between similar stimuli that vary only as a function of their meaning, be it as cue (i.e., "A") or noncue (i.e., "B" through "H"), and does not include the actual motor response to the target. Simultaneously, we tested the impact of the α_{2a} receptor agonist, guanfacine on cue utilization.

This study tested three competing hypotheses regarding the effects of guanfacine on alerting. Specifically, that guanfacine will produce either:

1. Decreases in alerting via α_2-autoreceptor inhibition of LC. This hypothesis predicts that guanfacine stimulation of α_2-autoreceptors in LC will attenuate evoked responses to salient stimuli, resulting in less NA release in terminal regions, which will be reflected in i) reduced signal-to-noise ratio or activation of thalamus, LPi, LPs, CMA, SMA, and PFC and ii) a selective increase in RT to cued targets but not uncued targets;

2. Increases in alerting via α_2-heteroceptor facilitation of PFC. This hypothesis predicts that guanfacine stimulation of postsynaptic α_2-heteroceptors will increase the signal-to-noise ratio and attentional functions of the PFC, which will be reflected in i) increased activation of PFC and ii) a selective decrease in RT to cued targets but not uncued targets;

3. A general sedative effect. This hypothesis predicts that guanfacine stimulation of α_2-autoreceptors in LC will inhibit both basal and evoked firing, resulting in less synaptic NA, as reflected in i) global decreases in activation ii) a nonselective increase in RT to both cued and uncued targets.

Method

Participants

Sixteen right-handed young adult volunteers (nine female) were recruited by word of mouth and fliers placed at Queens College of CUNY and Mount Sinai School of Medicine. The mean age was 25.44 years (sd = 4.37). There was ample representation of diverse ethnic groups with 50% Caucasian, 25% Black/non-Hispanic, 18.75% Hispanic, 6.25% Asian or Pacific Islander. Three additional individuals signed consent forms, but did not partake in the scanning portion of the study (two lost to follow-up, one screen failure due to suspected attention-deficit/hyperactivity disorder). The institutional review boards of Queens College, City University of New York, and Mount Sinai School of Medicine approved the study. Written informed consent was obtained from each participant prior to conducting any study procedures. Participants were compensated for their time and travel following each scanning session.

Exclusionary criteria included contraindications for MRI; history of head injury neurological or cardiovascular disease, or other conditions that affect brain function; and contraindications for guanfacine, including abnormal findings on electrocardiogram, and blood pressure readings. Findings of mental illness assessed during a mental status exam performed by a psychiatrist. Scores ≥ 15 on the Beck Anxiety Inventory (BAI) (Beck & Steer, 1990), depression using the Beck Depression Inventory – II (BDI-II) (Steer, Ball, Ranieri, & Beck, 1999), T-scores 1.5 standard deviations (SD) above the mean on the DSM-IV Inattention, Hyperactive-Impulsive, or Total ADHD Symptoms scales of the Conners' Adult ADHD Rating Scale (Conners, 1998), and an estimated IQ < 80 based on

the two-subtest version of the Wechsler Abbreviated Scale of Intelligence (WASI) (Psychological Corporation, 1999). Sample characteristics are presented in Table 1.

Table 1. Sample Characteristics

	Mean	SD	Range
Age	25.44	4.37	21 – 35
CAARS Inattentive Index	42.44	8.81	35 – 64
CAARS Hyperactive/Impulsive Index	39.69	5.44	33 – 52
CAARS ADHD Index	39.44	8.05	31 – 57
BDI-II	1.75	2.52	0 – 9
BAI	2.38	3.59	0 – 14
WASI Vocabulary	57.81	7.48	47 – 71
WASI Matrix Reasoning	58.38	6.57	43 – 68
WASI Estimated FSIQ	113.69	9.62	99 – 132

Note. CAARS, Conners' Adult ADHD Rating Scale; BDI-II, Beck Depression Inventory - II; BAI, Beck Anxiety Inventory;WASI, Wechsler Abbreviated Scale of Intelligence; FSIQ, Full-scale Intelligence Quotient.

Measures

Wechsler Abbreviated Intelligence Scale (WASI, Psychological Corporation, 1999). The WASI is an abbreviated version of the standard Wechsler intelligence scales that was developed to estimate IQ scores when administration of a full battery is not feasible. The test consists of four subtests that closely parallel their full Wechsler counterparts: Vocabulary, Similarities, Block Design, and Matrix Reasoning. General intellectual ability can be reliably estimated with a two-subtest form that includes the Vocabulary and Matrix Reasoning subtests. Raw scores for each subtest were converted

to T-scores based on norms for gender and age, and were then combined to provide a Full Scale IQ score. Full Scale IQ was estimated from the two-subtest form.

Conners' Adult ADHD Rating Scale (CAARS, Conners, 1998). The CAARS is a 66-item rating scale that required informants to indicate, using a four-point scale the degree to which ADHD symptoms and associated behavioral difficulties have recently been present. The scale provides nine subscale scores: three correspond directly to DSM-IV ADHD behaviors, while the remaining six provide a dimensional index of ADHD symptoms and related behaviors. Raw scores for each subscale were converted to T-scores based on norms for gender and age (in 10-year increments) derived from a normative sample of 839 adults. Internal consistency and test-retest reliability coefficients for individual subscales ranged from .88 - .91 and .80 - .91, respectively. Discriminative validity yielded a correct classification rate of 85%. The entire scale can be completed in 10 minutes.

Beck Depression Inventory-II (BDI-II, Steer, Ball, Ranieri, & Beck, 1999). The BDI-II consists of 21 multiple choice items measuring the presence and degree of depression in adolescents and adults. Each of the 21-items of the BDI corresponds to a specific category of depressive symptom and/or attitude. Each category purports to describe a specific behavioral manifestation of depression and consists of a graded series of four self-evaluative statements. The statements are rank ordered and weighted to reflect the range of severity of the symptom from neutral to maximum severity. Numerical values of zero, one, two, or three are assigned each statement to indicate degree of severity. Test-retest reliability and internal consistency coefficients were above 0.90 and 0.86 for individual test items, and the Spearman-Brown correlation for the

reliability of the BDI-II yielded a coefficient of 0.93. Face and content validity of the inventory are high. Finally, correlations between ratings on the BDI-II and other self- and physician-reported scales ranged from 0.65 -0.77. Participants with scores above 15 on the BDI-II were excluded.

Beck Anxiety Inventory (BAI, Beck & Steer, 1990). The BAI is a 21-item multiple choice inventory that assesses symptoms of anxiety and reliably discriminates anxiety from depression. Each item describes a common symptom of anxiety that the respondent is asked to rate over the past week on a 4-point scale ranging from zero to three. The items are summed to obtain a total score that can range from 0 to 63. The scale obtained high internal consistency and item-total correlations ranging from 0.30 to 0.71 (median=.60). Test-retest reliability was 0.75. The scale takes approximately 5 minutes to complete. A score above 15, indicative of moderate anxiety, was exclusionary.

Stay Alert Task: The task was run in 4 blocks of 300 seconds each. Each block begins and ends with a 30 second fixation period and comprises a series of 120 stimuli (i.e., capital letters). Letters were presented for duration of 200 millisecond with a jittered inter-stimulus interval (ISI) that varied from 1550 to 2050 milliseconds. The ISI was pseudorandomized so that the mean length is 1800 milliseconds in each block. Within each block there was 24 (20%) target trials (i.e., the letter X), with 50% of them preceded by a cue (i.e., the letter A). The remaining targets were preceded by letters other than A, and the letter A did not precede a non-target stimulus. In total, across the four blocks there were 48 cued and 48 non-cue targets. The behavioral dependent measure was reaction time to cued and uncued targets. The total time required to complete this task was 20 minutes.

The Stay Alert task was compiled and run on an IBM compatible personal computer using E-Prime™ software (Psychology Software Tools, Inc., Pittsburgh, PA) (Schneider, Eschman, & Zuccolotto, 2000a, 2000b). Stimuli were projected via an SVGA projector system onto a rear-projection screen mounted at the head of the magnet bore. Participants viewed the stimuli through a mirror on the head coil positioned above their eyes and responded with the right hand using the BrainLogics fiber optic button system (Psychology Software Tools, Inc., Pittsburgh, PA). Responses were recorded on a personal computer.

Procedure

Following the informed consent procedure, each participant underwent physical and psychological screening and two subsequent scanning sessions scheduled approximately one week apart (mean = 8 days). The screening consisted of a physical exam, mental status exam, and several questionnaires. Finally, the participants performed one block of the Stay Alert task on an office desktop.

On the days of the fMRI scans, blood pressure was checked and participants received 1mg oral guanfacine or placebo 90 minutes prior to the scheduled scan. Treatment order was randomized and double-blind. Following administration of the treatment, participants again practiced, on an office desktop, one block of the Stay Alert task.

For the scanning procedure, participants were positioned in a head-dedicated 3.0 Tesla scanner. A three minute T2-weighted anatomical scan was acquired, after which gradient echo echoplanar images (EPI) sensitive to blood-oxygen level dependent (BOLD) contrast were acquired while the participants performed the Stay Alert task. The

scanning procedure required approximately one hour to complete. Following each scan, participants' blood pressure was measured once again, compensation was given, and the participants' were released.

Structural and Functional Magnetic Resonance Image Acquisition. Scan sessions began with shimming and sagittal localization, followed by a high-resolution T2-weighted anatomical volume of the brain was acquired with a turbo spin-echo (TSE) pulse sequence with a repetition time (TR) of 4500 ms, echo time (TE) of 99 ms, flip angle of 170°, 21-cm field of view (FOV), and 512 x 336 matrix. Forty-two axial slices were acquired at a thickness of 4 mm with no gap and an in-plane resolution of 0.41 x 0.41 mm. This sequence was obtained to register and align the functional images with a reference brain. Functional T2*-weighted images depicting the blood oxygenation level-dependent (BOLD) signal were acquired at the same 42 slice locations using gradient-echo echo-planar images with a TR of 3000 ms, TE of 27 ms, flip angle of 85°, FOV of 21 cm, and an acquisition matrix of 64 x 64. Each functional image comprised a brain volume of 42 axial slices with 3-mm thickness with no gap and an in-plane resolution of 3.75 x 3.75 mm. All images were acquired with slices positioned parallel to the anterior commissure – posterior commissure line. The participants all completed 8 runs of 300 seconds each, yielding 100 time points per participant. The scan session was repeated.

Behavioral Analysis. A repeated measures analyses of variance (ANOVA) was performed with Time (pre-scan versus post-scan) and Treatment (Guanfacine versus Placebo) as within subjects variables to separately assess the impact of treatment on blood pressure and heart rate, measured in beats per minute. To test treatment effects on task performance, mean reaction time to cued targets and uncued targets for each

participant were entered into a repeated measures ANOVA for which Treatment (Guanfacine versus Placebo) and Condition (Cue versus Non-cue) served as within-subjects factors. The alpha level for these analyses was set at $p < 0.05$ due to the small sample. Results of these analyses are presented in Figure 1.

Functional Magnetic Resonance Imaging Analysis. Image preprocessing and analyses were conducted using statistical parametric mapping (SPM2; Wellcome Department of Cognitive Neurology, University College of London, London, England) implemented on a MatLab platform (version 6.5, Mathworks, Natick, MA). Four placebo and four guanfacine functional time series were acquired from each subject. Slice scan timing correction, realignment, and coregistration to T2 images were performed separately for the placebo and guanfacine functional series, after which the placebo scans were coregistered to the guanfacine scans. All the functional scans were spatially normalized to a standard template (Montreal Neurological Institute) using parameters estimated from the first scan T2 image, and then resampled using a sinc interpolation, resulting in a voxel size of 2×2×2 mm. Finally, the functional scans were spatially smoothed with an isotropic 8 mm full width at half maximum (FWHM) Gaussian kernel.

Slice Timing Correction. Interleaved slice acquisition was utilized, such that all odd slices were collected, followed by all even slices. This acquisition has the advantage of limiting the influence of the excitation pulse on contiguous slices. However, the disadvantage of this method is that each slice in a volume is collected at a different time point. SPM statistics assume that all voxels in a single volume are obtained at the same time point; therefore a temporal correction must be performed to account for differences in slice acquisition timing. First, a reference slice must be selected. We selected the first

slice. Then, all other slices in the volume are shifted in time with an interpolation method. Temporal interpolation estimates the value of the signal at a time point that was not originally collected, using data from nearby time points.

Spatial Realignment and Unwarping. Although participants are instructed to stay perfectly still during the scanning session, and their heads are stabilized with foam, some head movement is inevitable. If there is movement of the head, voxels that are assumed to represent the same area of the brain are no longer aligned (Ashburner & Friston, 2003; Ashburner & Friston, 2000; Brammer, 2001). Moreover, movement that is correlated with task performance results in false-positive increases in BOLD signal, and even movement that is not correlated with task performance add noise to the signal decreasing statistical power (Ashburner & Friston, 2003; Ashburner & Friston, 2000; Brammer, 2001).

Movement correction is completed for each participant using the mean image of the time series as a reference image. All images are repositioned until they match the reference volume. Spatial realignment is also known as rigid body transformation, which assumes that each image can be superimposed on the other images in the series by a combination of three translations (x-, y-, and z-planes) and three rotations (x-y, x-z, and y-z planes). The size and shape of the images are not changed, only their position. An algorithm is used that ensures that for each volume the least squared difference between the volume in the time series and the reference volume is minimized. Realignment corrects for movement as well as artifacts that can occur over the course of a session as the scanner becomes progressively hotter.

Following realignment susceptibility artifacts might still be present. Small

inhomogeneities in the magnetic field can cause distortions, particularly at tissue-air and tissue-bone boundaries. If the head moves, these distortions change in a non-linear manner. This is known as a susceptibility-by-movement interaction. This type of artifact can be minimized by unwarping the dataset. The realignment process is used to determine if and to what extent the participant moved. By figuring in how the magnetic field changes when the participant moves the distortions in the image can be estimated, and the variance in the dataset that is a consequence of the interaction between field inhomogeneities and movement can be estimated and removed (Andersson, Hutton, Ashburner, Turner, & Friston, 2001).

Spatial Coregistration. In addition to ensuring that all voxels line up temporally and spatially, results of analyses have to be applicable to specific brain regions. Functional images have relatively little spatial detail. However, higher resolution structural scans make it possible to delineate great detail in sulcal and gyral patterns across the cortex, as well as differentiation of subcortical structures. Therefore, coregistration procedures are used to map functional images onto higher resolution structural images for each participant (Ashburner & Friston, 2003; Jenkinson, 2001). For the current study, functional images from scan 1 and scan 2 were coregistered to their respective T2 images separately, after which the scans from both sessions were coregistered together.

Spatial Normalization. Individual brains vary greatly in morphology. Normalization is a form of coregistration that compensates for differences in morphology across participants by mathematically stretching, squeezing, and warping each brain so that they all match the size and shape of a standard brain (e.g., the template of the

Montreal Neurological Institute) (Ashburner & Friston, 2003; Ashburner & Friston, 2000). In the current study coregistered images from placebo and guanfacine scans were spatially normalized together.

Spatial Smoothing. The effects of interests in fMRI are changes in blood flow, which are expressed in low spatial frequency bands, alongside noise at higher spatial frequency bands. Spatial smoothing increases the signal to noise ratio by removing high spatial frequency noise. Smoothing also removes small frequency differences, facilitating comparisons across participants. Finally, smoothing the dataset satisfies the requirement for applying Gaussian Field Theory to correct for multiple comparisons in statistical analysis by making the data more normally distributed (Smith, 2001). Each 3D volume is convolved with a 3D Gaussian kernel, such that every data point is multiplied by a curve in the shape of a 3D normal distribution. The shape of the smoothing curve is defined by the Full Width Half Maximum (FWHM). This is the width of the curve at half of the maximum, defined in millimeters. The FWHM chosen for the smoothing curve is usually two or three times the voxel size.

First-Level Analysis: The General Linear Model. First-level analyses were performed separately for each participant to test the neural effects of guanfacine on alerting. A general linear model (GLM) was conducted to determine the relationship between the observed event-related BOLD signals and regressors that represented expected neural responses to trial events. Regressors were created by convolving a train of delta (δ) functions that represented the individual trial events with the default SPM basis function, which consisted of a synthetic hemodynamic response function, composed of two gamma (γ) functions and their derivatives (Friston., Fletcher, Josephs, Holmes,

Rugg & Turner, 1998).

Four regressors were entered into the model for each participant, including vectors representing cues, distracters, targets, and error events. Error events included distracter trials with commission errors and both the cue and target for omission errors. In addition, six parameters (*x*, *y*, and *z* translations and rotations) generated during motion correction were entered as covariates of no interest (Johnstone, Ores Walsh, Greischar, Alexander, Fox, Davidson, Oakes, 2006).

Linear contrasts separately accounting for BOLD signal changes in response to Cues, Visual Stimulation (cues, noncues, and distracters), and Targets were applied to the parameter estimates, which regressed out activation due to each of the other contrasts. Three contrast maps were generated each for Cues, Visual Stimulation, and Targets, representing, i) regions of significant BOLD signal change with placebo, ii) regions of decreased BOLD signal change with guanfacine compared to placebo (Placebo minus Guanfacine), and iii) regions of increased BOLD signal changes with guanfacine compared to placebo (Guanfacine minus Placebo).

Second-Level Analysis. The three contrast maps for Cues, Visual Stimulation, and Targets for all participants were entered into separate second-level group analyses conducted with random-effects statistical models that account for intersubject variability and permit population-based inferences. The resultant voxel-wise statistical maps were thresholded for significance using a cluster-size algorithm that protects against false-positive results in spatially extended continuous data (Hayasaka et al., 2004). The height (intensity) threshold of each activated voxel was set at an uncorrected *p* value of 0.05. A Monte Carlo simulation of the brain volume in the current study that assumed an

individual voxel type I error of $p < 0.05$ established that a cluster extent of 100 contiguous resampled voxels (2 x 2 x 2 mm^3) was necessary to correct for multiple voxel comparisons at $p < 0.05$ (Slotnick and Schacter, 2004).

Coordinates of activation for cue-related BOLD signal changes were converted to Talairach and Tournoux (1988) coordinates using a nonlinear transformation (http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace. html). Percent BOLD signal change for Visual Stimulation and Targets was calculated for regions of interest identified in the results of the Cue-related BOLD signal change contrasts to demonstrate the specificity of BOLD-signal changes in response to cues. Cytoarchitectural areas were designated according to the conventions of Vogt, Nimchinsky, Vogt, and Hof (1995) for the cingulate gyrus and according to Brodmann (1909) for the rest of the brain.

Results

Blood Pressure and Heart Rate Measurements

Means and standard deviations for blood pressure and heart rate measurements are presented in Table 2. There was a significant Treatment X Time interaction for systolic blood pressure, $F(1,15) = 7.66$, $p = .01$, $\eta^2 = .94$, such that systolic blood pressure was lower on average before the placebo scan compared to the guanfacine scan and post scan measurements increased for placebo, while they decreased for guanfacine (Figure 1). There were no significant main effects of Treatment, $F(1, 15) = .37$, $p > .1$, $\eta^2 = .06$ nor time, $F(1, 15) = .01$, $p > .1$, $\eta^2 = 0$ for systolic blood pressure. For diastolic blood pressure there was no significant interaction, $F(1, 15) = .03$, $p > .1$, $\eta^2 = .06$; nor significant main effects of Treatment, $F = .16$, $p > .01$, $\eta^2 = .35$, or Time, $F(1, 15) = .34$, $p > .10$, $\eta^2 = .58$ (Figure 2).

The was a significant Treatment X Time interaction for heart rate, $F(1,15) = 6.96$, $p = .02$, $\eta^2 = .25$, and a significant main effect of Time for heart rate $F(1,15) = 8.00$, $p = .01$, $\eta^2 = .75$, such that post-scan heart rate measurements were lower for both placebo and guanfacine, but decreased more for guanfacine than for placebo (Figure 3). There was no significant main effect of Treatment, $F(1, 15) = .03$, $p > .01$, $\eta^2 = 0$.

Table 2. Treatment Effects on Blood Pressure Measures

	Placebo				Guanfacine			
	Pre-scan		Post-scan		Pre-scan		Post-scan	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Diastolic BP (mmHG)	70.8	9.8	70.0	8.0	70.1	8.4	69.8	9.1
Systolic BP (mmHG)[a]	114.4	11.3	118.2	10.1	119.1	9.9	115.4	12.2
Pulse Rate (beats per minute)[b]	73.7	8.8	71.6	7.7	70.8	9.8	70.0	8.0

Note. BP, blood pressure.

[a] Significant Treatment X Time interaction ($F(1,15) = 7.66$, $p = .01$).

[b] Significant main effect of Time ($F(1,15) = 8.00$, $p = .01$) and Treatment X Time interaction ($F(1,15) = 6.96$, $p = .02$).

Behavioral Results

There was a strong alerting effect for both Placebo and Guanfacine conditions, $F(1, 15) = 262.82$, $p < .0001$, $\eta^2 = .99$, such that reaction times to cued targets were shorter than reaction times to uncued targets (Figure 1). However, there was no significant main effect of drug, $F(1, 15) = .12$, $p = .72$, $\eta^2 = .01$, nor was there a significant Drug X Cue interaction $F(1, 15) = .06$, $p = .81$, $\eta^2 = 0$. The hit rate was > 95% and the false alarm rate was < 1% across all conditions.

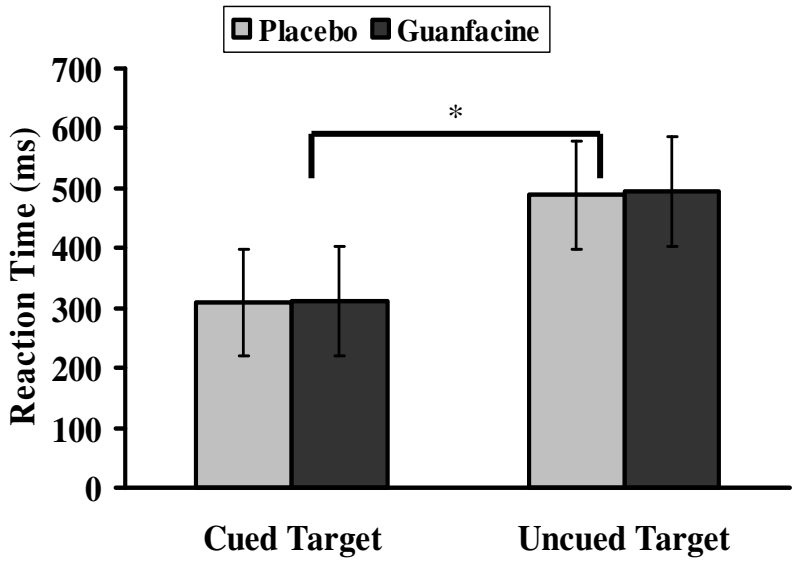

Figure 1. Treatment effects on reaction time for cued and uncued targets. The warning cue reduced reaction time to targets across treatments. Error bars represent 1 SEM. *** main effect of cue, *p* < .05**

For the standard deviation of reaction times, a separate two-way within-subjects ANOVA revealed no significant interaction of Drug X Cue, $F(1, 15) = .65$, $p > .10$, $\eta^2 = .99$, nor main effects of Drug, $F(1, 15) = .65$, $p > .10$, $\eta^2 = .42$, or Cue, $F(1, 15) = .93$, $p > .10$, $\eta^2 = .48$ (Figure 2).

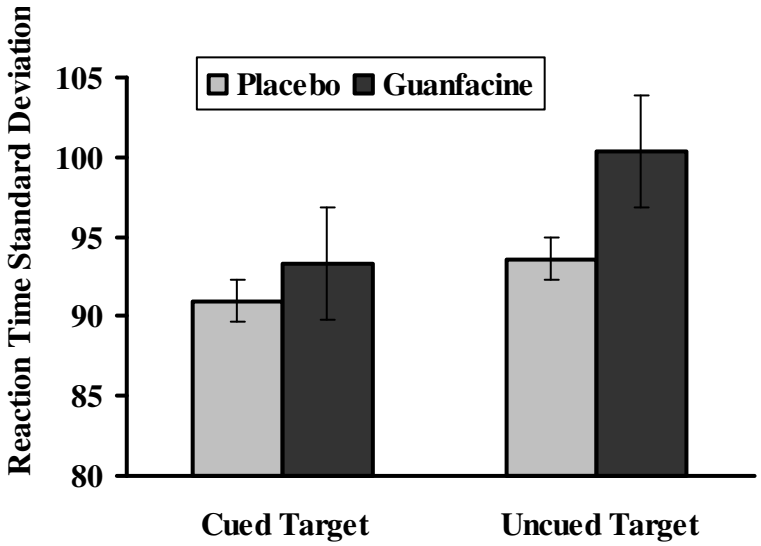

Figure 2. Treatment effects on reaction time standard deviation for cued and uncued targets. No significant effects were found, p's > .10. Error bars represent 1 SEM.

Imaging Results

Cue-related BOLD Signal Changes: Coordinates of the peaks of BOLD signal change for the placebo condition are presented in Table 3. Cue related BOLD signal changes in regions of the alerting network were similar for both placebo and guanfacine. There were significant cue-related activations in bilateral dorsolateral PFC (BA 46), and right lateralized temporoparietal junction (TPJ, BA 40) at the posterior end of the lateral fissure (Figure 3). Significant activations for both treatments were also observed in bilateral thalamus and putamen, with left-lateralized peaks (Figure 4). Activation in the left putamen extended to the insular cortex with placebo, but not with guanfacine. Finally, activation of the posterior rostral cingulate zone (RCZp, BA 24) extended laterally to right dorsal premotor area (PMd, BA 4) for placebo and left PMd for Guanfacine, and bilateral activation of the cerebellum was also observed for both treatments (Figure 5).

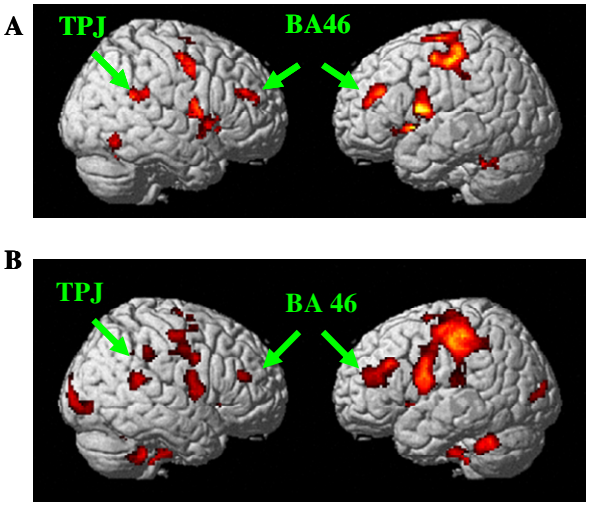

Figure 3. Rendered images of Cue-specific BOLD signal increases for placebo **(A)** and Guanfacine **(B).**

Table 3. Peaks of Cue-Related Bold Signal Changes with Placebo.

				Talairach Coordinates				
Region	Side	BA	Cluster Size	x	y	z	*t*	p
Dorsolateral prefrontal cortex	L	9/46	228	-39	36	24	4.01	.001
	R	9	151	30	47	16	5.80	<.001
Dorsal premotor area	R	4	244	40	-2	39	4.90	<.001
Posterior rostral cingulate zone	L	24	3090	-12	7	33	6.07	<.001
Temporoparietal junction	R	40	184	46	-42	22	4.14	<.001
Intraparietal sulcus	L	7	187	-16	-62	45	2.76	<.01
Putamen	L		4272	-26	-4	-2	6.25	<.001
Thalamus	L		3090	-12	-16	1	6.07	<.001
Cerebellum	L		130	-34	-54	-23	3.95	.001
	R		184	14	-47	-16	4.14	<.001

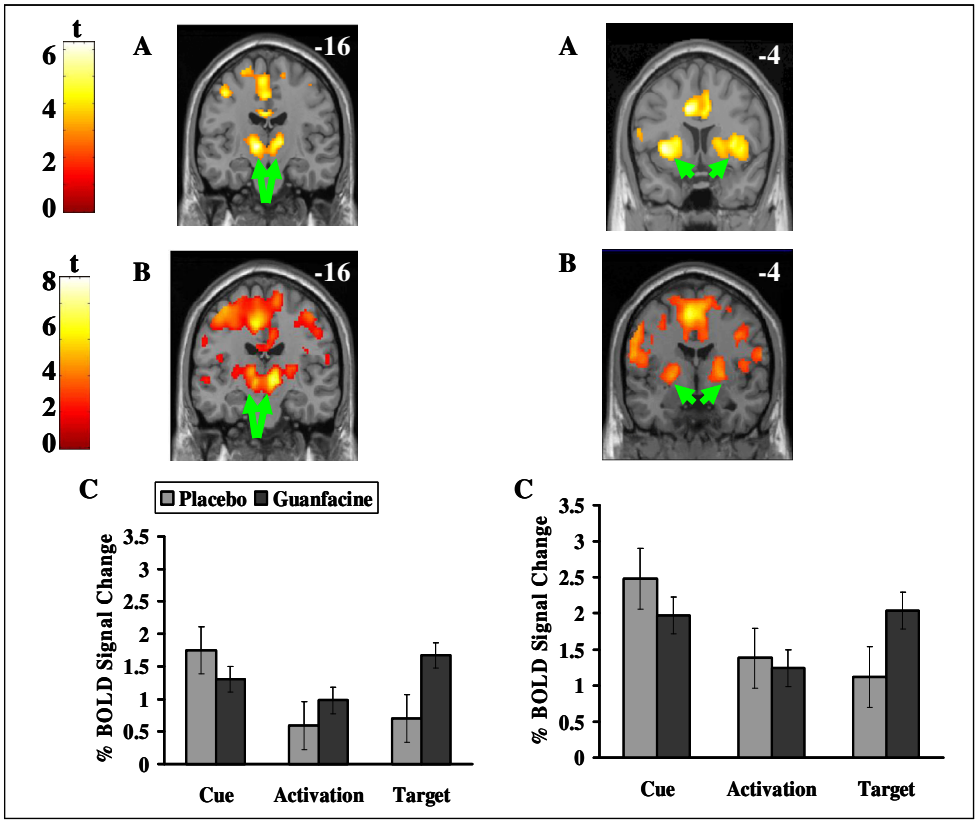

Figure 4. Cue-related BOLD signal increases for placebo **(A)** and guanfacine **(B)** in thalamus (**left panel**), putamen, and insula (**right panel**). There were no significant effects of treatment on cue-related activation **(C)** in thalamus or putamen. Error bars represent 1 SEM.

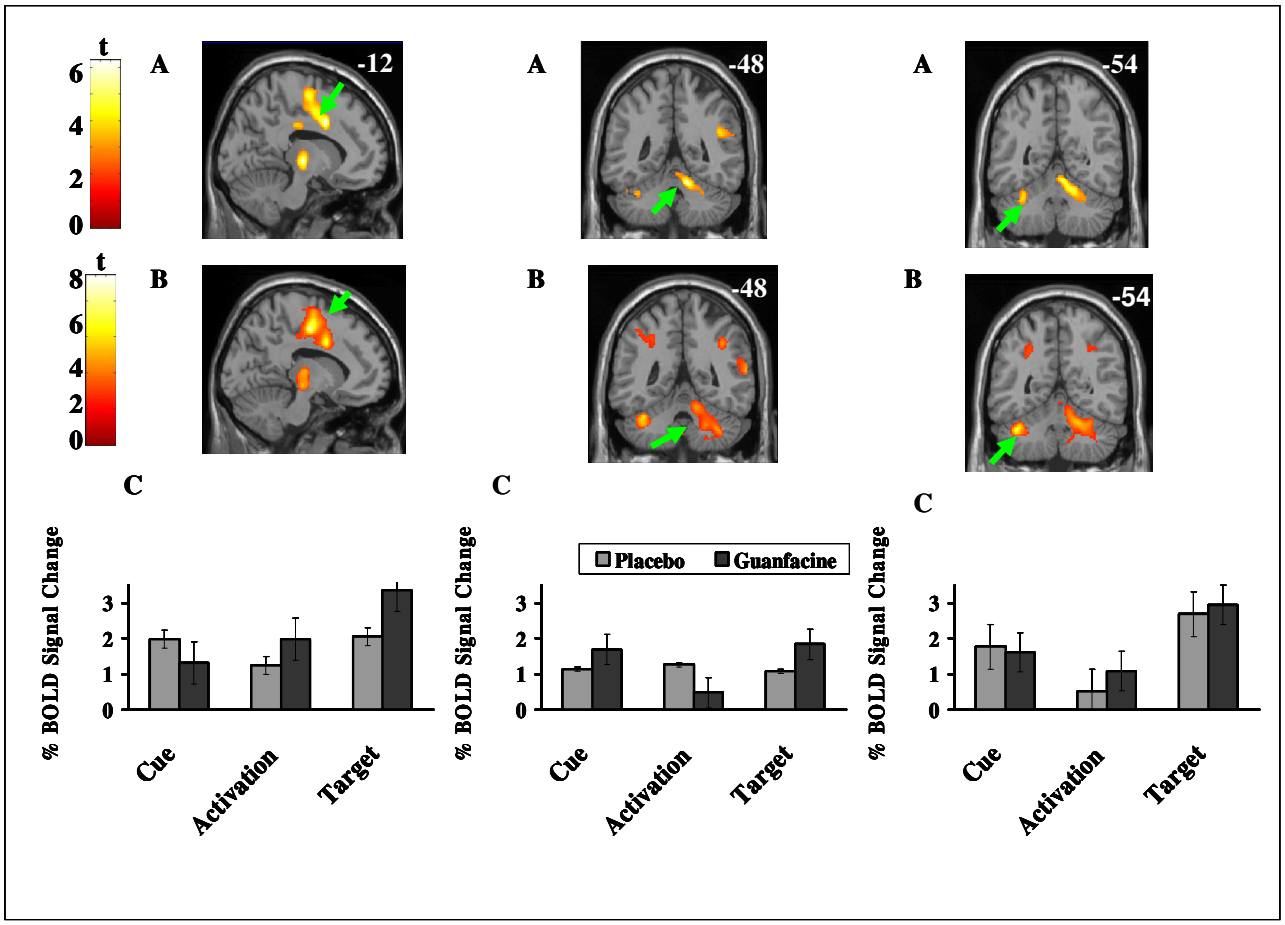

Figure 5. Cue-related BOLD signal increases for placebo **(A)** and guanfacine **(B)**, in right dorsal premotor area (**left panel**), right cerebellum (**middle panel**), and left cerebellum **(right panel)**. There were no significant effects of treatment of cue-related activation **(C)** in these areas. Error bars represent 1 SEM.

Comparisons of Cue-Related Activation Following 1mg Guanfacine Challenge Compared to 1 mg Placebo:

Rendered images comparing cortical regions of increased and decreased cue-related activation with guanfacine are presented in Figure 6. Coordinates of peak activation for the Placebo-Guanfacine contrast are presented in Table 4, and coordinates of peak activation for the Guanfacine-Placebo contrast are presented in Table 5.

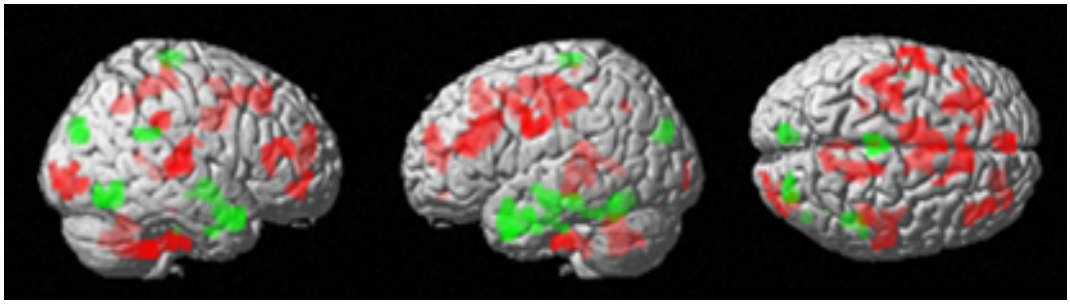

Figure 6. Rendered images of regions of increased cue-related activation in the guanfacine – Placebo **(Red)** and the Placebo – Guanfacine Contrast **(Green)**.

Comparison of treatment effects on cue-related BOLD signal increases revealed significantly greater cue-related activation in bilateral DLPFC (BA 46) with guanfacine compared to placebo (Figure 7). Post-hoc t-tests indicated overlapping clusters of comparable magnitude for guanfacine and placebo in right DLPFC, t (15) = .926, $p > .10$, and left DLPFC, t (15) = .151, $p > .10$. However, adjacent clusters of significant BOLD signal changes were present for guanfacine, but not placebo in both right DLPFC, t (15) = -2.73, $p < .05$, left DLPFC t (15) = -2.35, $p < .05$. Accordingly, the mean volume of cue-related activation was greater for guanfacine than placebo in right (868.40 ± 170.34 mm^3 versus 438.12 ± 182 mm^3) and left DLPFC (1091.88 ± 282.18 mm^3 versus 420.26 ± 139.90 mm^3). These treatment effects were specific to cue-related activity and were not seen for activation related to visual stimuli or targets.

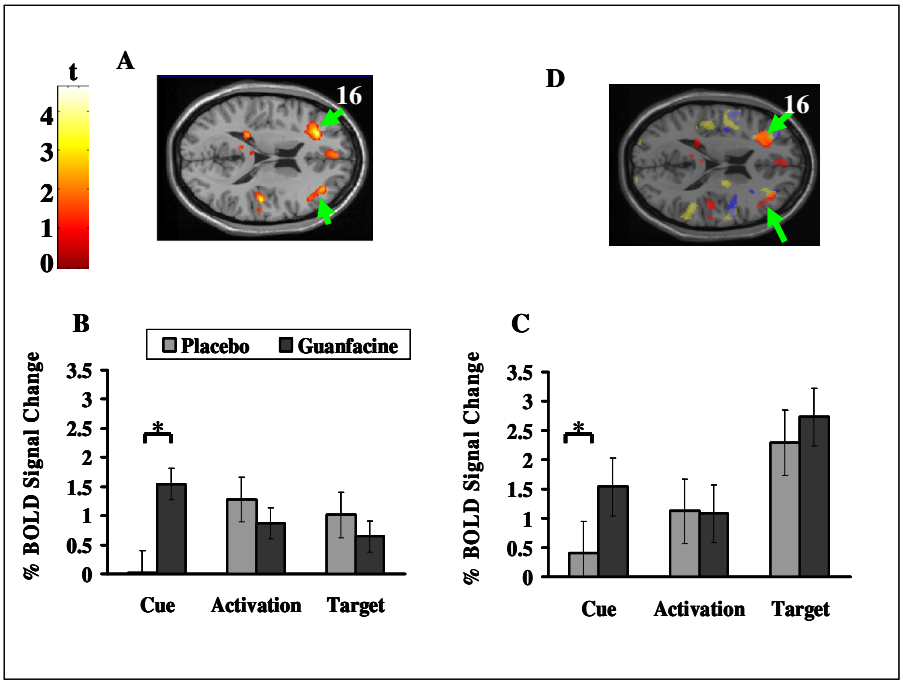

Figure 7. Greater cue-related BOLD signal changes **(A)** in left DLPFC **(B)** and right DLPFC **(C)** with guanfacine compared to placebo. Increased cue-related BOLD signal changes were characterized by an overlapping cluster of comparable magnitude for both treatments, with additional activation in adjacent clusters with guanfacine **(D,** blue, placebo; yellow, guanfacine; red, guanfacine – placebo**)**. Error bars represent 1 SEM. * $p < .05$.

Table 4. Peaks of Cue-Related BOLD Signal Differences for the Placebo – Guanfacine Contrast

Region	Side	BA	Cluster Size	Talairach Coordinates			*t*	*P*
				x	Y	Z		
Precentral Gyrus	R	4	106	4	-28	64	2.85	<.01
Temporal pole	L	21	381	-51	4	-29	3.96	<.01
Inferior temporal gyrus	L	20	383	-40	-30	-12	3.06	<.01
	R	37	234	40	-60	-4	4.68	<.001
Temporoparietal junction	R	40	101	44	-43	24	3.70	<.01
Amygdala	R		649	24	-6	-8	3.97	<.01
Parahippocampal gyrus	L	35/36	108	-28	-17	23	2.76	<.01
Occipital Lobe	R	19	127	30	-78	26	4.04	.001
	L	19	110	-12	-84	30	2.23	.01
Cerebellar Vermis	L		100	-2	-61	-9	3.45	.01

Table 5. Peaks of Cue-Relate BOLD Signal Differences for the Guanfacine - Placebo Contrast

Region	Side	BA	Cluster Size	Talairach Coordinates			*t*	*p*
				X	*y*	*Z*		
Ventromedial prefrontal cortex	L	9	282	-2	54	21	3.76	<.01
Dorsolateral prefrontal cortex	L	46	678	-26	36	13	4.07	.001
	R	46	206	36	40	15	3.05	<.01
Lateral Orbitofrontal cortex	R	47	164	20	42	-9	3.25	<.01
Dorsal premotor cortex	L	6	712	-59	-1	28	3.64	.001
Precentral Gyrus	R	4	374	12	-28	59	3.41	<.01
Post Central Gyrus	R	2	408	48	-19	16	3.82	.001
Anterior rostral cingulate zone	R	32	317	4	27	37	3.14	<.01
Posterior rostral cingulate zone	L	32	831	-4	10	42	4.09	<.001

Table 5 Continued. Peaks of BOLD Signal Differences for the Guanfacine - Placebo Contrast

Region	Side	BA	Cluster Size	Talairach Coordinates			*t*	*p*
				X	*y*	*Z*		
Retrospenial cingulate gyrus	R	29	903	2	-44	11	4.61	<.001
Temporoparietal junction	R	40	122	44	-31	31	4.20	<.001
Cerebellar vermis	L		282	-2	-55	-21	3.76	.001
Inferior cerebellum	R		579	26	-43	-37	4.62	<.001
Occipital Lobe	R	18	374	28	-93	0	3.41	<.01

Guanfacine-induced increases in cue-related BOLD signal change compared to placebo were observed in additional prefrontal regions, including left ventromedial prefrontal cortex (area 9), right lateral orbitofrontal cortex (area 47) extending into BA 10/11 (Figure 8).

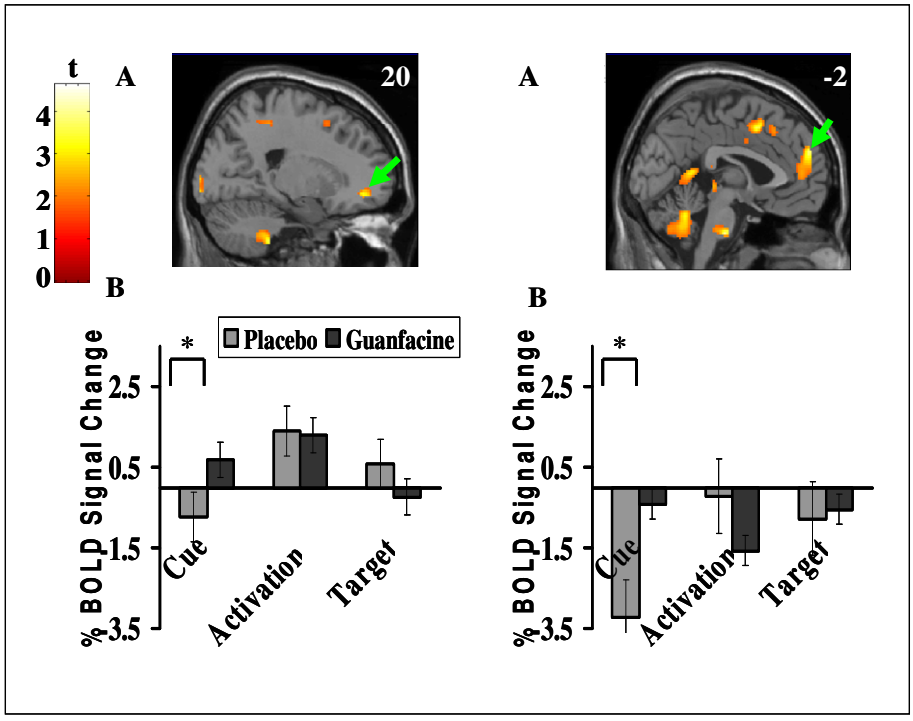

Figure 8: Cue-related BOLD signal increases with guanfacine compared to placebo in right lateral orbitofrontal cortex (BA 47) (**left panel, A, B**), and left ventromedial prefrontal cortex (BA9) (**right panel, A, B**). Error bars represent 1 SEM. * $p < .05$.

In cingulate cortex, significantly greater cue-related activation was also observed in RCZp for guanfacine compared to placebo (Figure 9). Post-hoc t-tests revealed that this result was a difference in signal magnitude in a cluster activated by both treatments in left RCZp, $t(15) = -2.23$, $p < .05$, as well as a difference in the extent of activation, such that with guanfacine there were greater BOLD signal changes in adjacent voxels that were not activated by placebo $t(15) = -2.53$, $p < .05$. The mean volume of cue-

related activation in bilateral RCZp was greater for guanfacine than placebo (482.88 ± 1053.58 mm^3 versus 2299.00 ± 450.40 mm^3), and this increase in extent accounted for the cluster of greater activation in RCZa for guanfacine.

Cue-related activation changes extended to the left PMd (area 6) and primary motor areas in right precentral (area 4) and postcentral gyrus (area 2), and were not seen in response to visual stimulation or targets ($p > .10$).

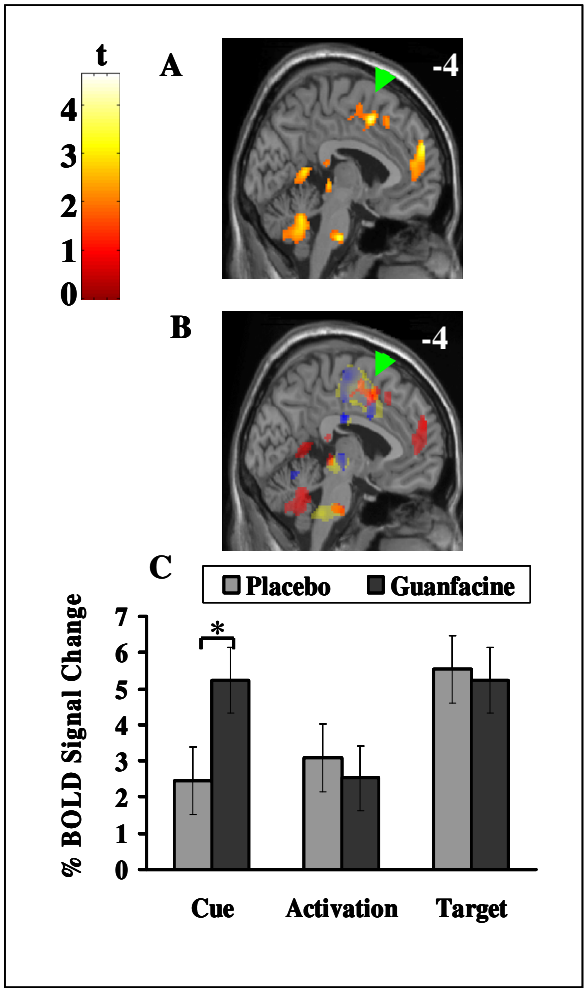

Figure 9. Cue-related BOLD signal increases in magnitude **(A)** and extent **(B)** of activation in RCZp with guanfacine. There was a cluster within RCZp of increased magnitude **(A)** and extent **(B,** blue, placebo; yellow, guanfacine; red, guanfacine – placebo) of activation compared to placebo. Differences in BOLD signal were only observed in response to the cue **(C).** Error bars represent 1 SEM. * $p < .05$.

Guanfacine induced increased and decreased cue-related activation in distinct clusters of right TPJ. Specifically, compared to placebo, guanfacine increased activation in a cluster situated along the anterior bank of the lateral fissure, and decreased activation in a cluster located along the posterior bank of the lateral fissure (Figure 10). There was no difference in the volume of activation for the two treatments, t (15) = -1.41, p > .10, (902.76 ± 162.12 mm^3 versus 654.62 ± 234.56 mm^3). Significant treatment effects were not observed for visual or target related activation (all *p's* > .10).

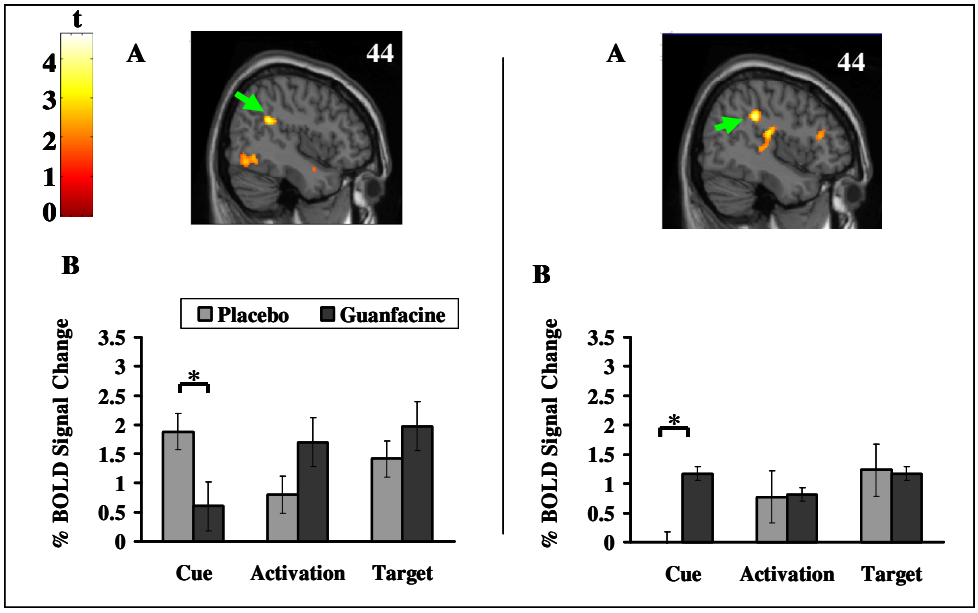

Figure 10: Guanfacine induced cue-related BOLD signal increases and decreases in temproparietal junction. Contrasting cue-related increases in BOLD signal with placebo compared to guanfacine in the right temporoparietal junction. Compared to placebo, guanfacine induced distinct clusters of cue-related BOLD signal decreases **(Left panel, A and B)** and increases **(Right panel, A and B)** in the right temporoparietal junction (TPJ). Error bars represent 1 SEM. * p < .05.

Guanfacine also had dissimilar effects on superior and inferior regions of the occipital cortex. Compared to placebo, guanfacine decreased cue-related activation in right superior occipital gyrus (BA 18/19), and increased cue-related activity in right inferior occipital gyrus (Figure 11).

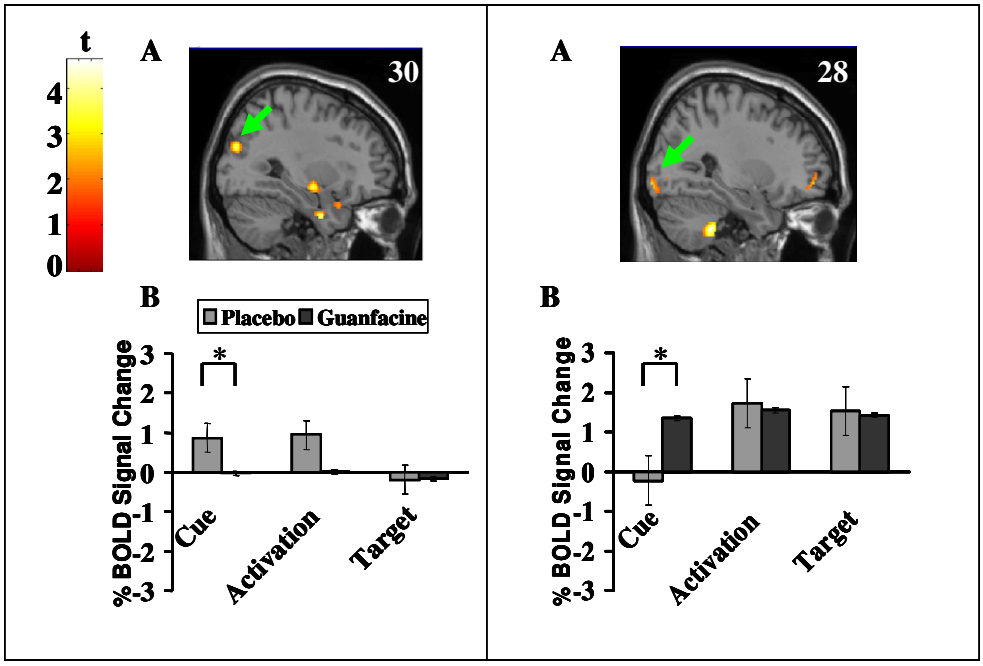

Figure 11: Guanfacine induced cue-related BOLD signal decreases **(left panel, A and B)** and increases **(right panel, A and B)** in the right occipital lobe. Error bars represent 1 SEM. * $p < .05$.

Contrasting effects of guanfacine on cue-related BOLD signal change in distinct regions of the cerebellum were also observed. Superior left cerebellar vermis demonstrated decreased cue-related activity with guanfacine compared to placebo, while right ansiform lobule demonstrated increased cue-related activity with guanfacine compared to placebo (Figure 12). Post-hoc *t*-tests revealed that guanfacine also decreased target-related activation in the superior vermis ($p < .05$). Cue-related activity for the two treatments did not overlap in the vermis (all $p > .10$). There was also a large

cluster of significantly greater activation for guanfacine than placebo in the inferolateral hemisphere of the right cerebellum, which primarily reflected greater BOLD signal magnitude increases for guanfacine than placebo in clusters that were activated by both treatments ($p = .02$). The volume of activation in the bilateral cerebellum did not differ for guanfacine and placebo (2,940 ± 325 mm^3 versus 2,968 ± 507 mm^3; $p > .10$).

Lastly, areas outside of the alerting network that demonstrated decreases in cue-related activation during the guanfacine condition compared to the placebo condition included left-lateralized temporal areas (BA 20, 21) and right inferior temporal gyrus (BA 37), left parahippocampal gyrus (BA 35/36) extending into the hippocampus, and the right amygdalahippocampal region extending into the temporal cortex (Figure 13).

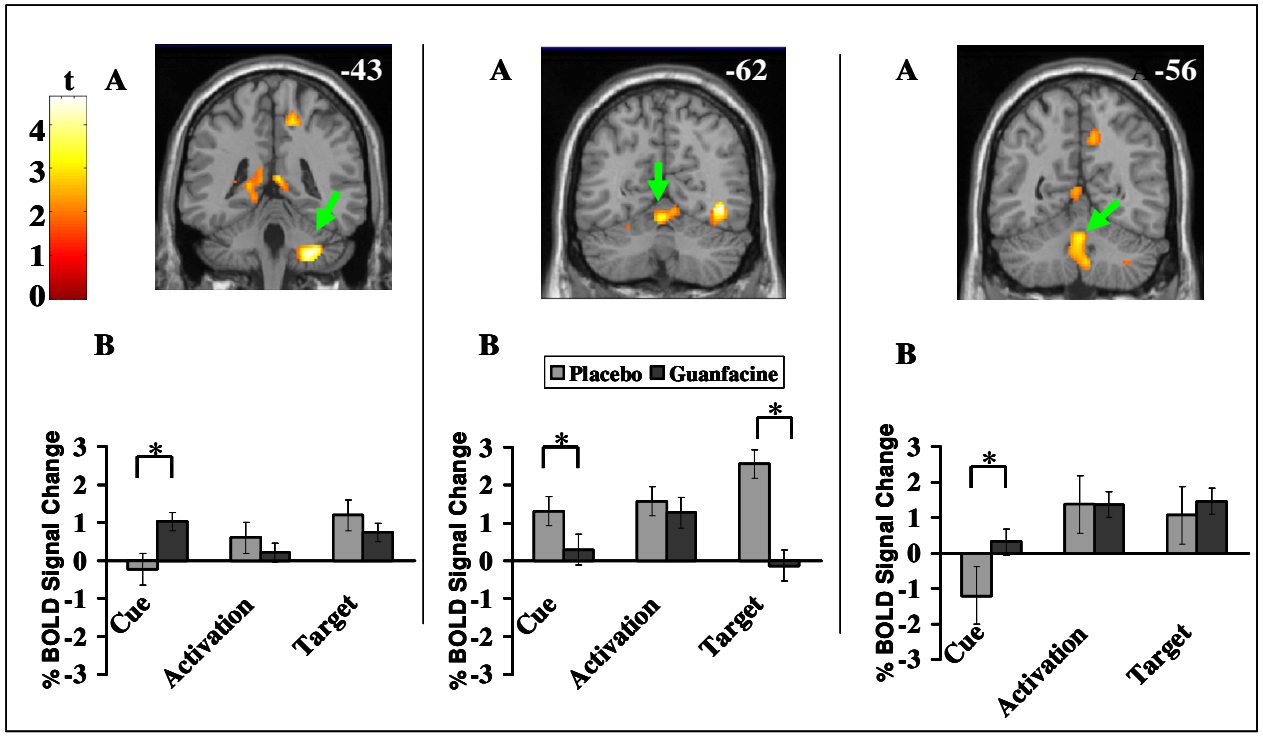

Figure 12. Guanfacine induced Cue-related BOLD signal changes in the cerebellum. Guanfacine induced greater cue-related activation in right inferior cerebellum compared to placebo **(left panel, A and B)**. Guanfacine induced distinct clusters of cue-related BOLD signal decreases in left cerebellar vermis **(middle panel, A and B)** and increases in the right posterior cerebellum **(right panel, A and B)**. Error bars represent 1 SEM. * $p < .05$.

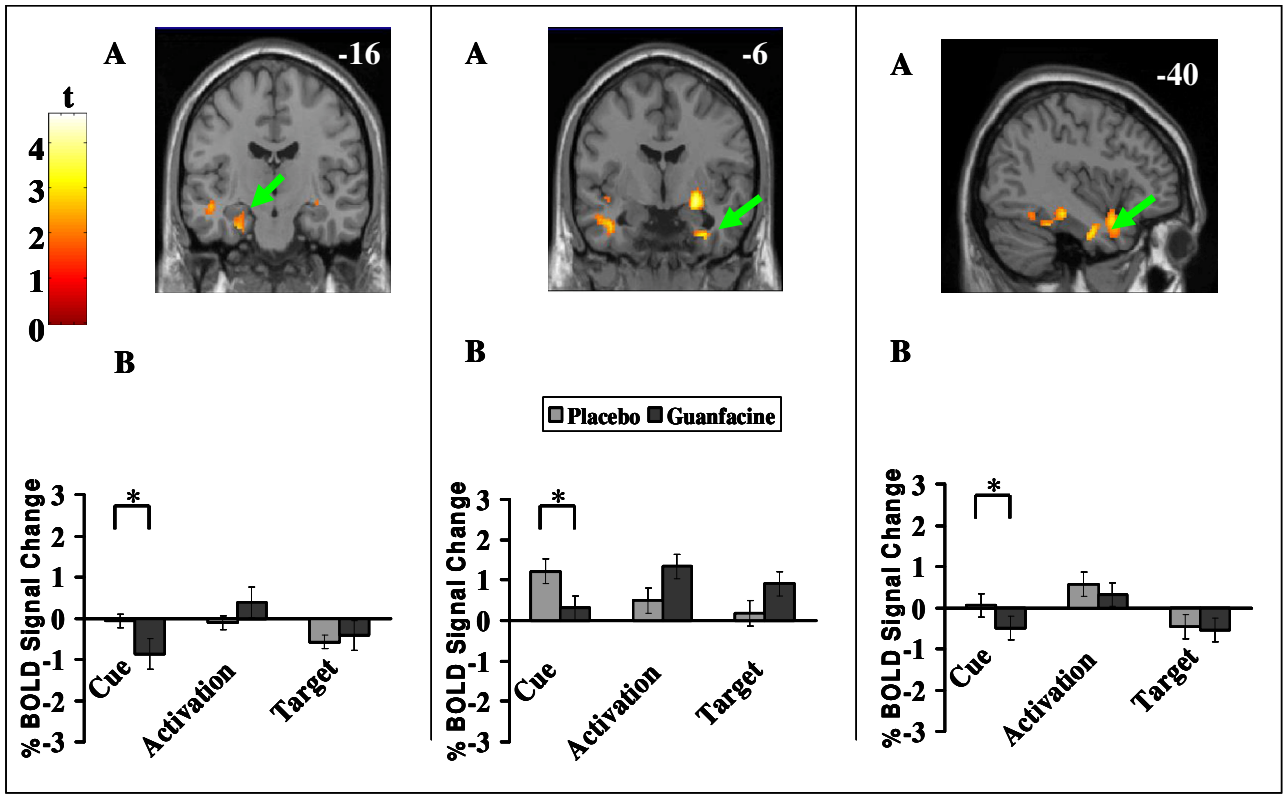

Figure 13. Guanfacine induced BOLD signal changes in the amygdalohippocampal region. Guanfacine induced distinct clusters of cue-related BOLD signal decreases in left parahippocampal gyrus (**left panel, A and B**), right amygdalohippocampal region **middle panel, A and B**), and left temporal cortex (BA 20/21, **right panel, A and B**). **Error bars represent 1 SEM.** * $p < .05$.

Discussion

The current study was an attempt to delineate the role of α_{2a}-adrenoceptors in the process of alerting through use of fMRI and a 1 mg guanfacine challenge. Three competing hypotheses were tested, specifically, a decrease in the alerting effect via α_2-adrenoceptors inhibition of NA release from the LC; a facilitation of alerting through post-synaptic α_{2a}-adrenoceptors binding in the PFC; and finally, a general sedative effect of guanfacine. None of these hypotheses were completely supported by behavioral and fMRI data.

Physiological and Behavioral Data

Blood Pressure and Heart Rate Measurements: Consistent with prior research (Coull et al., 2001) there were mixed effects of guanfacine on blood pressure. There were no significant effects of guanfacine on diastolic blood pressure; however we did find decreases in systolic blood pressure and heart rate (BPM) over time, which differed according to treatment. Mean systolic blood pressure was higher prior to the scans for guanfacine than for placebo. Systolic blood pressure tended to increase post-scan for placebo and decrease post-scan for guanfacine. Similarly, there was an interaction between BPM and Treatment, as well as a main effect of Time, such that BPM decrease for both placebo and guanfacine, but significantly more for guanfacine. These physiological measures indicate that guanfacine might have had a depressant effect on blood pressure and heart rate as was expected.

The Stay Alert Task and the Behavioral Alerting Effect: The alerting effect was defined as the difference between reaction time to cued targets minus reaction time to uncued targets. The Stay Alert task was successful in measuring the alerting across both

treatment conditions. A robust alerting effect was observed following placebo and guanfacine, such that reaction times to cued targets were consistently shorter than reaction times to uncued targets. Contrary to our hypotheses, reaction time to cued and uncued targets were unaffected by guanfacine. Reaction time standard deviation, which is thought of as an index of arousal and activation (O'Connell, Bellgrove, Dockree, Lau, Fitzgerald, & Robertson, 2008), was also unaffected by guanfacine administration.

There is only one available report of guanfacine effects on reaction time to cued targets. Witte & Marrocco (1997) reported a decrease in the alerting effect following guanfacine administration compared to saline in rhesus monkeys. Guanfacine increased reaction times to cued targets, suggesting that guanfacine decreased cue utilization in preparation for impending targets. However, it is difficult to generalize the results of this study since the sample consisted of only three subjects. Further, there are no reports of behavioral effects of guanfacine on human alerting (Coull et al., 2001). The tasks used to test the alerting effect involve relatively simple perceptual and motor responses, for which there might not be much room for improvement in healthy adults. It is possible that behavioral performance in the current study and other studies of alerting reflect a ceiling effect. It is also possible that no study to date has utilized the optimal dose of guanfacine to illicit an impact on reaction time without sedation confounding the results. A dose-response curve would be useful in determining the dose at which behavioral effects a likely without confounding sedative effects.

Sedative Effects of Guanfacine: Based on our physiological, behavioral, and neuroimaging results, it is unlikely that the 1 mg guanfacine challenge produced a general sedative effect. We did not observe a non-selective increase in reaction time with

guanfacine compared to placebo. In contrast to the less selective α_{2a}-adrenoceptor clonidine, which most likely exerts sedating effects through actions at α_{2b}-adrenoceptors (Buzsaki, Kennedy, Solt, & Ziegler, 1991) and hypotensive effects through imidazoline receptors (Ernsberger, Giuliano, Willette, & Reis, 1990), guanfacine has much lower affinity for these receptor types, and is therefore much less sedating (Arnsten et al., 1998). Guanfacine is also10 times weaker in lowering blood pressure than clonidine (Fischetti et al, 1994; Sorkin & Heel, 1986). Further, neuroimaging results discussed below do not indicate a global decrease in BOLD signal, which would be expected had guanfacine induced global changes in vasculature.

Neuroimaging

The Stay Alert Task and Alerting: The Stay Alert task was successful in activating a thalamo-cortical-striatal network previously associated with alerting and response anticipation (Fan et al., 2007; Posner & Petersen, 1990; Theil, 2007). Similar changes in BOLD signal were observed for both placebo and guanfacine in cortical and subcortical components of the alerting network (Posner & Petersen, 1990; Fan et al., 2005; Fan et al., 2007), which speak to the reliability of the Stay Alert task as a neuroimaging paradigm. In addition, direct comparison of the guanfacine and placebo conditions revealed treatment-specific increases in areas of the alerting network with guanfacine, including DLPFC and RCZp, and decreases in regions not commonly associated with alerting, but containing α_{2a}-adrenoceptors, including the amygdalohippocampal region, and temporal lobe.

The Prefrontal Cortex and Anterior Cingulate Cortex: Significant cue-related activation was observed for both placebo and guanfacine in DLPFC, VMPFC, and PMd,

as well as RCZp. Notably, activation in DLPFC and RCZp was augmented with guanfacine. In DLPFC, there was no significant difference in magnitude of activation in clusters that were commonly activated by both treatments, but there was increased extent of activation with guanfacine. Similarly, both guanfacine and placebo induced cue-related BOLD signal changes in RCZp. However, compared to placebo, the magnitude of activation within the RCZp cluster was increased with guanfacine. In addition, there was a greater extent of activation in RCZp that extended to PMd and RZCa with guanfacine compared to placebo.

Both DLPFC and RCZp are dense with post-synaptic α_{2a}-adrenoceptors. In recent years, the intracellular mechanisms by which α_{2a}-adrenoceptors facilitate efficient processing in PFC microcircuits have been delineated (Wang et al., 2007). α_{2a}-adrenoceptors are colocalized with HCN1 or HCN1/HCN2 heteromers on spines of distal pyramidal cell dendrites in primate PFC (Wang et al, 2007). Pyramidal cells have a negative resting potential (~ -65 mv), and HCN channels are thought to remain open in the presence of cAMP (Nolan et al., 2004). Blockade of HCN channels, stimulation of α_{2a}-adrenoceptors, or inhibition of cAMP all increase delay-related firing of PFC pyramidal neurons (Wang et al., 2007). When guanfacine binds to α_{2a}-adrenoceptors, cAMP production is reduced and HCN channels are closed, thereby strengthening PFC circuits. Although the relationship between neural processes and the BOLD signal remains unclear, recent evidence suggests that the BOLD signal reflects perisynaptic activity and intracortical processing (Logothetis, 2007; Viswanathan & Freeman, 2007).

Therefore, it is possible that guanfacine-induced increases in activation in DLPFC were indicative of changes in α_{2a}-cAMP-HCN signaling and increased efficiency of local

circuit processing. Subsequently, increases in extent of activation in DLPFC might be attributed to guanfacine-induced activation in clusters that were quiescent with placebo. Functionally, this augmentation of activation might facilitate response anticipation in a manner similar to PFC facilitation of working memory. Following a cue, the PFC works to hold information on-line, including information about proper responding and whether or not a response should be made. The results of this decision process provide a means of top-down control of the alerting network through direct connection with LC (Aston-Jones et al., 2000).

RCZp also provides top-down control of the LC. Top down projections, in addition to connections premotor and primary motor areas of the frontal cortex, allow RCZp to play a role in higher-order integration of cognitive and motor systems (Isomura, Ito, Akazawa, Nambu, & Takata, 2003). RCZp activation has been associated with response anticipation (Fan et al., 2007), task set maintenance and implementation (Petit, Courtney, Ungerleider, & Haxby, 1998; Quintana, Wong, Ortiz-Portillo, Marder, & Mazziotta, 2004), motor planning (Deiber, Honda, Ibenez, Sabato, & Hallet, 1999), and response selection (Pickard & Strick, 1997).

In summary, the LC receives dense innervation from DLPFC and cingulate cortex (Arnsten & Goldman-Rakic, 1984; Aston-Jones et al., 2000). Both regions monitor task performance and response utility, and ultimately drive LC discharge to increase or decrease the probability of response generation (Aston-Jones et al., 2000). Guanfacine-induced increases in magnitude and/or extent of cue-related BOLD-signal changes compared to placebo might reflect changes in α_{2a}-cAMP-HCN signaling that increased

efficiency of processing in local microcircuits and produced activation in clusters that previously did not reach significance.

The Temporoparietal and Amygdalohippocampal Region: Cue-related activation in right TPJ and IPS was observed for both guanfacine and placebo. Regions of the temporal and parietal lobes are integral in the allocation of attention (Posner & Petersen, 1990). The TPJ and areas along the intraparietal sulcus (IPS), which separates the superior and inferior parietal lobules, have also been implicated in cue utilization in alerting and orienting paradigms (Coull et al., 2001; Theil & Fink, 2007). It has been suggested that regions along IPS help prepare and guide actions in response to cues (Snyder, Batista, & Anderson, 1997), and are involved in preparation for finger movements (Adam et al., 2003).

Direct comparison of treatment effects indicated that guanfacine increased activation in a small cluster on the anterior bank of the lateral fissure and decreased activation in a small cluster on the posterior bank of the lateral fissure. Available literature does not indicate an abundance of α_{2a}-adrenoceptors in the TPJ region. Changes in top-down control of this region might have induced changes in the activation pattern.

Decreased activation with guanfacine compared to placebo was observed in regions of the left temporal lobe, including the temporal pole (BA 21), inferior temporal gyrus (BA20), and parahippocampal gyrus extending into the hippocampus. We also observed decreased activation with guanfacine in right-lateralized temporal lobe, parahippocampal gyrus and hippocampus, as well as amygdala. Air cavities in the amygdalohippocampal region can lead to distortion, which might account for activation in this region appearing lateral and superior to the amygdala. Decreased activation in

temporal areas could presynaptic α_{2a}-adrenoceptor binding in the amygdalohippocampal region.

The amygdalohippocampal region is rich in α_{2a}-adrenoceptors, and NA has been implicated in kindling in the amygdala (Corcoran & Mason, 1980) and the hippocampus (Barry, Wanschner, Kragh, Bolwig, Kokaia, Brundin, Bjorklund, Lindvall, 1989). Both endogenous NA and α_2-adrenoceptor agonist, clonidine, reduce excitatory post-synaptic potentials in the hippocampus (Curet & de Montingny, 1988a, b). Agonists, such as clonidine exert their effects via presynaptic α_{2a}-adrenoceptor binding (Curet & de Montigny, 1988a), which leads to inhibition of voltage-gated Ca^{2+} currents at the somata of glutamatergic neurons (Boehm, 1999). Agonist-induced decreases in hippocampal activity might provide the mechanism by which guanfacine can impair spatial reference memory (Sirvio, Riekkinen, Vajanto, Koivisto, & Riekinen, 1991). Alternatively, during performance of a cued-reaction time task, where there is little demand on hippocampal memory functions, α_2-adrenoceptor agonists might prime appropriate circuits to respond to task demands by decreasing activity in non-essential amygdalohippocampal regions, while facilitating neural processes in areas that serve more essential functions (e.g., DLPFC).

Differences in guanfacine effects in temporal regions might be attributable to noradrenergic modulation of anterior and posterior attention systems. While α_2-adrenoceptor binding has been shown to enhance PFC function, it can impair functions of posterior cortical areas, including temporal regions, and regions involved in stress responses, such as the amygdala. For example, in situations of low to moderate stress, moderate levels of NA transmission favors top-down decision-making and attentional

control through α_{2a}-adrenoceptor binding in regions such as DLPFC, allowing for thoughtful and controlled actions. On the contrary, in situations of high stress, or danger, when bottom-up processing of relevant stimuli and more reflexive responses might have survival value, higher levels of NA transmission tend to interrupt PFC processing through α1-adrenoceptor binding, while processing in the hypothalamus, amygdala, and posterior cortex is enhanced via NA binding to α1-adrenoceptors (Ramos & Arnsten, 2007). Therefore, these results support a modulatory role of NA in cognitive processes.

The Thalamus and Striatum: The thalamus, receives dense input from the reticular core, particularly through the intralaminar and midline nuclei (Van der Werf, Witter, & Goenewegen, 2002). The thalamus has long been linked to general arousal levels and levels of wakefulness (Sturm et al., 1999) and the importance of its cortical projections in the maintenance of vigilance has also been recognized (Paus et al, 1997). Given the strong connections from LC to thalamus (Steriade & Glenn, 1982), we hypothesized that changes in activation in the thalamus would be indicative of changes in presynaptic noradrenergic release in the LC. If guanfacine impacted the alerting network through presynaptic α_{2a}-adrenoceptors, we predicted a decrease in NA tone through out the LC-NA system, which would be observable in decrease activation of thalamus in response to cues.

Consistent with previous research (Coull, et al., 2001), we did not find differences in thalamic activation in response to cues as a function of guanfacine. In contrast, the less selective α_{2a}-adrenoceptor agonist, clonidine has been shown to decrease thalamic activation in response to cues (Coull et al., 1997). There are at least two possible neurochemical explanations for these results: i) guanfacine is 10 times less potent than

clonidine in reducing presynaptic NA release in LC or inhibiting LC firing (Engberg & Eriksson, 1991); ii) post-synaptic effects of guanfacine in thalamus are also unlikely, due to an absence of α_{2a}-adrenoceptors in human thalamus (Pascual et al., 1992), while clonidine also demonstrates high affinity α_{2b}-adrenoceptors receptors, which are prominent within human thalamus.

We did not have specific hypotheses about striatal activation. The striatum acts in motor preparation through its connections with cortical motor areas (Middleton & Strick, 2002). There was significant cue-related activation in putamen for placebo and guanfacine, with no differences between the treatment conditions. The putamen lacks α_{2a}-adrenoceptors (Berridge & Waterhouse, 2003); therefore direct effects at the putamen were not likely to be demonstrated with the guanfacine challenge. However, alteration of connections between putamen and other areas, such as PFC, due to guanfacine administration cannot be ruled out. Further analyses exploring functional connectivity would be necessary to determine such effects.

The Occipital Lobe: Compared to placebo, guanfacine-induced increased activation in inferior occipital gyrus, and decreased activation in superior occipital gyrus. Prior reports indicated cue-related BOLD-signal increases in occipital regions (Theil et al., 2004; Coull et al, 2001). Top-down processes are thought to modulate stimulus representations in visual cortex (Desimone & Duncan, 1995), providing attentional biases that facilitate preparation for behavioral responses to impending targets. It is possible that the cue-related changes found in the current study were mediated by such changes in top-down control.

The Cerebellum: Traditionally, the cerebellum has been viewed as a center for motor control (Courchesne & Allen. 1997); however, evidence is mounting for a role of the cerebellum in attention (Allen et al., 1997), and learning associations between stimuli and subsequent events (Courchesne & Allen. 1997). Functional connections between the cerebellum and the brainstem (Moruzzi & Magoun, 1949) and cortical areas, whereby the cerebellum modulates activity of reticular nuclei and EEG (Siegel & Wepsic, 1974) have led theorists to posit a general preparatory function of the cerebellum that sets the stage for appropriate behavioral responding (Courchesne & Allen, 1997). The importance of NE and α-adrenoceptors to cerebellar development and function has been demonstrated by studies of brain development in animals (Podkletnova & Algo, 1998; Sievers & Klemm, 1982) and pathological conditions in humans (Courchesne, 1997).

The cerebellum has reciprocal connections with the LC. Activation of the cerebellum in advance of sensory information is followed by increased signal-to-noise ratio in the brainstem, thalamus, and cortical areas (Crispino & Billock, 1984). The LC-NA system innervates all layers of the cerebellar cortex (Moore & Card, 1984) and is the only source of cerebellar NA afferents. Further, α_{2a}-adrenoceptors are abundant in cerebellar cortex, where they serve as post-synaptic heteroceptors on non-catecholaminergic cells (Aoki, Go, Venkatesan, & Kurose, 1998; Glass Huang, Aicher, Milner, & Pickel, 2001) and possibly as presynaptic receptors on GABAergic and/or Glutamatergic axon terminals (Schambra et a, Mackensen, Staffod-Smith, Haines, & Schqinn, 2005).

Mossy fibers and climbing fibers are the major afferents to the cerebellar cortex (Haines, 2002). Mossy and climbing fibers transmit timing and error information through

glutamate signals that are modulated by GABAergic interneurons. The major cell types of the cerebellar cortex include granule, Golgi, basket, stellate, and Purkinje cells. Granule cells are excitatory neurons, which receive excitatory input from mossy fibers. Granule cells bifurcate to form parallel fibers and synapse with Purkinje, Golgi, basket and stellate cell dendrites. Golgi cells are inhibitory interneurons, which extend through all layers of the cerebellar cortex and receive excitatory input from mossy, parallel, and climbing fibers. Purkinje cells also inhibit Golgi cells. Granule and golgi cells are reciprocally innervated and the strength of their synchronous firing depends on the strength of mossy fiber stimulation, and might be modulated by α_{2a}-adrenoceptors.

Both guanfacine and placebo activated regions of the cerebellar cortex. However, guanfacine induced significantly greater cue-related BOLD signal changes in right inferior cerebellum. In human cerebellum, α_{2a}-adrenoceptors are densely expressed on Purkinje, golgi, and stellate cells, and moderately expressed on basket cells (Schambra et al., 2005). NA enhances signal-to-noise ratio of glutamatergic and GABAergic synapses of cerebellar interneurons and depletion of NE impairs motor performance (Watson & McElligot, 1984). Stimulation of LC or iontophoretic application of NA at low concentrations in rat increases spontaneous Purkinje cell firing through β-adrenoceptor mechanisms. In contrast, moderate concentrations of iontophoretic application of NA activate α_{2a}-adrenoceptors, and increase the frequency of spontaneous firing of stellate cells, which depresses spontaneous Purkinje cell firing, (Basile & Dunwiddle, 1984; Mori-Okamoro & Tatsuno, 1998; Kondo & Marty, 1988). NA can also potentiate inhibitory activity of stellate and basket cells (Llano & Gerschenfeld, 1983; Saitow & Konishi, 2000).

With both guanfacine and placebo, bilateral areas of cerebellar cortex exhibited activation in response to cues. However, a region of left cerebellar cortex demonstrated increased cue-related activation with guanfacine compared to placebo. This is possibly due to NA effects at α_{2a}-adrenoceptors on interneurons. As described, at proper concentrations, NA can bind to α_{2a}-adrenoceptors on stellate cells, which inhibit Purkinje cell inhibition of deep cerebellar nuclei, which in turn have connections with premotor cortical regions. An anterior and superior region of the cerebellar vermis demonstrated decreased activation with guanfacine compared to placebo. In contrast, a cluster situated over the ansiform lobule demonstrated increased activation with guanfacine. A possible explanation lies in the distribution of α_{2c}-adrenoceptors in the cerebellar vermis. Inhibitory α_{2c}-adrenoceptors and α_{2a}-adrenoceptors are located on Purkinje cells in the anterior lobe vermis, but α_{2c}-adrenoceptors are absent in the ansiform lobule (Schambra et al., 2005). Guanfacine actions at these α_{2c} adrenoceptors may have caused the pronounced inhibition of both cue- and target-related activation in the superior vermis (Basile and Dunwiddie, 1984).

Neuronal Effects in the Absence of Behavioral Effects:

While guanfacine produced robust increases and decreases in brain regions, we did not observe changes in behavior as a result of guanfacine administration. These results are consistent with many other pharmacological challenge studies (Coull et al., 1997, 1999; Williams & Goldman-Rakic, 1995). A lack of change in behavioral measures of alerting and arousal/activation might be indicative of guanfacine's greater impact on prefrontal regions of the brain compared to brain stem nuclei. Presynaptic effects of guanfacine were hypothesized to decrease in NA throughout the brain via autoreceptor

inhibition of LC firing and was expected to impart either increased RT to cued target (indicative of decreased cue utilization) or a nonselective decrease on RT (general sedative effect). Our results do not support such effects. Rather, guanfacine had effects in prefrontal regions without behavioral effects. While it might be possible to increase RT to cued stimuli through sedative effects, it might be very difficult to decrease RT to cued stimuli, given the simplicity of the task.

Some have theorized that not only can pharmacological challenges induce differences in brain function that enable increased efficiency of processing without changing behavior, but in some circumstances it might be preferential to utilize a task that does not generate behavioral difference across conditions (Price & Friston, 1999). Behavioral differences between experimental groups can make it difficult to determine if changes in brain activation are a reflection of the change in behavior, rather than a change in cognitive processes. Others argue that neuroimaging data might be more informative than behavioral data because they provide information that would not necessarily be evident in behavioral data (Fink et al., 2002; Wilkinson & Halligan, 2004).

Changes in BOLD signal in the absence of changes in behavior can be indicative of different processes (Price & Friston, 1999). For example, a non-dominant system might begin to take over or facilitate the functioning of a dominant system. These changes in neuronal function can occur without noticeable changes in behavioral measures, and therefore uncover adaptive neuronal mechanisms. Neuronal changes can also reflect a change cognitive strategy that can drive differences in neuronal activity while preserving behavioral performance. Alternatively, a change in neuronal implementation, leading to changes in connectivity between regions, can occur, and may

be reflected in a greater extent of activation in regions known to subserve the behavior in question.

It is difficult to know which of the aforementioned alternatives account for the results in the current study. The task employed required relatively simple perceptual and motor responses; therefore, a change in cognitive strategy might not be likely. More likely are changes in neuronal activity that reflect compensatory responses or changes in top-down control from frontal brain regions. Future analyses, discussed below can explore these options.

Clinical Implications:

A selective pharmacological profile and low incidence of side affects makes guanfacine a preferred substance for treatment in psychological disorders compared to less selective α_2-adrenoceptor agonists such as clonidine. Guanfacine has previously demonstrated strong affinity for α_{2a}-adrenoceptors in the PFC, and is therefore being explored as a treatment option for individuals who exhibit varying degrees of PFC dysfunction.

Attention deficit/hyperactivity disorder is one example of a psychological condition that is characterized by symptoms commonly associated with PFC dysfunction, including poor attention span, inhibitory control, working memory, set-shifting, and inappropriate social behavior. Current theory posits a role of the PFC in the development (Barkley, 1997) and/or recovery (Halperin & Schulz, 2006) from symptoms of ADHD. Lesion studies have provided strong evidence of PFC involvement in the symptoms of ADHD. Right inferior PFC is associated with the ability to withhold a prepotent response (Aron, Robbins, & Poldrack, 2004), dorsolateral PFC regulates divided attention and set-

shifting (Manes, Sahakian, Clark, Rogers, Antoun, Aitken, & Robbins, 2002), while ventromedial PFC regulates emotional responses and enables socially appropriate behavior (Anderson, Bechara, Damasio, Tranel, & Damasio, 1999; Price, Carmichael, & Drevets, 1996).

Traditional stimulant ADHD treatments have targeted the dopamine and noradrenergic systems (Arnsten, 2006). Considerable evidence suggests a role of NA in the pathophysiology of ADHD (Biederman & Spencer, 1999). Proper levels of NA within PFC are critical to working memory, inhibitory control, and social behavior. Blockage of α_{2a}-adrenoceptors in PFC reproduces symptoms of ADHD, including poor impulse control (Ma, Qi, Peng, & Li, 2003), hyperactivity (Ma, Arnsten, & Li, 2005), and increased distractibility (Li & Mei, 1994). Immediate release guanfacine has been tested in ADHD patients, and has been linked to a decrease commission and omission errors on Continuous Performance Tests (Chappell, Riddle, Scahill, Lynchm Schultz, Arnsten, Leckman, & Cohen, 1995; Scahill, Chappell, Kim, Schultz, Katsovich, Shepherd, Arnsten, Cohen, & Lechman, 2001), produce significant improvements in ratings of inattention and hyperactivity (Scahill, 2001), and improvements in tic severity (Scahill et al., 2001). We have demonstrated that guanfacine acts in critical PFC regions to process relevant cues and enact appropriate behavioral responses. Guanfacine's actions at multiple PFC regions to enhance cognitive and behavioral responding may prove especially helpful in the treatment of individuals with ADHD and comorbid disorders, such as tic disorders, anxiety disorders, and conduct disorder.

Limitations and Future Directions:

There are several methodological limitations of the current study. First, it was difficult to properly assess blood pressure and heart rate changes due to the lack of data collection immediately prior to the scan. We first collected these physiological measurements shortly after participants arrived, which was immediately before administering the treatment, and then again immediately following the scan. An additional measurement immediately before the scan may have clarified the effects of guanfacine on heart rate and blood pressure. Second, we did not collect any measures to systematically assess perceived sedation. Participants were informally asked whether they felt tired after each scan. This informal survey revealed that most participants felt equally tired following each scan, and did not report being able to decipher which treatment they had received.

This study also utilized a single low dose of guanfacine. Based on prior use of guanfacine in a challenge study (Coull, 2001), as well as the Physician's Desk Reference, we chose a 1 mg dose to decrease the likelihood of sedative effects. However, a higher does might be necessary to induce behavioral effects of guanfacine. In addition, guanfacine might have different effects on attention systems with repeated use over time. Further, while consistent with other imaging studies (Coull, 1997, 2001; Theil et al., 2004, Theil & Fink, 2007) our small sample size also decreases our power in behavioral analyses. Although our behavioral and imaging results suggest that the Stay Alert task is a reliable and valid paradigm for studying the alerting effect, reliability and validity analyses with a large normative sample have not been conducted for this task.

Finally, it is possible that guanfacine enhanced efficiency of neuronal processing through increased strength of connections between areas of the alerting network, while inhibiting other areas. Changes in connectivity have been found in response to clonidine (Coull et al., 2001). However, the analyses conducted in this study cannot address these possibilities. Future analysis can explore the impact of guanfacine on the functional connectivity of the alerting network. For example, Dynamic Causal Modeling (DCM, Friston, Harrison & Penny, 2003) is a method of analysis that can be used to explore the strength of connections between brain regions. DCM utilizes a dynamic input-state-output model, and assesses interactions between neuronal states of multiple interconnected regions (Fan, Hof, Guise, Fosella, & Posner, 2007). Such sophisticated analyses might elucidate how changes in prefrontal cue-related BOLD signal impact are related to BOLD signal changes in other areas of the brain (i.e., TPJ, occipital lobe, and amygdalohippocampal areas)

Conclusions:

Although none of our hypotheses were directly supported by the results, we observed many interesting findings. The Stay Alert task was useful in activating a fronto-striatal-parietal network previously associated with alerting (Coull et al., 2001; Fan et al., 2007; Posner & Petersen, 1990; Theil et al., 2007). Many of the areas thought to be critical to alerting (i.e., thalamus, PFC, TPJ, ACC) demonstrated significant activation with both placebo and guanfacine. This was the first study to demonstrate changes in cue-related BOLD signal following a guanfacine challenge compared to placebo. Perhaps we were able to uncover these guanfacine-induced changes due to our isolation of the alerting system in with the Stay Alert task; our comparison of activation

in response to cues versus distracters, rather than activation in response to cues versus activation in the absence of a stimuli prior to the target; or our statistical analyses, which explored whole-brain changes rather than limited region of interest analyses (Coull et al., 2001).

Most notable among our imaging results was the apparent guanfacine-induced augmentation of DLPFC and RCZp activation in response to cues. These responses are possibly a reflection of changes in local microcircuits that facilitate response preparation, and possibly provide top-down control of the alerting system. It also seems that regions not directly involved in the alerting system, such as temporal regions and the amygdalohippocampal region were impacted by guanfacine. This is consistent with the suggestion that noradrenaline has the ability to augment inhibitory as well as excitatory responses, as well as the hypothesis that when necessary, NA stimulation of PFC α_{2a}-adrenoceptors can enhance PFC functions while dampening less adaptive effects in regions associated with stress responses (e.g., amygdala) that can interfere with proper response selection. In the current study, guanfacine might have acted on frontal regions to increase efficiency of preparatory responding, while inhibiting regions that were not necessary to task performance. The ability of guanfacine to act on multiple brain regions to prime appropriate responding suggests further exploration of this medication in the treatment of psychological disorders with associated prefrontal symptoms.

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