NORADRENERGIC MODULATION OF FUNCTIONAL BRAIN NETWORKS UNDERLYING EXECUTIVE CONTROL

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ABSTRACT

Executive control, the goal directed deployment of cognitive resources, depends critically on a class of neurotransmitters called catecholamines. One such catecholamine, norepinephrine, is theorized to facilitate cognition through shaping patterns of synaptic communication within neural networks. Major questions remain, however, if and how norepinephrine regulates the network structure of the human brain. Two studies were conducted to investigate this question. The first study simultaneously measured brain activity with functional magnetic resonance imaging (fMRI) and pupil diameter (a proxy for norepinephrine signaling). This study found that fluctuations in pupil diameter were synchronous with spontaneous brain activity in many canonical brain networks. Furthermore, individuals less prone to distractibility in everyday behavior demonstrated stronger positive coupling between the cingulo-opercular network and pupil diameter. These results suggest that networks important to executive control likely do not act in isolation, but coordinate with the NE system in a behaviorally relevant manner. Study II investigated how potentiated NE signaling at alpha-2a receptors altered distributed brain networks when executive control was engaged. A NE alpha-2a receptor agonist, guanfacine, was administered and brain activity was measured with fMRI during two cognitive states: a cognitively unconstrained resting state and a working memory task. This study found that NE signaling at alpha-2a receptors during working memory modulated the strength of every functional network examined. Machine learning analyses utilizing FC patterns across the brain
predicted drug vs. placebo state with high accuracy (75%, p < .001) when working memory was engaged, but with only modest accuracy (60%, p = .13) during the resting state. Furthermore, FC changes during working memory were not specific to any one network. When SVM analyses were restricted to each functional network in isolation, each network was sufficient to predict drug state at levels above chance, but no network achieved accuracy as high as that with whole-brain FC. Collectively, these FC alterations increased the overall modularity of brain networks during working memory engagement, indicating greater network segregation. Together, these studies demonstrate that norepinephrine shapes the spatio-temporal structure of functional networks in the human brain, and suggest that this may be an important neural mechanism enabling executive control.
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CHAPTER I: GENERAL INTRODUCTION

Introduction

Understanding how distributed collections of neurons interact in a networked fashion is critical to elucidating the neural basis of behavior. In recent years, this endeavor has been greatly advanced by the ability to map large-scale networks in vivo in the human brain with a technique called functional connectivity magnetic resonance imaging (fcMRI). The concept behind fcMRI is simple—areas whose neural activities are highly correlated over time (termed “functional connectivity”) are thought to be interacting, either through mono- or poly-synaptic connections. While conceptually straightforward, this idea is validated by animal models (Schölvinck et al., 2010; Wang et al., 2012; Shen et al., 2015), and computational models suggesting that neural synchrony may be an important aspect of the neural code (Fries, 2005). By employing fcMRI in conjunction with mathematical methods such as independent component analyses (Beckmann et al., 2005) or graph-theory (Power et al., 2011), the functional anatomy of the brain can be decomposed into distributed networks (termed “functional networks”) whose components are highly functionally connected to themselves, and sparsely functionally connected to the rest of the brain.

Strikingly, functional networks resemble the spatial distribution of regions that often co-activate during common tasks (Smith et al., 2009; Gordon et al., 2012), and remain highly functionally connected even when someone is in a task-free “resting” state (Fox et al., 2005; De Luca et al., 2006). For instance, areas that co-activate during cognitive tasks (i.e. fronto-parietal or cingulo-operuclar regions) also remain highly functionally connected during rest and thus are said to form “intrinsic” functional networks termed the fronto-parietal and cingulo-operuclar
networks. These networks may also subserve discrete cognitive functions (Dosenbach et al., 2008), suggesting that networks uncovered with fcMRI are fundamental, functionally meaningful sub-units of the human brain.

Historically, uncovering inherent levels of brain organization such as cortical layers and columns, up through areas and systems (Churchland & Sejnowski, 1988; Felleman & Essen, 1991), permitted greater understanding of brain-behavior relationships. Similarly, the discovery of large-scale functional networks has enabled a better understanding of the circuits underlying processes as diverse as stress (Hermans et al., 2011), attention (Castellanos et al., 2008), and memory (Sami et al., 2014). Network level analyses are particularly advantageous to the study of complex behaviors, because these behaviors require integration of information that spans multiple cognitive domains and is distributed across cortical areas (Siegel et al., 2015).

Open questions regarding the neurobiological basis of functional networks

Despite recent breakthroughs, basic questions remain regarding the neurobiological basis of large-scale functional networks. The reliable spatial distribution of functional networks (Chen et al., 2008; Van Dijk et al., 2010) suggests that they may be based upon anatomical connections. This assertion is backed up by both human (Honey et al., 2007) and animal studies (Wang et al., 2013) demonstrating that resting state connectivity patterns and the distribution of anatomical connections are broadly consistent (although functional connections can be present in the absence of direct structural connections (Honey et al., 2007)). While the overall spatial pattern of functional networks may be stable, however, the strength of functional connections within these networks is highly dynamic at short time-scales (i.e., tens of seconds) (Chang and Glover, 2010; Hutchison et al., 2013). This dynamism is too rapid for the spatio-temporal structure of
functional networks to be explained by anatomy alone; it is improbable that new connections can form in the second to minute timeframe. Even within resting state periods, where participants are typically asked only to hold still, there are ebbs and flows in connectivity patterns that produce recurring, quasi-stable brain-state configurations (Allen et al., 2012). Rather than noise, these functional connectivity dynamics may be a core mechanism that allows the brain to adapt to changing task demands (Bassett et al., 2011; Braun et al., 2015). When participants are asked to cycle through various tasks like math, memory, and movie watching, their cognitive state is predictable with almost perfect accuracy on the basis of brain-wide functional connectivity patterns (Gonzalez-Castillo et al., 2015). Even more subtle variations in cognitive demands produce reliable, widespread shifts in functional connectivity (Cole et al., 2012; Braun et al., 2015). This highlights the distributed nature of cognitive processing. It also raises a critical question: how do anatomical connections that are largely static at short time scales give rise to varied and dynamic configurations of functional networks in the service of cognition?

The role of neuromodulation

The motivation of this dissertation capitalizes on the framework provided by animal models and receptor-level studies designed to answer analogous questions in small circuits. Current theoretical models propose that a class of neurotransmitters called neuromodulators quickly “reset” distributed cortical networks (Bouret & Sara, 2005; Bargmann & Marder, 2013). Rather than directly excite or inhibit their post-synaptic targets, neuromodulators often change synaptic efficacy, and thus regulate neural interaction patterns (Arnsten et al., 2010). Numerous findings bolster this concept; including that neuromodulation can functionally “silence” present anatomical connections, can alter intrinsic excitability, and can change the frequency and phase
relationship within entire functional circuits (Bargmann & Marder, 2013). Collectively, this suggests that anatomical connections provide a minimal structure, and neuromodulation creates functional circuits in the service of behavior (Marder, 2012). This dissertation aims to expand this literature based on small circuits, to also understand how neuromodulation impacts large-scale functional networks in the human brain.

**Neuromodulation and executive control**

In addition to regulating neural circuits, neuromodulation is also critical for executive control, a constellation of processes—including working memory, attentional or task-set switching, and inhibitory control—that support the goal-driven deployment of cognitive resources. Understanding the neural basis of executive control is important not only to the basic science of brain networks, but also because executive deficits are common to many psychiatric conditions (Raffard & Bayard, 2012; Rosenthal et al., 2013; Shanmugan et al., 2016) and are highly detrimental to adaptive function (Szatmari et al., 1989; Pugliese et al., 2016). In certain disorders, such as autism spectrum disorders, even when primary symptoms are ameliorated, executive deficits still persist and cause marked impairment (Troyb et al., 2014). Despite being a source of major behavioral impairment, there remains a dearth of effective treatment options for executive dysfunctions, further highlighting the importance of understanding their brain bases.

As with any cognitive process, executive control is likely subserved by numerous and distributed neurotransmitters acting in conjunction. In particular, however, the neuromodulatory influence of one class of neurotransmitters called catecholamines—which include dopamine (DA), epinephrine (E), and norepinephrine (NE)—are critical to the functioning of the networks linked to executive control. Indeed, depletion of catecholamines from the dorsolateral prefrontal
cortex (dIPFC) can be as detrimental to its function as lesions to the tissue itself (Brozoski et al., 1979). While DA’s role in executive control has been more extensively studied, recent work points to a distinct role of NE (Arnsten & Contant, 1992; Arnsten, 1997, 2011; Sara, 2009; Gamo et al., 2010; Enge et al., 2012). Varying levels of NE signaling from the brainstem locus coeruleus (LC), that occur across different behavioral states, can flexibly regulate executive circuits to bring them online and strengthen their top-down influence for goal-directed behavior, or to lessen their influence and facilitate a higher fidelity of bottom-up signals that promote task disengagement (Arnsten, 2009, 2011).

The best studied example of NE’s role in executive control comes from studies demonstrating NE’s effect on the capacity to temporarily store and manipulate information, termed working memory (Arnsten, 1997, 2011). Working memory is enabled by local dorsolateral prefrontal (dIPFC) networks that maintain mental information through recurrent activation (Goldman-Rakic, 1995; Wang et al., 2013). NE facilitates this process by acting at alpha-2a receptors to increase the local synaptic efficacy of dIPFC networks, and promotes their sustained activity during working memory (Arnsten, 2011). This has led to the use of noradrenergic agonists such as guanfacine for the treatment of disorders with executive deficits, such as attention deficit hyperactivity disorder.

An important theoretical advancement has been the recognition that NE does not just facilitate local processing; it is also thought to regulate the overall state of distributed cortical networks (Bouret & Sara, 2005; Corbetta et al., 2008; Hermans et al., 2011; Eldar et al., 2013; Schwarz et al., 2015). NE neurons project diffusely throughout most of the cortex (Schwarz et al., 2015) and are theorized to gate a broader network of connections in the service of cognition.
(Arnsten et al., 2010). This concurs with evidence that the NE-dependent executive control functions, such as working memory, require precise synchronization and processing across distributed networks (Palva et al., 2010; Salazar et al., 2012). While there is strong theoretical and experimental evidence to support the fact that NE exerts large-scale influence across cortical networks, major questions remain regarding how it regulates the network architecture of the human brain. These questions are a major barrier to understanding the neurobiological origin of large-scale functional networks and the behaviors they subserve, including executive control.

**Dissertation Goals**

This dissertation includes two studies investigating how NE modulates brain networks that are important for executive control. The first study tested for basic relationships between resting brain activity and the LC-NE system. It also tested if noradrenergic coordination of activity within functional networks relates to individual differences in inattentiveness, which is associated with executive dysfunction. While investigating human neurotransmitter function *in vivo* often requires more invasive procedures such as positron emission tomography, or pharmaco-neuroimaging, pupil diameter can non-invasively index LC-NE activity. There is a close link between non-luminance mediated changes in pupil diameter and the LC-NE system (Aston-Jones & Cohen, 2005; Murphy et al., 2014), which may be established through common brainstem efferents that influence both the LC and autonomic pupil control centers (Nieuwenhuis et al., 2011) or through a direct pathway (Samuels & Szabadi, 2008). This has been specifically corroborated in the context of resting-state activity, wherein spontaneous fluctuations in pupil diameter are reliably preceded by LC spiking activity (Joshi et al., 2016). Study I thus utilized concurrent pupillometry and resting state fMRI, as well as trait-level behavioral questionnaires to
address two important gaps in the literature. Firstly, a limited number of studies (Fan et al., 2012; Murphy et al., 2014; Yellin et al., 2015) have shown that functional networks may couple with psychophysiological measures – such as galvanic skin response and pupil diameter – but this fundamental relationship is not well validated. Study I is the largest, multi-site investigation to date validating this proposal. Secondly, it is unknown if noradrenergic coordination of functional network activity relates to executive control as demonstrated in everyday behavior (such as trait-level inattentiveness). Study I therefore also tested for relationships between pupil-brain coupling and individual differences in self-reported attentional abilities.

While Study I could make important strides towards delineating the spatial extent and behavioral relevance of LC-NE related networks, its reliance on pupil diameter precludes causal inference. Pupil diameter in constant luminance is sensitive to LC-NE signaling, but is likely not specific to it. Furthermore, Study I cannot disambiguate if pupil-brain correlations resulted from top-down regulation of the LC-NE system, or this system’s bottom up regulation of brain activity. Hence, to more causally demonstrate noradrenergic impacts on large-scale brain networks, Study II was a double-blind pharmaco-fMRI investigation utilizing the NE alpha-2a receptor agonist guanfacine, a pharmaceutical agent commonly used to treat attentional and executive dysfunction. Because brain-wide connectivity patterns (Gonzalez-Castillo et al., 2015; Siegel et al., 2015) and their collective effects on overall network topology (Stevens et al., 2012; Sadaghiani et al., 2015) are both thought to be important to cognition, we tested for distributed impact on functional connectivity and network topology. Furthermore, NE neurons’ projection patterns are highly consistent with a role in regulating overall brain state. A single NE neuron, originating in the LC, can integrate synaptic inputs from, and project back to, numerous brain
regions (Schwarz et al., 2015). Here we examined NE’s brain-wide impact during the resting state and a working memory task. We utilized machine learning and graph-theory techniques which are capable of meaningfully capturing distributed patterns of functional connectivity. Together, studies I and II provide convincing evidence that NE regulates the spatio-temporal structure of functional networks in the human brain, and suggest that this may be an important neural mechanism enabling executive control.
CHAPTER II: COUPLING BETWEEN SPONTANEOUS PUPILLARY FLUCTUATIONS AND BRAIN ACTIVITY RELATES TO INATTENTIVENESS

Introduction

Dynamic interactions between the central and autonomic nervous systems are hypothesized to be essential for attentional function. Salient environmental cues evoke a cascade of autonomic changes, including pupil dilation, increased skin conductance and changes in heart rate, which are posited to optimize adaptive behavior by facilitating response preparation (Nieuwenhuis et al., 2011). Pupil diameter, in particular, which is controlled by sympathetic and parasympathetic inputs (Loewenfeld, 1999), has been used to study diverse attentional processes (Geva et al., 2013; Unsworth & Robison, 2014). Select regions of the central nervous system such as the anterior cingulate cortex (ACC) are thought to regulate pupil-linked arousal systems to facilitate task performance. The ACC is part of a broader network called the cingulo-opercular network that is a convergent site of attentional and autonomic control (Menon & Uddin, 2010). This network is thought to modulate autonomic reactivity (Menon & Uddin, 2010) and alertness (Sadaghiani & D’Esposito, 2015) in response to attentionally demanding stimuli, perhaps by interfacing with systems indexed by the pupil such as the sympathetic nervous system (Beissner et al., 2013) and the locus-coruleus norepinephrine (LC-NE) system (Aston-Jones & Cohen, 2005). While previous studies have demonstrated that functional connectivity between cingulo-opercular regions relates to individual differences in attentional abilities (Vaidya & Gordon, 2013), it remains unclear if these regions’ coordination with autonomic/arousal systems also relates to individual differences in attentional abilities.
Coupling between central and autonomic systems appears to be maintained in the absence of a directed task, called the resting state. Both pupil diameter (Murphy et al., 2014; Yellin et al., 2015) and galvanic skin response (Fan et al., 2012) correlate with resting activity in brain regions associated with cognitive, attentional, and autonomic functions. Resting-state brain activity is organized into networks (De Luca et al., 2006) that recapitulate task-evoked neural activity, possibly reflecting a lifetime of co-activation (Dosenbach et al., 2007; Harmelech et al., 2013). Furthermore, the connectivity strength of specific networks (e.g., cingulo-opercular, fronto-parietal) predicts both task performance in the cognitive domain that the networks subserve (e.g., executive control) and properties of that domain in everyday life (e.g., trait-level inattentiveness) (Vaidya & Gordon, 2013). Therefore, if coupling between select central brain regions and lower autonomic systems is characteristic of adaptive attentional control, its strength may reflect the history of coordinated usage of central and autonomic systems. Because attentional abilities rely on adaptive regulation of autonomic activity, we predicted that they should relate to the strength of resting state coupling between central brain regions regulating attention and lower autonomic/arousal systems.

We tested this prediction by measuring functional coupling between pupil diameter and concurrent resting fMRI brain activity, and examining its relationship to behavioral traits symptomatic of attentional dysfunction. Since the pupil is controlled by both sympathetic and parasympathetic subdivisions of the autonomic nervous system (which may diverge in their relationships to both functional networks [Beissner et al., 2013] and to attentional function [Negrao et al., 2011]), we examined relationships with inattentiveness separately within brain regions relating to each autonomic subdivision.
Materials and Methods

Participants

Fifty-one healthy volunteers were drawn from two sites, Georgetown University (N = 23) and University of Pittsburgh (N = 28). Sixteen participants were excluded for quality control issues described later, leaving a final sample of 35 (10 males, 25 females; Age Mean ± SD = 29.2 ± 10.3). Participants were screened by self-report for the use of psychotropic medication, MRI contra-indications, and psychiatric or neurological disorders. The Georgetown sample (N = 20) completed the Adult ADHD Self-Report Scale v1.0 (ASRS) (Kessler et al., 2005) to provide a trait-level measure of attention (Inattention Scale mean = 12.3 ± 3.3; range = 8-21; Hyperactive/Impulsive Scale mean = 11.2 ± 4.9; range = 0-20); these data could not be collected on the Pittsburgh sample. The protocols and consent procedures were approved by the Georgetown University Institutional Review Board and the University of Pittsburgh Institutional Review Board; data were aggregated at the Georgetown site with no transfer of identifying information. All study procedures conformed to World Medical Association Declaration of Helsinki.

Data Acquisition

We combined data from the Pittsburgh and Georgetown sites to create a pupil-linked map delineating regions related to pupil diameter. All participants included in analyses of trait-level attention were scanned at Georgetown University with identical scanning parameters to ensure that these analyses were not biased by scanner type or scanning parameters. Participants were scanned while viewing a central fixation cue (black cross at Georgetown, red dot at Pittsburgh) on a gray background (E-Prime, Psychology Software Tools) while maintaining constant
luminance to eliminate pupillary light reflexes. Participants were told to relax, keep their eyes open, and fixate on the central cue.

The scans acquired at Georgetown University included 288 functional images acquired on a Siemens Trio 3T scanner (Erlangen, Germany) using a gradient echo pulse sequence with the parameters: 47 slices with 3.2 mm thickness, TR = 2500 ms, TE = 30 ms, 90° flip angle. The first two images were discarded to allow for signal stabilization. Further, a high-resolution $T_1$-weighted structural scan (magnetization prepared rapid gradient echo) was acquired with the parameters: 176 sagittal slices with 1.0 mm thickness, TR/TE = 1900/2.52 ms, TI = 900 ms, 9° flip angle. Pupil diameter was recorded continuously at 60 Hz with MR compatible goggles equipped with an integrated infrared camera over the right eye (Mag Design and Engineering) and ViewPoint EyeTracker® software (Arrington Research, Inc.).

The scans acquired at the University of Pittsburgh included 280 functional images acquired on either a Siemens Trio 3T scanner (Erlangen, Germany), or a Siemens Allegra 3T scanner (Erlangen, Germany). The scanning parameters were: 29 slices with 3.2 mm thickness, TR = 1500 ms, TE = 25 ms, 73° flip angle. The first two images were discarded to allow for signal stabilization. A high-resolution $T_1$-weighted structural scan was acquired with the parameters: either 224 sagittal slices with 1.0 mm thickness, TR/TE = 1630/2.48 ms, TI = 800 ms, 8° flip angle; or 175 axial slices with 1.0 mm thickness, TR/TE = 2100/3.31 ms, TI = 1050 ms, 8° flip angle. Pupil diameter was recorded continuously at 60 Hz with a wall-mounted infrared camera recording from the left eye via a hot-mirror on the coil (Applied Science Laboratories (ASL) 5000 Eyetracker with long range optics, collected via ASL EyeTracker® software).
Data Analysis

Pupil Preprocessing

Pupil data were preprocessed with custom Matlab software (Siegle, 2000-2015) as described in (Siegle et al., 2003, 2008). Similar to Siegle et al. (2003), the proportion of each participant’s maximal dilation (95th percentile) was measured, rather than absolute pupil diameter, because the distance between participants’ eyes and the infrared camera / hot-mirror varied slightly. First, eye blinks and other artifacts were identified as pupillary changes too large to represent actual dilation or contraction (criteria described in Siegle et al, 2008), and replaced by linear interpolation. Data were then smoothed by averaging each time point with the preceding and following time points. Regressors for subsequent analyses relating pupil and brain data were created by convolving the artifact-corrected pupillary time courses with a canonical hemodynamic response function (HRF) and downsampling to one value per fMRI volume. fMRI volumes acquired during periods of greater than 20% pupil artifact were identified for later removal. Thirteen participants with less than 100 usable fMRI volumes due to pupil artifact were eliminated from further analysis. Within the final sample the mean usable fMRI volumes was 183.1 ± 55.8.

fMRI preprocessing

Functional images were preprocessed using SPM 8 (Wellcome Department of Cognitive Neurology, London, UK). Images were realigned, slice time corrected, coregistered to T1 anatomical scans, normalized using parameters calculated during segmentation of T1 scans, and smoothed with a Gaussian kernel with full-width at half-maximum of 8 mm. Any participant showing high mean frame-wise displacement (FD) (> 0.5) in the included fMRI volumes was
excluded from the final sample. Three participants violated this criterion and were removed from further analysis. The average mean FD in the final sample (N = 35) was 0.16 ± 0.1 mm. Average FD did not relate to the amount of useable pupil data (r = 0.02 p = .89) or standard deviation of pupil time courses (r = -.01, p = .95).

Creation of pupil-linked map

To identify a pupil-linked map, two general linear models (GLMs) were performed for each participant in SPM12 – an initial GLM to remove artifacts due to motion, physiological noise, and estimate serial auto-correlations; and, using the residuals from the initial GLM, a subsequent GLM in order to identify voxels significantly co-varying with pupil diameter. The initial GLM contained nuisance regressors including the mean ventricle and white-matter signals extracted from subject-specific masks from T1 segmentation, and the six realignment parameters generated during image realignment. Low-frequency drifts were removed using a high-pass filter with a 128 s cutoff. Serial auto-correlation was estimated using an autoregressive AR(1) model. Grand mean scaling was applied with global normalization to remove nonspecific noise (Van Dijk et al., 2010). To ensure appropriate filtering and estimation of serial auto-correlations, all fMRI images were included in the initial GLM. The subsequent GLM tested the effects of pupil-time course against the fMRI volumes retained after removing those occurring during periods of excessive pupil artifact (described above). We refer to the beta value created at each voxel from this GLM as pupil-brain coupling. In order to define a group-level pupil-linked map, each participant’s individual pupil-linked map was included in a second-level one sample t-test, with site as a covariate of no interest.
Correlation with individual differences in attention

Across the 20 participants from the Georgetown site, we assessed the correlation between pupil-brain coupling strength and inattention and hyperactivity/impulsivity ASRS scores (higher scores indicate worse function), separately within brain regions relating to sympathetic and parasympathetic subdivisions, using anatomical templates provided by (Beissner et al., 2013). These templates were derived by ALE meta-analysis of previous studies using sympathetic (e.g., skin conductance) and parasympathetic (e.g., high frequency heart-rate variability) measures in conjunction with neuroimaging. We created masks that included the overlap between our group level pupil-linked map, and template sympathetic or parasympathetic related brain regions. We then averaged the pupil-brain coupling separately within each mask and tested for correlation with ASRS scores. We then assessed whether p-values survived Bonferroni correction for the number of templates and ASRS sub-scales tested. To ensure these results were not driven by the amount of useable pupil data or head motion, the analyses were also repeated after entering the amount of usable data and average FD as covariates of no interest. We also tested if number of usable fMRI volumes or head motion independently related to pupil-brain coupling. Finally, we assessed potential confounds posed by individual differences in mental state during fMRI scanning, by using metrics derived from the pupillary signal. Given evidence that variability in spontaneous pupil fluctuations may relate to mind wandering (Grandchamp et al., 2014) we tested if the standard deviation of pupil time-courses related to pupil brain coupling or inattention scores. Additionally, given evidence that the spectral power of pupil fluctuations in specific frequencies relates to alertness (Wilhelm et al., 1998), we tested if the power of pupil time courses related to pupil brain coupling or inattention scores. We used a modified version of the
pupillary unrest index (PUI; Lüdtke et al., 1998), a validated measure of alertness. Ludke et al (1998) have shown similarities of the standard PUI to an index derived using power in the 0-0.6Hz range via a Fourier Transform. To establish a dynamic analog of this measure, we applied a continuous wavelet transform (Morelet) to the 60Hz data, smoothed with a 3.86Hz filter (equivalent of a 10 point moving average applied twice to yield a center-weighted moving average), and summed the power in the 0-0.6 Hz range at each sample. All TRs with less than 20% pupil artifact were then averaged to create an overall measure of alertness. In other comparably large samples from our lab, the mean value of this index across an entire resting state study has correlations of r>0.75 with the standard PUI index.

**Results**

**Group pupil-linked map**

The second-level analysis revealed a positive relationship between resting-state brain activity and pupil diameter in bilateral frontal, parietal, and temporal lobes, cerebellum, and thalamus (FDR corrected p < .05) (Figure 1.1, Table 1.1). Frontal lobe regions included regions included dorsolateral (BA 9), ventrolateral including anterior insula and inferior frontal gyrus, and mid cingulate extending into anterior cingulate (BA 24, 31, 32) cortex. Parietal lobe regions included inferior parietal lobe (BA 40), and bilateral medial regions including posterior cingulate (BA 30) extending into precuneus (BA 31). Temporal lobe regions included middle and inferior temporal regions (BA 37), and hippocampus. A negative correlation was observed in primary visual areas (BA 17), extending laterally along the ventral pathway into extrastriate areas (BA 18, 19), fusiform gyrus (BA 37, 19), and middle temporal gyrus (BA 21, 22, 37). The negative
correlation was also observed in sensorimotor (BA 3, 4), parietal (BA 7) and cerebellar regions (Figure 1.1, Table 1.1).

**Table 1.1. Regions positively and negatively related to pupil diameter.** Brodmann areas (BAs) are labeled to the nearest gray matter, when available.

<table>
<thead>
<tr>
<th>Region Name</th>
<th>Brodmann Area</th>
<th>Extent (voxels)</th>
<th>t-value</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regions Positively Related to Pupil Diameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L Middle Cingulate</td>
<td>31</td>
<td>23678</td>
<td>10.6572</td>
<td>0</td>
<td>-42</td>
<td>40</td>
</tr>
<tr>
<td>L Middle Cingulate</td>
<td>31</td>
<td></td>
<td>4.4068</td>
<td>-16</td>
<td>-30</td>
<td>42</td>
</tr>
<tr>
<td>L Middle Cingulate</td>
<td></td>
<td></td>
<td>8.8812</td>
<td>-8</td>
<td>-8</td>
<td>6</td>
</tr>
<tr>
<td>R Middle Cingulate</td>
<td>24</td>
<td></td>
<td>4.9289</td>
<td>2</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td>R Thalamus</td>
<td></td>
<td></td>
<td>9.7115</td>
<td>16</td>
<td>-12</td>
<td>12</td>
</tr>
<tr>
<td>R Putamen</td>
<td></td>
<td></td>
<td>7.6425</td>
<td>14</td>
<td>4</td>
<td>-10</td>
</tr>
<tr>
<td>L Anterior Cingulate</td>
<td>32</td>
<td></td>
<td>6.4617</td>
<td>2</td>
<td>28</td>
<td>32</td>
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<tr>
<td>R Middle Frontal</td>
<td>24</td>
<td></td>
<td>6.3036</td>
<td>24</td>
<td>6</td>
<td>40</td>
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<tr>
<td>R Middle Frontal</td>
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<td></td>
<td>3.6307</td>
<td>24</td>
<td>46</td>
<td>36</td>
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<tr>
<td>R Precuneus</td>
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<td>5.7705</td>
<td>20</td>
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<tr>
<td>R Precuneus</td>
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Figure 1.1. Resting-state brain activity is associated with fluctuations in pupil diameter (FDR corrected p < .05). The red color indicates voxels positively related to pupil diameter, the blue color indicates voxels negatively related to pupil diameter.

Correlation with Attention Traits

Applying anatomical templates for sympathetic and parasympathetic related brain regions from Beissner et al. (2014) to our positive group pupil-linked map, the sympathetic division overlapped with right supramarginal gyrus, middle and anterior cingulate, thalamus and anterior insula/inferior frontal gyrus (total k = 1,706 voxels), whereas the parasympathetic division overlapped with posterior cingulate, precuneus, and inferior parietal lobe (total k = 800 voxels) (Figure 1.2A). Applying templates to our negative group linked-pupil map revealed minimal overlap with the sympathetic division (in right postcentral gyrus, k = 97 voxels) or the parasympathetic division (in left fusiform gyrus, k = 75 voxels). Therefore, in order to reduce the
number of comparisons, we only tested for correlation with attentional traits within regions positively related to pupil diameter.

Participants with overall stronger pupil-brain coupling in positive sympathetic related-regions reported lower inattention on the ASRS ($r = -0.72$, $p = 0.0003$) (Figure 1.2B), but not significantly different hyperactivity/impulsivity ($r = -0.30$, $p = 0.20$). A partial correlation, controlling for each participants’ amount of useable data and mean frame-wise displacement in head motion, showed a similar relationship with inattention ($r = -0.67$, $p = 0.002$). By contrast, pupil-brain coupling within positive parasympathetic regions did not significantly correlate with inattention ($r = -0.15$, $p = 0.52$), or hyperactivity/impulsivity ($r = -0.38$, $p = 0.10$). A test of dependent correlations (Steiger, 1980) revealed that coupling strengths in sympathetic and parasympathetic regions significantly differed in their relationship to inattention $t(17) = -3.10$, ($p=.003$). Neither mean frame-wise displacement in head motion, nor the amount of usable data significantly related to pupil-brain coupling in sympathetic regions or parasympathetic regions (all $p$s $> 0.1$). Furthermore, the standard deviation of pupil time-courses did not relate to pupil-brain coupling in sympathetic related regions ($r = -0.24$, $p = 0.31$) or inattention ($r = 0.17$, $p = 0.48$). Finally, individual differences in alertness, as measured by the pupillary unrest index, did not relate to pupil-brain coupling in sympathetic related regions ($r = 0.12$, $p = 0.61$) or inattention ($r = -0.09$, $p = 0.71$). All statistically significant correlations survived Bonferroni correction for multiple comparisons.
**Figure 1.2. Pupil-brain coupling correlates with trait-level attention in sympathetic related brain regions.** A) The group level map of regions positively associated with pupil diameter was subdivided into regions that overlapped with sympathetic (red) and parasympathetic (blue) brain areas, as defined by a recent meta-analysis (Beissner et al., 2013). For each participant, the mean strength of relationship between brain activity and pupil diameter was computed separately within these sub-divisions. B) Coupling between the pupil and sympathetic regions related to ASRS inattention scores.

**Discussion**

We found a positive association between spontaneous fluctuations in pupil diameter and the activity of widespread brain regions, and the strength of this positive coupling in regions associated with the sympathetic system predicted inattentiveness. Positive pupil-linked coupling spanned regions involved in both externally oriented processing (ACC, dIPFC, anterior insula, supramarginal gyrus) (Fox et al., 2005) and regions of the default mode network (PCC, IPL) often linked to internally oriented processing (Buckner et al., 2008). All these regions have been associated with autonomic indices during the performance of tasks in cognitive or emotional domains (Beissner et al., 2013). Parsing our pupil-linked map by Beissner et al.’s (2013) sympathetic/parasympathetic anatomical subdivision, we observed that stronger positive coupling in regions associated with the sympathetic system, including ACC, anterior insula, and
supramarginal gyrus, was associated with lower inattentiveness. Together, these results indicate that central brain regions known to interface with autonomic systems during task performance (Beissner et al., 2013) maintain their coupling to pupil diameter during the resting-state, and this coupling within specific regions is behaviorally relevant. We also found a negative association between spontaneous pupil diameter and resting-state activity in primary visual and extrastriate cortices, and primary sensorimotor areas.

While pupil diameter has long been known to spontaneously fluctuate at low frequencies (Stark et al., 1958), its association with spontaneous brain activity has been noted only recently. One study found positive pupil-linked coupling in regions similar to ours (Murphy et al., 2014) and another in slightly different regions, including IPL/precuneus, but not the ACC, insula, or thalamus (Yellin et al., 2015). Direct neural recordings from the ACC, however, also find spontaneous coupling with pupil diameter (Joshi et al., 2016). Furthermore, markedly similar regions also couple with spontaneous fluctuations in skin conductance (Fan et al., 2012), indicating that resting-state central-autonomic coupling is not limited to pupil diameter. Our data extend these findings to demonstrate that central-autonomic coupling within specific regions is behaviorally relevant. Correlation with inattentiveness was observed in only regions associated with the sympathetic system, which largely comprise the cingulo-opercular resting-state network (Dosenbach et al., 2007; Seeley et al., 2007). The cingulo-opercular network is associated with the maintenance of tonic alertness (Sadaghiani & D’Esposito, 2015), monitoring the external environment for salient cues and, in response, initiating global network control signals in the service of attention (Sridharan et al., 2008; Menon & Uddin, 2010). Cingulo-opercular regions are also critical for the monitoring and control of autonomic activity (Critchley et al., 2011).
Thus, stronger intrinsic coordination between autonomic systems and the cingulo-opercular network may enable adaptive autonomic shifts in response to salient environmental cues, and the maintenance of alertness during focused attention. Such attentional functions have been associated with task-evoked pupillary responsivity (Kristjansson et al., 2009; Geva et al., 2013). Indeed, whether individuals with stronger resting-state pupil-brain coordination also demonstrate more adaptive task-evoked pupillary responses would be of great interest to examine in future work.

Resting-state brain-autonomic coupling can be viewed at three related but different levels. First, at a phenomenological level, visceral autonomic information is thought to be constantly relayed to regions of the pupil-linked map such as ACC and insula, as a necessary component of any subjective experience (Park & Tallon-Baudry, 2014). By this view, the coupling in these regions may be intrinsic to subjective experience, regardless of the resting/task state of the individual. Second, the coupling may reflect the nature of mental activity. Mind wandering, which is commonly reported in the resting state (Diaz et al., 2013), is associated with pupil diameter (Grandchamp et al., 2014). Since spontaneous mind wandering is associated with ADHD symptoms, even in healthy participants (Seli et al., 2015), our observed association with inattentiveness may reflect individual differences in mind wandering. We did not measure mind wandering, but pupil variability, which is associated with mind wandering (Grandchamp et al., 2014), did not correlate with inattentiveness or pupil-brain coupling in the present sample. Additionally the pupillary unrest index, a validated measure of alertness, did not relate to pupil brain coupling or inattention. Thus, greater or lesser mind wandering/alertness across subjects during the resting state are likely not the source of the observed correlation of pupil-brain
coupling and inattention. Third, the strength of coupling may reflect the accumulated history of brain-autonomic interactions through a lifetime of task-evoked activity. Prior task-evoked activity shapes subsequent resting-state network coupling at short (Stevens et al., 2010; Gordon et al., 2014) and long (Ma et al., 2011) time-scales. As central-autonomic coupling is inherent to the attentional processes one engages in throughout life experience, its strength may reflect the history of coordinated usage of central and autonomic systems.

There are multiple potentially concurrent pathways that may underlie pupil-brain coupling. Insular, prefrontal, and cingulate areas are thought to contribute to the generation of autonomic activity patterns to meet behavioral demands (Cechetto & Saper, 1990; Critchley et al., 2011). These regions communicate – possibly directly (Ward & Reed, 1946; Lowenstein, 1955), or through the thalamus, hypothalamus, and brainstem (Loewenfeld, 1999; Andreassi, 2000) – with the sympathetic with parasympathetic nuclei mediating pupil diameter. Additionally, visual areas are speculated to send direct excitatory input to the Edinger-Westphal nucleus, the parasympathetic nucleus whose activity constricts the pupil (Loewenfeld, 1999). This may be the reason we observed negative pupil-brain coupling in visual areas, which was also reported by (Yellin et al., 2015). Direct neural recordings in mice help contextualize our negative coupling; in the absence of visual stimulation V1 cells decrease their firing rate during pupil dilation (Vinck et al., 2015). Luminance was held constant in the present study, thereby minimizing visual stimulation, and V1 activity may have been suppressed when pupils dilated, yielding the observed negative coupling.

In addition to direct anatomical pathways, locus-coeruleus norepinephrine (LC-NE) system may also contribute to pupil-brain coupling. A close link between non-luminance
mediated changes in pupil diameter and the LC-NE system has been posited (Aston-Jones & Cohen, 2005; Murphy et al., 2014), which may be established through common brainstem efferents that influence both the LC and autonomic pupil control centers (Nieuwenhuis et al., 2011) or through a direct pathway (Samuels & Szabadi, 2008). In any case, the LC acts in close conjunction with the autonomic nervous system (Sara & Bouret, 2012), has a pronounced neuromodulatory influence on the cortex (Bouret & Sara, 2005; Castro-Alamancos & Gulati, 2014), and may optimize neural gain to meet attentional demands (Aston-Jones & Cohen, 2005).

Given that spontaneous fluctuations in pupil diameter are reliably preceded by LC spiking activity (Joshi et al., 2016; Varazzani et al., 2015) and co-vary with LC activity measured with resting-state fMRI (Murphy et al., 2014), the strength of pupil-brain coupling may reflect the efficacy of noradrenergic modulation of brain activity. Pupil-brain coupling may have predicted trait-level inattention in part due to individual differences in the LC-NE system. This is consistent with the suggestion that pupil-brain coupling within regions such as the ACC reflects LC-mediated coordination of brain activity (Joshi et al., 2016), and with theories that autonomic disruptions in disorders like ADHD relate to noradrenergic dysregulation (Beauchaine, 2001). The fact that our attentional correlation was specific to sympathetic-related brain areas also supports this notion, because the sympathetic nervous system and the LC-NE system are thought to often act in an integrated manner (Nieuwenhuis et al., 2011).

We attempted to account for multiple potential confounds that may influence pupil-brain coupling. Specifically, fMRI activity is affected by physiological processes like respiration and heartbeat, and pupil diameter is exogenously influenced by luminance and eye fixation. To control for physiological processes, we included mean white matter and CSF signal regressors
(Dagli et al., 1999), a common procedure in resting-state studies, and our pupil-linked map was similar to regions co-varying with pupil diameter, after measuring and controlling for those functions (Murphy et al., 2014). To control for exogenous influences on pupil diameter, we kept screen luminance constant and asked participants to maintain gaze on a central fixation. In previous work, pupil-brain coupling was still observed when no fixation stimuli was present, and manipulating luminance did not induce fMRI activations in pupil related regions (Yellin et al., 2015), indicating that slow fluctuations in screen luminance and eye fixation are unlikely to drive pupil-brain coupling. Finally, our observed correlation with inattentiveness is also unlikely to be an artifact of head motion or useable pupil data because our findings persisted after controlling for these factors.

Overall, our results suggest that resting-state brain-autonomic coupling may prove a useful index in understanding the broader neural networks underlying cognition. A growing body of resting state studies has shaped current understanding of coordinated central brain circuits, and how these circuits subserve behavior. Psychophysiology research, however, has long indicated that autonomic changes are also important components of cognition. Our results emphasize the contribution of brain-autonomic system coordination to individual differences in cognitive processes subserving behavior such as attention. Additionally, our results indicate that studying resting-state brain-autonomic coupling may also help elucidate the neural basis of autonomic abnormalities, which are common to many psychiatric and neurological disorders. Individuals with autism spectrum disorders exhibit decreased resting-state coupling between skin conductance and cingulo-opercular areas (Eilam-Stock et al., 2014), regions that our results indicate have behaviorally meaningful links to pupil diameter. Hence, functional disconnection
between higher brain areas and autonomic systems may be a contributing factor to autonomic abnormalities (Williams et al., 2004, 2007). Resting-state coupling may be a viable way to measure this functional disconnection and track outcomes of behavioral or pharmacological interventions targeting autonomic dysfunction, or attention more broadly.
**Introduction**

Emerging work suggests that cognitive processes—from basic sensory awareness (Godwin et al., 2015) to higher level attention (Rosenberg et al., 2016)—rely on highly diffuse neural interactions. This non-localized account of brain function is supported by studies showing that cognitive state can be reliably predicted using patterns of neural synchrony (termed functional connectivity, FC) across the whole brain (Shirer et al., 2012; Cole et al., 2013; Gonzalez-Castillo et al., 2015; Milazzo et al., 2016). When participants are asked to cycle through various tasks like math, memory, and movie watching, their cognitive state is predictable with near perfect accuracy on the basis of brain-wide FC patterns (Gonzalez-Castillo et al., 2015). Spatially restricting these analyses—even by excluding the least predictive brain regions—decreases their accuracy. This raises the possibility that brain-wide FC patterns may play a role in cognitive processing.

Working memory, the active maintenance of mental information, may be especially reliant on distributed FC patterns. Lesion (Butters & Pandya, 1969), single-unit recording (Funahashi et al., 1989), and functional Magnetic Resonance Imaging (fMRI) (Owen, 1997) studies have traditionally linked working memory to the dorsolateral prefrontal cortex (dIPFC). By modeling the whole brain as a network, however, recent FC fMRI and MEG studies reveal that engaging working memory adjusts the overall network topology of the brain (Kitzbichler et al., 2011; Vatansever et al., 2015; Liang et al., 2016). When working memory tasks increase in difficulty, FC changes occur across multiple canonical networks such as default-mode,
executive, and salience networks (Liang et al., 2016). Large-scale FC patterns not only dynamically change during working memory, they also reliably distinguish individuals who perform better on working memory tasks (Stevens et al., 2012; Stanley et al., 2015; Vatansever et al., 2015) and improve their performance after training (Yamashita et al., 2015). Whole-brain network interactions are thought to enable working memory by facilitating the integration of disparate information (Kitzbichler et al., 2011; Vatansever et al., 2015; Liang et al., 2016). This aligns with theoretical accounts of a “global mental workspace” underlying working memory (Baars, 2002). Together, these studies raise the possibility that working memory may rely on task-specific and widespread patterns of FC. The neurochemical mechanisms that adaptively regulate large-scale FC patterns during working memory, however, remain unclear. This poses a barrier not only to the understanding of working memory, but also to the utilization of FC network measures to inform clinical decision making when working memory is impaired.

Norepinephrine (NE) signaling is one potential mechanism that regulates both distributed neural interactions and working memory. Theoretical models and the anatomy of NE neurons suggest that NE modulates overall state of cortical networks in the service of arousal, attention, and mood (Sara & Bouret, 2012). A single NE neuron, originating in the locus-coeruleus (LC), can integrate synaptic inputs from, and project back to, diverse brain regions (Schwarz et al., 2015). Models such as the “network reset” theory of NE posit that the signaling of these LC projections can interrupt the activity of neural networks and reorganize them into configurations that determine behavioral output (Bouret & Sara, 2005). In the context of working memory, animal models show NE acts at diffuse (Scheinin et al., 1994; Tavares et al., 1996) post-synaptic alpha-2a receptors to quickly alter their synaptic weights and thereby regulate the strength of
neural interaction patterns (Wang et al., 2007; Arnsten et al., 2010). Blocking these receptors leads to profound impairments in working memory (Li & Mei, 1994), which suggests they play a critical role in working memory. These findings have led to the use of NE alpha-2a agonists such as guanfacine for the treatment of disorders with attentional and cognitive control deficits, such as attention deficit hyperactivity disorder (ADHD) (Hnatko, 2002). NE’s role in working memory, taken together with network models of NE function, lead us to hypothesize that NE signaling at alpha-2a receptors plays an important role in regulating large-scale patterns of FC during working memory.

We tested this hypothesis in a double-blind pharmaco-fMRI study utilizing the NE alpha-2a receptor agonist guanfacine. On two separate visits, a group of 20 healthy adult volunteers were scanned with fMRI during a resting state (to determine NE alpha-2a receptors’ impact on baseline network architecture) and a verbal N-back task (to determine NE alpha-2a receptors’ impact when working memory is required). Prior to scanning, participants received 1.5 mg guanfacine on one of the visits, and placebo on the other visit, in counterbalanced order. To test for changes in FC we used univariate approaches, and two techniques that are well-suited for examining whole-brain patterns of FC – multivariate machine learning and graph theory. Applying univariate approaches is important as they are commonly applied and therefore allow comparison with past findings. It is conceivable, however, that NE may have subtle whole-brain impact that require higher sensitivity to detect. Previous work has demonstrated that meaningful overall patterns of FC are readily detectable with high sensitivity using multivariate (Cole et al., 2013; Gonzalez-Castillo et al., 2015; Milazzo et al., 2016), and graph-theory methods (Giessing & Thiel, 2012). We therefore used machine learning (support vector machine; SVM) to predict
drug vs. placebo state based on the FC between a set of previously defined ROIs that covered the entire cortical surface (Gordon et al., 2016). To test for the specificity of these findings we also derived data-driven functional networks with community detection (Blondel et al., 2008) and conducted two types of follow up SVM analyses. Firstly we conducted one SVM per network that included only that network’s connections (i.e. the connections of the default mode network only), and secondly we conducted one SVM per network with that network removed from consideration (i.e. the connections of all other networks except the default mode network). This allowed us to test if guanfacine’s network effects were restricted to specific networks. Based on theories that working memory is subserved by multiple functional networks, as well as the diffusivity of the noradrenergic system, we hypothesized that drug state would be best detected on the basis of multivariate patterns of FC across all networks.

We next tested for changes in overall topology of FC patterns caused by guanfacine using modularity, a metric from graph theory, which quantifies the overall balance of integration and segregation between distributed brain networks. While there are numerous graph theoretic measures that capture neurobiologically meaningful whole-brain network parameters, we utilized modularity because it has been associated with cognitive functions, particularly working memory. Intra-individual changes in brain-wide network modularity are associated with variations in WM performance between days (Stevens et al., 2012), differences in working memory load (Vatansever et al., 2015), and trial-to-trial variations in stimulus detection (Sadaghiani et al., 2015). We therefore further hypothesized that alpha-2a receptor related alterations in FC ought to collectively increase brain network modularity.
Results

Guanfacine’s effect on behavior and physiology

Working memory performance was measured with accuracy and reaction time on a verbal N-back task with four loads (1-back through 4-back) performed during fMRI scanning. Drug (placebo, guanfacine) x Load (1-, 2-, 3-, 4-back) repeated-measures ANOVAs revealed no main effects of guanfacine on accuracy, $F(1,19) = 1.1, p = .31$, or reaction time, $F(1,19) = 1.2, p = .28$, and no significant Drug x Load interactions on accuracy, $F(3,57) = 1.8, p = .20$, or reaction time, $F(3,57) = 1.5, p = .22$. As expected, there were significant main effects of N-back load on accuracy, $F(3,57) = 19.1, p = .00$, and reaction time, $F(3,57) = 11.0, p = .00$ (Figure 2.1A&B); participants showed less accurate and slower performance at higher loads. These results are in line with previous work demonstrating a lack of working memory differences on guanfacine in healthy adults (Müller et al., 2005 although see Jäkälä et al., 1999). Guanfacine has more pronounced impact on WM, however, in those with lower working memory ability, such as individuals with traumatic brain injury (McAllister et al., 2011). In our sample, task accuracy was high in all loads, but it is feasible that those with lower accuracy on placebo may have accrued benefits. Consistent with this possibility, there was a significant negative relationship between placebo accuracy and drug-related accuracy changes (guanfacine - placebo accuracy across all loads), $B=-.54, p=.01$, indicating that individuals with lower placebo accuracy showed a larger magnitude of performance improvement on guanfacine (Figure 2.1C). To ensure this was not driven by order effects, order of visits (i.e. placebo or drug first) was included as a covariate of no interest. As evident in Figure 2.1A, drug-related performance change was small in magnitude and differed among the sample with only a subset showing improvement. Across
the full sample, however, the lack of significant drug-related change in average performance allows for interpretation of our brain network findings without confounds of performance differences.

![Graph showing accuracy and reaction time for different N-back loads with guanfacine and placebo.](image)

**Figure 2.1. Guanfacine did not Alter N-back Performance.** (A-B) Mean reaction time and accuracy for all N-back loads. Error bars represent standard error of the mean. (C) The relationship between placebo accuracy and the change in accuracy on guanfacine (guanfacine – placebo).

We also tested for basic physiological effects of guanfacine. Systolic and diastolic blood pressure, and pulse rate were not significantly different from placebo before or after dosing (all ps >.25; Supplementary Figure 2.1). The lack of physiological side effects suggests that our brain network findings were not due to hemodynamic effects driven by blood pressure or pulse rate.
Guanfacine’s effects on FC detected with univariate methods

We next tested if guanfacine induced brain-wide changes in functional connectivity using univariate methods. Functional connectivity was defined as the fisher-transformed temporal correlation between a set of 333 previously defined ROIs that encompassed the entire cortical surface (Gordon et al., 2016). Guanfacine’s effects were assessed using paired-t tests (drug vs. placebo) conducted on all functional connections, false discovery rate (FDR) corrected for multiple comparisons. No connections during the resting state or N-back task survived correction for multiple comparisons.

Uncorrected results, however, suggested that, during both the resting state and N-back task, guanfacine altered distributed functional connectivity patterns in a manner that was not spatially restricted to any specific region. Results at the p < .001 uncorrected level are shown in Figure 2.2, and the t-scores of all comparisons are shown in figure 4E&F.

To determine if guanfacine’s effects during working memory varied by load, for each connection we also conducted a repeated measures Drug X Load ANOVA. To increase power, we averaged across 1- and 2-back loads to create a low load condition, and 3- and 4-back loads to create a high load condition. Significant Drug x Load interactions (Figure 2.2) revealed that the effect of guanfacine on many connections was load-dependent. Again, no connections survived FDR correction for multiple comparisons. Overall, univariate analyses revealed widespread effects of guanfacine during both the resting state and WM. No connections survived statistical correction for multiple comparisons.
Figure 2.2. Guanfacine Induced Wide-Spread FC Changes. The top panels show connections significantly altered by guanfacine, detected with paired t-tests (p<.001, uncorrected). The bottom panel shows connections whose drug effects depended on N-back load, detected with repeated measures ANOVAs (p<.001, uncorrected). The color of connections indicates $t$ or $F$ values. The color of nodes indicates their network affiliation, as determined with community detection.

Guanfacine’s effects on FC detected with multivariate methods

Our univariate analyses suggested that NE alpha-2a receptor activation induced distributed and complex changes in FC, consistent with our hypothesis and the known distribution of NE alpha-2a receptors across the entire brain (Scheinin et al., 1994; Tavares et al., 1996). These analyses were difficult to definitively interpret, however, because no changes survived correction for multiple comparisons.
Previous work has demonstrated that meaningful overall patterns of FC are readily detectable with high sensitivity using multivariate methods (Cole et al., 2013; Gonzalez-Castillo et al., 2015; Milazzo et al., 2016). We utilized linear support vector machine (SVM) to predict drug vs. placebo state using all ROI-to-ROI FC strengths as features. Although machine learning analyses are sometimes restricted to connections expected to best differentiate the condition of interest, we included FC strengths from across the brain to address our hypothesis regarding noradrenergic influence on wide-spread networks.

We trained and validated SVM models using leave one out cross validation (LOOCV); for each person, a model was trained excluding their drug and placebo data, and the trained model was then used to predict their drug vs. placebo state from connectivity strengths. SVM analyses detected drug vs. placebo during the resting state with 60% accuracy (55% sensitivity, 65% specificity; Figure 2.3A). Although this result was above chance accuracy (50%), testing for statistical significance against a null distribution (LOOCV accuracy computed 1,000 times with drug and placebo labels randomly switched), revealed that SVM accuracy during rest did not reach statistical significance (p=.13). SVM analyses during the N-back task, however, detected drug state with 75% accuracy (75% sensitivity, 75% specificity; Figure 2.3A) and with high statistical significance (p < .001). A direct comparison of rest vs. N-back SVM accuracies through permutation testing revealed a marginally significant accuracy difference between the conditions (p = .055). Although our univariate analyses above suggested that drug effects during the N-back may have been load dependent, LOOCV accuracy was low when restricting SVM
analyses to either the low load blocks (50%), or high load blocks (55%), perhaps due to lower power.

**Figure 2.3. SVM Analyses Confirm Wide-Spread FC Modulation by Guanfacine.** (A) The prediction accuracy (drug vs. placebo) of SVM analyses utilizing all functional connections. (B) Specificity of N-back SVM results was assessed by restricting analyses to, and excluding, connections detected with univariate analyses at p thresholds of .001-.05. (C) Specificity was also assessed by systematically restricting analyses to, and excluding, each network. (D) The connections most significantly driving overall SVM analyses were detected through permutation testing on SVM feature weights. The color of connections indicates their SVM feature weight. The color of nodes indicates their network affiliation. (E) The distribution of feature weights amongst networks.

**Follow up analyses assessing the specificity of FC changes during working memory**

We tested for the specificity of our working memory findings in several ways. Firstly, we tested if guanfacine’s effects were limited only to the regions detected with traditional univariate
approaches. Restricting the SVM to only include significant connections from univariate analyses decreased LOOCV accuracy, and this was true across many different p-value thresholds (Figure 2.3B). Furthermore, excluding significant connections from the SVM entirely did not result in marked decreases in LOOCV accuracy (Figure 2.3B). Together, these results suggest that NE alpa-2a activation altered diffuse patterns of functional connectivity even beyond connections detected with traditional univariate methods.

We next tested if select networks or connections drove our N-back SVM classifier results. We derived data-driven functional networks that were common across participants and visits during the N-back task. We utilized the Louvain community detection algorithm (Blondel et al., 2008) on weighted functional connectivity matrices (Rubinov & Sporns, 2011), which subdivides brain areas into partitions that are highly connected within themselves, and sparsely connected to other partitions. We used this method because it has been previously used to define networks both during rest (Rubinov & Sporns, 2011) and working memory (Vatansever et al., 2015; Liang et al., 2016), and it also quantifies how modular these networks are – the graph-theory metric we utilized in later analyses. Defining a common set of networks used in both follow-on SVM and later graph theory analyses facilitated clearer interpretability of both analyses. It allowed us to first to assess the specificity of network changes, and then quantify the collective effects of guanfacine on these same networks.

The results of the Louvain algorithm can vary from run to run, so we also used a consensus clustering approach (Lancichinetti & Fortunato, 2012). For each participant, we generated 1,000 network partitions, and used the consensus approach to group nodes together that were commonly assigned to the same network across the 1,000 runs. We then used the
consensus approach again to find networks present across participants and visits (Figure 2.4A). These networks corresponded well to known functional networks: including a network encompassing the posterior cingulate, inferior parietal lobe, and medial frontal cortex (default mode network, red in Figure 2.4A); a network encompassing sensory and motor regions (sensorimotor network, gray in Figure 2.4A); a network encompassing lateral frontal, parietal, and cingulo-insular regions (cognitive control network, yellow in Figure 2.4A); and a network encompassing primary and secondary visual areas (visual network, blue in Figure 2.4A).

After deriving these networks, we removed all of the ROIs of each network, one network at a time, and conducted a SVM on the remaining connections. The removal of each network resulted in a decrease in LOOCV accuracy (Figure 2.3C), indicating that each network contributed to overall classification accuracy. Conversely, conducting SVM analyses on the connections of each network in isolation revealed that each network alone was sufficient to predict drug state with above chance accuracy (Figure 2.3C), but no single network achieved accuracy as high as when all networks were included. Together, these results suggest that the effects of NE alpa-2a activation were not restricted to any large-scale network.

In linear SVM analyses, feature weights are sometimes used to provide a more detailed interpretation of SVM models (although see Haufe et al., 2014). Therefore, lastly, to confirm if functional connectivity differences were confined to restricted networks, we computed an SVM model from all participants’ data, and summed feature weights by network (Figure 2.3E). While the summed weights indicated which networks were more important in an absolute sense, larger networks may have contributed more because they contained more features. We therefore also averaged feature weights by network (Figure 2.3E). Both the summed and averaged feature
weights were distributed amongst networks, again indicating that guanfacine induced widespread connectivity changes that were not specific to any network.

For a more fine-grained analysis of which connections were most strongly weighted in the SVM model, we created a null feature weight distribution for each connection. We randomly permuted drug and placebo labels 15,000 times, and obtained SVM feature weights for each iteration. The true feature weight of each connection was then compared against its own null distribution to compute a p-value. The most strongly predictive connections (p < .001) are shown in Figure 2.3D. Again, the connections most strongly altered by guanfacine were distributed across the brain.

**NE alpha-2a receptor activation’s effects functional network topology**

SVM analyses revealed that NE alpha-2a receptor activation induced distributed changes in functional connectivity that were more readily detectable when cognitive control was required. We next tested if, cumulatively, these changes altered overall brain network topology. Because network modularity is a topological feature thought to be important for cognitive control, we tested if guanfacine altered network modularity during the resting state and N-back task. The Louvain method above that was used to generate functional network partitions (Figure 2.4A) was also used to quantify how modular these network partitions were. We averaged modularity values across each participant’s 1,000 iterations of the Louvain algorithm for drug and placebo during each state (rest and N-back) and conducted paired t-tests to examine the effect of guanfacine. Guanfacine did not alter modularity during rest (guanfacine: Q = .32; placebo: Q = .32), t(19) = .07, p = 0.943, but significantly increased modularity during working memory (guanfacine: Q = .32; placebo: Q = .34), t(19) = 2.42, p = 0.037 (Figure 2.4B&C). A repeated
measures ANOVA did not reveal a significant drug x state interaction, F(1,19) = 1.3, p = .25. Furthermore, changes in modularity did not correlate with changes in N-back accuracy (r = -.20, p = .40).

We reasoned that two different scenarios were capable of producing this pattern of results; modularity during the N-back could have differed amongst fundamentally re-organized networks, or the same networks could have become more modular. To adjudicate between these possibilities, we tested if the networks during the N-back re-organized between drug and placebo visits. We derived networks separately for each visit using the consensus clustering approach, and quantified how different these network structures were using the normalized variation of information (VIn). This metric quantifies how much information is lost and gained when comparing two network partitions (Meilă, 2007). Next, to determine if network structure was significantly altered by guanfacine, we constructed a null distribution by randomly permuting drug and placebo labels 1,000 times, creating consensus network partitions, and comparing these partitions with VIn (Dwyer et al., 2014). This analysis revealed that network structure on guanfacine and placebo visits was not significantly different (p = .38) (Figure 2.4D). Collectively, these findings indicate that the same core functional networks became more modular on guanfacine, and this effect was specific to when cognitive control was engaged.
Figure 2.4. Guanfacine Increased the Modularity of Brain Networks. (A) Consensus clustering applied to the results of Louvain community detection revealed four networks that were present across participants and drug and placebo visits. (B) Spring-graph visualizations of N-back network structure in a representative participant. Colors represent network affiliation of each node. (C) Comparison of network modularity values across all drug and task states. (D) A box plot showing VIn values. Network structure between drug and placebo visits was similar for all participants. (E) N-back functional connectivity matrices on placebo and guanfacine. (F) A matrix of t-values for paired t-tests of guanfacine vs. placebo.

Discussion

The present findings demonstrate that NE alpha-2a receptor activation induces widespread FC changes that are more readily detectable during working memory. We found that drug state (guanfacine vs. placebo) was predictable at above chance levels during both the resting state (60%) and working memory (75%) on the basis of whole-brain FC. Only predictions during working memory, however, reached statistical significance upon permutation testing.
Furthermore, we found that FC changes were not restricted to any one network during working memory. The FC of each functional network in isolation was sufficient to predict drug state at levels above chance, but no network achieved accuracy as high as that with whole-brain FC. We also found that FC changes extended beyond regions detected with traditional univariate analysis; drug state could be predicted even when exclusively utilizing connections not showing significant univariate drug vs. placebo differences. Finally, we found that the FC changes induced by NE alpha-2a activation collectively altered the overall topology of functional brain networks. Guanfacine increased whole-brain network modularity during working memory, a metric which indexes the overall balance of network integration and segregation, and has been linked to better working memory abilities (Stevens et al., 2012). Although guanfacine did not affect average working memory performance, its effects varied across individuals such that those with lower performance on placebo showed more drug-related improvement. Together, these findings indicate that NE alpha-2a receptor activation during working memory engagement has brain-wide effects that drive networks into a more modular configuration, a state thought to facilitate executive control.

**NE neuromodulation shapes FC across the brain**

FC fMRI studies have increasingly linked a variety of cognitive processes to whole brain network structure. When cognitive functions—such as working memory—are engaged, there are subtle but distributed changes in network FC (Cole et al., 2014). It is important to consider what neurochemical mechanisms may drive these changes. Animal models suggest that the anatomical connectome provides a stable structure whose activity is constantly modified by neuromodulation (Marder, 2012). NE is one such neuromodulator that is thought to interrupt the
activity of neural networks and reorganize them into configurations that determine behavioral output (Bouret & Sara, 2005). Our data is consistent with this notion, demonstrating that, when working memory is engaged, NE alpha-2a receptors modify FC across the cortex. Other neuromodulators such as acetylcholine (Giessing et al., 2013) and dopamine (Carbonell et al., 2014) also produce whole-brain network changes. Our data, in conjunction with these studies, suggests that neuromodulation may allow for flexible and adaptive modification of human large-scale brain network structure.

We utilized multivariate methods to study noradrenergic modification of network structure in the present investigation for several reasons. We utilized modularity because this metric may reveal whole-brain communication patterns, and therefore capture cognitive processes that rely on signaling across broad extents of sensory and association cortex (Godwin et al., 2015), like working memory. It has also been argued that whole brain metrics like modularity are well suited to studying cholinergic and noradrenergic systems, which affect broad cognitive processes and whose neural impact are subtle and highly non-focal (Giessing & Thiel, 2012). Hence, our finding that guanfacine increased modularity during working memory is an important first step to confirming our hypothesis that large-scale network communication is altered by noradrenergic signaling. However, a disadvantage of this metric is that it cannot reveal which specific patterns of FC were modulated by the drug, as distinct patterns could produce similar modularity values. Therefore, future studies are needed to characterize the precise nature of the observed noradrenergic effects. Hence, while our graph theory analyses are well targeted to capture distributed noradrenergic effects, follow-up studies are needed to probe granular noradrenergic changes within specific networks or regions. Our SVM analyses were capable of
producing network specific effects, however, these also indicated that guanfacine induced widespread FC changes in all networks. Furthermore, SVM analyses can detect complex patterns, but their output makes it difficult to parse the specific nature of these patterns. One potential method to gain insight into the patterns underlying SVM accuracy is to examine feature weights, as we have done here. It is not possible to unequivocally assign neurobiological relevance to SVM feature weights, however, because non-zero feature weights can be observed for regions or connections that are statistically independent of the examined brain process (Haufe et al., 2014). Therefore, while our SVM analyses confirmed the widespread nature of noradrenergic effects, the difficulty of interpreting the pattern underlying their accuracy reinforces the importance of future studies aimed at probing granular noradrenergic changes within specific networks or regions.

It should also be noted that, while our results indicate that NE alpha-2a receptor activation induces large-scale network changes, we cannot definitively ascribe behavioral relevance to all of these changes. There are several possible reasons why we did not observe a significant working memory performance difference on guanfacine. Firstly, repeated or higher dosing may be needed to produce behavioral effects. Daily guanfacine treatment is sometimes required before behavioral effects manifest in ADHD populations (Scahill et al., 2001). Secondly, heterogeneity in behavioral drug response may necessitate larger sample sizes to detect effects, whereas functional brain measures may be more sensitive. Our results indicate some heterogeneity may result from baseline performance differences, because placebo accuracy was predictive of drug effects on working memory performance. Hence, those with lower baseline working memory may accrue more cognitive benefits from guanfacine. Thirdly, our N-
back task may be have been insensitive to working memory differences because of ceiling
effects, or insufficient difficulty of our cognitive loads.

The absence of a behavioral finding makes it difficult to determine potential cognitive
changes related to the observed functional connectivity modulations. It is possible that our
functional connectivity effects were specific to working memory processes, and with a more
difficult task or higher sample size, behavioral differences would have emerged. It is also
possible that guanfacine altered more general cognitive processes, such as alertness or attention,
during the working memory task. Therefore, it is important for future studies to determine if
guanfacine induces large-scale network changes non-specifically during cognition, or its effects
are specific to tasks requiring working memory.

**NE’s network effects depend on behavior state**

In the present study, we examined whole brain networks during two behavioral states in
the same participants. Permutation testing revealed that drug state was significantly better
predicted by FC during working memory compared to the resting-state, and our graph theory
findings were also specific to working memory, although our design was limited in detecting a
significant drug x state interaction. It is possible that a larger sample size would be needed to
find graph theory effects that were specific to cognitive state. The fact that our SVM accuracy
was significantly higher during working memory, however, suggests that NE’s network effects
can differ by cognitive state. There are likely several reasons for this. Firstly, different NE
receptors are activated in different behavioral states, and these receptors can have very different
cognitive and neural effects (Arnsten, 2009, 2011). Secondly, neurmodulatory influence on
neural networks depend on numerous exogenous and endogenous factors (Giessing & Thiel,
including the prior history of the network and the other neuromodulators present in the network (Marder, 2012). Collectively, this indicates that studies seeking to study the large-scale network effects to neuromodulators, or predict neuromodulatory drug response from network measures, should consider cognitive state.

The dependence of network effects on cognitive state also indicates that a more complete model of NE action requires consideration of other behavioral domains. Previous human neuroimaging work has shown that NE also adjusts functional networks during stress (Hermans et al., 2011), attention (Coull et al., 1999), emotional control (Schulz et al., 2014), and motor control (Wang et al., 2011). It remains unclear, however, how NE’s impact on networks differs as a function of behavioral state. One reason for this uncertainty is that most previous studies examined isolated networks or behavioral states. Future studies examining different behavioral domains should test if NE alters global network parameters in a fashion similar to our working memory findings, or has more restricted effects.

Implications for future pharmaco-neuroimaging studies

In addition to furthering understanding of NE’s network effects, guanfacine’s global network impacts also have important implications for future pharmacological neuroimaging studies. Studies combining neuroimaging with acute or long-term drug dosing have lent important insights into mechanisms of psychiatric drug action and helped predict heterogeneous clinical responses (Schaefer et al., 2014; Sarpal et al., 2016; Whitfield-Gabrieli et al., 2016). Like the LC-NE system, however, many neuromodulatory systems targeted by common pharmaceutical agents – such as the serotonin system (Frazer & Hensler, 1999) – are also widely dispersed. This suggests that other psychiatric medications may also have global network effects.
Furthermore, the fact that guanfacine’s network effects extended beyond connections detectable with univariate methods, suggests that univariate methods may be insufficient to capture whole-brain network changes. For this reason, machine learning and graph theory techniques, which are suitable for detecting distributed and multivariate patterns of change, may prove useful in future pharmacological neuroimaging studies.

**Mechanisms of guanfacine induced network changes**

Although it can be difficult to assign mechanisms of action to neuropsychiatric drugs, several mechanistic considerations provide important context in interpreting our results. Firstly, while guanfacine has high binding affinity for NE alpha-2a receptors (Uhlén & Wikberg, 1991), these receptors can be located both pre-synaptically and post-synaptically (Aoki et al., 1998). This is important to consider because the neural and behavioral effects of these receptors differ based on their synaptic location. Whereas guanfacine’s cognitive effects are attributed to post-synaptic alpha-2a receptors, pre-synaptic receptors can down-regulate the LC (Arnsten, 2011). In comparison with other alpha-2a receptor agonists like clonidine, however, guanfacine has 10 times weaker effects on pre-synaptic LC inhibition (Engberg & Eriksson, 1991), and 10-100 times stronger effects on post-synaptic actions (Arnsten et al., 1988). Hence, there is evidence that guanfacine’s network effects may be ascribed predominantly to the cognitively relevant post-synaptic alpha-2a receptors, but we cannot definitively test this possibility with the present data.

The second important mechanistic consideration is the possibility that not all FC changes were caused by direct drug actions within the altered networks. Localized neural activity changes can propagate widely throughout many networks (Gratton et al., 2013). Guanfacine may have
caused distributed network changes in part through local impact on key network nodes. Animal (Arnsten et al., 2010) and fMRI studies (Clerkin et al., 2009; Schulz et al., 2013) indicate that alpha-2a activation may have a particularly strong impact in the dlPFC, where it closes Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels, strengthening the functional connectivity of local PFC networks (Wang et al., 2007). Furthermore, local processing in PFC networks is thought to influence behavior through top-down modulation of other brain regions (Munakata et al., 2011). It is thus possible that guanfacine altered distributed networks directly, or indirectly through its actions in the PFC and other key network nodes.

The final important mechanistic consideration is that guanfacine may also have other effects that indirectly affect fMRI network measures. Given that guanfacine is often prescribed to treat attention deficits and hyperactivity (Hnatko, 2002), it is conceivable that reduced head motion during on-drug scanning may relate to the observed drug-related FC changes. Even small head-motion differences can bias estimates of FC (Power et al., 2012). In the present sample, however, head motion did not differ by drug state and therefore unlikely to have contributed to FC differences. Additionally, guanfacine can also alter performance on cognitive tasks (Jäkälä et al., 1999). If task performance was changed by guanfacine, the drug’s impact on the brain could be attributable to either direct action, or to indirect effects due to performance differences. N-back performance, however, was not significantly altered on drug, which enables us to interpret drug effects on the brain, without the confounds of performance differences (Casey, 2002; Clerkin et al., 2009). Finally, guanfacine can have basic physiological effects, which may influence the fMRI signal. None of the physiological parameters we measured, including heart rate and blood pressure, however, were significantly altered by the drug.
Conclusion

In this report we found that NE alpha-2a receptor activation during working memory affects the overall network structure of the human brain. This lends support to models such as the network reset theory of NE action, which posits that the LC-NE system can reset brain networks and reorganize them in a manner that supports behavioral output. More broadly, these results contribute to a growing body of work demonstrating that neuromodulation may allow for flexible and adaptive modification of human large-scale brain network structure in the service of cognition.

Materials and Methods

Participant Screening and Demographics

20 (7 male, 13 female) participants, aged 21-37 (mean 27 years) were recruited from a Washington, DC area community sample. All participants provided informed consent. The research protocol and consent procedures were approved by the Georgetown University Institutional Review Board. Prior to fMRI scanning visits, participants were screened for exclusionary criteria; including heart, lung, kidney, neurological or psychological disorders; currently smoking cigarettes; blood pressure or resting heart rate values outside of the 5th - 95th percentile range (by gender and age); or abnormal EKG results. Screening data were collected by the study research team and trained nursing staff, and reviewed by our study physician. Participant characteristics are shown in Table 2.1.
Table 2.1. Participant demographics.

<table>
<thead>
<tr>
<th></th>
<th>Mean (S.D.)</th>
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<tr>
<td>Height (cm)</td>
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</tr>
<tr>
<td>Weight (kg)</td>
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<tr>
<td>Age (years)</td>
<td>27.05 (4.72)</td>
</tr>
<tr>
<td>Male:female ratio</td>
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FMRI scanning protocol and medication dosing

Participants were scanned with fMRI on two separate visits (average of 12 days apart), and were asked to abstain from caffeine for at least 2 hours prior to each visit. 90 minutes prior to fMRI scanning, participants once received placebo and once 1.5 mg guanfacine. Order of dosing was randomized, counter-balanced and double blind. Physiological monitoring was conducted by trained nursing staff 30 minutes prior to dosing, 45 minutes post-dosing, and after fMRI scanning (mean time post-dosing = 150 minutes) (Supplementary Figure 2.1).

FMRI scanning was conducted on a 3T Siemens Trio (Erlangen, Germany). 288 functional images were collected during the resting state scan, and 209 functional images were collected during the N-back task. Both functional scans were collected with gradient echo pulse sequences with the parameters: 47 slices with 3.2 mm thickness, TR = 2500 ms, TE = 30 ms, 90° flip angle. The first two images from each functional run were discarded to allow for signal stabilization. Further, a high-resolution $T_1$-weighted structural scan (magnetization prepared rapid gradient echo) was acquired with the parameters: 176 sagittal slices with 1.0 mm thickness, TR/TE = 1900/2.52 ms, TI = 900 ms, 9° flip angle.

Task parameters

During the resting state scan, participants were asked to stay awake and keep their eyes focused on a central fixation cross. The N-back task consisted of twelve 30 second N-back
blocks (3 blocks each of 1-, 2-, 3-, and 4-back loads, in pseudorandomized order). Blocks began with 3000 ms of instructions indicating the N-back condition, and all blocks but the last were followed by a 14 second fixation. During each block, a series of 9 black consonants were displayed for 500 ms in the center of a gray background, with an inter-stimulus-interval of 2500 ms. Participants were told to press a button in their right hand if the letter currently displayed matched n letters ago, and a button in their left hand if it did not match. 18% of all trials were targets, with 1-2 targets per block. Stimuli were presented with E-prime (Psychology Tools Inc., Pittsburgh, PA). Participants practiced the N-back task outside of the scanner on both visits. Both accuracy and reaction time measures were created by first computing separate means within target and non-target trials, and then averaging these.

**MRI data preprocessing and functional connectivity calculation**

Using SPM12 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (Mathworks, Inc., Sherborn, MA), fMRI images were realigned, slice-time corrected, normalized using parameters calculated through segmentation of EPI images, and smoothed with a 8 mm FWHM Gaussian kernel. Anatomical volumes were segmented into grey matter, white matter, and cerebro-spinal fluid, and the resulting masks were eroded by one voxel to minimize partial volume effects.

The conn toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012) was used to linearly detrend fMRI time series and to remove confounding effects by nuisance regression; which included 3 principle components each from subject specific white matter and cerebro-spinal fluid masks; 6 motion parameters and their first-order temporal derivatives; and point-regressors to censor time points with mean frame-wise displacement (FD) > .5 mm (one covariate per
censored volume, consisting of a one at the censored time point and zeros for all other time points). For N-back data, a regressor for each task condition (1-4 back load) was convolved with a hemodynamic response function and included as an additional nuisance regressor, along with its first order temporal derivative. Residual time-series were band-pass filtered (0.008 Hz < f < 0.09Hz). Importantly, during both rest and the N-back conditions, the mean FD and number of time points censored due to motion were not significantly different on guanfacine (all ps > 0.21). The mean FDs ±SD (range) for all conditions were; rest placebo: 0.13 ± 0.05 (0.07-0.25), N-back placebo: 0.10 ± 0.05 (0.04-0.22), rest guanfacine: 0.15 ± 0.12 (0.06-0.53), and N-back guanfacine: 0.10 ± 0.06 (0.05-0.25). The mean censored time points ±SD (range) for all conditions were; rest placebo: 4.95 ± 7.12 (0-29), N-back placebo: 1.55 ± 2.04 (0-8), rest guanfacine: 11.65 ± 24.88 (0-97), and N-back guanfacine: 1.70 ± 4.37 (0-19).

Following nuisance regression and band-pass filtering, mean time courses were extracted from a previously defined set of 333 ROIs (Gordon et al., 2016). These ROIs were created through a parcellation of resting state data that yielded highly homogenous parcels with a network structure similar to canonical functional networks. Functional connectivity was computed as the fisher-transformed pearson’s correlation coefficient between ROI time series.

**Univariate Analyses**

Univariate functional connectivity differences on drug were detected with 2-way drug x load repeated measure ANOVAs and paired-t tests (drug vs. placebo) conducted in MATLAB. Analyses were conducted separately for each functional connection from all 333 ROIs to every other ROI.
Support Vector Machine Analyses

Linear support vector machine analyses were conducted in MATLAB. We trained and validated SVM models using leave one out cross validation; for each person, a model was trained excluding their drug and placebo data, and the trained model was then used to predict their drug vs. placebo state from connectivity strengths. Before SVM analyses, each feature (i.e. functional connection) was standardized by subtracting the mean and dividing by the standard deviation across visits and participants. During LOOCV, to avoid biasing the training model, features were standardized only on the training data. The same standardizations were then applied to withheld data (i.e. again using the means and standard deviations only from the training data). When features were restricted to connections based on univariate paired t-tests, these tests were conducted only on the training sample to avoid biasing the training model.

The statistical significance of SVM prediction accuracies was assessed through permutation testing, wherein a null distribution was created by computing LOOCV accuracy 1,000 times with drug and placebo labels randomly switched. A null distribution of the accuracy differences between rest and working memory conditions was created by computing LOOCV accuracy 1,000 times with rest and working memory labels randomly switched. Post-hoc analyses of SVM feature weights were conducted by randomly permuting drug and placebo labels 15,000 times, and obtaining SVM feature weights for each iteration. The true feature weight of each connection was then compared against its own null distribution to compute a p-value.
Graph Theory Analyses

Networks were detected and modularity values were computed using the Louvain algorithm implemented with the Brain Connectivity Toolbox (Rubinov & Sporns, 2010). This algorithm was applied to full weighted functional connectivity matrices, with asymmetric weighting of positive and negative connections (as suggested by Sporns et al. 2011). For weighted and signed FC networks, modularity can be calculated as:

\[
Q^* = Q^+ + \frac{v^-}{v^+ + v^-} Q^- = \frac{1}{v^+} \sum_{ij} (w_{ij}^+ - e_{ij}^+) \delta_{M_iM_j} - \frac{1}{v^+ + v^-} \sum_{ij} (w_{ij}^- - e_{ij}^-) \delta_{M_iM_j}
\]

Where \(w_{ij}\) is the weight of connection \(ij\), \(e_{ij}\) is defined as \(s_i^+ s_j^+ / v_i^\pm\), and \(\delta_{M_iM_j} = 1\) when \(i\) and \(j\) are in the same module and \(\delta_{M_iM_j} = 0\) otherwise. A default resolution parameter of 1 was used. As described in the results section, we used a hierarchical consensus clustering approach to detect group level networks, and compare network structure between visits. We first applied network detection 1,000 times within each participant, which produced a network partition for each participant across all conditions (i.e. drug or placebo visit and rest or working memory task) The same network detection algorithm was then run on a matrix indicating how often each pair of nodes was classified in the same network, across the 1,000 times. This provided stable within-subject network partitions. We then conducted the consensus clustering approach again at the group level. The network detection algorithm was run on a matrix indicating how often each pair of nodes was classified in the same network across participants. This yielded one overall set of functional networks that was consistent across participants and visits (Figure 4A). Network partitions were compared with the normalized variation of information (VIn), an information
theory metric which quantifies how much information is lost and gained when comparing two network partitions (Meilä, 2007).
CHAPTER IV: GENERAL CONCLUSION

Conclusion

The study of large-scale brain networks has permitted a deeper understanding of the neural basis of executive control. A more complete model of network function, however, must not only link network structure to executive control, but also elucidate the mechanisms regulating this network structure. The studies presented in this dissertation provide a framework for understanding one potentially significant regulator of large-scale networks – the LC-NE system. Study I indicated that, in the resting state, many networks that are important to executive control couple with the LC-NE system in a behaviorally relevant manner. Hence, networks contributing to executive control likely do not act in isolation, but coordinate with lower brain-stem neuromodulatory systems. Furthermore, work in animal models suggests that this coordination may influence the level of NE signaling, and thereby shape the overall state of cortical networks (Sara & Hervé-Minvielle, 1995; Sara & Bouret, 2012). Indeed, study II indicated that NE alpha-2a receptor signaling influenced the overall topology of brain networks during executive control. Taken together, these studies can be thought of as investigating different portions of a broader executive circuit; with study I examining the baseline communication between executive networks and the LC-NE system, and study II examining how the LC-NE system in turn regulates the communication within these networks. This unified framework maps well onto anatomical and electrophysiological studies examining LC-NE function, which suggest that the LC integrates disparate synaptic input from across the cortex, and broadcasts back out to the entire cortex, regulating overall brain-state (Sara & Bouret, 2012; Schwarz et al., 2015). The present findings help contextualize past work, and have important
implications for future work. Most directly, Studies I and II have relevance for understanding how NE acts on brain networks to facilitate executive control. They also have implications for studies investigating disorders where executive control is impaired. Finally, they have methodological implications for how psychiatric medications can be better understood using neuroimaging. Each of these topics is considered below.

**Functional networks important for executive control**

Despite the advances presented in studies I and II, many questions remain regarding how NE and other neurmodulators regulate the functional networks underlying executive control. Firstly, it is an ongoing debate if NE regulates non-specific network and behavioral parameters such as arousal (Samuels & Szabadi, 2008; Sara & Bouret, 2012), or if NE’s behavioral and network effects are far more specific (Yu & Dayan, 2002; Clayton et al., 2004). Models of non-specific NE action point to the fact that—in response to environmental cues—the LC is often activated in parallel with basic physiological systems, such as the autonomic nervous system. This is thought to facilitate mental and physical arousal in preparation for an adaptive behavioral response (Sara & Bouret, 2012). By this view, NE mediates cognition through its influence on general physiological and behavioral state (i.e. arousal, effort, etc.).

Study II indicated that NE’s network effects may vary depending on the general behavioral context (i.e. rest vs task). This behavioral distinction is was too uncontrolled, however, to determine if NE’s network effects vary by general or specific behavioral parameters. Future studies should also attempt to assess the specificity of NE’s network effects by separately manipulating two factors: tonic arousal and behavioral domain (i.e. perceptual, executive, etc.). Previously studies have separately manipulated these factors (Sadaghiani & D’Esposito, 2014),
and a similar approach can be taken while delivering an NE agonist, antagonist or reuptake inhibitor. If NE’s network effects are similar across different behavioral domains when arousal levels are comparable, this will validate non-specific accounts of NE’s network effects. Conversely, if NE’s network effects differ by both behavioral domain and arousal, this will suggest that NE’s network effects may be more complex than accounted for by models of arousal alone.

In addition to assessing NE’s network impacts on executive networks, it is also important to understand how the cortex regulates evoked LC response during executive tasks. A common conceptual framework in network studies is that the baseline/intrinsic state of a network constrains its evoked activity (Luczak et al., 2009; Deco et al., 2011). It is interesting to consider if an analogous concept is also true of the reciprocal networks formed between the cortex and the LC (Jodo et al., 1998; Schwarz et al., 2008). Study I found that the strength of baseline network coupling between the LC-NE system (as indexed by pupil diameter) and cingulo-opercular regions related to trait-level inattentiveness. Given that optimal attention in everyday life is thought to rely on appropriate recruitment of the LC, it is conceivable that stronger intrinsic network coupling between the LC and the cingulo-opercular network may enable adaptive evoked LC responses. By this view, the intrinsic fidelity of cortical regulatory networks which maintain resting and evoked LC tone (Jodo et al., 1998) may constrain the ability of the LC to optimally react to attentionally salient stimuli. We did not directly test this assertion, however, so future studies should investigate this hypothesis. One way to test this experimentally would be to assess if individuals with stronger resting-state pupil-brain coordination also demonstrate more adaptive task-evoked pupillary responses.
Finally, creative multi-modal investigations are needed to delineate other neuromodulatory impacts on executive networks. NE is only one of numerous neuromodulators that likely regulate these networks. Furthermore, neuromodulators like NE and acetylcholine can have highly divergent cortical effects. For instance, acetylcholine shifts neurons into tonic firing model, while NE suppresses spontaneous firing (Castro-Alamancos & Gulati, 2014). These divergent neural effects make it important to explore the shared and unique network impacts of different neuromodulatory systems. Pharmaco-neuromiaging studies such as study II are a promising avenue to explore this question. Other study designs are also promising. Simultaneous PET/fMRI studies (Schlemmer et al., 2008; Wehrl et al., 2013) can map endogenous activity at norepinephrine, dopamine, serotonin or acetylcholine receptors (Wernick & Aarsvold, 2004), and link it to network structure (Roffman et al., 2016), both at rest and during executive tasks. Magnetic resonance spectroscopy (MRS) is another promising approach. Although many neuromodulators cannot be resolved with MRS, cholinergic regulation of the networks underlying executive control can be studied by measuring localized changes in acetylcholine with multi-voxel MRS (Mountford et al., 2010) during executive tasks. Network structure can then be measured with fMRI within the same individual, albeit not simultaneous to MRS acquisition. While more stable neural features, such as fixed anatomical connections, are an important basis for functional networks, neuromodulation is also likely an important contributor. Studies such as those described above are therefore critical for a true mechanistic understanding of human large-scale brain networks.
**Functional connectivity dynamics**

In addition to studying static network structure, many recent investigations have focused on the dynamics of human brain networks. Dynamic adjustments on a core intrinsic network structure are thought to facilitate adaptive cognition (Cole et al., 2014). The sources of these dynamics, however, remain unclear. While study II did not directly examine brain network dynamics, it did reveal that potentiated signaling at NE alpha-2a receptors can modify the overall architecture of functional brain networks. This finding – taken together with evidence of the varying levels of LC-NE signaling that occur across general behavioral state (Arnsten, 2009), as well as in rapid phasic alignment with behaviorally relevant stimuli (Aston-Jones & Cohen, 2005) – suggest that endogenously fluctuating levels of NE signaling continually modify network structure. For instance, during focused attention or executive control, moderate NE levels act at high-affinity alpha2-a receptors, which study II indicates drives the brain into a more modular network configuration. During stress, however, another study found that higher NE signaling at lower-affinity beta receptors prompts FC changes in the salience network (Hermans et al., 2011). Hence, the LC-NE system may make context-specific adjustments to the network structure of the human brain based on general behavioral context and its level of signaling. This concept aligns with network reset theory of NE function, which posits that NE acts as a rapid “reset” signaling that reorganizes functional networks to meet the behavioral context at hand (Bouret & Sara, 2005).

The network reset framework holds important implications for dynamic network studies: it suggests that NE may facilitate the network dynamics accompanying NE-dependent behavioral states, including arousal, mood, executive control, and stress. For instance, a recent study found
that dynamic changes in brain network modularity across a stimulus detection task predicted if participants would correctly complete a trial (Sadaghiani et al., 2015). The mechanisms regulating ongoing shifts in network modularity, however, were unknown. Study II’s finding that NE alpha-2a receptors increase modularity, taken together with importance of NE to basic stimulus detection, (Ikeda et al., 2015), suggests it is conceivable that NE contributes to ongoing network modularity adjustments. While the results of study II argue in favor of this point, they cannot directly validate it. Future studies can directly test this assertion by delivering antagonists rather than agonists, and determining if network dynamics are decreased. This would indicate that NE signaling at the blocked receptors’ is necessary for network adjustments. NE alpha-2a antagonists such as yohimbine, however, have much less selective receptor action than guanfacine (Millan et al., 2000). This type of investigation may thus rely on the future development of more specific NE alpha-2a antagonists, and thorough safety and proof of concept testing in animal models. More selective antagonists are available for other NE receptors (Lowe et al., 2002; Ladage et al., 2013), however, enabling more near-term investigations of their network effects.

**Animal models of noradrenergic modulation of executive control**

Animal models of NE’s contribution to executive control have focused on its action on post-synaptic alpha-2a receptors in the PFC. More specifically, they have focused on NE’s effect on the sustained delay-related firing of dIPFC networks during working memory (Arnsten, 2011). Several lines of evidence suggest that these models can be expanded. Firstly, NE-dependent executive control functions, such as working memory, require precise synchronization and processing across distributed networks beyond the PFC (Palva et al., 2010; Salazar et al.,
Secondly, study II demonstrates that NE also modulates distributed brain networks during executive control. Thirdly, recent evidence suggests that discrete oscillatory dynamics, rather than sustained neural activity, underlies working memory (Lundqvist et al., 2016). Together, this evidence indicates that future animal models of NE’s contribution to working memory may profit from multi-electrode array recordings in an effort to delineate NE’s contribution to oscillatory dynamics in local PFC networks, and long-range neural synchronization across distributed cortical regions.

**Attention Deficit Hyperactivity Disorder (ADHD)**

While neither study I nor II directly investigated ADHD—a psychiatric disorder defined by inattentiveness, hyperactivity, and impulsivity—both have important implications for future studies of the disorder. It is posited that an inability to maintain appropriate levels of arousal may be an important contributor to the inattentiveness observed in ADHD (Barkley, 1997; Becker, 2013). Study I suggests an interesting testable hypothesis regarding the source of these arousal deficits: they may result from altered coordination between the cingulo-opercular network and the LC-NE system. Study I found that inattentiveness, a defining symptom of ADHD, relates to the coupling between the LC-NE system (as indexed by pupil diameter) and the cingulo-opercular network. Interestingly, both the cingulo-opercular network and the LC-NE system are thought to modulate arousal. Hence, optimal attention might rely on appropriate coordination between the brain networks and neuromodulatory systems underlying arousal. Given evidence of both cingulate (Bledsoe et al., 2013) and arousal (Becker, 2013) dysfunction in ADHD, it is conceivable that coupling between the cingulo-opercular network and the LC may be compromised. This hypothesis can be tested by determining if pupil-brain coupling is lessened in
ADHD populations. It would also be interesting to test if pupil-brain coupling is normalized by pharmacological interventions that aide executive functions, such as with stimulant and non-stimulant medications (including guanfacine).

Study II indicated that guanfacine, a medication commonly prescribed for ADHD, induces widespread neural changes. This has important implications for future studies of the pharmacology of ADHD, as well as the disorder itself. Based on animal work showing guanfacine’s effect on the sustained delay-related firing of dlPFC networks, the dlPFC is often assumed to be the locus of guanfacine’s therapeutic action (Arnsten & Jin, 2012). Several lines of evidence suggest that the drug may also have important actions outside the PFC. Firstly, study II revealed widespread action of the drug, but could not definitively ascribe behavioral relevance to all of these changes. In another recent pharmaco-fMRI study in ADHD participants, however, the neural changes most predictive of guanfacine’s clinical effects were outside of the dlPFC, in areas like the mid and posterior cingulate cortices (Bédard et al., 2015). Guanfacine also has non-cognitive benefits, whose neural sources are unknown, including anxiety reduction and the amelioration of stress induced drug cravings (Fox & Sinha, 2014). Together, this evidence suggests that guanfacine may have more distributed neural and behavioral effects than current animal models would suggest.

In addition to an evolving understanding of the neural locus of guanfacine’s action, a less-PFC centric model of ADHD itself has also recently been proposed (Castellanos & Proal, 2012). This model suggests that many networks may be implicated in ADHD; including the fronto-parietal, dorsal attention, motor, and default networks. It is thought that ADHD pathophysiology, rather than originating solely in the PFC, may stem from aberrant interplay
between default, cognitive control and limbic networks. Given that study II showed that guanfacine alters the interplay between these networks, this further underscores the importance of understanding if the drug’s clinical efficacy results from wide-spread or local action. Future studies in ADHD populations should attempt to link overall network measures which index large-scale network interaction, such as modularity, to drug-induced behavioral improvements.

**Psychiatric translational studies**

Large-scale network measurement as a tool in the broader push for personalized medicine ("Precision Medicine Initiative", 2016) is relatively new, but holds great promise. Recently, baseline brain network architecture has been used to predict drug response in disorders such as schizophrenia (Sarpal et al., 2016) and social anxiety disorder (Whitfield-Gabrieli et al., 2016). A new framework also suggests the potential utility of predicting long-term clinical efficacy from how brain networks reorganize in response to acute drug dosing. Network measures were recently used to detect whole-brain and immediate effects of selective serotonin reuptake inhibitors (Schaefer et al., 2014). These acute network changes may be useful in predicting long-term clinical efficacy for depression, which can often take weeks or longer to manifest (Sugrue, 1983; Harmer et al., 2009). It remains to be seen if acute network changes are related to eventual clinical efficacy, but this is an intriguing prospect for future studies to investigate.

While promising, many of these studies utilized standard univariate techniques, which study II suggests may be insufficient to capture the entirety of drug related network change. Furthermore, the ultimate translational aim of these studies is to provide effective predictions of which medication, or combinations of medications, a patient will best respond to. It is likely that this problem will require complex modeling with high sensitivity. Thus, machine learning and
graph theory measures, which can capture meaningful and distributed patterns of network change with high sensitivity, will likely be useful methodological additions to translational psychiatric neuroimaging studies.

**Conclusions**

The discovery of large-scale functional networks has enabled a better understanding of the circuits underlying processes as diverse as stress, attention, and memory. It has also enabled a re-conceptualization of many psychiatric disorders as fundamentally disorders of neural connectivity (Di Martino et al., 2014). A more complete model of both network function and dysfunction, however, requires a deeper understanding of the neurobiological mechanisms regulating network structure. The studies presented in this thesis suggest NE as one potentially significant regulator of large-scale networks. As future studies continue to delineate the neurobiological mechanisms controlling network structure, this will enable a deeper appreciation for the brain bases of cognition, as well as expand the translational potential of brain network science.
Supplementary Figure 2.1. Guanfacine Did Not Change Blood Pressure or Heart Rate

Blood pressure was measured 30 minutes pre-dosing, 45 minutes post-dosing, and after fMRI scanning (mean time post-dosing = 150 minutes). Dashed lines represent dosing time.
REFERENCES


program


