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Coupling between spontaneous pupillary fluctuations and brain activity relates to inattentiveness

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Abstract

Autonomic activity in neurological and psychiatric disorders is often dysregulated, particularly in the context of attentional behaviors. This suggests that interplay between the autonomic nervous system and aspects of the central nervous system subserving attention may be disrupted in these conditions. Better understanding these interactions and their relationship to individual variation in attentional behaviors could facilitate development of mechanistic biomarkers. We identified brain regions defined by trait-sensitive central-autonomic coupling as a first step in this process. As spontaneous neural activity measured during the resting-state is sensitive to phenotypic variability, unconfounded by task performance, we examined whether spontaneous fluctuations in brain activity and an autonomic measure, pupil diameter, were coupled during the resting state, and whether that coupling predicted individual differences in attentional behavior. By employing concurrent pupillometry and fMRI during the resting-state, we observed positive coupling in regions comprising cingulo-opercular, default mode, and fronto-parietal networks as well as negative coupling with visual and sensorimotor regions. Individuals less prone to distractibility in everyday behavior demonstrated stronger positive coupling in cingulo-opercular regions often associated with sympathetic activity. Overall, our results suggest that individuals less prone to distractibility have tighter intrinsic coordination between specific brain areas and autonomic systems, which may enable adaptive autonomic shifts in response to salient environmental cues.

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These results suggest that incorporating autonomic indices in resting-state studies should be useful in the search for biomarkers for neurological and psychiatric disorders.

Keywords

Pupil Diameter; Attention; Autonomic; Locus Coeruleus; Cingulo-Opercular

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, 2015). 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 and 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-coeruleus 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 (reviewed in 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 $\pm$ 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. This multi-site approach was utilized to increase the statistical power of the pupil-linked map. 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 (voxel size = $3.2 \times 3.2 \times 3.2$ mm³, slice thickness = 3.2 mm), TR = 2500 ms, TE = 30 ms, 90° flip angle. The first two images were discarded to allow for signal stabilization. Further, a high-resolution T1-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 (MRA, Washington, PA) 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 (voxel size = $3.12 \times 3.12 \times 3.2 \text{ mm}^3$, slice thickness = 3.2 mm), TR = 1500 ms, TE = 25 ms, 73° flip angle. The first two images were discarded to allow for signal stabilization. A high-resolution T1-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 in the 60 Hz pupillary time-series 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 (3 from the Georgetown sample, 10 from the Pittsburgh sample). 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 from the Pittsburgh sample violated this criterion and were removed from further analysis. The average mean FD in the final sample ($N = 35$) was $0.16 \pm 0.1 \text{ mm}$. 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, the continuous time-series of 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). 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. Data acquisition site (Georgetown or Pittsburgh) was included as a covariate of no interest.

Correlation with individual differences in attention—Across the 20 participants from the Georgetown site (no participants from the Pittsburgh sample were included), 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 at 60 Hz. 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 $p < .05$) (Genovese *et al.*, 2002) (Figure 1, Table 1). Frontal lobe 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, Table 1).

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 2A). The resulting sympathetic and parasympathetic masks contained minimal overlap with each other (including the left brainstem extending into the amygdala, $k = 40$, and right inferior parietal lobe, $k = 9$). 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 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 ($t(17) = -3.10$, $p = .003$). Within participants included in attentional analyses, 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.

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, dlPFC, 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. Pupil variability, however, 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 or baroreflex rhythms, 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). It is important to note, however, that this common procedure may not rule out the influence of all physiological processes on our results. 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. Additionally, although the screen color was different between the Pittsburgh and Georgetown sites, only the Georgetown sample was included in attentional analyses. It is therefore unlikely that screen color drove our attentional findings. 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, reduced functional connectivity 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.

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Abbreviations

ACC	anterior cingulate cortex
ASL	applied science laboratories
ASRS	Adult ADHD Self-Report Scale v1.0
dIPFC	dorsolateral prefrontal cortex
FMRI	functional magnetic resonance imaging
GLM	general linear model
HRF	canonical hemodynamic response function
IPL	inferior parietal lobe
LC-NE	locus-coeruleus norepinephrine

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Figure 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.

Figure 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 (green) 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.

Table 1

Regions positively and negatively related to pupil diameter

Brodman areas (BAs) are labeled to the nearest gray matter, when available.

			MNI Coordinates			
Region Name	Brodman Area	Extent (voxels)	t-value	x	y	z
Regions Positively Related to Pupil Diameter						
L Middle Cingulate	31	23678	10.6572	0	-42	40
L Middle Cingulate	31		4.4068	-16	-30	42
L Middle Cingulate			8.8812	-8	-8	6
R Middle Cingulate	24		4.9289	2	10	42
R Thalamus			9.7115	16	-12	12
R Putamen			7.6425	14	4	-10
L Anterior Cingulate	32		6.4617	2	28	32
R Middle Frontal	24		6.3036	24	6	40
R Middle Frontal	8		3.6307	24	46	36
R Precuneus	29		5.7705	20	-46	12
R Precuneus	31		3.9389	10	-62	30
R Precuneus	7		2.6139	6	-68	50
L Insula			5.5935	-28	12	-4
L Hippocampus			5.2046	-28	-22	-8
R Hippocampus			3.0758	34	-14	-10
R Inferior Parietal	40		4.7056	46	-52	38
L Inferior Frontal			4.5144	-30	2	32
R Brainstem			4.281	3	-28	-22
L Middle Frontal	8	546	5.697	-30	34	36
Cerebellum (vermis)		297	4.5515	-2	-68	-12
L Cerebellum			4.2041	-16	-56	-20
Middle Frontal		393	4.0147	30	52	-12
R Inferior Temporal	37	179	3.9373	56	-48	-12
L Middle Frontal		23	3.6213	-28	42	-4

Region Name	Brodmann Area	Extent (voxels)	t-value	MNI Coordinates		
				x	y	z
L Inferior Parietal	40	150	3.4478	-50	-54	48
R Anterior Cingulate	24	180	3.3912	12	40	-4
L Anterior Cingulate	24	23	3.3428	-10	34	-4
R Insula		21	3.2644	46	2	14
L Middle Frontal	9	45	3.2638	-28	52	30
L Cerebellum		30	3.1061	-24	-38	-22
L Precuneus	19	28	2.9632	-42	-80	34
L Inferior Frontal	46	12	2.8149	-44	30	10
R Middle Frontal	46	11	2.7537	46	36	18
L Insula		8	2.7059	-34	24	10
Regions Negatively Related to Pupil Diameter						
L Fusiform Gyrus	19	11866	-11.0163	-32	-84	-10
L Fusiform Gyrus	37		-4.6885	-38	-54	-14
R Fusiform Gyrus	37		-6.1609	40	-52	-14
L Middle Occipital	17		-9.2198	-20	-86	18
L Middle Occipital	19		-6.8927	-48	-78	8
R Middle Occipital	18		-7.4165	26	-86	20
R Inferior Occipital	18		-8.6605	40	-84	2
R Lingual Gyrus	18		-7.0746	8	-70	2
R Cerebellum			-5.2967	16	-48	-4
R Superior Occipital	19		-4.9172	26	-78	46
R Postcentral Gyrus	40	289	-5.579	50	-24	52
R Inferior Parietal	7	127	-5.3195	30	-54	56
L Superior Parietal	7	151	-5.0968	-28	-54	56
L Postcentral Gyrus	3	643	-4.7076	-46	-16	44
L Middle Temporal	22	569	-4.6467	-62	-34	4
L Middle Temporal	22		-4.0755	-60	-10	-4
R Superior Temporal	22	220	-4.1114	54	8	-14

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MNI Coordinates				
Region Name	Brodmann Area	Extent (voxels)	t-value	$\bar{x}$ $\bar{y}$ $\bar{z}$
R Superior Temporal	22		-3.6781	66 -14 0
R ParaHippocampal		22	-3.8877	28 -16 -22
R Middle Temporal	21	11	-3.294	62 -6 -16