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Spontaneous pupil dilations during the resting state are associated with activation of the salience network

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ABSTRACT

Resting state functional magnetic resonance imaging (rs-fMRI) is increasingly applied for the development of functional biomarkers in brain disorders. Recent studies have revealed spontaneous vigilance drifts during the resting state, involving changes in brain activity and connectivity that challenge the validity of uncontrolled rs-fMRI findings. In a combined rs-fMRI/eye tracking study, the pupil size of 32 healthy subjects after 2 h of sleep restriction was recorded as an indirect index for activity of the locus coeruleus, the brainstem's noradrenergic arousal center. The spontaneous occurrence of pupil dilations, but not pupil size per se, was associated with increased activity of the salience network, thalamus and frontoparietal regions. In turn, spontaneous constrictions of the pupil were associated with increased activity in visual and sensorimotor regions. These results were largely replicated in a sample of 36 healthy subjects who did not undergo sleep restriction, although in this sample the correlation between thalamus and pupil dilation fell below whole-brain significance. Our data show that spontaneous pupil fluctuations during rest are indeed associated with brain circuitry involved in tonic alertness and vigilance. Pupillometry is an effective method to control for changes in tonic alertness during rs-fMRI.

Introduction

Over the last decades, resting state functional magnetic resonance imaging (rs-fMRI) has been increasingly applied to identify functional biomarkers for mental disorders and brain pathology. During rs-fMRI, subjects are typically instructed to rest inside the scanner for 5 to 10 min and to think of nothing in particular. Despite promising new insights yielded by this method, experimental control over the subject's actual behavior during the resting state is low. For instance, it is unclear how well subjects manage to keep their eyes open and, if a fixation cross is used, how stable their gaze remains over time. It has been shown that the blood oxygen level dependent (BOLD) signal in occipital regions differs systematically between an eyes-closed and fixation rs-fMRI condition (Bianciardi et al., 2009; Marx et al., 2004). Furthermore, a recent study about rs-fMRI connectivity patterns by Tagliazucchi and Laufs (2014) indicated that a considerable portion of (healthy) subjects falls asleep during typical resting state experiments, involving changes in cortical and subcortical connectivity (Sämann et al., 2010; Spoormaker et al., 2010). Such vigilance-dependent changes may become particularly problematic when comparing rs-fMRI findings between healthy subjects and (psychiatric) patients with hypo- or hyper-arousal symptomatology.

Simultaneous eye gaze tracking and pupillometry allows for better experimental control of such confounds. Previous studies have shown that the fluctuations in the size of the pupil reflect activity of the locus coeruleus (Alnaes et al., 2014; Aston-Jones and Cohen, 2005; Murphy et al., 2014; Rajkowski et al., 1993), a cluster of brainstem nuclei involved in the regulation of vigilance through noradrenergic modulation (Aston-Jones et al., 1991; Berridge and Waterhouse, 2003). The locus coeruleus (LC) is located in the dorsolateral pons and, being part of the ascending reticular activating system (ARAS), has extensive wakefulness-promoting projections throughout the brain (Aston-Jones and Cohen, 2005). It is reciprocally innervated by the orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC), which modulate LC function based on factors like goal-relevance (Aston-Jones and Cohen, 2005), motivational relevance (Mohanty et al., 2008) and conflict between competing responses (Sheth et al., 2012). The LC features both an excitatory connection to the sympathetic innervation pathway of the pupil (originating in the intermediolateral cell column of the spinal cord) and an inhibitory connection to the parasympathetic pathway (originating in the midbrain Edinger-Westphal nucleus; Szabadi, 2013). Consequently, increased LC activity involves increased sympathetic and decreased parasympathetic pupil innervation, causing a relative size increase (referred to as *dilation*) of the pupil (Szabadi, 2012). During low vigilance levels, the mean pupil diameter is reduced (Henson and Emuh, 2010; Ranzijn and Lack, 1997), reflecting low tonic firing rates of LC neurons and a dominant parasympathetic innervation of the pupil. Furthermore, during the transition from

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wakefulness to drowsiness, spontaneous fluctuations in pupil size have been reported to increase (Lowenstein et al., 1963; Wilhelm et al., 1998). This behavior of the pupil has been referred to as "pupillary unrest" or "pupillary fatigue waves" and attributed to an imbalance between the sympathetic and parasympathetic innervation (Loewenfeld, 1993; Warga et al., 2009; Wilhelm et al., 2001). The extent of these sleepiness-related pupil fluctuations is quantified by the "pupillary unrest index" (PUI), which has been proposed to indicate the subject's level of vigilance (Ludtke et al., 1998; Wilhelm et al., 1998).

To our knowledge, only two studies have addressed the link between pupil size and the blood oxygen level dependent (BOLD) signal during rs-fMRI: Murphy et al. (2014) included a regressor for pupil size together with the hemodynamic response function (HRF) derivatives in their analysis and reported activity in the ACC, right insular cortex, visual cortex and medulla. These HRF derivatives are typically included to capture the BOLD response despite unknown variations in its timing and duration (Wall et al., 2009). Yellin et al. (2015) found positively correlated activity in regions of the default mode network (DMN), as well as negatively correlated activity in visual and sensorimotor areas. In clinical, non-fMRI studies employing pupillometry, the dynamic increase and decrease in pupil size is frequently analyzed in addition to pupil size alone. For instance, a recent study by Muppidi et al. (2013) provided evidence that several indices from the constriction and dilation phases of the pupillary light reflex can be used to disentangle parasympathetic and sympathetic pupil function, enabling a distinction between patients with autonomic dysfunction and healthy controls.

The aim of this study was therefore to investigate the neural correlates of pupil size and of *changes* in pupil size observed during spontaneous pupil fluctuations in resting healthy subjects. Simultaneous electroencephalography (EEG)/fMRI studies focusing on vigilance fluctuations have observed activity decreases in thalamus with increasing drowsiness (Olbrich et al., 2009; Sadaghiani and D'Esposito, 2015; Sadaghiani et al., 2010), short duration sleep periods referred to as micro-sleeps (Poudel et al., 2014), and light sleep stage 1 (Kaufmann et al., 2006). Moreover, neural correlates of EEG-defined tonic alertness extend to dorsal ACC (dACC) and anterior insula (Sadaghiani and D'Esposito, 2015; Sadaghiani et al., 2010). Activity increase associated with increasing drowsiness is typically reported for visual and sensorimotor areas (Olbrich et al., 2009; Sadaghiani et al., 2010), regions that also have a negative correlation with eye closure (Marx et al., 2003; Ong et al., 2015; Poudel et al., 2010) and pupil size (Yellin et al., 2015). We therefore hypothesized that activity in thalamus, dACC and anterior insula (salience network) is positively associated with pupil size and/or change (spontaneous dilations), whereas activity in visual and sensorimotor regions will be negatively correlated to pupil size and/or change (spontaneous constrictions). Finding such tonic alertness correlates would support the notion that pupillometry is an alertness/arousal marker with a less complicated setup (and data post-processing) than simultaneous EEG/fMRI, a higher sensitivity than manual scoring of eye closures, and an established link to a well-defined neurobiological system (Aston-Jones and Cohen, 2005).

Additionally, given the evidence for vigilance-dependent changes in DMN connectivity (Ong et al., 2015; Sämann et al., 2010) and the proposed link between pupil size and DMN activity (Yellin et al., 2015), we further expected pupil size to have an influence on functional connectivity within the DMN. Furthermore, previous studies have shown that thalamo-cortical connectivity undergoes widespread decreases during the transition from wakefulness to light sleep (Spoormaker et al., 2010; Tagliazucchi et al., 2013). Hence, we aimed to examine whether such changes in functional connectivity can already be observed when subjects become drowsy, as indicated by a reduction in mean pupil size and the occurrence of sleepiness-related fluctuations. To increase variation in drowsiness and fluctuations in pupil size, a mild sleep restriction procedure was employed. Finally, we applied the same pupil/fMRI analyses to a replication sample of 36 healthy subjects (who did not undergo sleep restriction).

Materials and methods

Subjects

The study protocol was in line with the Declaration of Helsinki and was approved by a local ethical review committee. Subjects provided their written informed consent after the study protocol had been fully explained, and were reimbursed for their participation. Thirty-two non-smoking, right-handed participants (mean age: 25.9 ± 4.1 years, range: 18–35 years, 17 female) underwent a general medical interview and clinical MRI to exclude present and past neurological, psychiatric, and sleep disorders. Additional exclusion criteria were regular intake of medication (except contraceptives), a change of time zone or night shift work in the last four weeks, current pregnancy, and generally acknowledged contraindications to MRI. During the MR sessions, short-sighted subjects with less than −3.0 diopters wore contact lenses to ensure normal vision.

Subjects were asked to refrain from caffeine consumption on the day of the experiment and to get up 2 h earlier than they would on a regular weekday. The aim of this mild sleep restriction procedure was to increase the variance of drowsiness levels among subjects. To assure an intact circadian rhythm, participants filled out a sleep diary for at least four nights before the MR session. The sleep diary assessed, among others, bed time, time of falling asleep, wake-up time and total sleep time. For each subject, sleep diary parameters (bed time, time of falling asleep and sleep duration) were averaged across the nights preceding the sleep restriction night and compared to the corresponding parameters for the sleep restriction night using paired *t*-tests.

Procedure

All subjects completed two resting state scans of 12 min each during which they were instructed to think of nothing in particular, to maintain their gaze on a red fixation dot presented on a black background, and to not fall asleep. If subjects had their eyes continuously closed over a period of more than 1 min without reacting to verbal instructions ("Please keep your eyes open"), the session was aborted. Sessions which required such verbal interventions were later excluded from analysis.

Pupil fluctuations as a marker of vigilance level are usually measured in total darkness in order to avoid the simultaneous occurrence of similar-appearing, so-called *light-induced* pupil oscillations (Loewenfeld, 1993; Warga et al., 2009) which do not relate to vigilance level. This type of pupil fluctuation can be observed at constant light intensities and has been associated with feedback mechanisms of the pupillary light reflex pathway (Longtin and Milton, 1989; Longtin et al., 1990; Warga et al., 2009). Since recordings in total darkness might not always be feasible in the MRI environment, we used two rs-fMRI sessions that differed only in their illumination settings to assess the potential influence of light-induced fluctuations on sleepiness-related ones: one run was conducted in darkness with lights in the scanner room and inside the scanner switched off ("dark condition"), while the other was conducted in an illuminated scanner environment, that is, lights in the scanner room and inside the scanner were switched on ("light condition"). The order of illumination condition was counterbalanced across subjects and established at least three to 4 min before the start of each run to ensure accommodation of the pupil. The fixation dot was presented with Presentation Software (version 16.3, Build 12.20.12, Neurobehavioral Systems Inc., Berkeley, California, USA) using a projector outside the MRI scanning room that displayed the stimuli onto a translucent screen located at the end of the scanner bore. Participants viewed the fixation dot through a first-surface reflecting mirror attached to the head coil. The color and luminance of the fixation dot were chosen in a way that subjects were able to see it clearly in both lighting conditions.

MR sessions were conducted during the afternoon. Participants were asked to rate their sleepiness level on the Karolinska Sleepiness Scale