

COGNITIVE NEUROSCIENCE

Slow fluctuations in eye position and resting-state functional magnetic resonance imaging brain activity during visual fixation

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Abstract

The neuronal circuitry that supports voluntary changes in eye position in tasks that require attention-driven oculo-motor control is well known. However, less is known about the neuronal basis for eye control during visual fixation. This, together with the fact that visual fixation is one of the most commonly used baseline conditions in resting-state functional magnetic resonance imaging (fMRI) studies, prompted us to conduct a study in which we employed resting-state fMRI and concurrent recordings of eye gaze to investigate the relationship between spontaneous changes in eye position during passive visual fixation and intrinsic brain activity. As a control experiment, we recorded fMRI brain activity related to cued voluntary vertical and horizontal changes in eye position in a block-related task-evoked fMRI experiment. Our results for the voluntarily performed changes in eye position elicited brain activity in the bilateral occipitotemporal cortex, supplementary motor cortex and frontal eye fields. In contrast, we show that slow fluctuations in eye position during passive visual fixation are linked to intrinsic brain activity, foremost in midline cortical brain regions located in the posteromedial parietal cortex and the medial prefrontal cortex, brain regions that act as core cortical hubs in the brain's default mode network. Our results suggest that subconscious and sustained changes in behavior are tied to intrinsic brain activity on a moment-by-moment basis.

Introduction

Since the seminal result presented almost 20 years ago that resting-state functional magnetic resonance imaging (fMRI) activity is correlated in time across the bilateral sensorimotor cortex (Biswal *et al.*, 1995), research on intrinsic fMRI activity has steadily gained interest. In the last decade, research on slow, task-unrelated fluctuations in blood oxygen level-dependent (BOLD) fMRI activity has flourished and its applicability in neurological disorders, aging and brain development has increasingly been targeted (Fox & Raichle, 2007; Zhang & Raichle, 2009). Although previous studies have shown that the resting brain is hierarchically organized into large-scale cortical networks encompassing long-range anatomical pathways of functional relevance (Greicius *et al.*, 2003; Fransson, 2005; Damoiseaux *et al.*, 2006), the physiological and behavioral significance of the brain's so-called 'dark energy' (Raichle, 2010) has remained elusive. Previous work has shown that baseline spontaneous fMRI activity influences the trial-to-trial variability of BOLD responses to evoked stimuli (Fox *et al.*, 2007) and that ongoing intrinsic activity biases perceptual decision-making (Hesselmann *et al.*, 2008). However, the experimental support for a sustained coupling between spontaneous fMRI brain activity and physiological responses during resting-state conditions is so far scarce.

The large majority of resting-state fMRI studies published have typically employed baseline conditions where the subjects are either instructed to rest with their eyes closed or told to fixate their vision on a centrally positioned cross-hair on a blank screen. In this study, using a methodological platform that involves concurrent recording of eye position and resting-state fMRI scanning during visual fixation, we tested the hypothesis that slow and involuntary executed changes in eye position during passive visual fixation are linked to resting-state fMRI activity in the human brain.

To test our hypothesis, we simultaneously recorded fMRI activity and eye position of the right eye in 17 subjects during two separate resting-state sessions for which subjects were asked to fixate on a centrally displayed cross-hair. In addition, we recorded brain responses elicited by externally guided changes in eye position in 15 of the participants. Our results show that slow, spontaneously performed changes in eye position during visual fixation are linked to intrinsic spontaneous activity in the medial posteromedial parietal cortex and the medial prefrontal cortex. Furthermore, our results suggest that brain activity related to unconscious neuronal processes produces a behavioral response that can be measured in a laboratory setting.

Materials and methods

Participants

A total of 24 subjects participated in the study (mean age: 26.7 ± 4.9 years, 12 females). All subjects had normal or corrected

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vision and had no prior or present history of neurological or psychiatric disease. The study was approved by the local ethics committee in Stockholm (DNR 2012/693-31/2) and all subjects gave their written informed consent in accordance with the declaration of Helsinki (1964).

MRI

MRI was performed on a 3T General Electric 750 MR scanner installed at the MR Research Center, Karolinska Institute, Stockholm. Anatomical MRI included a T2-weighted FLAIR sequence as well as a high-resolution BRAVO 3D T1-weighted image sequence ($1 \times 1 \times 1 \text{ mm}^3$ voxel size). In each subject, four fMRI sessions were performed (echo-planar imaging, TR/TE = 2500/30 ms, flip = 90° , 49 slices, 96×96 matrix size, field of view = 288×288 mm, slice thickness = 3 mm, interleaved slice acquisition). Two of the fMRI sessions probed slow spontaneous changes in eye position that each lasted for 8 min and 20 s (200 image volumes, in total 16 min and 40 s of passive fixation fMRI for each subject). The remaining two fMRI sessions investigated externally cued changes in eye position in the horizontal (X) and vertical (Y) direction respectively (4 min and 24 s, 106 image volumes). In total, 612 functional image volumes were acquired in each subject. During fMRI scanning, breathing and peripheral pulse were measured using parts integrated with the General Electric MR scanner. Breathing was recorded at a sampling rate of 40 ms using a pneumatic belt around the subject's chest. The peripheral pulse was sampled at a rate of 10 ms using a pulse-oximeter attached to the subject's right index finger. Four dummy scans were acquired before each fMRI recording to reach T1-relaxation equilibrium conditions.

Pre-processing realignment estimates of echo planar imaging (EPI) showed substantial head movement in one subject and data from this subject were excluded from analysis. The eye-recording equipment malfunctioned in one subject. In five subjects, marked signs of sleepiness and drowsiness were observed throughout the scanning sessions that resulted in poor quality of the eye position data as the eye lids covered too much of the eye. Hence, data from these subjects were not used in the analysis. In eight subjects, parts of the data were excluded from analysis for the same reason. In summary, passive fixation resting-state fMRI data and the corresponding recordings of eye position from 17 subjects were used in the analysis and the amount of data from each subject included in the analysis is given in the first column in Supporting Information Table S1.

Visual presentation setup and recording of eye position

During fMRI sessions, changes in eye position in the horizontal (X) and vertical (Y) direction were recorded from the subject's right eye using an MRI-compatible eye tracking camera mounted directly onto a visual presentation fMRI goggle system (NNL, Nordic Neuro Lab, Bergen, Norway). Position changes of the eye in the X - Y plane was computed online and sampled at 60 Hz using the image-analysis software ViewPoint from Arrington Systems (Scottsdale, AZ, USA) that is integrated with the visual presentation/eye tracking hardware. Data from the right eye were calibrated, recorded and saved separately for offline analysis. The vision of the subject's left eye was occluded by an eye-patch. The visual presentation system provided 800×600 -pixel resolution with a field-of-view that nominally spans 23.5° in the vertical direction and 30° in the horizontal direction. The ViewPoint software saves the eye's gaze position in X and Y coordinates in a screen reference format. With regard to quantifying changes in eye position, we decided to keep the screen

coordinate format when reporting magnitude and direction changes in eye position. Our reason for doing so is that the amplitude/angle of eye movements expressed in degrees will depend on the distance as well as the angle between the infrared (IR) camera and the subject's eyes, which in turn depends on the flexibility of the IR recording system mounted on-top of the head coil to fit to the geometry of each subject's head size and position inside the MR head-coil. Given this added uncertainty in estimating changes in eye position in angular units, we opted to report magnitude changes of eye position in the screen reference system provided by the eye tracker system.

In the passive fixation sessions, the subjects were instructed to keep their vision focused on a black cross-hair positioned centrally on a gray background. For the externally guided changes in eye position sessions, the fMRI paradigm followed a standardized block-related paradigm in which blocks of fixation (centrally positioned cross-hair, 24 s) were interleaved with blocks of either horizontal or vertical changes in eye position, akin to the experimental design used by Alkan *et al.* (2011). During blocks of externally cued changes in eye position, the fixation cross-hair was shifted in steps either to the left (up) or right (down) relative to the central fixation point of reference in a sequential order (i.e. upward shift, central position, downward shift, central position, etc.). The duration of each visual shift was randomized between 0.5 and 2 s in intervals of 0.5 s so that the subjects could not anticipate when a shift in fixation was to occur. In each cued block of change in eye position, 22 visual step stimuli were presented during which the position of the fixation cross on the screen was changed sequentially by -250 , 0 or $+250$ pixels horizontally or vertically, corresponding to a nominal change in saccade magnitude of approximately 9° . The achieved precision in our experimental set-up to record changes in eye position during fixation inside the MR scanner was estimated by sampling the X and Y position for a stationary object. In our case we used a schematic drawing of an eye that was positioned adjacent to the lens of the goggle/IR camera (see Fig. S1). The measured signal variability expressed as the standard deviation of the position of a stationary eye was 0.0005 and 0.0015 in the X and Y direction, respectively (6.5 min recordings during which 23 500 sampling points were acquired).

Image pre-processing

All MRI pre-processing, image modelling and analysis of changes in eye position were done in MATLAB 11a (Waltham, MA, USA) using publicly available software packages and custom built routines. fMRI volumes were realigned, co-registered and normalized to the MNI (Montreal Neurological Institute) EPI template within SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>, Wellcome Trust Center for Neuroimaging, London, UK) with a voxel size of $3 \times 3 \times 3 \text{ mm}^3$. The normalized functional EPI volumes were spatially smoothed using a kernel of 8 mm full width half maximum.

Analysis of recordings of changes in eye position

Data from the eye tracker camera related to changes in position for the right eye in the vertical and horizontal direction during the fixation sessions were processed as follows. First, the time-series data were visually inspected and the temporal first derivative of the eye tracker signal was computed. Based on the visual inspection as well as the amplitude of the first derivative of the signal, spikes related to eye blinks or brief elapses of a lost tracking of the pupil by the eye tracking software were detected and subsequently removed by linear

interpolation. The amount of data in each subject affected by the presence of spikes and subsequently linearly interpolated is given in Table S1 (column DD). Second, the signal time series for each component of eye position were de-trended to account for baseline signal drift (second-order polynomial regression). Next, the time series were convolved with the canonical hemodynamic response function as defined in SPM. Subsequently, the X and Y components of change in eye position were down-sampled to 0.4 Hz to match the fMRI sampling frequency using the Matlab function 'interp'. As we did not have any *a priori* hypothesis regarding the directionality of spontaneous changes in eye position during fixation, we performed a principal component analysis (PCA) (see also Friston *et al.*, 1993) of the X and Y components in each data set. The validity of using a PCA on the X and Y components to capture changes in eye position during fixation in a single regression vector is also supported by the large degree of correlation between the X and Y components of the change in eye position that was found in most subjects (see also last column in Table S1). Averaged across subjects, the first PCA component explained 87% of the total variance related to slow fluctuations of the position of the eye during visual fixation. Consequently, we henceforth used the first PCA component to represent change of eye position in the X - Y plane as our parameter of interest in the analysis.

Analysis of brain activity related to slow, spontaneous change in eye position

To test our hypothesis that slow, spontaneous change in eye position is linked to resting-state fMRI brain activity, the first PCA component of change of position in the X - Y plane recorded during visual fixation was inserted into a general linear model (GLM) of fMRI brain activity as implemented in the framework of SPM8. Residual signal variance due to translational and rotational head movement was modeled by including six movement regressors of no interest in the model. Moreover, it is known that intrinsic, spontaneous fMRI activity might be confounded by signal variance related to spurious signal components residing foremost in cerebrospinal fluid (CSF), vascular space and white matter (see Birn, 2012 for a recent review). To account for non-neuronal signal contributions in our resting-state fMRI analysis, we adopted a strategy to use a PCA-based noise reduction method (CompCor; Behzahi *et al.*, 2007) recently instantiated in the CONN toolbox in MATLAB (Chai *et al.*, 2012; Whitfield-Gabrieli *et al.*, 2012). The CompCor method corrects for signal variability linked to non-neuronal sources by regressing out principal signal components that stem from voxels inside white matter and CSF based on the segmentation of the individual T1-weighted anatomical MR image volumes (Whitfield-Gabrieli *et al.*, 2012). In our analysis, we chose to retain the three largest principal components for white matter and CSF space respectively as co-variables of no interest in the GLM analysis. We also opted to include the mean global signal in the model as recent research has shown that global signal regression is a powerful procedure to eliminate motion-related variance (Power *et al.*, 2014). To ensure that our results were not biased by global mean signal regression, we additionally conducted all analyses without removal of the mean global signal. In the analysis without the global signal, we obtained very similar results in terms of brain activity patterns. Besides the potential influence from global, CSF and white matter tissue signal contributions in the BOLD signal, effects from respiration and cardiac pulsations might degrade the BOLD activation maps. We therefore constructed regressors of no interest related to respiration and cardiac pulsations that were calculated from the sampled physiological

parameters collected during fMRI scanning. Based on the RETROICOR method, we used the PhLEM package (Verstynen & Deshpande, 2011) running in MATLAB to construct three regressors that modeled respiration and cardiac pulsations during passive visual fixation. In total, the GLM to estimate intrinsic brain activity related to slow, spontaneous changes in eye position contained one regressor of interest and 16 co-variables of no interest. All statistical maps of brain activity were corrected for multiple comparisons with a significance level of $P < 0.005$ uncorrected at peak level and $P < 0.05$ FDR-corrected (false discovery rate) at cluster level.

Subject movement and spontaneous change in eye position

fMRI signal change related to residual head movement is an issue of methodological concern that needs to be taken into account in every fMRI study. Using a comprehensive set of regions of interest positioned throughout the brain, Power *et al.* (2012) showed that seed-based correlation maps of functional resting-state connectivity are vulnerable to head displacement and rotation. Importantly, their study suggested that residual movement-related fMRI signal variance leads to an under-estimation of long-range connectivity and over-estimation of local network resting-state connectivity. Although the present study does not rely on seed-based correlation resting-state analysis, we nevertheless investigated to what degree residual movement-related image signal variance co-varied with recorded X and Y position of the right eye during visual fixation as well as during the cued task. Overall, the degree of estimated subject movement during both the resting-state passive fixation condition and the externally guided tasks was small in all 17 subjects. The maximal translational movement in each subject was on average less than 1 mm in all directions and the rotational movement less 1° in all directions for all subjects. In detail, the mean and standard deviation of the maximal translational and rotational head movement across subjects for the cued task-evoked fMRI sessions along the vertical axis were (corresponding estimates for the horizontal saccade task are given in parentheses): $\max(x) = 0.18 \pm 0.11$ (0.19 \pm 0.11) mm, $\max(y) = 0.19 \pm 0.18$ (0.21 \pm 0.21) mm, $\max(z) = 0.45 \pm 0.36$ (0.36 \pm 0.14) mm, $\max(\text{rot}(x)) = 0.70 \pm 0.58$ (0.57 \pm 0.39)°, $\max(\text{rot}(y)) = 0.26 \pm 0.16$ (0.28 \pm 0.19)°, $\max(\text{rot}(z)) = 0.34 \pm 0.33$ (0.22 \pm 0.23)°. The mean and standard deviation of the maximal translational and rotational head movement across subjects for the passive fixation sessions across subjects were: $\max(X) = 0.34 \pm 0.21$ mm, $\max(y) = 0.38 \pm 0.24$ mm, $\max(z) = 0.72 \pm 0.53$ mm, $\max(\text{rot}(x)) = 0.97 \pm 0.56$ °, $\max(\text{rot}(y)) = 0.46 \pm 0.35$ °, $\max(\text{rot}(z)) = 0.51 \pm 0.30$ °.

For the passive visual fixation sessions, there was a small but significant correlation (Fischer r -to-log transformation, Student's t -test) between the vertical component of the eye position and image translation in the z -plane ($r = 0.078$, $P < 0.0035$, $T = 2.3$) as well as between the vertical component of eye position and rotation of head with respect to the x -axis ($r = -0.10$, $P < 0.00017$, $T = 4.87$). In addition, there was a correlation between the horizontal component of eye position and rotation of the head along the Z -axis ($r = -0.15$, $P < 0.0004$, $T = 4.44$). All other correlations between head movement (translation in x -, y - and z -planes, rotational movement around x -, y - and z -axes) and the measured changes in eye position in X and Y were non-significant. Moreover, for the externally guided horizontal changes in eye position, we found significant correlations between the horizontal component of eye position and head displacement in the x - ($r = -0.07$, $P < 0.04$, $T = 2.3$) and y -planes ($r = 0.11$, $P < 0.02$, $T = 2.65$) as well as between the X component and head rotation around the x -axis ($r = -0.29$,

$P < 0.01$, $T = 2.97$). For the vertical cued eye position change fMRI session, we observed a significant correlation between head rotation around the x -axis ($r = -0.34$, $P < 0.0008$, $T = 4.41$). Our finding of co-variability between head and changes in eye position suggests that, regardless of whether changes in eye position take place spontaneously or are externally guided, subjects perform small movements of their head that follow the direction of eye gaze. Thus, one has to consider the possibility that brain activity patterns during passive fixation can partly arise due to minuscule changes in head movement. However, for several reasons we find this scenario to be unlikely. First, if the brain activation pattern observed during passive viewing were simply an effect of head movement, the same activation patterns should also appear during the externally guided sessions, which in fact displayed very similar correlations between changes in eye position and head movements. However, this was not the case. Second, we conducted group-level analyses on statistical parametrical maps based on individual single subject GLM analysis in which estimates of subject head motion in the z -plane and rotation around the x -axis were used as regressors of interest. The resulting group-level brain activity pattern related to translative motion along the z -axis as well as rotational movement around the x -axis did not spatially overlap with the activation patterns related to slow changes in eye position shown in Fig. 3D. Third, we performed permutation analyses in which the subject-specific regressors related to spontaneous change in eye position were randomly

assigned to different subjects in the cohort and subsequently analysed at a second level. None of the group results based on permuted regressors produced any significant brain activity pattern at the chosen threshold ($P < 0.005$ uncorrected at peak level and $P < 0.05$ FDR-corrected at cluster level). These observations, together with the fact that the overall amplitude of head movements was small, favor the idea that the change in eye position observed during passive visual fixation is related to resting-state brain activity and not simply due to small changes in head position.

Results

The position of the right eye was recorded simultaneously with EPI BOLD imaging of the brain during resting-state conditions (passive fixation) as well as during task sessions that were composed of blocks of externally guided changes of eye position interleaved with blocks of fixation. The task sessions included blocks of either vertical or horizontal displacement of a cross-hair, which elicited marked change in the position of the right eye as shown for one subject in Fig. 1A and B. Brain activity related to cued externally guided changes in eye position were assessed at the individual level by comparing the level of brain activity during blocks of task compared with fixation in a GLM. At a group level, task-evoked fMRI of guided changes in eye position in the vertical direction showed a widespread pattern of brain activity that encompassed the primary

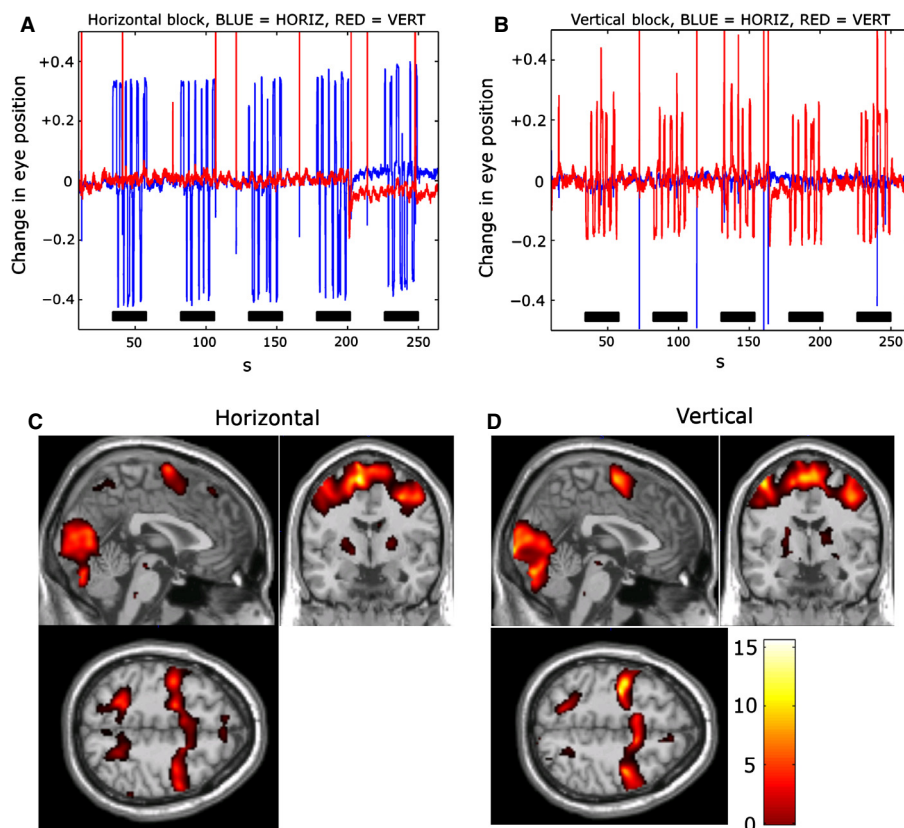


FIG. 1. Change in eye position and fMRI responses during externally guided horizontal (left/right, A and C) and vertical (up/down, B and D) changes in eye position. (A) The cued change in horizontal (BLUE) and vertical (RED) position for the right eye in a single subject in response to horizontal displacements of a cross-hair in a block-related fMRI design (task periods marked with filled boxes). Similarly, (B) shows the cued change in horizontal (BLUE) and vertical (RED) position of the right eye (same subject) in response to vertical displacement of the cross-hair. The time-series shown in A and B are the mean-centered and de-trended time-series. The degree of movement (Y -axis in A and B) is given in the reference coordinate system provided by the eye tracking software. (C, D) (horizontally cued eye position change, vertically cued eye position change) fMRI activity at a group level (15 subjects, $P < 0.005$ uncorrected peak level, $P < 0.05$ FDR-corrected at cluster level) in the occipitotemporal cortex, frontal eye fields, supplementary motor area and cerebellum.

and extrastriatal areas of the visual cortex (BA 17/18/19, local peak maxima at $[X,Y,Z] = [0, -80, -22]$, $Z = 4.72$), bilateral occipito-temporal cortex (BA 37, $[44, -60, 8]$, $Z = 4.39$; $[-48, -80, 2]$, $Z = 4.33$), left and right frontal eye fields (BA6, $[-32, -12, 50]$, $Z = 5.22$; $[38, -8, 48]$, $Z = 5.06$), supplementary motor area ($[-4, -6, 64]$, $Z = 5.30$) as well as the left and right cerebellum ($[-36, -60, -32]$, $Z = 4.53$; $[34, -58, -34]$, $Z = 4.21$) as shown in Fig. 1C. Guided change in eye position along the horizontal (left/right) axis (Fig. 1D) yielded a very similar network of brain activity compared with guided change of eye position along the vertical direction. The reversed contrast, i.e. brain regions that are more activated during fixation compared with task revealed activity in the default mode network (DMN), foremost in its posterior sub-network, including the bilateral posterior parietal cortex and the precuneus/posterior cingulate cortex. Our results from the task-evoked fMRI sessions are in good agreement with what has previously been reported for cued change in eye position (Berman *et al.*, 1999; Alkan *et al.*, 2011).

Next, we computed the spectral power diagram for the pre-processed time-series of the vertical and horizontal changes in eye position separately. The individual spectral power profiles were subsequently averaged across subjects and the result from the group analysis is displayed in Fig. 2. Low-frequency fluctuations in eye position, recorded during visual fixation and BOLD image data acquisition, are substantially larger along the vertical axis (marked in red in Fig. 2) than along the horizontal axis (marked in blue). The larger spectral power of oscillatory changes in eye position along the vertical axis is supported by the fact that the variance of the recorded time-series of eye position changes along the vertical axis is on average about seven times larger than for the horizontal axis (see Table S1). The substantially larger variability of change of eye position in the Y -plane compared with the X -plane is also well in line with the result that the first component yielded from PCA of the change in eye position in the X - Y plane is on average able to capture 87% of the signal variance. Moreover, the spatial asymmetry of the spectral power of changes in eye position for subjects laying in a supine position inside the MR scanner is well in line with previous observations that have measured slow fluctuations in eye position in subjects who were examined in an upright, sitting position conducted in a behavioral laboratory setting (Zhang *et al.*, 2011).

The unfiltered as well as the de-spiked and filtered eye tracking recordings during fixation are shown in Fig. 3A and B (one subject),

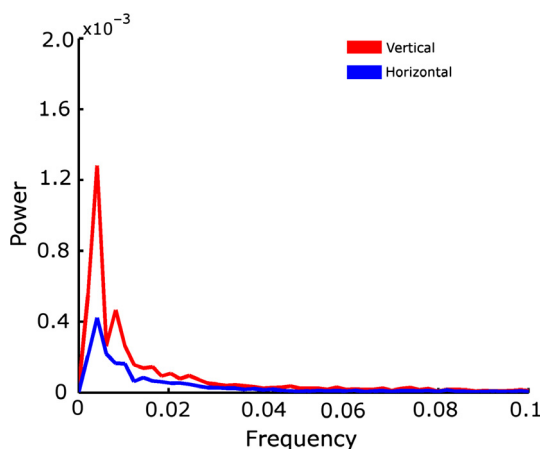


FIG. 2. Spectral power plot for slow, spontaneous fluctuations in eye position (horizontal changes in eye position shown in BLUE, vertical changes in RED) during passive visual fixation in the frequency interval 0–0.1 Hz. The plot shows the average power spectrum (arbitrary units) for 17 subjects.

together with a 3D graph that shows the trajectory of eye position change in space–time (Fig. 3C). Apart from spikes due to eye blinks (shown in blue) the eye position trajectories presented in Fig. 3A–C show that the eye during resting-state conditions exhibits slow fluctuations in position. Note that an increase of the signal signifies an upward shift of the eye position along the vertical (Y) axis (Fig. 3B) whereas an increase in the signal along the horizontal axis (X) denotes a leftward shift in eye gaze (Fig. 3A). Similar spontaneous fluctuations of eye position during resting-state and visual fixation were observed in all participants, as shown in Fig. S2. The de-trended, de-spiked, low-pass-filtered and down-sampled eye-tracking signal time-courses (X,Y) recorded during each passive fixation resting-state fMRI session were used as input to a PCA. Subsequently, the first principal component was used as a regressor of interest in a GLM of brain activity related to spontaneous change in eye position during visual fixation. In addition to the eye position-related regressor, non-neuronal signal sources such as subject movement, breathing, cardiovascular pulsations, and signal contributions from the cerebrovascular space and white matter were included in the model as regressors of no interest (see also Materials and methods). Figure 3D shows that spontaneous fluctuations in eye position during passive fixation significantly correlate with brain regions located in the posterior–medial parietal cortex, including the precuneus/posterior cingulate cortex (BA 31/7, $[-2, -46, 38]$, $Z = 3.08$; $[-4, -46, 24]$, $Z = 3.0$) and the medial prefrontal cortex (BA 9, $[-20, 42, 22]$, $Z = 3.58$; $[2, 54, 20]$, $Z = 3.47$). Additionally, there was a supra-threshold activation in the left dorso-lateral prefrontal cortex (BA 46/45, $[-560, 16, 40]$, $Z = 3.33$, $P < 0.005$ uncorrected).

Discussion

Our study showed marked differences in brain activity that are involved in externally guided changes in eye position versus spontaneous fluctuations in eye position during passive fixation. Externally cued changes in eye position elicited brain activity in occipital, parietal and frontal cortices, a brain network that is well known to be involved in visual attention and oculomotor planning (Kastner & Ungerleider, 2000). Intriguingly, slow fluctuations in eye position during visual fixation were correlated with brain activity in midline brain structures that are known to be key nodes in the default mode network, including the posteromedial parietal cortex and the medial prefrontal cortex (Raichle *et al.*, 2001; Buckner *et al.*, 2008; Fransson & Marrelec, 2008). It has previously been shown that slow oscillations in the BOLD signal are synchronized among the nodes in the default network during rest (Greicius *et al.*, 2003; Fox *et al.*, 2005; Fransson, 2005). Our results suggest that intrinsic BOLD fMRI activity in the midline regions of the default mode network are linked to an oculomotor response in the form of slow fluctuations in eye position during passive visual fixation.

A central question is whether slow ocular fluctuations serve a specific purpose from a low-level visual processing point of view? In this context, it is noteworthy that slow fluctuations in eye position show similar characteristics to the so-called fixational drifts that occur between microsaccades (Schulz, 1984; Martinez-Conde *et al.*, 2004), albeit at a much slower time scale. During fixation, the drift moves the stationary object of interest across the retina and hence prevents it from perceptual fading due to retinal adaptation. Thus, drifts are for this purpose of great importance for normal visual function. The question of the underlying neuronal control of drift is currently unknown and it has received relatively little attention in the past, perhaps due to the fact that it was long thought to be random (Ditchburn & Ginsborg, 1953). However, drifts are assumed to be

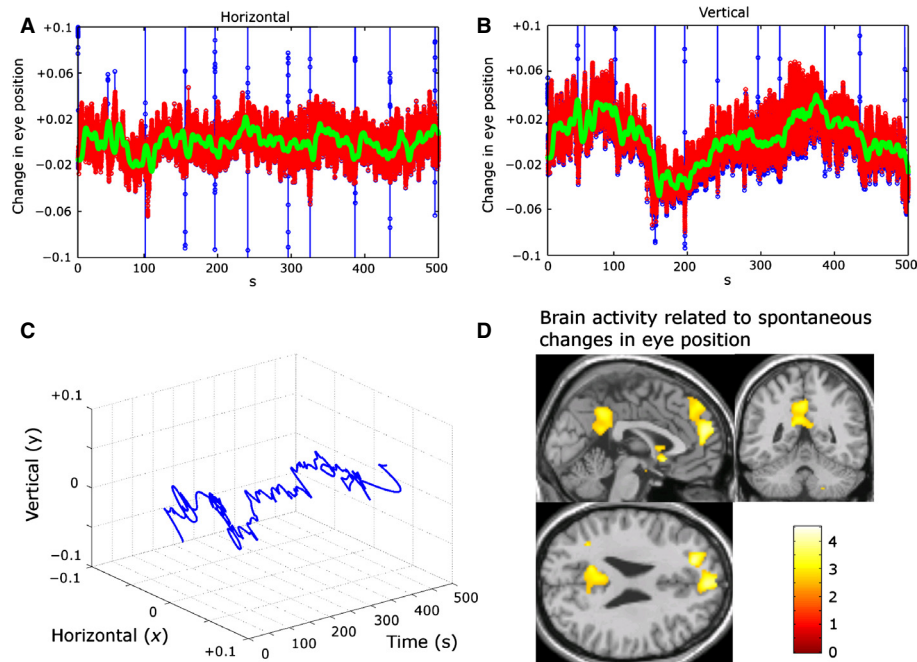


FIG. 3. Change in horizontal (left/right, A) and vertical (up/down, B) eye position for the right eye in a single subject during passive visual fixation (same subject as shown in Fig. 1). The time-series in blue shows the unfiltered eye tracker data (mean-centered, linear and quadratic trends removed). The signal shown in red depicts the recording with signal spikes removed whereas the signal shown in green is the de-spiked signal after convolution with the hemodynamic response function. The amplitude of eye position change (Y -axis in A and B) are given in the reference coordinate system provided by the eye tracking system. (C) The trajectory of the position of the right eye in a 3D-plot for which the convoluted time-series shown in green A and B are plotted against time (z -axis). (D) Brain activity elicited by slow, spontaneous changes in eye position during passive fixation at a group level. Activity is present in the postero-medial parietal cortex and the medial prefrontal cortex ($P < 0.005$ uncorrected peak level, $P < 0.05$ FDR-corrected at cluster level).

under 'slow-control' as a six-fold increase in variability (Skavenski & Steinman, 1970) and a five- to ten-fold increase in velocity of drifts (Skavenski & Steinman, 1970; Becker & Klein, 1973) has been observed in the absence of a fixation target, which presupposes a hitherto unknown inhibitory neuronal control mechanism of drift during active vision. Interestingly, a similar fixational instability has also been observed in the afoveate rabbit (Collewijn, 1970). Following this line of reasoning, the suggestion would then be that resting-state cortical activity in the midline regions of the default network acts as a control mechanism for the fixational drift component. All sensory systems habituate over time and need to be continuously updated. From a brain system level perspective, a coupling to resting-state brain activity would ensure that the eyes never stop moving and hence optimizing retinal stimulation and visual function during normal conditions. Moreover, resting-state network activity has a temporal profile that fits this purpose in that an ocular movement is generated that on the one hand is not too fast so that it interferes with visual perception [contrast vision $>5^\circ/s$ (Burr & Ross, 1982); visual acuity $>2.5^\circ/s$ (Westheimer & McKee, 1975)] and on the other hand is sufficiently fast to prevent retinal suppression.

However, other candidates for the neuronal mechanism behind the observed coupling between resting-state fMRI activity and slow fluctuations in eye position during passive viewing need to be considered. The suggestion that brain activity in the midline hubs of the default network is coupled to aspects of low-level visual processing is at odds with the fact that a large body of previous work has shown that the default network is foremost involved in higher-order cognition with a preference for self-referential cognitive processes (e.g. Buckner *et al.*, 2008). Thus, at first glance it would seem more likely to expect the neuronal control for slow changes in eye position during fixation to be localized to visual cortices and attention-related cortical networks. However, a recent study has

implicated altered functional resting-state connectivity between midline regions of the DMN in a task that involve saccadic eye movement recorded in Parkinson's disease (PD) (Gorges *et al.*, 2013). They reported a decrease in functional connectivity within nodes of the DMN that correlated with oculomotor performance in PD patients. Specifically, PD patients showed a negative correlation between accuracy in main saccades and functional connectivity between the posterior cingulate cortex/precuneus and medial temporal lobe (Gorges *et al.*, 2013). Furthermore, the authors conclude that functional resting-state connectivity within the DMN is involved in PD-associated saccadic hypometria, in particular in the vertical direction. Moreover, it is important to keep in mind that slow, spontaneous changes in eye position are minuscule oscillations in eye position that we most likely do not experience consciously. Its subconscious nature and its anatomical localization to the midline nodes of the default network are two major facets of slow fluctuations in eye position that suggest that they represent a behavioral manifestation of the temporal dynamics of the brain's large-scale intrinsic network architecture at a subconscious level.

Interestingly, a recent study showed that brain activity in the default mode network increases after spontaneous, involuntary eye-blinks that occurred while viewing videos (Nakano *et al.*, 2013). Moreover, a study by Ramot *et al.* (2011) recorded electro-ocular activity during resting-state fMRI when the subjects kept their eyes closed. By using the absolute value of the amplitude of the horizontal electro-ocular activity as a regressor in their model of brain activity, they showed that spontaneous electro-ocular activity elicits distributed patterns of activity in occipital and parietal cortices. Taken together, these findings argue for the notion that spontaneous changes in behavior are linked to spontaneous fluctuations in resting-state brain activity. Further, it raises the interesting possibility that there exists a functional interaction between intrinsic brain

activity and moment-by-moment changes in subconscious behavior that is manifested as slow oscillatory changes in eye position, electro-ocular motor activity and spontaneous eye blinks. Whether such interactions are an epiphenomenon of the dynamics of intrinsic large-scale neuronal network activity or represent a behaviorally meaningful aspect of the computational architecture of the brain as recently suggested (Sadaghiani & Kleinschmidt, 2013) is an open question that needs to be further investigated.

Although we explicitly tested for the influence of head motion on the BOLD activation maps related to spontaneous changes in eye position during visual fixation, recent research suggests that head motion does not necessarily in its entirety lead to artificial changes in intrinsic connectivity. Interestingly, results from two recent studies suggest that head movement can be linked to genuine changes in functional connectivity that is system-specific to several resting-state networks, particularly so for the sensorimotor networks and the DMN (Pujol *et al.*, 2014; Zeng *et al.*, 2014). Thus, we cannot rule out the possibility that slow changes in eye position that occur during fixation at least in part are synchronous with small movements of the head, movements which in themselves have a neuronal origin.

In summary, our results suggest that subconscious behavioral responses that continuously vary over time are linked to intrinsic brain activity. Specifically, our findings suggest that intrinsic activity in midline regions of the brain's default mode network play a part in mediating slow, unconscious changes in eye position during visual fixation.

Supporting Information

Additional supporting information can be found in the online version of this article:

Fig. S1. Horizontal (*X*) and vertical (*Y*) position measurements for a stationary object (schematic drawing of a human eye) attached to the lens of the goggle eye tracking device inside the MR scanner during 6.5 min of continuous recording.

Fig. S2. Change in horizontal and vertical eye position during passive visual fixation for all 17 subjects. The time-series shown was de-spiked, de-trended and convoluted with the hemodynamic response function as defined in SPM8.

Table S1. Statistics for pre-processing and data sampling of horizontal (*X*) and vertical (*Y*) eye movements during visual fixation.

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Abbreviations

BOLD, blood oxygen level-dependent; CSF, cerebrospinal fluid; EPI, echo planar imaging; FDR, false discovery rate; fMRI, functional magnetic resonance imaging; GLM, general linear model; IR, infrared; PCA, principal components analysis; PD, Parkinson's disease.

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