Brain mechanisms of self-control: A neurocognitive investigation of reward-based action control and error awareness

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Chapter 7

Pupil-dilation during error awareness predicts insula activation and shifts between task-focused and default-mode brain networks

This investigation aims to further our understanding of the brain mechanisms underlying the awareness of one’s erroneous actions. While all errors are registered as such in the rostral cingulate zone, errors enter awareness only when the anterior insula cortex is activated. Aware but not unaware errors elicit autonomic nervous system reactivity. Our aim is to investigate the hypothesis that activation in the insula during error awareness is related to autonomic arousal and to inter-regional interactions with other areas of the brain. To examine the role of the anterior insula in error awareness, we assessed its functional connectivity to other brain regions along with autonomic nervous system reactivity in young healthy subjects who underwent simultaneous pupil-diameter and functional magnetic resonance imaging measurements while performing a complex and error-prone task. Error blindness was associated with failures to engage sufficient autonomic reactivity. During aware errors increased pupil-diameter along with increased task-related activation within, and increased connectivity between anterior insula and task-related networks suggested an increased capacity for action-control information transfer. Increased pupil-diameter during aware errors was furthermore associated with decreased activation of the default-mode network along with decreased insular connectivity with regions of the default mode system, possibly reflecting decreased task-irrelevant information processing. This shifting mechanism may be relevant to a better understanding of how the brain and the autonomic nervous system interact to enable efficient adaptive behavior during cognitive challenge.

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Introduction
Here we seek to understand the brain- and autonomic mechanisms underlying the awareness of one’s erroneous actions. The relevance of insight into the conditions under which error awareness arises is probably most readily apparent in pathologic conditions that are associated with deficits in error awareness. Deficits in error awareness occur in health as well as in pathology. Impaired error processing abilities have for instance been suggested to mediate poor insight in one’s deficits after in traumatic brain injury (Hart, Seignourel, & Sherer, 2009; O’Keeffe, Dockree, & Robertson, 2004). This represents a key obstacle to rehabilitation, and is a significant predictor for overall long-term outcome, return to community living, and productive lifestyle (see for a review Klein et al., 2013).

The recent literature in the field of error awareness can be characterized by an increasing attempt to explore mechanisms and conditions under which error awareness occurs. The anterior insula cortex has been found to activate selectively to aware errors, whereas the rostral cingulate zone shows no difference between unaware and aware errors (Hester et al., 2005; Klein et al., 2007). However, during error awareness, the insula’s variety of operating characteristics within several contexts, such as autonomic processes (Critchley, Tang, Glaser, Butterworth, & Dolan, 2005; Critchley, Wiens, Rotshstein, Ohman, & Dolan, 2004), interoception (Craig, 2009, 2011), visceral sensory and motor processes, limbic integration (Augustine, 1996), and large-scale brain network shifts (Menon & Uddin, 2010; Seeley et al., 2007; Sridharan, Levitin, & Menon, 2008), has yet prevented a clear distinction of its precise functional contributions to error awareness. Limited data is available on the networks of the human insula cortex, and reports on arousal signals mediating insula networks are largely lacking.

By articulating the insula’s functional network and autonomic function, we aim to gain more insight into the nature of the insula’s activity during error awareness. This approach was specifically motivated by the observation that the insula cortex plays a principal role in error awareness, in the mapping of autonomic and visceral functions (Critchley, 2005), and is involved in neural networks dedicated to the evaluation of motivational salience (Seeley et al., 2007; Sridharan et al., 2008). Moreover, initial studies have placed increasing emphasis on the changes in the autonomic nervous system during error awareness (O’Connell et al., 2009; Wessel et al., 2011). Traditionally, the use of terms like vegetative or involuntary to describe the function of the autonomic nervous system implied that the autonomic nervous system has little to do with cognitive or voluntary actions. However, studies of autonomic activity that accompanies attention, cognitive effort and the orienting to surprising events have demonstrated that the autonomic nervous system is not simply a ‘non-cognitive’ part of brain function (Hugdahl, 1996). Autonomic arousal is commonly thought to prepare the organism to respond to changed internal and external requirements, by recruiting the necessary mental as well as physical effort (Ullsperger et al., 2010).
Gilzenrat and colleagues have recently suggested that pupil diameter constitutes an indirect index for the tonic and phasic modes of locus coeruleus-norepinephrine (LC/NE) function, that can be linked to lapses of task engagement and poorer performance (Gilzenrat, Nieuwenhuis, Jepma, & Cohen, 2010). Within cognitive tasks, baseline pupil diameter (before a stimulus) and evoked pupil diameter (after a stimulus) can serve as indices for tonic and phasic modes of LC/NE function, respectively (Aston-Jones, Raijkowski, Kubiak & Alexinsky, 1994). Large pupil diameter before a task-relevant event (baseline pupil diameter) and reduced task-evoked pupil dilations have been associated with overt indications of task disengagement as indexed by reaction times, as observed in a simple oddball task (Gilzenrat et al., 2010). On the other hand, low baseline pupil diameter and increased phasic task-evoked pupil dilations have been proposed to correspond to effortful processing. In line with these findings, pupil dilation has been shown to increase after aware errors, but not after unaware errors (Wessel, Danielmeier & Ullsperger, 2011). These findings on LC/NE function and pupil dilation emphasize the link between autonomic function and cognition. Recently, researchers have begun to investigate the link between brain function and autonomic function during cognitive operations. Although intriguing, thus far these new studies leave open the question to what extent autonomic signals during cognitive operations are related to brain function during error awareness, and to inter-regional brain network communication.

Here, in order to capture the error awareness state in relation to autonomic and neural activation and neural network activity, participants performed an antisaccade task, known to yield both aware and unaware errors (Endrass, Reuter & Kathmann, 2007; Klein, Endrass, Kathmann, Neumann, von Cramon & Ullsperger, 2007, Nieuwenhuis, Ridderinkhof, Blom, Band & Kok, 2001), while undergoing simultaneous fMRI, oculomotor and pupil diameter measurements. We quantify anterior insula’s functional activation and functional connectivity and its relation to changes in pupil diameter during error awareness. The aim is to investigate the hypothesis that activation in the insula during error awareness is related to autonomic arousal and to inter-regional interactions with other areas of the brain. We examine moreover if these inter-regional interactions of the insula cortex during error awareness are mediated by autonomic arousal. We hypothesized that pupillary responses during error awareness are related to activity in the anterior insula and its related functional networks. Such a link would yield direct evidence that a crucial function of the anterior insula is to integrate homeostatic regulation and neural network functions in order to enable the aware processing of an error.
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**Material and Methods**

**Participants**

23 healthy right-handed volunteers (17 females, mean age $21.5 \pm 2.0$) with normal or corrected-to-normal vision participated in the experiment after giving written informed consent according to the Helsinki Declaration. They were paid 50 Euros for participation. None of the participants had a history of neurological or psychiatric disorders or eye problems, and none were taking medication influencing the central nervous system or cardiovascular systems. Participants were selected beforehand in a task-session outside the scanner. Selection was based on a minimum of 15 errors in the aware/unaware condition with a false alarm rate lower than the aware/unaware error rates and based on a post-experimental self-rating of uncertainty in performance evaluation (maximum 5% on a analogue scale 1-100%). 2 participants were excluded due to motion-correction estimates above 2mm and anatomical deviations evident after medical inspection of structural scans. Insufficient number of errors for fMRI analysis led to exclusion of a third subject.

**Task**

We examined unaware and aware errors in an antisaccade task commonly used to study error awareness (Endrass et al., 2007; Klein et al., 2007; Nieuwenhuis et al., 2001). Participants were instructed to fixate on a central target and generate an immediate eye movement away from an abrupt peripheral target to its mirror location on the opposite side of the screen without making an eye movement to the peripheral target itself. The temporal order of stimulus presentation is displayed in Figure 1a. The trial started with a central fixation dot surrounded by two square outlines (each subtending 3.8° of visual angle; distance from fixation 12.4°; display duration 1000 ms). After a 150-300 ms jittered fixation gap, the peripheral target (a white circle subtending 2.9°) was presented for 117 ms in the left or the right square. To induce erroneous responses a precue was presented in 50% of the trials (Klein et al., 2007; Nieuwenhuis et al., 2001) briefly (50 ms) thickening the outlines of the square at the opposite side of the target and validly indicating the target location. After a response window of 880 ms, a cross appeared for 500 ms in the correct square, indicating the correct gaze direction.

Immediately after each eye-response and after de correct gaze direction had been indicated by the cross in the correct square, participants were to evaluate their performance (within 1500 ms) by pressing one of two buttons. They had been instructed that each initial eye movement towards the peripheral target was classified as an error even when they ended up moving their eyes in the correct direction. With the button press they were to indicate whether their antisaccadic response was correct (no initial eye movement toward the target) or incorrect (an initial eye movement toward the target). The erroneous responses that participants
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had rated as incorrect were classified as aware errors, and erroneous responses rated as correct were classified as unaware errors. If the erroneous eye movement was redirected to the correct (opposite) side of the screen, the response was labeled “corrected error”.

On trial numbers 20, 40, 60 and 80, an instruction screen (duration: 2 s) appeared, reminding participants to keep saccading at fast pace. A black screen with jittered duration (16, 500, 1000, 1500 ms) was displayed between trials and 10% of the trials were ‘null events’ (fixation-only trials of 5952 ms). Participants completed 3 blocks of 100 antisaccade trials, each lasting 11 minutes. For assessment of the pupil response, light flux was calibrated to equal luminance across trials with the program Colorfacts 7 and the color calibration system “EyeOneMonitor” (www.datacolor.eu) and tested for equal pupil luminance response across precue conditions. There was no significant difference in pupil dilation between trials with (0.4 ± 1.1) and without precue (0.4 ± 1.2; t(22)=.01, p<.995).

Light in the scanning environment was constrained to video presentation of stimuli against a black background. The projecting screen for the stimuli was placed in front of the window to the adjacent scan-operator room, such that window was not visible for the participant. The adjacent scan-operating room, was lighted with constant, non-varriing ambient light, as pupil size is sensitive to change in ambient light flux.

**Behavioral data acquisition and analysis**

Oculomotor, pupil and button-press responses were recorded with 2 interconnected PCs: An eye-tracker PC (ViewPoint EyeTracker, Arrington Research, www.arringtonresearch.com) and a presentation PC (Neurobehavioral Systems, www.neurobs.com, Albany, USA). Both PCs were connected to the MRI-scanner allowing for the time locking of stimuli, responses and fMRI image acquisition. The participant’s left eye was continuously monitored with an MRI-compatible infrared oculographic limbus tracker (Resonance Technology, Inc., www.mrivideo.com) attached to the head coil and placed 3 cm beneath the participant’s left eye. The eye-tracker registered eye movements, aspect ratio and diameter of the pupil with a sampling rate of 60 Hz along with scanner pulses and stimulus onsets. Before the scan, a 9-point calibration was performed and calibrated eye position was slip corrected during the task to eliminate slow drifts. Calibration and stimuli were presented on a 66 cm x 88 cm screen, placed at a 4-m viewing distance at the front end of the scanner and seen through a mirror above the participants’ heads.

Saccade onsets, amplitudes and directions were detected with in-house Java-based software (www.java.com) using minimum amplitude (>1.5°) and velocity (>30°/s) criteria and were subsequently double-checked by 2 raters. In line with common definitions (Fischer et al., 1993) we excluded trials in which subjects initiated saccades faster than 80 ms after target appearance (3.3 ± 4.1% (s.d.) of all trials), trials in which subjects were looking away from fixation during target
presentation (2.7 ± 3.9%), blinked during target appearance (0.6 ± 1.2%) and trials for which the eye-movement data were not interpretable due to poor quality of the eye-tracker signal (5.0 ± 4.3%).

To compute pupil diameter, data were cleaned using Matlab algorithms (Mathworks, Natick, MA, www.mathworks.com). Artifacts, including blinks, were defined as points greater than 2 standard deviations above or below the mean, occurring too rapidly to signify dilation. These points were replaced using spline interpolation from the preceding and following points. Next, the data were converted to percent signal change from the entire time series mean. This was done to facilitate cross-subject (i.e., different pupil sizes across subjects) and cross-condition comparisons, without the need to normalize using a pre-stimulus baseline correction. Hence, pupil diameter results, as illustrated in figure 1a, are given in % signal change relative to the mean pupil diameter across the entire experiment and are presented for two immediate time windows, a period of 1 second before and a period of 1 second after the presentation of the peripheral target. To test for statistically significant differences in pupil diameter between aware and unaware errors a paired-samples t-test was computed. For individual difference covariation analyses, pupil data were aligned to each event of interest, specified for 2 time windows (1 second before, 1 second after the error) averaged across time windows and trials to produce an average pupil diameter percent signal change value for each participant in the 2 time windows for each of the two trials types: aware and unaware errors.

Power of low-frequency oscillations of pupil diameter (3hz-6hz) was computed by convolving the single-trial pupil diameter of cleaned pupil data with a family of Morlet wavelets. Slow pupillary oscillations are a characteristic of decreasing subjective alertness due to unstable fluctuations in central sympathetic activity (Wilhelm, 2008). A paired-samples t-test was computed to test for statistically significant differences in the power of low-frequency oscillations between aware and unaware errors. Pupil oscillation results, as illustrated in figure 1b, are given in dB for a period of 4 seconds around peripheral target presentation (−2 to +2 seconds) with grey areas indicating significant differences at p=.05.

**fMRI Acquisition, GLM and Functional Connectivity**

Acquisition. Functional images were acquired on a Philips (Philips, the Netherlands) 3 T MRI system equipped with echo planar imaging (EPI) capabilities using a standard head coil for radio frequency transmission and signal reception. Functional scans of the entire brain were acquired with a single-shot, gradient-recalled EPI sequence parallel to the AC–PC plane (TE/TR = 28/2000 msec; 30 axial slices; slice thickness 3 mm; interslice gap 0.3 mm; voxel size 3 x 3 x 3 mm; FOV = 222 x 2 mm; 96 x 96 in-plane resolution/matrix size, 90° flip-angle). The first 2 volumes were discarded to allow for T1 equilibration effects. The duration of the antisaccade task
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was three times 11 minutes (335 scans per scanblok). High-resolution anatomical images were subsequently acquired using a 3-D T1-weighted scan in steady state sequence (TE/TR = 4.6/9.69 ms; 182 sagittal slices; slice thickness 1.2, interslice gap 0.3 mm; voxel size 1x1x1 mm cubic; FOV = 25 x 2 cm; 256 x 2 in-plane resolution, 8 degrees flip angle, sagittal orientation).

**Preprocessing and GLM**

Preprocessing of the functional data and calculation of the contrast images for statistical analysis was done with FEAT (FMRI Expert Analysis Tool) Version 5.63, a part of FSL (FMRIB’s Software Library; www.fmrib.ox.ac.uk/fsl). Functional images were realigned to compensate for small head movements, slice-time corrected, spatially smoothed with a 5-mm full-width half-maximum Gaussian kernel, filtered in the temporal domain using a high-pass filter with a cutoff frequency of 1/50 Hz to correct for baseline drifts in the signal and prewhitened (Woolrich et al., 2009). For each experimental run of each participant, the overall activity evoked by correct responses and error commission was modeled (2 levels: aware errors vs. unaware errors) and each regressor was convolved by a prototypical synthetic hemodynamic response function and its first derivative. To remove any artifactual signal changes due to head motion, six parameters describing the head-movements (three translations, three rotations) were included as confounds in the model. In the second-stage analysis participants were treated as a fixed factor to concatenate the three experimental runs. Contrasts pertaining to the main effects constituted the data for the third-stage (mixed effect) analysis, where the significance of observations was determined across the group of 23 subjects using FLAME 1 and 2 (FMRIB’s Local Analysis of Mixed Effects, Smith et al., 2004). For each whole brain comparison of aware versus unaware trials, a cluster-corrected threshold of p < .001 corrected for whole-brain multiple comparisons was set using Gaussian random field theory (GRFT). In subsequent whole brain covariance analysis with individual pupil diameter values we report cortical regions with a threshold of p < .05 cluster-corrected for whole-brain multiple comparisons (using GRFT).

**Functional Connectivity**

For functional connectivity analysis, the psycho-physiological interaction (PPI) method (Friston et al., 1997) was applied. PPI makes inferences about regionally specific responses co-varying with the interaction between the psychological factor and the physiological activity in a specified seed area. Bilateral anterior insula represented an a priori region of interest as error awareness seems to place particularly strong demands on bilateral anterior insula (Klein et al., 2007). The seed anterior insula subtended the three principal short insular gyri (anterior, middle, posterior) and the accessory and transverse insular gyri, all anterior to the insular sulcus. Definition was based on the MNI structural atlas of the FSL-atlas
Chapte
toolbox and literature on neurosurgical landmarks (Eickhoff et al., 2007; Mazziotta et al., 1995; Ture, Yasargil, Al-Mefty, Yasargil, 1999). The entire time course of activity in bilateral AIC seeds was extracted for each subject and activity during 6 TRs following each aware and unaware error was used as an independent variable in a GLM. AIC time course was multiplied with a condition vector that was ones for 6 TRs following the error, and zeros otherwise. These resulting vectors were then used as regressors, in a separate regression, which included the aware and unaware vectors as the independent variables of interest. For each individual, this procedure yielded a functional connectivity map identifying areas where BOLD signal changes were temporally coupled with signal changes derived from bilateral AIC seeds as induced by aware as compared to unaware errors. The three experimental runs were concatenated and third level group analyses were conducted with a cluster-corrected statistical threshold of $z < 2.3$ and $p<0.05$, correcting for whole-brain multiple comparisons.

**Individual differences covariance analysis**

Individual, normalized mean pupil diameters for the two trial types, aware and unaware errors in time windows 1 s before and 1s after the commitment of the error were orthogonalised with the group mean separately for each trial type. Demeaned condition-related mean differences in pupil dilation for each subject were incorporated as a covariate in two separate GLM regression models, one for each time-window and all voxels exceeding the cluster-corrected threshold of 0.05 in the mean $z$-map were determined. The same demeaned pupil diameter values were subsequently entered as covariates into two separate PPI analysis (pupil diameter before and after aware errors) and all parameter estimates at each voxels exceeding the cluster-corrected threshold of 0.05 $z > 2.3$ in the mean $z$-map were determined. Thus, apart from explaining changes in functional connectivity on the basis of the error class alone, it was assessed whether changes in functional connectivity during aware versus unaware errors can be predicted by individual differences in pupil diameter.
Results

**Figure 1:** A. Antisaccade task: Participants were instructed to fixate on a central target and generate an immediate eye movement away from an abrupt peripheral target to its mirror location on the opposite side of the screen (correct response) without making an initial eye movement to the peripheral target itself (incorrect response). After the participants made an eye movement, a cross appeared in the correct square, indicating the correct gaze direction. Subsequently, participants were to evaluate their performance (correct/incorrect) by pressing one of two buttons. The erroneous responses participants had rated as incorrect were classified as aware errors and erroneous responses rated as correct were classified as unaware errors. B. Antisaccade results: Aware errors and unaware errors occurred equally often and were similar in mean latency. C. Pupil dilation results: Signs of autonomic reactivity in pupil diameter vary with awareness. C. left panel: Mean (±SE) pupil diameter in the 1-second period before errors and in the 1-second period after errors, plotted relative to
baseline (mean dilation across the whole task) across all subjects (N=23). Pupil diameter was significantly larger, reflecting reduced vigilance, before unaware compared to aware errors. Pupil dilated significantly in response to aware errors, indicative of autonomic engagement, but not to unaware errors. C. right panel: Power of low-frequency oscillations (3-6 Hz) for a period of 4 seconds around peripheral target presentation, plotted relative to baseline across all subjects. A stable decrease in low-frequency oscillations during the visual appearance of the fixation point in both aware and unaware errors reflects increased vigilance caused by the fixation point. The decrease in low-frequency oscillations with aware errors was greater than with unaware errors, suggesting greater autonomic disengagement from the task during unaware errors.

**Behavior results**

The mean error rate was 28.1 ± 13.7 % (s.d.), and the majority of errors were made on trials with a precue (80.2 ± 15.0%). Pair-wise comparisons indicated that aware errors and unaware errors occurred equally often (13.5 ± 10.4 % vs. 14.6 ± 8.7 %; t(22)= .43; p = .67; see Figure 1b), and there was no significant difference between the occurrence of aware or unaware errors on precue trials (81.9 ± 17.1% vs 73.5 ± 23.6%); t(22)= 1.53; p = .14). Unaware errors were corrected significantly more often than were aware errors (94.2 ± 14.9% vs. 66.3 ± 31.3%; t(22)= 3.9; p<.001). False alarm rates below 5.1% indicated that participants rarely reported an error when they made a correct antisaccade.

Erroneous responses were initiated faster than correct responses (191 ms vs. 284 ms (s.d); t(22)= 10.3; p<.001). Unaware and aware errors were similar in mean latency (186 ms vs. 195 ms; t(22)= .71; p=.49; see Figure 1b). There was no significant post-error slowing, as indicated by a nonsignificant difference between onset latencies on trial following corrects versus those following errors (263 ms vs. 255 ms; t(22)= .17; p = .10). Post-error onset latency was also not different following aware versus unaware errors (255 ms vs. 254 ms; t(22)= .13; p = .899).

There was no significant difference in pupil dilation between trials with (0.4 ± 1.1 % signal change) and without precue (0.4 ± 1.2 % signal change, t(22)=.007, p = .99), suggesting that precue luminance did not influence our results.

**Pupil diameter: Signs of autonomic task engagement on aware errors and disengagement on unaware errors**

Effortful processing and engagement is associated with intermediate pre-stimulus pupil diameter and large stimulus-evoked dilations; conversely, task disengagement is associated with large pre-stimulus dilated pupil diameter and a small stimulus-evoked pupil response (Gilzenrat et al., 2010; Rajkowski et al, 2004; Usher, Cohen,
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Servan-Schreiber, Rajkowski & Aston-Jones, 1999). To test the hypothesis that unaware errors are associated with autonomic signs of task disengagement and that aware errors are associated with autonomic signs of effortful processing, we examined pupil diameter immediately before and after aware errors. Results are given in % signal change, relative to mean pupil diameter across the entire time series.

Consistent with previous results (Wessel et al., 2011) pupil diameter was significantly larger before unaware (1.6 ± 4.1%) compared to aware errors (0.2 ± 2.0%; t(22)= 1.71; p<.05). Pupil size did not change after unaware errors (1.5 ± 3.9%), but increased significantly following aware errors (0.8 ± 2.3%; t(22)= 1.79; p<.04; Fig. 1c).

Low-frequency fluctuations in pupil diameter index autonomic dysregulation during decreases in self/reported vigilance (Wilhelm, 2008; Wilhelm, Wilhelm, Ludtke, Streicher & Adler, 1998). To examine pupil diameter responses for signs of autonomic dysregulation during unaware errors we computed the power of low frequency oscillations (3-6 Hz) of pupil diameter in a period of 2 seconds before and 2 seconds after unaware- and aware errors relative to mean pupil diameter across the entire timeseries (baseline). Oscillatory power between unaware and aware errors was considered significantly different when at least 156 contiguous ms (40 time points) survived a paired-samples t-test at p < 0.05. Significantly stronger power in slow oscillations were observed preceding and following unaware errors compared to aware errors. Gray regions in figure 2 reflect time windows with significantly higher power in low frequency pupillary oscillations on unaware errors than on aware errors (Fig. 1c).

**Aware versus unaware errors: FMRI activation and functional connectivity**

Aware compared to unaware errors yielded significantly increased activation in right anterior insula, bilateral somatosensory cortex, thalamus, areas in the anterior cingulate, frontal eye fields and intraparietal sulcus (Fig. 2a and Supplementary Table 1). PPI analyses showed increased functional connectivity of the anterior insula with bilateral somatosensory cortex and bilateral intraparietal sulcus (Fig. 2b and Supplementary Table 2).
Figure 2. BOLD activation and functional connectivity of anterior insula for aware errors. (a) Statistical parametrical map of difference in BOLD activation between aware and unaware errors. Red and yellow voxels represent clusters of significant BOLD signal increase. (b) Statistical parametrical map of difference in functional connectivity of anterior insula cortex between aware and unaware errors. Red and yellow voxels represent clusters of significant BOLD signal correlation between the seed region anterior insula cortex and all other voxels in the brain during aware errors as compared to unaware errors. For a full list of activated regions (z = 2.3, whole-brain cluster-corrected, p = 0.05), see Table 1 and Table 2. Note. L/R = left/right, oculomotor control structures (IPS = intraparietal sulcus, FEF = frontal eye fields), salience structures (AIC = anterior insula cortex, ACC = anterior cingulate cortex, S1 = somatosensory cortex).

Pupil diameter predicts shifts in engagement between default mode and task-focused brain networks.

To link the BOLD response to the pupil response, we correlated functional activation (aware>unaware) across subjects with % signal change in pupil diameter in the 1-second period before and after errors. Pre-aware error pupil diameter correlated with activation in the right anterior insula, dorsal anterior cingulate, right somatosensory cortex and in oculomotor task control structures (frontal eye fields, intraparietal sulcus). Negative correlations with pre-error pupil diameter were observed in structures associated with the default mode network, including anterior medial prefrontal cortex, frontal pole, precuneus and posterior cingulate (Fig. 3a and Table 1a).

Pupil diameter after aware errors correlated with activation in the right anterior insula, right somatosensory cortex and in oculomotor task control
Pupil-dilation during error awareness predicts insula activation and shifts between task-focused and default-mode brain networks structures (frontal eye fields, intraparietal sulcus). Negative correlations were observed in structures of the default mode network, including anterior and subcallosal medial prefrontal cortex, frontal pole, precuneus and posterior cingulate (Fig. 3b and Table 1b).

**Figure 3.** Links between BOLD activation and pupil diameter for aware errors. Increased activation in the task control structures and decreased activation in the default-mode network are predicted by pupil diameter immediately (a) before and (b) after aware errors. Statistical maps of the correlation between individual differences in pupil diameter and corresponding BOLD contrast (aware > unaware errors). The spatial distribution of correlation coefficients shows both positive correlations (red and yellow voxels) and negative correlations (blue voxels) at cluster-corrected statistical thresholds of $z= 2.3$, $p= .05$. For a full list of activated regions, see Table 3. Note. L/R= left/right, oculomotor control structures (IPS= intraparietal sulcus, FEF= frontal eye fields), (AIC= anterior insula cortex, ACC= anterior cingulate cortex, S1= somatosensory cortex), default mode (PCC= posterior cingulate cortex, aMPC= anterior medial prefrontal cortex).
Table 1. Brain regions in which the aware > unaware error contrast covaried with pupil diameter (cluster corrected at $z=2.3$, $p=.05$).

<table>
<thead>
<tr>
<th>(a) BOLD effect varying with pupil diameter before aware errors</th>
<th>(b) BOLD effect varying with pupil diameter after aware errors</th>
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<tr>
<td>Brain region</td>
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<tr>
<td>Positive covariance</td>
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<tr>
<td>R AIC</td>
<td>30</td>
</tr>
<tr>
<td>R MIC</td>
<td>40</td>
</tr>
<tr>
<td>R S1</td>
<td>48</td>
</tr>
<tr>
<td>R dorsal ACC</td>
<td>6</td>
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<tr>
<td>L dorsal ACC</td>
<td>-2</td>
</tr>
<tr>
<td>R suppl.motor cortex</td>
<td>6</td>
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<tr>
<td>R IFG</td>
<td>56</td>
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<tr>
<td>R FEF</td>
<td>34</td>
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<td>R IPS</td>
<td>56</td>
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<tr>
<td>L IPS</td>
<td>-40</td>
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<tr>
<td>R V2</td>
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<td>R aMPC</td>
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<td>L frontal pole</td>
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<td>L frontal pole</td>
<td>-10</td>
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<td>R PCC</td>
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<td>R precuneus</td>
<td>8</td>
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<td>L precuneus</td>
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</table>

Coordinates are given in MNI space.

Note. L/R= left/right, AIC= anterior insula cortex, MIC= medio insula cortex, S1= somatosensory cortex, ACC= anterior cingulate cortex, FEF= frontal eyefields, IPS=...
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intraparietal sulcus, IFG= inferior frontal gyrus, V1= primary visual cortex, V2= secondary visual cortex, PCC= posterior cingulate cortex, aMPC= anterior medial prefrontal cortex.

To link functional connectivity of the anterior insula to pupil diameter, we correlated individual differences in the anterior insula-seeded PPI with pre- and post-error pupil diameter. Here we found positive correlations (i.e., larger pupil diameter predicts stronger insula-seeded functional connectivity) in oculomotor control structures including intraparietal sulcus and left parietal-occipital junction, and somatosensory cortex. Negative correlations (larger pupil diameter predicts weaker insula-seeded functional connectivity) were observed in default mode network regions, including anterior medial prefrontal cortex and frontal pole (Fig. 4 and Table 2).

Figure 4. Links between anterior insula’s functional connectivity and pupil diameter for aware errors. Increased functional connectivity of anterior insula with oculomotor task control structures and decreased functional connectivity of anterior insula with areas of the default-mode network are predicted by pupil diameter immediately before aware errors. The spatial distribution of correlation coefficients (z-scores) shows both
positive correlations (red and yellow voxels) and negative correlations with preparatory pupil diameter (blue voxels) at cluster-corrected statistical thresholds of $z=2.3$, $p=.05$. For a full list of activated regions, see Table 2. Note. L/R= left/right, S1= somatosensory cortex, oculomotor control structures (IPS= intraparietal sulcus), default mode (aMPC= anterior medial prefrontal cortex).

**Table 2.** Brain regions in which functional connectivity with anterior insula cortex during aware errors varied with pupil diameter before aware errors.

<table>
<thead>
<tr>
<th>Brain regions in which functional connectivity of AIC during aware errors &gt; unaware errors, covaried with pupil diameter before aware errors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive covariance</strong></td>
</tr>
<tr>
<td>cluster corrected at $z=2.3$, $p=.05$</td>
</tr>
<tr>
<td>Brain region</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>R Postcentral gyrus (primary somatosensory cortex BA2R, BA1R, BA3bR)</td>
</tr>
<tr>
<td>R Anterior intraparietal sulcus</td>
</tr>
<tr>
<td>L Parietal occipital junction (superior parietal lobe/lateral occipital lobe)</td>
</tr>
<tr>
<td><strong>Negative covariance,</strong></td>
</tr>
<tr>
<td>cluster corrected at $z=2.3$, $p=.05$</td>
</tr>
<tr>
<td>R Anterior medial prefrontal cortex</td>
</tr>
<tr>
<td>L Medial prefrontal cortex</td>
</tr>
<tr>
<td>L Frontal pole</td>
</tr>
</tbody>
</table>

Coordinates are given in MNI space

**Discussion**

The goal of the current study was to capture the awareness state in the neural activation and neural networks centering on the insula in relation to autonomic activity while participants performed the antisaccade-awareness task.

**Pupil diameter findings**

To date pupil diameter changes during cognitive control tasks have to our best knowledge not yet been addressed in studies of functional brain connectivity. Therefore, for a thorough understanding of the relation between the pupil-diameter
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patterns during error awareness and the associated changes in the insula cortex and its related networks, we will start the discussion with an interpretation of the observed pupil-dilation pattern.

We found that a change in awareness state involved concomitant changes in autonomic activity as indexed by changes in pupil diameter. The observed changes in pupil diameter during aware errors are consistent with evidence from previous studies (Wessel et al., 2011).

Pupil diameter was significantly larger before unaware compared to aware errors. Pupil size did not change after unaware errors, but increased significantly following aware errors. These findings are in line with the notion that within cognitive tasks, baseline pupil diameter (before a stimulus) and evoked pupil diameter (after a stimulus) can serve as indices for tonic and phasic modes of LC/NE function, respectively (Aston-Jones, et al., 1994; Gilzenrat et al., 2010; Murphy, Robertson, Balsters & O’Connell, 2011). Tonically enlarged pupil diameter (as compared to normalized baseline) before a task-relevant event and reduced phasic task-evoked pupil dilations have been associated with overt indications of task disengagement. On the other hand, baseline pupil diameter prior to the event, and increased phasic task-evoked pupil dilations have been proposed to correspond to effortful processing. In the context of this literature, the currently observed enlarged baseline pupil diameter prior to unaware errors, and the unchanged pupil diameter after the unaware error point to disengagement from the task. In contrast, the observation of baseline pupil diameter prior to aware errors, and the increase of pupil diameter in reaction to aware errors, seem to index task engagement. Moreover, in line with this “engagement/disengagement” interpretation of the pupil pattern prior to and after aware errors and unaware errors respectively, and with previous observations in the field of sleep research (Wilhelm, Wilhelm, Ludtke, Streicher, & Adler, 1998), we observed that unaware compared to aware errors were associated with larger low-frequency oscillations from 2 seconds prior to 2 seconds after the unaware error. Although pupil-oscillations are largely unexplored within cognitive tasks, during states of sleep deprivation, slow pupillary oscillations have been found to be associated with decreased levels of alertness and increased daytime sleepiness. The underlying cause of the increased pupillary oscillations observed in less alert and sleepy subjects is presumably an unstable drift of central sympathetic activation (Wilhelm, 2008, Wilhelm et al., 1998).

The current results show that low frequency pupil oscillations are not only related to subjectively reported decreased vigilance but also to unaware errors. Taken together, the absence of “task-appropriate” pupil-modulation during unaware errors was evident in two measures of pupil diameter: in the baseline and error-evoked pupil diameter results (as previously shown by Wessel et al., 2011), and in the oscillatory pupil diameter results, with more power in low-frequency oscillations immediately before and after unaware errors (Fig. 1b). The pupil pattern prior and subsequent to aware errors can be interpreted as task-synchronized arousal levels. These task-synchronized arousal levels reflect that the
participant is engaging in the task at hand. Unaware errors in contrast were characterized by unstable desynchronized arousal levels before and after committed errors, reflecting disengagement from the task, perhaps due in part to attentional lapses, to which continuous attention tasks are particularly vulnerable.

**BOLD activation, functional connectivity, and their relation to pupil diameter changes during error awareness**

Aware compared to unaware errors yielded significantly increased activation in right anterior insula, bilateral somatosensory cortex, thalamus, areas in the anterior cingulate, frontal eye fields and intraparietal sulcus. Analyses of functional connectivity showed increased functional connectivity of the anterior insula with bilateral somatosensory cortex and bilateral intraparietal sulcus. In a covariation analysis, the pupil measures during error awareness explained variance in the neural data. Individual differences in pupil diameter before the aware error predicted increased BOLD activation in salience processing areas (anterior insula, rostral cingulate zone, somatosensory cortex) and in oculomotor control areas (frontal eye fields, intraparietal sulcus) as well as increased functional connectivity of the anterior insula with other salience processing areas (somatosensory cortex) and with oculomotor control areas (intraparietal sulcus). Individual differences in pupil diameter before the aware error predicted decreased BOLD activation in areas of the default mode network (viz. anterior medial prefrontal cortex / frontal pole, posterior cingulate cortex, precuneus) and a decrease of functional connectivity of the anterior insula cortex with default mode network areas (anterior medial prefrontal cortex / frontal pole).

This suggests that, when added to the neural analyses, the pupil patterns can be interpreted as revealing signs of task engagement in the BOLD signal (decrease of default mode areas and increase of oculomotor areas during aware errors as compared to unaware errors). The decrease in default mode areas during error awareness was not manifest when computing the contrast aware versus unaware errors without taking pupil patterns into account. This widespread “neuro-autonomic pattern” with increase in task-related oculomotor- and salience processing areas and decrease of activation in default mode network areas, seems well suited to prepare the individual to respond to the changed requirements after the detection of an error, by recruiting the necessary mental as well as physical efforts.

Based on the postulated mechanisms underlying pupil dilation during changes in cognitive control state (Gilzenrat et al., 2010), the observed neuro-autonomic pattern during error awareness seems to reflect a widespread whole-brain pattern of increased task-engagement and increased alertness during aware as compared to unaware errors. Signs of pupillary disengagement during unaware errors on the other hand, may be associated with a failure in neural systems to disengage the
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default mode network and generate a state of heightened activity in task-related structures. This observed widespread neuro-autonomic pattern is line with the postulated role of the LC in the ascending reticular activating system (Kayama & Koyama, 1998), which controls the level of activity of the whole brain, and has been shown to mediate transitions from relaxed wakefulness to periods of increased alertness and attention (Kinomura, Larsson, Gulyas, & Roland, 1996).

Overall, the current data provide evidence that the insula engages in large-scale networks during shifts in awareness states, and that these inter-regional interactions of the insula cortex during error awareness are mediated by autonomic arousal. Autonomically mediated changes in brain connectivity have previously been observed in human attentional fronto-parietal brain systems (Coull, Buchel, Friston, & Frith, 1999). For example, a noradrenergic challenge produced differential effects on brain connectivity during rest (when low arousal is presumed) compared to during task performance. The current results illustrate that this relationship between autonomic processes and functional brain connectivity extends to cognitive operations on small timescales within a task.

Anterior insula’s neural networks during error awareness

Overall, the results suggest a relation of changes in the bodily periphery during error awareness with changes on several neural levels (activation and functional connectivity). The observed functional connectivity between the anterior insula and the parietal oculomotor structures (intraparietal sulcus) is in line with human diffusion tensor imaging tractography demonstrating fiber tracts between anterior insula and parietal cortex, specifically the intraparietal sulcus (Uddin, et al., 2010). The observed dichotomous functional connectivity-profile of anterior insula cortex (positive relation with task-related networks/negative relation with areas of the DMN) during aware as compared to unaware errors is consistent with a recently proposed network model of anterior insula function (Menon et al., 2010). Menon c.s. suggested that the anterior insula regulates the balance between default mode and task-specific control networks. They found that during effortful task engagement, functional connectivity of the anterior insula increased with task-specific control networks and decreased with the default-mode network (Dosenbach, et al., 2008; Dosenbach, et al., 2007; Seeley, et al., 2007; Sridharan, Levitin, & Menon, 2008; Uddin & Menon, 2009). Task-focused control networks are generally thought to be engaged when attention is focused on the task. The default-mode network, in contrast, is believed to participate in a self-referential state of brain function that is suspended during task engagement (Dosenbach, Fair, Cohen, Schlaggar, & Petersen, 2008; Fox, Zhang, Snyder, & Raichle, 2009; Greicius, Krasnow, Reiss, & Menon, 2003; Gusnard, Raichle, & Raichle, 2001; Raichle, et al., 2001). This balance between the default mode and task-focused control networks has been found to predict attentional lapses and performance variability (Eichele et al., 2008; Kelly, Uddin, Biswal, Castellanos, & Milham, 2008; Uddin et al., 2009; Weissman, Roberts, Visscher,
& Woldorff, 2006). The current results extend the previously observed shifts between task-related and default mode networks during variations in performance efficiency (specifically Eichele et al., 2008; Kelly et al., 2008; Weissman et al., 2006) by suggesting that such shifts vary with task related changes in the body-periphery as indexed by pupil diameter.

**The role of the anterior cingulate cortex**

The awareness of errors engendered co-activation of the anterior insula and the anterior cingulate associated with awareness-related pupil dilation. This is in line with previous observations in a Stroop task (Critchley et al., 2005). In that study, pupil dilation and corresponding activity in the anterior insula, mediofrontal cortex, and pre-supplementary motor area increased during the commitment of performance errors. The neural pattern was interpreted by the authors as potentially reflecting the awareness that the performance error. This interpretation was confirmed by direct evidence in the current study. Together these results support a model postulating that the anterior insula integrates salient events with autonomic information (Craig, 2002; Craig, 2009). Both findings also show more activation of dorsal anterior cingulate with more autonomic engagement during aware error processing. Indeed, patients with dorsal anterior cingulate damage are impaired at regulating sympathetic outputs with increasing task effort (Critchley et al., 2001).

In a review of 107 studies, Paus and colleagues (1998) found the dorsal anterior cingulate activated during non-specific behavioral effort. Similarly, Raichle and colleagues (2001) pointed in the same behavioral context to decreases in activation of the ventromedial prefrontal cortex and subgenual cingulate. Dorsal and subgenual portions of the anterior cingulate may be functionally dissociated with respect to autonomic drive and cognitive effort: Whereas the dorsal anterior cingulate is associated with autonomic up-regulation during increased task effort when people are engaged in demanding tasks (Critchley et al, 2009), the subgenual cingulate has been related to resting state and to autonomic control centers involved in sleep (Critchley, 2004). In line with these findings, awareness-related pupil responses in the current study were inversely linked to the dorsal and subgenual parts of the anterior cingulate (Fig. 3). The current results suggest that this functional dissociation of dorsal and subgenual cingulate with respect to autonomic arousal also applies to rapid cognitive operations when cognitive engagement and its associated autonomic nervous system activity fluctuate.

**Limitations and strengths of the current study**

Multiple limitations may apply to the present study. While this study suggests a relationship between changes in arousal level and the neural networks underlying error awareness, the directionality of the effect is not clear. The methodology used
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cannot disentangle afferent and efferent contributions to the observed changes in arousal, nor does it provide information on the directionality of the neural network pathways, or provide firm conclusions about temporal relationships between arousal and neural responses. Generating the appropriate level of preparatory autonomic activity might be viewed as a goal state (Jennings & van der Molen, 2005), serving to disengage the default mode network and energize control structures to render the system ready to process and respond to salient information. Thus, physiological arousal may mediate differences in large-scale network changes between aware and unaware errors. Alternatively, a transient state of task disengagement might incur a failure of timely network configuration and, consequently, a failure to mobilize task-adequate levels of bodily arousal. Thus, neural activity/connectivity changes may mediate the changes in autonomic state. The causal direction of this effect remains to be determined.

For functional connectivity, the shift in balance varied with patterns of pupil diameter before the aware error. For functional activation, network shifts co-varied with pupil diameter both before and after the aware error. This difference may be due to the fact that functional connectivity is measured in a longer time window than the local BOLD activation. In combination with the pupil data this may have the consequence that the baseline pupil explains more variance during this longer window, as the phasic pupil reaction to the error extends over a relatively shorter period of time than the baseline pupil pattern. Given that the timing of the BOLD response is not very informative about the timing of the neural responses, it is difficult to disentangle whether the pupil diameter 1s before the aware error precedes or follows error-related BOLD changes or whether they started concurrently. Additional studies, e.g., using MEG, are needed in order to further address the differential timing of autonomic responses with respect to anterior insula function and network changes.

One strength of the current approach is the combination of pupil diameter with functional brain networks, which has to our knowledge not been undertaken before. As discussed previously, pupil dilation changes have previously been related to error-commission and insula activation (Critchley et al., 2005). However, the impact of changes in arousal levels on cognitive tasks has only been considered in few functional neuroimaging studies such, and mainly in selective attention tasks (Chee et al., 2008; Czisch et al., 2012). The simultaneous objective acquisition of vigilance and arousal levels during neuroimaging studies has up to now mainly been the domain of sleep research, or research on sleep deprivation. Another strength is the covariation with activation and insula networks at the whole-brain level, revealing the potential of the pupil measurement to explain not only variance in activation increases in task-related local BOLD activation within the insula, but also variance in activation decrease in areas of the default mode network.
Chapter 7

Clinical implications

Based on our findings in healthy volunteers, one could propose that synchronized behavior-pupil relations might not be present in patients with deficits in error awareness. This hypothesis is in line with investigations of error awareness in ADHD, where autonomic arousal has been shown to be blunted during error awareness (O’Connell et al., 2009). One may speculate that deficits in error awareness may be associated with a deficit in the ability to synchronize autonomic and cognitive states. Desynchronized arousal may be reflected in the loss of strict negative correlation between task-positive and task-negative default mode activation. Such default-mode brain dysfunction has been observed in pathologies including ADHD, schizophrenia, addiction, and fronto-temporal dementia (Broyd et al., 2009; Di Martino et al., 2009; O’Connell, et al., 2009; Tian et al., 2008; Uddin & Menon, 2009). The relevance of knowledge on not local but widespread neural networks underlying error awareness can be particularly appreciated when considering neural findings on deficit unawareness. According to patient reviews, the observed differences in the anatomical localization of lesions causing anosognosia for hemiplegia suggest that neural substrates subserving these monitoring processes are located in separate brain areas, forming pathways and circuits that, when damaged, give rise to deficit awareness (Moro, Pernigo, Zapparoli, Cordioli, & Aglioti, 2011; Pia, Neppi-Modona, Ricci, & Berti, 2004). Whether desynchronized pupil-behavior relationships may constitute an index for disintegrated relations between task-related processes and processes in areas of the default mode network would be an intriguing question for future research.

Conclusion

To conclude, the current data provide a direct link between the awareness state, the neural activity and connectivity of insular cortex, and the associated variability in peripheral autonomic response measures. Anterior insula networks shifted between task-related brain structures and default mode systems and co-varied with the physiological arousal system. These results advance our understanding of anterior insula network function and of dissociations between aware and unaware processing. During events that require our increased attention and awareness the peripheral processes of the autonomic nervous system seem to relate to large-scale network changes. If we interpret the data based on the postulated mechanisms underlying pupil dilation during cognition, the observed widespread neuro-autonomic pattern during error awareness seems to reflect increased task-engagement during aware as compared to unaware errors. This widespread neuro-autonomic pattern in task-related areas and the suppression of DMN areas, seems well suited to prepare the individual to respond to the changed requirements after the detection of an error, by recruiting the necessary mental as well as physical effort.
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