Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control

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The dorsal anterior cingulate cortex (dACC) is a core structure for the governing of cognitive control, and recent studies have shown that interindividual differences in dACC anatomy are associated with corresponding differences in the ability for cognitive control. However, individuals differ not only in anatomical features of dACC, but also exhibit substantial variability regarding the biochemical characteristics of the dACC. In this study, we combined magnetic resonance spectroscopy (H-MRS) and functional magnetic resonance imaging (fMRI), finding that interindividual differences of glutamate levels in the dACC during resting-state predict the strength of the blood-oxygen level-dependent (BOLD) response to a task requiring cognitive control. This relationship was observed in the retrosplenial cortex, the orbitofrontal cortex, the inferior parietal lobe, and the basal ganglia. More specifically, individuals with low resting-state glutamate levels in the dACC showed an increased BOLD response when the task demands were high, whereas high-glutamate individuals showed the opposite pattern of an increased BOLD response when the task demands were low. Thus, we show here that individual variability of glutamate levels is directly related to how the brain implements cognitive control.

Results

**Imaging Results.** The statistical analysis of the fMRI data revealed a significant interaction between attention instruction and IID [P < 0.05, familywise error correction, extent threshold k = 20 voxels]. This interaction was found bilaterally in the lateral frontal cortex (lIFC), stretching into the bilateral anterior dACC and presupplementary motor area (preSMA), and also bilaterally in the inferior parietal lobe (IPL), the right precentral gyrus (MTG), and the right dorsolateral prefrontal cortex (DLPFC) (Table 1 and Fig. 1B). Post hoc analyses showed that the above regions displayed higher BOLD responses when the task demanded higher levels of cognitive control, i.e., when the attention was directed toward the less salient stimulus (see Fig. 1C for a representative post hoc analysis from the dACC). These results support the notion that the task probes cognitive control mechanisms.

More importantly, the above interaction was significantly modulated by the rsGlu level, as indicated by a three-way interaction (P < 0.001, uncorrected, k = 20 voxels) of attention instruction, IID, and rsGlu level. This interaction was found in several brain regions, located in the left retrosplenial cortex (RSC), the right orbitofrontal cortex (OFC), the left IPL, and bilaterally in the basal ganglia (BG) (Table 2 and Fig. 2). For the post hoc analyses and visualization of the BOLD response, the subjects were evenly split into three groups based on their individual rsGlu concentrations, in a high- (n = 13, rsGlu = 1.82 ± 0.07), medium- (n = 14, rsGlu = 1.62 ± 0.04), and low-rsGlu group (n = 13, rsGlu = 1.49 ± 0.05). This approach allowed for a comparison of how the BOLD response for the three groups varied over the four conditions. The results showed that the BOLD response was differentially modulated dependent on the rsGlu levels; low levels of rsGlu predicted an increase in BOLD response when the task demanded higher levels of cognitive control, whereas high rsGlu levels predicted higher BOLD response when the task was easier (attention aligned with the stimulus).
The analysis of the performance on the dichotic listening task did not show any particular pattern of BOLD modulation over the task conditions (Fig. 2).

**Behavioral Results.** The analysis of the performance on the dichotic listening task did not show a significant main effect or any significant interactions with rsGlu (Table 3). Other significant main effects and interactions were found but have been thoroughly discussed in a previous analysis of the data (12).

**Discussion**

The results from this study demonstrate that interindividual differences in rsGlu levels of the dACC significantly influence how the brain implements cognitive control. Individuals with lower rsGlu showed higher BOLD response when the task required higher levels of cognitive control in the IPL, OFC, RSC, and the BG. This pattern was contrasted by the high-rsGlu group, in which the recruitment of the same regions was enhanced when the task was easier to perform. BG and OFC have strong anatomical connections with the dACC (8) and are thought to be important for the flexible integration and gating of information, which is later evaluated by the dACC (13, 14). Notably, although the contribution of the BG in cognitive functioning mainly has been attributed to levels of dopamine and its actions (7), our results show the additional importance of the glutamatergic system in the modulation of BG response to a cognitive conflict. Furthermore, IPL and RSC are both considered part of the default-mode network (15). The results indicate that there is a recruitment of these brain regions during high cognitive control, but only in the low-rsGlu group. The high-rsGlu group, by contrast, displayed the reversed pattern, i.e., an increased BOLD response when there was a low demand on cognitive control. Thus, it is possible that high rsGlu in the dACC generates a more efficient processing strategy with regard of cognitive control through a higher capacity for energy turnover (1), thereby up-regulating the activity of the default-mode network when the task becomes too easy. Although the relationship between glutamate concentration and neural energy metabolism in the human brain is not entirely clarified, there are indications that these are related (16, 17). Lower rsGlu levels might necessitate additional resources for task processing or reduce the efficiency of the down-regulation of the default-mode network. Hence, as our results show a glutamate-related modulation of default-mode regions by a task-positive region, a previous study by Duncan et al. (18) found the reciprocal pattern: Glutamate levels in a default-mode region were related to the BOLD response in a task-positive region. Furthermore, we have here demonstrated that this relationship depends on individual rsGlu levels, because the BOLD response exhibited distinct patterns for individuals with high and low rsGlu levels. Together, these results indicate that glutamate is involved in the interaction between task-dependent response and the default-mode network, possibly contributing to the activation and deactivation of these networks (19).

Because of the glutamatergic contribution to energy metabolism and signal processing, it would not have been unreasonable to expect that rsGlu levels likewise predicted the BOLD response in the measured region itself. However, the lack of any results in the dACC from the three-way analysis rather tells us that the relationship between brain biochemistry and the BOLD response is more complex than the existing level of an excitatory neurotransmitter simply yielding higher or lower BOLD response. Projections from the anterior cingulate to the basal ganglia and cortical regions such as those found in this study are typically glutamatergic, so it is possible that the measured glutamate in this region rather exerts long-range influence on other regions than directly modulating the dACC during task

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**Table 1. Results from the fMRI analysis**

| Anatomical structure | MNI coordinates | Cluster size, voxels |
|----------------------|-----------------|---------------------|
| R latFC 8/9/44/47/48 | 42 14 37 11.6 | 2,339 |
| dACC/preSMA 6/8/24/32 | 6 23 52 10.72 | Subcluster |
| R IPL 21/39/40 | 48 -43 40 10.83 | 1,350 |
| L latFC 6/44/45/47/48 | -33 23 -8 8.4 | 1,326 |
| L IPL 39/40 | -48 -46 49 8.17 | 615 |
| R precuneus 7 | 9 -61 43 6.25 | 48 |
| L MTG 20/21 | -51 -40 -8 6.25 | 124 |
| R DLPFC 9 | 21 47 34 5.49 | 43 |

Clusters show a significant interaction of attention instruction and interaural intensity difference, i.e., cognitive control processing. Displayed clusters showed a significant interaction (P < 0.05, FWE, k = 20 voxels) between top-down (attention instruction) and bottom-up (interaural intensity difference) mechanisms. The MNI coordinates are for peak voxels, but due to their size the clusters include several BAs. The dACC/preSMA cluster in italics is a subcluster of the large R latFC cluster. BA, Brodmann area; dACC, dorsal anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; IPL, inferior parietal lobe; MTG, medial temporal gyrus; L, left; latFC, lateral frontal cortex; preSMA, presupplementary motor area; R, right.
performance. Furthermore, studies that have focused on the relationship between \( \gamma \)-aminobutyric acid (GABA) and BOLD response have shown that resting GABA concentrations predict negative BOLD response within the acquired MRS voxel both in the perigenual cingulate cortex (20) and in the visual cortex (21, 22). Because Duncan et al. (18) also did not find any relationship between glutamate and BOLD response in the measured region itself, it could possibly point toward a more global effect of glutamate on BOLD response, whereas GABA might be more responsible for local BOLD effects. It is nonetheless reasonable to assume that both glutamate and GABA and, possibly, other metabolites and neurotransmitters, contribute to the individual variations in the BOLD response. Thus, investigation of this interrelation with \(^1\)H-MRS protocols that are both GABA- and glutamate sensitive, such as MEGA-PRESS (point resolved spectroscopy) or J-resolved sequences, would be of great value in future combined fMRI/\(^1\)H-MRS studies. In addition, a more thorough MRS-based investigation of the regions revealed in the current study could be of interest to identify their specific relationship and contribution to cognitive control mechanisms.

We also found that the observed differences in neuronal implementation of cognitive control processes did not significantly alter task performance. This observation is in accordance with earlier findings from a similar task where pharmacologically reduced glutamate concentrations in healthy individuals did not influence the performance of the subjects (3). Although glutamate is substantial in neuronal signaling, one would not necessarily expect that behavior in general and the present task performance in particular is affected by only one neurotransmitter or metabolite. It is likely that our lack of a behavioral correlate is founded in the diversity of individual cerebral biochemistry, thus either requiring a higher degree of variation in glutamate or additional

### Table 2. Results from the combined fMRI/\(^1\)H-MRS regression analysis

| Anatomical structure | BA    | x    | y    | z    | t value | Cluster size, voxels |
|----------------------|-------|------|------|------|---------|---------------------|
| L RSC  23/26/29      | −9    | −46  | 19   | 4.61 | 31      |
| L BG                 | −15   | 2    | 1    | 4.21 | 104     |
| R BG                 | −9    | 8    | 1    | 4.01 | 48      |
| R OFC  11            | 24    | 50   | −5   | 3.91 | 20      |
| L IPL  39            | −48   | −61  | 28   | 3.88 | 45      |

Regions show a significant interaction of attention instruction, interaural intensity difference and rsGlu, indicating a modulation of rsGlu on the BOLD response in these regions during cognitive control processing. BA, Brodmann area; BG, basal ganglia; IPL, inferior parietal lobe; L, left; OFC, orbitofrontal cortex; R, right; RSC, retrosplenial cortex.

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Fig. 2. Regions showing a significant interaction (\( P < 0.001, \) uncorrected, extent threshold 20 voxels) of attention instruction, IID, and rsGlu. Individuals with lower levels of rsGlu display increased BOLD response when the demand for cognitive control is high (attention directed toward the less salient stimulus), whereas individuals high in rsGlu show the opposite pattern with higher BOLD response when the cognitive control demand is low (attention directed toward the salient stimulus). Vertical bars denote SE. BG, basal ganglia; FL, forced-left attention condition; FR, forced-right attention condition; IPL, inferior parietal lobe; L, left; LE+, left ear 18 dB louder; OFC, orbitofrontal cortex; R, right; RE+, right ear 18 dB louder; RSC, retrosplenial cortex.
significant findings are indicated in italics. $\omega^2$ indicates the effect size. ATT, attention instruction; IID, interaural intensity difference.

**Methods**

Subjects. Forty healthy volunteers, 20 male (mean age $\pm$ SD: 25.0 $\pm$ 3.6) and 20 female (26.0 $\pm$ 4.0) y native Norwegian speaking students from the University of Bergen participated in the study. All were right handed, as assessed with the Edinburgh Handedness Inventory (23). Normal hearing was ensured by using the Hughson-Westlake audiometric test (Oscilla USB-300; Inmedico) with frequencies of 250, 500, 1,000, 2,000, and 3,000 Hz. Subjects with an interaural acuity difference of $>10$ dB over all frequencies were excluded. The Regional Committee for Medical Research Ethics in Western Norway approved the study, and informed consent was obtained from all subjects before the experiment.

Auditory Cognitive Control Task. The experimental paradigm was a variant of the Bergen dichotic listening test (24), an auditory speech perception task using pairwise presentations of consonant-vowel syllables, whereby one syllable is presented to the left ear and the other one, simultaneously, to the right ear. The syllables were made from six stop consonants (b/l, d/l, g/l, k/l, p/l, t/l) together with the vowel /a/, resulting in three voiced (b/l, d/l, g/l) and three unvoiced (k/l, p/l, t/l) syllables. When combining the syllables into dichotic pairs, only syllables with the same voicing were paired (e.g., b/l-d/l, g/l-t/l), using 12 of 30 possible dichotic combinations. This restriction was applied because of the possible effect of voicing on ear advantage and performance (25, 26). The syllables had a duration of 400–500 ms and were spoken by an adult male Norwegian voice with constant intensity and tonation. The syllables were aligned to achieve a synchronous onset. To achieve systematic variation in cognitive control, a stimulus-driven (bottom-up) and an instruction-driven (top-down) component was added to the paradigm. The bottom-up component was varied by manipulating the salience of the stimulus through different levels of interaural sound intensity (27, 28). Five levels of interaural intensity differences (IID) were used—18 dB in favor of the left ear, 9 dB in favor of the left ear, no intensity difference, 9 dB in favor of the right ear, and 18 dB in favor of the right ear. In the condition with no intensity difference, the stimulus was presented at a 70 dB sound pressure level (SPL) at both ears. The other four conditions were presented with 70 dB SPL at the weaker stimulus, whereas the weaker stimulus was 11 and 52 dB SPL, respectively. The stimulus intensity in the headphones was controlled and adjusted by using the Head and Torso Simulator System and a sound level meter (2250 and 4128C; Bruel and Kjaer). The top-down instruction-driven component was added by selectively directing the subjects’ attention, giving instructions to focus their attention to, and report from, the right- (forced-right attention condition [FR]) or the left-ear stimulus [forced-left attention condition (FL)] (24, 29). Thus, focusing on the less-salient stimulus would induce a higher need for top-down cognitive control processes than focusing on the more salient stimulus, predicting a significant interaction between attention instruction and sound intensity manipulation (11, 12). Attention instructions were given by using goggles mounted to the head coil (NordicNeuroLab) and preceded the auditory stimulus by 1.5 s. The attention conditions were randomly intermixed, and which ear to attend was indicated on the screen by text (“Attend left/right ear”) and by an arrow pointing in the respective direction (left/right).

When combining the two attentional instructions with the five levels of IID, it resulted in 10 experimental conditions. Each of the 10 conditions contained 18 dichotic presentations, resulting in a total of 180 stimulus presentations that were pseudorandomly intermixed with 90 silent null events. This procedure created a stochastic event-related design for fMRI acquisition (30), which was recorded over a 20-mm FOV. The dichotic syllables were presented by using headphones (NordicNeuroLab) at the beginning of the silent gaps of the sparse sampling protocol for the fMRI acquisition (31). The participants responded orally by naming the syllable they heard immediately after the stimulus, thereby ensuring response termination by the start of the next scan and avoiding movement artifacts during data acquisition. Further, no responses were recorded for decoding of the on-line acoustic input into a compatible microphone and an mp3 recorder. The number of correct reports from the left and right ear was scored after testing and served as dependent variables for the behavioral analysis. Stimulus administration and synchronization of the stimuli with the fMRI acquisition was performed by using E-Prime software (version 2.0, Psychology Software Tools).

**MR Imaging and Analysis.** All imaging was performed on a 3.0 T GE Signa scanner. The subjects underwent an imaging protocol including a scout sequence and structural imaging before the fMRI and the 1H-MRS. Structural imaging was done by applying a T1-weighted pulse sequence (Fast Spoiled Gradient; repetition time (TR) = 7.9 ms; echo time (TE) = 3.1 ms; 11° flip angle) measuring 180 sagittal slices (field of view, FOV = 256 mm $\times$ 256 mm; 256 $\times$ 256 scan matrix) of 1-mm thickness.

The fMRI and preprocessing. Functional imaging was performed by using an echo-planar imaging (EPI) sequence (TE = 30 ms; 90° flip angle) and was oriented to the structural image. A sparse sampling protocol was used [TR = 5.5 s, acquisition time (TA) = 1.5 s] with silent gaps of 4.0 s between scans for task performance, thereby avoiding interfering scanner noise and movement artifacts through overt speech. All EPI volumes covered the cerebrum and most of the cerebellum and contained 25 axial slices (0.5 mm interslice gap; FOV = 220 $\times$ 220 mm, 64 $\times$ 64 scan matrix) of 5-mm thickness, resulting in a voxel size of 3.44 $\times$ 3.44 $\times$ 5.0 mm.

Preprocessing and statistical analysis of the data were performed by using Statistical Parametrical Mapping (SPM8) analysis software package (Wellcome Department of Cognitive Neurology) running under MATLAB R2010a (Mathworks). The EPI images were realigned intrasubjectively to the first image in each time series and unwarped to correct for head movements and related artifacts. The images were then normalized into a standard stereotactic space by using the MNI template and resampled with a cubic voxel size of 3 mm. To remove remaining interindividual anatomy differences and increase the signal-to-noise ratio, the normalized images were smoothed by using a 6-mm full-width-at-half-maximum (FWHM) Gaussian filter. The first-level individual statistical analysis of the fMRI data was set up as a model including a predictor for each of the 10 experimental conditions. The predictors were convolved with the canonical hemodynamic response function and applied with a temporal high pass filter (cutoff at 128 s). The resulting individual beta maps were used for the second-level group analysis.

$^1$H-MRS and preprocessing. An axial T2-weighted image was obtained before the $^1$H-MRS to position the voxels. In vivo $^1$H spectra were obtained from the left and right dACC by using a single voxel PRESS sequence (TR = 1,500 ms, TE = 35 ms, voxel size 24 $\times$ 20 $\times$ 20 mm, 128 averages). See Fig. 1A for voxel localization. The average line width was 0.038 ppm (SD 0.04) for the left hemisphere and 0.044 ppm (SD 0.007) for the right hemisphere, and the signal-to-noise ratio (SN) was averaged at 22.47 (SD 3.4) for the left hemisphere and 20.77 (SD 2.79) for the right hemisphere. The spectra were analyzed by using

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**Table 3.** Behavioral analysis showing the results of a four-way ANOVA with the factors rsGlu, attention instruction, interaural intensity difference, and ear.

|                     | $\delta_{effect}$ | $\delta_{error}$ | $\omega^2$ |
|---------------------|-------------------|-----------------|-----------|
| rsGlu               | 0.09              | 2.55            | 0.07      |
| ATT                 | 0.00              | 27.61           | 0.25      |
| ATT * rsGlu         | 0.36              | 1.05            | 0.00      |
| ID                  | 0.67              | 0.18            | 0.00      |
| ID * rsGlu          | 0.69              | 0.38            | 0.00      |
| Ear                 | 0.00              | 11.54           | 0.12      |
| Ear * rsGlu         | 0.29              | 1.29            | 0.01      |
| ATT * ID            | 0.00              | 10.92           | 0.06      |
| ATT * ID * rsGlu    | 0.20              | 1.68            | 0.01      |
| ATT * Ear           | 0.00              | 13.36           | 0.07      |
| ATT * Ear * rsGlu   | 0.19              | 1.73            | 0.01      |
| IID * Ear           | 0.00              | 40.92           | 0.20      |
| IID * Ear * rsGlu   | 0.51              | 0.68            | 0.00      |
| ATT * IID * Ear     | 0.37              | 0.83            | 0.00      |
| ATT * IID * Ear * rsGlu | 0.31          | 1.22            | 0.00      |

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LCModel software (Version 6.2–1A; ref. 32), allowing for quantification of metabolite levels (Fig. 51). Glutamate values relative to creatine were used, because this ratio is commonly used externally as a reference. Because no task was presented during the 1H-MRS sequence, the obtained measures is referred to as resting-state glutamate (rsGlu) levels. The average of the left- and right-hemispheric measurement was used for statistical analysis. Control of possible confounding factors. The level of glutamate in a voxel has been shown to depend on the amount of gray and white matter within the investigated voxel (see, e.g., ref. 33), and interindividual differences in gray matter of the dACC have been reported (34). We were concerned that the rsGlu levels measured here might be confounded by differences in the proportion of gray matter within the measurement voxels. To test for this possibility, we used the T1-weighted anatomical images to estimate the gray matter content of the dACC region in which the 1H-MRS measures were performed. For this purpose, bilateral template masks of the dACC (created in MIPAV) were fitted to each individual’s brain by using SPMMB, (i) by estimating the transformation parameters for the normalization of each individual T1-weighted image to the MNI template, and then (ii) by inverting transformation to transfer the dACC mask into each individual’s native space (unnormalized) brain image. The proportion of gray matter within the dACC region was then estimated based on gray matter probability maps (in native space) as obtained by the segmentation procedure implemented in SPMB (35). The mean gray matter content for the left- and right-hemispheric regions was then estimated based on gray matter probability maps (in native space) after transformation to transfer the dACC mask into each individual’s native space. 

Statistical analysis of the combined fMRI1H-MRS data. In the statistical analysis of the fMRI data, only 4 of the 10 conditions were included to investigate the BOLD response during cognitive control. This reduced design involved only the four conditions when the IBD was 18 dB in favor of one of the ears in the FL and attention conditions, contrasting the two conditions with the highest (FL with +18 dB to the left ear, and FL with +18 dB to the right ear) and lowest (FL with +18 dB to the left ear, and FL with +18 dB to the right ear) demands for attention. These two conditions were thought to most reliably reflect a high and low degree of cognitive conflict, hence showing the need for administration of cognitive control processes when contrasted. The analysis was set up within the framework of the general linear model (full factorial design), including predictors coding the main effects of attention instruction (categorical, within-subjects factor), IBD (categorical, within-subjects factor), and rsGlus (continuous, between-subjects factor; z-standardized), and all possible interactions of these three predictors (36). Brain regions showing a significant interaction of attention instruction and IBD was interpreted as being involved in cognitive control processes (11, 12). The effect of interest was the three-way interaction of all three predictors, indicating the modulation of cognitive control processes by the rsGlus levels. All analyses were performed by using SPMB. Post hoc exploration of significant clusters was performed by using the SPM toolbox MarsBaR(37) and MRICron software (www.cabiatl.com/mricron). The anatomical location of each cluster was determined by using Automated Anatomical Labeling (38).

Behavioral Analysis. The behavioral data from the dichotic listening task were analyzed with a four-factorial ANOVA with the repeated-measures factors ear (two levels; right ear and left ear), attention instruction (two levels; FR and FL), and IBD (two levels; +18 dB right ear and +18 dB left ear), and rsGlus (as a continuous predictor, between-subjects factor; z-standardization). Post hoc analysis was done by using Fisher’s Least Significant Difference and effect-size measures were calculated as percentage of explained variance ($\omega^2$).

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