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GABA concentrations in the human anterior cingulate cortex predict negative BOLD responses in fMRI

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The human anterior cingulate cortex (ACC) is part of the default-mode network that shows predominant negative blood oxygen level–dependent (BOLD) responses in functional magnetic resonance imaging (fMRI). We combined fMRI during emotional processing and resting-state magnetic resonance spectroscopy measurements and observed that the concentration of GABA in the ACC specifically correlated with the amount of negative BOLD responses in the very same region. Our findings show that default-mode network negative BOLD responses during emotions are mediated by GABA.

Studies using fMRI have shown pervasive negative BOLD responses rather than positive BOLD responses during various emotional-cognitive tasks in the human ACC[1–5]. Together with cortical regions (ventro- and dorsomedial prefrontal cortex, posterior cingulate cortex, lateral parietal cortex and superior temporal gyrus), the ACC is therefore considered to be part of the default-mode network that is characterized by high neuronal resting-state activity[1,2,6]. However, the neurochemical nature of negative BOLD responses in the default-mode network remains unclear. Recent studies demonstrate that negative BOLD responses in the visual cortex are tightly coupled to decreases in neuronal activity, which may be traced back to increased neuronal inhibition[7–10]. Decreases in neuronal activity are assumed to be mediated by inhibitory neurotransmitters such as GABA. GABAergic substances such as lorazepam modulate neuronal activity in medial prefrontal cortical regions such as the ACC in both humans and animals[11,12]. One would consequently hypothesize that the concentration of GABA predicts the amount of negative BOLD responses in these regions. The current study's aim was to determine whether negative BOLD responses in the ACC (when compared with resting state) are specifically associated with the concentration of GABA (at rest) in the very same region.

To address this question, we combined fMRI with magnetic resonance spectroscopy (MRS) at rest to measure negative BOLD responses and GABA in the right ACC and in the right paracentral cortex as a control region in 12 healthy human subjects. Carrying out reliable MRS measurements of GABA at magnetic field strengths of 1.5–3 T is, in contrast to glutamate and glutamine, particular challenging, as the signal is very small and is overlaid by other metabolite signals. In addition, the ACC is prone to magnetic susceptibility artifacts. To overcome these difficulties, we applied an adapted point-resolved spectroscopy–localized two-dimensional J-resolved MRS sequence to assess GABA and glutamine concentrations at rest (**Supplementary Methods** online).

This approach allows for a substantial reduction of spectral overlap by spreading multiplet resonances along two frequency axes instead of along only one axis, as in conventional one-dimensional MRS (**Supplementary Fig. 1** online). In addition, a maximum echo-sampling scheme avoids the distortion of metabolite signals of interest by the tail of the water signal. This is of special interest in the ACC, where susceptibility problems may hamper the quality of water suppression[13]. The acquisition time of one PRESS-localized two-dimensional J-resolved MRS sequence measurement was 16 min. Quantification of 19 brain metabolites, including GABA and excitatory glutamine transmitters, was carried out using ProFit[13], with the ACC as the main region of interest and the right paracentral cortex as the control region. In contrast to former quantification methods, ProFit can access the full information content of two-dimensional J-resolved spectra by fitting a linear combination of two-dimensional basis spectra to it (**Supplementary Methods** and **Supplementary Fig. 1**). We conducted tests for reliability to validate sequence and quantification before this study[13]. Negative BOLD responses in our brain sampling regions, the ACC and the paracentral cortex, were obtained in fMRI during affective picture viewing, affective picture judgments and the expectancy period of emotional pictures by the corresponding contrasts against rest (**Supplementary Fig. 1** and **Supplementary Methods**). Our approach focused on the resting state, as, relying on the default-mode network theory, the level of resting-state neuronal activity (and neurochemical state of rest) may influence (and even at least partially determine) the amount of signal changes that are nonspecifically induced by emotional (or cognitive) tasks (**Supplementary Methods**). We obtained informed written consent for all subjects.

Both picture judgment and perception yielded negative BOLD responses in the ACC and in the parietal and superior temporal cortex (**Fig. 1** and **Supplementary Table 1** online) when compared with the resting state. As demonstrated in time courses (**Fig. 1b**) and bar diagrams (**Fig. 1c**), negative BOLD responses occurred in the ACC for both emotional perception and judgment, the latter (–0.153 ± 0.064) showing significantly stronger ($T = 2.93$, degrees of freedom = 11, $P < 0.05$) negative BOLD responses than the former (–0.103 ± 0.045). In contrast, we did not observe negative BOLD responses in the ACC during the expectancy periods whose neural activity remained close to the resting state (**Fig. 1b** and **Supplementary Table 1**). No effects of age were found for BOLD responses in the ACC. Neither

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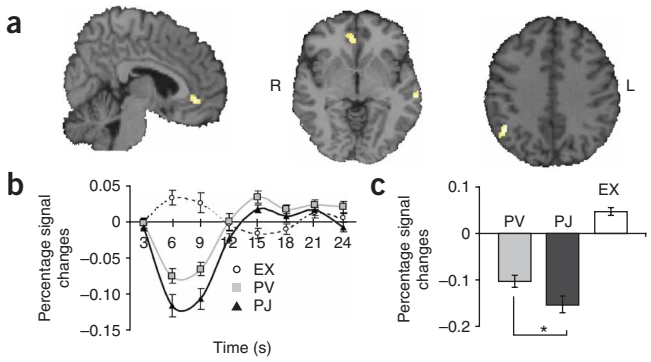

Figure 1 Negative BOLD responses in the default-mode network during emotional conditions. (**a**) Signal changes in the default-mode network obtained in fMRI whole-brain analysis during all picture conditions. The level of statistical significance was set to $P < 0.001$, family-wise error corrected. L, left; R, right. Regions showing negative BOLD responses included the right ACC, the left superior temporal gyrus and the right lateral parietal cortex (for further details, compare **Supplementary Table 1**). (**b**) Time courses (percentage signal changes) for emotional picture viewing (PV), picture judgment (PJ) and expectancy period of pictures (EX) in our region of interest, the ACC. (**c**) Separate analysis of effect sizes (percentage signal changes) in PV, PJ and the EX, indicating PV or PJ in our region of interest, the ACC. PJ revealed significantly stronger negative BOLD responses than PV (*$P < 0.05$). Error bars indicate s.e.m.

the mean concentration of GABA nor that of glutamine differed significantly between the ACC (GABA, 0.22 ± 0.07; glutamine, 1.60 ± 0.39) and the control region (GABA, 0.17 ± 0.04; glutamine, 1.41 ± 0.19) ($P > 0.05$). Metabolite concentrations of GABA or glutamine did not correlate with age in either ACC or control region.

Using a Spearman correlation analysis, we observed significant correlations of GABA-concentrations with negative BOLD responses in the ACC. This was observed for all emotional pictures (including both picture viewing and picture judgments, $P < 0.01$, **Fig. 2a**) as well as separately for picture judgments ($P < 0.05$) and for picture viewing (marginally significant, $P < 0.061$, **Fig. 2b**). The stronger the negative BOLD responses in the ACC, the higher the GABA concentration measured in the very same region. In contrast, no correlation with GABA was observed for signal changes during the expectancy period (**Fig. 2a** and **Supplementary Table 2** online). Furthermore, signal changes in the control region did not correlate with GABA concentration in the control region (**Supplementary Table 2**). Because neurotransmitter concentrations are substantially higher inside gray matter compared with white matter, it can be assumed that mainly cortical regions contribute to the observed GABA-negative BOLD response correlation in the ACC. Therefore, the partial volume effects that were primarily attributable to the large MRS voxel size did not obscure the observed correlations.

To further confirm these correlational findings and to directly account for partial volume effects common to both fMRI and MRS, we carried out a single-voxel regression analysis with the individual subjects' level of GABA in the ACC as the regressor in the design matrix for the contrast rest versus all emotional pictures (**Supplementary Methods**). Peak voxels for the significant correlation between negative BOLD responses and GABA were observed in the ACC (x, y, z, Z value: 12, 38, 8, 2.89, respectively, $P < 0.05$) and were restricted to gray matter inside the selected volume of interest, which corresponded to the spectroscopy volume (**Fig. 2c**). No significant effects of age were observed in fMRI-MRS correlation patterns of GABA ($P > 0.05$).

The reported correlations of GABA with negative BOLD responses in the ACC also remained significant when including glutamine and gluthathione concentrations in the ACC as covariates in partial correlation analysis ($P < 0.05$). In contrast to GABA, no correlation with glutamine in either the ACC or the control region was observed (**Supplementary Table 2**).

In our combined *in vivo* fMRI and MRS investigation, we could demonstrate that the concentration of GABA at rest predicts the amount of negative BOLD responses in the human ACC during emotional stimulation. The higher the total concentration of GABA in the ACC, the stronger the negative BOLD responses that were induced in the very same region. In contrast, we did not observe any correlation with other metabolites such as glutamine, nor did we detect any confounding effects of glutamine and gluthathione on the correlation with GABA. Recent investigations demonstrate that negative BOLD responses, as observed in fMRI, are tightly coupled to decreases in neuronal activity[7–10]. Neuronal activity decreases may be either related to decreased excitatory transmission, as mediated by glutamate, or to increased inhibition by transmitters such as GABA. We found that neuronal activity decreases coupled to negative BOLD responses are probably mediated by increased GABA transmission. This would agree with the direction of the negative BOLD response-GABA correlation observed here. Finally, our observation complements previous findings of neurochemical modulation of the ACC and medial prefrontal cortical regions by GABAergic administration in both humans and animals[11,12,14].

The correlation with GABA was most visible in emotional judgment, implying the highest cognitive load as reflected in reaction times, that

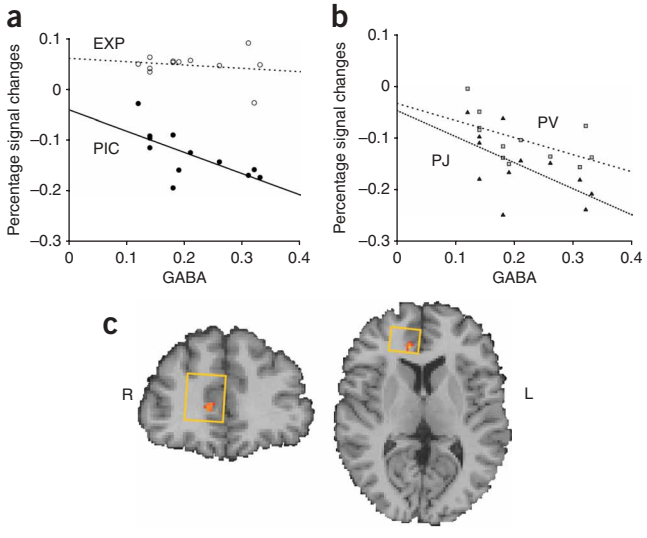

Figure 2 Correlation between GABA concentration and negative BOLD responses in the ACC. (**a**) Correlation between GABA concentrations (relative to creatine levels) and negative BOLD responses in the ACC for all pictures (PIC, $r = –0.713$, $P < 0.01$, filled circles and black line) and the EX (not significant, $P > 0.9$, open circles and dotted line). (**b**) Separate correlations for PV ($r = –0.554$, $P < 0.1$, open squares and open dashed line) and PJ ($r = –0.635$, $P < 0.05$, solid triangles and dense dashed line) (**Supplementary Table 2**). (**c**) Local maxima of the voxel-wise simple regression analysis of the contrast [Rest > all pictures] with GABA levels in the ACC region of interest. Shown are the coronal (left) and transversal (right) sections. Orange boundaries indicating region of interest size and location correspond to the MRS voxel. Negative BOLD responses correlating with GABA concentrations are clearly restricted to the medial prefrontal gray matter in the ACC even on an uncorrected level of significance ($P < 0.005$).

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showed the highest amount of negative BOLD responses. Although weaker, only a marginally significant correlation ($P < 0.061$) was observed during emotional picture viewing, and no correlation at all was observed with signal changes (for example, positive bold responses) during the expectancy period. This indicates that GABA specifically modulates negative BOLD responses and suggests that neuronal inhibition may be crucial in human negative BOLD responses, which agrees with previous findings in animals[9]. Moreover, our results indicate that the strength of the GABA-negative BOLD response relationship depends psychologically on the cognitive load. Our results thus complement previous observations that default-mode network negative BOLD responses are neurochemically engaged by increasing cognitive load[4,5,15].

Our results should, however, be interpreted cautiously. Future studies combining measurement of all three factors, GABA, negative bold response and neuronal activity, will be necessary to lend further support for our conclusion. Finally, although we did not obtain any correlation of negative BOLD responses with glutamine, the role of excitatory transmission on negative BOLD responses and positive bold responses needs to be clarified in detail. In this context, it is problematic that the measured total glutamine concentrations do not directly correspond to the pools of glutamate used in excitatory neurotransmission. Glutamate is also involved in other processes, such as the malate-aspartate shuttle and therefore the energy metabolism of neuronal and glial cells. Furthermore, GABA is directly synthesized from glutamate. Hence, although there is no relevant evidence in our data, we cannot exclude that the excitation-inhibition balance producing negative BOLD responses in the ACC is affected, in part, by a reduction in glutamergic drive. Our correlation results indicate a direct modulation of negative BOLD responses by GABA, rather than an indirect one mediated by glutamate.

In conclusion, our data indicate that the resting-state concentration of GABA predicts the strength of negative BOLD responses during emotional processing in human ACC as crucial part of the default-mode network. Although the demonstrated positive correlation does not prove causation, our results indicate GABAergic, and thus inhibitory, modulation of negative BOLD responses during emotions in the default-mode network as distinguished from glutamatergic excitatory mediation.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

G.N. designed the study, co-analyzed the data, wrote the manuscript and supervised the whole project. M.W. analyzed the fMRI-MRS correlation data in collaboration with G.N., S.G. and H.B., carried out the fMRI experiments and analyzed the fMRI data. R.F.S. designed and implemented the software for MRS data acquisition and for MRS data analysis. R.F.S. and J.B. carried out the MRS experiments. U.D. and A.H. supervised the MRS part of the study, and contributed to the interpretation of the data and preparation of the manuscript. P.B. took the technical and financial responsibility for the combined MRI-MRS study and revised the manuscript.

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