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Electrophysiological measurements of anterior cingulate function

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Summary. Based on recent findings from various areas of brain research the anterior cingulate cortex (ACC) within the prefrontal cortex is increasingly considered as a brain region activated during tasks requiring conflict-monitoring and allocation of attention. In the present study with event-related potentials (ERPs) the question has been addressed, whether the NoGo-condition of the Continuous Performance Test is associated with enough conflict-monitoring and allocation of attention in order to activate the ACC in healthy controls. Low Resolution Electromagnetic Tomography (LORETA), a new three-dimensional source localization method, revealed significantly increased brain electrical activity during the NoGo-ERP as compared to the Go-ERP with its maximum located exactly within the ACC in four independent samples of healthy subjects. These results relate the conflict-monitoring requirements associated with inhibition of a prepared motor response (NoGo-condition) to a powerful brain electrical ACC-activity. This non-invasive, easy to perform and inexpensive electrophysiological measurement, therefore, provides a new method for the assessment of ACC-function in healthy subjects.

Keywords: event-related potentials, ERP, Continuous Performance Test, CPT, anterior cingulate, ACC, centroids, NGA, LORETA.

Introduction

The anterior cingulate cortex (ACC) is a brain region forming a ring around the rostrum of the corpus callosum and having multiple projections to various other brain areas. In the classical neuroanatomical literature, the ACC has been considered as a pivotal part of the limbic system, primarily involved in the regulation of emotion (MacLean, 1993). However, an activation of the ACC has been reported in recent investigations during tasks requiring executive brain functions associated with motor responses (Vogt et al., 1992; Devinsky et al., 1995). In particular, the ACC has been addressed as a brain region activated during tasks like the Continuous Performance Test (CPT)

where errors are likely to occur (Bench et al., 1993; Cabeza and Nyberg, 2000; Carter et al., 1997; Corbetta et al., 1991; Posner et al., 1988; Posner and Dehaene, 1994; Taylor et al., 1994). Notably, an activation of the ACC has been reported especially during the inhibition of goal-directed behaviors (George et al., 1994; Carter et al., 1998). Moreover, work on non-human primates has pointed to an ACC source during inhibitory brain activity (Sasaki and Gemba, 1986). Based on these and many more studies, the ACC has been associated with either "strategic" or "evaluative" brain functions: Following the "strategic" theory, the ACC provides resources for attentional processes (attention-to-action; Posner et al., 1988). The "evaluative" view primarily relies on the error negativity (N_e; Falkenstein et al., 1991), later termed error related negativity (ERN; Gehring et al., 1993), which is an event-related brain potential associated with the occurrence of erroneous responses with a supposed generator in the midline prefrontal cortex in the vicinity of the ACC (Dehaene et al., 1994; Holroyd et al., 1998). Based on results of PET and fMRI-studies, the "evaluative" interpretation of ACC-function has been modified from strict error detection to conflict-monitoring. This more integrative theory suggests that the ACC is active during each cognitive task requiring the decision between conflicting responses in order to strategically allocate additional attentional resources (Carter et al., 1998, 1999, 2000; MacDonald et al., 2000).

Principally, in the assessment of brain function, electrophysiological methods have two advantages as compared to PET, SPECT and fMRI: at first, they allow a direct measurement of electrical, neuronal function and do not rely on secondary metabolic changes in substrates and blood flow only indirectly related to neuronal activity. The second advantage is that electrophysiological methods reflect neuronal function almost in real time, while the metabolic response assessed with PET, SPECT and fMRI usually has a delay of several seconds. However, the spatial resolution of at least fMRI and PET is clearly superior to electrophysiological methods.

In our laboratory we focussed on the P300 time range (positive going ERP-component about 300 ms after stimulus presentation) and employed a cued version of the Continuous Performance Test (CPT; Rosvold et al., 1956) combined with a simultaneous EEG-recording as an experimental paradigm for the assessment of cognitive response control (Fallgatter et al., 1997). The applied CPT-version required both the execution (Go-condition) and the inhibition (NoGo-condition) of a prepared motor response and, therefore, reflects the fundamental processes underlying response control mechanisms. A spatial analysis of the event-related potentials (ERPs) with the centroid-method (Lehmann, 1987) revealed that the gravity center (centroid) of the individual NoGo-ERPs was located more anterior as compared with the respective Go-ERPs. This finding was termed NoGo-anteriorisation (NGA) and was quantified with values derived from a simple anterior-posterior axis. The NGA-phenomenon was found in every single of 49 healthy subjects investigated in 3 subsequent studies (Fallgatter et al., 1997, 2000; Fallgatter and Strik, 1999). Furthermore, a high short-term (Fallgatter et al., 2001) and long-term test-retest reliability (Fallgatter et al., in press) of the NGA

has been shown. Moreover, evidence has been presented that the NGA-phenomenon is not affected by neither age nor gender (Fallgatter et al., 1999). Therefore, this electrophysiological parameter qualifies for a first topographical standard-index in electrophysiology (Fallgatter et al., 1997, 2000; Fallgatter and Strik, 1999). A source localization analysis of the original data-set (Fallgatter et al., 1997) with the first version of the LORETA-method without implementation of the Talairach space (Low Resolution Electromagnetic Tomography; Pascual-Marqui et al., 1994) explained the NGA-phenomenon as an effect of a significantly increased electrical activity in prefrontal brain areas during the NoGo- as compared to the Go-condition (Strik et al., 1998). However, sophisticated neuroimaging methods ought to yield a more precise localization of the electrophysiological correlates of cognitive processes. Recently, the LORETA-method has been substantially improved by means of implementing normalizations to the space of Talairach coordinates. Thus, LORETA sources of electrical activity can be located three-dimensionally and quite precisely within the space of a reference brain (Montreal Neurologic Institute) by means of the x-, y- and z-coordinates.

The cued version of the CPT applied in the current investigation also requires the decision between two contrary responses (execution of an anticipated motor response in the Go- and its inhibition in the NoGo-condition), i.e. this task requires conflict-monitoring with the need to provide attentional resources. On an electrophysiological level, the inhibition of a prepared motor response (NoGo) is supposed to be more demanding and, therefore, associated with a higher level of conflict-monitoring as well as allocation of attention than its execution (Go). This view is supported by the finding of significantly longer latencies of the NoGo- as compared to the Go-ERPs (Fallgatter et al., 1997, 2000; Fallgatter and Strik, 1999). Based on these considerations, we hypothesized that NoGo-ERPs (i.e., response inhibition) would be characterized by more brain electrical ACC-activity as compared to Go-ERPs (i.e., response execution). This topographical hypothesis was tested in four independent samples of healthy subjects by means of LORETA source analyses.

Materials and methods

In this study the ERPs elicited by Go- and NoGo-conditions of the CPT in four independent samples of healthy controls (Berg et al., 2001; Fallgatter et al., 1997, 2000; Fallgatter and Strik, 1999) were analysed by LORETA (Pascual-Marqui et al., 1994) with an implementation of the neuroanatomical space. This LORETA-version (LORETA-Key-01 FreeBrainWare) calculates x-, y- and z-coordinates which permit a three-dimensional localization of LORETA-sources in the structures of a normalized reference template. All four studies were reanalysed separately, but in an identical manner.

Subjects

The samples consisted of 14, 10, 27 and 12 subjects (Berg et al., 2001: n = 14, 8 female, 6 male, mean age 50.4 ± 11.0 years, range 29–70 years; Fallgatter et al., 1997: n = 10, 5 female, 5 male, mean age 29.1 ± 2.8 years, range 25–33 years; Fallgatter and Strik, 1999: n = 27, 12 female, 15 male, mean age 40.3 ± 10.7 years, range 22–60 years; Fallgatter et al., 2000: n = 12, mean age 28.8 ± 4.0 years, range 24–41 years). All subjects were healthy, medication-free and, besides one, right-handed according to Oldfield (1971). None had a

history of psychiatric or neurologic illnesses. Vision was normal or corrected to normal in all subjects.

Continuous Performance Test

The Continuous Performance Test (CPT) was described in more detail elsewhere (Fallgatter et al., 1997). Briefly, it consisted of 400 stimuli which were presented in a pseudorandomized order on a computer screen 120 cm in front of the subjects for 200 ms each with an interstimulus interval of 1,650 ms. The letters on the screen were 12 mm high and 11 mm wide resulting in a visual angle of 1.15° horizontally and 1.05° vertically. Subjects were instructed to press a response button as fast and accurate as possible, whenever the letter "X" was preceded by the letter "O". For all other letters following the letter "O", the prepared motor response had to be suppressed. The letter "X" followed the letter "O" (Go condition) 40 times, at 40 occasions another letter followed the letter "O" (NoGo condition). Besides these 80 presentations of the letter "O" (priming condition), 240 different letters were presented as distractors.

EEG recordings

The EEG was recorded at 21 scalp sites positioned according to the international 10–20 system, using gold-cup-electrodes (NICOLET, Madison, WI; 3 mm diameter). Linked mastoids with compensating resistors of 10 kΩ were used as reference electrodes. Three additional electrodes were placed at the outer canthi of both eyes and below the right eye to monitor eye blinks and movements. The bandpass was set to 0.1–70 Hz, the EEG was sampled continuously at a rate of 256 Hz. Impedance values were kept at 5 kΩ or below. For recording a 32 channel DC-amplifier (BRAINSTAR, Schwind, Erlangen, Germany) and an acquisition software (NEUROSCAN, Sterling, VA, version 3.2) were used.

Data analysis

The procedure of the EEG-analysis method has been described in detail in previous publications (Fallgatter et al., 1997, 1998, 1999, 2000, 2001; Fallgatter and Strik, 1999; Fallgatter and Herrmann, 2001). In brief, after visual as well as computerized artifact rejections leaving only amplitudes below 98 μV in all EEG- and artifact-channels within the first 500 ms after stimulus presentation, at least 20 artifact-free single trials were obtained for every single subject in Go- and NoGo-conditions. Only trials with correct responses were included in the ERP-analysis. The so-defined trials were averaged to one Go- and one NoGo-ERP for every subject. In these ERPs, the individual Global Field Power peak (GFP; Lehmann and Skrandies, 1980) in the time window lasting from 277 ms to 434 ms was calculated (Fig. 1). The GFP corresponds to the standard deviation of all measured potential values at a given time point and, therefore, is considered as a one-number estimator of the electrical field strength in multi-channel recordings. The borders of the applied P300 time segment were derived in a data-driven manner based on the local minima of the GFP of the difference ERP (Go-NoGo) in the first sample of healthy subjects (Fallgatter et al., 1997). In order to allow comparisons, the identical time segment was applied in all four samples of healthy subjects. A mean GFP peak latency for the Go- and the NoGo-ERP was computed for each of the four samples (see Table 1). Finally, the LORETA analyses were performed at these individual time points.

LORETA analysis

LORETA (Pascual-Marqui et al., 1994, 1999) calculates the current density at each of 2,394 voxels in the gray matter and the hippocampus of a reference brain (Brain Imaging Centre, Montreal Neurologic Institute) as a linear, weighted sum of the scalp electric potentials. LORETA chooses the smoothest of all possible current density configurations

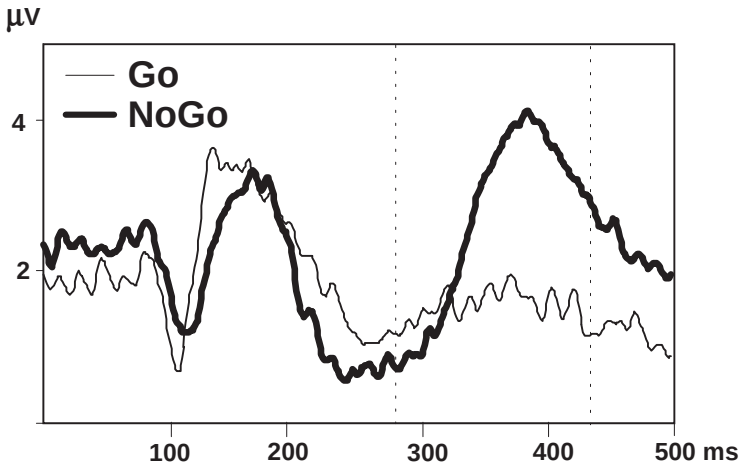


Fig. 1. Global Field Power (GFP) curves for the first 500 ms of the ERP in the conditions Go (thin lines) and NoGo (heavy lines) of the Berg et al. study. The vertical dotted lines indicate the beginning (277 ms) and the end (434 ms) of the P300 time segment applied for the topographical analyses

Table 1. Omission errors (number of missed responses), commission errors (number of false alarms), reaction times, mean GFP latencies and amplitudes with respective standard deviations in the conditions Go and NoGo of the four studies

	Berg et al. (2001)	Fallgatter et al. (1997)	Fallgatter and Strik (1999	Fallgatter et al. (2000)
Omission errors (n)	0.1 ± 0.3	1.1 ± 1.9	0.5 ± 0.9	0.2 ± 0.4
Commission errors (n)	2.4 ± 2.6	0.9 ± 0.9	0.4 ± 0.7	0.2 ± 0.4
Reaction times (ms)	467.9 ± 113.2	381.2 ± 80.1	499.9 ± 125.4	445.5 ± 89.9
GFP-latency Go (ms)	343.8 ± 49.6**	347.7 ± 27.3*	365.5 ± 39.7**	325.2 ± 41.3**
GFP-latency NoGo (ms)	385.2 ± 22.2**	379.7 ± 25.8*	379.5 ± 25.3**	371.7 ± 27.2**
GFP-amplitude Go (µV)	4.1 ± 1.4*	5.62 ± 1.11	5.47 ± 1.67°	5.16 ± 1.22
GFP-amplitude NoGo (µV)	5.2 ± 1.2*	5.62 ± 3.17	6.15 ± 1.38°	5.26 ± 5.29

° = $p < 0.10$; * = $p < 0.05$; ** = $p < 0.01$

throughout the brain volume. This procedure only implicates that neighboring voxels should have a maximally similar activity, no other constraints are used. LORETA-images represent the electrical activity at each of the 2,394 voxel as squared magnitude (i.e. power) of the computed current density. The applied version of LORETA used a three-shell spherical head model registered to the Talairach space (Talairach and Tournoux, 1993).

Statistical analyses

The method to analyse differences for Go and NoGo condition in LORETA corresponds exactly to the statistical non-parametric mapping described by Holmes et al. (1996). The differences in localization between conditions Go and NoGo were computed by voxel-by-voxel t-tests for dependent measures of the LORETA-images, based on the subject-wise

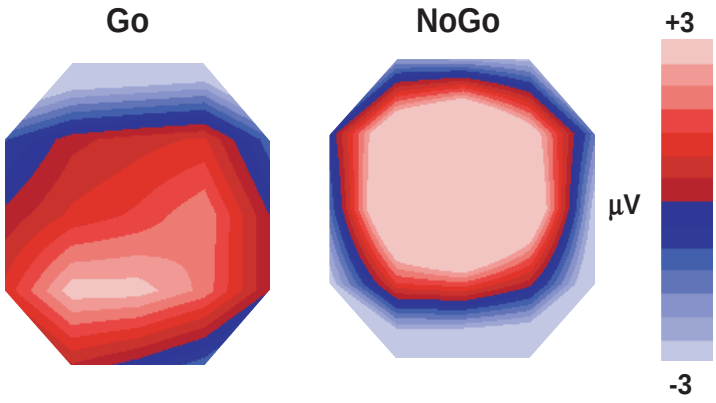


Fig. 2. Two-dimensional grand average field maps of the conditions Go (left) and NoGo (right) in a P300-range (277–434 ms), displaying the NoGo-anteriorisation phenomenon. Red colours indicate positive brain electrical field areas, blue colours stand for negative areas

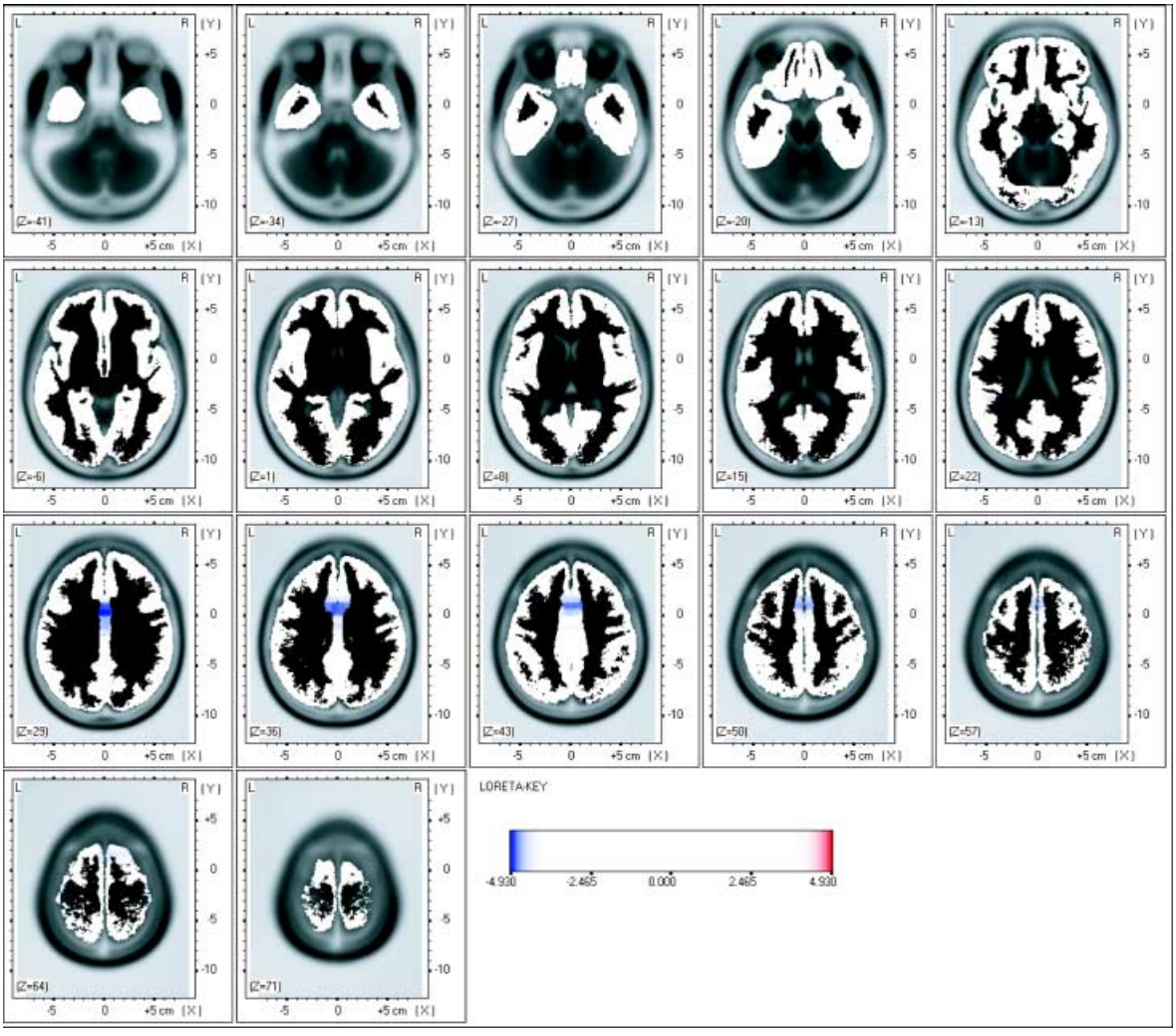


Fig. 3. 17 consecutive transversal LORETA-slices from the data set of the study Berg et al. (2001) are displayed, blue colour indicates an increased NoGo-activity, and red colour significantly more Go-activity. L refers to left side, R to the right

normalized and log-transformed power of the estimated electric current density. The statistical LORETA-analysis relies on a bootstrap method with 5,000 randomised samples (LORETA-Key-01 FreeBrainWare; Pascual-Marqui et al., 1999). This procedure gives the exact significance thresholds regardless of non-normality, and corrected for multiple comparisons. Corresponding z-values are shown in the Figs. 3 and 4.

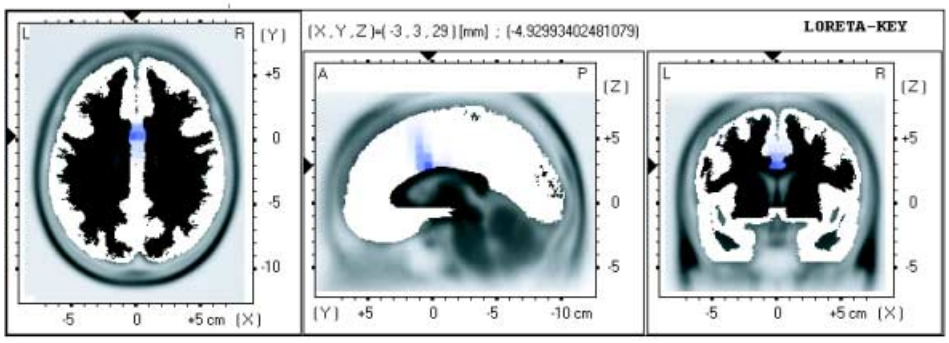
Results

The performance parameters and the mean latencies of the GFP peaks for all four studies are summarised in Table 1. GFP Latency in the NoGo condition was significantly higher than in the Go condition (385.2 ms ± 22.2 ms vs. 343.8 ms ± 49.6 ms; $t = 3.11$, $p < 0.01$). Moreover, GFP amplitude was significantly higher in the NoGo- as compared to the Go-condition (5.2 μV ± 1.2 μV vs. 4.1 μV ± 1.4 μV; $t = 2.98$, $p < 0.05$). Table 2 displays all significant LORETA solutions with a threshold level of $p < 0.05$. Figure 1 illustrates the GFP curves of the ERPs in the conditions Go and NoGo of the Berg et al. study. The two-dimensional grand average brain electrical field maps at the time point of the GFP peak within the P300-window are given in Fig. 2. In Fig. 3, all transversal LORETA-projections of the study Berg et al. (2001) contrasting Go- and NoGo-ERPs at the time point of the mean latencies of the respective GFP peaks are displayed. A significantly increased NoGo-activity (blue colour) was found in the anterior cingulate (Brodman Area (BA) 24, $X = -3$, $Y = 3$, $Z = 29$) and, less pronounced, in the middle frontal gyrus ($X = 11$, $Y = 10$, $Z = 43$; Table 2). The LORETA analysis in Fig. 4 shows the regions of maximal statistical difference between conditions in three orthogonal views for the four studies. Blue colour indicates more NoGo-activity, red colour less NoGo-activity as compared to Go-ERPs. In the first study (Fallgatter et al., 1997) the higher activity for NoGo- as compared to Go-condition did not withstand a correction for multiple testing. In the study of Fallgatter and Strik (1999) significantly higher activity for NoGo- as compared to Go-condition was found in BA 24 and BA 23, both located in the cingulate gyrus (Table 2). In the study of Fallgatter et al. (2000) significantly higher activity for NoGo- as compared to Go-condition was found only in BA 24.

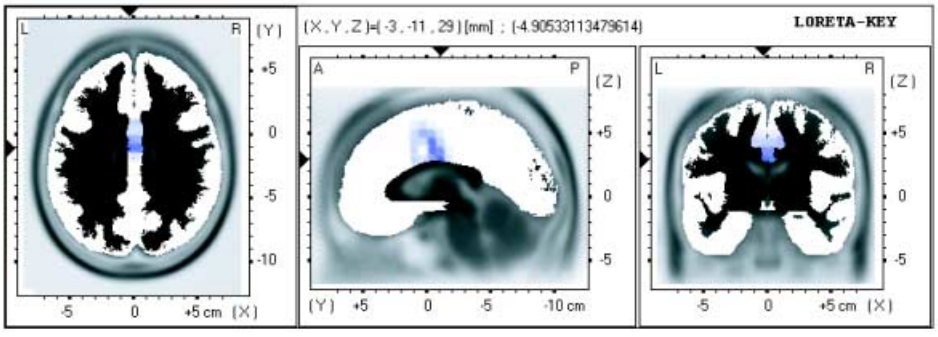
Discussion

In confirmation of the hypothesis, LORETA source analysis of event-related potentials elicited by a response control paradigm (CPT) revealed significantly more electrical ACC-activity in NoGo- as compared to Go-conditions. This was the case in three of four independent samples of healthy controls (Figs. 2 and 3; Fallgatter et al., 1997, 2000; Fallgatter and Strik, 1999). In the study of Fallgatter et al. (1997) the maximal difference between Go and NoGo was also localised in the anterior cingulate cortex, but as only 10 subjects were analysed, we guess that the power of this analysis was to weak to withstand a correction for multiple comparisons ($p = 0.14$). Regarding the specificity of these results, it is remarkable that 10 of 12 regions with a significantly increased brainelectrical NoGo-activity were located in the anterior cingulate

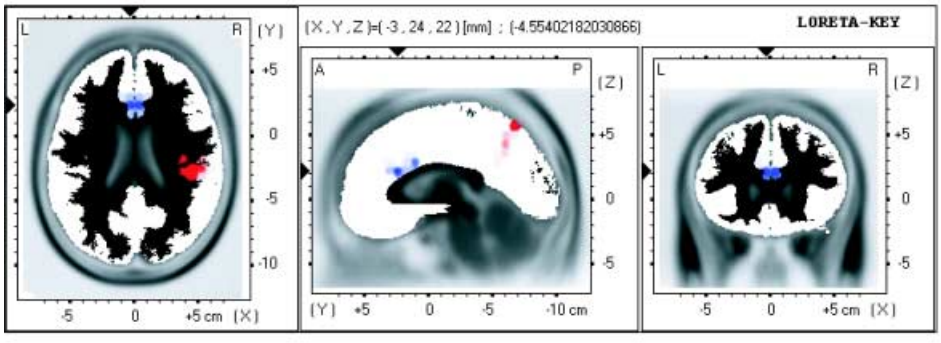
Berg et al., 2001 (n=14)



Fallgatter and Strik, 1999 (n=27)



Fallgatter et al., 1997 (n=10)



Fallgatter et al., 2000 (n=12)

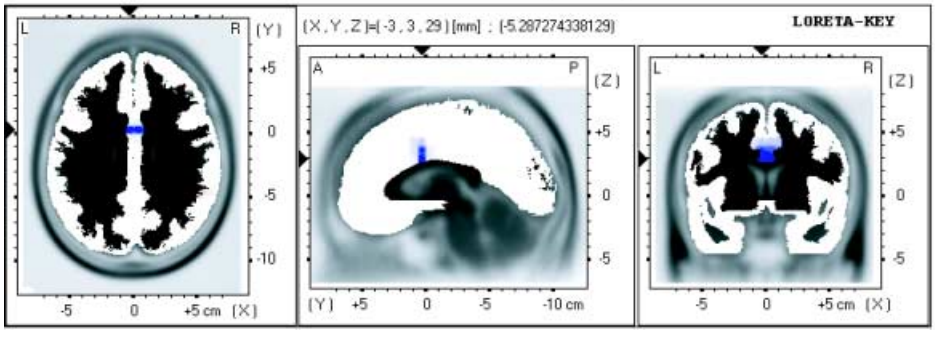


Table 2. *BA* Brodman area; coordinates: exact three-dimensional localisation of the increased NoGo-activity according to the Talairach atlas; z-values: corresponding z-values for each coordinates; *p min* minimal p values for the statistical comparison between Go and NoGo condition, corresponding to the highest z-value of each study; $p < 0.05$: threshold for significant z values

	Localisation	Coordinates	z-value	p min	$p < 0.05$
Berg et al. (2001)	BA 24	X = −3, Y = 3, Z = 29	−4.93	0.04	−4.71
	BA 32	X = 11, Y = 10, Z = 43	−4.77		
Fallgatter et al. (1997)	BA 24	X = −3, Y = 24, Z = 22	−4.55	0.14	−5.64
Fallgatter and Strik (1999)	BA 24	X = −10, Y = −4, Z = 43	−4.91	0.03	−4.01
	BA 23	X = −3, Y = −11, Z = 29	−4.91		
	BA 24	X = 11, Y = −4, Z = 43	−4.87		
	BA 24	X = 4, Y = −4, Z = 43	−4.87		
	BA 24	X = 4, Y = 10, Z = 36	−4.63		
	BA 24	X = −3, Y = 10, Z = 29	−4.63		
	BA 24	X = 4, Y = 10, Z = 29	−4.63		
Fallgatter et al. (2000)	BA 24	X = −3, Y = 3, Z = 29	−5.29	0.01	−4.99
	BA 24	X = −3, Y = 3, Z = 36	−5.29		
	BA 24	X = 4, Y = 3, Z = 29	−5.29		

(BA 24) and the remaining two were in neighbouring brain regions (BA 23 and BA 32; Table 2). The peculiarity of these results is that nearly identical brain regions were identified as the sources of a specific brain function in four independent samples. This high stability and replicability contributes to the validation of LORETA as a reliable electrophysiological source location method.

Furthermore, a significantly longer GFP-latency and also a significantly higher GFP-amplitude in the NoGo- as compared to the Go-ERP have been found in the sample of Berg et al. (2001). These results indicate that the inhibition of a motor response (NoGo) is a more demanding process requiring more time (latency of the ERP) and the activity of larger neuronal assemblies (amplitude of the ERP) as its execution (Go). A trend for higher GFP-amplitudes in the NoGo- as compared to the Go-condition has been reported in the largest of the other studies (Fallgatter and Strik, 1999) while in the two smaller samples the GFP-values did not differ significantly between conditions (Fallgatter et al., 1997, 2000). It has to be concluded that regionally raised NoGo-activity in the ACC is not consistently reflected in a general

Fig. 4. Transversal (left), sagittal (middle) and coronar (right) LORETA-slices are displayed for each of the four studies, selected for maximal NoGo-activity. Blue colour indicates an increased NoGo-activity, red colour less NoGo-activity. L refers to the left side, R to the right side, A to anterior and P to posterior. The localization of the maximum of the respective NoGo-activity is marked by black triangles. The Talairach are shown at the top of each Fig. with x, y, z values, as well as the respective z-statistic in brackets

amplitude measure like the GFP, which mirrors the global neuronal activity as measured with all electrodes. However, these electrophysiological findings are well in line with the hypothesis that higher levels of conflict-monitoring in the NoGo-condition require more attention and, therefore, are associated with more ACC activity.

It has to be mentioned, that several fMRI studies emphasise an (additional) activation of the ventrolateral prefrontal cortex, in particular on the right hemisphere, during inhibition elicited by means of different Go-/NoGo tasks (Garavan et al., 1999; Konishi et al., 1999; Liddle et al., 2001; Rubia et al., 2001). In contrast, in none of the four LORETA analyses with the described characteristics (evaluation at the mean GFP peak of a certain group of subjects, subject-wise normalization and log-transformation of the data, threshold for statistical analysis), the activation in the ventrolateral prefrontal cortex survived the applied statistical threshold. This may be due to various reasons, ranging from differences in investigated samples, employed tasks, differences between neuronal vs. metabolic responses and also to the specific design of the present LORETA analysis. Furthermore, the findings of the current study may be interpreted in a way, that an increased electrical activity of the ACC during the NoGo-condition of the CPT (response inhibition) forms only a part of the neuroelectric basis of the NGA-phenomenon described in previous studies (Fallgatter et al., 1997, 2000; Fallgatter and Strik, 1999; Strik et al., 1998). It might well be the case that the two-dimensional NGA not only originates from ACC-activity during conflict-monitoring but also reflects a specific inhibitory process in the NoGo-condition involving probably the right ventrolateral prefrontal cortex. These hypothetical different contributions to the NGA-phenomenon could optimally be disentangled in future studies investigating the same subjects during various Go/NoGo tasks with fMRI and electrophysiological methods simultaneously.

Moreover, systematic investigations of populations suffering from different psychiatric diseases together with a sophisticated assessment of the underlying psychopathology are warranted in order to clarify ACC-functioning under pathological conditions. In this context, the outlined topographical analysis of ERPs related to the Go- and the NoGo-conditions of the CPT (two-dimensional NGA and three-dimensional LORETA-analyses) might be a valuable extension of the above described PET- and fMRI approaches measuring function of the ACC and other regions of the prefrontal cortex.

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