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Real-time neural activity and connectivity in healthy individuals and schizophrenia patients

Andreas A. Ioannides,[a,*] Vahe Poghosyan,[a] Jürgen Dammers,[b] and Marcus Streit[c,1]

[a]*Laboratory for Human Brain Dynamics, BSI, RIKEN, Wako-shi, Saitama, 351-0198, Japan*
[b]*Institute of Medicine, Research Centre Jülich, D-52425 Jülich, Germany*
[c]*Department of Psychiatry, University of Düsseldorf, D-40605 Düsseldorf, Germany*

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Processing of facial information is distributed across several brain regions, as has been shown recently in many neuroimaging studies. Disturbances in accurate face processing have been repeatedly demonstrated in different stages of schizophrenia. Recently, electroencephalography (EEG) and tomographic analysis of average magnetoencephalographic (MEG) data were used to define the latencies of significant regional brain activations in healthy and schizophrenic subjects elicited during the recognition of facial expression of emotions. The current study re-examines these results using tomographic analysis of single trial MEG data. In addition to the areas identified by the analysis of the average MEG data, statistically significant activity is identified in several other areas, including a sustained increase in the right amygdala activity in response to emotional faces in schizophrenic subjects. The single trial analysis demonstrated that the reduced activations identified from the average MEG signal of schizophrenic subjects is due to high variability across single trials rather than reduced activity in each single trial. In control subjects, direct measures of linkage demonstrate distinct stages of processing of emotional faces within well-defined network of brain regions. Activity in each node of the network, confined to 30 to 40 ms latency windows, is linked to earlier and later activations of the other nodes of the network. In schizophrenic subjects, no such well-defined stages of processing were observed. Instead, the activations, although strong were poorly linked to each other, managing only isolated links between pairs of areas.

Keywords: Schizophrenia; Neural activity; Connectivity

Introduction

The strong association of schizophrenia with changes of activation in multiple brain regions including frontal cortex, temporal cortex, thalamus, hippocampus, basal ganglia, and cerebellum tend to link schizophrenia with connectivity and communication disruption within the neural circuitry (Andreasen et al., 1997; Bunney and Bunney, 2000; Fletcher et al., 1996; McGlashan and Hoffmann, 2000; Weinberger et al., 1992). Tasks involving the recognition of facial information are known to excite specific areas spread widely over the cortical mantle (Allison et al., 1999; Haxby et al., 2000) and to be associated with relatively specific deficit in schizophrenia (Mandal et al., 1998).

The analysis of average magnetoencephalographic (MEG) signals elicited from the brain in response to facial emotions identified weaker activations within a spatially and temporally well-defined network of brain regions in schizophrenic patients (Streit et al., 2001). The analysis of the average signal could not however distinguish whether this was due to weaker activation in each single trial or due to higher variability across single trials. Disturbed connectivity between brain regions is often associated with schizophrenia, but it has not yet been demonstrated directly in terms of how areas fail to link as stimuli are processed millisecond-by-millisecond.

The availability of time courses of single trial regional activations in this study allowed us to address the question of variability across trials versus the strength in each trial directly and to identify the timing of significant linkage between areas in healthy subjects and the way these links are modified in schizophrenia patients.

We organize the paper by focusing on the contrast in activations between healthy and schizophrenia individuals. We addressed three specific questions. Do weaker average regional activations in patients correspond to reduced activity in each single trial or, alternatively, to more variability across single trials? What, if any, additional areas to the ones identified by the analysis of averaged data show differential activation in healthy subjects and patients in the single trials? Do measurements of linkage between regions and/

* Corresponding author. Laboratory for Human Brain Dynamics, BSI, RIKEN, 2-1 Hirosawa, Wako-shi, Saitama, 351-0198, Japan. Fax: +81 48 467 9731.
E-mail address: ioannides@postman.riken.go.jp (A.A. Ioannides).

[1] Dr. Marcus Streit has tragically died in 2003. We dedicate this paper to the memory of his life and the long methodical work he has pioneered in the investigation of the responses of schizophrenic subjects to facial expressions of emotions with EEG and MEG.

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or their timing reveal differences in connectivity between the two groups?

Methods

Subjects

Fifteen inpatients with partly remitted DSM-IV schizophrenia (six women and nine men; mean age = 34.5 years, SD = 9.8) and a comparison group of 12 healthy subjects (seven women and five men; mean age = 35.8 years, SD = 11.6) participated in the study. Only right-handed subjects were included. Written informed consent, approved by the University of Düsseldorf's institutional ethics committee was obtained from potential participants who demonstrated that they were able to comprehend the study procedures, as well as the risks and potential benefits of study participation. None of the healthy subjects had a history of any significant CNS disorders or substance abuse. The diagnosis of patients was based on clinical examination by two psychiatrists who independently agreed the classification of DSM-IV (partly remitted at the time of the MEG study). Symptoms were rated in patients by the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984). At the time of the study all patients were receiving antipsychotic medication (mean dose = 675 mg/day, SD = 414 in chlorpromazine equivalents). Exclusion criteria for patients were substance abuse, neurological disorders, and treatment with additional anticholinergic medication or benzodiazepines. We used strict criteria to identify single trials contaminated by external noise or excessive eye movements or muscular activity and these trials were removed from further analysis. To assure getting unambiguous single trial based results, we selected only those subjects who had at least 90% artifact-free single trials in each run of each condition. This selection reduced the number of subjects to 12; 7 controls (3 female) and 5 patients (1 female). The subject-by-subject single trial results for these 12 subjects indicated an important role for the amygdala in the comparison between healthy individuals and schizophrenic subjects. This comparison however was further complicated by differences in the regional activations and linkage measures between male and female subjects within each diagnostic group, consistent with recent reports (Cahill et al., 2001). Since there were not enough female subjects in the two groups we restricted the comparison to the male subjects. The results reported in the present study are therefore based on single trial analysis of four schizophrenia and four healthy individuals, all right handed males, with ages 35 ± 11.21 years for controls and 33.5 ± 6.8 years for patients. Table 1 provides some background information for the four patients used in this study.

Table 1
Brief clinical history for the four right-handed male schizophrenic subjects that participated in the study

Patient	Age/MEG (years)	Age/onset (years)	Episodes #	Hospitalization #	Dosage (mg/day)
P1	34	31	3	3	1342
P2	36	20	6	6	667
P3	40	27	11	11	500
P4	24	12	12	12	1333

The dosage is given in mg/day in chlorpromazine equivalents.

Stimuli and tasks

Fig. 1 outlines the tasks and shows examples of stimuli used in our study. The stimuli, subtending a visual angle of 11×15^0 were back-projected via optical fibers to a screen approximately 45 cm from the subject.

The first, object recognition task, used images of six categories of objects (chairs, lorries, flowers, birds, horses and neutral faces; 30 images each). The second, the emotional face recognition task used 30 Ekman and Friesen's "pictures of facial affect" (Ekman and Friesen, 1976) (5 faces, three male, two female, each displaying the six basic emotions: happiness, fear, anger, surprise, disgust, and sadness). The results reported here were derived from the tomographic analysis of MEG data elicited by emotional faces in the emotion recognition task and the neutral faces in the object recognition task.

Data collection and tomographic analysis

The BTi whole head system at the Research Center Jülich, Germany (148 channels) was used to record the MEG signal with 500 Hz sampling rate. The data were high-pass filtered above 1 Hz off-line. Heartbeat and eye-blink artifacts were removed from the MEG signal using independent component analysis.

Four separate, partially overlapping, source spaces ($17 \times 17 \times 11$ grid points in each) completely covering left and right hemispheres and back and top of the brain were defined. Magnetic field tomography (MFT) (Ioannides et al., 1990) was used to obtain three-dimensional distributions of the primary current density, J, at each grid point of each source space. The solutions from all four source spaces were combined together into one big source space, which covered the whole brain, using the values of the MFT solutions and the sensitivity profile of the sensors, from the nearby points of each of the four separate source spaces (Ioannides, 2002).

Post-tomographic analysis

For each subject, voxel by voxel statistical parametric maps (SPMs) were used to identify statistically significant differences in the brain activity between different tasks (emotional and neutral faces) and between post- and pre-stimulus periods of each task. Specifically, the Student's t test was used to compare two distributions of values extracted from single trials. The contribution of each single trial to the corresponding distribution of a given latency, t, and voxel was the integral of the modulus of current density at the given voxel over a window of latencies around t. For SPMs contrasting different tasks reported in this paper, we used 100 ms window and stepped the center latency by 50 ms to compute the next SPM. The long latency window captured changes in activity that were sustained and/or poorly time-locked to the stimulus onset. For the post vs. pre-stimulus comparisons, shorter windows were used to probe changes at finer temporal resolution. Specifically, a post-stimulus (active) distribution was made up of the single trial values for the current density modulus averaged over an 8-ms window centered at a given latency. Each active distribution was contrasted with each of three distributions derived from identical 8 ms windows, randomly selected from the same set of single trials from the 100-ms period preceding stimulus onset. The worst result (highest p value) was retained for each latency. The next SPM was

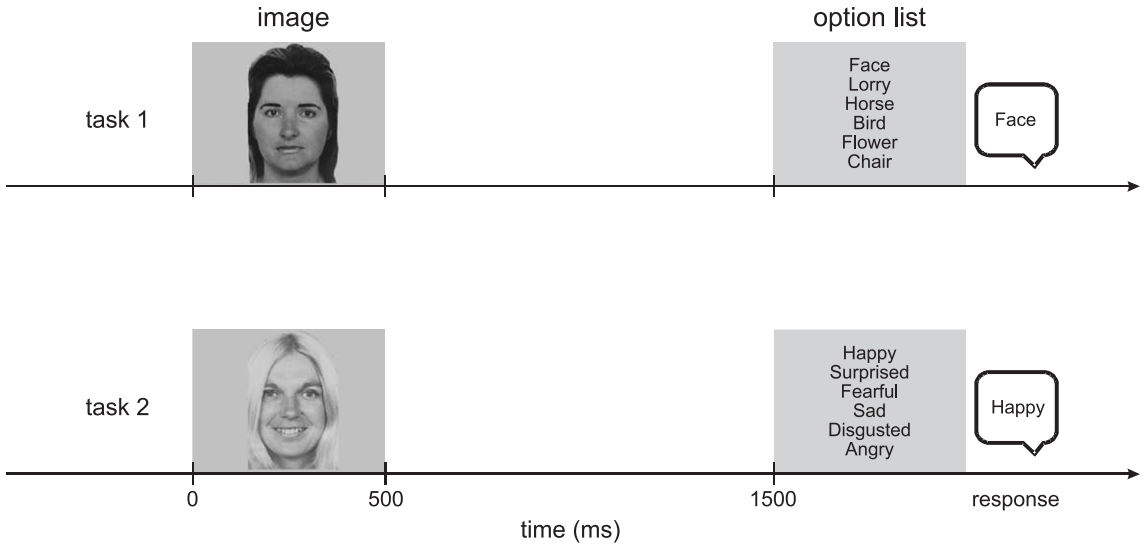

Fig. 1. Two tasks were used. The first, object recognition task, used images of six categories of objects (chairs, lorries, flowers, birds, horses and neutral faces). The second, the emotional face recognition task used images of faces displaying six basic emotions (happiness, fear, anger, surprise, disgust and sadness). In both tasks the stimuli were displayed for 500 ms, 1000 ms after the offset of stimuli the option list appeared on the screen and the subjects had to verbally report the category. The 1-s pause between the offset of the stimulus and the appearance of the option list was used to avoid contribution to the MEG signal from motor-related activity.

computed by stepping by 4 ms the center of the latency window of the active distribution.

The SPMs generated for each subject were transformed to the Talairach space (Talairach and Tournoux, 1988) to facilitate the identification of similar patterns of activation across subjects. The transformation allowed the comparison of voxels showing statistically significant change in activity across subjects. The resulting grand-SPMs identified, for each latency, consistent changes in activity across the four subjects of each diagnostic group.

Comments on localization accuracy

MEG is often portrayed as a method with limited resolution but excellent temporal resolution. We have nevertheless repeatedly demonstrated that the application of SPM analysis to the tomographic MFT solutions leads to remarkably accurate localization while maintaining an excellent temporal resolution (Ioannides, 2001). More recently, a stringent validation of the method was attempted in a protocol where known neurophysiology could provide guidance as to the expected general area of activation. Specifically, we compared the SPM foci obtained from the MFT reconstructions with fMRI localizations for pattern onset stimuli confined to part of the lower quadrant of visual field, selected so that the estimated V1 activation was well separated from the V2 representation of the same stimulus (Moradi et al., 2003). The study showed excellent agreement between tomographic estimates of activity (3–5 mm), for the very first entry to the visual system, typically 40 ms after the stimulus onset. The accuracy deteriorates with depth, but only slightly unless one is too close to the center of the head when model inaccuracies become very important. An eye movement study has recently demonstrated remarkably good reconstructions for deep sources (but still well away from the center of the head) at the level of the amygdala and the brainstem (Ioannides et al., 2004).

Region of interest analysis

For each individual subject the foci of high statistically significant changes in SPMs were consistent with the local maxima in the MFT reconstructions derived from the average signal, whenever such clear maxima could be identified in the corresponding instantaneous MFT solutions. For consistency, we identified the centers of regions of interest (ROI) from the individual subject's SPMs. For each identified area, the center of the SPM activation was used to define a ROI with radius of 1 cm. This value for the radius ensured that the ROI represented activity at a few grid points, since the actual distance between grid points for each of the four original and the final combined source spaces was a little under 1 cm. Given the fairly large size of the stimuli and their foveal presentation we used a radius of 2 cm for the V1/V2 ROI to include the cortical V1–V2 representation of all four quadrants of the visual field. For each ROI, latencies showing statistically significant changes were also identified. For across subject comparisons the coordinates of the centers of all ROIs were transformed to Talairach coordinates. For each ROI the instantaneous current density vector, $\boldsymbol{J}$, was averaged across the single trials, at the latency of the most statistically significant change, and the direction of the resulting average vector was defined as the main direction for the ROI. For each trial, i, at each latency, t, the component of $\boldsymbol{J}$ along its main direction and its modulus was computed and integrated over the ROI volume to produce two measures for the instantaneous single trial activation, $J_1^i(t)$ and $|J_1^i(t)|$, respectively. For each condition, the activity of the ROI across trials was summarized by computing the average of $J_1^i(t)$ and $|J_1^i(t)|$ across trials, denoted, respectively, as $J_1(t) = \langle J_1^i(t) \rangle$ and $J_1^{||}(t) = \langle |J_1^i(t)| \rangle$. For each ROI, we also computed the instantaneous value for the current density vector, $J_{1av}(t)$, derived from the MFT analysis of the average MEG signal. Although MFT relies on a non-linear algorithm to solve the biomagnetic inverse problem, the estimates $J_1(t)$ and $J_{1av}(t)$ are remarkably similar in general (Ioannides, 2001) and specifically so in the present study.

This similarity justifies the use of $J_1(t)$ computed from the single trial analysis in this study as a link to $J_{1av}(t)$, the measure of activity used in our previous study (Streit et al., 2001). The availability of the single trial time series makes possible powerful pattern analysis methods to characterize the time dependence of activations across single trials (Laskaris and Ioannides, 2001). In this study, we computed the signal-to-noise ratio, SNR(t) using a conventional estimator (Raz et al., 1998) from single trial event patterns in a 20 ms latency window. The same definition was used for each ROI throughout, that is, for all computations for $J_1(t)$, $J_1^{||}(t)$ and SNR(t) and for the responses to emotional and neutral faces.

Each one of the three measures, $J_1(t)$, $J_1^{||}(t)$ and SNR(t), probes different aspect of the response and its variability across single trials. The instantaneous measures $J_1(t)$ and $J_1^{||}(t)$ measure the time-locked variation of the current density vector and modulus. High activations do not necessarily lead to high values for $J_1(t)$, they require in addition that the phase and direction of the current density vector are both well time-locked to the stimulus onset. Positive and negative large values for $J_1(t)$ can sum up to very small values. In contrast, $J_1^{||}(t)$ is not sensitive to the current density direction so it attains high values when the activity in single trials is high, even in the presence of variability in the phase relative to the stimulus onset. A latency jitter will reduce the value of the average, but since $J_1^{||}(t)$ is always positive, there will be no destructive interference of positive and negative contributions. The SNR is sensitive to both the phase and the amplitude of the single trial activations (Laskaris and Ioannides, 2001) and can be considered a more sophisticated than any one instantaneous measure because it uses information from a set of instantaneous activations (from a 20-ms latency window in our case).

For each subject, peak values of $J_1(t)$, $J_1^{||}(t)$ and SNR were identified for the latency windows where each ROI showed statistically significant change in activity, separately for the neutral and emotional face presentations. The resulting 8 numbers for each diagnostic group (4 subjects × 2 conditions = 8 numbers) were used to compute the average and standard deviation (Fig. 2). The paired t test was used to test whether the difference between the distribution of values for healthy subjects and patients was significant.

Mutual information analysis

Mutual information (MI) was used to study the connectivity between brain areas (Ioannides et al., 2000). MI is a non-linear measure of relatedness of two time series based on information theory (Shannon, 1948). A MI value was computed between two time series extracted from the single trial ROI time courses of two brain regions. Each time series contained the values of the current density modulus in a window of length W_L ms and centered,

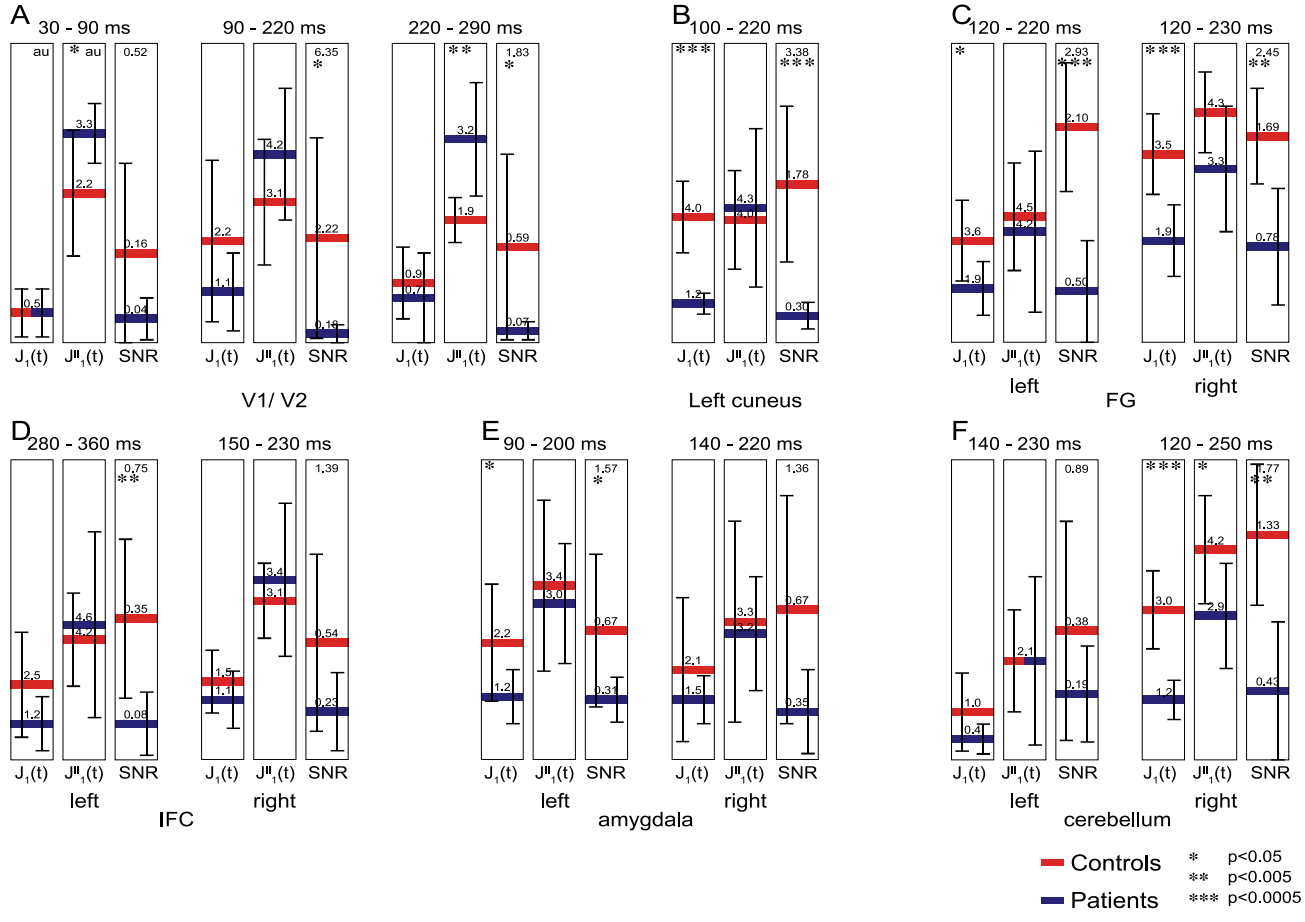

Fig. 2. The averages and standard deviations of peaks of $J_1(t)$, $J_1^{||}(t)$ and SNR across each diagnostic group for different ROIs and latency ranges. The asterisks indicate p values when the distributions of peak values for each measure for healthy and schizophrenic subjects were contrasted using paired t test. The maximum SNR value across all subjects is given at the top of each SNR bar plot. The values of $J_1(t)$, $J_1^{||}(t)$ are given in the same arbitrary units.

respectively at latency t_{ROI1} of the first ROI and t_{ROI2} of the second. The MI values, $M(t_{ROI1},t_{ROI2})$, were computed separately for each (t_{ROI1},t_{ROI2}) pair, for all t_{ROI1} and t_{ROI2} values in the range −100 to 300 ms using equidistant steps for each ROI of Δt ms. We have tested different window lengths and steps and obtained similar results for the range $20 < W_L < 60$ ms. In all the results reported here we used $W_L = 40$ ms $\Delta t = 4$ ms. With these choices the MI value, $M(t_{ROI1},t_{ROI2})$, identifies how the activity of the first ROI in the 40-ms latency window centered at t_{ROI1} is linked to the activity in a similar 40 ms latency window of the second ROI but centered at t_{ROI2} latency. It is important to realize that this linkage does not necessarily imply a causal influence. The link identified by a high MI value may be due to common interaction with a third area via links with different time delays. However, since the link is identified across single trials, the most parsimonious explanation is that some influence (direct or indirect) is exerted from the area with the earlier latency to the other area. After normalizing $M(t_{ROI1},t_{ROI2})$ values for each subject and ROI pair, (t_{ROI1},t_{ROI2}) pairs with the $M(t_{ROI1},t_{ROI2})$ values less than 20% of the normalized maximum were eliminated. The remaining pairs were used to identify the ones that were common to all four subjects. This identification was made separately for each diagnostic group (healthy, schizophrenia) in each condition. To show these common pairs in compact and informative way, the MI maps are displayed using t_{ROI1} on the horizontal axis and $(t_{ROI2}–t_{ROI1})$ as the vertical axis. In this way, the timing of an island of high MI values can be immediately identified by the latency of the first ROI and the relative delay of the second ROI. However, to visualize the overall contribution from even a small set of areas, the MI map for each ROI pair is needed. We simplify the representation in two steps. We first combine the MI maps involving one area (X) by showing on the same MI map the MI values from all pairs involving X. A different color is used to discriminate between different areas (Fig. 3 provides an example of a set of MI maps superimposed on one diagram).

In the second step, we use the MI maps from all pairs linking a set of brain areas to construct influence diagrams, where an area is represented by a node and an "influence" by a directed arrow between nodes. Influence diagrams provide a view of the network structure through a comprehensive visualization of the history of directed linkage between areas that MI analysis reveals.

Methods summary

In summary, for each subject we computed a full tomographic estimate of activity for each timeslice of each single trial. The resulting MFT solutions were used for a voxel by voxel SPM analysis that in turn was used to define the centers of ROIs. The full MFT solutions were then used to define the main direction for each ROI. The single trial time course for each ROI was computed directly from the corresponding single trial MFT solution. The resulting ROI time series were used for computing the three time-dependent measures $J_1(t)$, $J_1^{\|}(t)$ and SNR(t). This set of robust, compact and informative measures allowed quantification of the activity and variability across single trials providing in turn estimates for the strength and coherence, $J_1(t)$, for the mean energy, $J_1^{\|}(t)$, and for the relative size of amplitude and variance, SNR(t). The same time series provided the input for the MI computations that probed the "interactions" between areas.

Results

Visual inspection of single trial instantaneous MFT solutions revealed a web of activations throughout the brain, including "candidate evoked responses" (CER), that is, activations at expected areas and latencies. Without prior knowledge about when and where to look, there was little to distinguish CERs from other activations in the pre- and post-stimulus period. Using prior knowledge, some CERs were easily identified, for example, in

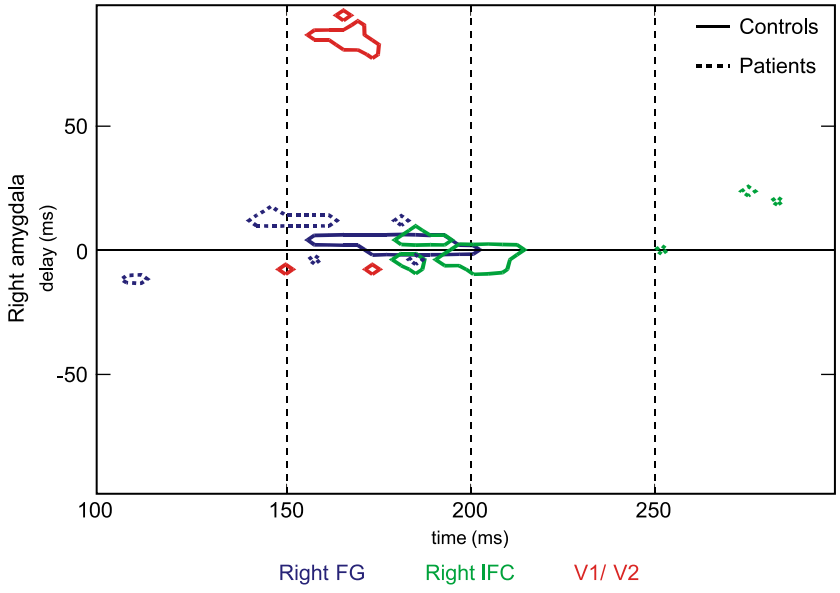

Fig. 3. The display shows MI maps computed from pairs of time courses between the right amygdala and V1/V2 (red), right FG (blue), right IFC (green). The horizontal axis shows the latency of the first ROI (V1/V2, right FG, right IFC) and the vertical axis the relative delay of the second ROI (right amygdala) relative to the first ROIs. The MI islands for control (patients) are shown by solid (dotted) contours. The area within each island contains latency-delay pairs for which the normalized MI value of each subject in the diagnostic group exceeds 20%.

early visual areas and the right fusiform gyrus for face stimuli at latencies in the second 100 ms after stimulus onset. CERs were identified more often and consistently in control compared to schizophrenic subjects. In agreement with our earlier studies, all CERs, including the ones from normal subjects, were spatially extended with considerable jitter in latency from trial to trial (Ioannides, 2001). The slightly more consistent CER activations across trials produced recognizable maxima in the average signal and highly statistically significant foci in the SPMs for each subject across different contrasts, again more frequently and consistently for control rather than schizophrenic subjects. SPMs at the threshold of significance ($p < 0.05$) usually started at well-circumscribed foci but were then quickly spread in the next 8 ms windows over fairly wide regions. SPMs with high significance ($p \ll 0.05$) activated the same well-circumscribed regions seen at the low significance level, often a little later in time. The individual ROI centers were identified from the SPMs and named based on their projection on the individual MR image and their Talairach coordinates. Table 2 lists the names and Talairach coordinates of the areas identified consistently across subjects. Fig. 4 shows the contours for common SPM activations across control subjects for the right FG for $p < 0.001$ and $p < 0.0005$ at 175 ms after stimulus onset. These contours correspond to the contrast of post- and pre-stimulus periods of responses to emotional faces and they are shown together with the ROI centers for each subject.

The single trial based measures $J_1(t)$ and SNR(t) confirmed the marked hypo activity identified as reduced $J_{1av}(t)$ in patients in our earlier analysis of the average signal (Streit et al., 2001). In both studies, the left cuneus was identified as one of the areas with strongest consistent signal across runs for control subjects, but with much reduced activity for patients. Fig. 5 shows for each subject the two new measures generated from single trials $J_1(t)$ with SNR(t) for the left cuneus ROI in the latency range 100–200 ms. Both neutral and emotional faces show a clear separation between healthy subjects and schizophrenia patients.

Grand-SPM maps were computed to identify voxels with statistically significant change in all subjects for each diagnostic group. In addition to the areas identified in the analysis of the averaged responses (Streit et al., 2001), one more area was identified from the single trial SPM. Right amygdala showed

Table 2
Talairach coordinates of main ROIs

	X	Y	Z	SD
V1/V2	0	−73	1	8
Left				
Cuneus	−9	−61	10	10
FG	−39	−50	−16	9
IFC	−46	22	−1	3
Amygdala	−21	−1	−25	6
Cerebellum	−26	−56	−30	7
Right				
FG	32	−46	−9	3
IFC	41	10	−4	4
Amygdala	17	−2	−26	5
Cerebellum	28	−44	−21	4

Mean (across subjects) Talairach coordinates of main identified areas. The last column shows the standard deviation of Talairach coordinates across subjects. All coordinates are in millimeters.

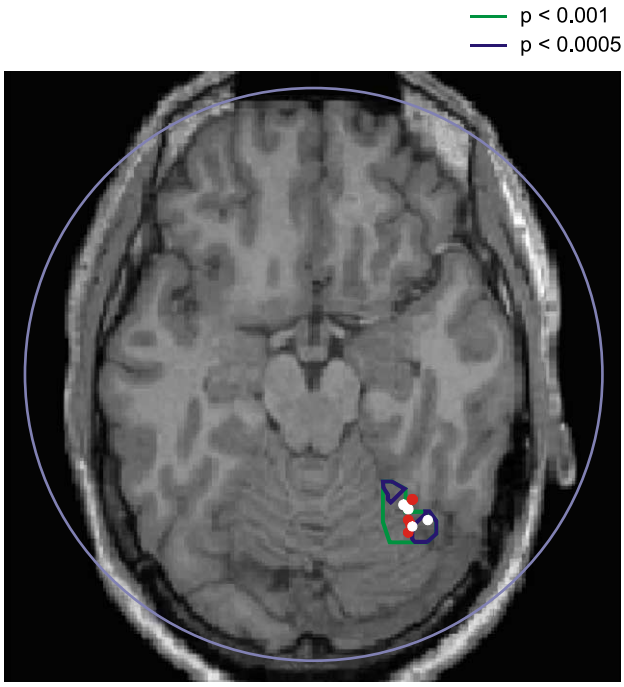

Fig. 4. Grand-SPMs showing increase activity at 175 ms after stimulus onset in the right FG. Green and blue contours encompass the area where the statistical significance was $P < 0.001$ and $P < 0.0005$ in all four-control subjects. White dots show the centers of right FG ROIs of control subjects, red dots of schizophrenic patients. Centers of ROIs are within 2 mm of the displayed axial MR slice.

different patterns of activation between healthy and schizophrenic individuals, when responses to emotional and neutral faces were contrasted using 100 ms window. Intermittent activity was identified in the amygdala of the patients in both pre-stimulus and post-stimulus periods. This activation was in general highly variable from trial to trial and thus produced low SNR values and no obvious features in the average signal. In the first 100 ms, however, and for each patient, highly significant increases in amygdala activity were identified by the SPM analysis when single trial responses elicited by emotional faces were contrasted with the corresponding responses elicited by neutral faces (Fig. 6). In controls, the same SPM comparison produced no statistically significant results for the amygdala activity at any latency. In summary, our results reveal that the presentation of the emotional faces in the emotional face recognition task induces an irregular, persistent, and sustained change in the right amygdala of patients, but not in control subjects.

We use MI analysis to address the question of connectivity between the areas, stressing however that the words “linked activity” and “influence” are used as parsimonious descriptors for high mutual information values without implying that a causal interaction based on direct anatomical connection is present. We have computed the MI between all pairs of identified areas, but we will focus the remainder of the results on the linkage between V1/V2 and the right hemisphere areas that were particularly active in tasks requiring the recognition of facial expression, namely the right FG, right IFC and right amygdala. Fig. 7 shows the influence diagram constructed from the (t_{ROI1},t_{ROI2}) pairs identified from MI computations made between all possible pairs that can be formed from these areas for the emotional face recognition task. The

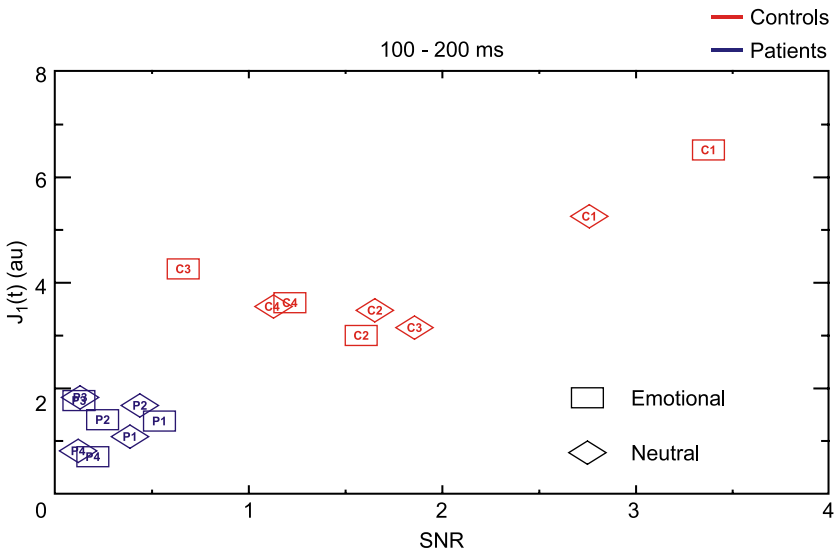

Fig. 5. The peak values of $J_1(t)$ and SNR(t) plotted against each other for the left cuneus ROI, between 100 and 200 ms, are displayed for each subject and task separately.

influence diagrams are constructed separately for healthy subjects (Fig. 7A) and patients (Fig. 7B). Each influence diagram represents the summary of several MI maps like the one in Fig. 3. The results show higher connectivity for normal subjects compared to patients.

In healthy individuals, there is a well-organized sequence of interchanges leading to a "synergy" of activations: influences converge onto an area at well-defined latency ranges as the activity from that same area begins to influence other areas. Influences from V1/V2 and the right amygdala converge to the right FG between 160 and 200 ms, and at about the same time the right FG activity influences the right IFC. At the right amygdala, influences converge from the right FG and the right IFC between 170 and 215 ms while the amygdala activity beginning at 170 and 180 ms is influencing the right FG and right IFC, respectively. The influences already described from the right FG and the right amygdala converge to the right IFC between 190 and 220 ms. Between 170 and 180 ms and at 150 and 175 ms influences from activity in the right FG (beginning at 120 ms) and the amygdala (140 and 165 ms), respectively, converge onto V1/V2. Around the same period, activity in V1/V2 (beginning at 160 ms) influences the right amygdala at 250 ms. These synergies are marked by solid arrows in the diagram to distinguish from isolated links marked by dashed arrows. For healthy subjects, such isolated links are encountered at the end of what appears to be a processing cycle, at rather late

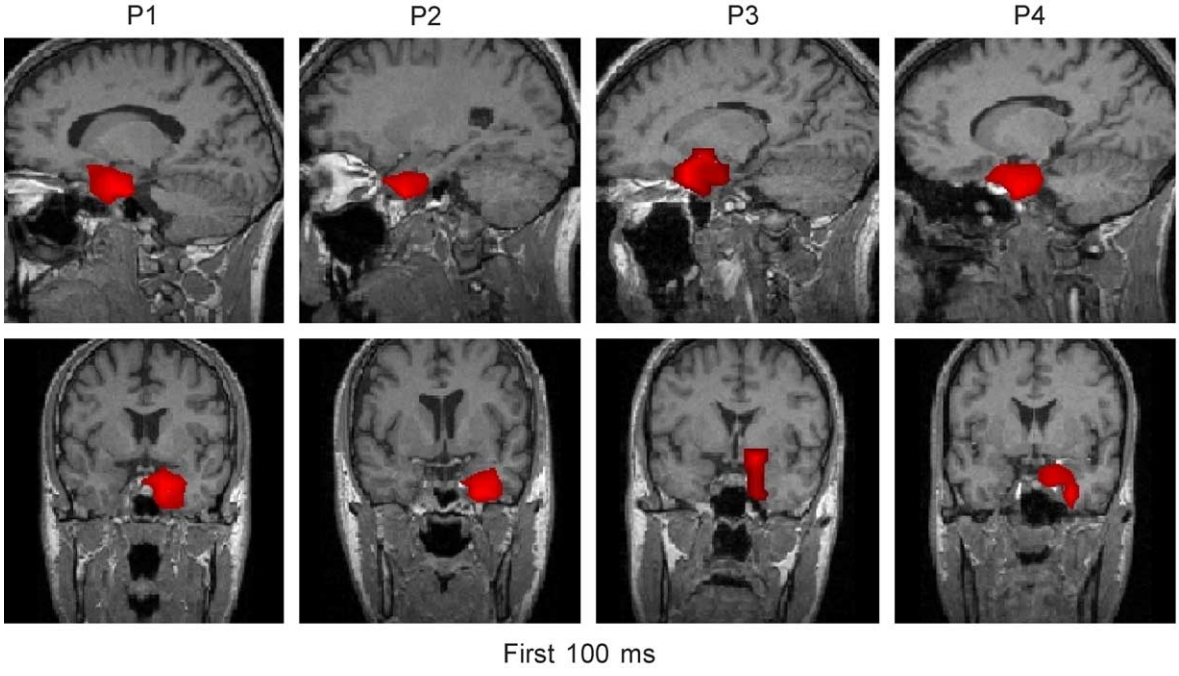

Fig. 6. SPMs for the contrast between emotional faces and neutral faces during the first 100 ms after stimulus onset. The SPM maps show right amygdala activation for each schizophrenic subject. Sagittal (upper) and coronal (lower) views are shown.

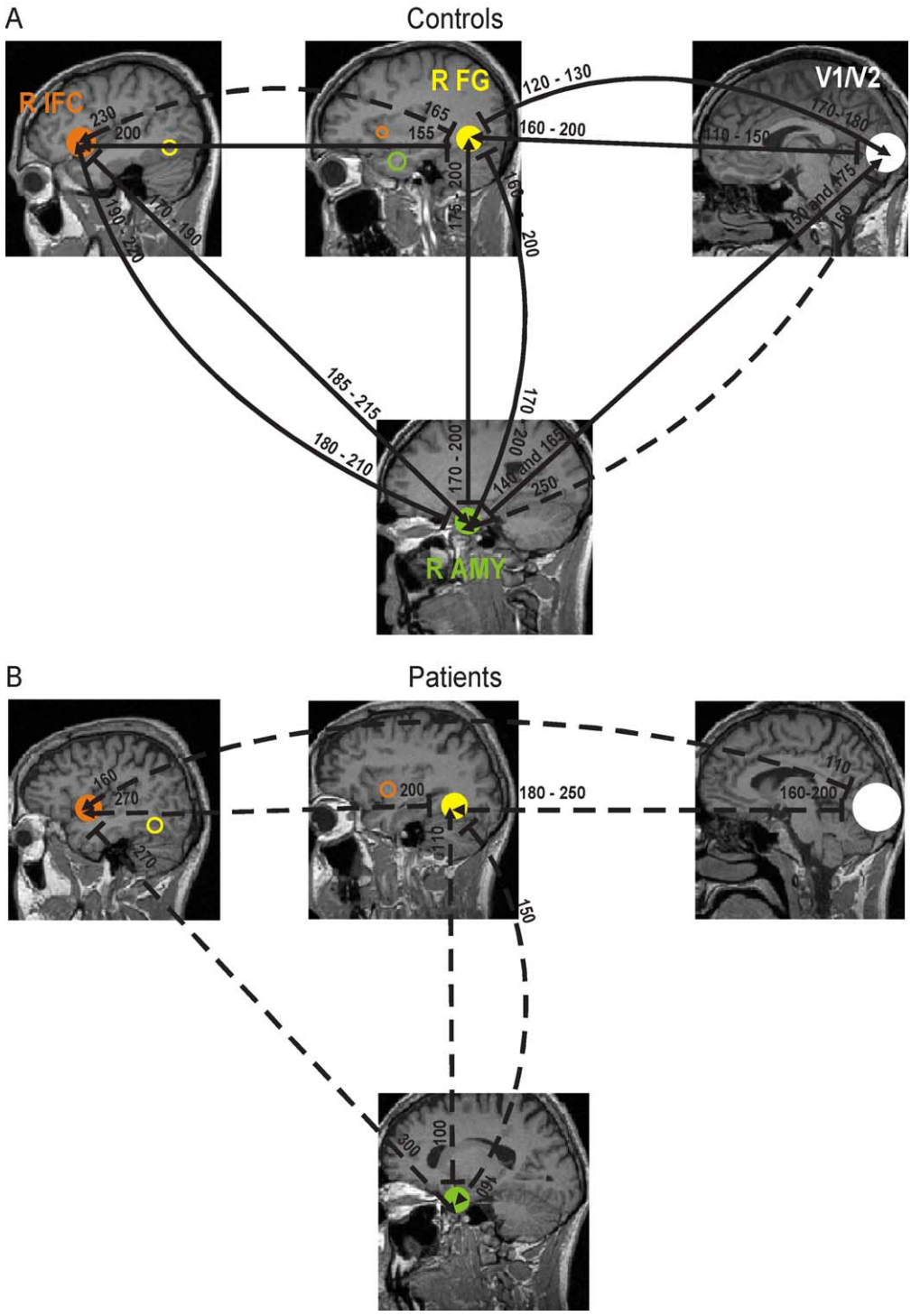

Fig. 7. Influence diagrams derived from MI maps computed from pairs of time courses between all possible pairs formed between any two of the following areas: right amygdala, V1/V2, right FG and right IFC. Solid arrows identify links that converge with the links from other areas in the target area. Dashed arrows identify isolated links. For each link, the latency range printed at the beginning of the arrow identifies the earliest activation in the first area (modulating area) and the latency range at the end of the arrow defines the activity in the second area (modulated area). (A) Controls, (B) patients. All the times are given in ms after the stimulus onset.

latencies, at 230 ms in right IFC (influence from right FG) and 250 ms in right amygdala (influence from V1/V2).

The influence diagram for schizophrenia patients shows weak linkages with many of the influences identified in controls missing. Note the missing link between right amygdala and V1/V2 and an additional link from V1/V2 to right IFC, which was not identified in controls. The chain of influences V1/V2 → right FG → right IFC → right amygdala is delayed for patients while the influence from the right amygdala to the right FG is earlier compared to controls. In controls, the link between right IFC and right amygdala

is bidirectional, but in patients, it is unidirectional, starting from right IFC and ending in right amygdala. In patients, isolated influences are either very brief or very extended (between V1/V2 and right FG). These influences do not combine into the kind of synergies seen in healthy subjects and form only isolated links.

Discussion

Both this and our previous studies identified changes in the well-defined areas already known to be involved in the emotional evaluation of faces (Morris et al., 1998). Our current single trial analysis revealed group differences in the right amygdala that were not evident in our earlier analysis of average data. Two other important changes in brain mechanisms in schizophrenia patients were also identified. First, the reduced activity found in the analysis of average data of schizophrenic patients is due to much higher variability and not reduced activation in each single trial. Second, the variability in schizophrenic patients is accompanied by connectivity dysfunctions.

The comparison between emotional and neutral faces has revealed persistent right amygdala activity in patients and a statistically significant increase in the SPM contrast between emotional and neutral faces in the first 100 ms after stimulus onset. No such effect was identified in the responses of the controls. We note here that the subjects were performing two completely different tasks and it is therefore unclear whether the difference relates to the different stimuli (neutral versus emotional) or the different task requirements.

The results reported here provide a refinement of earlier studies because they explicitly deal with variability at the level of regional activations in the brain rather than macro-electric surface measures (Winterer et al., 2000) and they do so using the full information in the single trial MEG signal rather than just the average (Streit et al., 2001). The first of our single trial based measure of activity $J_1(t)$ is almost identical to the instantaneous value of the current density vector, $J_{1av}(t)$, extracted from the average MEG signal. It is therefore the quantity most closely related to the earlier results using only average MEG data (Streit et al., 2001). The more robust SNR measure also relies on the current density vector and it is also reduced when the single trial variability is high. These two measures showed consistently reduced values for patients compared to control subjects and used together provide a good discrimination between the two diagnostic groups (Fig. 5). The results in Fig. 5 cannot however distinguish whether the reduced average response is due to reduced activity in single trials or high variability (low SNR). The third measure, $J_1^{||}(t)$, relies on the modulus rather than the vector properties of the current density and it is therefore sensitive to overall changes in the energy of the activation rather than phasic time-locked properties. This measure does not show the same type of differences between control and schizophrenic subjects, except for occipital visual areas where it is actually higher for schizophrenics. The implication of these results is that the reduction in the average signal observed in patients compared to healthy subjects is due to more variable, but not necessarily reduced regional activity in single trials, in agreement with a recent EEG study (Winterer et al., 2000).

For normal subjects, activity contributing to strong MI links lasted typically for 20–30 ms, consistent with the notion that a good deal of the analysis in regions specializing in object and face recognition is completed in stages lasting a few tens of milliseconds (Tovee et al., 1993). For patients, the regional activations are usually very brief, and in one occasion (link between V1/V2 and right FG) much longer than 20–30 ms.

The MI analysis for normal subjects showed a very organized pattern of linkages, with convergence in timing marking concerted activity across many areas. In a recent study, "sensor of interest analysis" was used to identify stages of processing in the average MEG signal patterns elicited by face stimuli (Liu et al., 2002). The synergy of activations identified in the present study for each one of the healthy subjects is consistent with a division of the processing of face information into relatively well-defined stages. Our results show that stages identified at the level of the average MEG signal (Liu et al., 2002) do not correspond to activations in any one area but rather to synergies of activation in several areas in the relevant network. For example, around 180 ms activity is identified in the right FG, the right amygdala, the right IFC, and V1/V2. The end of processing is marked by isolated late activations that reach the right IFC at 230 ms from right FG and the right amygdala at 250 ms from V1/V2.

For the same brain areas, MI analysis shows overall disturbed linkage for schizophrenia patients, despite the strong activity present in the single trial activations. There is no convergence or bi-directional activations, especially from higher areas in the visual hierarchy to lower ones. The only candidate for bi-directional link in patients is a transient early MI increase between right amygdala and right FG (100 to 110 ms). This connectivity disturbance in patients may lead to incomplete stages of processing. These findings are consistent with the notion that key brain areas like the amygdala may lose their functional focus in schizophrenia (Stevens, 1999).

We stress again that high MI values do not necessarily correspond to a direct influence from one area to another. Our observations can equally well be interpreted as a cooperative interaction between each area and the network. Elaborations of the collective network computations can lead to replay of similar patterns, expressed at different stages of processing by activity in a subset of areas, leading to high MI values across single trials. It is perfectly feasible that the pattern of activations and their timing may be different from trial to trial. What may be an invariant across single trials for each task may be just a reoccurrence of ripples of activity in at least some of the nodes of the network at roughly similar latencies with similar relative timing across single trials. The MI will increase as these elusive transient linked activations reoccur across single trials, despite the large fluctuations that will largely eliminate their contribution to the average signal. Therefore, MI computation across single trials can capture influences between areas that do not lead to significant activations in the average signal or even the SPMs of any one area.

In summary, we have identified well-defined regional activations and relations between activations in different areas. Before summarizing the comparison between the two diagnostic groups, we acknowledge that the sample size of our study was small and therefore put forward these results as preliminary, until they are replicated with a larger pool of subjects. With this cautionary remark stated, we note that differences in the two diagnostic groups point to specific functional deficits in facial affect recognition in schizophrenia. Our results provide evidence for disturbances in functional connectivity in schizophrenia based on real time data analysis and they therefore contribute substantially to the definition of the so-called endophenotype of the disease (Andreasen, 2000). In conclusion, this study demonstrates the potential of MEG in

investigating brain mechanisms related to schizophrenia and/or other mental disorders.

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