

Resting-state fMRI study of patients with fragile X syndrome

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Abstract. The study aimed to assess the neural activity of different brain regions in patients with fragile X syndrome (FXS) and the healthy volunteers by resting-state functional magnetic resonance imaging (fMRI) on a 1.5 T MRI Achieva scanner (Philips). Results: The fMRI study showed a DMN of brain function in patients with FXS, as well as in the healthy volunteers. Furthermore, it was found that a default mode network of the brain in patients with FXS and healthy volunteers does not have statistically significant differences ($p > 0.05$), which may indicate that the basal activity of neurons in patients with FXS is not reduced. In addition, we have found a significant ($p < 0.001$) increase in the FC within the right inferior parietal and right angular gyrus in the resting state in patients with FXS. Conclusion: New data of functional status of the brain in patients with FXS were received. The significant increase in the resting state functional connectivity within the right inferior parietal and right angular gyrus ($p < 0.001$) in patients with FXS was found.

1. Introduction

Fragile X syndrome (FXS) is one of the most common causes of hereditary mental retardation. The main mechanism of pathogenesis associated with the expansion CGG repeats in the promoter region of the FMR1 (fragile mental retardation 1) gene, resulting in suppression of production of protein FMRP (fragile mental retardation protein), which is essential in the normal development of the central nervous system [1]. The importance of early diagnosis is determined by the increasing severity of the disease from generation to generation, which is associated with the accumulation of the number of repeats in the mutant site of the X-chromosome. During the last decade, researchers have been actively using resting-state (fMRI) for the study of brain physiology and pathological changes. This technology allows practitioners to evaluate the low-frequency (0.01–0.1 Hz) fluctuations in BOLD signal, and it is most frequently used to study patients with neurological or psychiatric disorders. BOLD signal is based on changes in hemodynamic parameters, and it indirectly detects the neuronal activity of the various brain regions both during task completion and in resting state. Resting-state fMRI allows the assessment of functional connectivity and the identification of the default mode network (DMN). In contrast to other networks, DMN has elevated levels of neuronal activity during



rest, compared to when tasks are performed. It is accepted that the activities of the network show the basal activity of neurons of the human brain.

In the present study, we used a new approach to the diagnosis of diseases, such as FXS, based on neuroimaging techniques. This gives hope that the data obtained from the study of this disease can help in the search of mental retardation mechanisms. Furthermore, the data obtained from the study are used as a prediction of mental retardation and to select strategies of cognitive therapy. Usually, when performing routine MRI in patients with FXS, in most cases any abnormalities are not observed. Therefore, to study the functional organisation of the brain and changes in neuronal activity, we used fMRI.

2. Materials and methods

This study was performed at the Institute International Tomography Centre of the Russian Academy of Sciences (Novosibirsk). A total of 16 male subjects were included in this study, consisting of 8 patients with FXS and 8 healthy volunteers. The study was approved by the local ethical committee. The whole experimental process accorded with the Declaration of Helsinki. The basic procedures and requirements of the experiments were explained to the participants or their parents, and they signed written informed consent forms. MRI exclusion criteria included in a group of healthy volunteers were: metallic implants such as surgical prostheses; history of traumatic brain injury or surgery; history of any chronic, mental, or nervous system disease; availability of permanent pacemakers; claustrophobia.

2.1. FMRI data acquisition

The fMRI was performed on an Achieva (Philips) scanner with a magnetic field strength of 1.5 T by using an 8-channel head SENSE coil. FMRI data were acquired using an echo-planar imaging sequence with the following imaging parameters: matrix = 64×64 , 35 slices, voxel size = $4 \times 4 \times 4$ mm, repetition time TR = 3500 ms, echo time TE = 50 ms). Reference anatomical images were obtained by T1-TFE (turbo field echo) sequence with the following imaging parameters: matrix = 256×256 , 64 slices, voxel size = $1 \times 1 \times 3$ mm in three orthogonal projections.

The study design involved registration of the BOLD signal in the resting state. Resting-state fMRI could not be performed in awake patients with FXS owing mental retardation. Therefore, patients with FXS were investigated under sedation with constant control of respiratory and circulatory functions. Propofol[®] was entered by titration (2.3 mg/kg) and a supporting dose at an infusion rate of 9–15 mg/kg/h. Healthy volunteers were instructed to rest with eyes closed.

2.2. Data analysis

FMRI data were processed with FSL (<http://www.fmrib.ox.ac.uk/fsl>). Independent component analysis and seed-based correlation analysis were used.

The following pre-statistics processing was applied: slice-timing correction; registration to high-resolution structural and standard space images using FLIRT [2] with further refinement using FNIRT non-linear registration [3]; motion correction using MCFLIRT [2]; and high pass temporal filtering.

Seed-based fMRI data processing was performed using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL. Regions of interest (ROI) time courses were extracted from preprocessed data and then entered into GLM analysis as regressors along with nuisance regressors, which included motion parameters and global, white matter, and CSF average signals. This way it was possible to use GLM to produce whole brain statistical maps of functional connectivity, excluding the nuisance factors mentioned previously.

ROIs were selected from the automatic anatomical labeling (AAL) atlas [4]. Regions related to the cerebellum were not used, resulting in 90 ROIs analysed. Each ROI analyses was performed independently. Resulting statistic images for group comparisons were thresholded using clusters determined by $Z > 3.09$ ($p < 0.001$) and a corrected cluster significance threshold of $P = 0.05$ [5].

Independent component analysis (ICA) was performed using probabilistic independent component analysis [6] as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) Version 3.14, part of FSL. DMN was manually selected out of all identified components. Group comparisons were performed using dual regression procedure [7] to generate subject-specific versions of the spatial maps of the components with further testing for group differences using FSL's randomise permutation-testing tool.

3. Results

3.1. *FMRI results Independent Component Analysis*

Independent component analysis (ICA) is useful in identifying neural networks, DMN. ICA separates the signal into spatial and temporal components, allowing better removal of noise and the identification of functionally connected submodules within the whole brain.

The DMN subnetwork was successfully identified in both the FXS group and the control group. Three orthogonal projections of DMN identified in the group of patients with FXS are shown in Figure 1. Comparison of results for the two groups showed no statistically significant differences in DMN organisation between patients with FXS and the control group ($p < 0.05$).

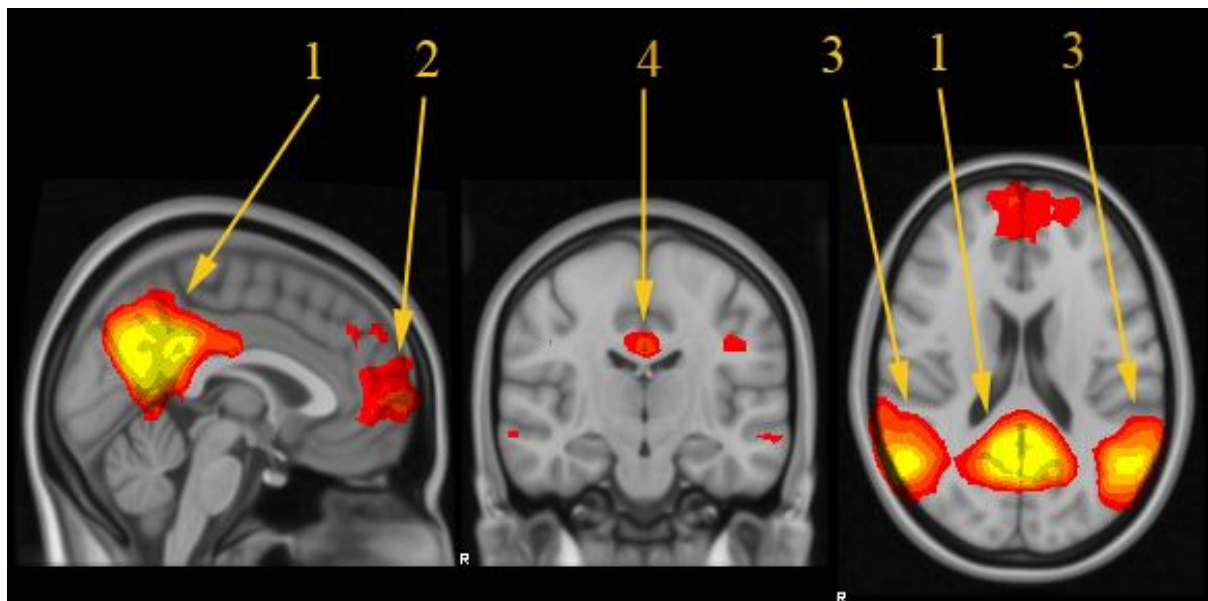


Figure 1. Regions of spontaneous neuronal activity within the default mode network in three projections in patients with FXS. 1 –posterior part cingulate, precuneus, 2 –medial frontal lobes, 3 – parietal lobe, 4 – cingulate gyrus.

3.2. *Results of Seed-Based Analysis*

Using FSL software, the maps of functional connectivity (FC) for 90 anatomical regions of the brain were obtained for patients of both groups. Statistically significant between-group differences were observed in FC maps for right inferior parietal gyrus and right angular gyrus.

3.2.1. *Right inferior parietal gyrus*

When comparing the functional connectivity between the groups, it was observed that patients with FXS showed a significantly higher ($p < 0.001$) FC between the right inferior parietal and the following brain regions: postcentral, supramarginal gyrus, Brodmann area 2 (which is located in the area of the postcentral gyrus) and Brodmann area 40 (which is located in the area of the supramarginal gyrus).

The resulting map of between-group comparison of the functional connectivity of the healthy volunteers and the FXS group is presented in Figure 2.

3.2.2. Right angular gyrus

When comparing the functional connectivity between the groups, it was observed that patients with FXS showed a significantly higher ($p < 0.001$) FC between the right angular gyrus and the following brain regions: cingulate gyrus, limbic lobe, paracentral lobule, medial frontal gyrus, Brodmann area 24 (is part of the anterior cingulate), and Brodmann area 31 (also known as dorsal posterior cingulate area 31). The resulting map of between-group comparison of the functional connectivity of the healthy volunteers and the FXS is presented in Figure 3.

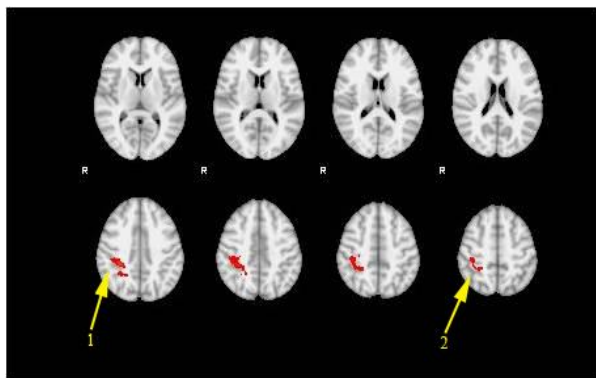


Figure 2. Comparison of functional connectivity in patients with FXS and healthy volunteers. Red colour marks regions in which the spontaneous activity during rest was in higher coherence with that of the region of interest in patients with FXS than in healthy volunteers. As a region of interest - right inferior parietal. 1 – supramarginal gyrus, 2 – postcentral gyrus. $p < 0.001$.

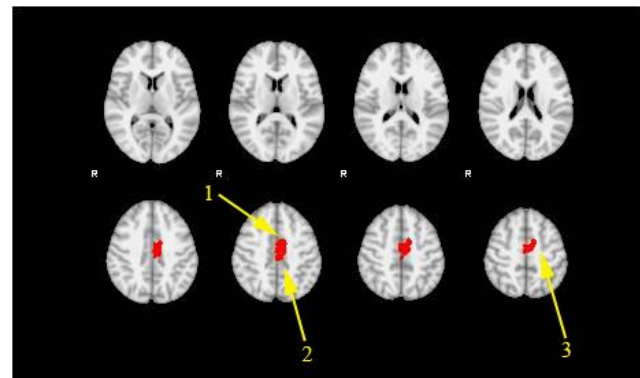


Figure 3. Comparison of functional connectivity in patients with FXS and healthy volunteers. Red colour marks regions in which the spontaneous activity during rest was in higher coherence with that of the region of interest in patients with FXS than in healthy volunteers. As a region of interest - right angular gyrus. 1 – cingulate gyrus, 2 – paracentral lobule, 3 – medial frontal gyrus. $p < 0.001$.

4. Discussion

The high prevalence of FXS, character of inheritance, and development of persistent disabling mental retardation make this disease one of the most important medical and social problems. There have been a number of publications on functional neuroimaging in patients with FXS, but they have all been performed using task-based fMRI paradigms. Also, adult women in the permutation with adult males have been investigated in previous studies [8].

Resting-state fMRI evaluates low-frequency oscillations (0.01-0.1 Hz) in BOLD signal, which is based on the change in hemodynamic parameters that indirectly determines the spontaneous neuronal activity of the different regions that form the neuronal network. When processing resting-state data, temporal similarity and frequency characteristics of oscillations in BOLD signal are estimated for all the voxels of the observer image. It should be noted that the signal of interest for resting-state fMRI analysis is low-frequency oscillations (0.01-0.1 Hz) of time sequences. Currently, there is significant evidence that these low-frequency vibrations are not artefacts at rest (due to respiratory movements and heartbeat) and reflect the important integrative indicator of brain activity—basal activity of neurons. As already stated, DMN is characterised by its activity increase at rest and, conversely, decrease when performing cognitive tasks. This phenomenon represents the occurrence of basal activity of brain neurons in the resting state. It is believed that DMN is involved in cognitive processes and the formation of awareness of one's personality, inner speech, spontaneous thoughts, and dreams. Thus, DMN may affect the formation of consciousness. In addition, in patients with mild cognitive

impairment, a reduction in functional relationships within DMN is described compared with healthy volunteers. This fact may have clinical significance if we assume that the functional connectivity between the regions of DMN is a marker of cognitive abilities; the strength of this connection may be of diagnostic value for a variety of clinical conditions and diseases.

Our study using the independent component analysis of resting-state fMRI demonstrates the presence of a DMN in both groups of patients. In our study, DMN brings together the prefrontal, anterior cingulate, posterior cingulate cortex, inferior temporal gyrus, and the superior parietal lobule, which is consistent with previous studies [9]. In this study using group analysis, it was found that the observed DMN did not differ ($p > 0.05$) between the two groups of patients, which may indicate the presence of basal neuronal activity in patients with FXS similar to that of control group patients.

When comparing the functional connectivity between the groups, it was observed that patients with FXS showed higher functional connectivity of the right inferior parietal gyrus with postcentral and supramarginal gyri, and Brodmann areas 2 and 40. In addition, it was observed that patients with FXS showed higher functional connectivity between the right angular gyrus and areas located in the cingulate gyrus, limbic lobe, middle frontal gyrus, paracentral lobule, and Brodmann areas 24 and 31. Probable cause of these findings may be a failure in mechanisms of deactivation of certain neural circuits in patients with FXS.

5. Conclusion

New data of functional status of the brain and genetics in patients with FXS were received. Fragile site FRAXA in the patients' blood samples was determined. Presence of the DMN was demonstrated using resting-state fMRI in both the control group and in patients with FXS. Furthermore, it was found that the two groups do not show statistically significant differences ($p > 0.05$) in DMN, which may indicate that the basal activity of neurons in patients with FXS is not reduced. Also, an increase in functional connectivity of the right inferior parietal and right angular gyrus ($p < 0.001$) in the resting state in patients with FXS was found. Data from this study help shed light not only on the genetic basis, but also on the state of functional connections, in the brain of patients with FXS.

Acknowledgments

The work was financially supported by Russian Science Foundation (the project № 15-15-10001 regarding molecular genetic part, the project №14-35-00020 for works of MRI and the project № 14-45- 00040-II for manuscript preparation and publication) and FASO Russia (project № 0333-2014-0003) regarding theoretical part.

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