Complex study of hallucinatory-paranoid syndrome in patients with schizophrenia.

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Comprehensive research method

1-st level
- Anatomy
- Structures of the brain

2-nd level
- Biochemistry
- Epigenetics
- Gene RELN

3-rd level
- Physiology
- ERP
- MRI

4-th level
- Psychology
- Affective sphere

5-th level
- Psychopathology
- Positive symptoms
Hypotheses of schizophrenia

- **Glutamatergic**
  - Glutamate

- **GABAergic/gamma-wave**
  - Gamma-aminobutyric acid (GABA)

- **Dopaminergic**
  - Dopamine
Temporolimbic system is the basis of affective perception dysfunction
Instinct and basic emotions

- A complex behavioral act involving a specific sequence of several components.
- It is triggered by internal signals or external stimuli.
- Stimuli play a role of triggers that cause a reaction of «all or nothing».
Kandinsky-Clérambault syndrome
Syndrome of mental automatism, alienation syndrome-a kind of hallucinatory-paranoid syndrome

Thought disorder
delusional ideas of influence, persecution, mastery

Perceptual disorder
pseudo-hallucinations (open mindedness)

Ideomotor automatism
feeling of estrangement, self-made thoughts, movements
Subjects and methods

45 patients (F20.0) ICD-10 (25 m., 20 f.), aged 28.39±0.91 yrs., The total score of the severity of psychopathological symptoms was determined by the PANSS scale, in patients it was 98.1 ± 2.1. 40 matched healthy subjects (23 m., 17 f.), aged 32.55±1.98 yrs. All subjects were right-handed, without somatic diseases, brain injuries or any other co-morbid brain pathology.

EEG: patients and matched healthy subjects were presented at random order with emotional negative (threatening) and neutral visual stimuli with IAPS system.

MRT: 15 patients and 12 healthy subjects were presented at random order with emotional negative (threatening) and neutral visual stimuli with IAPS system.

Epigenetics: 45 patients and 40 matched healthy subjects.
Electroencephalography (EEG and ERPs)

Recording, processing and analysis Used were neutral (60 stimuli) and threatening (60 stimuli). Stimulus was presented in random order. Time of presentation of the stimulus was 1000 ms, interstimulation period was from 1.5 to 3 ms. Electrode is placed on the international circuit of 10-20%.

ERP were recorded from 19 leads: Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, T5, T6, P3, P4, O1, O2, Fz, Cz, Pz.

Components P100, N170, P200, P300, N400 were detected. P100 and N170 reflected early stage, P200 – middle stage, P300 and N400 – late stage of perception.
In individual potentials with the step of 5ms the peaks of maximal amplitude closest to the averaged ones were detected. Interval for early stage 60-150ms, middle 150-250, late 270-440.
Affective stimuli

Threat

Neutral
### Activation of early ERP components to threatening stimuli compared to neutral

<table>
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<tr>
<th></th>
<th>P100</th>
<th>N170</th>
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<tr>
<td></td>
<td>N</td>
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<td>T6,O2</td>
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<td>F7,F4</td>
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- **N** — neutral stimuli
- **Th** — threatening stimuli
- **N/Th** — difference between neutral and threatening stimuli
- **HC** - Healthy control
- **SCH** - Schizophrenia

**Activation**

**The paradoxical effect**
Activation of late ERP components to threatening stimuli compared to neutral stimuli

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<tr>
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<th>P300</th>
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<tr>
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**Legend:**
- **N** — neutral stimuli
- **Th** — threatening stimuli
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- **HC** - Healthy control
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**Activation**

**The paradoxical effect**
Paradoxal effect
fMRI Method

**Threatening set**
- 10 stimuli - 3 sec for one

**Pause**
- 30 sec

**Neutral set**
- 10 stimuli - 3 sec for one

Repeat 3 times

Tomography and fMRI data was obtained on 3T tomograph Magnetom Verio, Siemens. The regions are defined according to the MNI atlas. ERPs were recorded to the same participants and stimuli according to standard protocol.
Healthy control
Main regions of fMRI activation at fMRI on stimuli of different affective significance

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<tr>
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<th>SCH</th>
<th>HC</th>
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<tbody>
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p=0.001; voxel size 2x2x2mm
DTI method

- **SIEMENS Verio scanner 3T**
- **64_DIR_2mm AP**
- **TE=101 ms**
- **TR=13700 ms**
- The slice thickness was 2 mm.
- A deterministic fiber tracking algorithm (Yeh et al., PLoS ONE 8(11): e80713) was used.
- A seeding region was placed at whole brain.
- The angular threshold was 60 degrees.
- The step size was 1 mm.
- A total of 100000 tracts were calculated.
Tracts - schizophrenia (number of tracts between areas)

- Hippocampus_L: 163
  - Talamus_L: 70
  - Amigdala_L: 222
- Hippocampus_R: 54
  - Talamus_R: 171
  - Amigdala_R: 190
  - Cuneus_R: 237
Tracts - healthy control (number of tracts between areas)

- Hippocampus_L: 343
  - Talamus_L: 214
    - Amigdala_L: 702
  - Cuneus_L: 702

- Hippocampus_R: 196
  - Talamus_R: 544
    - Amigdala_R: 486
  - Cuneus_R: 486

Total: 688
Comparison of fractional anisotropy (FA) values in different brain white matter regions on diffusion-tensor tractography (DTT). Compared with the control group, the FA values in the observation group – schizophrenia – shown by DTT were significantly lower in the Thalamus_R, Thalamus_L (P<0.01).
Molecular genetics

Dysfunction of methylation of the RELN gene
Why reelin?
Methylation was studied by bisulfate transformation of DNA samples. The resulting DNA-transformed bisulfite preparations were subjected to a (nested) PCR.

- DNA panel screening.
- DNA was isolated using hemolysis techniques and using magnetic nanoparticles.
- PCR amplification of fragments of the promoter region of the RELN gene.
Methylation of the gene RELN obtained from peripheral blood (promoter region -415 to -530), site -442 in patients with schizophrenia is completely absent in 100% of cases, and in healthy controls, 95% is present in all CG pairs.
Methylation difference blood/brain of patients with schizophrenia
General scheme

- **Biochemistry & Epigenetics**
  - 1-st level: Gene RELN hypomethylation

- **Anatomy**
  - 2-nd level: Structures of the brain

- **Physiology**
  - 3-rd level: High general activation, paradoxal effect

- **Psychology**
  - 4-th level: Affect

- **Psychopathology**
  - 5-th level: Positive symptoms

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**Ontogenesis**

**Behavior**
Further research plans

- Epigenetic therapy and diagnostics
- Crispr Cas9
- Electrophisiology
- ERP for stimuli, associated with delusion
- MRI
- Dynamic causal modeling
Thank you for your attention.