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

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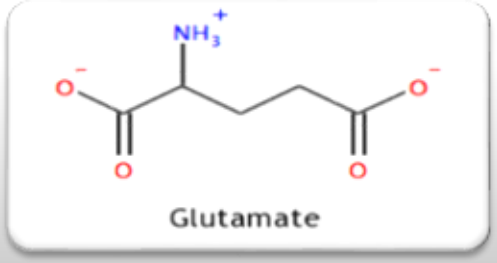
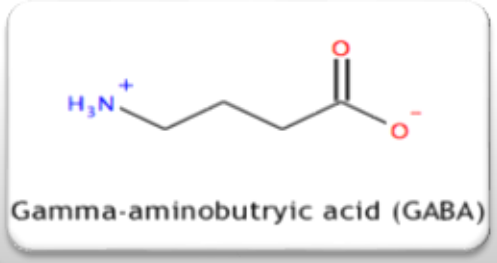
Comprehensive research method

Anatomy	Biochemistry Epigenetics	Physiology	Psychology	Psycho-pathology
1-st level	2-nd level	3-rd level	4-th level	5-th level
Structures of the brain	Gene RELN	ERP MRI	Affective sphere	Positive symptoms

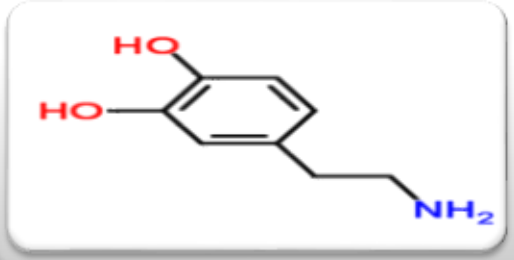
Hypotheses of schizophrenia



Glutamatergic

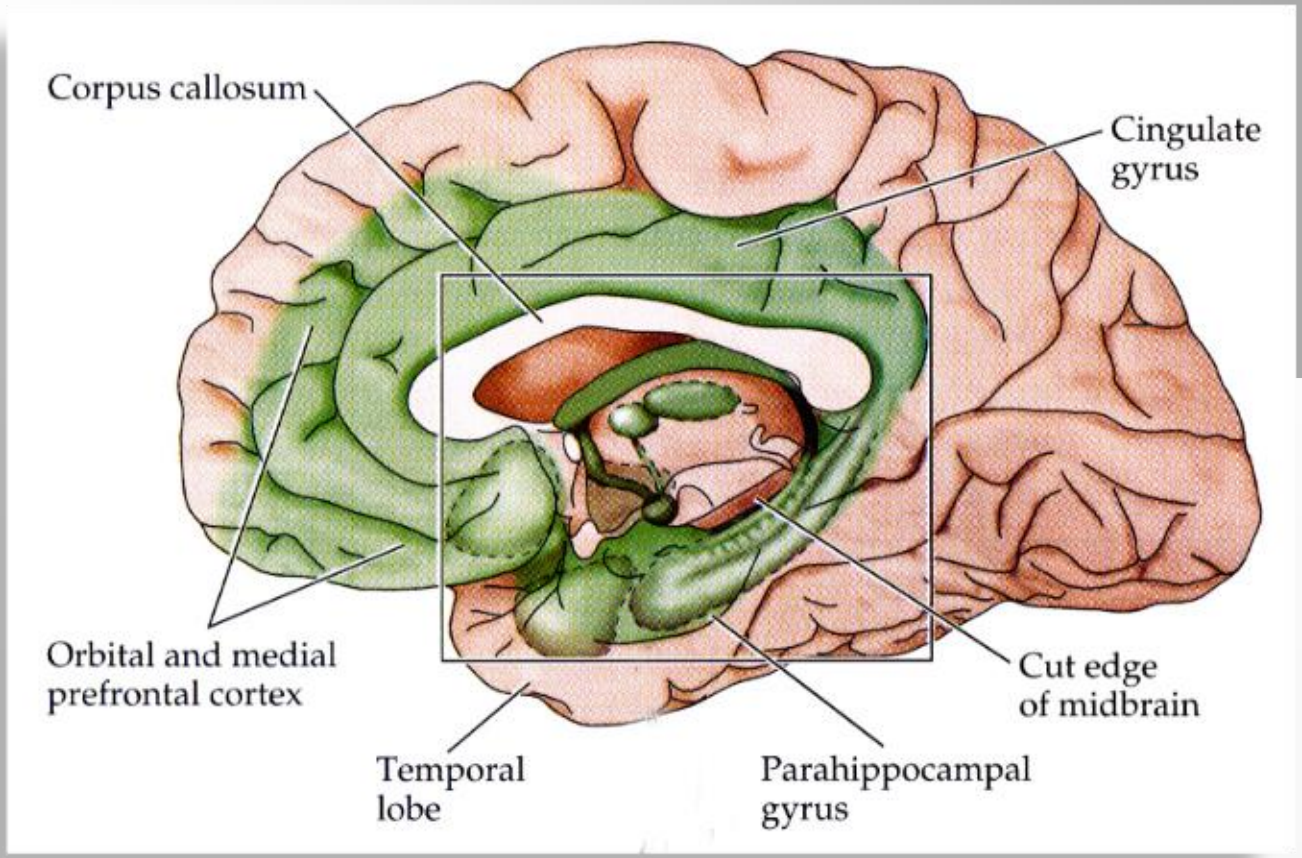


GABAergic/gamma-wave

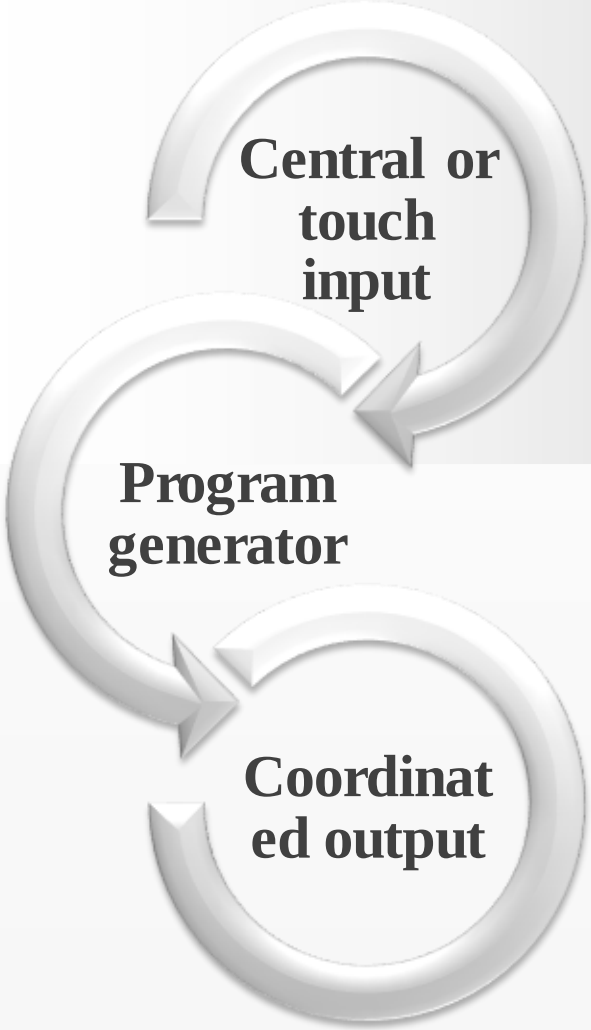


Dopaminergic

Temporolimbic system is the basis of affective perception disfunction



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».

Psychophathology

Kandinsky-Clérambault syndrome

Syndrome of mental automatism, alienation syndrome- a kind of hallucinatory-paranoid syndrome

Thought disorder	Perceptual disorder	Ideomotor automatism
delusional ideas of influence, persecution, mastery	pseudo-hallucinations (open mindedness)	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

	P100			N170			P200		
	N	Th	N/Th	N	Th	N/Th	N	Th	N/Th
HC									T6,O2
SCH									T6,O2
									F7,F4

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

	P300			N400		
	N	Th	N/Th	N	Th	N/Th
HC			O1,T5			T6,O2
SCH			O1,T5			T6,O2
			F7,Fp1, F8			Fp1,Fp2

N — neutral stimuli
Th — threatening stimuli
N/Th — difference between neutral and threatening stimuli
HC- Healthy control
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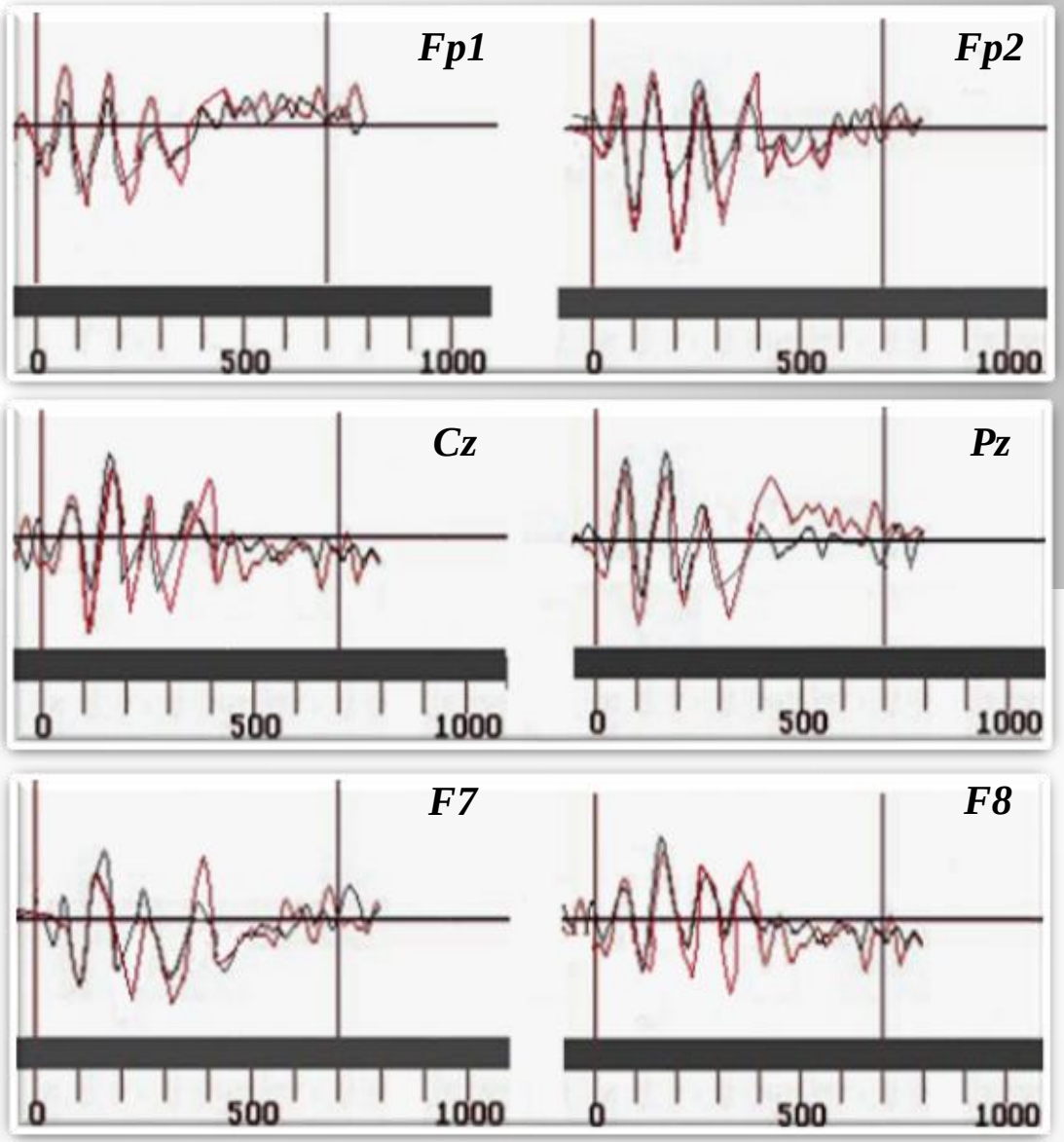
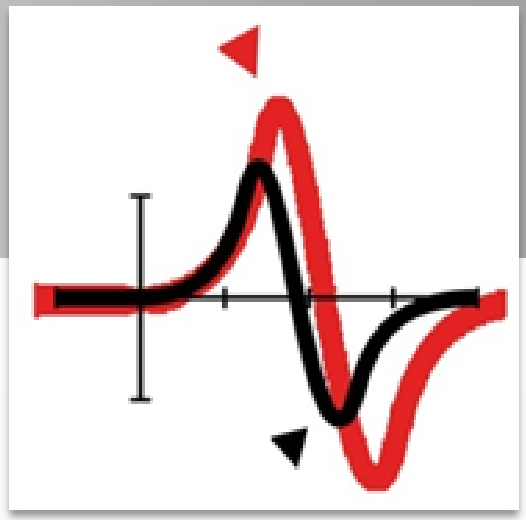
Activation

The paradoxical effect

Paradoxal effect

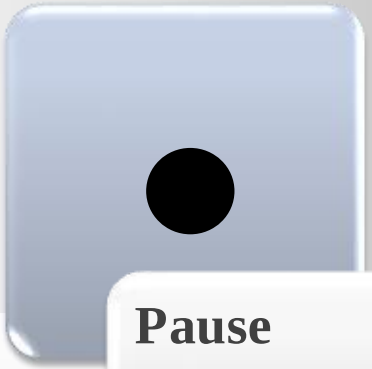




fMRI Method

Threatening set

- 10 stimuli - 3 sec for one



Pause

- 30 sec

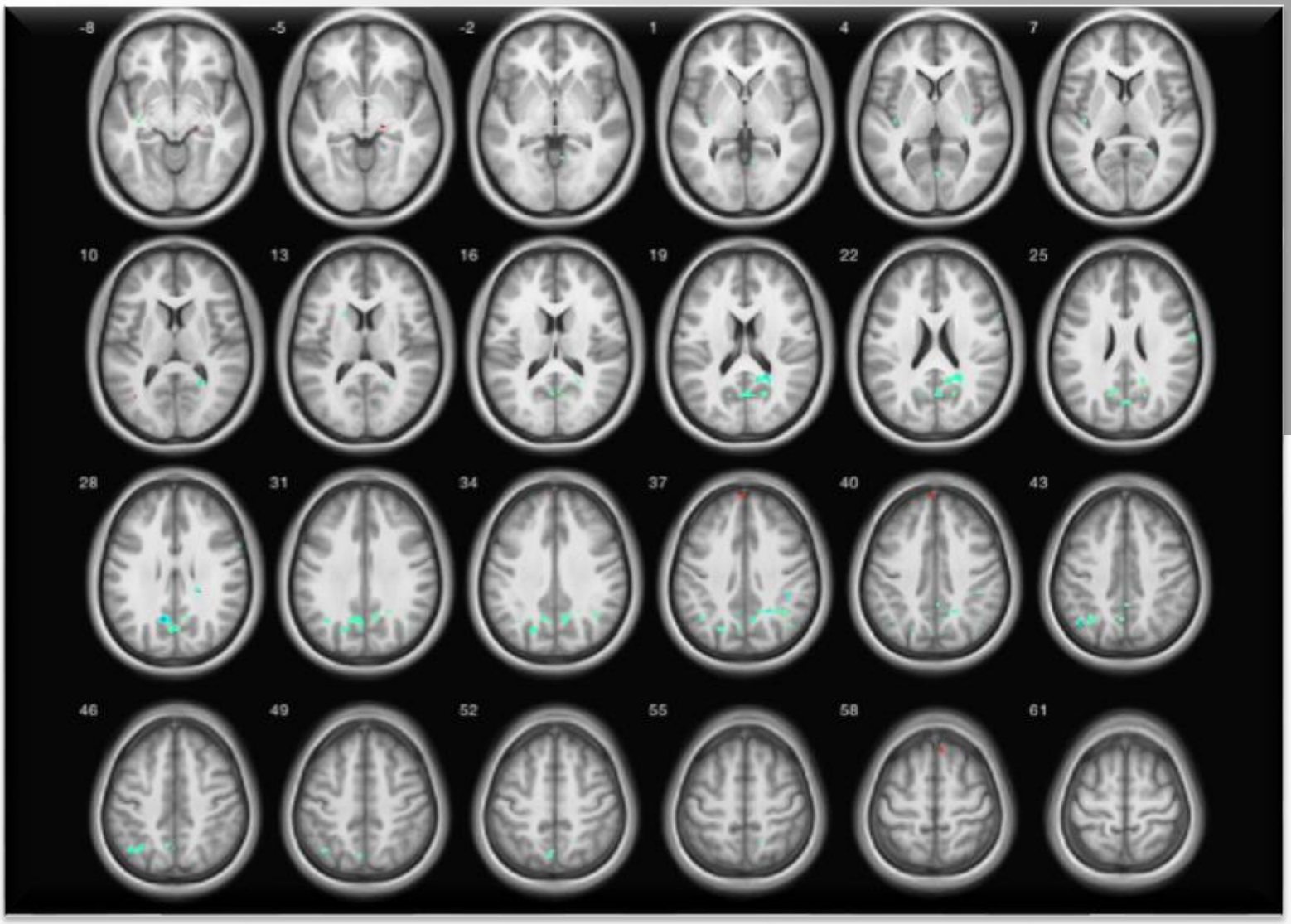
Neutral set

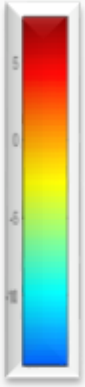
- 10 stimuli - 3 sec for one

Repeat 3 times

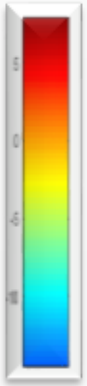
Tomography and fMRI data was obtained on 3T tomograph Magnetom Verio, Siemens. The regions are defined according to the MNI atlas.
ERPs were recorded to the same participants and stimuli according to standard protocol.

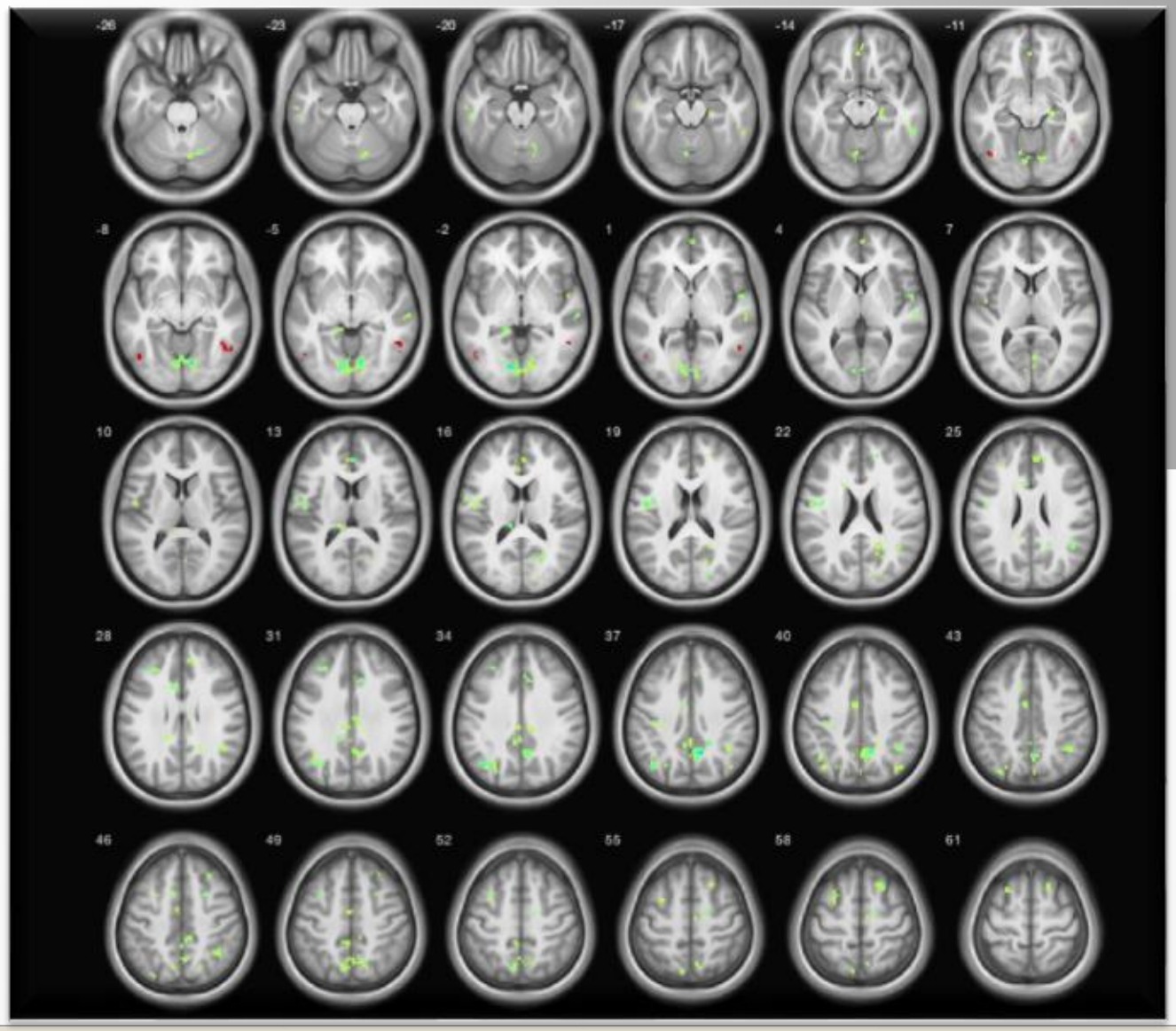
SCH





Healthy control





Main regions of fMRI activation at fMRI on stimuli of different affective significance

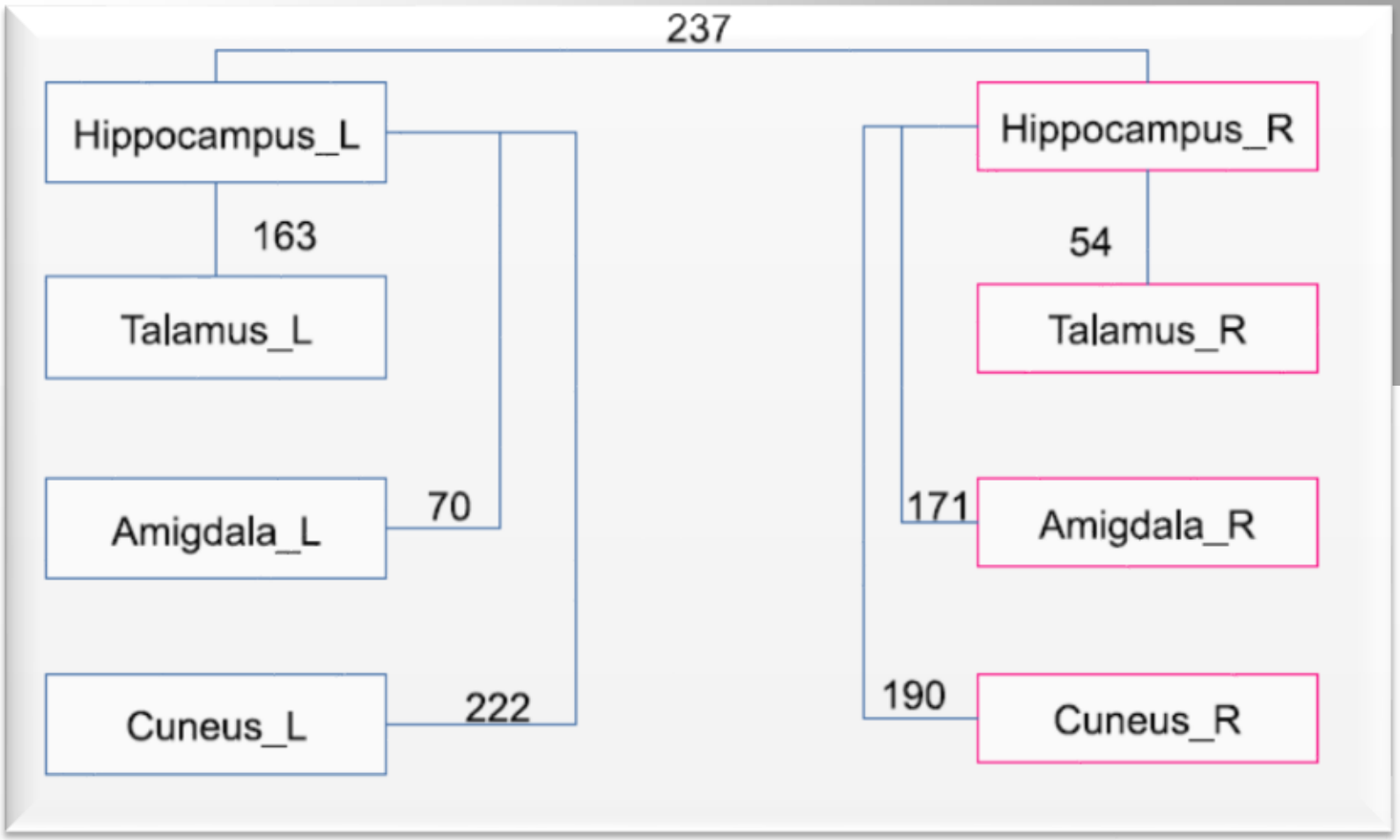
	SCH	HC
«Threatening» category	Hippocampus_R Precuneus_R Angular_R Calcarine_R Cingulum_Post_R Parietal_Inf_R Frontal_Sup_Medial_L Frontal_Sup_L Frontal_Sup_Medial_R	Occipital_Inf_L Temporal_Inf_L Temporal_Inf_R Temporal_Mid_R Temporal_Mid_L Occipital_Mid_L
«Neutral» category	Precuneus_R Angular_R Calcarine_R Cingulum_Post_R Parietal_Inf_R Precuneus_L Cuneus_L Angular_L Parietal_Inf_L Parietal_Sup_L	Calcarine_R Precuneus_R Precuneus_L Parietal_Sup_L

p=0,001; voxel size 2x2x2mm

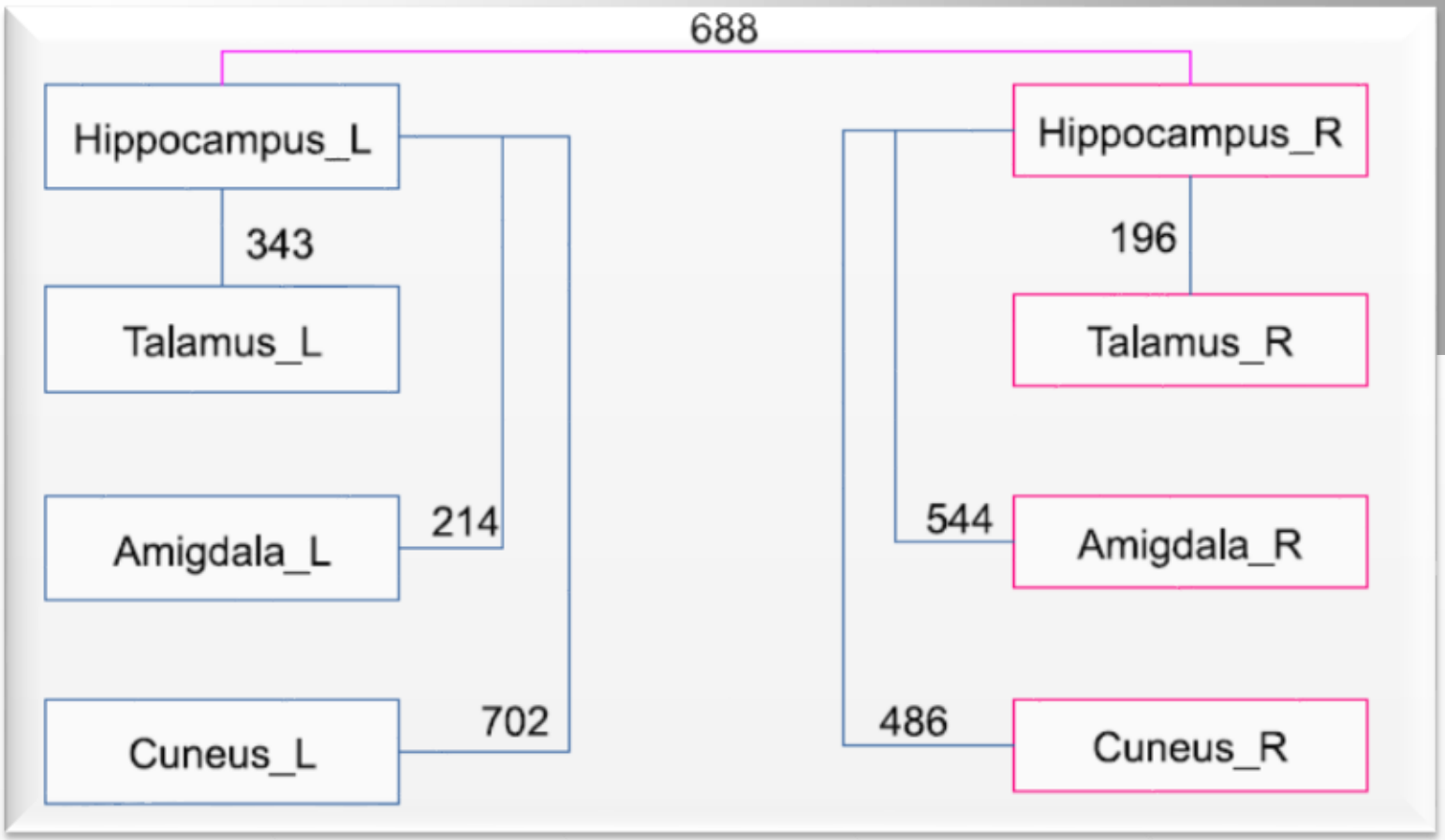
DTI method

- 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.

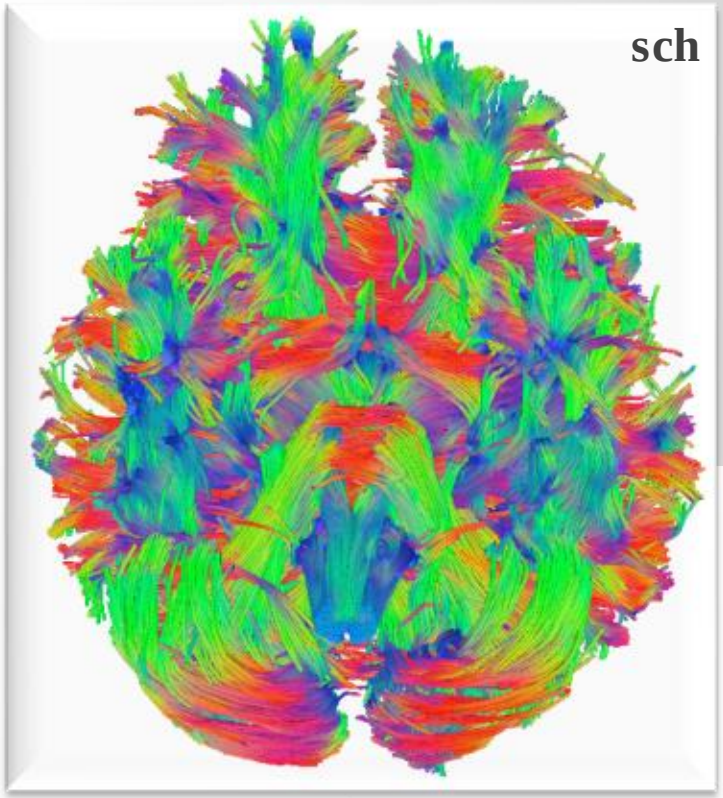
Tracts - shizophrenia (number of tracts between areas)

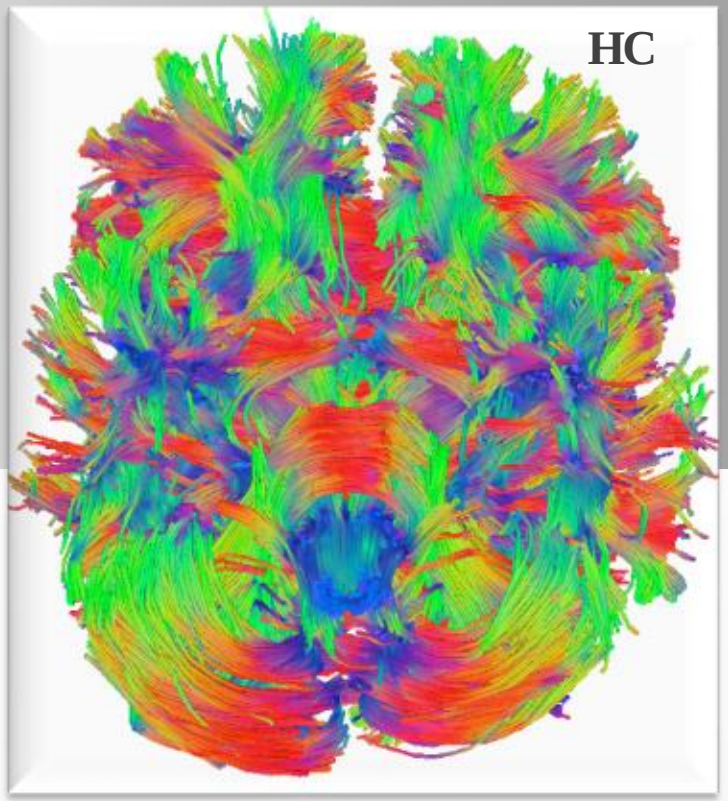


Tracts - healthy control (number of tracts between areas)



DTI

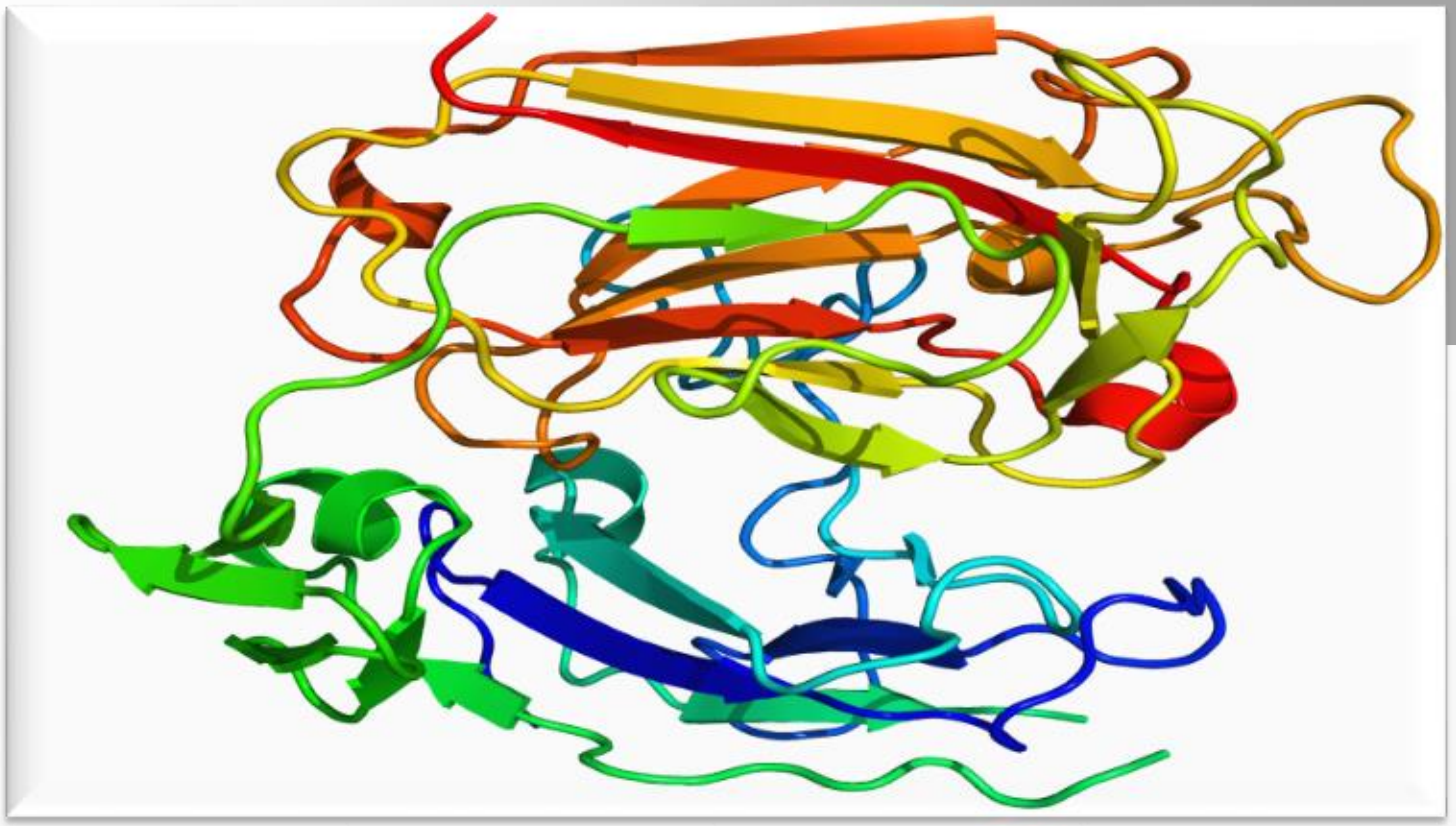




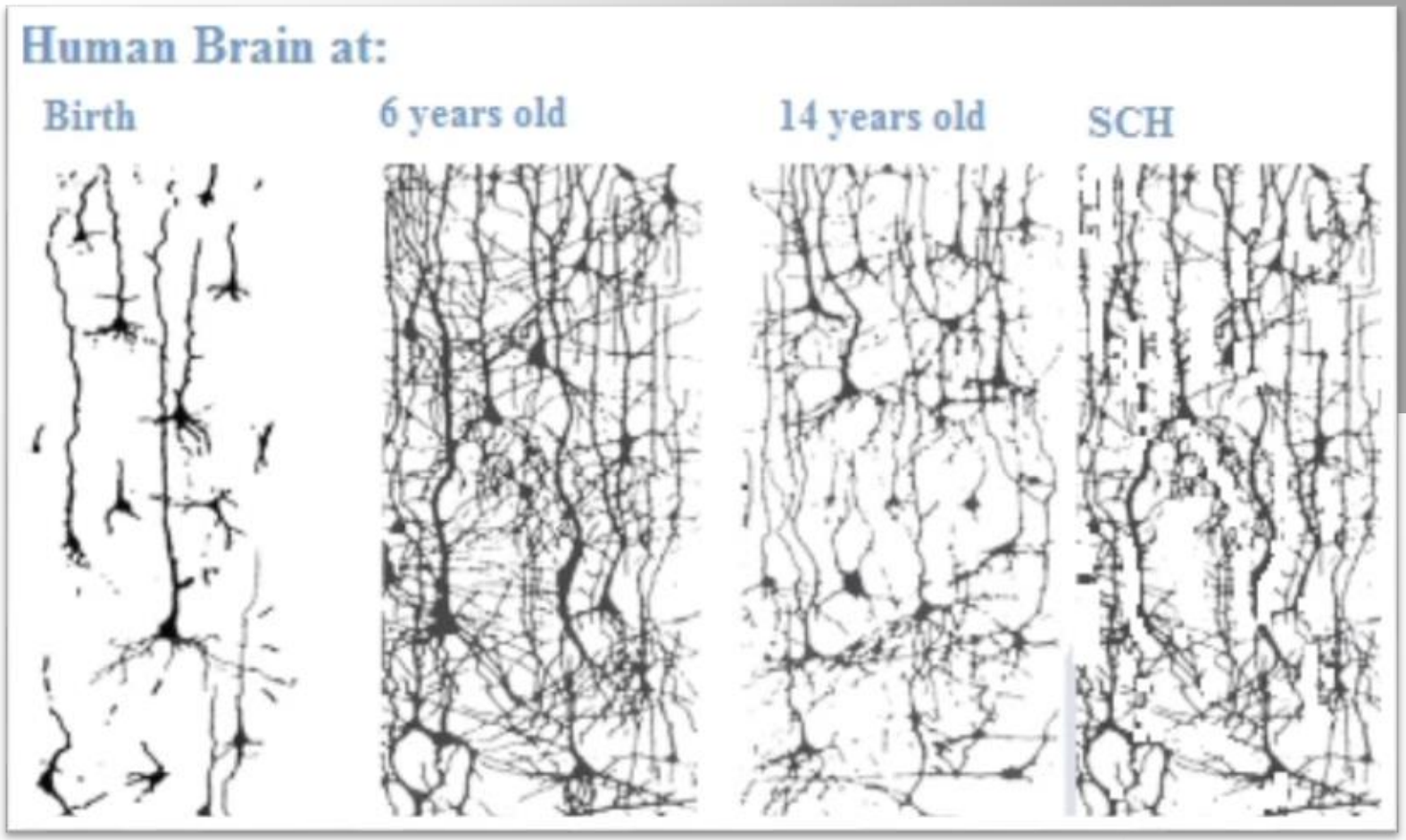
Comparison of fractional anisotropy (FA) values in different brain white matter regions on diffusion-tensor tractography (DTT). Compared with the control group, the FA values in the observation group – schizophrenia – shown by DTT were signifcantly lower in the Thalamus_R, Thalamus_L (P<0.01).

Molecular genetics

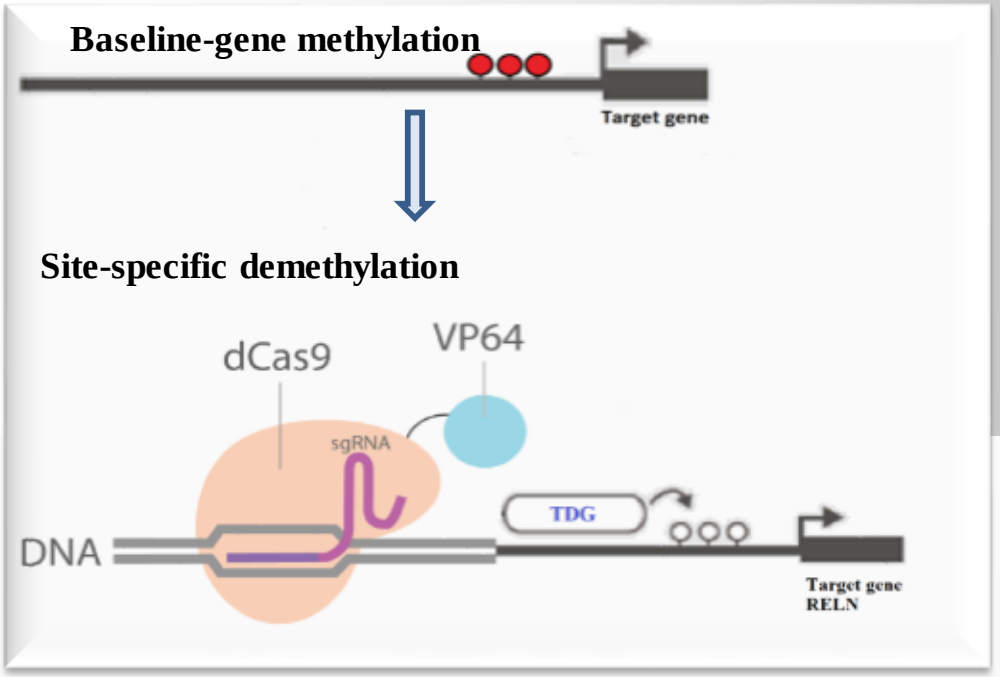
Dysfunction of methylation of the RELN gene



Why reelin?



Method of methylation



- 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 was studied by bisulfate transformation of DNA samples.
- The resulting DNA-transformed bisulfite preparations were subjected to a (nested) PCR.

Methylation results RELN

-415

cggccgtccc tgccgcccct ctccttccct cacgcatcct cccaggaaaa acagggcaca

ctgacggcca aaggggctgg ccttccccttt **tagatagttg gatgggaggt gtttttgtg**

gggtttgat gtttttgta gaagagttgt gggtttagtg gtttttgata gtgtttttgt

site -442 **tttgttcccc ggtgggtgtt tttttttgtt ttttgggtg tgaattgggc gttggttggg**

gatttgggg atgtgtgtgt tttttgttgt gtgaggtgcc gccgagccag cccgagaggg

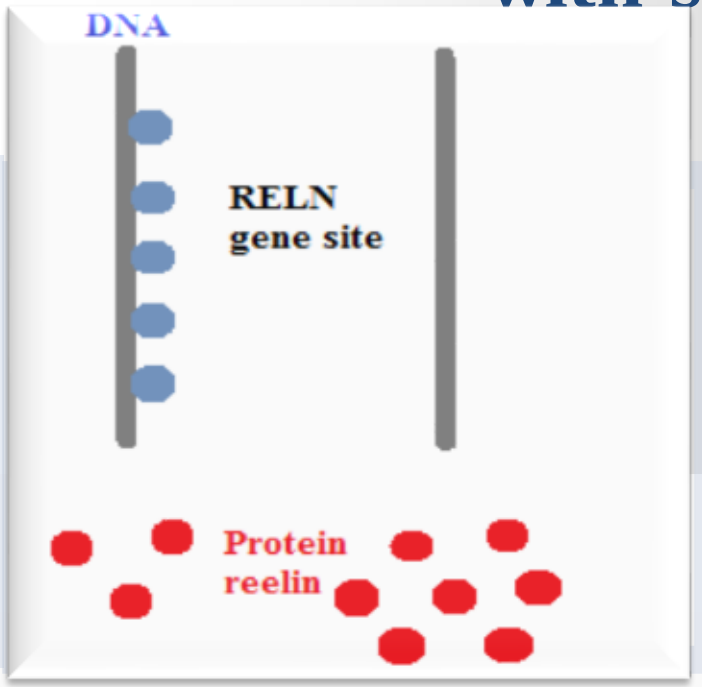
cgggggcgg gcggggcggc gcgcgggggc gggggagcgg ccgggacacg tgtggcggcg

-530

promoter

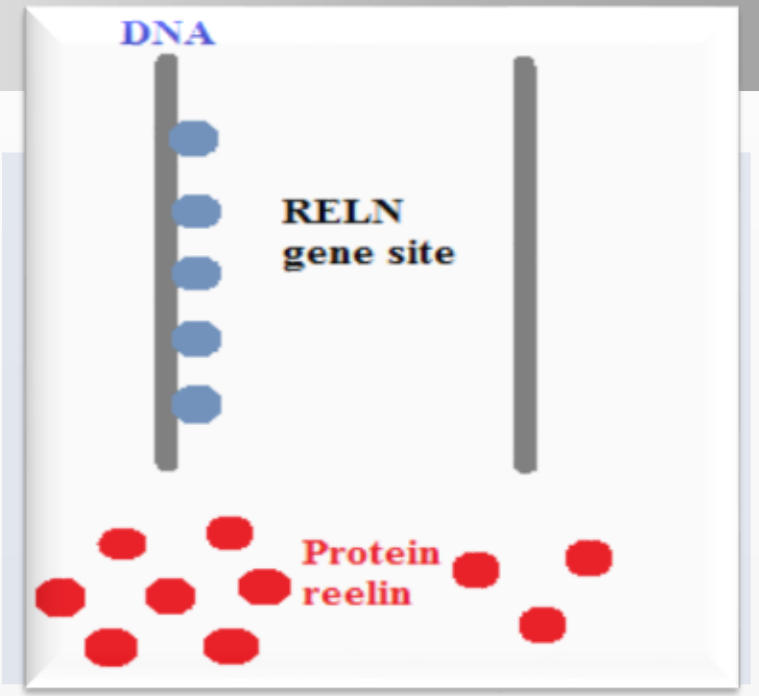
Methylation of the gene RELN obtained from peripheral blood (promoter region -415 to -530), site -442 in patients with schizophrenia is completely absent in 100% of cases, and in healthy controls, 95% is present in all CG pairs.

Methylation difference blood/brain of pations with schizophrenia



← Peripheral blood

Brain →



General sheme

Biochemistry Epigenetics	Anatomy	Physiology	Psychology	Psycho-pathology
1-st level	2-nd level	3-rd level	4-th level	5-th level
Gene RELN hypomethylation	Structures of the brain	High general activation, paradoxal effect	Affect	Positive symptoms

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.