Metabotropic glutamate receptors in the hippocampus and the prefrontal cortex in rats during neurodegeneration caused by trimethyltin chloride.

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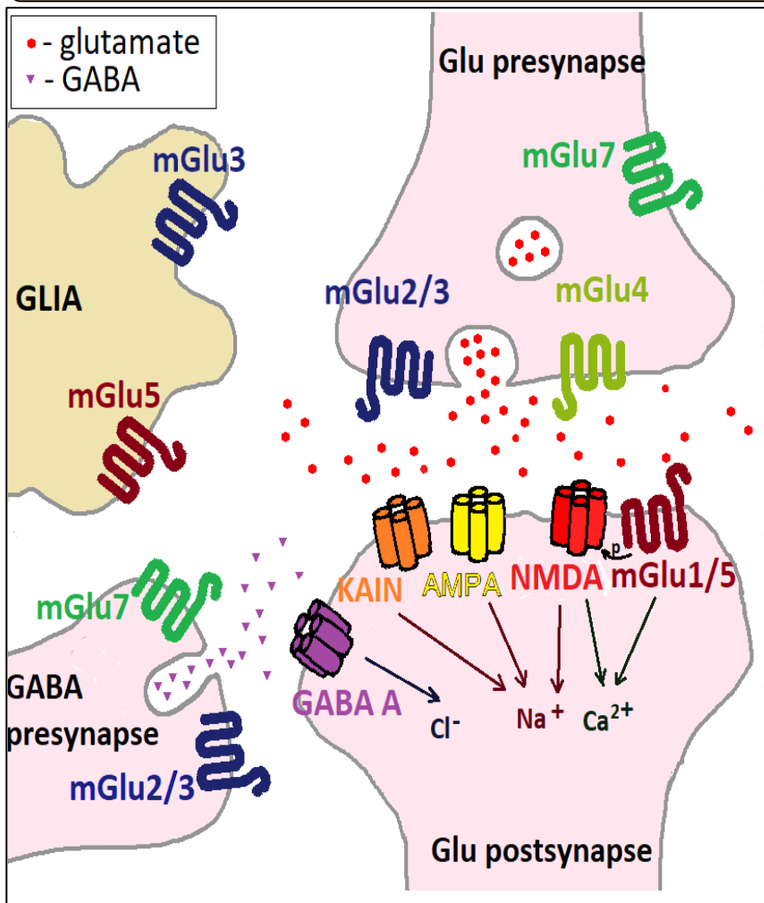
I. Introduction



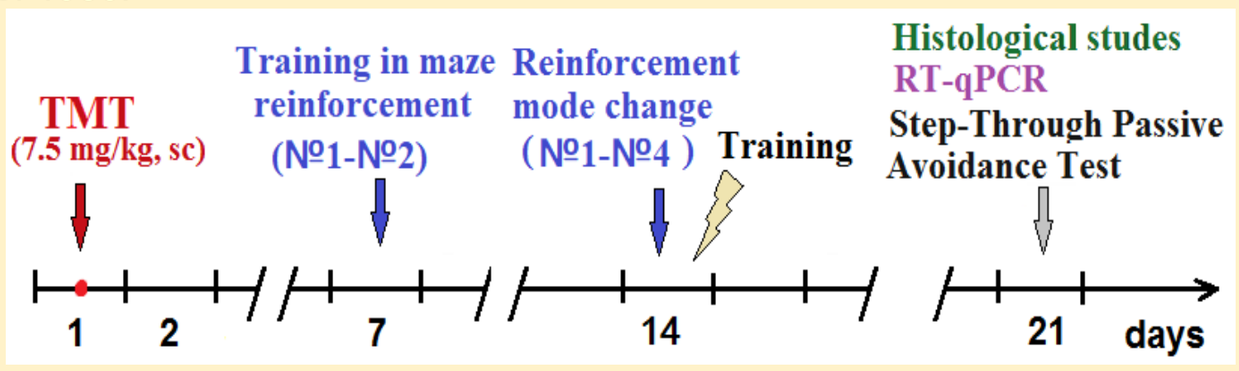
The metabotropic glutamate receptors (mGluRs), are G-protein-coupled receptors which act via secondary messengers. There are 8 types of mGluRs divided into three groups:

mainly postsynaptic
•Group I: mGluR1, mGluR5 (Gq, ↑PC, ↑IP3, ↑Ca2+)
mainly presynaptic
•Group II: mGluR2, mGluR3 (Gi/o, ↓AC, ↓cAMP)
•Group III: mGluR4, mGluR6, mGluR7,mGluR8 (Gi/o, ↓AC, ↓cAMP).

In this work, the involvement of mGluR in neurodegeneration was studied using a neurotoxic model based on the action of neurotoxicant trimethyltin chloride (TMT).

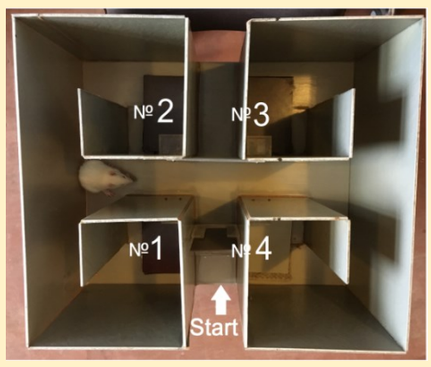
II. Materials and methods

Male Wistar rats (210-220g) were used as subjects. Experiments were carried out in accordance with the guidelines of the Institutional Animal Care and Use Commission of ITEB RAS and the Council EC Directive of 1986.



Behavior tests: locomotor activity, grooming, time spent in the dark and light zones, step-through passive avoidance test was carried out in the complex «Shelter»(Neurobotics, Moscow)

Learning of rats in the maze (food reinforcement goal shelf №1-№2) began 7 days after TMT injection. Study of the task retrieval after change of reinforcement goal shelf (№1-№4) and the number of times the animal entered the no longer reinforced compartment №2 was recorded as errors.



Histological studies were carried out on Epon sections (9 nm) with osmium staining (2%).

qRT-PCR: Isolation total RNA→ Reverse transcription → qPCR. The threshold cycle (Ct) value was determined using DTmaster software (DNA-technology, Russia). The signal was normalized to that obtained for the gene of cytoskeletal protein beta-actin (*Actb*). Amplicon quality and sizes were verified by gel-electrophoresis in 3% agarose gel. The amount of mRNA was estimated by the 2-ΔΔCt method [Schmittgen, 2008]

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III. Results

Behavior tests:
Rats after TMT treatment showed increased anxiety and a reduced orienting response. Locomotion, grooming, and time spent in dark areas increased, and vertical movements decreased compared to rats in the control group.

Step-through passive avoidance test: 7 days after learning the animals from the control group demonstrated a freezing behavior for 3 min. In contrast, rats in the TMT group showed amnesia and entered the dark compartment of the box after 26 ± 24 s.

In the maze, learning dynamics of rats after TMT treatment didn't differ from animals in the control group. Stable reproduction of the task was observed after 5 days of training.However, after changing shelves with reinforcements, rats in the TMT group made more mistakes than in the control group. We suggest that this indicates impaired hippocampal function.

Histological studies:

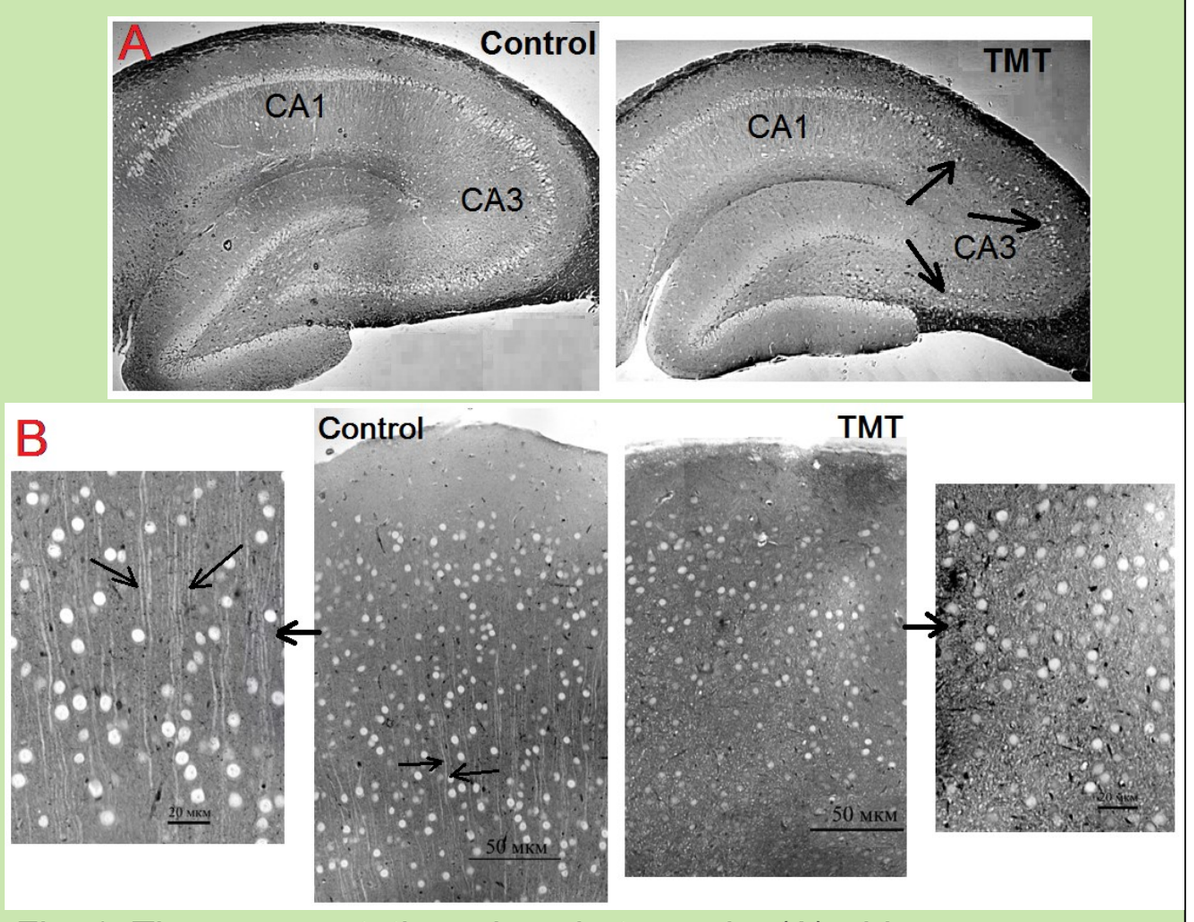

Fig. 1. The representative microphotographs (A) - hippocampus and (B) - prefrontal cortex of rat in control and TMT groups.

qRT-PCR:

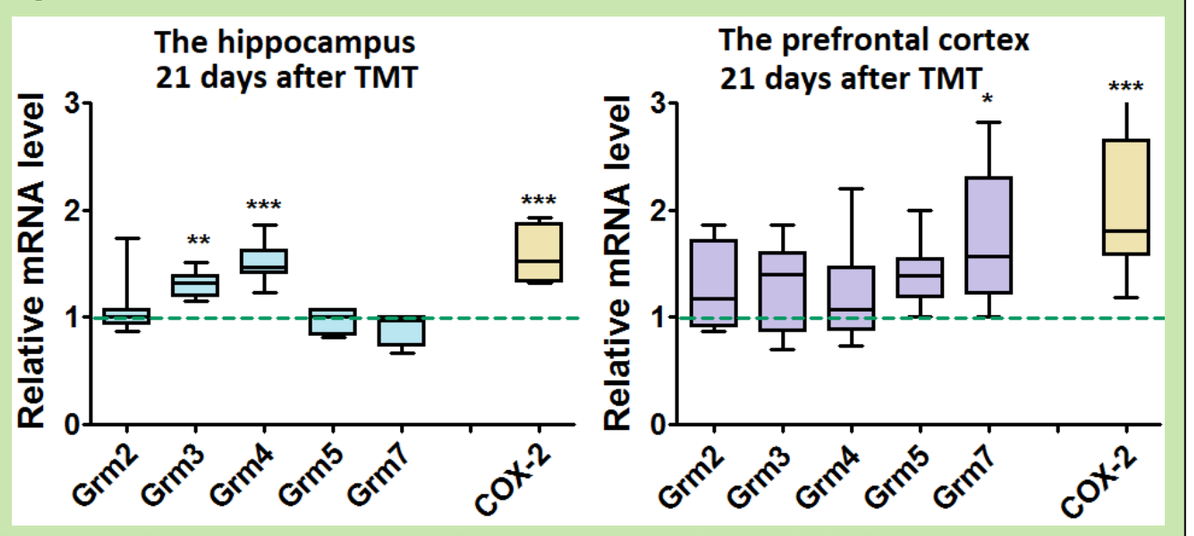

Fig. 2. mRNA level of mGluRs and COX2 (n=16) (mRNA level of control animals was taken as 1, horizontal line);*- p<0.05,**- p< 0.001, ***- p< 0.0001(ANOVA followed by multiple comparisons, Dunnet test).

IV.Conclusion

TMT treatment leads to an increase in neuronal death, mainly in the rat hippocampus. Therefore, the difference in mGluR gene expression level in the prefrontal cortex and hippocampus may indicate a different contribution of mGluR subtypes to neuroprotection. We suggest that mGlu7 is a promising target for a neuroprotective effect.