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Egr3-/- mice, a mouse-model of schizophrenia, show decreased levels of *Htr2a* mRNA in the anterior frontal cortex after sleep deprivation compared to WT mice.

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Abstract

- In the U.S., 1 in 25 adults experience serious mental illness each year.
- Despite ongoing research efforts, the pathogenesis of schizophrenia remains unknown.
- The aim of this study is to answer the question "Do *Egr3-/-* mice, a mouse-model of schizophrenia, show decreased levels of *Htr2a* mRNA in the prefrontal cortex (PFC) region of the brain after sleep deprivation (SD) compared to wild type (WT) mice?"
- Data resulting from the study will shed light on the pathogenesis of such a disabling mental disorder.
- Our study investigates the interaction between two of the genes linked to increased risk of schizophrenia, the early growth response (*Egr*) 3 gene and *Htr2a*, which encodes the serotonin 2a receptor ($5HT_{2A}R$) in response to SD, a form of stress.
- We used a cohort of age-matched pairs of C57BL/6 *Egr3-/-* and WT male mice. Half of these underwent a SD protocol, while the other half served as a control group.
- *Htr2a* mRNA was quantified in four different brain regions via densitometry after it was visualized using *in-situ* hybridization.
- Our findings that *Egr3-/-* mice show statistically significant decreased expression levels of *Htr2a* mRNA in the PFC support our proposed biological pathway for schizophrenia risk.

Introduction

Schizophrenia is a debilitating brain disorder that affects approximately one percent of the global population. People with schizophrenia often experience hallucinations, delusions, thought and movement disorders and a lack of pleasure in everyday activities, which ultimately leads them to live non-productive lives. Today, the underlying cause of schizophrenia remains unknown, which makes it difficult to prevent and treat this disease. Twin studies, however, have strongly suggested that risk for schizophrenia is not only determined by genetics, but rather by interactions between genetic and environmental factors, and despite the fact that its complex genetic etiology has made it challenging to identify individual genes involved in its pathogenesis, recent studies have identified the early growth response *(Egr) 3* gene as a potential susceptibility gene for schizophrenia risk. In fact, mice lacking *Egr3* display characteristics that are often seen in schizophrenia animal models, such as hyperactivity and memory deficits. Interestingly, while *Egr3-/-* mice respond to the anti-aggressive effects of clozapine, an atypical antipsychotic, they are relatively resistant to its sedating effects. This tolerance to clozapine has also been documented in schizophrenia patients who are able to handle much greater doses of the drug compared to healthy individuals.

In Dr. Gallitano's lab, we used an *Egr3-/-* schizophrenia mouse model as a tool to identify that serotonin 2A receptors ($5HT_{2A}Rs$) were responsible for the sedating effects of clozapine in WT mice. Because the *Htr2a* gene encodes the $5HT_{2A}$ R, we now want to determine whether *Htr2a* expression is affected in the absence of *Egr3* at baseline and after sleep deprivation, a form of stress.

Methods

Sleep Deprivation (SD)
Eight age-matched pairs of *Egr3-/-* and WT mice were single-housed 5 d prior to SD for habituation. On the day of the study, mice from the experimental group were transferred to a procedure room at 8:00 A.M. were they were kept awake via olfactory and sensory stimuli for 6 h. Control mice undergoing a normal sleep cycle were kept in their home cages during this time period.

Collection and Processing of Tissue Samples
Immediately following SD, each age-matched pair was anesthetized and sacrificed at the same time. Mouse brains were then quickly removed from the skull, flash frozen in isopentane and stored at -80°C until sectioned. Coronal sections at 20µm were obtained and stored at 20°C until processing.

***In Situ* Hybridization**
A radioactive isotope (^{35}S) was used to label a riboprobe against target *Htr2a* mRNA. Tissue sections were prehybridized using a buffered formaldehyde solution. The hybridization reaction with the *Htr2a* radioactively labeled probe was then carried out at 55°C for 24 h. After hybridization, tissue slides were washed, dehydrated and air dried.

Imaging and Quantification
After visualization of radioactive signal via autoradiography, *Htr2a* mRNA expression was quantified in tissue sections via densitometry in four different brain regions (Figure 1).

Statistical Analysis
Htr2a mRNA expression in *Egr3-/-* and WT mice was analyzed using a two-way ANOVA and Sidak multiple comparisons tests.

Results

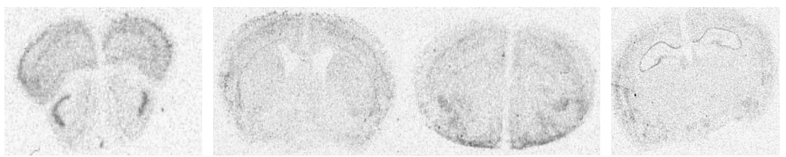
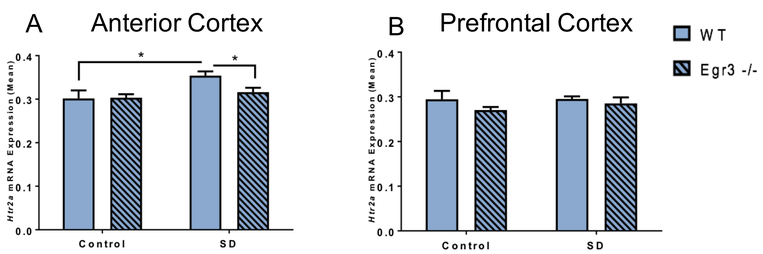


Figure 1. Representative images of Htr2a mRNA labeling in the A) anterior cortex (Bregma +2.96 to +2.10), prefrontal cortex (Bregma +2.10 to +0.14), and hippocampus and posterior cortex (Bregma +0.14 to -2.18).



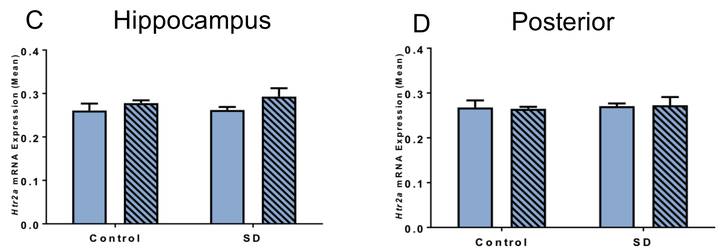


Figure 2: A) Egr3-/- mice show decreased expression of Htr2a mRNA in the anterior cortex of the brain compared to WT controls after 6 h of sleep deprivation. A two-way ANOVA showed a significant effect of genotype for the anterior cortex (F(1,17) = 8.391, p=0.01.

Conclusions

- Sleep deprivation, a form of stress, upregulates *Htr2a* mRNA expression levels in the anterior cortex of C57BL/6 mice.
- Upregulation of *Htr2a* mRNA expression levels is dependent on the presence of the immediate early gene transcription factor (IEG-TF) *Egr3* as demonstrated by the fact that *Egr3-/-* mice, showed decreased *Htr2a* mRNA levels in the anterior cortex of the brain after6 h of SD compared to WT mice.
- Our findings support our proposed biological pathway for schizophrenia risk, where *Egr3*, a transcription factor, directly regulates *Htr2a*, a schizophrenia-linked gene encoding the serotonin 2A receptor ($5HT_{2A}R$).
- Lastly, these findings show that environmental factors (i.e., stress) impact brain expression patterns of genes that have been linked to schizophrenia risk.

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