

The Role of Novel Chemokine-like Protein TFA2 in the Modulation of GABAergic Synaptic Transmission and Emotional Behavior

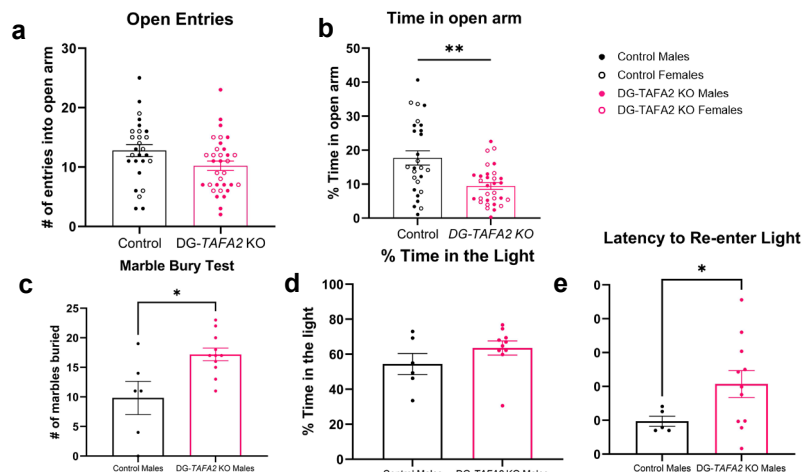
*Cindy Nunez¹, Gillian Kearney¹, Thomas Kelly¹, Kathryn Heaster¹, David Grau¹, Qing-song Liu^{1,2}, Sang H. Lee^{1,2};

¹Department of Pharmacology and Toxicology, ²Neuroscience Research Center, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226.

Introduction

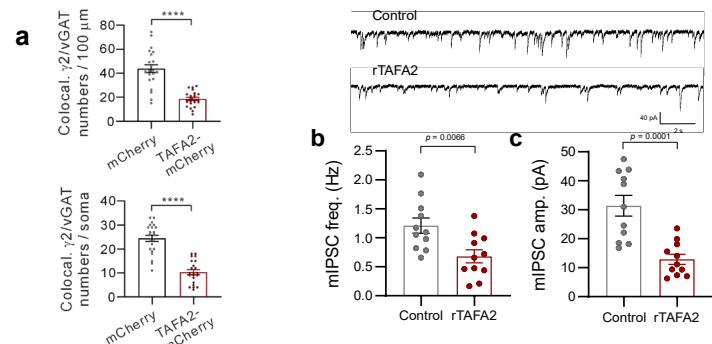
TFA2 is a secretory protein that is most highly expressed in the dentate gyrus (DG). Previous studies showed that global knockout of TFA2 increases anxiety and fear in mice and zebrafish, but the mechanisms that underlie these behavioral changes remain uncertain. In this study, we evaluate the role of TFA2 in modulating anxiety-like behavior via the DG-CA3 pathway using DG-specific TFA2 KO mice. The effect on behavior and changes in GABAergic transmission were evaluated in these DG-TFA2 KO mice. We additionally evaluated the effects of TFA2 on hippocampal cells *in vitro* and its mechanism of release. Overall, we sought to broaden our knowledge of neuromodulation in hippocampal pathways and how they influence behavior, opening the opportunity to identify new therapeutic targets in the treatment of psychiatric disorders.

TFA2 DG-KO increases anxiety-like behavior



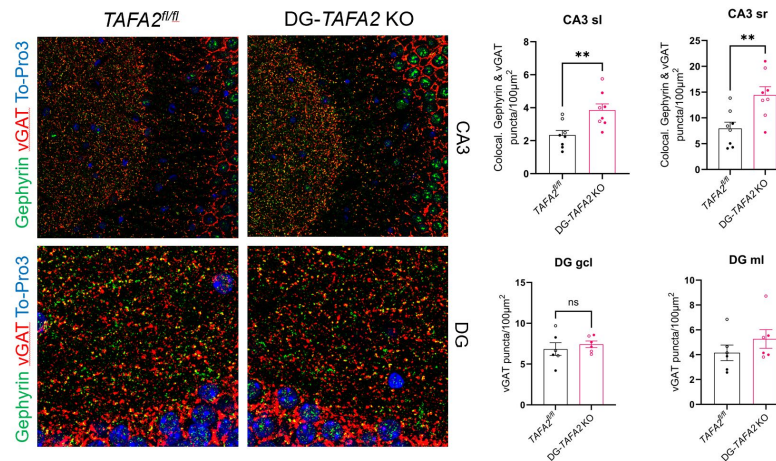
a) Number of open entries and b) time spent in open arms of elevated plus maze for DG-TFA2 KO mice. c) Number of marbles buried in marble bury test. d) Time spent in light and e) latency to re-enter the light in light-dark box test. Data are presented as mean \pm sem.

TFA2 reduces GABAergic synapse transmission



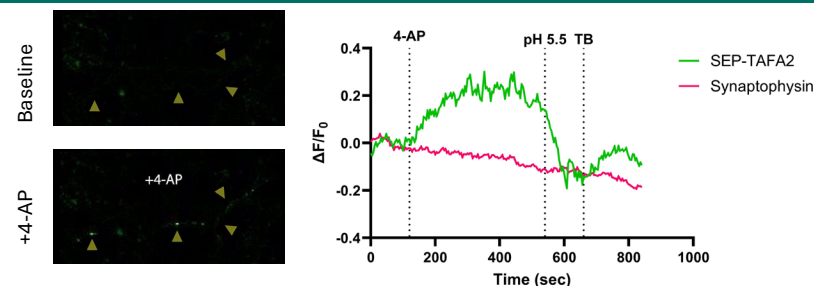
a) The effect of TFA2-mCherry overexpression on the number of colocalized GABA_AR₂ and vGAT puncta on the dendrites and soma of cultured rat hippocampal neurons. b) The effect of rTFA2 10 min treatment on mIPSC amplitude and c) frequency. Data are presented as mean \pm SEM.

TFA2 DG-KO increases GABAergic synapse numbers specifically in the CA3



The effect of TFA2 DG KO on individual and colocalized GABA_AR₂ and vGAT puncta in the stratum lucidum of CA3. Data are presented as mean \pm sem.

TFA2 is activity-dependently secreted from synapses



The effect of activation by 4-AP on TFA2 release from synapses. pH5.5 buffer was used to quench the SEP fluorescence signal. Synaptophysin was used as a presynaptic marker.

Conclusions

- DG-TFA2 KO mice showed increased anxiety in the EPM, marble bury, and light-dark box tests but not in the open field tests or in either contextual or cued fear conditioning (not shown).
- TFA2 overexpression reduces GABAergic synapse numbers on both dendrites and somata of cultured rat hippocampal neurons and TFA2 is sufficient to decrease GABAergic synaptic transmission *in vitro*.
- DG-TFA2 KO mice have increased GABAergic synapse numbers specifically in the stratum lucidum where DG mossy fibers synapse onto CA3 pyramidal neurons.
- TFA2 is released pre-synaptically following 4-AP induced activity.

In summary, TFA2 functions as a neuromodulator that is activity-dependently released from synapses, affecting GABAergic synaptic transmission and emotional behavior. We show a dentate gyrus-specific effect in controlling anxiety-like behavior, but not fear, and we saw that a DG-specific KO of TFA2 increases GABAergic synapses specifically in CA3. Taken together, these results support our hypothesis that **TFA2 plays a critical role in the regulation of anxiety-like behaviors via modulation of GABAergic synapses in the dentate gyrus-CA3 pathway.**

Acknowledgements

Financial support was provided by the National Institutes of Health, grant numbers RO1MH119105 and R21AG073610.