Impact of the Deletion of Tumor Necrosis Factor Receptor-Associated Factor 3 (Traf3) in Parvalbumin (PV)-Expressing Inhibitory Interneurons in Male and Female Mice

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INTRODUCTION

- Psychiatric disorders present a major global health challenge, yet the underlying biological mechanisms behind them remain under researched and thus, poorly understood.
- Inhibitory interneurons (INs) are a small but diverse subset of neurons that modulate the activity of principal neurons. The parvalbumin (PV)-expressing IN subtype are implicated in stress-related mood disorders [1].
- Genome-wide association studies have identified Tumor necrosis factor receptor associated factor 3 (Traf3) as a postsynaptic protein enriched in INs [2]. Traf3 has also been identified in genome-wide association studies to be associated with post-traumatic stress disorder (PTSD) [3], major depression disorder (MDD) [4], and suicide ideation [5].
- Traf3 has been researched in the immune system, but its function in the nervous system is not known.
- Our lab generated the transgenic mouse line to characterize Traf3's role in the nervous system: $Traf3^{F/F1}$: $vGat^{Cre/WT}$ (IN-Traf3 KO). IN-Traf3 KO results in a loss of Traf3 protein in all INs.

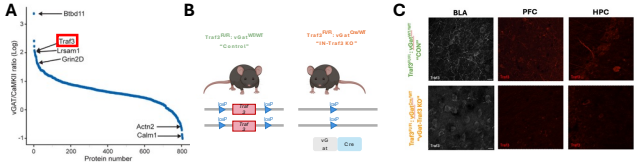

Figure 1. Transgenic mouse lines used as tools to study Traf3 as a synaptic protein in INs.
(A) Mass spectrometry data from postsynaptic density protein 95 (Psd95) complex pull-down from vGat-expressing (inhibitory) and CaMKII-expressing (excitatory) cells showed that Traf3 is enriched in inhibitory neurons compared to excitatory neurons. **(B)** Schematic of IN-Traf3 KO transgenic mouse model used for behavioral assays. **(C)** Marked reduction in Traf3 expression across the basolateral amygdala (BLA), prefrontal cortex (PFC), and hippocampus (HPC) in conditional knockout mice ("vGat-Traf3 KO") compared to controls ("CON").

PRELIMINARY DATA

IN-Traf3 KO Mice Exhibit Anxiety- and Depression-Like Behavior

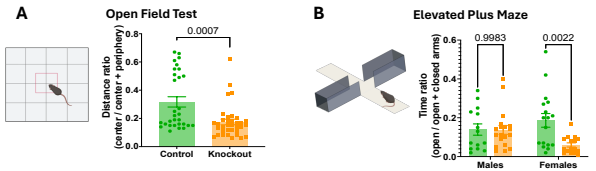

Figure 2. Behavior test results from IN-Traf3 KO animals.
(A) IN-Traf3 KO (vGat-Traf3 KO) animals spend significantly less time at the center compared to time spent in periphery during the first 10 minutes of open field test (OFT) (Unpaired two-tailed t-test (p=0.0007); n=65 (nCON = 30, nKO=35)). **(B)** Female IN-Traf3 KO (vGat-Traf3 KO) mice spent less time in the open arms during the elevated plus maze (EPM) compared to controls (2-way ANOVA (p=0.0211, genotype x sex); p-values in the figure from Sidak's post-hoc test; n=66 (nM CON= 14, nM KO = 19, nF CON = 17, nF KO = 16)).

IN-Traf3KO Animals Show Increased Freezing Behavior

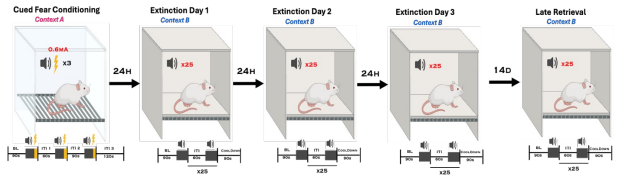

Figure 3. Fear conditioning and extinction paradigm. Schematic illustrates the fear conditioning and extinction paradigm followed by each mouse in the study. There was a waiting period of at least 1 week between the end of EPM and cued fear conditioning experiments.

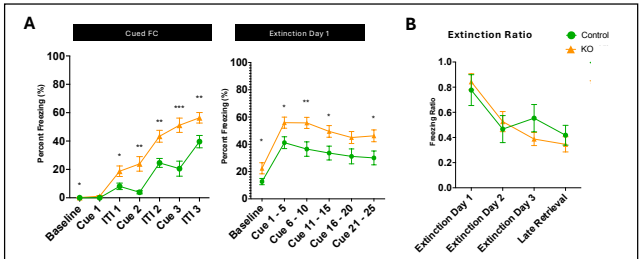

Figure 4. Animals with Traf3 knock-out in interneurons exhibit higher fear response during cued fear learning.
(A) IN-Traf3 KO (vGat-Traf3 KO) animals showed higher freezing behavior on Cued Fear Conditioning day and Extinction Day 1. (2-way ANOVA (p<0.0001, genotype x timepoint); Sidak's post-hoc test; n=33 (nM CON= 7, nM KO = 9, nF CON = 8, nF KO = 9)). **(B)** IN-Traf3 KO (vGat-Traf3 KO) and control animals do not show a difference in the ratio between % freeze at the last 5 cues compared to the first 5 cues on Extinction Day 1. (2-way ANOVA (p=0.2395), genotype x timepoint; n=33 (nM CON= 7, nM KO = 9, nF CON = 8, nF KO = 9)).

RESEARCH QUESTION

Are the behavioral phenotypes observed in IN-Traf3 KO mice driven by Traf3 disruption in PV-expressing INs specifically?

- This will be answered by performing a behavioral battery a conditional KO mouse line, PV-Traf3 KO, in which the Traf3 protein is deleted from PV Ins specifically.
- Mice will be tested at 12 weeks old and will be handled for one week prior to the behavioral battery.

BEHAVIORAL BATTERY RESULTS

Open Field Test

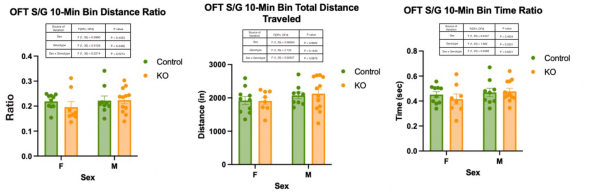

Figure 5. Open Field test activity by sex and genotype.
Comparison of OFT activity between control and PV-Traf3 knockout mice, separated by sex. The graphs show the same measures as mentioned above, for female (F) and male (M) mice. Individual points represent individual mice, bars indicate group means, and error bars represent variability. The statistical analyses shown above each graph assess the effects of sex, genotype, and the sex x genotype interaction with the corresponding F- and p-values displayed.

Elevated Plus Maze

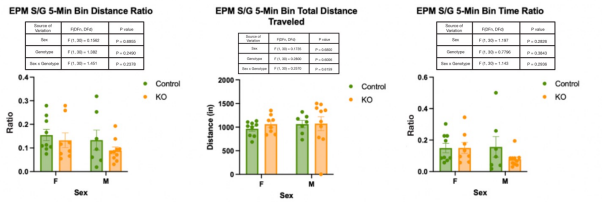

Figure 6. Elevated Plus Maze test activity by sex and genotype.
Comparison of EPM activity between control and PV-Traf3 knockout mice, separated by sex. The graphs show the 5-minute bin distance ratio (left), total distance traveled (middle), and 5-minute bin time ratio (right) for female (F) and male (M) mice. Individual points represent individual mice, bars indicate group means, and error bars represent variability. Statistical analysis was used to assess the effects of sex, genotype, and the sex × genotype interaction, with corresponding F-values and p-values shown above each graph.

Cued Fear Conditioning

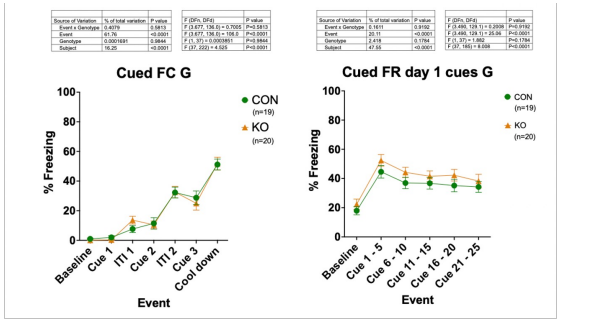

Figure 7. Fear conditioning and retrieval in control and knockout mice.
The same fear conditioning and extinction paradigm as demonstrated before was used for this study. Graphs show percentage freezing in control (CON, green) and PV-Traf3 KO (KO, orange) mice during fear conditioning (Cued FC G, left) and the first day of cued fear retrieval (Cued FR day 1 cues G, right). Data are presented as mean and their error bars. Statistical analyses examined the effects of event, genotype, and event × genotype interaction, with F-values and corresponding p-values shown above each graph.

DISCUSSION

1. In both the OFT and EPM, PV-Traf3 KO animals exhibited no significant differences (p-value > 0.05) compared to controls across total distance traveled, center time ratios, or open arm exploration. These findings indicate that Traf3 expression strictly within PV INs is not required for maintaining baseline anxiety levels.
2. While vGat-Traf3 KO mice showed elevated freezing during both conditioning acquisition and initial recall, the conditional PV-Traf3 KO did not follow this. Statistical tests confirmed that there were no statistically significant genotype or genotype-by-event interaction effects on either fear acquisition or extinction (p-values > 0.05)
3. These behavioral differences may be driven by distinct windows of gene deletion. While vGat expression begins early during embryonic interneuron development [6], PV expression occurs later in postnatal development (P13 - P14) [7]. Thus, it is possible that Traf3 plays a fundamental role in early neurodevelopmental processes, which would occur before PV gene expression switched on. Because Traf3 expression remains intact throughout embryonic and early postnatal development in the PV-Traf3 KO mice, critical circuit maturation would proceed normally during this window.
4. It could be that Traf3 disruption within another IN subtype drives these behavioral phenotypes, or that a concurrent disruption or loss across multiple IN populations is required to elicit phenotypes.

FUTURE DIRECTIONS

1. Confirm Traf3 was properly knocked out of PV INs in mice that are 12 weeks old.
   - If not, performing the behavioral battery in older mice may be suitable.
2. Explore effects of Traf3 disruption in other subsets of INs, such as VIP or SST INs.

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