Long Non-coding RNA *MIAT* Regulates Inflammation-induced Synaptic Deficits in Human iPSC-derived Dopaminergic Neurons

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Introduction

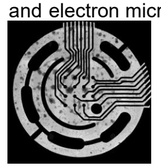
- 2/3 of major depressive disorder patients are female, suggesting sex gap in etiology [1].
- Inflammation contributes to depression and reduces dopamine (DA) concentration in the brain [1].
- Interleukin (IL)-6 induces DA neuron deficits primarily in females.
- LncRNA *MIAT* contributes to sex-specific responses to inflammation in DA neurons, while underlying mechanisms remain unclear.

Methods

Model: male human induced pluripotent stem cell (hiPSC)-derived DA Neurons [2].

Approaches:

- Inflammatory stimulus: 5 ng/mL IL-6 for 24 hrs, with vehicle (PBS) control
- Manipulate *MIAT*: knock out promotor region of *MIAT* by CRISPR editing [3]
- Evaluate DA function: ELISA, multielectrode assay (MEA), and electron microscopy (TEM)



16 electrodes in 1 well of cell culture plates for MEA

Key Findings

- Male DA neurons had higher expression of *MIAT* at baseline (Fig. 1A)
- IL-6 reduced DA concentrations (Fig. 2), neural activities (Fig. 3), synaptic vesicle docking and density (Fig. 4) in MIAT KO neurons, while these functions were unaltered in parental DA neurons.

Acknowledgements

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Results

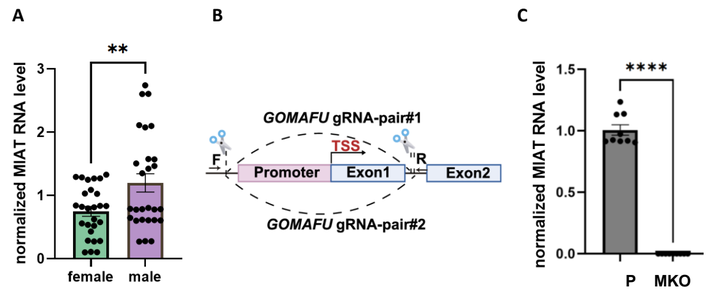

Fig. 1 Knocking out *MIAT* in male DA neurons. A) RT-qPCR results showing higher baseline MIAT expression in healthy control male DA neurons. **B)** Diagram illustrating target sites of two gRNA pairs for deletion of the MIAT promoter region [3]. C) MIAT KO efficiency verified by RT-qPCR. P: parental, MKO: MIAT KO. Unpaired t test (2-tail): ** p < 0.01, **** p < 0.0001.

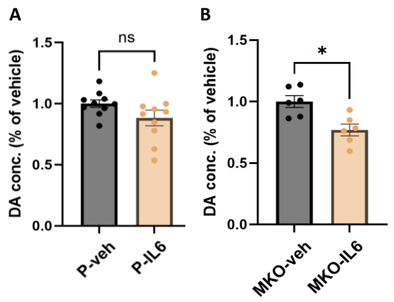

Fig. 2 DA concentration in cell culture medium detected by ELISA. A) DA concentrations in IL-6-treated and vehicle control parental DA neurons (male). **B)** DA concentrations in IL-6-treated and vehicle control isogenic MIAT KO DA neurons (male). P: parental, MKO: MIAT KO. Unpaired t test (2-tail): * p < 0.05, ns p >= 0.05.

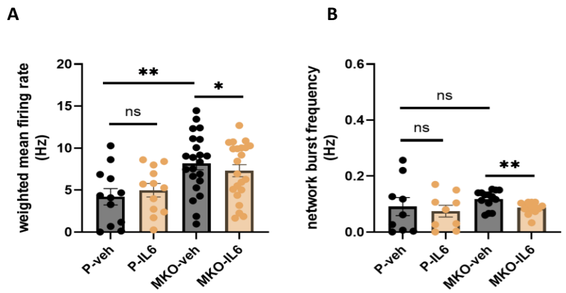

Fig. 3 Neural activity recorded by multi-electrode array (MEA). A-B) weighted mean firing rate (A) and network burst frequency of parental and MIAT KO DA neurons (male) before and after IL-6 treatment. P: parental, MKO: MIAT KO. Paired t test (2-tail) for IL-6 vs veh comparisons, Unpaired t test (2-tail) for other comparisons: * p < 0.05, ** p < 0.01, ns p >= 0.05.

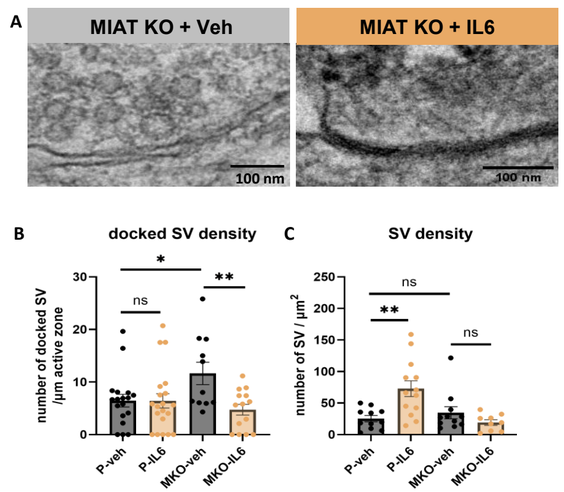

Fig. 4 Transmission electron microscopy (TEM) of synaptic vesicles. A) TEM images of synaptic terminal in IL-6-treated and vehicle control MIAT KO DA neurons. Ultrathin sections (60–70 nm) were cut using a Leica EM UC6 ultramicrotome and imaged in a Jeol JEM 1400 TEM operated at an accelerating voltage of 80KV. **B-C)** quantification of docked synaptic vesicle (B) and synaptic vesicle at terminals in parental and MIAT KO DA neurons. P: parental, MKO: MIAT KO. Unpaired t test (2-tail): * p < 0.05, ** p < 0.01, ns p >= 0.05.

Conclusions

Male DA neurons had higher expression of MIAT at baseline, compared to female DA neurons. Therefore, we knocked out MIAT in male DA neurons to investigate the role of MIAT in mediating responses to IL-6. Interestingly, IL-6 reduced DA release, neural activity, synaptic vesicle docking and density in MIAT KO neurons, while these functions were unaltered in parental (healthy control) male neurons.

Furthermore, these DA deficits observed in male MIAT KO neurons were similar to those in healthy control female DA neurons as suggested by our previous findings. To our knowledge, we provided the first evidence that lncRNA *MIAT* mediates differential responses to inflammation in female and male DA neurons via regulating synaptic functions. This role might be connected to MIAT's inhibitory effects on immune signaling pathways [2] and underlying molecular machinery will be an interesting future direction.

- This work is published in *Neuropsychopharmacology* (PMID: 41535632)
- This study was conducted with power analysis, blinding, and randomization.
- Shown data are mean ± SEM. *P* value < 0.05 was considered statistically significant.
- COI: AHM is a paid consultant for Freedom Biosciences, Cerevel Therapeutics, and Sirtsei Pharmaceuticals, Inc.

References

[1] Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016 Jan;16(1):22-34. doi: 10.1038/nri.2015.5. PMID: 26711676. **[2]** Nolbrant S, Heuer A, Parmar M, Kirkeby A. Generation of high-purity human ventral midbrain dopaminergic progenitors for in vitro maturation and intracerebral transplantation. Nat Protoc. 2017 Sep;12(9):1962-1979. doi: 10.1038/nprot.2017.078. Epub 2017 Aug 31. PMID: 28858290. **[3]** Teng P, Li Y, Ku L, Wang F, Goldsmith DR, Wen Z, Yao B, Feng Y. The human lncRNA GOMAFU suppresses neuronal interferon response pathways affected in neuropsychiatric diseases. Brain Behav Immun. 2023 Aug;112:175-187. doi: 10.1016/j.bbi.2023.06.009. Epub 2023 Jun 8. PMID: 37301236.