Striatal dysfunction in *SHANK3* overexpressing mice

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BACKGROUND

Genomic duplications involving *SHANK3* have been implicated in a variety of neuropsychiatric disorders, including Asperger's, ADHD, bipolar disorder, and seizure disorders. SHANK3 is a scaffolding protein in the post-synaptic density that connects membrane proteins to the cytoskeleton. SHANK3 is most abundantly expressed in the striatum which is known to be a critical center for control of motor activity. We previously discovered that mice overexpressing SHANK3 have a heightened sensitivity to amphetamine, a lower immobility in tail suspension, and spontaneous seizures, all implying behaviors consistent with the symptoms in patients with *SHANK3* Duplication Syndrome.

PURPOSE

The purpose of this work is to understand the role of the striatum in abnormal behaviors of *SHANK3* Duplication Syndrome and test the effectiveness of D2 dopamine receptor antagonist drugs, BRD4018 and BRD5814, on rescuing behaviors in *Shank3* TG mice.

METHODS

- We treated *Shank3* TG mice with D2 receptor antagonists that were developed as potential anti-psychotics. Open field activity, acoustic startle, prepulse inhibition, and tail suspension tests were conducted to evaluate for behavioral abnormalities.
- We normalized Shank3 abundance in D2 dopamine receptor neurons and performed behavioral analysis.
- Neurophysiological recordings were taken in striatal neurons.
- Golgi staining of *Shank3* TG mouse brains was performed to assess changes in dendritic spine amounts.

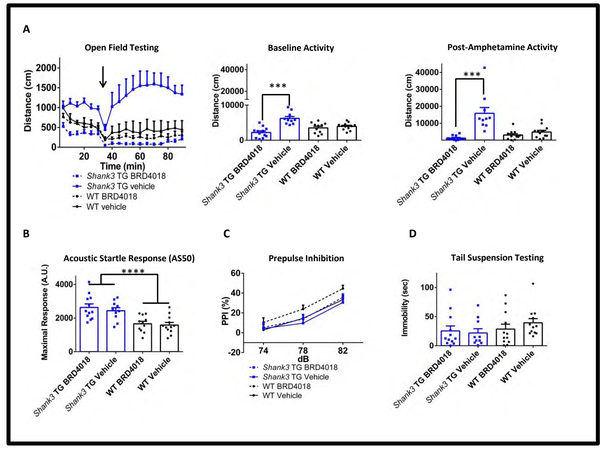

Fig. 1| D2 antagonist rescues hyperactivity in *Shank3* TG mice. Shank3 TG mice were treated with BRD4018, a highly selective D2 antagonist, and assessed for behavioral rescue. **a,** Baseline hyperactivity and accentuated AIH were rescued while all others (**b-d)** were not. All behaviors analyzed by two-way ANOVA with Tukey post-hoc analysis. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

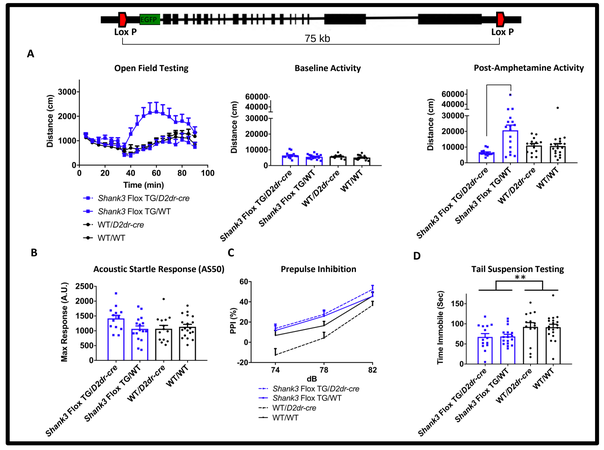

Fig. 2| Normalization of Shank3 in D2 neurons rescues accentuated AIH. Mice carrying a floxed Shank3 expression transgene were created and mated with D2-Cre mice. **a,** Baseline hyperactivity and accentuated AIH were rescued while all others **(b-d)** were not. All behaviors analyzed by two-way ANOVA with Tukey post-hoc analysis. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

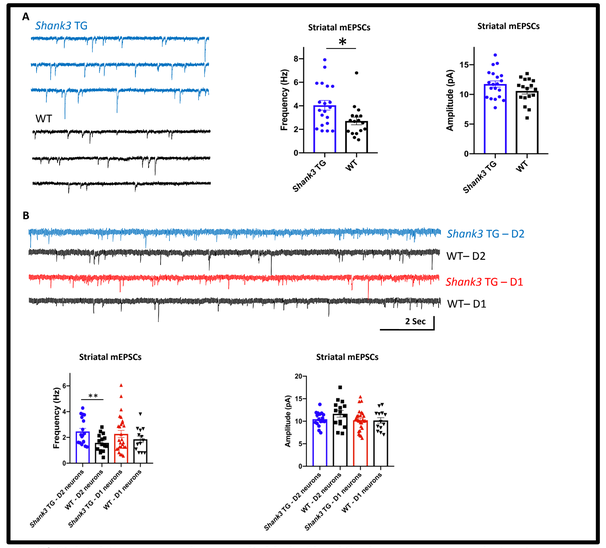

Fig. 3| Striatal Neurons are hyperexcitable in *Shank3* mice. a, Frequency, but not amplitude, of mEPSCs was increased in striatal neurons of transgenic mice. **b**, Frequency of mEPSCs was increased in striatal D2 neurons in transgenic mice. There was no significant difference in D1 neurons. Analyzed using two-way ANOVA with Tukey post-hoc analysis. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

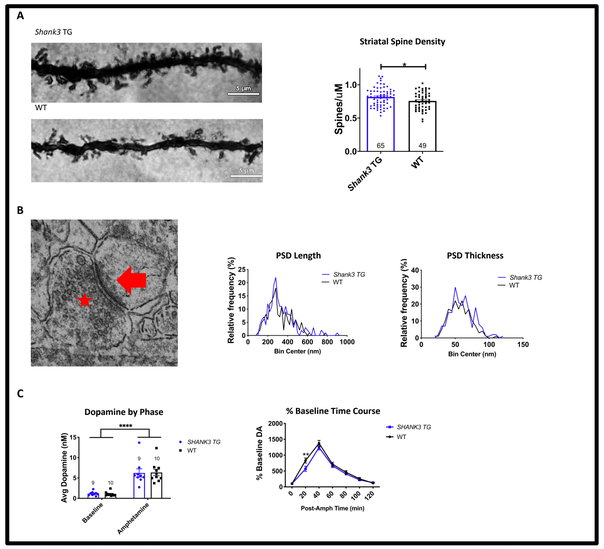

Fig. 4|*Shank3* mutant mice have changes in spine morphology. a, Shank3 TG mice had increased dendritic spine amounts in striatal neurons. **b,** Shank3 TG mice have normal synaptic structure by electron microscopy. Star indicates presynaptic compartment, arrow indicates PSD. **c,** In vivo synaptic dopamine content as measured by striatal dialysis**.** Analyzed using two-way ANOVA with Tukey post-hoc analysis. *p<0.05, ** p<0.01, *** p<0.001

RESULTS

- BRD4018 and BRD5814 (not shown) reversed baseline hyperactivity and amphetamine-induced hyperactivity without altering other behavioral abnormalities.
- Normalizing Shank3 in D2 dopamine receptor neurons reversed exaggerated amphetamine induced hyperactivity due to Shank3 overexpression.
- Shank3 TG mice have increased frequency of mEPSCs in striatal D2 neurons, with no change in amplitude. This suggests an increase in the overall functional synapse number.
- Golgi staining revealed an increase in density of dendritic spines in Shank3 overexpressing mice.
- Electron microscopy revealed no change in synaptic architecture in the striatum of Shank3 overexpressing mice.

CONCLUSION

Shank3 overexpression changes the morphology of dendrites of medium spiny neurons. Dysfunction of D2dr-expressing neurons in Shank3 TG mice contributes to the hyperactivity observed in the mice. D2 dopamine receptor antagonists reduce hyperactivity but are not effective in rescuing other symptoms associated with the duplication of *SHANK3*. Together, these data demonstrate a specific pharmacogenetic property of a subset of aberrant behaviors associated with Shank3 overexpression.

REFERENCES

1. Han K, Holder JL, Schaaf C, Lu H, Chen H, Kang H, Tang J, Wu Z, Hao S, Cheung SW, Yu P, Sun H, Breman A, Patel A, Lu HC, Zoghbi H (2013) SHANK3 overexpression causes manic-like behavior with unique pharmacogenetic properties. Nature.
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