Targeting Presynaptic Kappa Opioid Receptors to Normalize Molecular, Physiological and Behavioral Phenotypes in Mice Engineered to Express the ADHD and Autism-Associated Dopamine Transporter Coding Variant Val559

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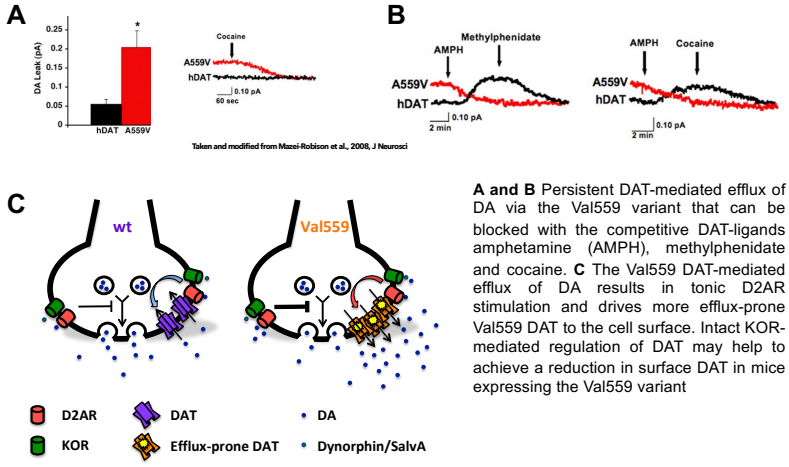
Summary

The presynaptic dopamine (DA) transporter (DAT) is a key regulator of dopaminergic signaling, dictating clearance of DA following release and providing for DA recycling in support of vesicular DA release[1]. The DAT Val559 coding variant was identified in subjects with attention-deficit hyperactivity disorder (ADHD), autism and bipolar disorder. This variant exhibits anomalous DAT-mediated DA efflux (ADE)[2], leading to increased extracellular DA levels that support tonic activation of pre-synaptic D2 receptors[3], driving efflux prone Val559 to the cell surface in the dorsal striatum[4]. Kappa opioid receptors on DA terminals also regulate DAT[5], providing a path of convergence of opioid and DA signaling, and raising the question as to whether the DAT Val559 variant impacts DAT regulation beyond that supported by synaptic DA autoreceptors. Using biotinylation approaches, we show that the regulation of DAT by kappa opioid receptors (KORs) remains intact in Val559 transgenic mice. Current efforts are focused on evaluation of the possibility that pharmacological manipulation of KORs may represent an attractive target to reduce the synaptic abundance of efflux-prone DAT Val559, thereby limiting the functional impact of ADE-induced hyperdopaminergia.

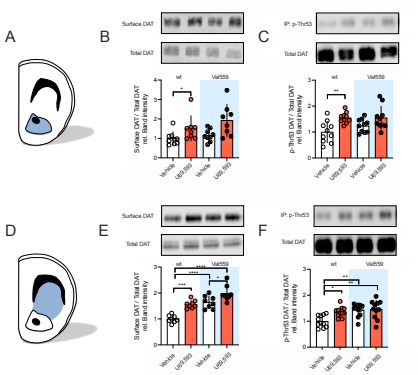
Background



Taken and modified from Mazei-Robison et al., 2008, J Neurosci

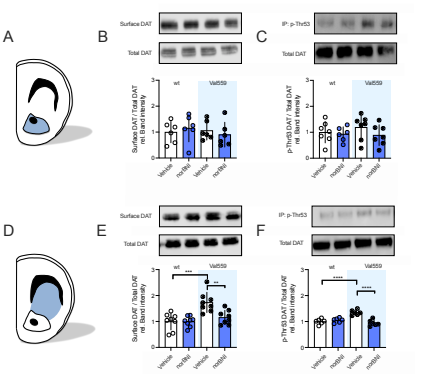
A and B Persistent DAT-mediated efflux of DA via the Val559 variant that can be blocked with the competitive DAT-ligands amphetamine (AMPH), methylphenidate and cocaine. **C** The Val559 DAT-mediated efflux of DA results in tonic D2AR stimulation and drives more efflux-prone Val559 DAT to the cell surface. Intact KOR-mediated regulation of DAT may help to achieve a reduction in surface DAT in mice expressing the Val559 variant

The Val559 DAT Variant Remains Amenable to KOR-mediated Regulation



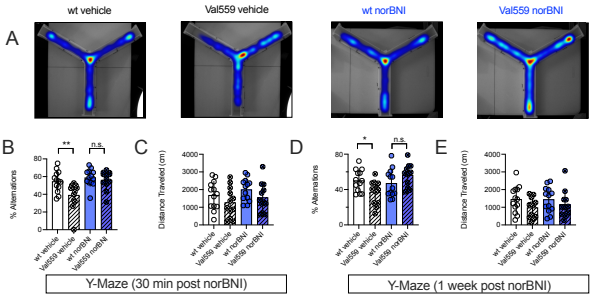
DAT-surface expression and phosphorylation at Threonine 53 (p-Thr53 DAT) was assessed in acute brain slices of wild-type (wt) and Val559 DAT knock-in mice. Treatment with the KOR-agonist U69,593 promotes increased significantly affected surface trafficking and phosphorylation in the ventral (**A-C**) and dorsal striatum (**D-F**). Two-way ANOVA revealed significant drug effects, regardless of brain region and genotype. *=P<0.05; **=P<0.01; ***=P<0.001; ****=P<0.0001; Šídák's multiple comparisons test

The KOR Antagonist norBNI Normalizes Val559 DAT Surface Trafficking and Phosphorylation



DAT-surface expression and phosphorylation at Threonine 53 (p-Thr53 DAT) was assessed in acute brain slices of wild-type (wt) and Val559 DAT knock-in mice. Treatment with the KOR-antagonist nor-Binaltorphimine (norBNI) normalizes the elevated surface trafficking and phosphorylation of Val559 DAT in the dorsal striatum without affecting wt DAT (**D-F**). No effect was observed in the ventral striatum (**A-C**).Two-way ANOVA revealed significant drug effects, regardless of brain region and genotype. **=P<0.01; ***=P<0.001; ****=P<0.0001; Šídák's multiple comparisons test

KOR antagonism normalizes the aberrant behavior of Val559 DAT knockin-in mice



Treatment with the KOR-antagonist nor-Binaltorphimine (norBNI) (10 mg/kg, i.p.) normalized the alternation deficit of male Val559 DAT knock-in mice in the Y-maze spontaneous alternation test, a measure of spatial working memory. (**A**) representative heat-maps of wild type (wt) and Val599 DAT knock-in mice, treated with either vehicle or norBNI. (**B**) acute treatment with norBNI restored the behavior of Val559 DAT knock-in mice to levels comparable to wt mice without affecting locomotor activity (**C**). In line with the long-lasting effects reported for norBNI, Val559 DAT knock-in mice revealed normal alternation patterns and locomotor activity 1 week after the administration of norBNI (**D-E**).

Summary and Conclusion

Both wild-type and Val559 DAT are amenable to regulation via KOR

Val559 DAT displays enhanced surface trafficking and phosphorylation at threonine 53 in the dorsal striatum of male mice

Treatment with the KOR-antagonist norBNI normalizes both the surface trafficking and phosphorylation of Val559 DAT in the dorsal striatum without affecting wild-type DAT

Systemic administration of the KOR-antagonist norBNI normalizes the behavior of Val559 DAT knock-in mice. Importantly, systemic norBNI remained without effect in wild-type mice

Our pre-clinical data emphasize the utility of KOR-antagonists as an alternative, non-addictive treatment strategy for disorders that arise from imbalances in dopaminergic signalling

References and Acknowledgements

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