Genetic and Epigenetic Target Validation for the Serotonin 2A Receptor for Treating Stimulant Abuse

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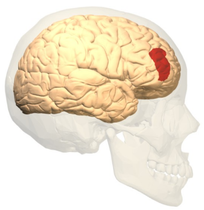
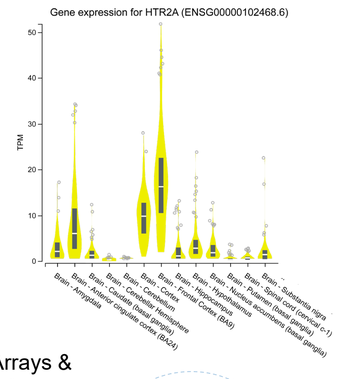
Background & Significance

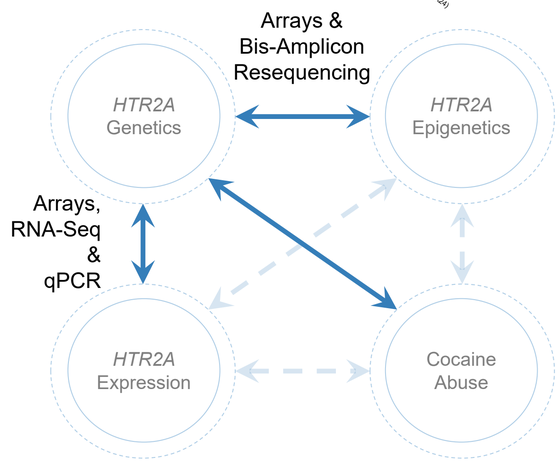
- Accidental cocaine overdose deaths up since 2010, partially due to combined drug toxicity with opioids
- No FDA-approved treatments for cocaine use disorder: identifying and validating new drug targets for cocaine abuse is a significant unmet medical need
- New drug targets have higher probability of success if they have:
  1. High druggability
  2. Preclinical evidence for efficacy
  3. Human genetic associations with disorder
- Serotonin 2A Receptor (encoded by *HTR2A*) has these properties

Samples & Methods



Postmortem human brain tissue from cocaine overdose subjects and matched controls dissected from dorsolateral prefrontal cortex (DLPFC; BA46) are used for the studies described below.

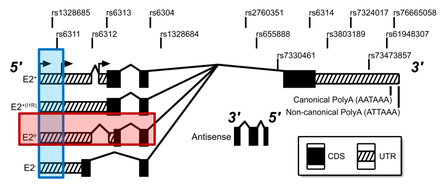




Measured total and allelic RNA expression & methylation

Results

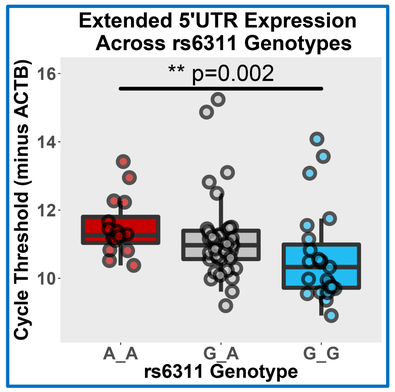
Genetic Variation and Cocaine Use is Associated with Changes in *HTR2A* Isoform Expression

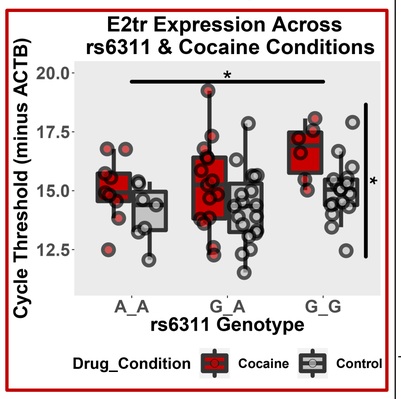


Top: HTR2A gene diagram shows multiple promoters and alternative splicing in the first two exons. Note: Blue and Red boxes correspond to data in Middle and Right Panels.

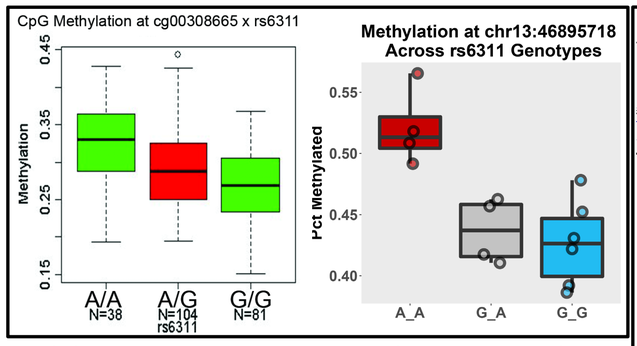
Middle: Expression of the extended 5'UTR is modulated by a common genetic polymorphism (rs6311) associated with addiction risk and impulsivity. From Smith et al. (2013).

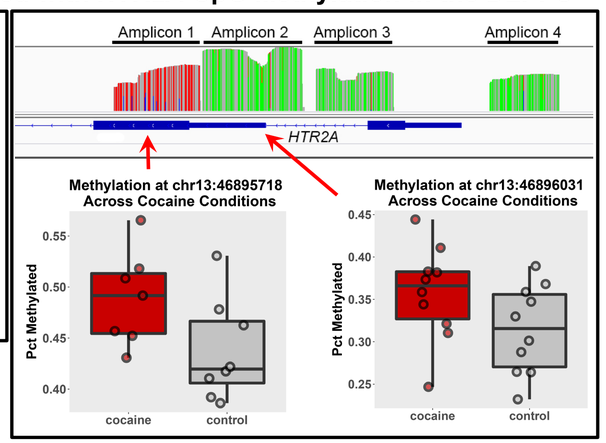
Right: Expression of an alternative spliceoform containing a truncated exon 2 (E2tr) is modulated by both genetics risk variants and cocaine use.





Genetic Variation and Cocaine Use is Associated with *HTR2A* CpG Methylation in the DLPFC

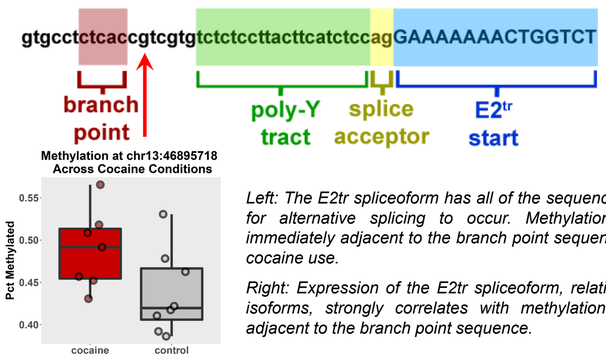




Top: CpG methylation at multiple sites in exon 2 (left – array, right – bisulfite-seq) is modulated by the common addiction risk variant rs6311.

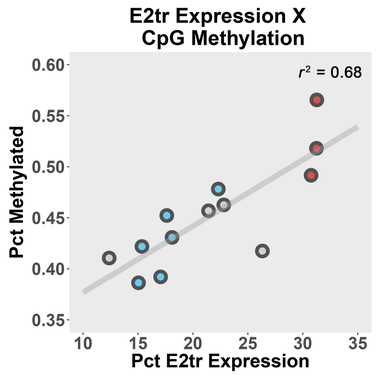
Right: Cocaine use is associated with increased CpG methylation at sites in and around exon 2, as measured by bisulfite amplicon resequencing.

CpG Methylation Correlates with Alternative Splicing of Exon 2 *HTR2A* mRNA

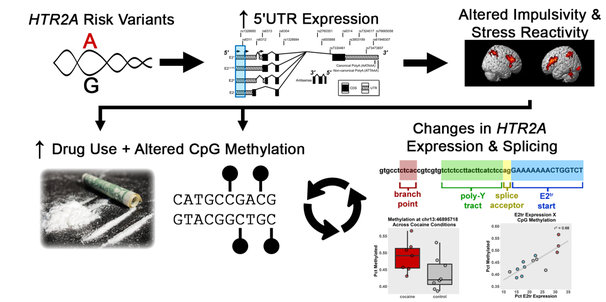


Left: The E2tr spliceoform has all of the sequence motifs necessary for alternative splicing to occur. Methylation at a CpG site immediately adjacent to the branch point sequence is modulated by cocaine use.

Right: Expression of the E2tr spliceoform, relative to other exon 2 isoforms, strongly correlates with methylation at the CpG site adjacent to the branch point sequence.



Model of *HTR2A* Regulation



Conclusions

- Genetic risk variants for substance use disorders are correlated with changes in *HTR2A* gene expression and CpG methylation
- Cocaine use is associated with changes in *HTR2A* gene expression and CpG methylation
- Changes in CpG methylation are correlated with expression of alternative *HTR2A* spliceoforms

Future Studies

- Determine the signaling potential of E2tr isoform and role in 5-HT signaling
- Examine cellular and subcellular expression of spliceoforms
- Look for corresponding expression and methylation changes in preclinical models & peripheral tissues
- Test pervasiveness of CpG methylation changes in other SUDs and childhood trauma

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