Dissecting the molecular mechanisms by which Lim-Only 4 regulates alcohol consumption and reward

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Background

- Previous work has indicated transcription factor Lim-Only 4 (LMO4) in the basolateral amygdala (BLA) plays a role in alcohol consumption and award
- The Kappa Opioid Receptor (KOR) has previously been shown to regulate excessive alcohol use.
- Preliminary studies show LMO4 knockdown results in 50% reduced KOR expression.
- KOR has been identified as a transcriptional target of LMO4 in the BLA, with expression of both colocalized in 50% of BLA neurons.
- In order to investigate the relation between LMO4 and KOR in the BLA further, LMO4 knockdown will be restricted to only KOR expressing cells using Cre-dependent small hairpin RNA's (shRNAs) against LMO4.
- This project will focus on designing and validating Cre-dependent shRNA's against LMO4.

Knockdown of Lmo4 in the BLA reduces alcohol consumption

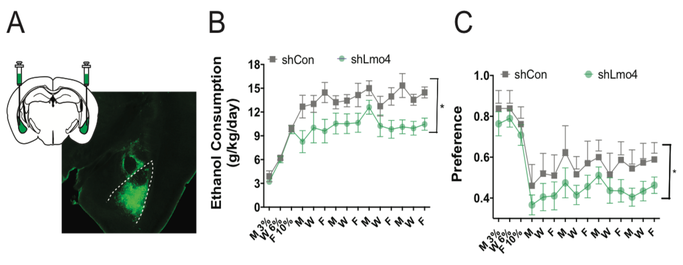

Figure 1. shRNA-mediated knockdown of LMO4 in the BLA reduces alcohol consumption and (**B**) and preference(**C**). Representative image of lentiviral infection in the BLA is shown (**A**). *, p<0.05 , n =13-15/group

Kappa opioid receptor expression is significantly reduced in the BLA of Lmo4-deficient mice

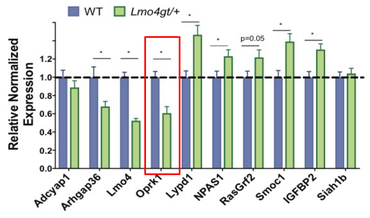

Figure 2. Whole genome sequencing of the BLA of WT and Lmo4-deficient mice revealed that *Oprk1* is a transcriptional target of Lmo4 . QPCR analysis of Oprk1 expression from WT and Lmo4gt/+ BLA. *. P>0.05, n =9-11/group

Lmo4 and *Oprk1* colocalization in the BLA

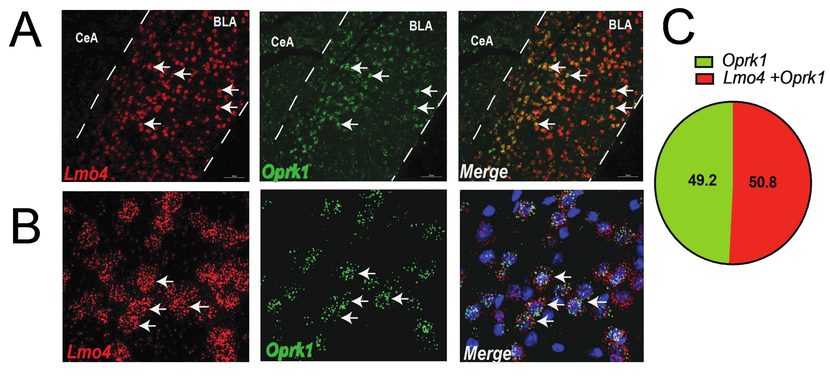

Figure 3. A. olocalization of *Oprk1* and *Lmo4* mRNA in the BLA. We used dual fluorescence RNAScope to ascertain colocalization of *Oprk1* and *Lmo4* expression in the BLA. Scale bar 200 µm. **B** Higher magnification image of colocalization is shown. Scale bar 50 µm. **C.** Colocalization was quantified using Image J. ~50% of *Lmo4* positive cells overlap with *Oprk1*. Arrows point to expression overlap.

LMO4 binds to *Oprk1* promoter elements in the BLA

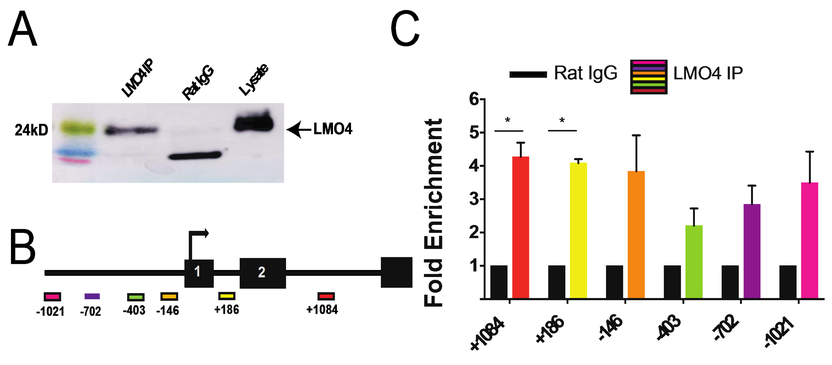

Figure 4: Chromatin immunoprecipitation experiments reveal that LMO4 binds to *Oprk1* promoter elements. A) The LMO4 antibody but not a nonspecific rat IgG was able to immunoprecipitate LMO4 from formaldehyde crosslinked forebrain lysates. **B)** Schematic of *Oprk1* promoter region with binding sites for QPCR primers. **C)** Significant enrichment of some of KOR promoter elements with LMO4 antibody compared to Rat IgG control.

Validation of Cre-dependent shRNAs against *Lmo4*

shRNA Sequences tested

1) L1 - ATGACTGAATATGAACATTAAG
2) L2 - TACTTCTAATTTGCTGAATGAA
3) L3 - TTGTGATAATTCTATCACAAAC

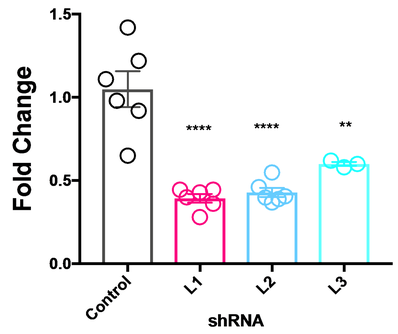

Figure 4: shRNA'S designed against 3'UTR of LMO4 cause robust knockdown of LMO4 in HEK 293 Cells. We transfected HEK293FT cells with shRNA's against LMO4 or a control shRNA and LMO4 cDNA containing the 3'UTR. We measured Lmo4 mRNA levels by QPCR 72h post transfection. All shRNA's produced robust and significant knockdown of LMO4. L1 was the best candidate and knocked down Lmo4by approximately 30%. ***, p<0.001, n =3/shRNA

Conclusions

- QPCR indicates the validity of all three shRNA's against LMO4.
- Results indicate L1 and L2 shRNA's are more effective in inhibiting LMO4 transcription than L3
- We have subcloned this shRNA in to an pAAV-mir30-FLEX-shRNA backbone that result sin cre-dependent expression.
- We are currently packaging this virus. These viruses will be injected into the BLA of Kappa-opioid Receptor-Cre mouse

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