Verification of AMPK Knockdown in Sprague Dawley Rats using a Vivo-Morpholino Antisense Oligonucleotide

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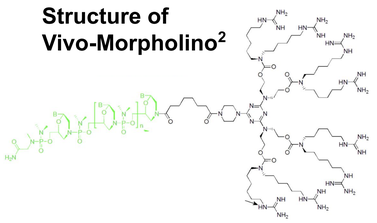
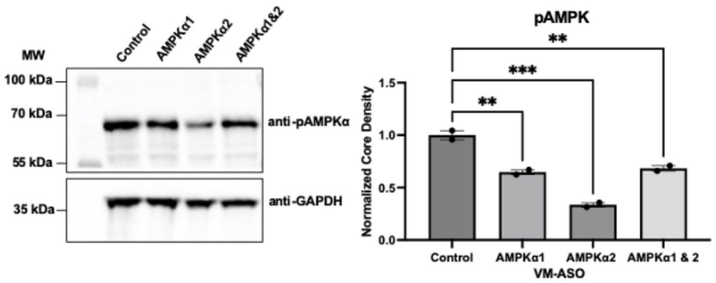
Introduction

- Cocaine is a highly addictive drug associated with nearly one in five overdoses. Relapse is common in those addicted to cocaine[3].
- There is no FDA-approved treatment for decreasing risk of relapse.
- Cocaine acts via the brains mesolimbic dopamine reward system, which includes the nucleus accumbens core (NAcC)[5].
- Cocaine causes the build up of dopamine in the synapse, and decreases phosphorylation levels of adenosine monophosphate-activated protein kinase (AMPK)[5].
- Metformin, a drug used for type II diabetes, has been shown to reduce cue-induced cocaine seeking given direct microinjections into the NAcC[1]. The proposed method of action for this effect is rescuing AMPK activity.
- A vivo-morpholino antisense oligonucleotide (VM-ASO) can be engineered to target specific localized proteins, preventing translation[6].
- A collaboration with Dr. Slosky and PhD student Maddi Moore has previously confirmed knockdown of AMPKα in HEK293 cells via VM-ASO.

Structure of Vivo-Morpholino[2]





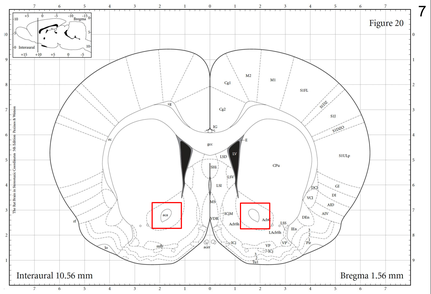
- In order to further validate the effect of metformin on cocaine use disorder (CUD) we need to validate the use of VM-ASO in knocking down AMPK in the NAcC.

Predictions

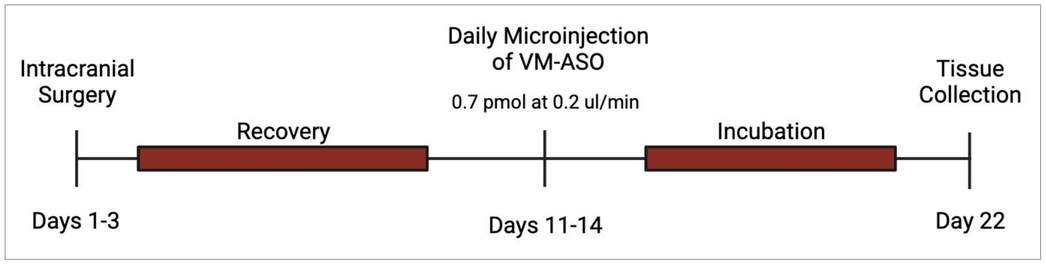
- We expect to see a decrease in pAMPK in the NacC in all rats treated with the VM-ASO compared to a scrambled control-treated group.

Methods

- This study was conducted using 8 (6 male, 2 female) Sprague Dawley rats with a start age of 10-12 weeks.
- Rats underwent stereotaxic intracranial surgery for the insertion of a cannula into the NAcC in each hemisphere (from bregma: AP +1.5; ML: +/- 1.8; DV: - 5.5mm).
- Following a week of recovery, 140 pmol of VM-ASO or scrambled control was delivered via microinjections into both hemispheres at a rate of 0.2 µl/minute for 4 days[2].
- Eight days after the last microinjection tissue samples were collected by way of decapitation and dissection of NAcC[4].
- Tissue then underwent whole cell lysate crude fractionation, determine levels of pAMPK present in the NAcC.



Experimental Timeline



Results

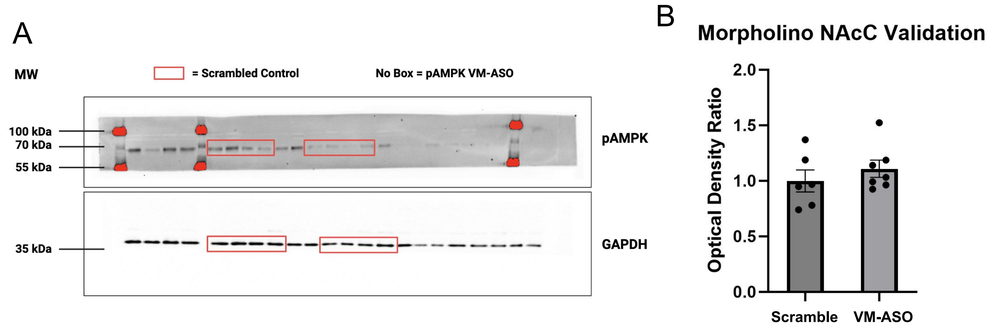


Figure 1: Summary data showing relative levels of pAMPK in NAcC as well as validation of western blot bands. **A)** A single western blot including; control (GAPDH), and experimental (pAMPK), with exposure times of <1 second for GAPDH and two minutes for pAMPK. Bands represent from left to right each rats right and left brain hemispheres. **B)** Representation of pAMPK in the NAcC given treatment of a scrambled control VM-ASO and a VM-ASO engineered to target AMPKα2 (p=0.3960).

Discussion

- Contrary to our expectations, bilateral microinjections of a VM-ASO in the NAcC did not significantly decrease the amount of pAMPK.
- These results suggest that the VM-ASO is not resulting in an AMPK knockdown. This may be due to exclusion of data.
- Eight of twenty-two samples were omitted from analysis due to either blockage of cannula during time of microinjections or no visualization from the Western blot.
- Cannula blockage was determined by the inability to completely push the microinjector through the cannula during microinjection days. This was then confirmed via the lack of ink staining on the day of tissue collection.

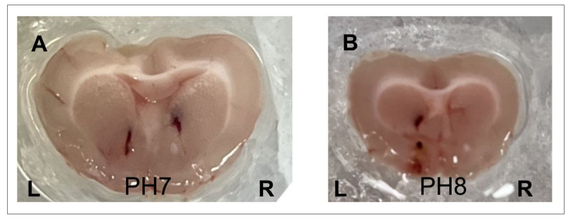


Figure 2: Brain slices taken from PH7 and PH8. **A)** PH7 represents a successful ink microinjection. Staining is observed on both the left and right NAcC, which was used to extract the specific tissue where the microinjection was performed. **B)** PHR8 was blocked during microinjections and later confirmed during tissue extraction. This confirmation is visualized by lack of ink diffusion into the expected brain region (PHR8). Successful microinjections on the Left was confirmed via ink stain.

- Failure to knockdown pAMPK via the VM-ASO could be attributed to biodistribution differences given rat tissue and HEK293, insufficient dosage, or too short incubation time.
- Throughout the experimental timeline, complications associated with microinjections and intracranial surgery may have decreased the VM-ASOs efficacy.

Future Directions

- Current results are yielded from one blot. Therefore, replications are necessary to confirm our findings.
- Repeat experimental timeline with various changes such as a larger VM-ASO dose or increased incubation time in order to determine if our methodology is the cause of failure to knockdown.
- Given confirmation of failure to knockdown AMPK with VM-ASO, pursue RNAi as a method of AMPK knock down and repeat steps to determine success or failure.
- Relate successful AMPK knockdown to the impact of metformin on the reduction of cue-induced cocaine seeking in rats in order to confirm AMPK as the target molecular mode of action.

References

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