The Role of Nucleus Accumbens Beta-catenin Expression In Alcohol Consumption

Roberto A. Aponte-Rivera[1], Efrain A. Ribeiro[2], Ezekiel Mouzon[2], Eric J. Nestler[2] & Cristina Velázquez-Marrero[3]

1. University of Puerto Rico Rio Piedras Campus, San Juan, PR
2. Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY
3. Institute of Neurobiology, University of Puerto Rico Medical Sciences Campus, San Juan, PR

Introduction:

The nucleus accumbens is a region of the basal forebrain that plays a significant role in the cognitive processing of reward and aversion. It receives dopaminergic inputs from the ventral tegmental area (VTA), which release dopamine in response to reward related stimuli. It also receives glutamatergic inputs from the basolateral amygdala, the hippocampus, and the medial prefrontal cortex, which control reward-related aspects of perception and memory (Russo & Nestler, 2013). This region is studied as a physiological substrate of addiction and depression, because it is highly involved in the processing of reward.

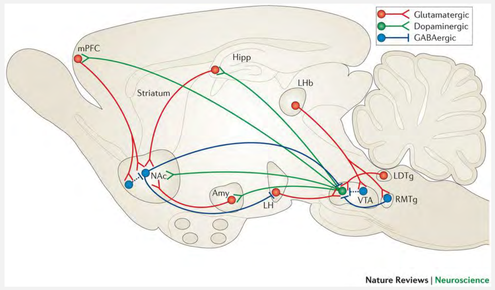


Figure 1. The VTA-NAc circuit: Russo S.J. & Nestler E.J. (2013)

β-catenin is a protein involved in cell to cell adhesion and gene transcription via the Wnt/β-catenin signaling pathway. Dysregulation of β-catenin expression and function within the NAc has been implicated in psychiatric diseases such as depression, anxiety, and drug addiction (Dias et al., 2014; Wilkinson et al., 2011). Recent work at our lab has shown that the Wnt/β-catenin signaling pathway is necessary for the internalization of the Big Potassium channel after ethanol exposure (Velázquez-Marrero et al., 2016). This prolonged ethanol exposure is necessary to produce persistent molecular tolerance (PMT) at the cellular level (Velázquez-Marrero et al., 2011). Furthermore, decrease in BK channel surface expression will cause an increase in intrinsic excitability within the striatum. Therefore, we wanted to explore whether β-catenin played a significant role in regulating alcohol consumption behaviors within the nucleus accumbens.

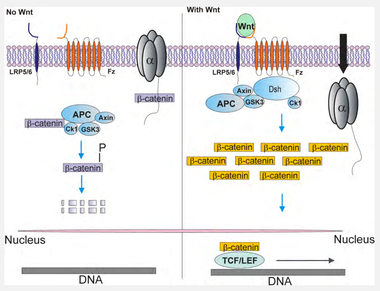


Figure 2. The Wnt/β-catenin pathway in the context of BK internalization: Velázquez-Marrero et al. (2016)

Methods :

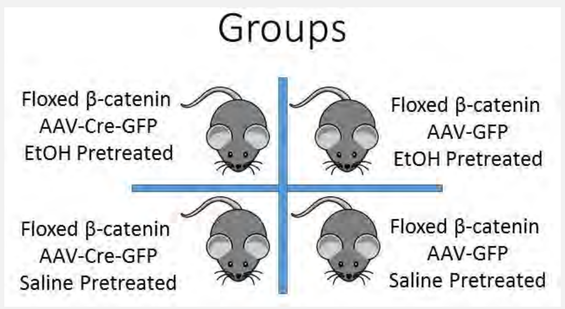


Figure 3. Mice were separated in groups according to viral mediated gene transfer and pretreatment. Adeno-associated virus (AAV) was used to mediate infection of cells in the NAc. (Dias et al., 2014)

Methods :

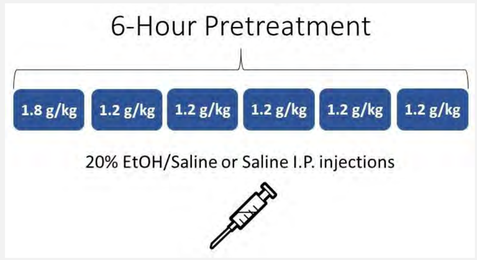


Figure 4. EtOH or Saline Pretreatment: Pretreatment was achieved via hourly i.p. injections. This protocol has been show to produce persistent molecular tolerance (PMT) at the cellular level. (Velázquez-Marrero et al., 2011)

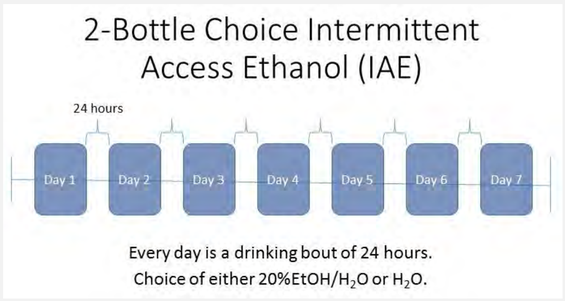
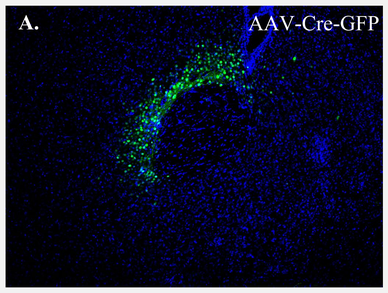


Figure 5. Intermittent Access Ethanol paradigm: IAE consists of 24 hour drinking bouts interspaced by 24 hours of ethanol deprivation. This achieves escalation in ethanol consumption. (Hwa et al., 2011; Melendez RI, 2011)

Results:



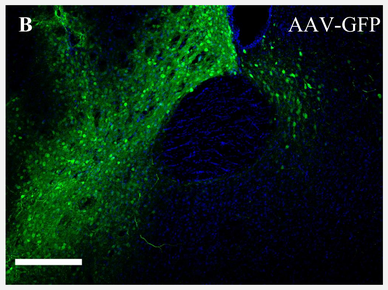


Figure 6. Viral-mediated gene knockout in NAC validated via immunohistochemistry

3 weeks after NAc stereotaxic surgery, brain slices containing NAc were prepared from perfused mice using a Vibratome. Both virus utilized (AAV-Cre-GFP & AAV-GFP) express Green Fluorescent Protein (GFP) upon successful infection. **A.** NAc of AAV-Cre-GFP mice (n=3) **B.** NAc of AAV-GFP mice (n=3) . Scale = 250um

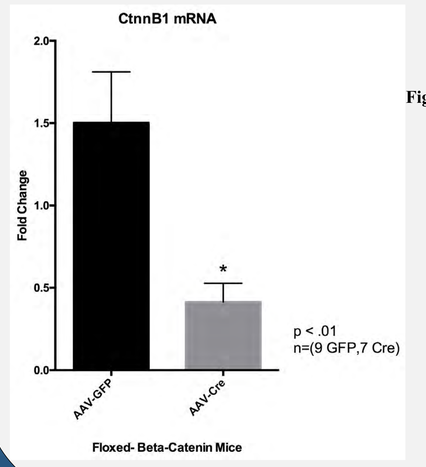
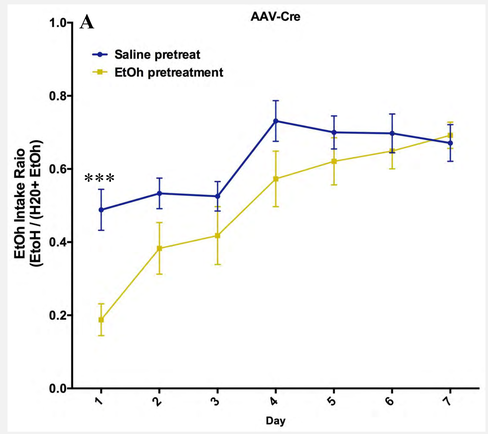
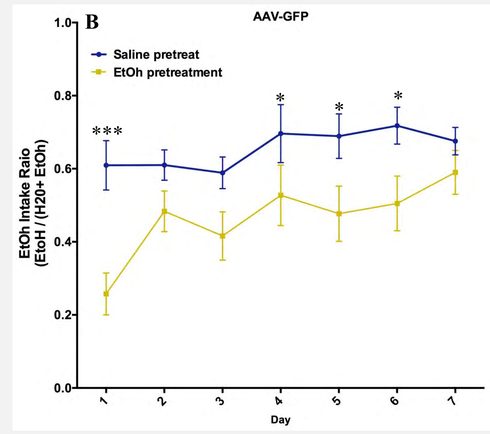


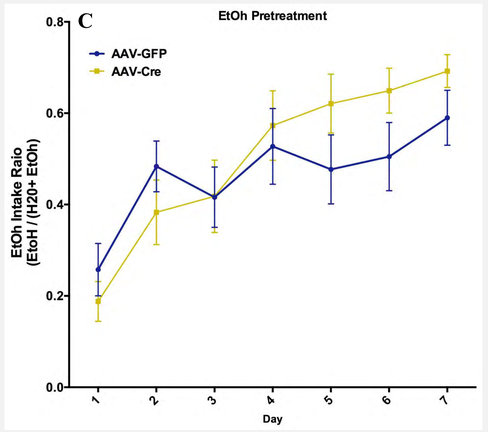
Figure 8. Validation of knockout via qPCR:

NAc punches were taken from both AAV-GFP and AAV-Cre mice. Ctnnb1 primers were validated on wild-type NAc tissue (data not shown). Results show that viral mediated knockout of β-catenin in the NAc was successful.

Results:







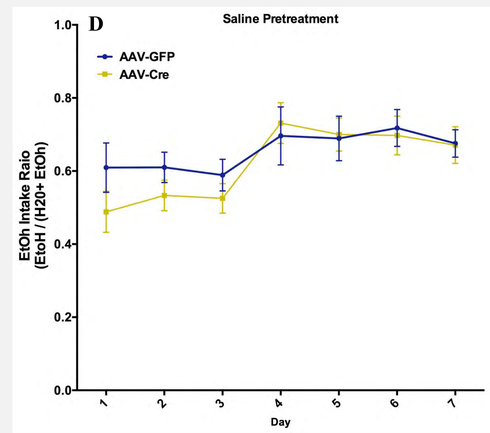


Figure 7. Ethanol preference ratios: A & B. Mice showed a difference in ethanol preference after EtOH vs Saline pretreatment regardless of genotype. **C & D.** When compared across genotypes, there was no significant difference between the groups regardless of treatment.

References:

- Dias, C., Feng, J., Sun, H., Shao, N. yi, Mazei-Robison, M. S., Damez-Werno, D., … Nestler, E. J. (2014). β-catenin mediates stress resilience through Dicer1/microRNA regulation. *Nature*. http://doi.org/10.1038/nature13976
- Hwa, L. S., Chu, A., Levinson, S. A., Kayyali, T. M., DeBold, J. F., & Miczek, K. A. (2011). Persistent escalation of alcohol drinking in C57BL/6J mice with intermittent access to 20% ethanol. *Alcoholism, Clinical and Experimental Research*, *35*(11), 1938–47. http://doi.org/10.1111/j.1530-0277.2011.01545.x
- Melendez, R. I. (2011). Intermittent (every-other-day) drinking induces rapid escalation of ethanol intake and preference in adolescent and adult C57BL/6J mice. *Alcohol Clin Exp Res*, 35(4), 652-658. 10.1111/j.1530-0277.2010.01383.x
- Russo, S., & Nestler, E. (2013). The brain reward circuitry in mood disorders. *Nature Reviews Neuroscience*, 14, 609–625. http://doi.org/10.1038/nrn3381
- Velázquez-Marrero, C., Wynne, P., Bernardo, A., Palacio, S., Martin, G., & Treistman, S. N. (2011). The relationship between duration of initial alcohol exposure and persistence of molecular tolerance is markedly nonlinear. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(7), 2436–46. http://doi.org/10.1523/JNEUROSCI.5429-10.2011
- Velázquez-Marrero, C., Burgos, A., García, J.O., Palacio, S., Marrero, H.G., Bernardo, A., Pérez-Laspiur, J., Rivera-Oliver, M., Seale, G., & Treistman, S.N. (2016) Alcohol Regulates BK Surface Expression via Wnt/β-Catenin Signaling. *Journal of Neuroscience*, 36(41), 10625-10639. http://dx.doi.org/10.1523/JNEUROSCI.0491-16.2016
- Wilkinson, M. B., Dias, C., Magida, J., Mazei-Robison, M., Lobo, M., Kennedy, P., … Nestler, E. J. (2011). A Novel Role of the WNT-Dishevelled-GSK3 Signaling Cascade in the Mouse Nucleus Accumbens in a Social Defeat Model of Depression. *Journal of Neuroscience*, *31*(25), 9084–9092. http://doi.org/10.1523/JNEUROSCI.0039-11.2011

Conclusions:

1. Knocking out β-catenin within the NAc does not seem to produce any changes in ethanol preference ratios throughout IAE.
2. A 6 hour ethanol pretreatment is sufficient to cause a lower ethanol preference 24 hours after. Whether this is caused by aversion to ethanol or increased sensibility to the effects of ethanol is still unknown.

Acknowledgements:

This research would not have been possible without the opportunity provided by the Summer Undergraduate Research Program (SURP) from the Icahn School of Medicine at Mount Sinai.

Microscopy was performed at the Microscopy CORE at the Icahn School of Medicine at Mount Sinai

Supported by:

NIH NIGMS 5P20GM103642 COBRE Center for Neuroplasticity grant awarded to Cristina Velazquez-Marrero, Ph.D. at the Institute of Neurobiology, Old San Juan.

NIH 1R25MH092912-01 Neuroscience Research Opportunities to Increase Diversity (NeuroID) program grant awarded to Roberto A. Aponte-Rivera at the University of Puerto Rico Rio Piedras Campus.