TrkB activity alters voluntary alcohol consumption in nondependant mice

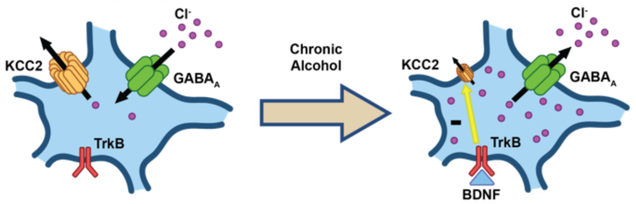
Amendolara AB[1], Payne AJ[1,2], Brundage JN, Park, HJ, and Steffensen SC[2]

1 Department of Biomedical Sciences. Noorda College of Osteopathic Medicine. Provo, Utah 84606

2 Department of Psychology and Center for Neuroscience. Brigham Young University. Provo, Utah 84602

Introduction

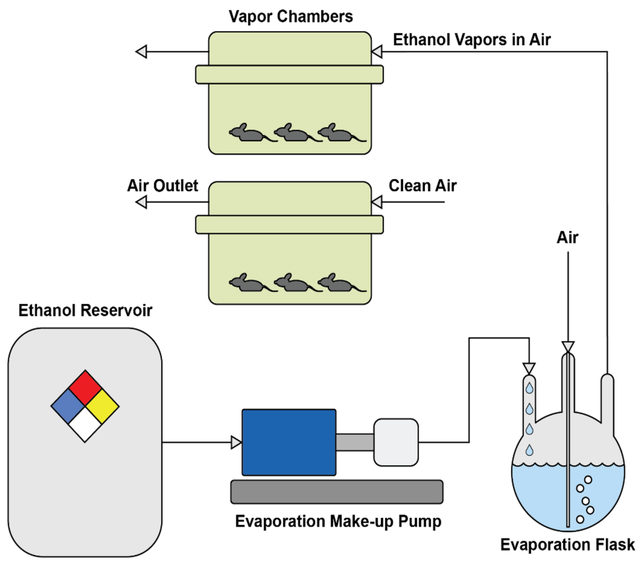
GABA neurons located in the ventral tegmental area of the midbrain are sensitive to ethyl alcohol (EtOH) and become hyper-excitable during withdraw. Some of this change may be mediated by brain-derived neurotrophic factor (BDNF) whose main receptor is tyrosine receptor kinase B (TrkB). TrkB can cause downregulation of chloride-exporting potassium-chloride cotransporter 2 (KCC2). Understanding this pathway, and validating the effect of TrkB modulation, opens the door to addiction therapies targeting KCC2.



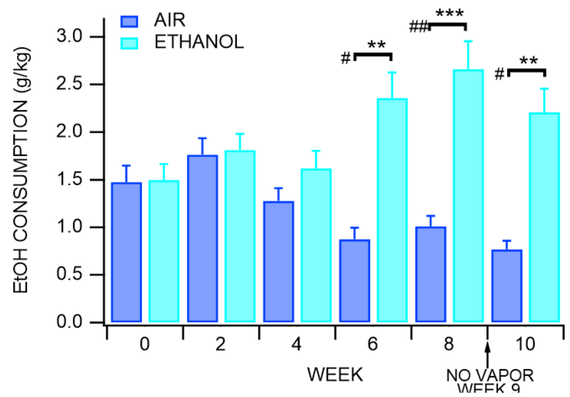
Methods

Drinking in the dark.
Mice were allowed to binge drink for a period of 2-4 hrs beginning at 13:00 hrs, 3 hrs into their inverted dark cycle.

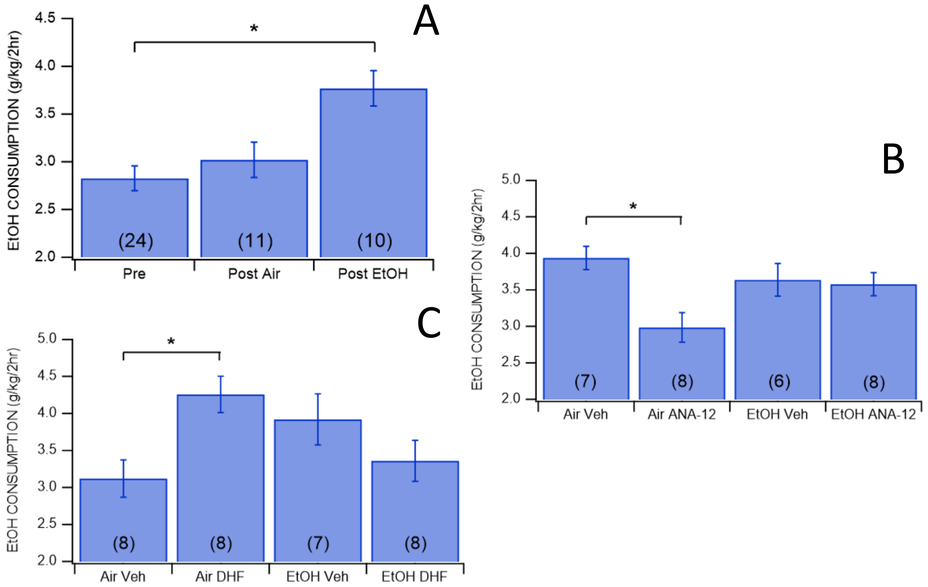
Alcohol vapor chamber system.
This system enables automation of chronic intermittent ethanol (CIE) exposure. For this study, animals were exposed to ethanol vapors or clean air for 16 hrs from 10:00 each morning to 02:00 hrs the next day. This was repeated four times per week.



Results



Validation of CIE
Drinking behavior, as measured by two bottle choice, showed an increase in the EtOH exposed mice and no increase in the air exposed mice. Baseline drinking was recorded followed by 1 week of CIE. This cycle repeated until week 9, when CIE was omitted and the animals were allowed to withdraw. This confirmed some amount of persistent dependance in the vapor exposed mice.



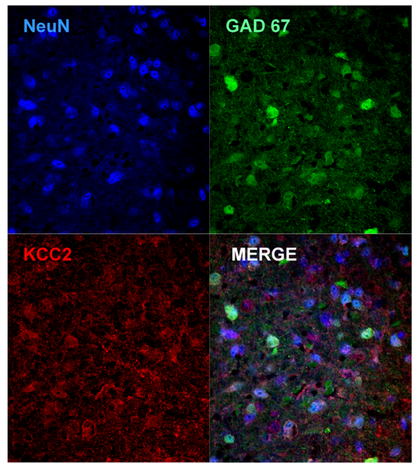
TrkB agonist and antagonist modulate drinking behavior in nondependant mice
Alcohol consumption in response to CIE and TrkB drugs after 6 week CIE period. Alcohol consumption before and after CIE for both the air-exposed mice and the EtOH-exposed mice (**A**). Consumption after CIE with an acute injection of a TrkB antagonist ANA-12 or vehicle (**B**). Consumption after CIE with an acute injection of TrkB agonist 7,8-DHF or vehicle (**C**).

Future Directions

In order to better understand the role of TrkB and KCC2 in EtOH addiction, further steps are planned:

1. Immunohistochemistry has been performed on mice brain slices. This data needs to be analyzed in depth to explore the current hypothesis that KCC2 channels are downregulated in the VTA during chronic EtOH exposure.

2. In order to investigate the role of KCC2 channels in chloride disregulation in GABAergic neurons, patch clamp experiments are planned. This may show evidence of GABA switching in the setting of chronic EtOH exposure. And it will provide a foundation to explore KCC2 agonists in theraputic roles.



Fluorescence imaging of brain slices
Transgenic GAD67-GFP marker in the green channel; NeuN neuronal stain in the blue channel; KCC2 stain in the red channel

Conclusions

Given that TrkB activity modulated drinking in nondependant mice, it appears that the BDNF/TrkB/KCC2 pathway may be involved in EtOH consumption. However, the fact that the TrkB antagonist did not decrease drinking in EtOH-dependant animals reduces its potential as a drug target for alcohol use disorder. But there is still potential for KCC2 to be used as a target. It is likely that a KCC2 agonist will have few side affects since KCC2 activation reduces intracellular chloride levels, which are already very low in healthy individuals.

Acknowledgements

This work was supported by PHS NIH grants AA020919 and DA035958 to SCS