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Selective GSK3B Deletion in Camk2a+ Forebrain Neurons or Inhibition Via Tideglusib, Decreases Ethanol Consumption in C57BL/6J Mice

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SELECTIVE GSK3B DELETION IN Camk2a^{+} FOREBRAIN NEURONS OR INHIBITION VIA TIDEGLUSIB, DECREASES ETHANOL CONSUMPTION IN C57BL/6J MICE

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INTRODUCTION

Nearly 6% of the adult population within the United States meets criteria for Alcohol Use Disorder (AUD) (SAMHSA, 2018). However, few treatments for AUD exist, with no new FDA-approved therapeutic agents within the last 15 years. To elucidate mechanisms underlying the neurobiology of AUD, the Miles laboratory has used genome-wide expression network profiling of brain regions in mouse genetic models following acute and chronic ethanol (EtOH) exposure. These studies identified glycogen synthase kinase-3 beta (*Gsk3b*) as a central member of a gene network highly regulated by acute EtOH in medial prefrontal cortex (mPFC) and associated with risk for alcohol dependence in humans (Wolen et al., 2012; Putman et al., 2016; van der Vaart et al., 2018).

Further, modulation of *Gsk3b* via stereotaxic injection of viral vectors alters EtOH consumption in rodent models. Viral-mediated overexpression of GSK3B within the mPFC was shown to increase EtOH consumption in male mice (Fig 1). Additionally, deletion of Gsk3b decreased EtOH consumption and preference in females (Fig 2) (van der Vaart et al., 2018). GSK3B could thus represent a potential new therapeutic target for the treatment of alcohol use disorder (AUD). However, the cell type specificity for GSK3B's actions on EtOH consumption have yet to be determined.

Here, we investigate *Gsk3b* specifically in Camk2a^{+} neurons in forebrain and its effects on EtOH consumption as well as report preclinical evidence for the selective GSK3B inhibitor, tideglusib, as a therapeutic agent for AUD.

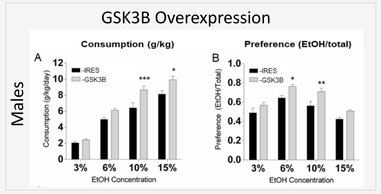

Fig 1. Viral-mediated overexpression of GSK3B in mPFC (A) increases EtOH consumption (g/kg) at higher EtOH concentrations, and (B) increases EtOH preference at medium concentrations in male mice (van der Vaart et al, 2018).

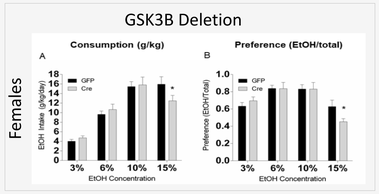

Fig 2. Viral-mediated deletion of GSK3B in mPFC (A) decreases EtOH consumption (g/kg) and (B) preference at high EtOH concentrations (van der Vaart, 2018).

HYPOTHESIS

Downregulation of GSK3B, either by deletion in Camk2a^{+} neurons or pharmacological inhibition with tideglusib, will decrease EtOH consumption

METHODS

Delete *Gsk3b:*

- **Mouse** – male and female transgenic Cre/Gsk3b fl/fl mice (n=5-7 females, 8-10 males per genotype)
- **Tamoxifen** – dissolved in corn oil at 20mg/ml and injected at 75mg/kg per body weight every 24 hours for five days. Control animals only receive corn oil injection
- **Drinking** – animals given continuous access to 15% w/v EtOH for 15 days, then switched to intermittent access for 5 weeks (Fig 3)

Inhibit GSK3B:

- **Mouse** – male C57BL/6J mice (n=48)
- **Tideglusib** – dissolved in corn oil at 20mg/ml and orally gavaged at 100mg/kg twice daily
- **Drinking** – animals given intermittent access to 20% v/v EtOH for 3 weeks, then continued drinking for 4 more weeks during tideglusib treatment or corn oil control

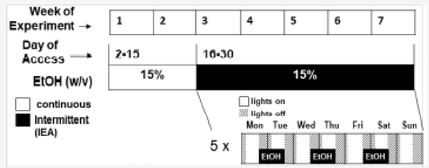

Fig 3. Schematic of ethanol access in Gsk3b deletion experiment

RESULTS

Gsk3b deletion in Camk2a^{+} cells decreases EtOH consumption and preference during both continuous and intermittent EtOH access.

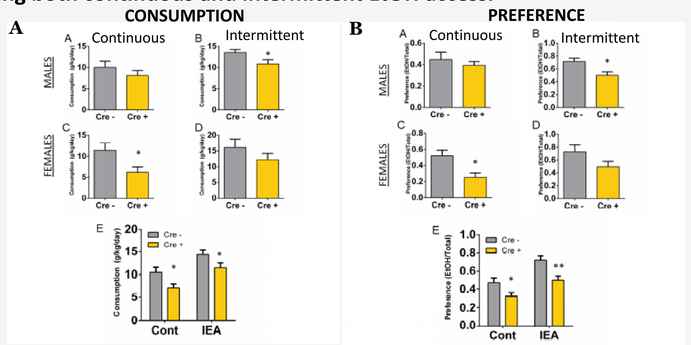

*Fig 4 . Deletion of Gsk3b significantly decreases EtOH (A) consumption and (B) preference in males during IEA and in females during continuous access. No main effect of sx or sex*genotype interaction, so sexes were collapsed, revealing a significant decrease in Cre+ animals in both conditions (*p<0.05) (** p<0.01) (van der Vaart, 2018).*

GSK3B inhibition with tideglusib decreases binge (2hr) and daily (24hr) EtOH consumption and preference during intermittent EtOH access following three weeks of administration.

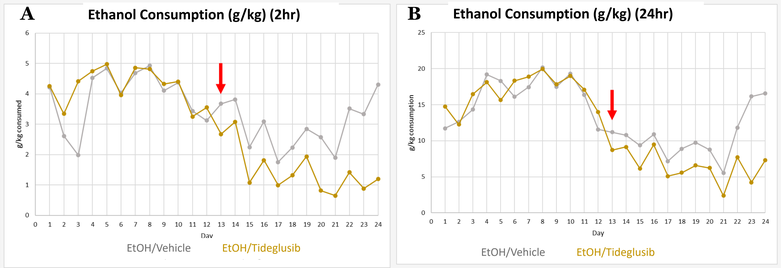

Fig 6. Arrow denotes beginning of tideglusib treatment. Two Way RM ANOVA revealed: (A) an effect of group (p=0.21), treatment day (p<0.001), and a significant interaction between the two (p<0.001) for the 2-hour binge reading. Tukey's posthoc analysis revealed significant differences between treatment groups during the last week of tideglusib treatment (Day 22 p=0.002; Day 23 p<0.001; Day 24 p<0.001) (B) an effect of treatment day (p<0.001) and a significant interaction between group x day (p=0.024) for the 24-hour reading. Tukey's posthoc analysis revealed significant differences between treatment groups during the last week of tideglusib treatment (Day 23 p<0.001; Day 24 p=0.001).

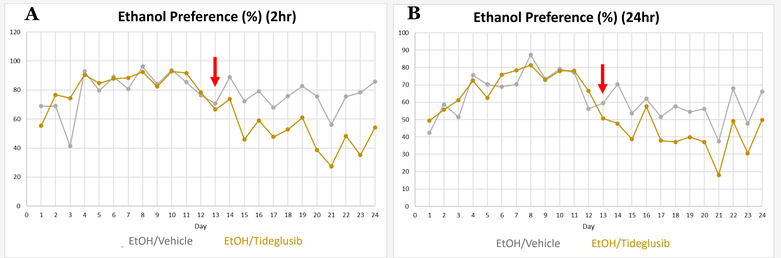

Fig 7. Arrow denotes beginning of tideglusib treatment. Two Way RM ANOVA revealed: (A) an effect of group (p=0.003), treatment day (p<0.001), and a significant interaction between the two (p<0.001) for the 2-hour binge reading. Tukey's posthoc analysis revealed significant differences between treatment groups during the last two weeks of tideglusib treatment (p<0.05) (B) an effect of group (p=0.032), treatment day (p<0.001) and a significant interaction between the two (p<0.001) for the 24-hour reading. Tukey's posthoc analysis revealed significant differences between treatment groups during the last two weeks of tideglusib treatment (p<0.05).

CONCLUSION & DISCUSSION

These results suggest GSK3B may be a therapeutic target for treatment of AUD. Deletion of Gsk3b in forebrain Camk2a-neurons showed a regional and cell-type specificity in GSK3B's modulation of EtOH consumption and preference, providing insight into the mechanisms of Gsk3b action in EtOH consumption. Importantly, previous work has shown *Gsk3b* modulation did not alter basal locomotor activity, anxiety-like behavior (light-dark box), taste preference for quinine or saccharin, or EtOH pharmacokinetics (Fig 8) (van der Vaart at al, 2018).

Targeting GSK3B using tideglusib, a selective GSK3B inhibitor, also produced a decrease in EtOH consumption and preference during week 4 of treatment, though initial gavage decreased total fluid consumption in all groups, regardless of EtOH drinking history or tideglusib treatment. Control studies showed no effect of tideglusib on liver fat accumulation in EtOH consuming animals.

These findings were consistent with previous work in our lab investigating the delivery of tideglusib through intraperitoneal injections (Fig 9), though these studies were limited to a shorter drug-administration period (van der Vaart, 2018). Here we have used a more therapeutically translatable route of administration via oral gavage and begun to investigate the longer-term effects of tideglusib on EtOH behaviors and toxicity. Tideglusib is a clinically available agent that warrants investigation in the treatment of AUD.

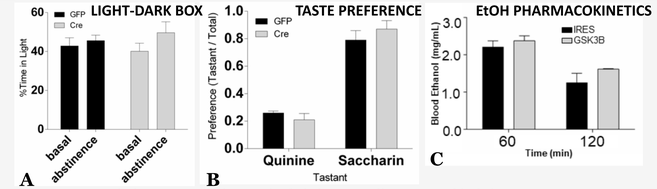

Fig 8. Previous work showing: (A) deletion of Gsk3b does not affect anxiety-like behaviors in EtOH naïve or 24hr abstinence mice or (B) taste preference for quinine or saccharin; and (C) overexpression of Gsk3b does not affect EtOH pharmacokinetics (modified from van der Vaart et al, 2018).

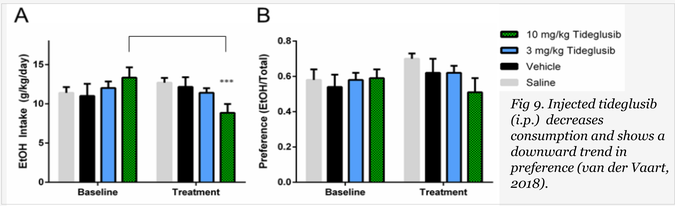

Fig 9. Injected tideglusib (i.p.) decreases consumption and shows a downward trend in preference (van der Vaart, 2018).

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