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Effects of the Selective GSK3B Inhibitor, Tideglusib, on Ethanol Consumption, Anxiety-like Behavior, Taste Preference, and Downstream Proteins

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INTRODUCTION

Glycogen synthase kinase 3 beta (*Gsk3b*) has previously been identified as a central member of a gene network highly regulated by acute ethanol in the mouse medial prefrontal cortex (mPFC). We have demonstrated modulations in GSK3B abundance influence ethanol consumption in rodent models, showing overexpression increased ethanol consumption *(fig. 1)* while knock-out produced a decrease (*fig. 2).*

Furthermore, we have shown pharmacological inhibition with the selective GSK3B inhibitor, tideglusib significantly decreased binge ethanol consumption and preference in a two-bottle choice, intermittent ethanol access (IEA) paradigm when administered prior to ethanol access (*fig. 3*).

GSK3B could be a target for treatment of alcohol use disorder (AUD), with tideglusib serving as a novel therapeutic. **Here we report tideglusib's actions on ethanol consumption in a drinking in the dark (DID) model and investigate tideglusib-induced responses in other behaviors to further assess its potential as a therapeutic agent for AUD. We additionally investigate tideglusib modulation of phosphorylation of downstream GSK3B targets involved in synaptic plasticity and neurotransmission.**

GSK3B Overexpression

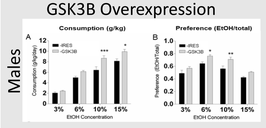


Fig 1. Viral-mediated overexpression of GSK3B in mPFC (A) increases EtOH consumption (g/kg) at higher EtOH concentrations, and (B) increases EtOH preference at medium concentrations in male mice (van der Vaart et al, 2018).

GSK3B Deletion

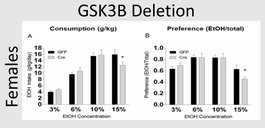


Fig 2. Viral-mediated deletion of GSK3B in mPFC (A) decreases EtOH consumption (g/kg) and (B) preference at high EtOH concentrations (van der Vaart, 2018).

GSK3B Inhibition with tideglusib

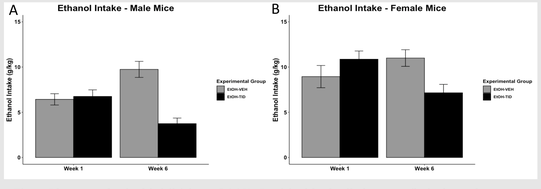


Fig 3. Pharmacological inhibition of GSK3B with tideglusib (200mg/kg) via oral gavage (i.g) decreases binge (2hr) ethanol consumption in (A) male ($p<0.0001$) and (B) female ($p<0.01$) mice

HYPOTHESIS

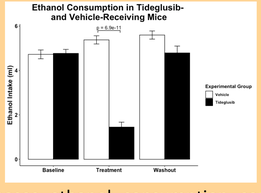
Tideglusib will decrease ethanol consumption while leaving other behaviors unaffected, suggesting utility as a therapeutic in treatment of AUD. Western blotting will reveal differential regulation of proteins downstream of GSK3B, providing evidence for a potential mechanism for tideglusib's effects.

METHODS

- C57BL/6J males and females received i.g. 200mg/kg tideglusib, except drinking-in-the-dark (males;100mg/kg i.p.)
- *Drinking-in-the-dark (DID)*: Mice given 20% ethanol 4-hours, 4-days/week x 3 weeks and then i.p. tideglusib or vehicle x 4 days in a Latin Square design with ethanol consumption measured daily
- *Light/Dark Box*: Mice gavaged with tideglusib or vehicle and i.p. injected with 1.8g/kg ethanol or saline then tested for 10-min.
- *Taste Preference*: Mice received tideglusib x 6 days and then tested daily for saccharin or quinine taste preference.
- *Western Blots*: Mice received tideglusib or vehicle i.g. 3x/week for 2-weeks and mPFC assayed for phosphorylated and total GSK3B, Dynamin1, and PSD-95.

ETHANOL CONSUMPTION

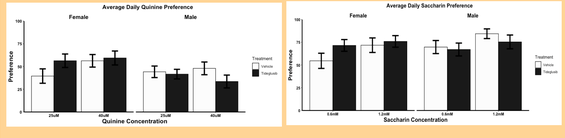
Tideglusib decreases ethanol consumption during drinking-in-the-dark



Tideglusib decreases ethanol consumption compared to control injected mice. Effects are quick to washout, with consumption returning to baseline within one week of no drug treatment

TASTE PREFERENCE

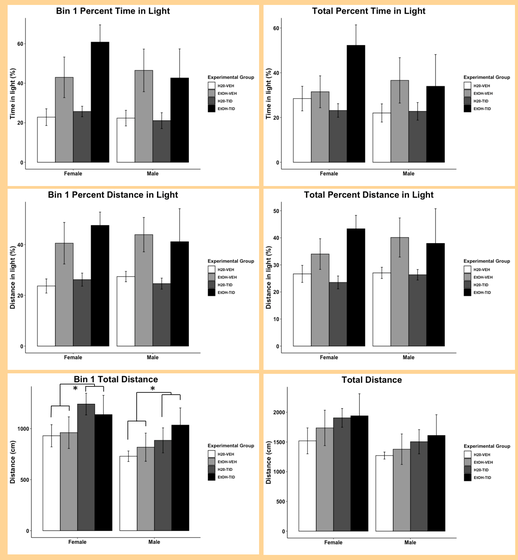
Tideglusib has no effect on taste preference for either quinine or saccharin



Tideglusib has no effect on taste preference for water over quinine nor saccharin over water. Each adulterant was tested at both a low and high dose

ANXIETY-LIKE BEHAVIOR

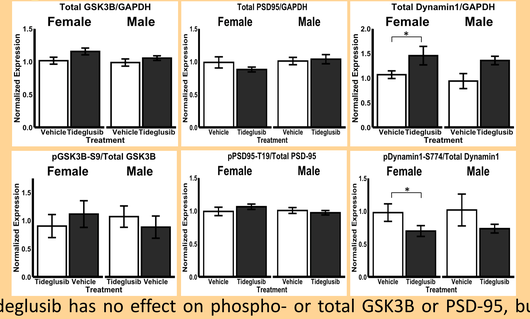
Tideglusib has no effect on anxiety-like behavior in light-dark box; locomotion transiently increases



Tideglusib has no effect on percent time spent in the light nor percent distance traveled in the light. However, tideglusib does produce a transient increase in total locomotion (*$p<0.05$) within the first 5-minutes of testing. This effect dissipates during the last 5-minutes of testing and is non-significant when the 5-minute bins are collapsed

DOWNSTREAM PROTEINS

Tideglusib increases total Dynamin1 levels in mPFC



Tideglusib has no effect on phospho- or total GSK3B or PSD-95, but increases Dynamin1 protein levels ($p=0.024$) in females, resulting in decreased pDynamin1 to total Dynamin1 ratio ($p=0.037$)

CONCLUSION

These data support use of tideglusib as a therapeutic in treatment of AUD.

Tideglusib is clinically available and is in phase II clinical trials for Alzheimer's disease, supranuclear palsy, and other neurological disorders.

Here we show **tideglusib significantly decreases ethanol consumption in the DID model of binge drinking.** Tideglusib is fast acting to decrease consumption, even in mice habituated to ethanol drinking, suggesting utility in lowering ethanol consumption prior to onset of a relapse event.

Tideglusib had **no influence on taste preference** for either adulterant. The perceived taste of ethanol is an important determinant of consumption, yet we show tideglusib is likely not reducing alcohol consumption by altering taste in the mouse.

GSK3B is ubiquitously expressed throughout the brain, making it vital to assess effects of global inhibition on behaviors outside of ethanol-consumption as well. There **were no actions of tideglusib on anxiety-like phenotypes**. However, tideglusib **transiently increased overall locomotion** in mice, likely through actions within the basal ganglia.

Total Dynamin1 levels are elevated with tideglusib treatment. Dynamin1 is central to the activity-dependent bulk endocytosis (ADBE) pathway, which recycles presynaptic vesicles following neurotransmitter release. ADBE relies on rephosphorylation of Dynamin1 by GSK3β to prepare it for the next round of ADBE. Our observation of increased total not phospho- Dynamin1 likely represents a compensatory response in Dynamin1 to account for loss of GSK3B activity and may provide insight to the mechanism through which tideglusib is carrying out its actions on ethanol behaviors.

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