RAPID ETHANOL EXPOSURE FACILITATES ALCOHOL CONSUMPTION

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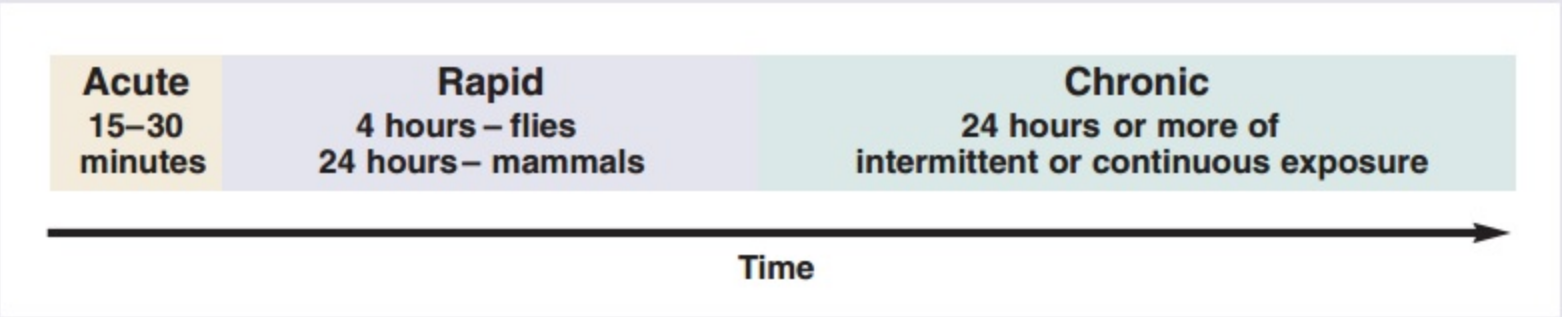
ALCOHOL TOLERANCE

3 Main Factors:

- Direct Tolerance
- Speed of Recovery
- Resistance

Types:

- Molecular
- Cellular
- Behavioral

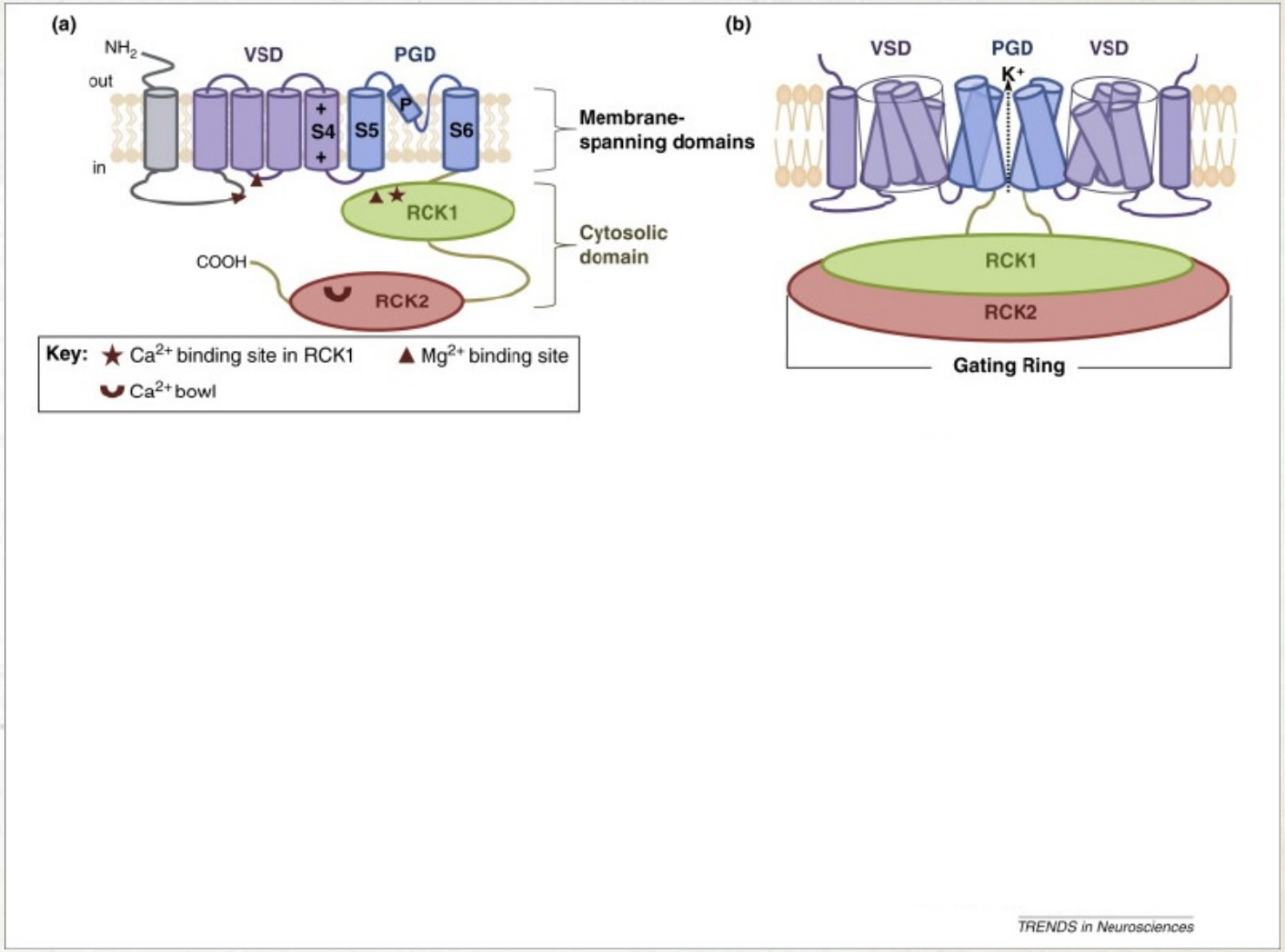


Pietrzykowski, AZ, & Treistman, SN. (2008)

BIG POTASSIUM (BK) CHANNEL

- Ion channels with large conductance of potassium
- Voltage and Ca^{2+} activated
- Tetrameric structure
- Modulatory subunits may associate with the channel.

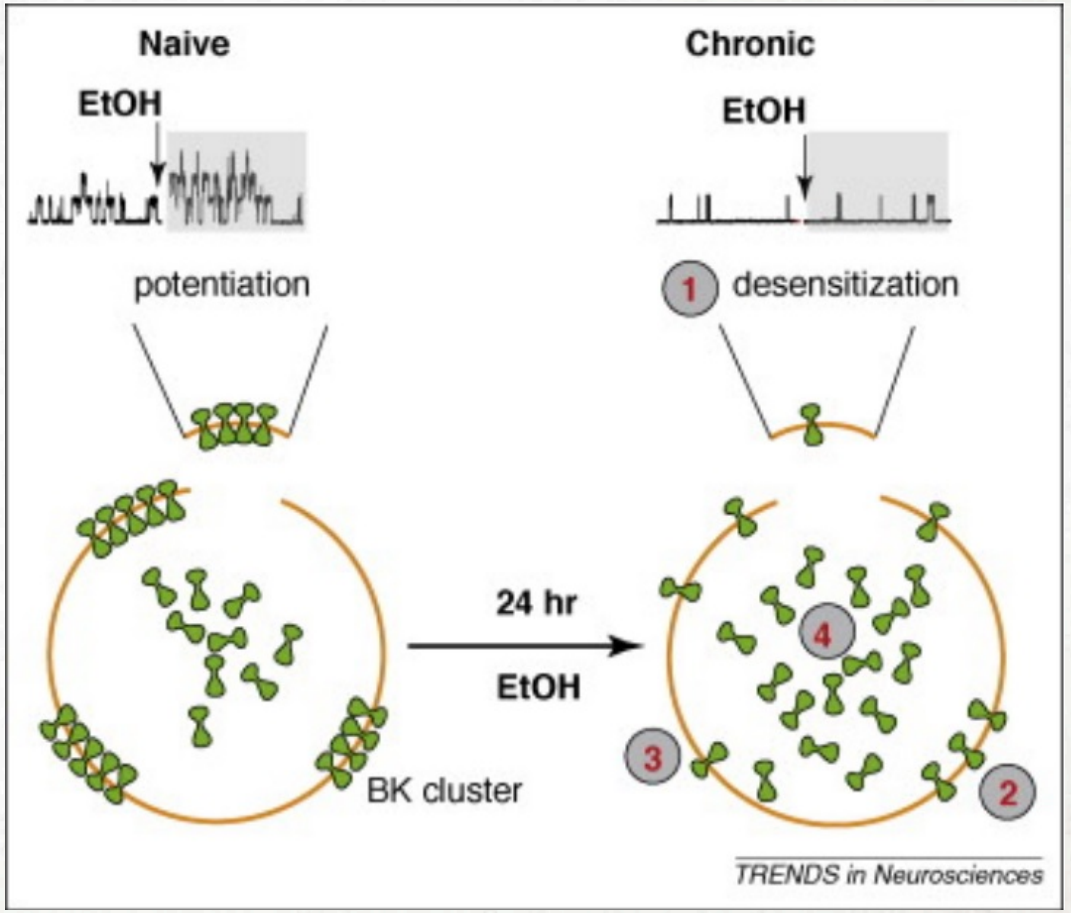
BK channels are essential for the regulation of several key physiological processes such as neurotransmitter release, action potential patterning and dendritic excitability.



Lee, US, & Cui, J. (2010)

INTERNALIZATION OF BK CHANNEL AS A COMPONENT OF PERSISTENT MOLECULAR TOLERANCE

- BK channels are very responsive to alcohol, generally, but not exclusively, exhibiting potentiated channel activity.
- Potentiation is elicited by alcohol concentrations as low as 10 mM (legal intoxication is 20 mM).
- Chronic exposure leads to desensitization, declustering, and internalization of the channel.



Treistman, SN, & Martin, GE. (2009)

ETOH-induced BK channel internalization is blocked by IWP-2:

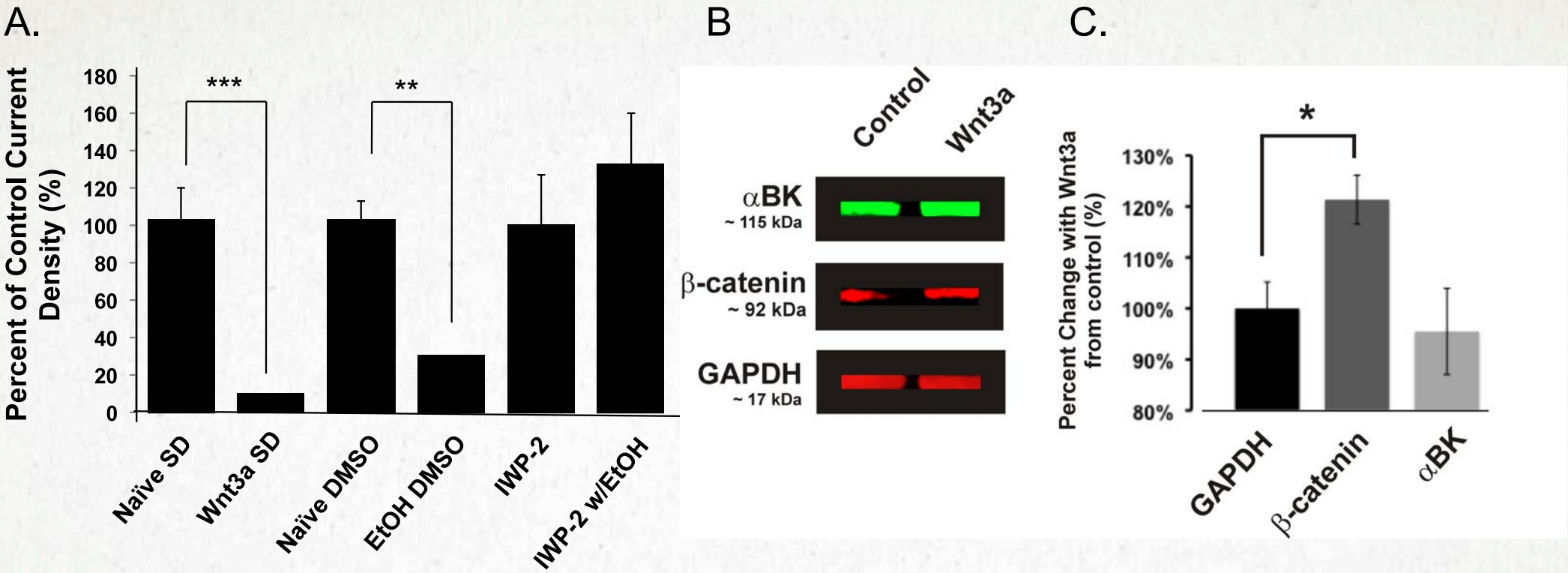
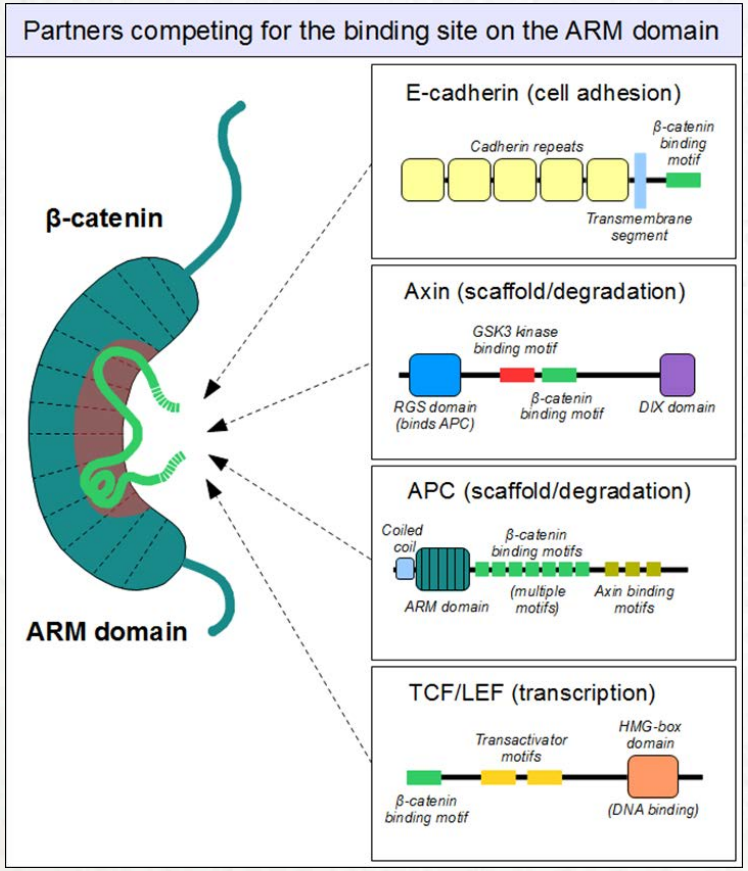
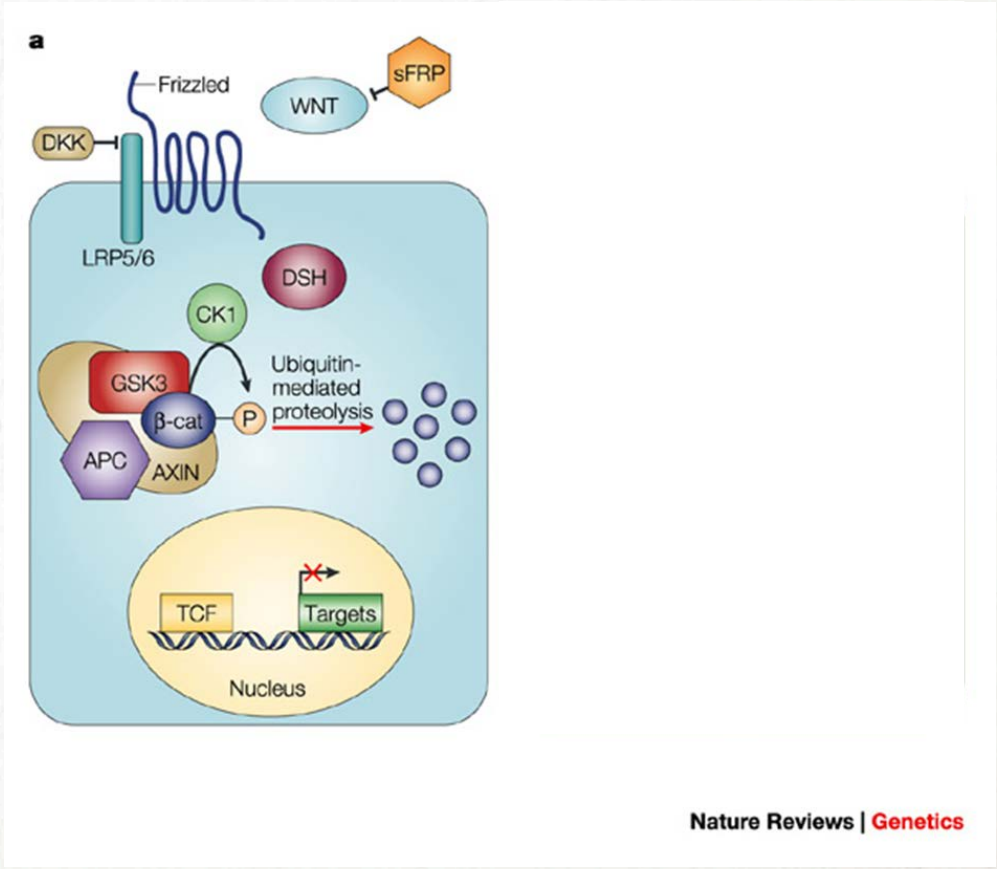


Figure . BK expressed in HEK 293 cells show a decrease in current density when treated with 200 ng/mL Wnt3A (Wnt/β-catenin pathway activator) and there is a block in the decrease in current density in the presence of 5 μM IWP-2 (Wnt/β-catenin inhibitor) when treated for 6 hr with 25 mM EtOH. A) Naïve, 200 ng.mL Wnt3A, 5 μM IWP-2 with and without 25 mM EtOH data are reported as mean ± SEM; n = representing the number of individual experiments. B) Representative Western Blot labeled for alpha-BK, b-catenin and GAPDH under control and after 6hr Wnt3a incubation. C) Quantification of all three proteins as percent change with Wnt3a from control (untreated). Asterisks represent statistical differences (*) $p \geq 0.05$ and (***) $p \leq 0.001$.

BETA-CATENIN FUNCTION



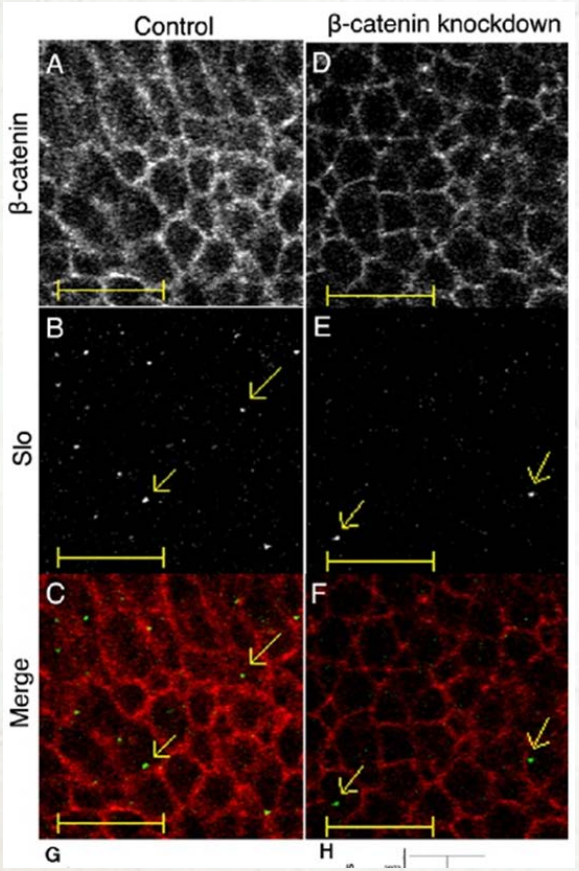
Wnt/β-Catenin Signaling Pathway



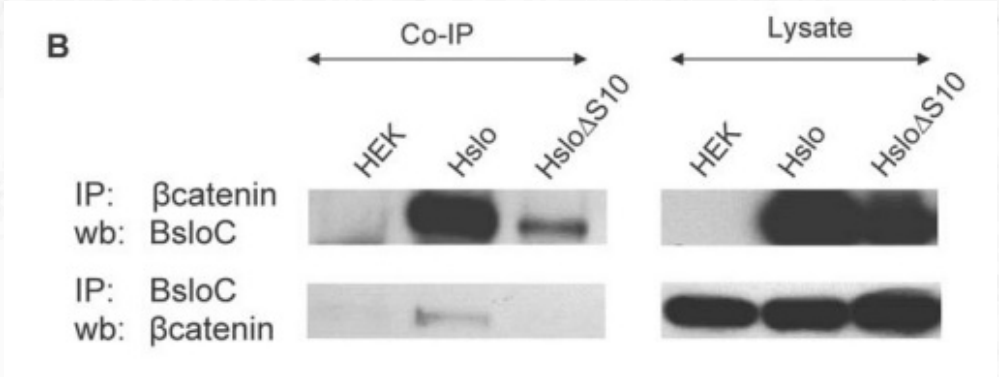
Moon, RT, et. al. (2004)

RELATIONSHIP BETWEEN B-CATENIN & BK CHANNEL

Co-localization



Co-Immunoprecipitation



•Both co-localization and co-immunoprecipitation support protein-protein interaction between BK channels and β-catenin.

•Deletion in the S10 region of BK , site for β-catenin interaction, impairs association.

- Bian, S , et. al. (2011)

DRINKING IN THE DARK (DID)

- Models human binge drinking.

- **National Institute on Alcohol Abuse and Alcoholism** (NIAAA) defines the term "binge drinking" as a pattern of drinking that brings a person's blood alcohol concentration (BAC) to 0.08 grams percent or above. (NIAAA, 2004)

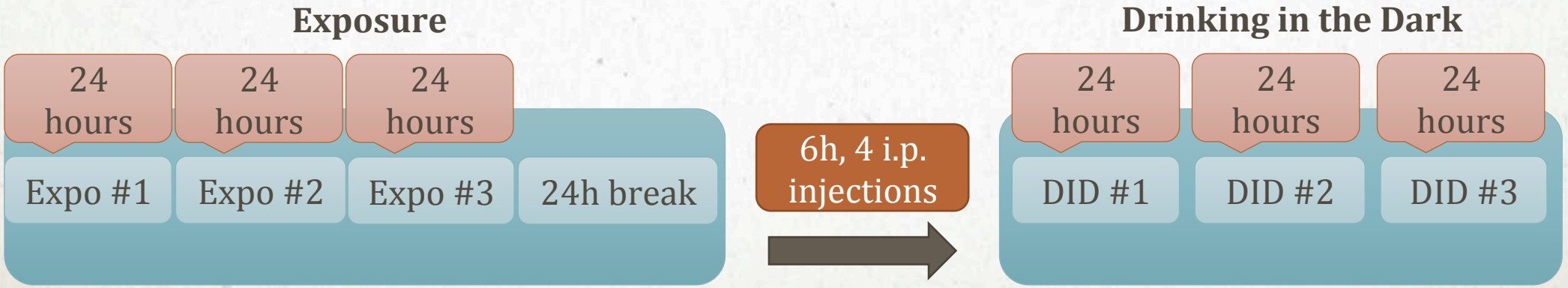
- "…entails giving C57BL/6J mice limited access (2- to 4-h) to a 20% (v/v) ethanol solution, in place of water, beginning 3-h into the dark phase of the circadian cycle." (Thiele et al., 2014)

Thiele et al. (2014)

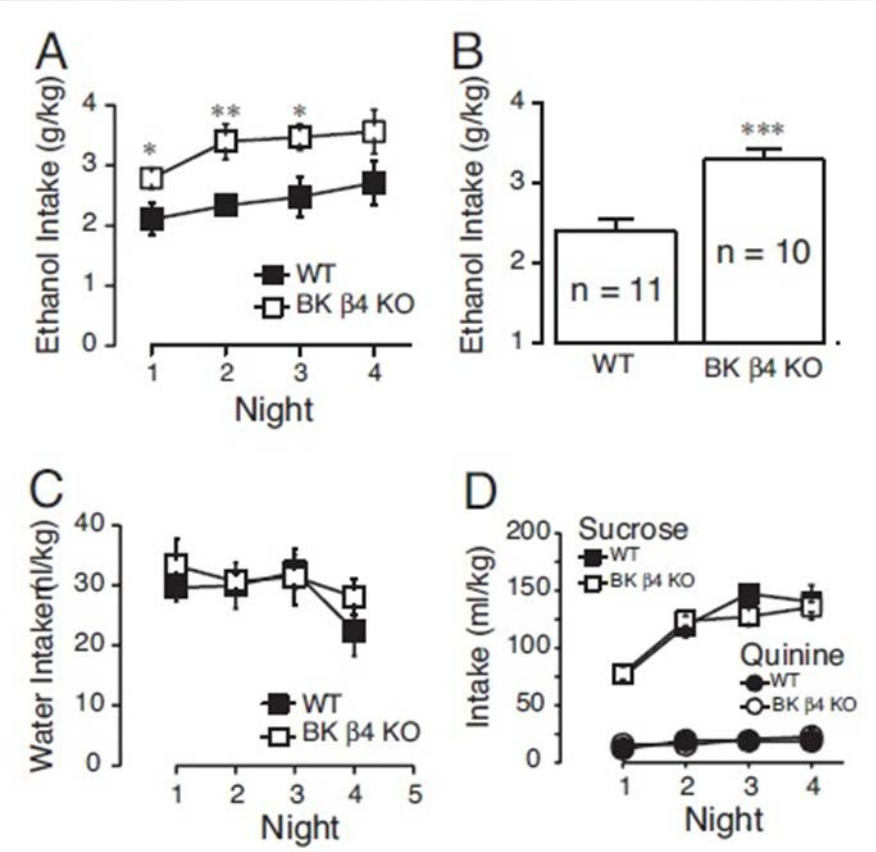
Facilitation Protocol

- 20% (v/v) ethanol was administered during a 2h period after a 2h water deprivation after "lights out"

Escalation Protocol



BETA-4 SUBUNIT ASSOCIATION WITH BK CHANNEL BLOCKS ACUTE ALCOHOL TOLERANCE



Martin, G , et. al. (2008)

- Used Drinking in Dark (DID)
- Showed that ethanol intake increased in β4 knockout mice.
- Also showed that in absence of β4, there was increased tolerance to ethanol potentiation.

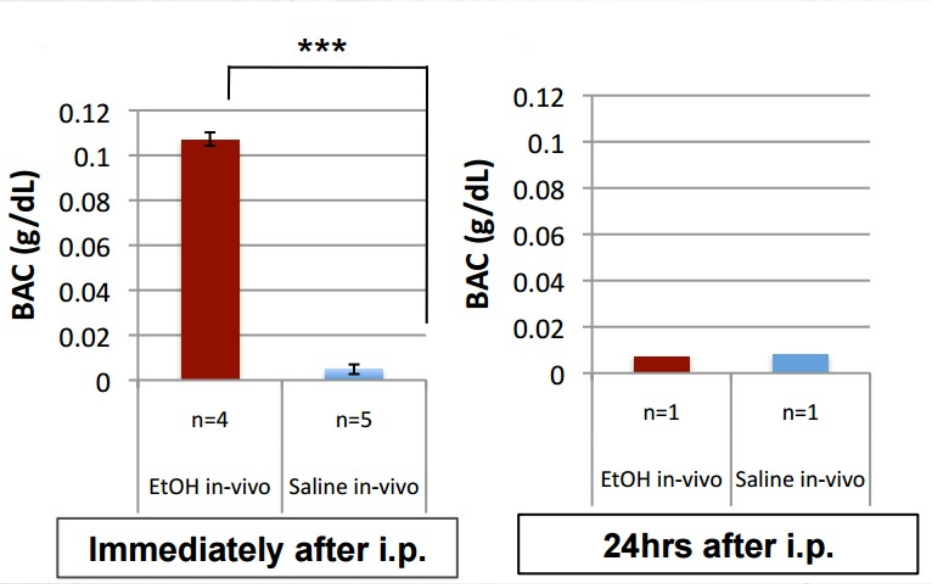
GOAL

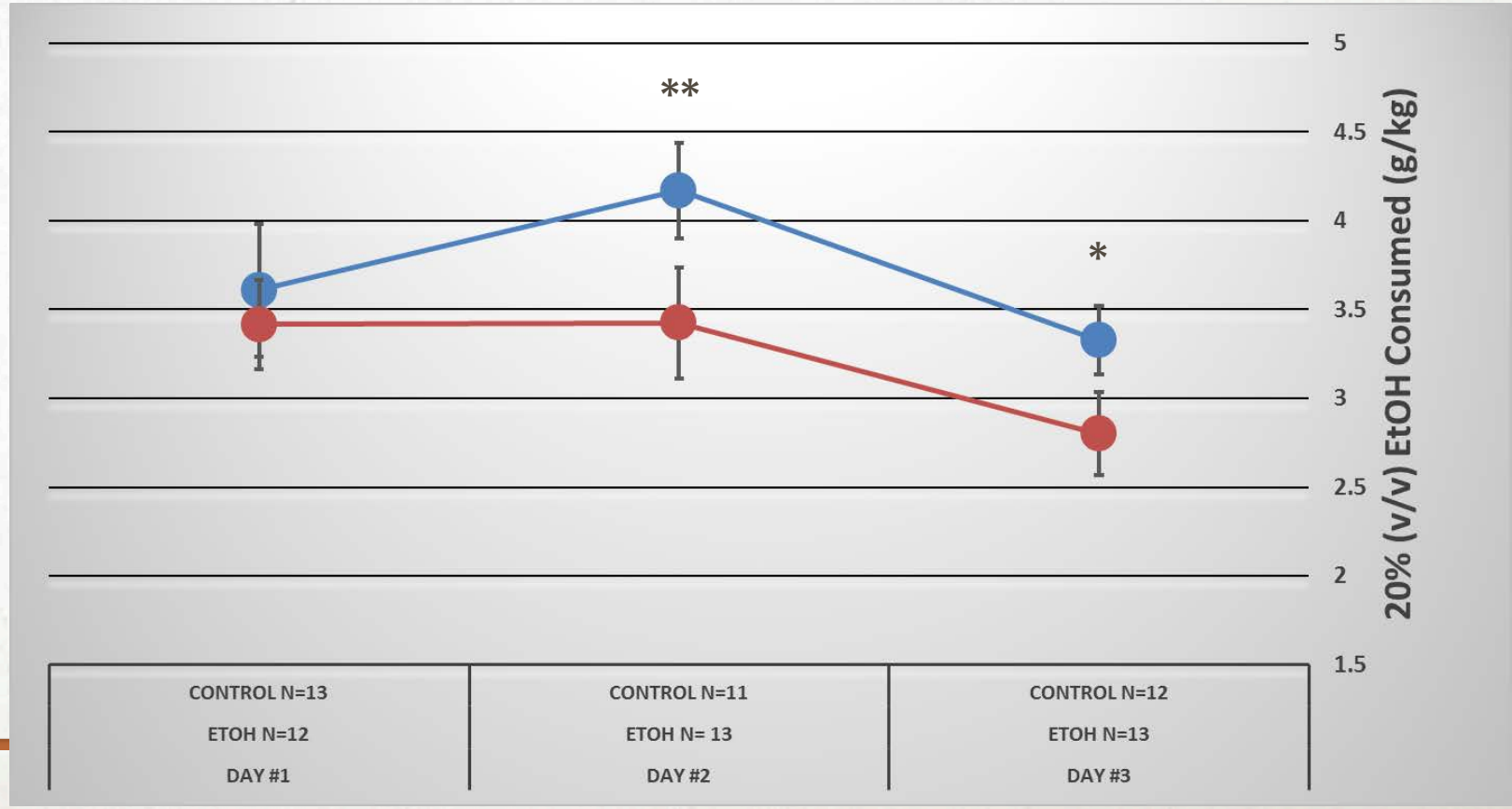
- Demonstrate that a rapid *in-vivo* ethanol exposure that induces BK alcohol tolerance at the neuronal level also influences subsequent alcohol voluntary consumption at the behavioral level.

FACILITATION

6h, 4 i.p. injections

24 hours	24 hours	24 hours
DID #1	DID #2	DID #3





ESCALATION

Exposure

24 hours | 24 hours | 24 hours

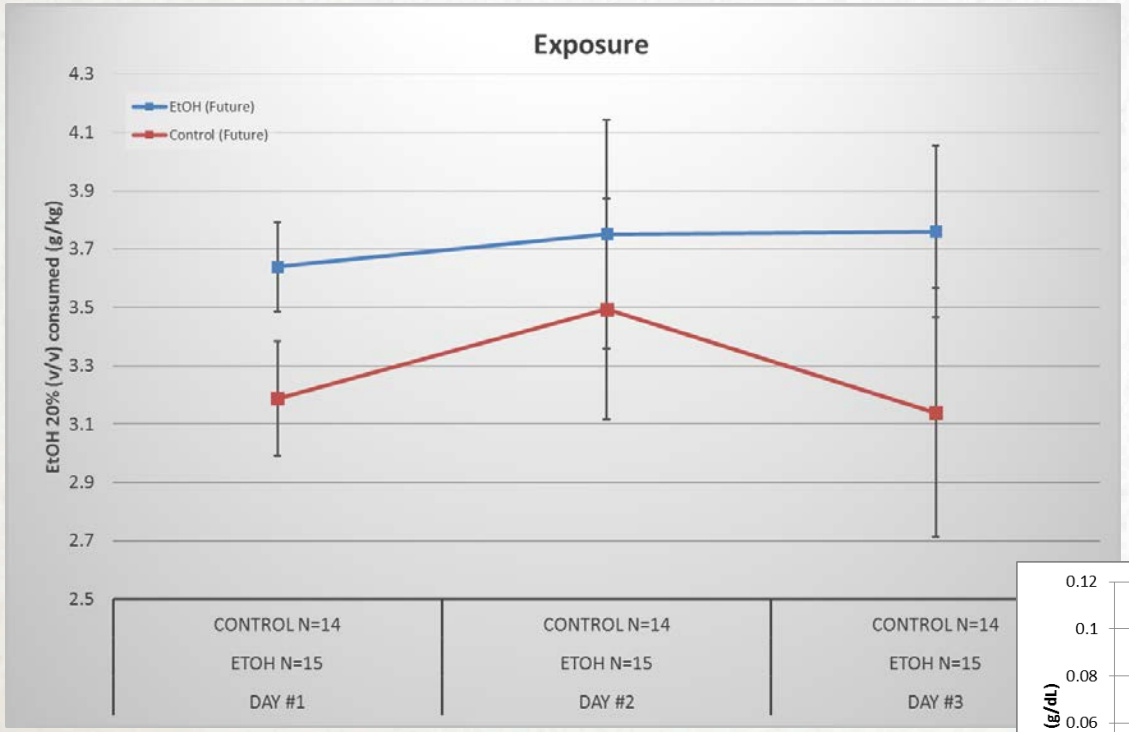
Expo #1 | Expo #2 | Expo #3 | 24h break

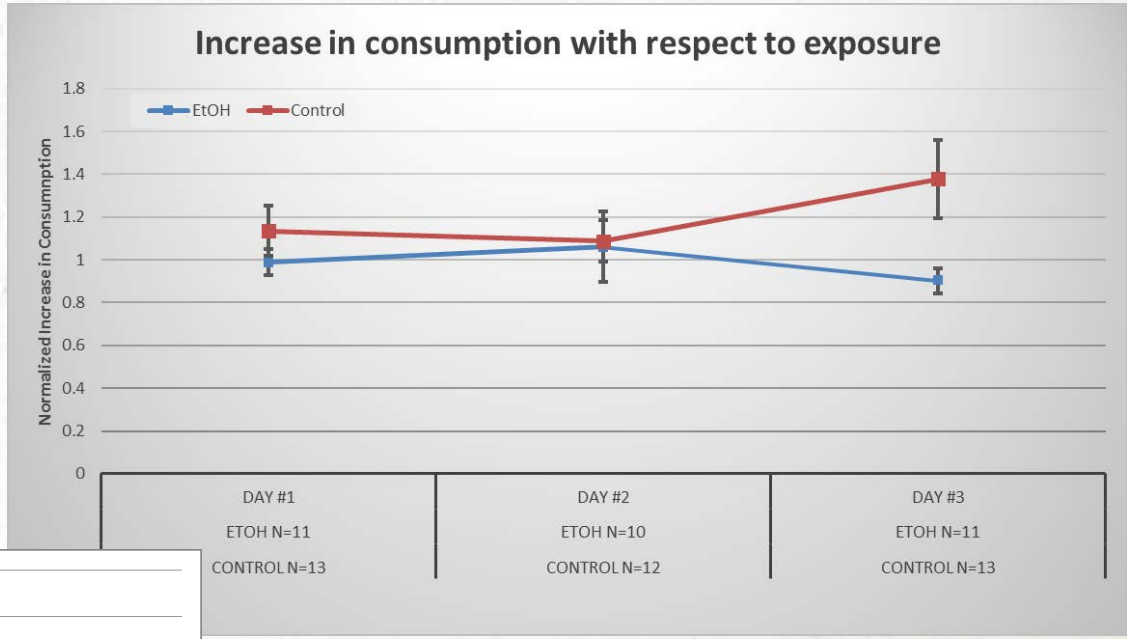
6h, 4 i.p. injections

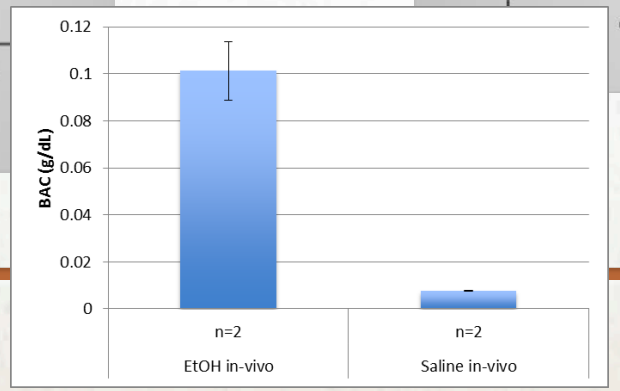
Drinking in the Dark

24 hours | 24 hours | 24 hours

DID #1 | DID #2 | DID #3







CONCLUDING REMARKS

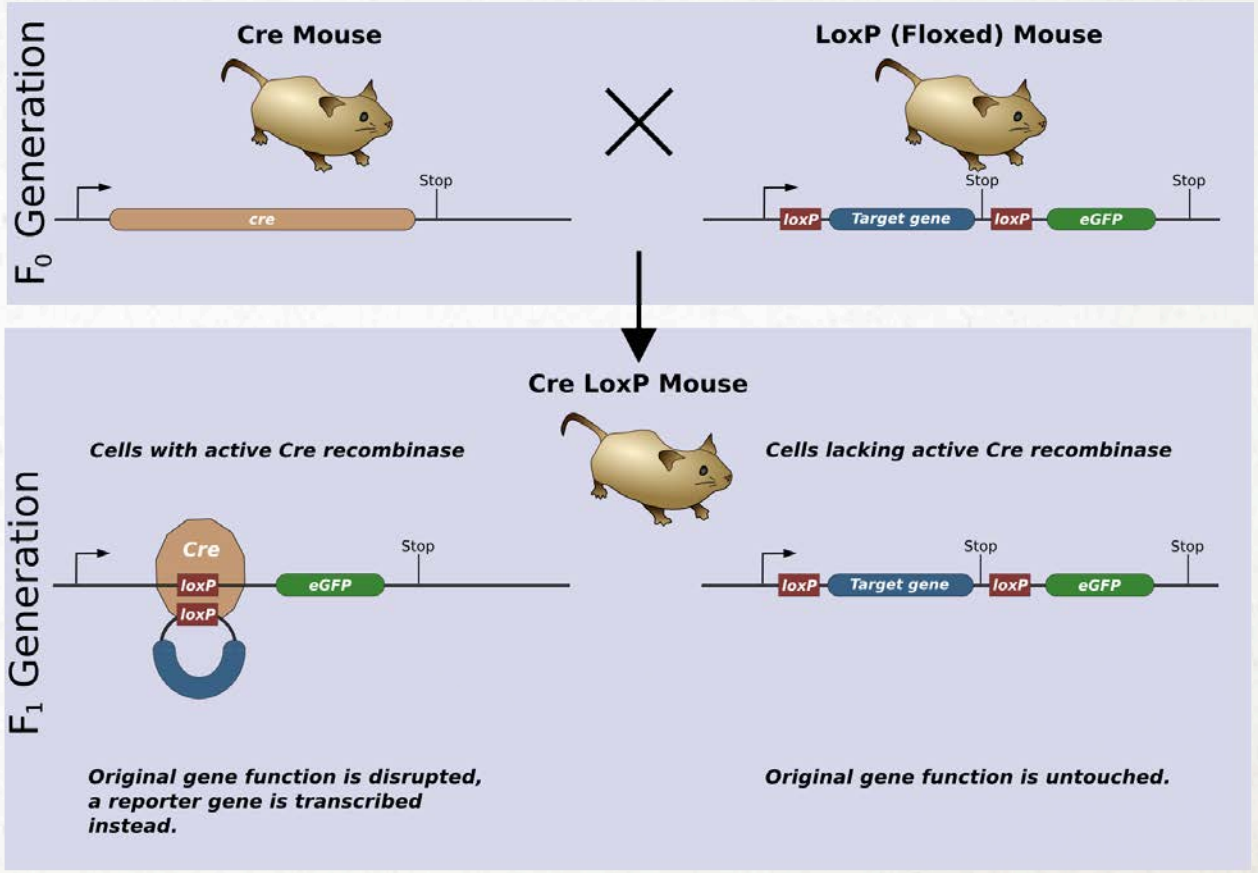
- **Patterns of *in-vivo* exposure that results in persistent molecular tolerance in wild-type C57 mice, further result in increased voluntary consumption.**

- **This does not occur as an escalation in consumption but rather a facilitation.**

- **Data suggests that a "binge-like" episode modeling moderate intoxication for 6 hours may be sufficient to not only induce molecular tolerance but result in subsequent increased consumption.**

FURTHER EXPERIMENTATION

- Beta-catenin Cre-loxed C57 mice will utilized.
- Stereotactic injections will be done in the ventral striatum of the experimental mice to induce a localized Beta-Catenin Knockdown.
- DID paradigm will be utilized to observe the effects of the rapid ethanol exposure on consumption, BK expression.



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THANK YOU FOR LISTENING!

Any Questions?