Binge drinking induces cross-species plasticity in the orbitofrontal cortex

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Background

~17% of adults in the U.S. have engaged in binge drinking behavior, excessive ethanol drinking in a short amount of time. The 'Drinking in the Dark' paradigm closely models human binge drinking with associated high blood ethanol concentration (BEC) (1). Binge drinking can induce alterations in the brain regions associated with executive function, specifically in the orbitofrontal cortex (OFC). Previous work on pyramidal neurons in the cortex shows that long-term binge drinking causes increased synaptic transmission (2). However, the effect of binge drinking on interneuron function has not been well established.

Here we examine the intrinsic properties, excitability, and synaptic drive in OFC interneurons (INs) of mice and rhesus macaques that have engaged in binge-drinking behavior using patch clamp electrophysiology.

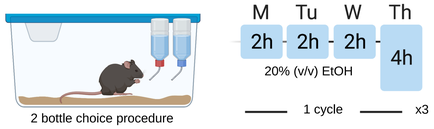
Methodology

Subjects

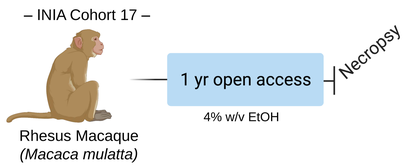
- Adult male and female C57BL/6J mice
- Adult male and female Rhesus Macaques

Drinking Protocols

- Mice were housed in a reverse light cycle room (12-hr) for the duration of a "Drinking in the Dark" (DiD) protocol. Mice were given access to both 20% v/v ethanol and H2O for three 2hr (M-W) and one 4hr (Thurs) session for 3 weekly cycles. Experimental mice were separated into either a 24-hour withdrawal group or a 7-day withdrawal group depending on when electrophysiology was performed.



- Rhesus macaques consumed 4% w/v under 22h/d open access over a one-year period and separated into low- vs. binge-level drinking groups (3).

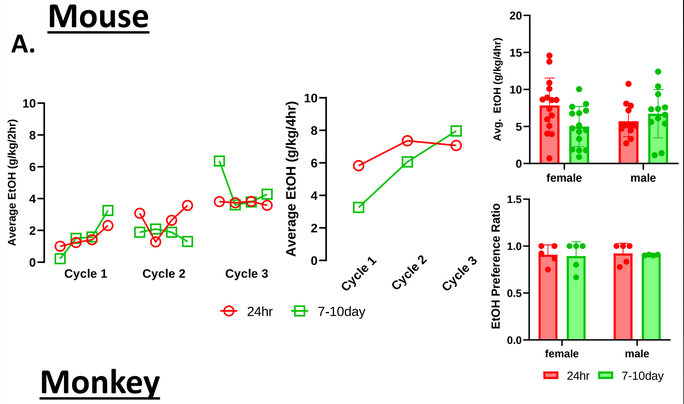


Patch-clamp electrophysiology

- Whole cell patch clamp recordings were performed in the lateral orbitofrontal cortex (lOFC). Interneurons were identified visually (size and shape) and by intrinsic properties (e.g., Cm < 70). For E/I measures, a cesium-based internal solution was used to record for 4 minutes at -55mV (sEPSC) and +10mV (sIPSC) in voltage clamp mode. sE/IPSC were analyzed using miniAnalysis software. Excitability measures were obtained in current clamp mode using a potassium-based internal solution. Resting membrane potential (RMP), action potential (AP) threshold, rheobase (minimum current to elicit firing), and firing over a series of 10 pA current steps (-30mV to 190mV) were assessed and analyzed using Clampfit.

Drinking

Mouse



Monkey

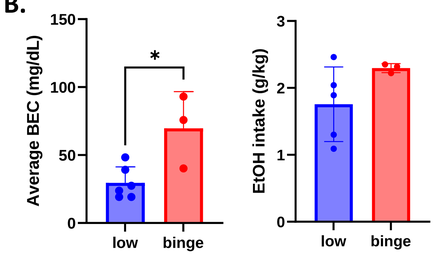
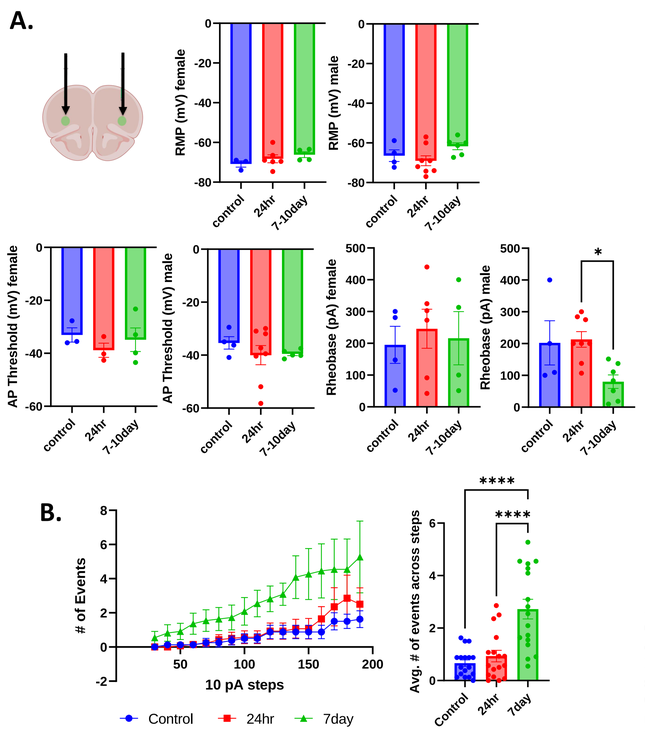


Figure 1. A) Mice in 24hr and 7-10day groups did not drink significantly different amounts of EtOH (g/kg). Mice of both sexes showed significantly increased preference for 20% EtOH over H2O during two bottle choice in the 'Drinking in the Dark' paradigm. There was no significant difference between sexes in EtOh (g/kg) consumed. B) Binge-drinking monkeys had significantly higher blood alcohol concentrations (BEC) compared to low-drinkers, but the EtOH (g/kg) was not significantly different over the 1-year drinking period *p< 0.05, **p< 0.01.

Excitability

Mouse



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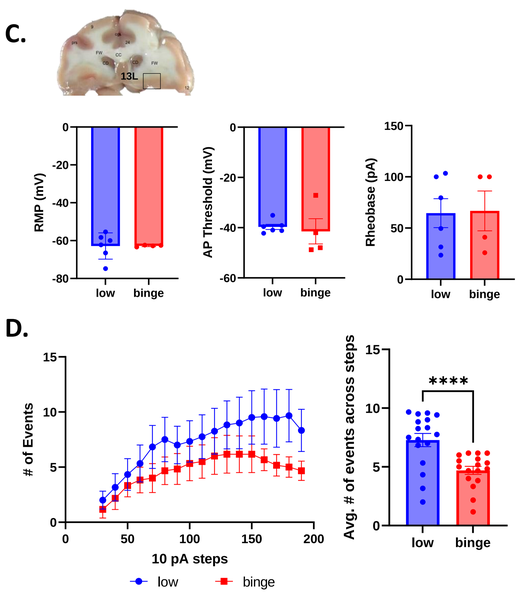
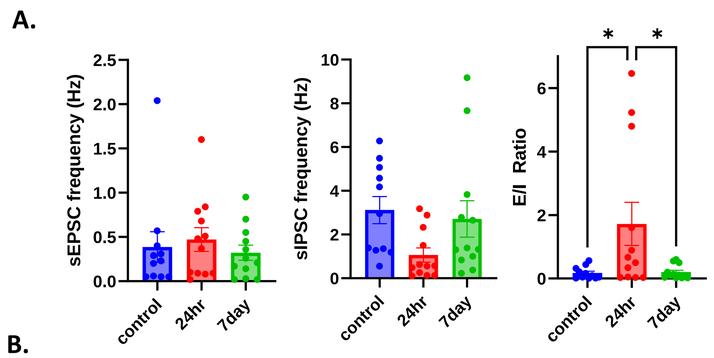


Figure 2. A) Male mice under 7-10 day WD from binge drinking had decreased rheobase compared to 24hr WD mice, but no differences in action potential (AP) threshold or resting membrane potential (RMP). B) Mice under 7-10 day WD had an increased amount of events over 10pA steps compared to control or 24hr WD mice in both male and female mice. C) There was no significant difference between low and binge-drinking monkeys in RMP, AP threshold, or rheobase. D) Binge-drinking monkeys had a significantly decreased amount of events over 10pA steps compared to low-drinking monkeys *p< 0.05, **p< 0.01, ***p< 0.001

Synaptic Transmission

Mouse



Monkey

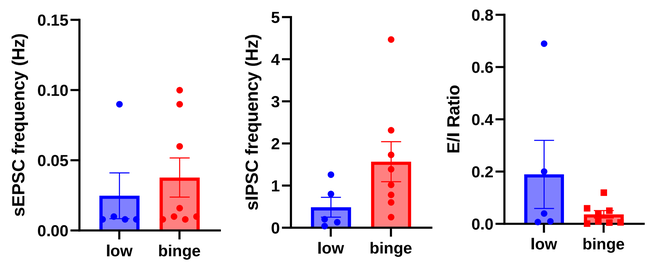


Figure 3. A) Mice in 24hr WD group had in increase in the E/I ratio compared to control and 7-10day WD groups. B) Monkeys had no alteration in sEPSCs, sIPSCs, or any subsequent change to E/I ratio *p< 0.05, **p< 0.01.

Conclusions

- OFC INs showed increased excitability 7-10 days following 'Drinking in the Dark (DID):
- 7-10 day WD mice had a higher amount of AP events during 10pA steps compared to controls or 24hr WD mice. Male 7-10 day WD mice also experienced decreased rheobase
- 24 hours after DID withdrawal, mice experienced increased synaptic drive onto OFC INs
- Binge-drinking monkeys had higher average BEC compared to low-drinking monkeys, though average intake did not differ. This may be due to the specific drinking type where large quantities were ingested in short bouts ('gulp' vs 'sip).
- Binge-drinking caused a reduction in excitability in OFC INs, as AP events were reduced. However, no alterations in RMP, rheobase, or AP threshold were observed
- Synaptic drive onto OFC INs did not seem to be altered in monkeys

References

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FUNDING: NIH/NIAAA R00 AA028298; UMSOM Dept of Neurobiology

ACKNOWLEDGMENTS: We'd like to thank Dr. Kathy Grant and Oregon National Primate Research Center for providing us with the opportunity to collect NHP data.