Novel Compounds for Treatment of Alcohol Addiction and Anxiety

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Shortfalls of current alcohol addiction drugs

- Opioid antagonists employed for the treatment of alcohol abuse can cause mood disorders, depression, anxiety, and dysphoria
- Addiction to the medications are common
- Anxiety and depression during withdrawal
- Patient compliance is a severe problem
- Classical benzodiazepines employed to treat alcohol abuse are sedating, ataxic, and amnesic with abuse liabilities
- SSRIs employed in treatment of alcoholism are limited because they cause sexual dysfunction in many patients and take 3-4 weeks to take effect; they also cause anxiety in some patients

Our Solutions

- The inventors have developed novel aza-beta carboline compounds useful in chemical addiction, anhedonia, anxiety, and other conditions associated with withdrawal from alcohol
- Compounds specifically target the alpha 1 subunit of the GABA(A) receptor
- Alpha 1-preferring antagonists have been shown to reduce ethanol intake, reduce craving (baboon study), and reduce any anxiety/anhedonia during withdrawal in P and HAD rats
- The latest compounds are easy to synthesize in 2 steps in larger amounts and can be easily scaled up

Market, Intellectual Property, and Partnering

Market

- Over 16 million people in the U.S. have an alcohol addiction and the condition affects more than 25% of the population at some point during life and binge drinking kills six people a day
- The costs of alcohol abuse in the U.S. reached $249B in 2010
- 90% of adults who are excessive drinkers binge drink and 90% consumed by youth is binge drinking
- Alcohol withdrawal delirium will result in the death of 1 out of every 5 alcoholics who stop drinking without professional medical intervention
- The U.S. market for substance abuse treatment and diagnosis should reach $12.7 billion in 2018 with a compound annual growth rate of 5.3%
- Recent health care reform has mandated that health insurance coverage for mental health illnesses include drug and alcohol disorders, making treatments more widely available to the public. In 2014 the Mental Health Parity Act's reach will also extend to include small group insurance plans and plans for individuals

Intellectual Property

- U.S. Utility Patent 8,268,854 issued 9/18/2012

Partnering

- Looking for a development partner to aid in the development of a final product

Novel $GABA_A$ subtype specific agents

- To elucidate the role of specific $GABA_A$/benzodiazepine receptor subtypes in regulating alcohol reinforcement, a number of active-carbolines have been synthesized and evaluated

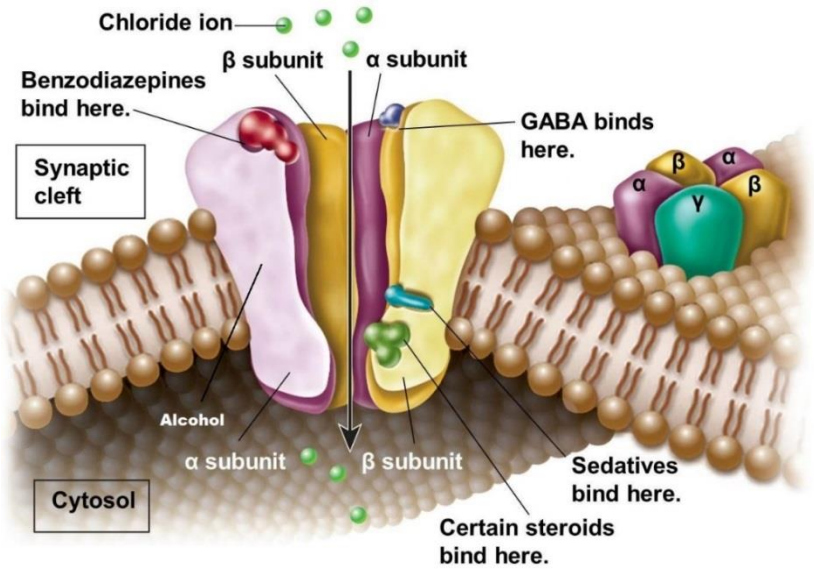
- A study with the benzodiazepine receptor antagonists, 3-propoxy-beta-carboline (3-PβC) and beta-carboline-3-carboxylic acid *t*-butyl ester (βCCt), was carried out to examine the role of α1 receptor subtypes within the ventral pallidum (VP) on alcohol self-administration

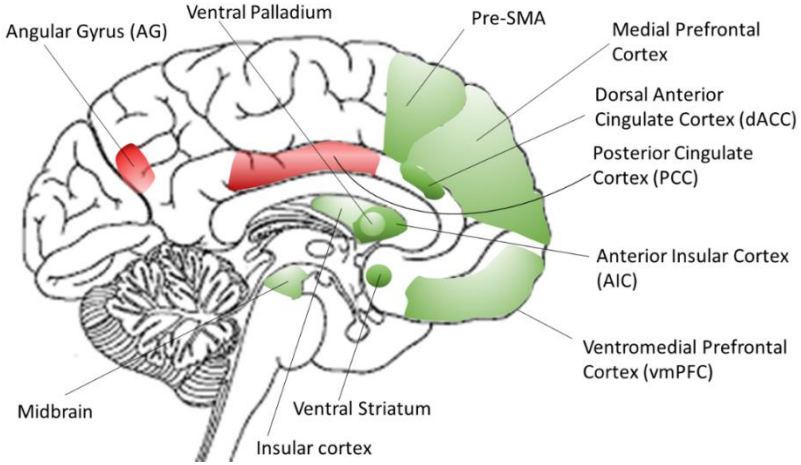
- 3-propoxy-beta-carboline (3-PβC) tested on maternal separation rats for binge drinking and impulsivity in adults and is mediated via a CRF/$GABA_A$ mechanism

- Examination of the data indicated a reduced rate in alcohol self-administration in P and HAD rats and reduced binged drinking in maternally separated rats

- These results provide a new avenue for the design of clinically safe and effective drugs for treatment of alcoholism

GABA/BzR chloride ion complex





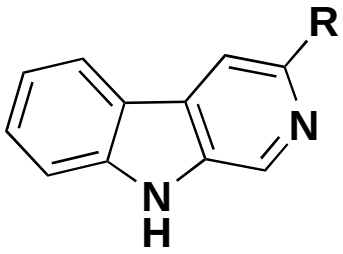
- Major inhibitory neurotransmitter in the CNS
- Ionotropic receptor and ligand-gated ion channel
- Pentameric structure
- The α1 subtype receptor in ventral pallidum plays a vital role in alcohol regulating behavior

Subunits	Associated Effect
α1	Sedation, anterograde amnesia, some anticonvulsant action, ataxia, some addiction
α2	Anxiolytic, hypnotic (EEG), maybe some muscle relaxation at higher doses, some anticonvulsant action
α3	Some anxiolytic action, some anticonvulsant action, maybe some muscle relaxation at higher doses
α4	Diazepam-insensitive site
α5	Cognition, temporal, and spatial memory, (Maybe memory component of anxiety)
α6	Diazepam-insensitive site

3-PβC, 3-ISOPβC and βCCT

- The γ-aminobutyric acid (GABA) system is implicated in alcohol reinforcement and consumption
- 3-PβC (3-Propyloxy-β-carboline) hydrochloride salt binds with 10- to 20-fold selectivity at benzodiazepine $GABA_A$ α-1 receptors
- 3-PβC and 3-ISOPβC function as a mixed benzodiazepine receptor agonist-antagonist
- Benzodiazepine $GABA_A$ receptor agonists can increase alcohol consumption, where as antagonists and inverse agonists decrease alcohol consumption
- βCCT (beta-carboline-3-carboxylic acid t-butyl-ester) is an alpha-1-subunit selective antagonist at BzR/GABA(A) receptors

Affinities of β-Carbolines at $\alpha_{1\text{-}6}\beta_{2/3}\gamma_2$

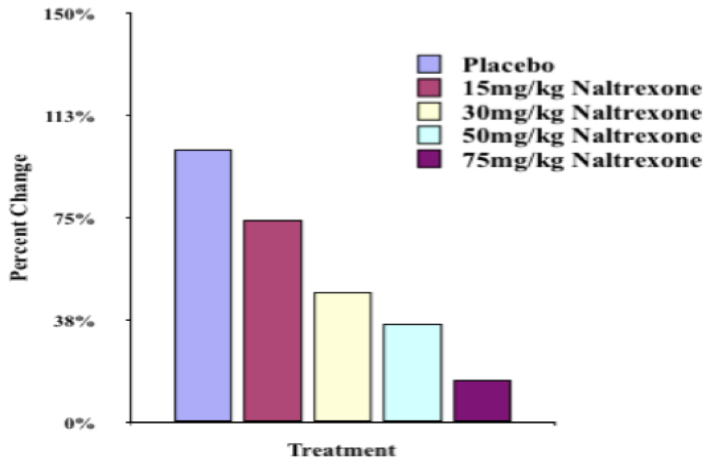


Compound	α1	α2	α3	α5	α6 nM
βCCt	0.72	15	18.9	111	>5,000
3-PβC	5.3	52.3	68.8	591	>1,000
3-EBC	6.43	25.1	28.2	826	>1,000
Zolpidem	26.7	156	383	10,000	>10,000
Diazepam	14	20	15	11	>3,000

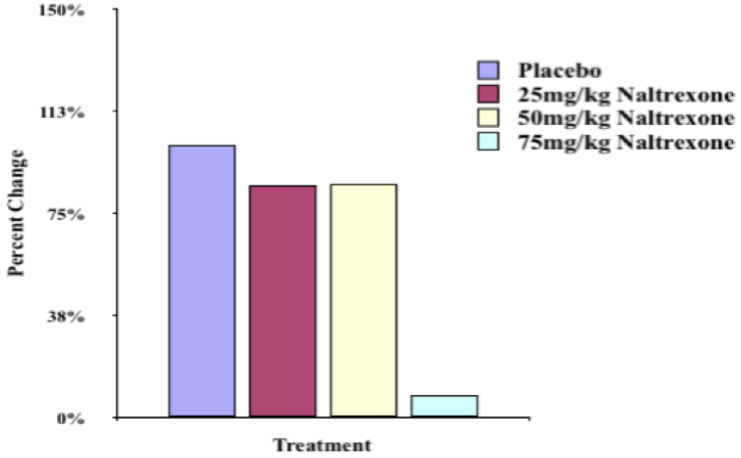
- [^{3}H] competition binding with flumazenil binding to Ltk- cells expressing human receptors
- βCCt and 3-PβC bind 10-fold or greater more selectively to the alpha-1 receptor ($\alpha_1\beta_{(2/3)}\gamma_2$)

Alcohol Self-Administration Studies in Rats: Naltrexone control tests

Percent Change of Responding for EtOH following Gavage of Naltrexone

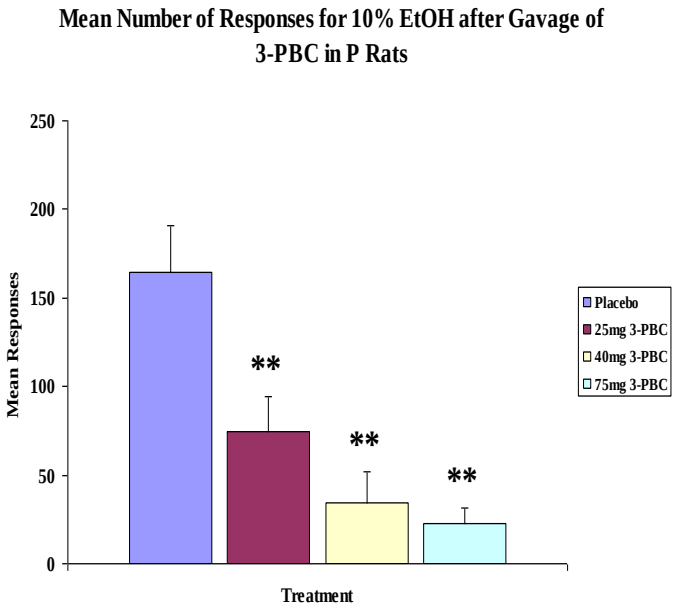


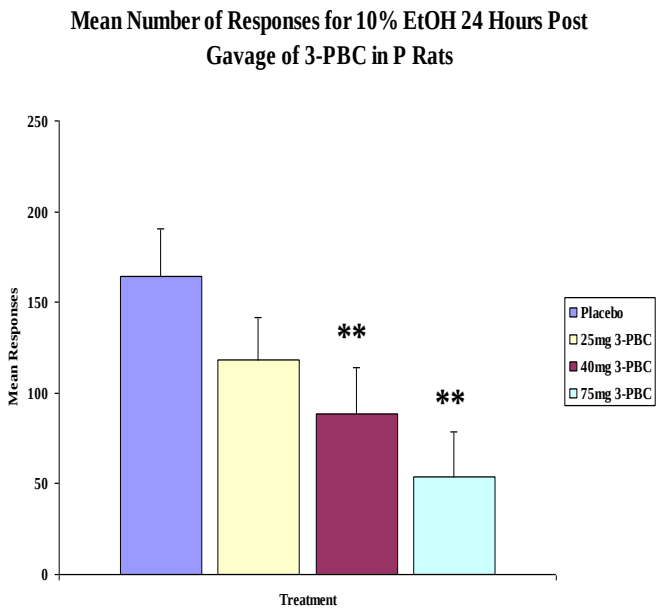
Percent Change of Responding for Sucrose following Gavage of Naltrexone



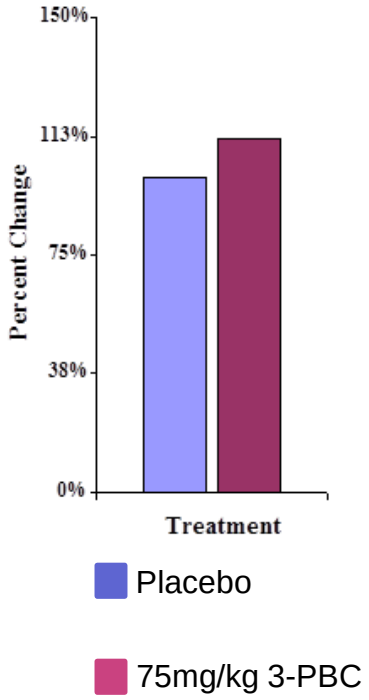
- Naltrexone is currently used as a treatment in alcohol addiction
- Increasing levels of naltrexone leads to less self-administration of ethanol by rats
- The highest dose of naltrexone also leads to less intake of sucrose, most likely due to the dysphoric and anxiogenic effects of the drug

Less Self-Administration with 3-PβC



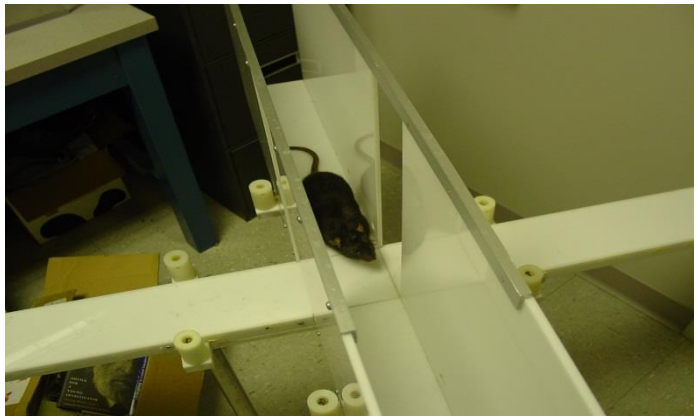


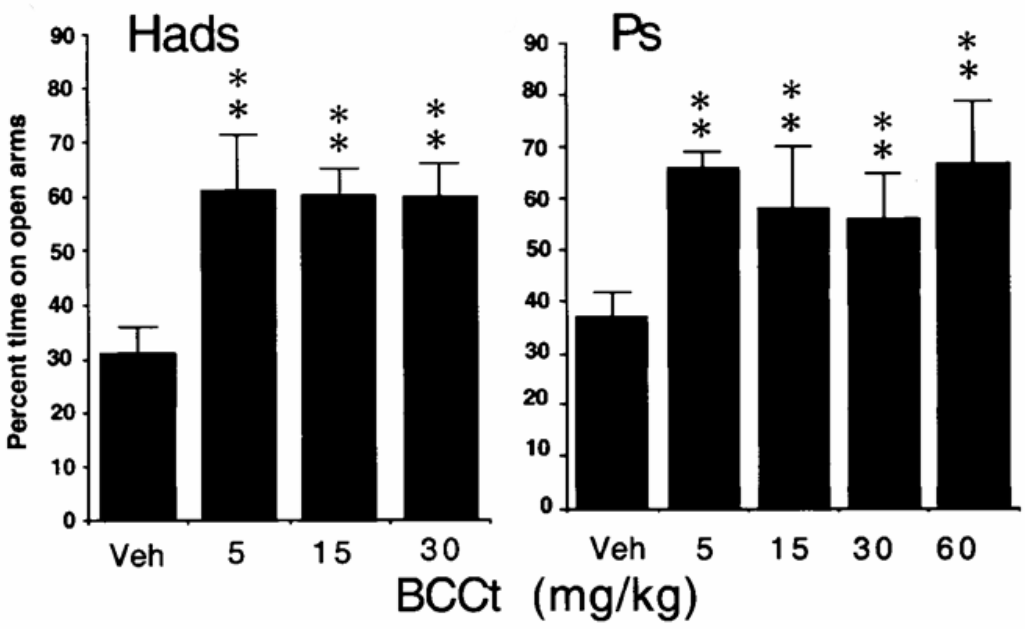
Control: % Change of response to sucrose



- The mean number of responses in rats to 10% ethanol after oral gavage of 3-PBC was significantly lower compared to the placebo treated rats
- No sedative effect was observed at the highest dose

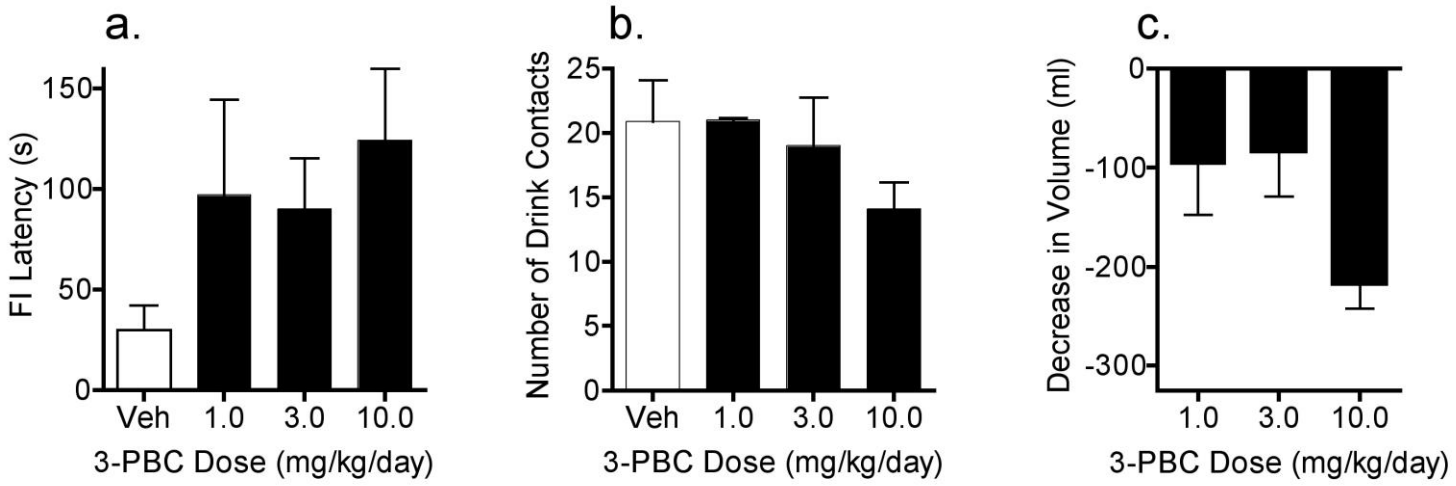
Reduced Anxiety with βCCt treatment





- In mouse EPM (elevated plus maze), systemic injection of βCCt led to mice spending more time crossing the open and unprotected arms of the maze indicating lower levels of anxiety
- (Had and P rats)
- βCCt had little effect on anxiety in normal, control rats

3-PβC Reduced Consumption of Alcohol



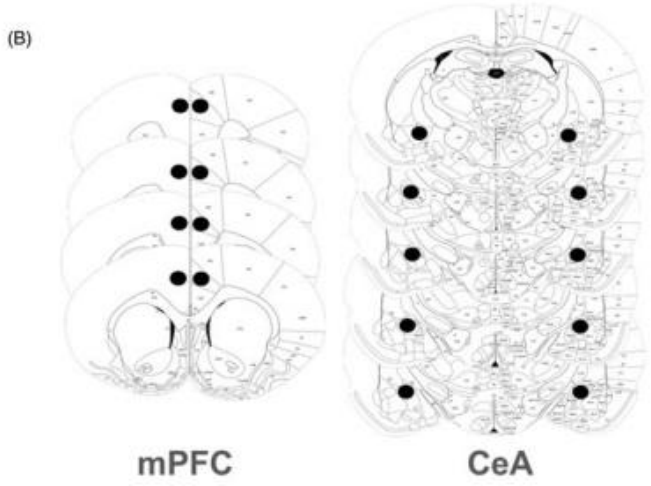
- Baboons treated chronically with 3-PβC · HCl consumed less alcohol compared to untreated controls
- Administration of 3-PβC reduced alcohol-seeking consumption and craving

Early Life Stress and Binge Drinking/Impulsivity

- The stress in the early life stages will have a huge impact on impulsive behavior and substance use disorders in the adulthood
- Maternally separated (MS) rats in the pre-weaning period are used a model to study the effect childhood stress on binge drinking and impulsivity
- Here we propose, MS increase the $GABA_A$ α2 receptor expression in addition to the elevated levels of corticotrophin releasing factor (CRF)
- To know the role played by these mechanism, novel $GABA_A$ α2 sub unit ligand 3-PβC were directly infused to central amygdala (CeA) and medial prefrontal cortex (mPFC) of the brain
- **3-PβC has shown a positive effect by decreasing the impulsive nature and binge drinking without effecting the sucrose responding**

Formulation and Drug administration

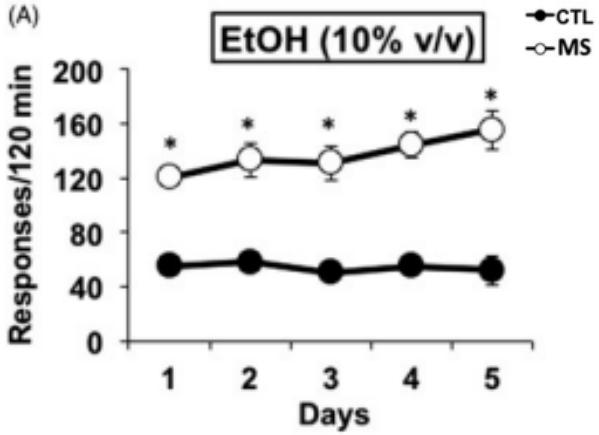
- MS rats are anesthetized with isoflurane/oxygen gas inhalation and were placed in stereotaxic apparatus for bilateral implantation of canulae through which the drug is given into the CeA or mPFC.
- Two drugs are mixed in 1 mL PBS and tween 20 was added drop wise until the drug dissolved
- Infused at a rate of 0.1 µL/min up to 5 minute
- Antalarmin 2 µg and 4 µg and 3-PBC 20 or 40 µg



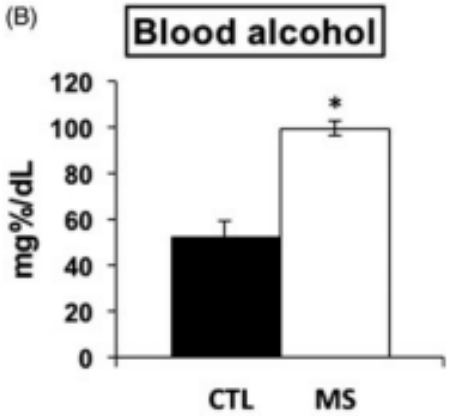
Cannula implantation location in mPFC and CeA

Gondre-Lewis *et al*. *Stress* **2016**, *19*, 235

MS Rats Drink More



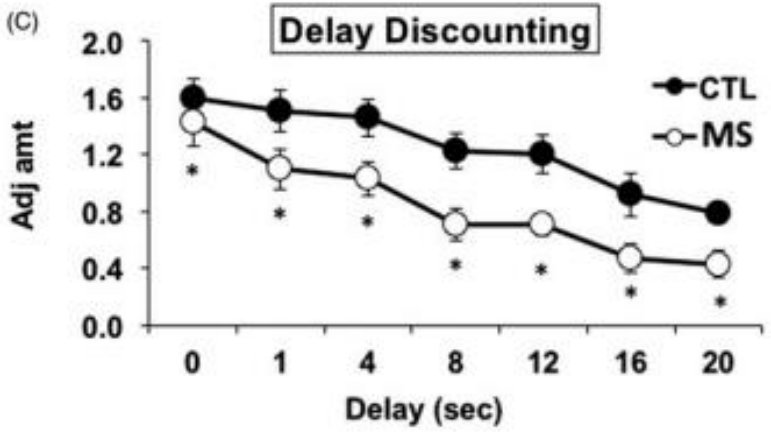
Responding for alcohol in MS and CTL rats



Blood Alcohol Concentration in rats after 2 hrs.

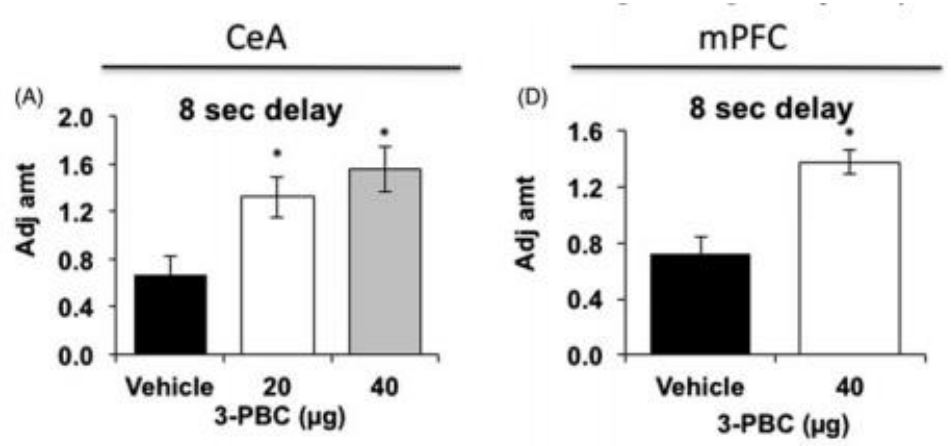
Control rats

Maternal separation rats

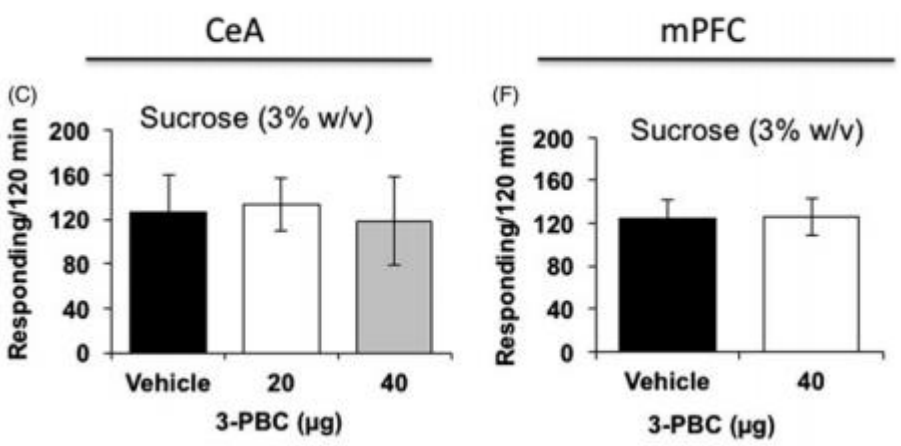


Operant responding to Delay Discounting

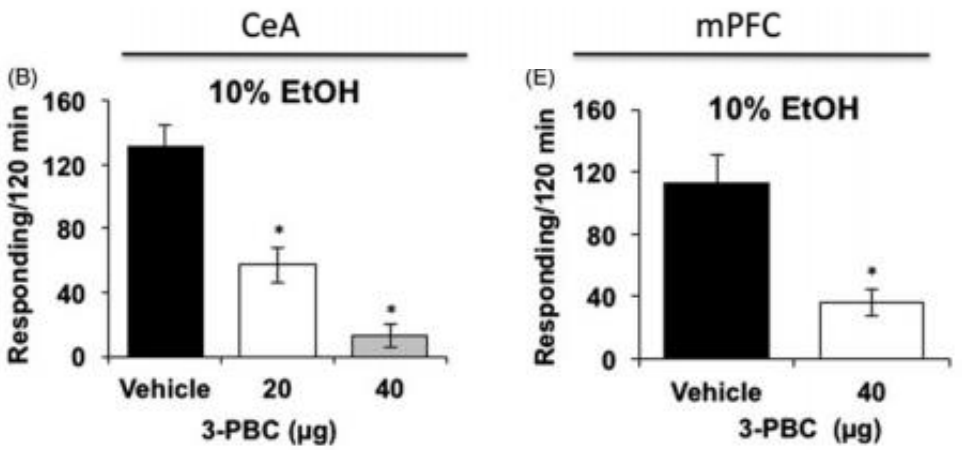
3-PBC Prevents Binge Drinking and Impulsivity



Delay discounting response for Impulsivity



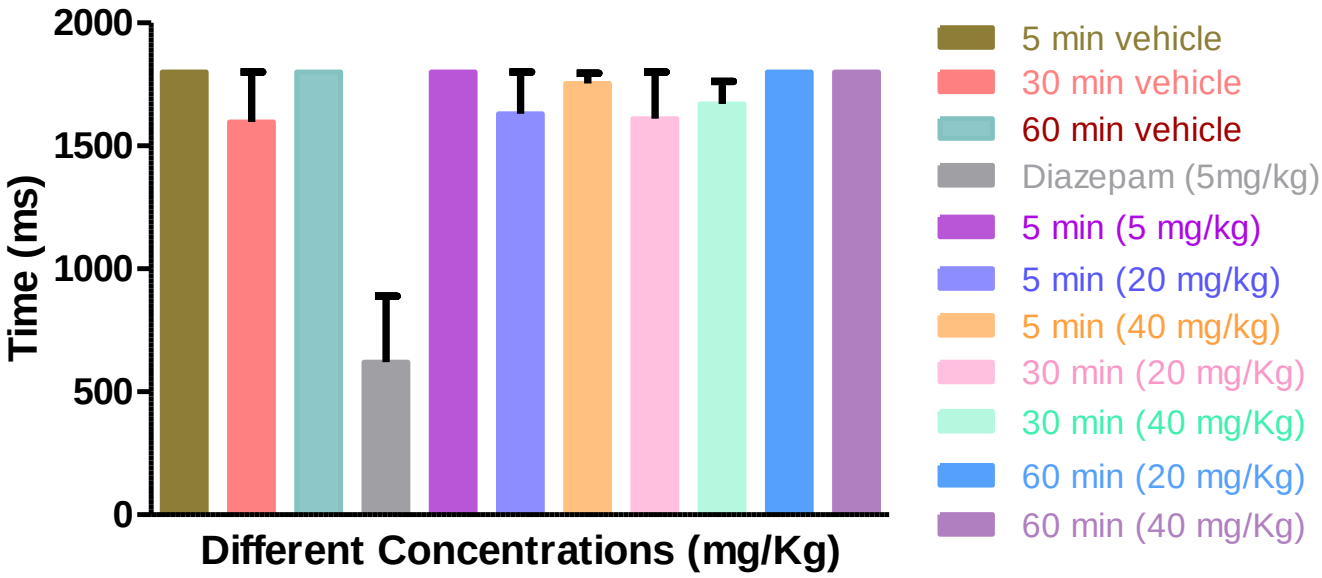
Effect of 3-PBC on operant responding for Sucrose

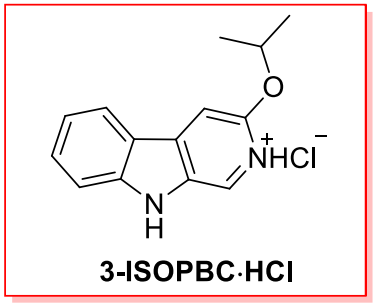


Effect of 3-PBC on operant responding for alcohol

- Increases the delay discounting time for impulsivity
- Decreases the response for alcohol without effecting the sucrose drinking response

Rotarod Results for 3-ISOPBC·HCl

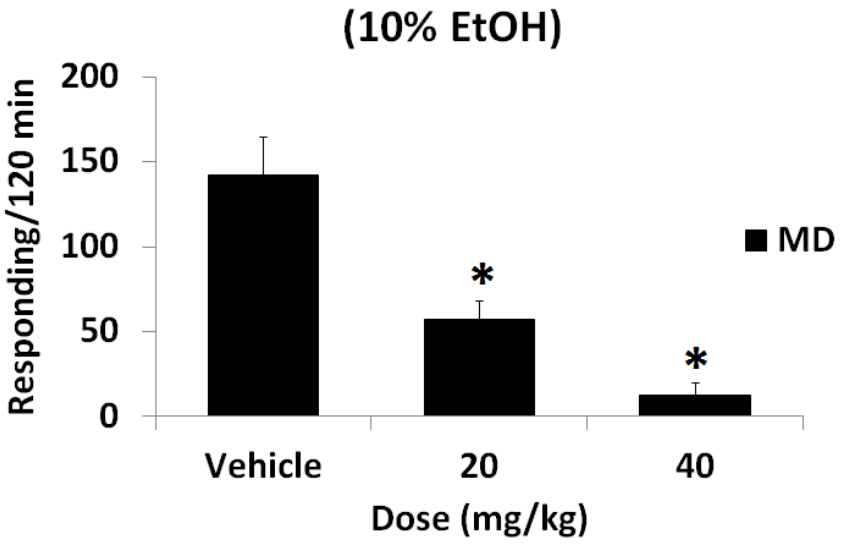




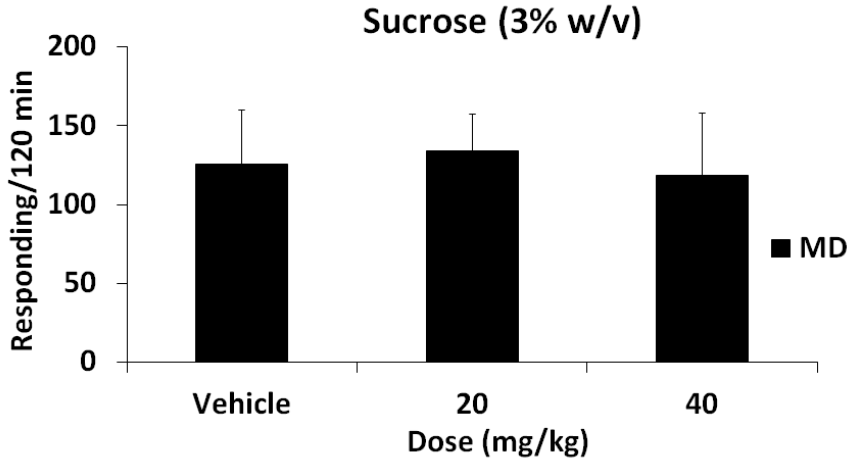
3-ISOPBC·HCl

- Rotorod tests for sedative effects of the compounds
- No failures observed even at higher doses of 20 and 40 mg/kg in mice versus diazepam control
- No adverse side effects were observed like sedation, ataxia and loss of righting reflex

3-ISOPBC·HCl Prevents Binge Drinking in MS Rats



Responding for alcohol is decreased by 3-ISOPBC·HCl in MD rats



Responding for sucrose is not affected by 3-ISOPBC·HCl in MD rats

Summary

- The results of the present study demonstrate that the α1 selective ligands βCCt, 3-PBC and 3-ISOPBC are highly effective in suppressing ethanol responding in P and HAD rats
- βCCt, 3-PBC, and 3-ISOPBC are highly effective anti-anxiety agents in P and HAD rats but not normal rats
- βCCt and 3-PBC are equally effective orally as Naltrexone when administered acutely; furthermore, βCCt and 3-PBC remain effective 24 hours post administration, unlike Naltrexone
- The α1 receptor clearly plays a role in alcohol-motivated behaviors; however, it also plays a role in normal ingestive behaviors
- **βCCt, 3-PBC and 3-ISOPBC may represent prototypes of novel pharmacotherapeutic agents that can be used to treat alcohol abuse and alcoholism**
- **This regiospecific two-step synthetic protocol is very useful to capable of scale-up to multigram quantities and were performed on 50 gram scale level for *in vivo* biology**

Next Steps

- New analogs have been designed and have resulted in the synthesis of aza-beta carbolines available as HCl salts
- Two lead aza-beta carbolines related to βCCt, 3-PβC and 3-ISOPβC also decrease alcohol self-administration in rats
- A series of aza-beta carbolines have been designed to execute an SAR and vary the cLogP to enhance the blood brain barrier permeability
- Collaborators at Johns Hopkins Univ. are conducting primate studies
- **Looking for a development partner to test the best current compounds in primate models of alcohol consumption and craving, and to expand the SAR to look for new effective agents or back-up compounds**