Standardized Laboratory Protocols
For INIAstress

• Chronic Ethanol Exposure by Inhalation Route and Dependence Testing (HIC)

• Ethanol Drinking Behavior: Home-Cage Limited Access Paradigm

• Operant Ethanol Self-Administration and Reinstatement Model
Features of Chronic Ethanol Exposure
By Inhalation Route

- √ dependence can be established in a relatively brief period of time
- √ health of subjects maintained during intoxication period (loss of body weight & body temperature moderate)
- √ duration of ethanol exposure and time of withdrawal more precisely controlled
- √ level of intoxication (BEC) can be maintained relatively stable during the course of intoxication, as well as from one cycle of exposure to another
Chronic Ethanol Exposure: Vapor Inhalation Chambers

~ Methods ~

• Plexiglas chamber holding individually housed mice

• Fresh air combined with vaporized ethanol

• Flow rate of EtOH vapor adjusted to attain blood EtOH concentration of 150-200 mg/dl
HANDLING-INDUCED CONVULSION (HIC) SCORING SCALE*

0 = no activity on tail lift or after gentle 360° spin
1 = facial grimace after 360° spin
2 = tonic convulsion after 360° spin
3 = tonic/clonic convulsion after 360° spin
4 = tonic convulsion on tail lift
5 = tonic/clonic convulsion on tail lift; delayed onset
6 = severe tonic/clonic convulsion; no delay
7 = severe tonic/clonic convulsion prior to tail lift

* modified after Crabbe and Kosobud (1990)
Multiple Cycles of Chronic Ethanol Exposure and Withdrawal Results in Exacerbation of Withdrawal-Related Seizure (HIC) Activity
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Ethanol Drinking Behavior: Home-Cage Limited Access Model

Training Procedure

* Mice are individually housed
* No food or water deprivation
* Access to ethanol in home cage 2 hr/day
* Daily limited access sessions begin 0.5 hr prior to dark cycle
* Sucrose fading procedure utilized to stabilize intake of 15% (v/v) EtOH
EtOH Intake Under Limited Access Conditions (2 hr/day) in C57BL/6J Mice

EtOH Intake = 2.9 ± 0.2 g/kg; BEC = 59.6 ± 14.1 mg/dl
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Operant Ethanol Self-Administration and Reinstatement Model

Training Procedure

* Mice are individually housed
* No food or water deprivation
* Mice trained to lever respond (FR1->FR4) for reinforcement in 15 min sessions
* Sucrose fading procedure used to stabilize responding/intake of 15% (v/v) EtOH
* EtOH reinforcement (20 µl) accompanied by tone and light
Ethanol Self-Administration in C57BL/6J Mice

Responses

Reinforcers

Session

BEC (mg/dl)

EtOH Reinforcers

(20 ul 15% EtOH)
Operant Ethanol Self-Administration and Reinstatement Model

Training Procedure

* Establish stable baseline FR-4 responding for 15% EtOH reinforcement in daily 15 min sessions

* Test under extinction conditions: responding with no cues or reinforcement delivery for 4-6 daily sessions

* Reinstatement testing: assess effects of cues and/or stress exposure on responding under extinction conditions
Conditioned Cue-Induced Reinstatement of EtOH Responding