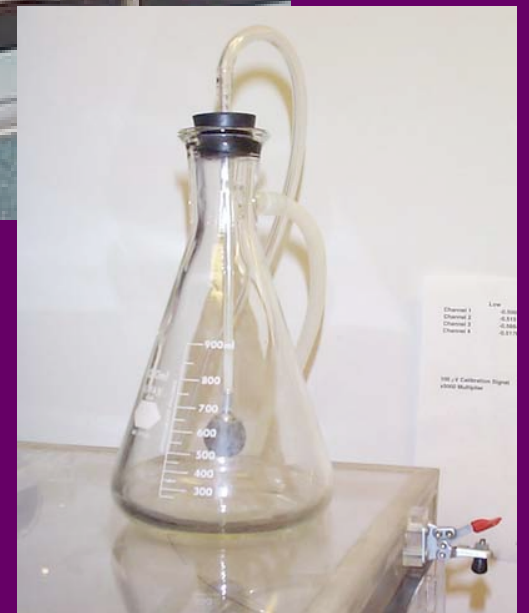


Standardized Laboratory Protocols For INIAstress

- **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

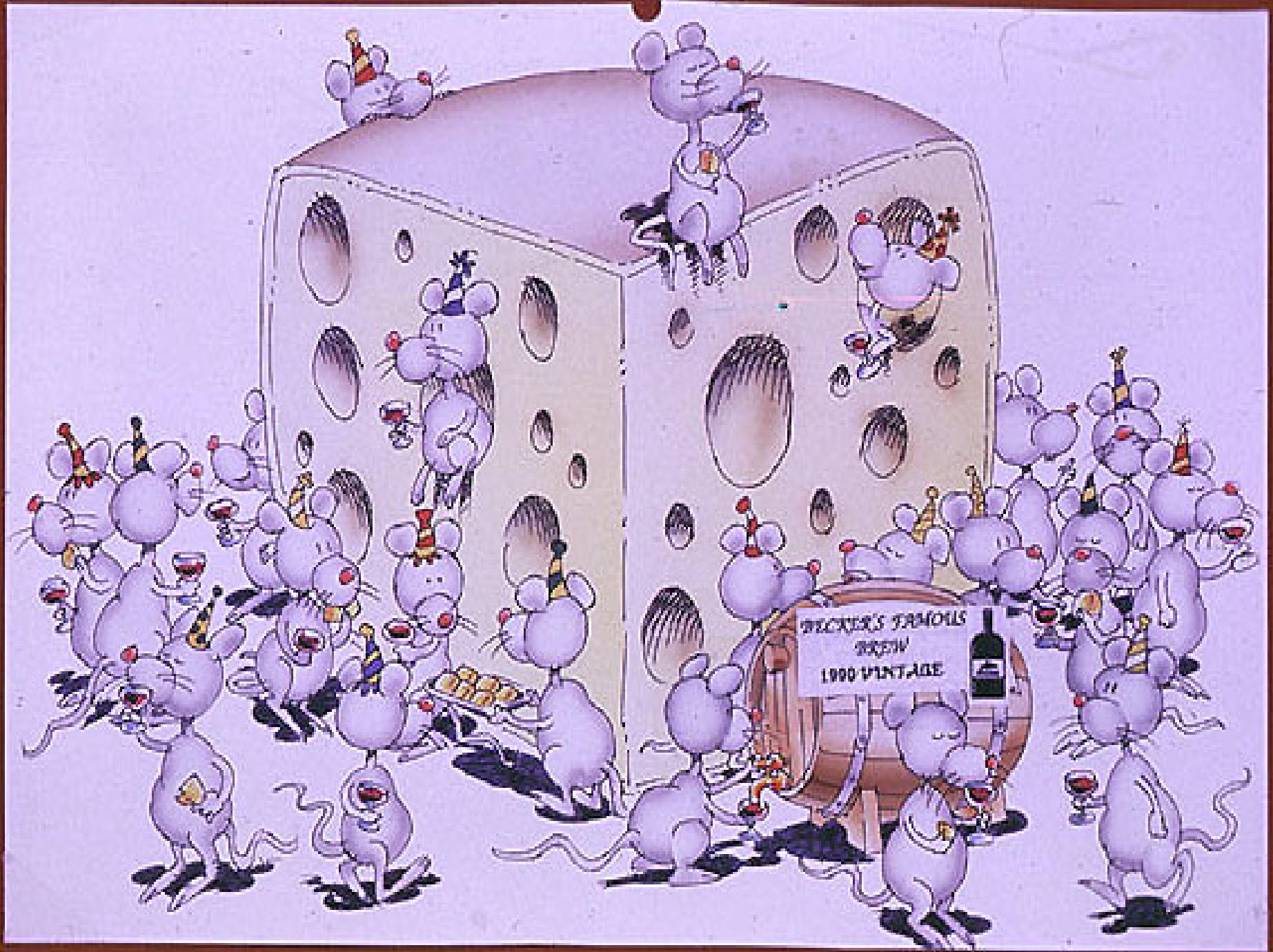
- ✓ **dependence can be established in a relatively brief period of time**
 - ✓ **health of subjects maintained during intoxication period (loss of body weight & body temperature moderate)**
 - ✓ **duration of ethanol exposure and time of withdrawal more precisely controlled**
 - ✓ **level of intoxication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



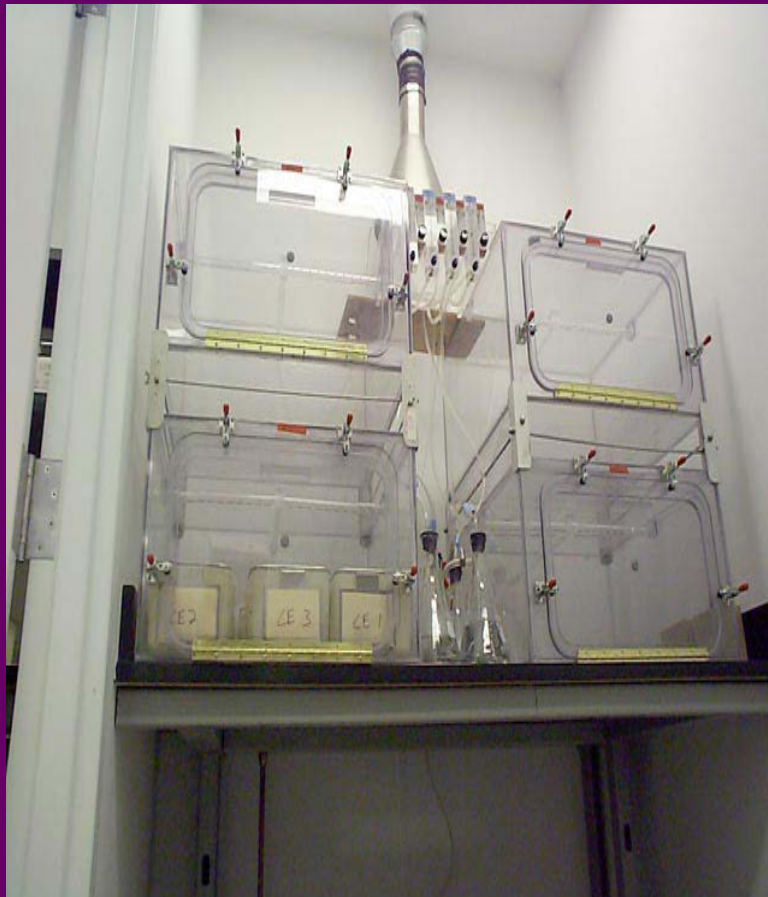
Channel	Low
Channel 1	0.000
Channel 2	0.010
Channel 3	0.020
Channel 4	0.030

100 µl Calibration Signal
1000 Multiplexer





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

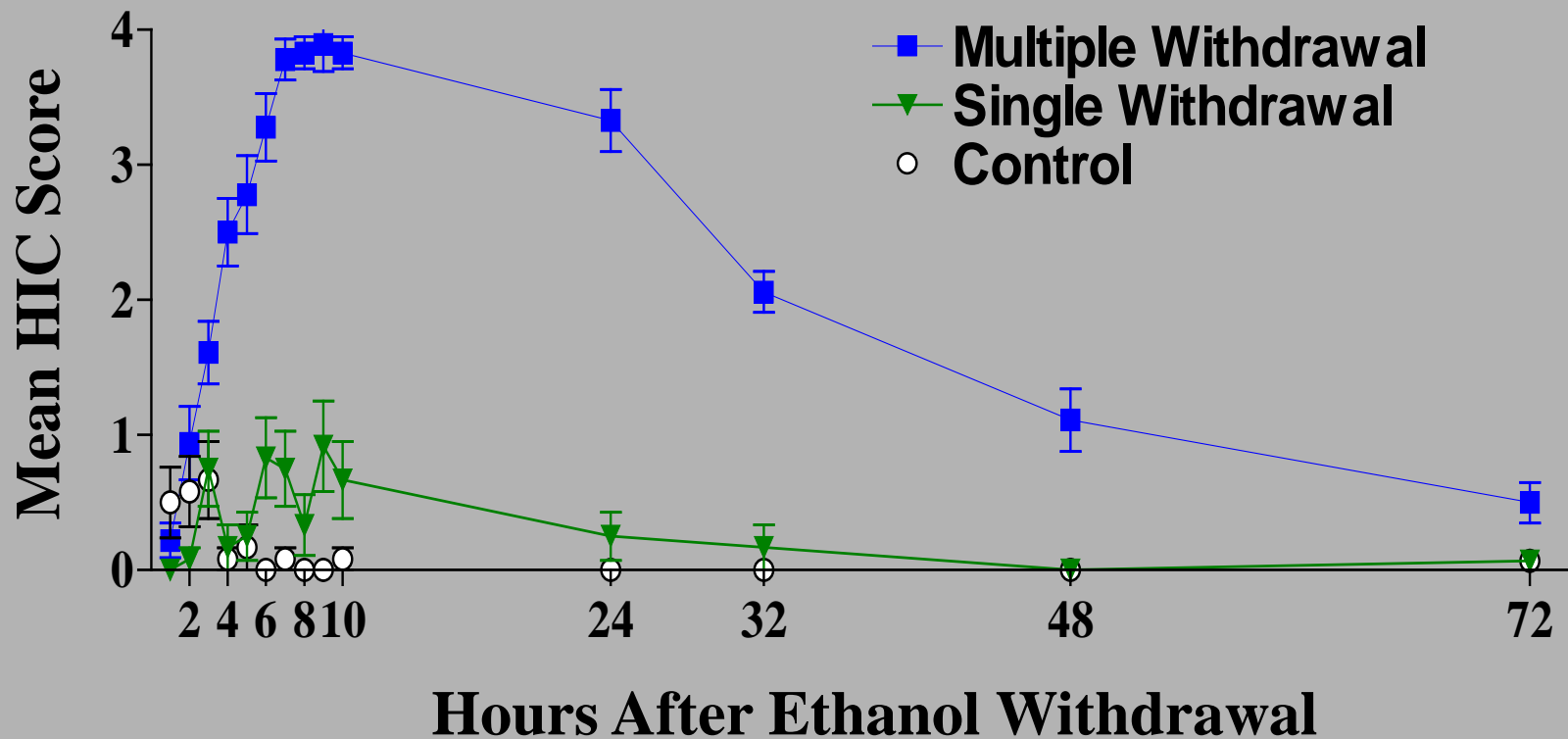
HANDLING-INDUCED CONVULSION (HIC) SCORING SCALE*



-
- 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



Standardized Laboratory Protocols For INIAstress

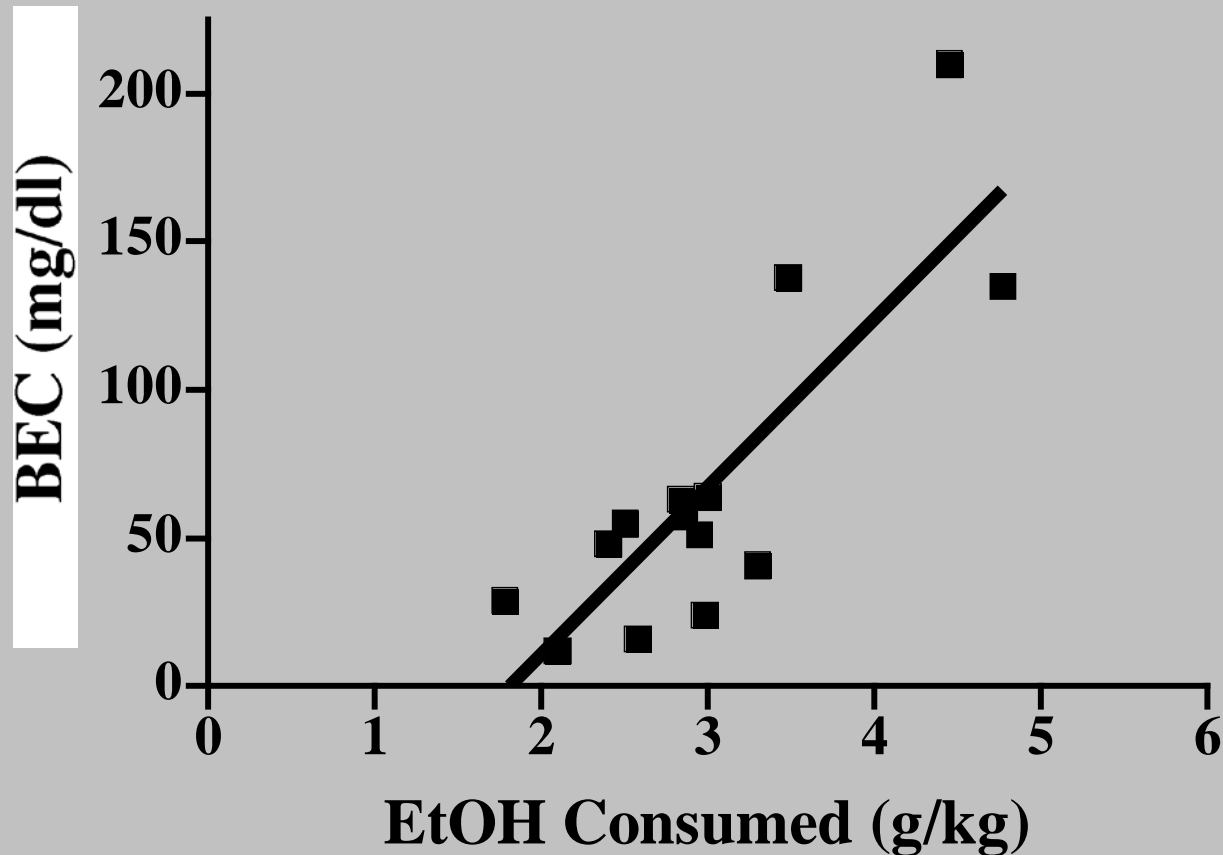
- **Chronic Ethanol Exposure by Inhalation Route and Dependence Testing (HIC)**
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Ethanol Drinking Behavior: Home-Cage Limited Access Model

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**
-
-

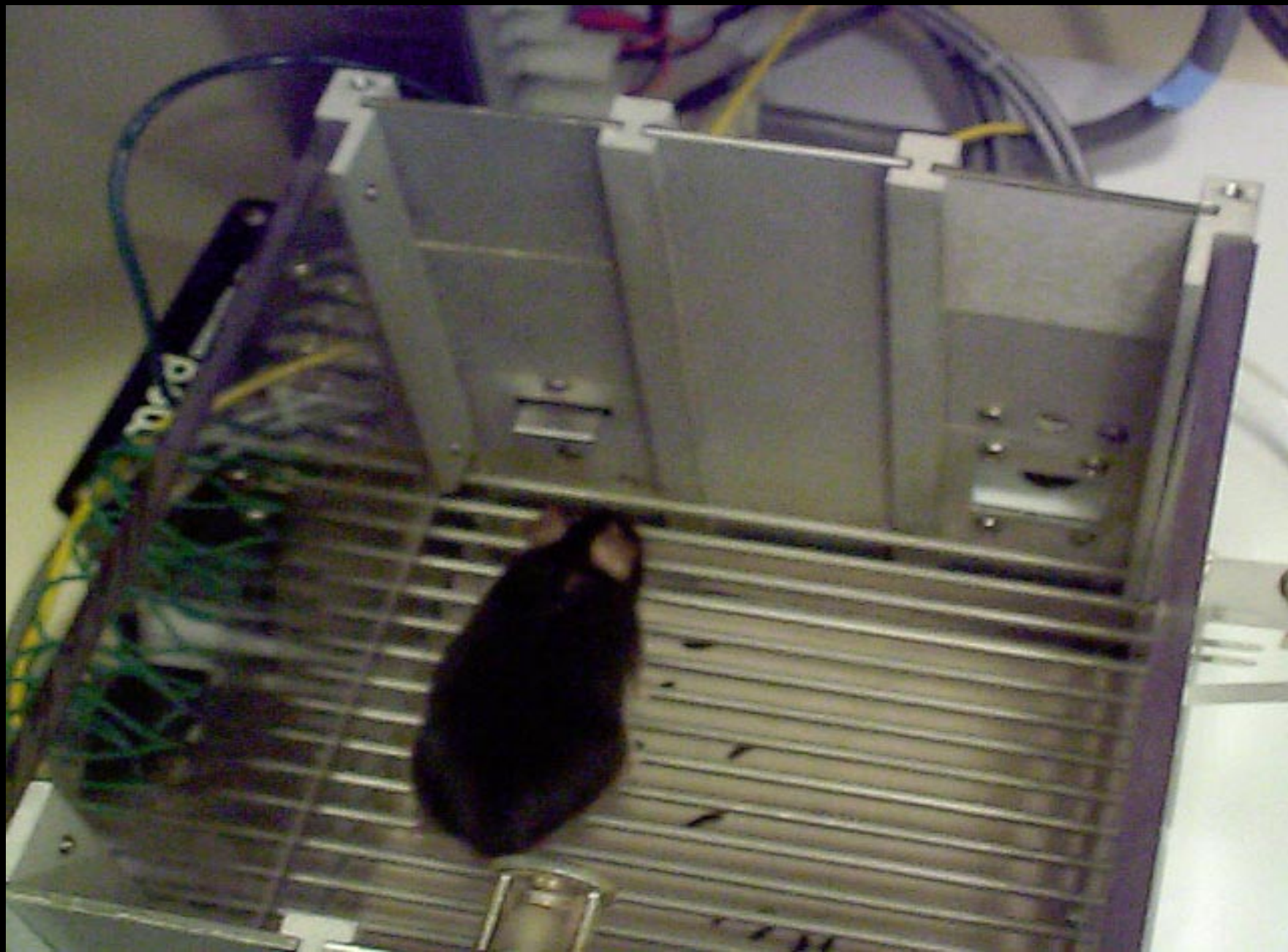
EtOH Intake Under Limited Access Conditions (2 hr/day) in C57BL/6J Mice



EtOH Intake= 2.9 ± 0.2 g/kg; BEC= 59.6 ± 14.1 mg/dl

Standardized Laboratory Protocols For INIAstress

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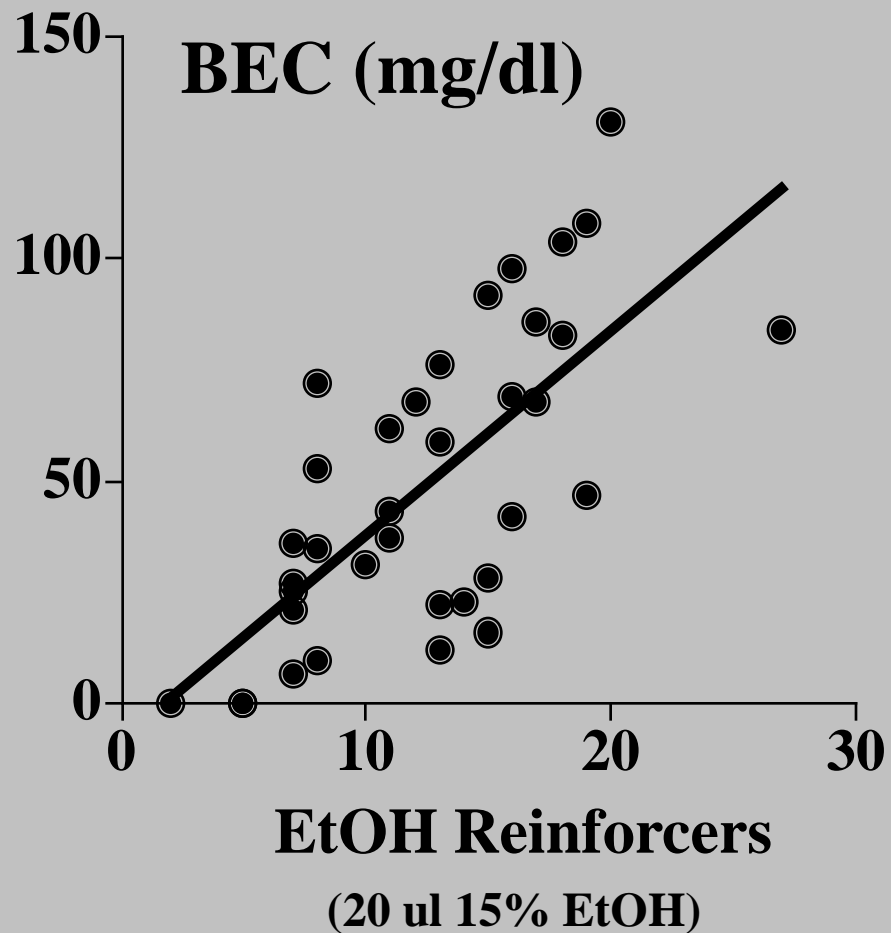
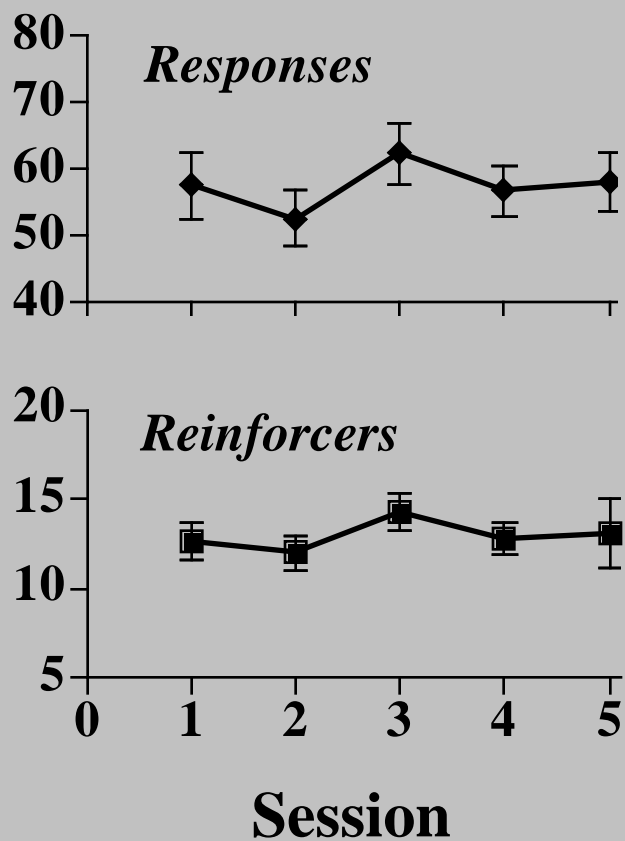


Operant Ethanol Self-Administration and Reinstatement Model

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 μ l) accompanied by tone and light
-
-

Ethanol Self-Administration in C57BL/6J Mice



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

