Changes in Striatal D_2 levels following chronic alcohol self-administration

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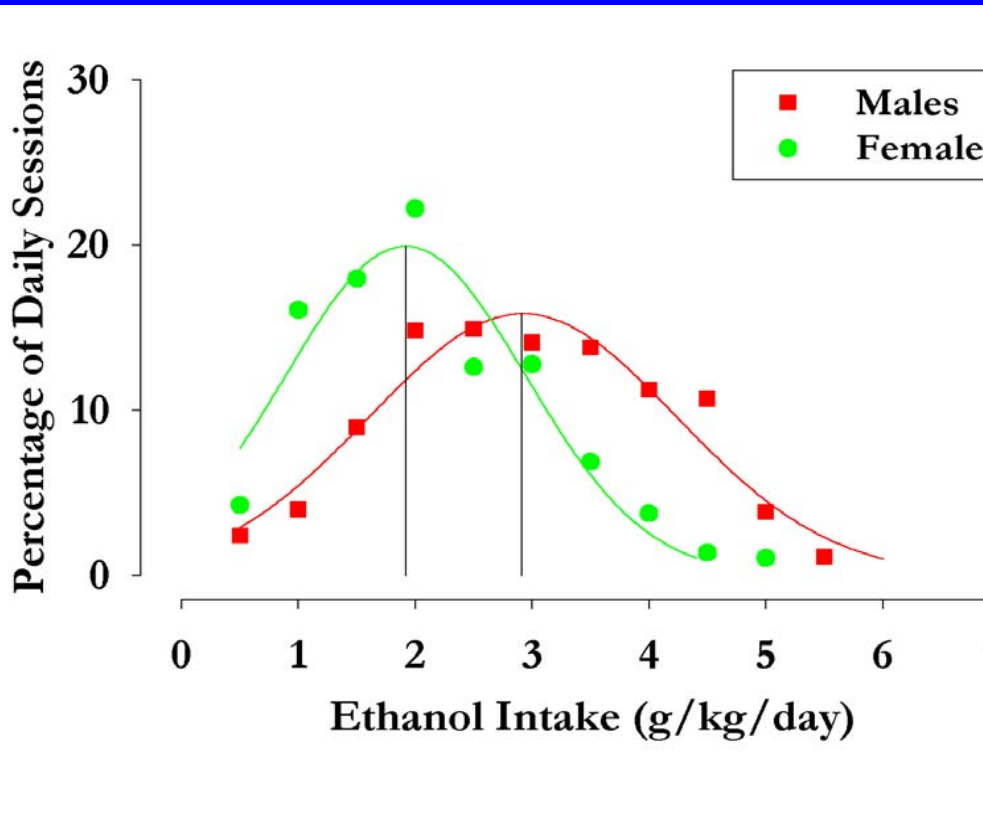
Introduction

Studies in human alcoholics have shown a decrease in dopamine D_2 receptors in the striatum as measured by positron emission tomagraphy (PET) of raclopride (Volkow et. al, 1996, Hietala et. al, 1994).

Explored hypothesis that binding potential of D_2 receptors would be lowered in population of heavy drinking cynomolgus monkeys

Model of Excessive Alcohol Drinking



- Population of adult cynomolgus monkeys (*Macaca fascicularis*) exposed to daily 22-hr oral ethanol self-administration sessions
- Effective method to identify individual monkeys that have ethanol intakes in the categorical level of heavy-to-abusive drinking

Experimental Design

Induction of ethanol

30 days water
30 days 0.5 g/kg ethanol
30 days 1.0 g/kg ethanol
30 days 1.5 g/kg ethanol

Choice between 4% (v/v) ethanol and water

16-22 hr sessions: 6 months

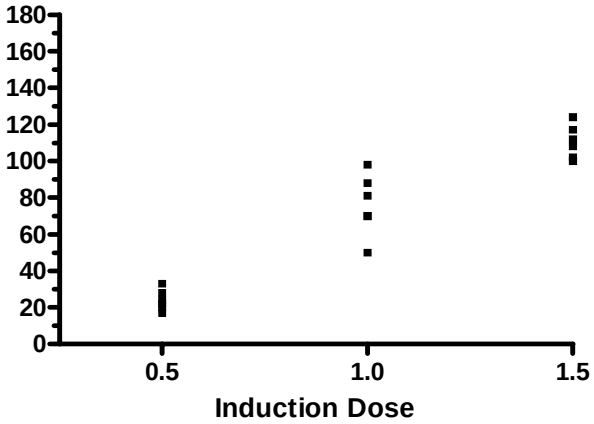
Abstinence: 12 months

22 hr sessions: 18 months

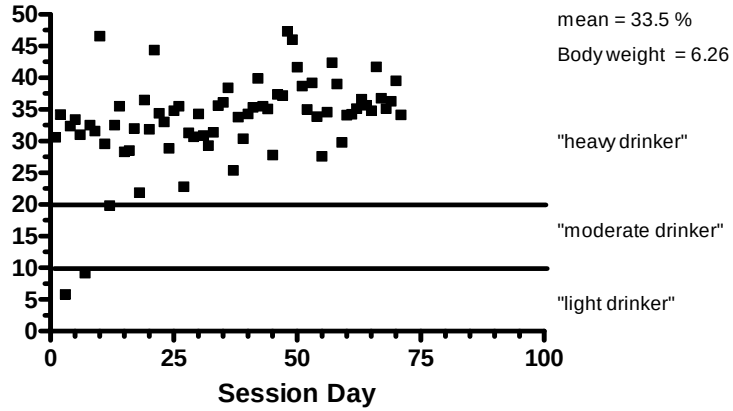
Necropsy

6887

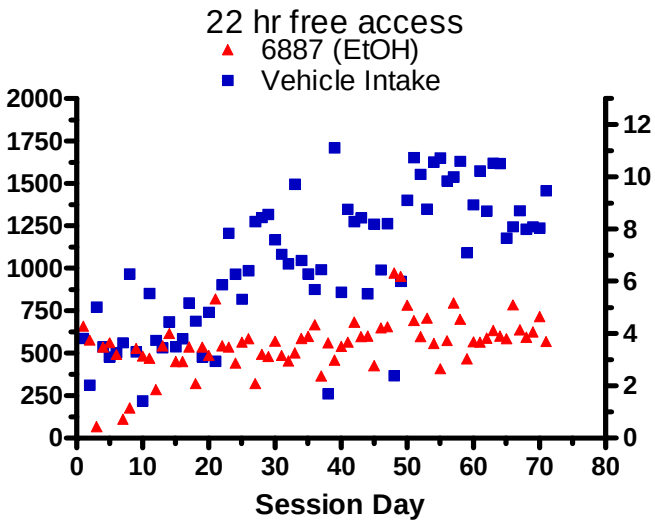
Blood Ethanol Concentration during Ethanol Induction



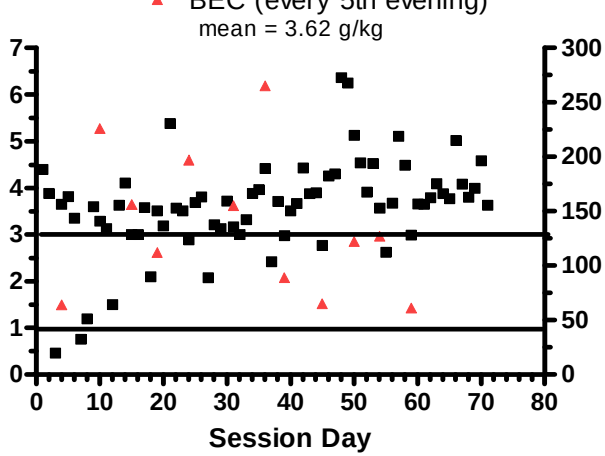
Percent of Calories from Ethanol during 22 hr access to EtOH

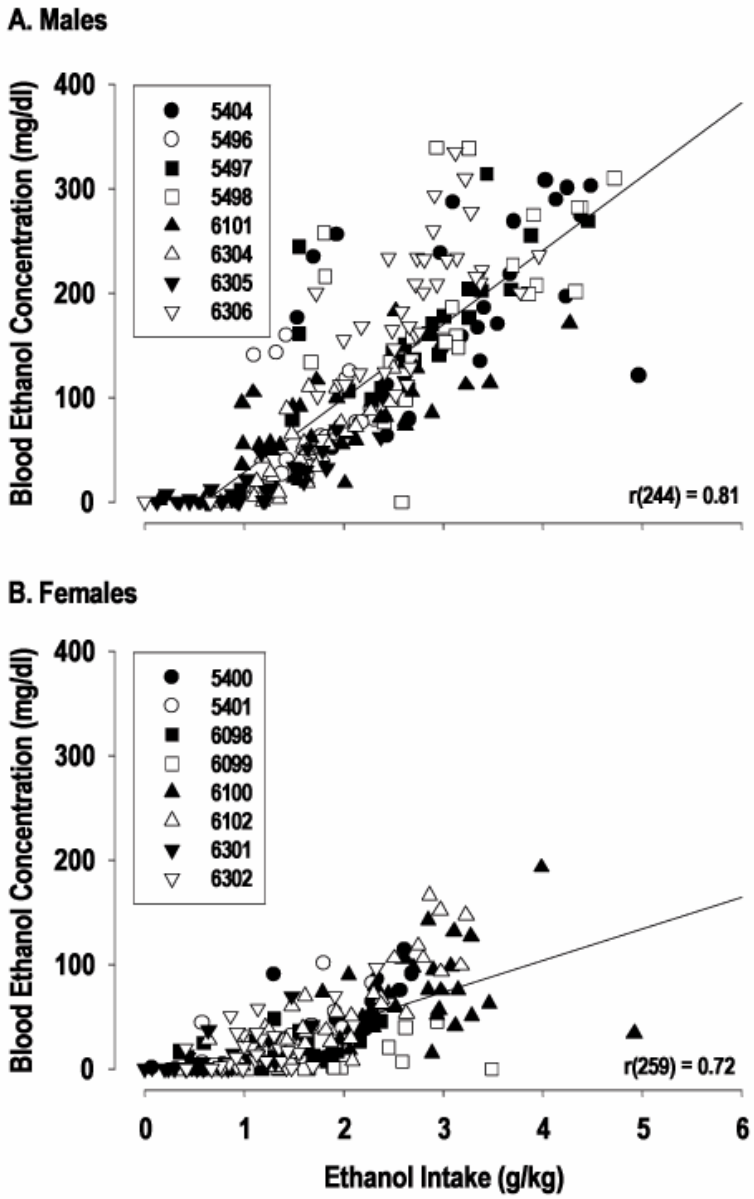


22 hr free access



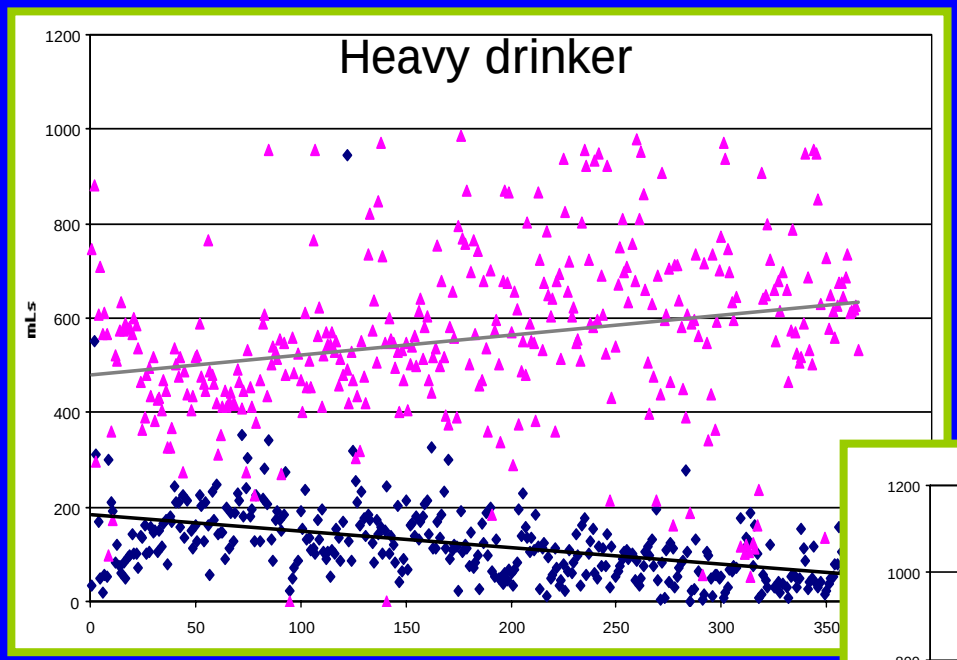
22 hr free access

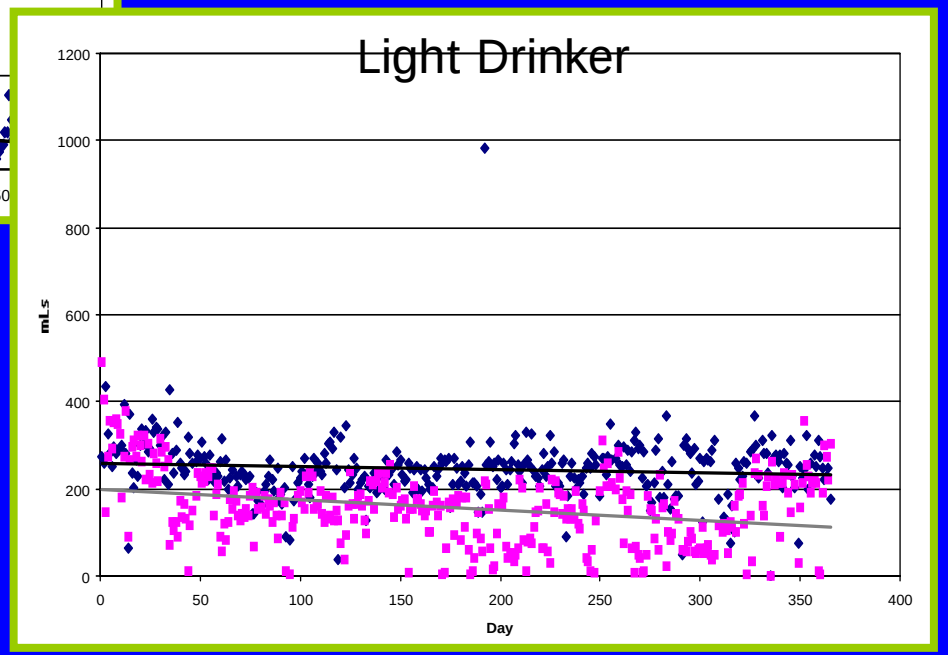



A. Males
Blood Ethanol Concentration (mg/dl)
400
300
200
100
0
5404
5496
5497
5498
6101
6304
6305
6306
r(244) = 0.81
B. Females
Blood Ethanol Concentration (mg/dl)
400
300
200
100
0
5400
5401
6098
6099
6100
6102
6301
6302
r(259) = 0.72
0
1
2
3
4
5
6
Ethanol Intake (g/kg)

Extreme Phenotypic Differences in Excessive Alcohol Drinking

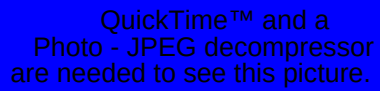
Volume consumed (mls)

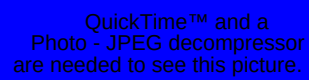




Daily drinking for 1 year

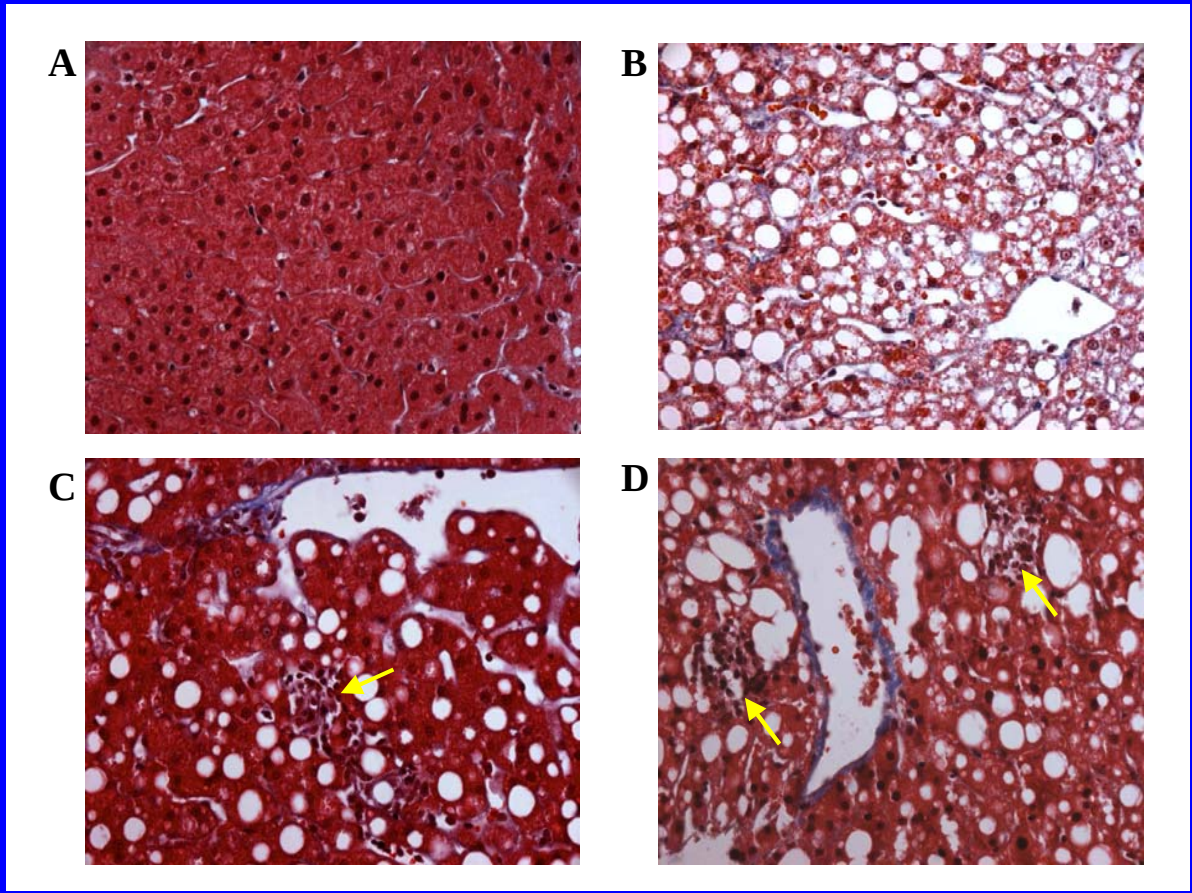
Control’s liver





Excessive Drinker’s liver

6 months abstinence

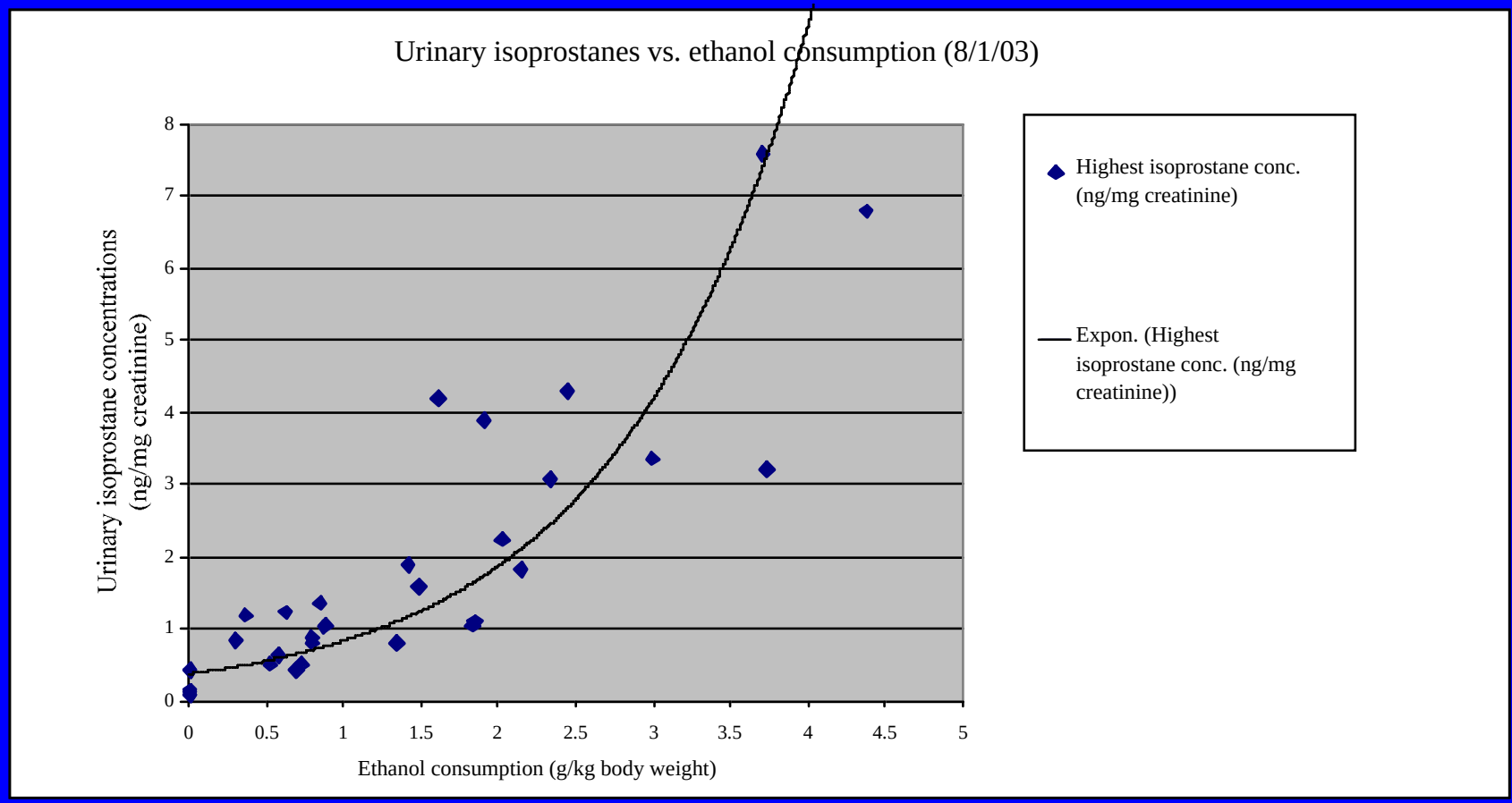


3 months Self-Admin

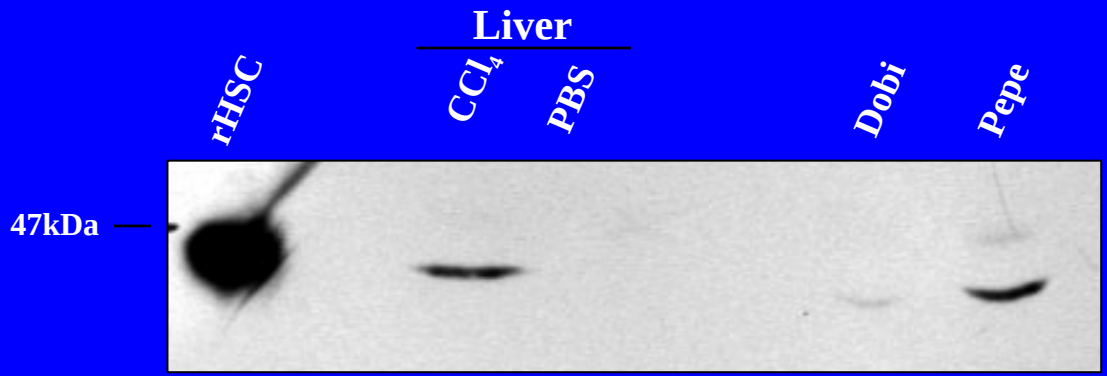
6 months Self-Admin

12 months Self-Admin

Figure 1. Progression of alcoholic liver disease in a self-administering male cynomologus monkey. These biopsy sections were stained with Masson's Trichrome and all are presented at 40x magnification. A. Abstinent from ethanol for 6 months. Liver has normal appearance. B. 3 months of ethanol consumption. Extensive fatty liver. C. 6 months of ethanol consumption. Yellow arrow indicates possible area of inflammation. Fat deposition was less than seen after 3 months consumption. D. 12 months of ethanol consumption. Yellow arrows indicate areas of possible inflammation and cellular necrosis. Fat deposition was less than seen after 3 months consumption.

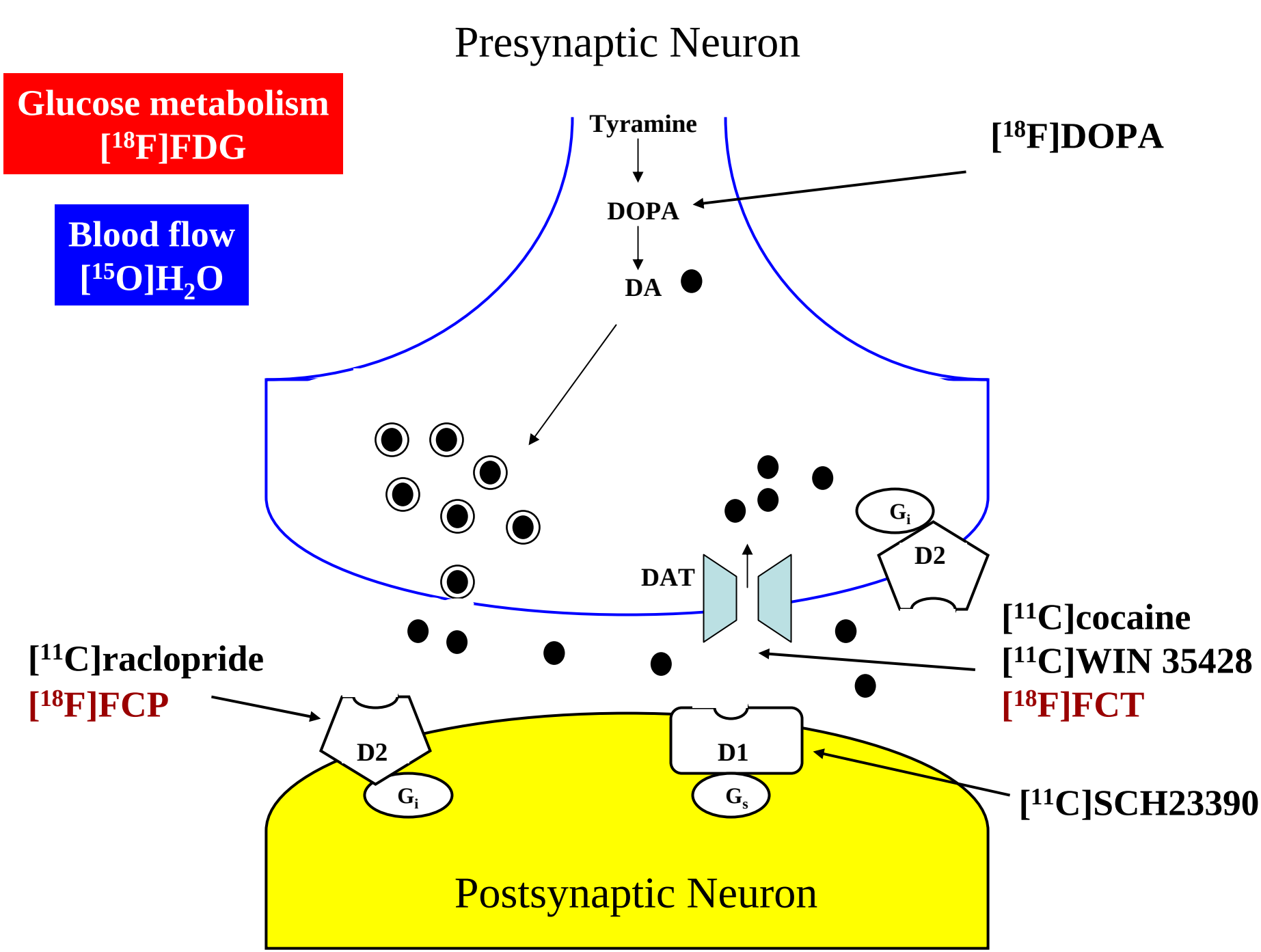


Smooth Muscle α-actin Expression



Methods

- Six male and six female cynomolgus monkeys (*Macaca fascicularis*)
- Monkeys scanned when they were:
  - naïve to ethanol (n=6)
  - actively drinking (n=12)
  - abstinent (n=12)
  - returned to 22-hr drinking (n=6)


Presynaptic Neuron
Glucose metabolism
[18F]FDG
Blood flow
[15O]H2O
Tyramine
DOPA
DA
[18F]DOPA
Gi
D2
DAT
[11C]cocaine
[11C]WIN 35428
[18F]FCT
[11C]raclopride
[18F]FCP
D2
Gi
D1
Gs
[11C]SCH23390
Postsynaptic Neuron

Methods

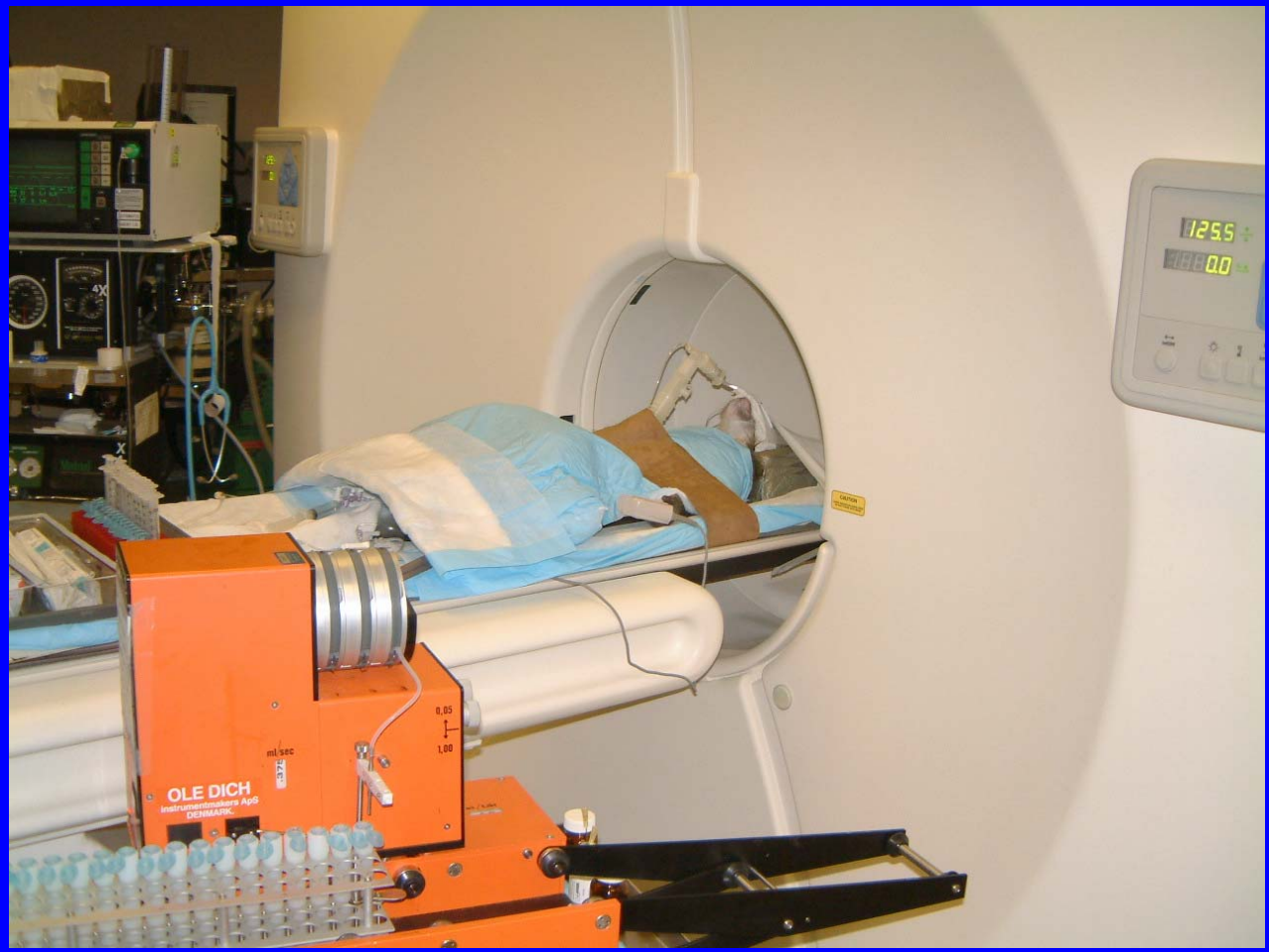
- D_2 receptor levels in the striatum measured using [^{18}F] flurocleboprideide
- Siemens CTI 951/31 PET scanner

Douglas Maynard

Mike Nader

Bob Mach
Don Gage
Richard Ehrenkaufer
Nancy Buckheimer

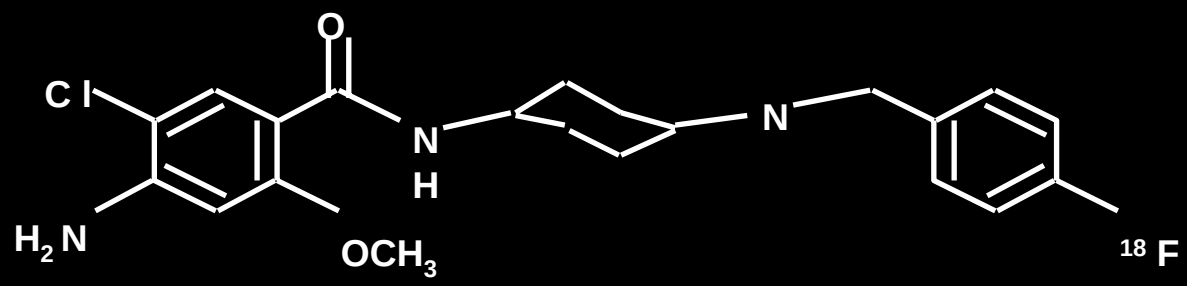
Mary Lou Voytko
Carol Shively
Jim Daunais



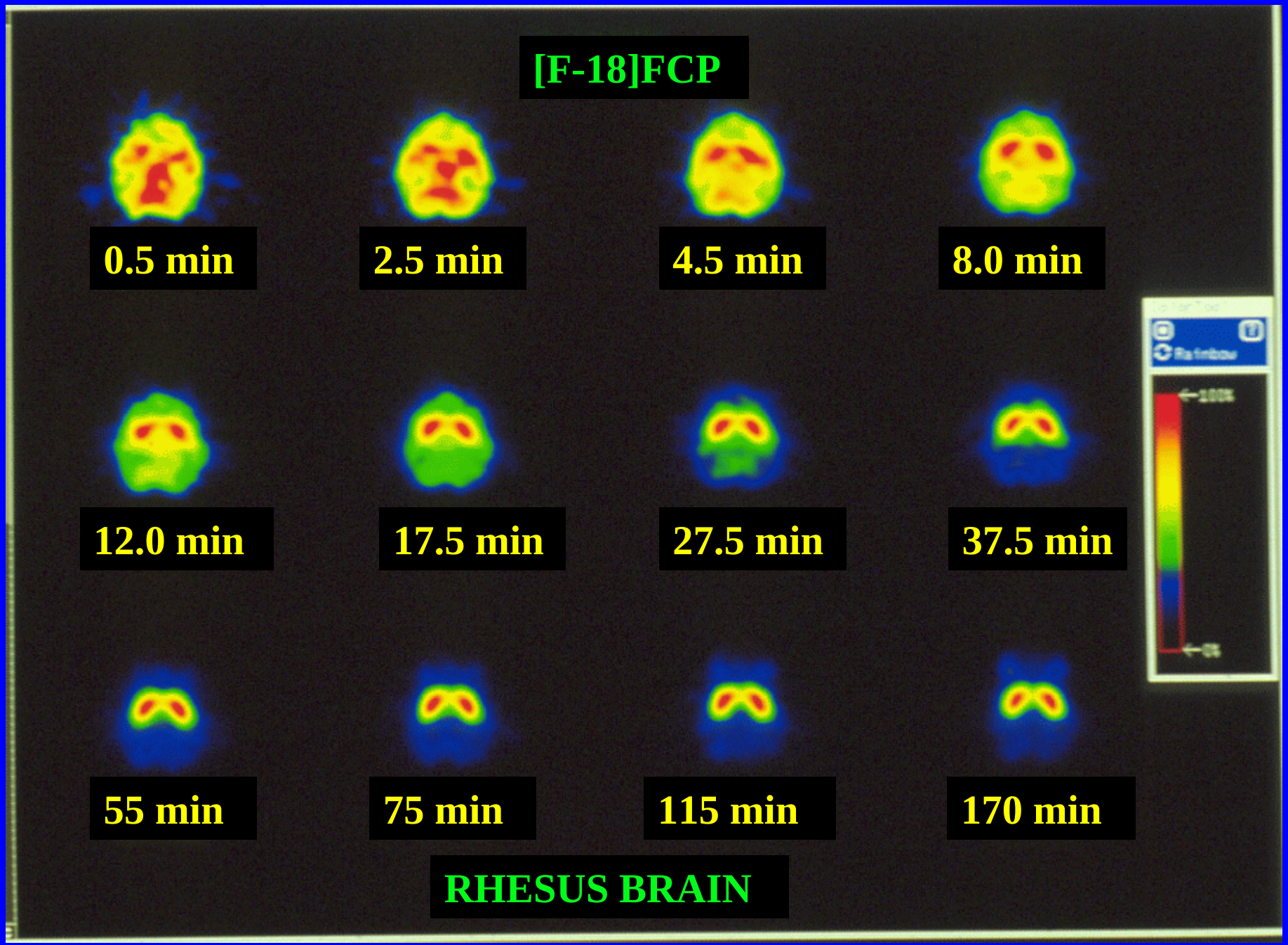
PET Radioligand

[^{18}F] Fluoroclebopride
D_2 receptor selective

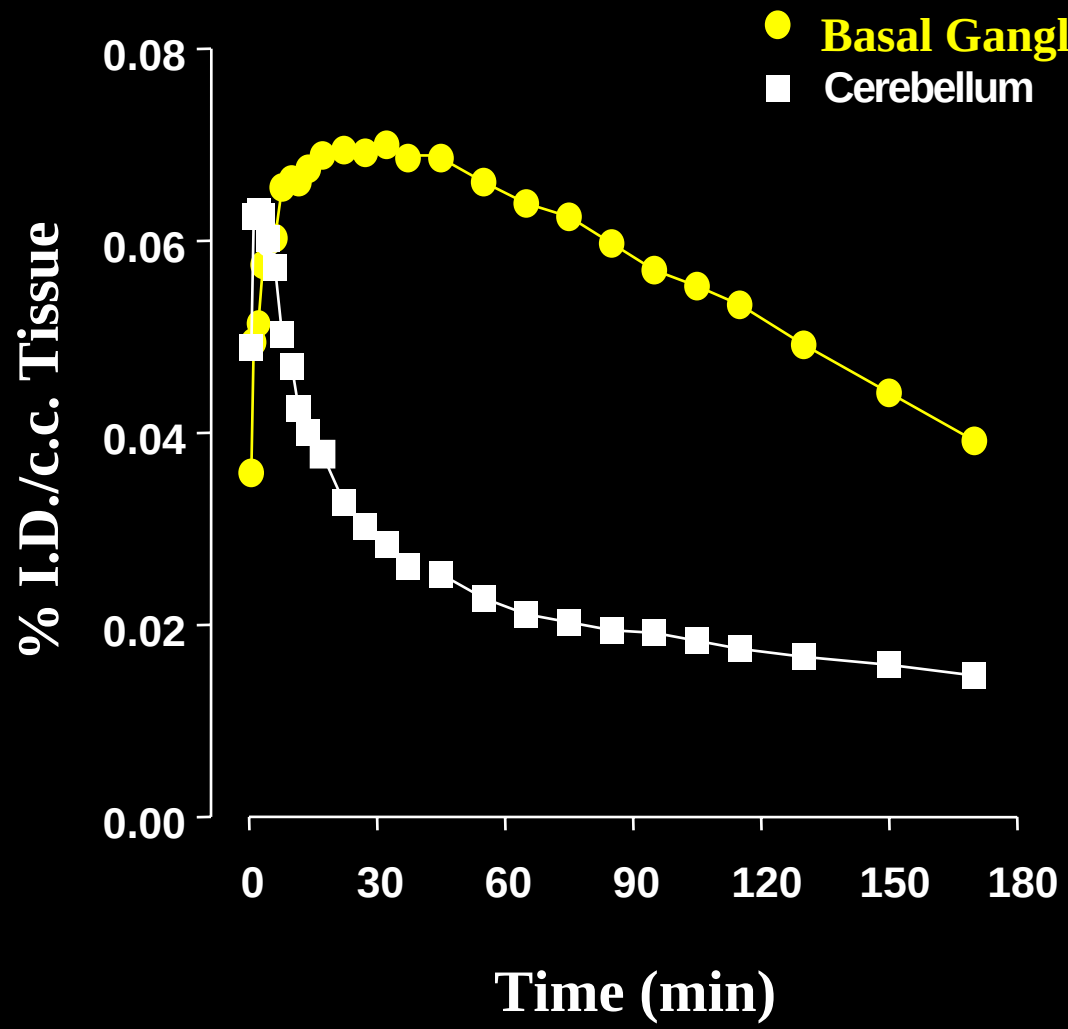
Reversible binding kinetics
Influenced by synaptic DA



Receptor	K_i [nM]
D_1	> 10,000
D_2	0.95 ± 0.22
D_3	5.46 ± 0.62
D_4	144 ± 21


[F-18]FCP
0.5 min
2.5 min
4.5 min
8.0 min
12.0 min
17.5 min
27.5 min
37.5 min
55 min
75 min
115 min
170 min
RHESUS BRAIN

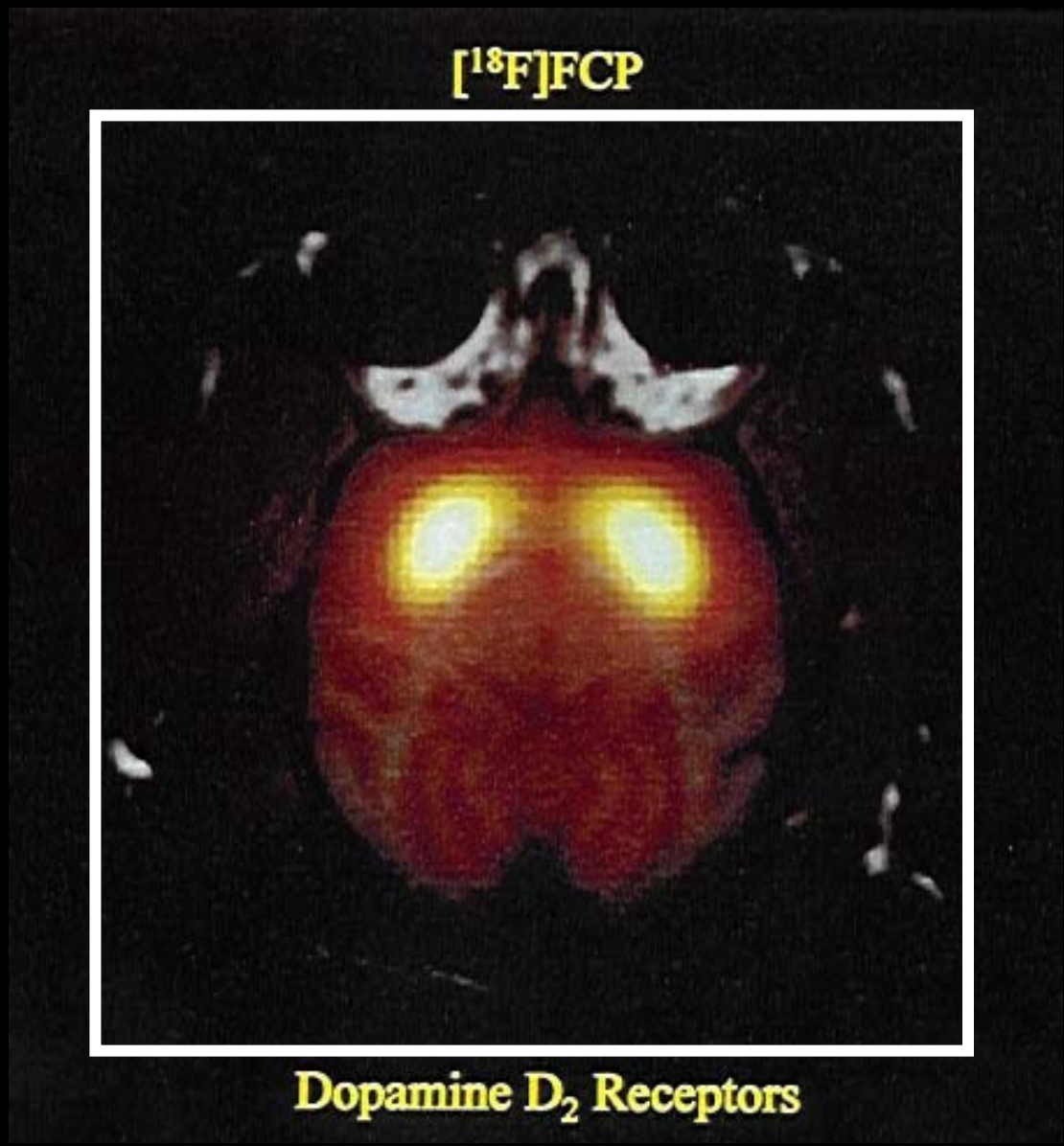
Tissue-time activity curve



Binding potential is the ratio of binding in the basal ganglia to the cerebellum.

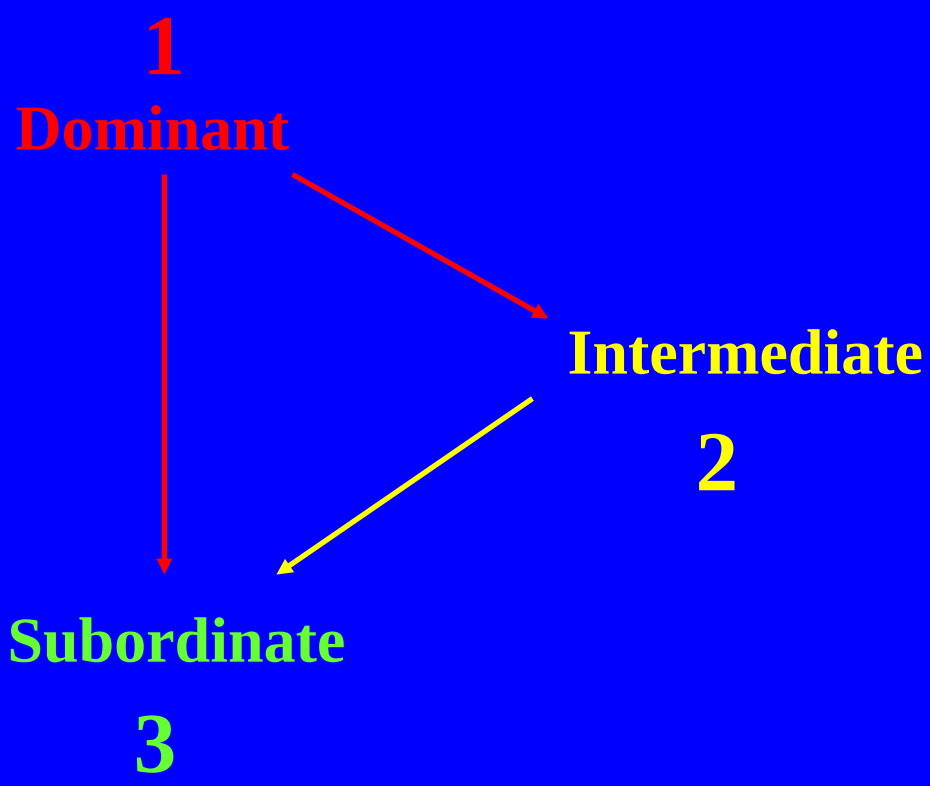
Higher the number, more radioligand is bound

PET and MRI co-registration

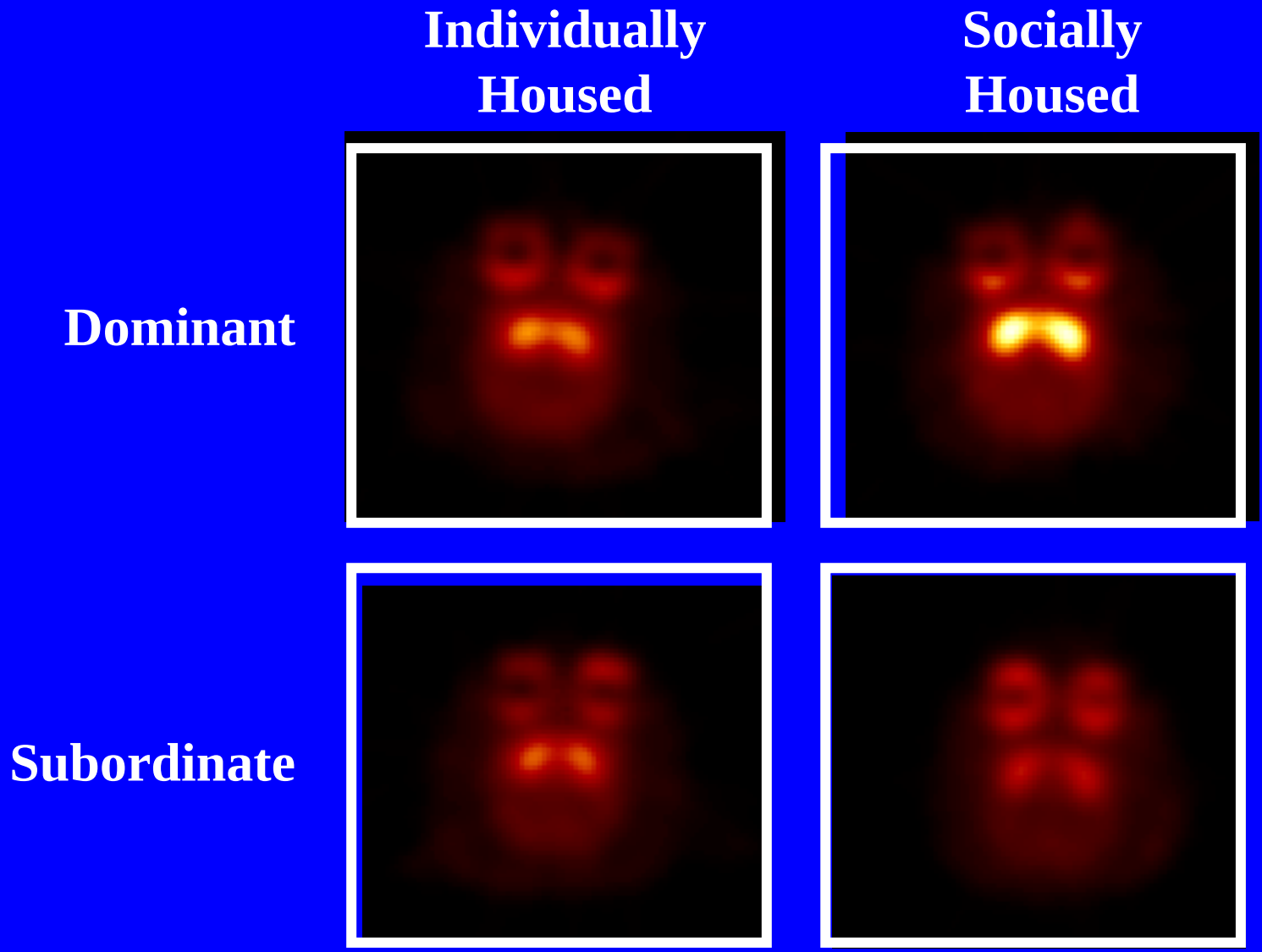


Social Rank in group-housed cynomolgus monkeys

Based on the outcomes of agonistic encounters (i.e. fights)



QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.

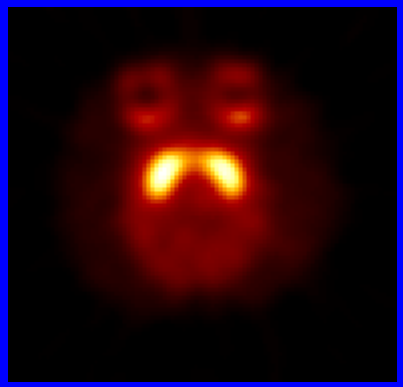


Morgan et al. (2002)

[^{18}F]FCP

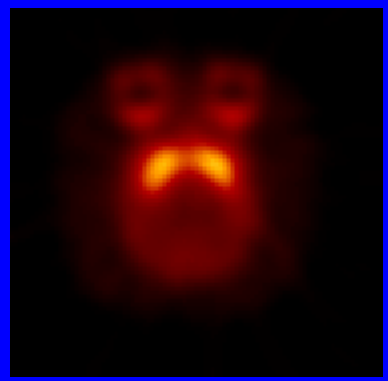
Monkey R-1241: Cocaine Self-Administration

2.93



Baseline

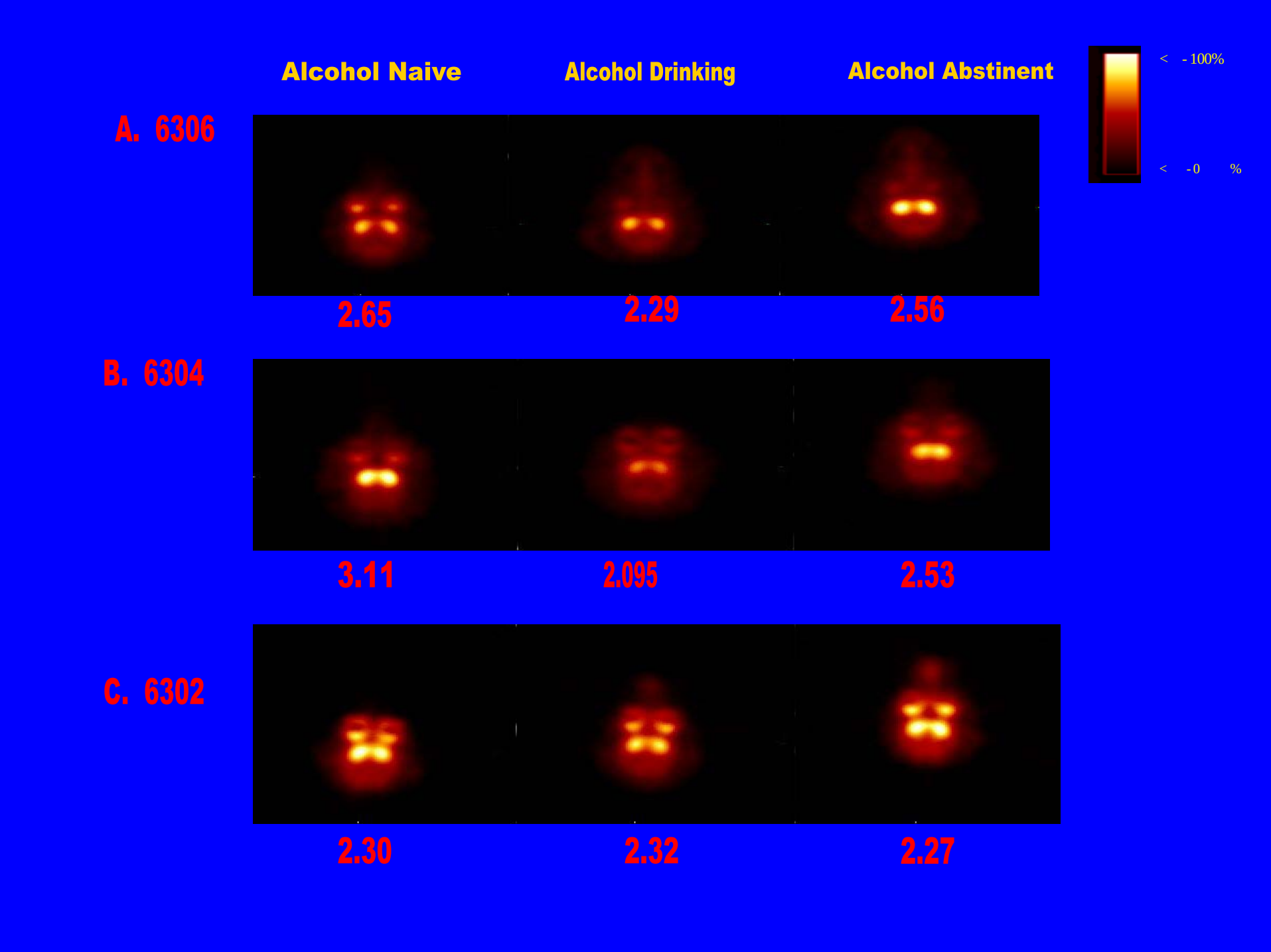
2.37

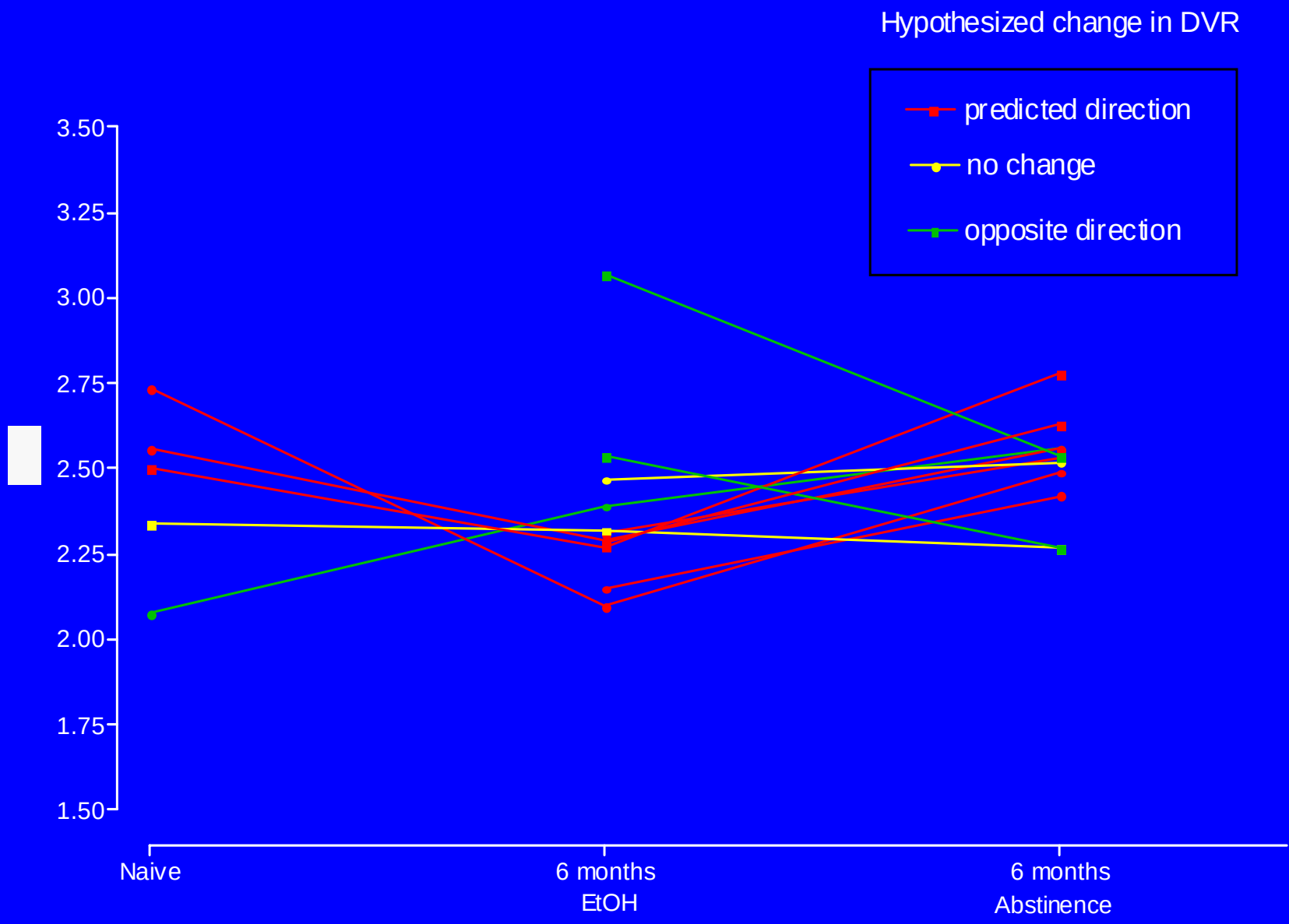


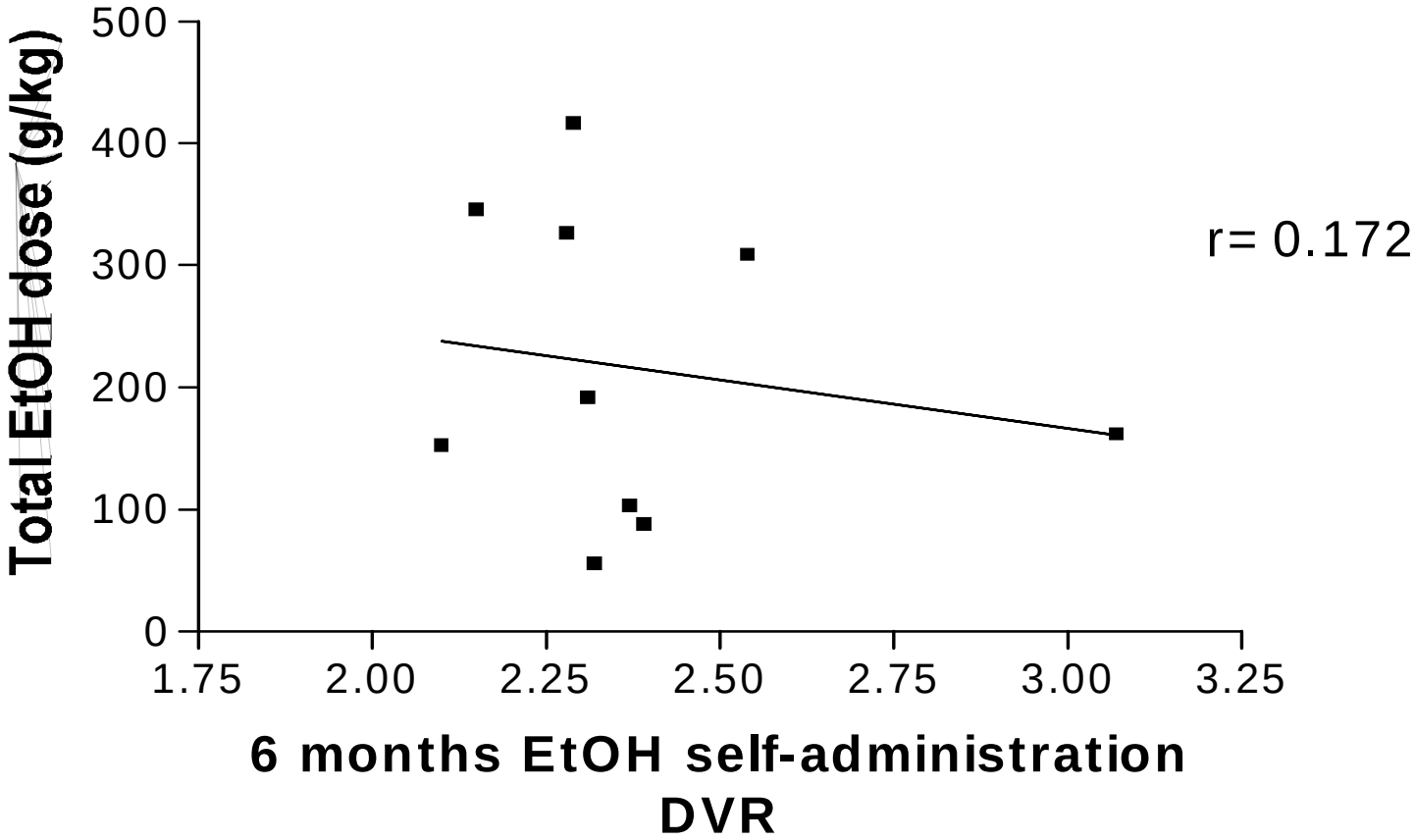
3 months
self-administration

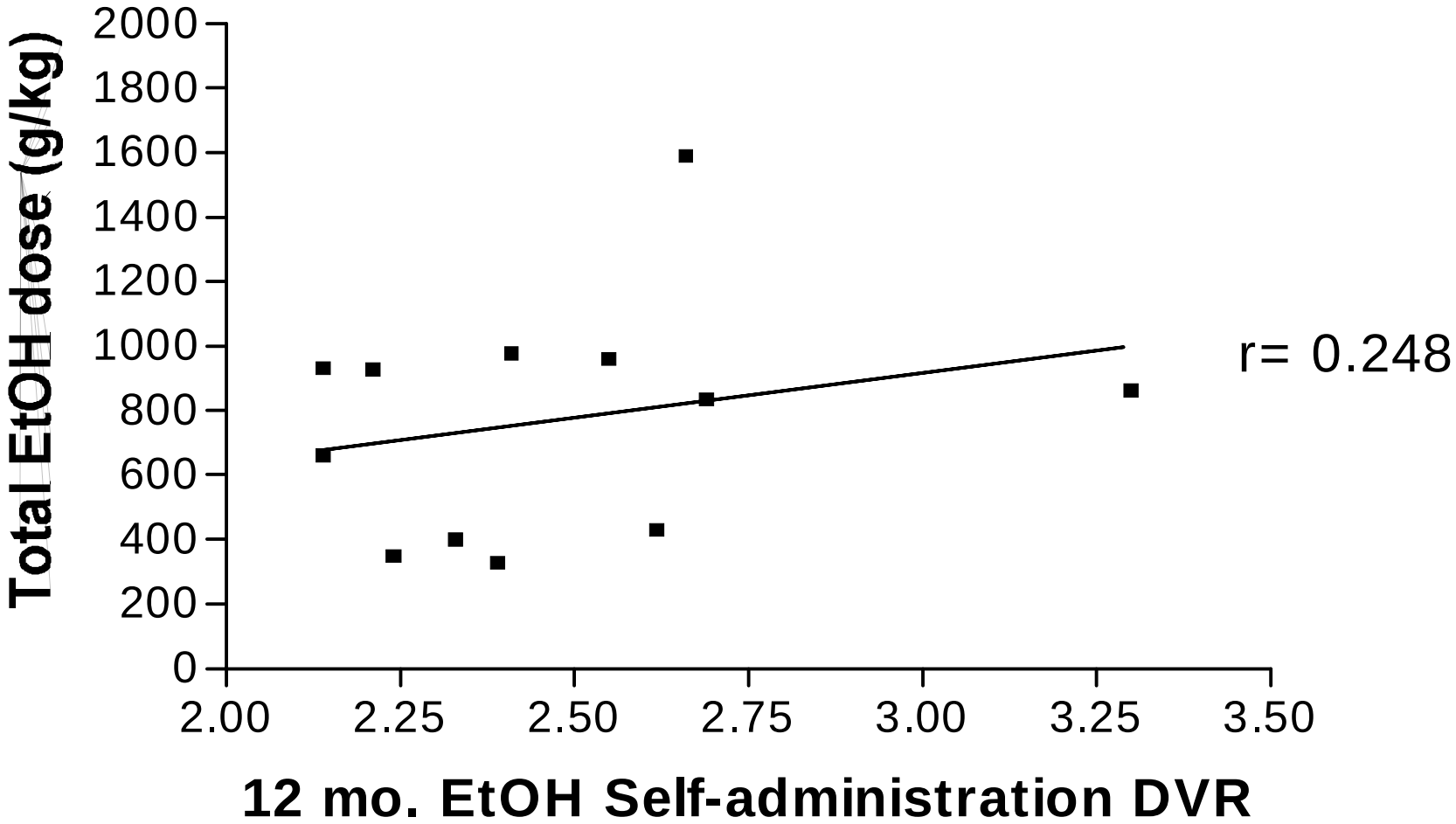
2.12

12 months
self-administration

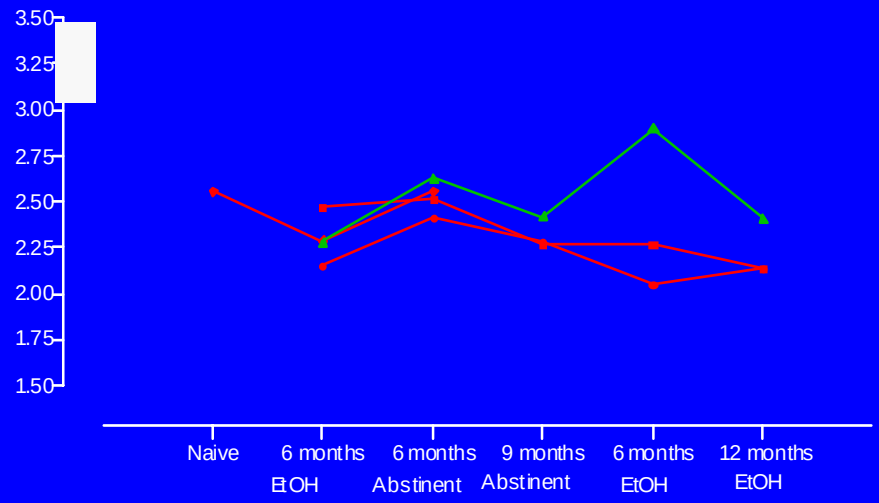

Alcohol Naive
Alcohol Drinking
Alcohol Abstinent
< - 100%
< - 0 %
A. 6306
2.65
2.29
2.56
B. 6304
3.11
2.095
2.53
C. 6302
2.30
2.32
2.27


Hypothesized change in DVR
predicted direction
no change
opposite direction
3.50
3.25
3.00
2.75
2.50
2.25
2.00
1.75
1.50
Naive
6 months
EtOH
6 months
Abstinence

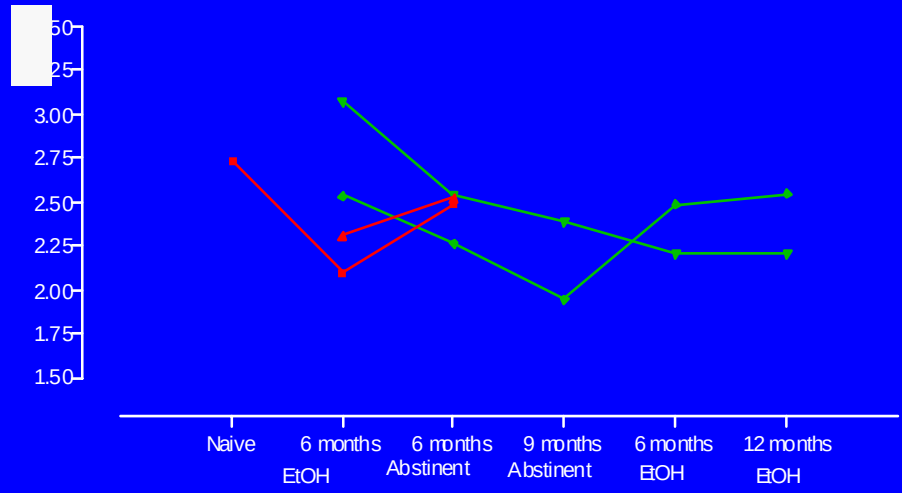

Total EtOH dose (g/kg)
500
400
300
200
100
0
1.75
2.00
2.25
2.50
2.75
3.00
3.25
r= 0.172
6 months EtOH self-administration
DVR


Total EtOH dose (g/kg)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
2.00
2.25
2.50
2.75
3.00
3.25
3.50
12 mo. EtOH Self-administration DVR
r= 0.248

Heavy Drinkers



Moderate Drinkers



Light Drinkers

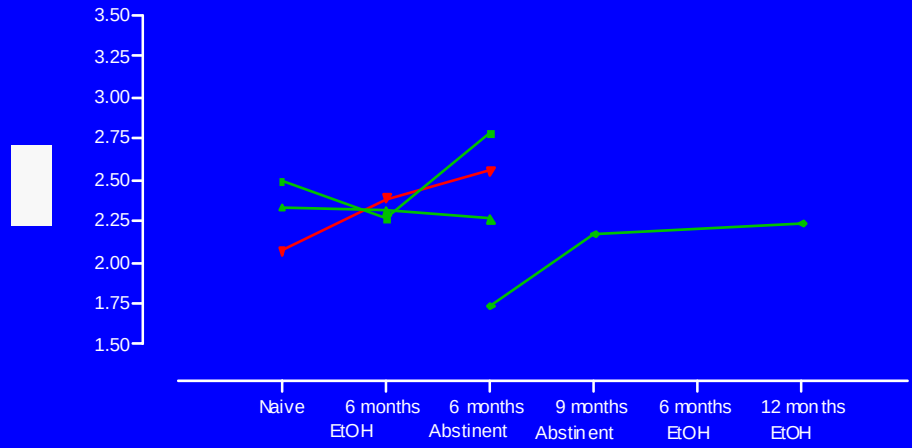


Table 1: Individual DVR's obtained from[18F]FCP PET scans during the longitudinal design.

Monkey #	Baseline	Baseline	6 months EtOH	6 months Abstinence	9 months Abstinence	6 months EtOH	12 months EtOH
6306	2.65	2.47	2.29	2.56			2.66
5404	-	-	-	2.52	2.26	2.27	2.14
6098	-	-	3.07	2.54	2.39	2.21	2.21
6100	-	-	2.28	2.63	2.42	2.90	2.41
6102	-	-	2.54	2.27	1.95	2.49	2.55
6101	-	-	2.31	2.53	-	-	3.30
5497	-	-	2.15	2.42	2.29	2.05	2.14
6305	2.04	2.11	2.39	2.49	-	-	2.69
6302	2.58	2.42	2.27	2.78	-	-	2.62
6304	3.11	2.36	2.10	2.53	-	-	2.33
4993	-	-	-	1.74	2.18	-	2.24
6301	2.30	3.65	2.32	2.27	-	-	2.39
mean	2.54 ± 0.40	2.60 ± 0.60	2.38 ± 0.27	2.44 ± 0.26	2.25 ± 0.17	2.38 ± 0.33	2.47 ± 0.33

Summary

Experimental Condition	Mean DVR
Naïve	2.43
Actively drinking for 6 months	2.38
6 months abstinent	2.44
9 months abstinent	2.25
6 months actively drinking	2.38
12 months actively drinking	2.28

–Previous study reported a naïve DVR range from 2.40-2.58 (n=20) for male cynomolgus monkeys (Morgan et. al, 2002)

Discussion

- Unable to find evidence that D_2 receptor binding potential was altered in response to prolonged ethanol self-administration
- Although there was a lack of changes in dopamine 2 receptor binding characteristics, there are profound physiological changes in other organ systems
  - Hepatic liver disease
  - Disruption of menstrual cycle
  - Alterations in HPA axis

Discussion

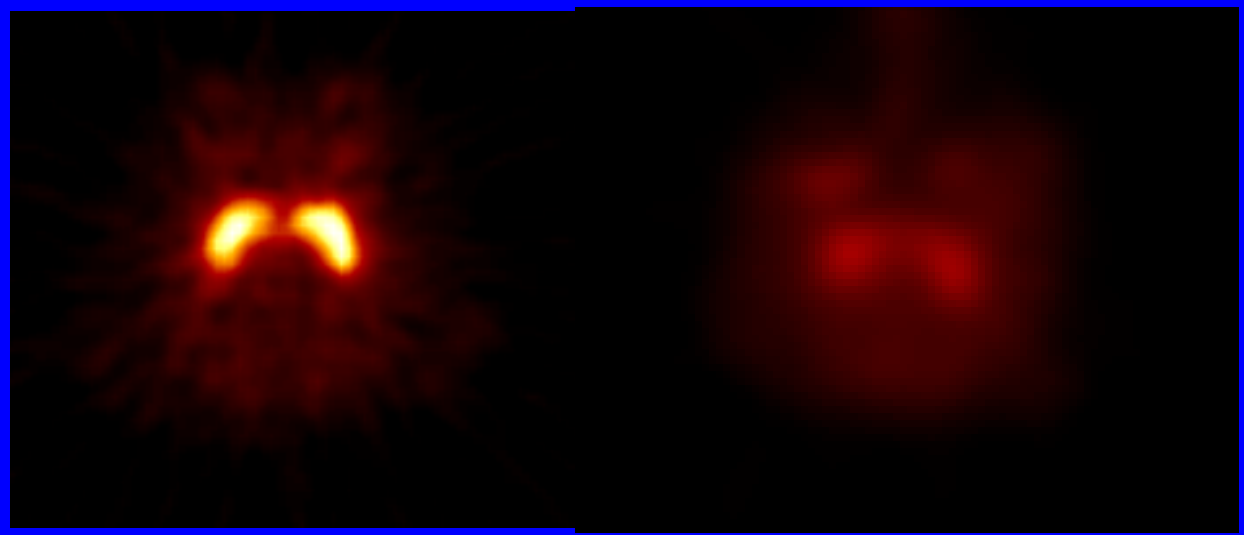
- Results in contrast to results found in human alcoholics
  - Age of humans (over 40 years) vs. age of monkeys (equivalent of 20-30 years)
  - Degree of chronic alcohol exposure
  - Smoking
  - Anesthesia
  - Length of Abstinence
- Other PET ligands that target 5-HT, GABA or glutamate receptor systems may be necessary to find neurochemical adaptations that correlate with early signs of heaving drinking in these animals.
- Data from voltametry studies indicate DA transporter activity is increased in these brains

Model of Excessive Alcohol Drinking

Steve Gonzales
Heather Green
Kristen Jordan
Dr. Mike Nader
Laura Rogers
Dr. Mary Kautz
Natalie Maners
Sarah Thronton
Dr. Robert Mach
Don Gage
Ken Szeliga
Nancy Buckheimer
Dr, Jef Vivian
Jennifer Young

NIAAA &WFUMS

[F-18] FCP: Dopamine D2 Receptors



Control

Alcohol

Cynomolgus Monkeys