

ASSESSING THE MECHANISTIC
OVERLAP OF SELF CONTROL AND
ALCOHOL CONSUMPTION USING
ACTIVITY-DEPENDENT
MANIPULATIONS OF
FRONTOSTRIATAL CIRCUITRIES

BACKGROUND / AIMS

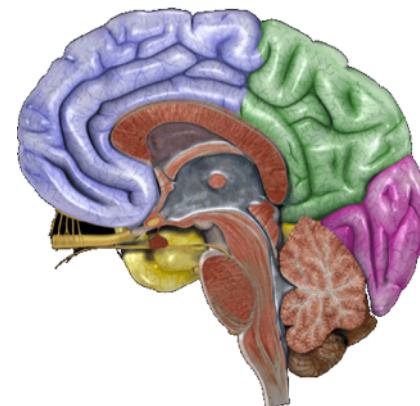
- Impulsive decision-making appears central for development and maintenance of the addiction cycle
- Common methods are either non-specific or allow only correlative links between behavior and brain function (typical optogenetic, pharmacogenetic, microinfusion or lesion methods) without exclusive access to cells activated during behaviours (ensembles).
- Lack of causative data on ensemble overlap of impulsivity and drug consumption

We aim to test possible overlaps of self control / alcohol consumption using
eGFP+/LacZ+ mice and delay discounting (DD) followed by ETH intake

cFos – LacZ enables targeting of behaviorally relevant activity

Anatomical target

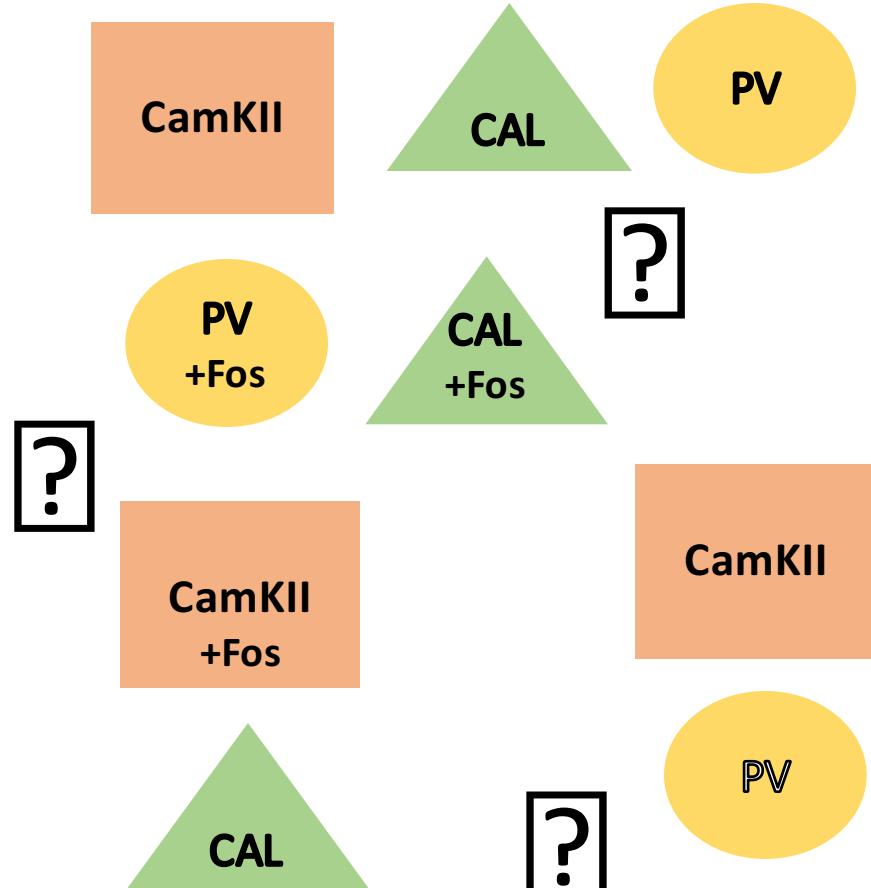
(e.g., local drug infusions/lesions)



Targets select region
irrespective of activity in
cognitive process

Molecular targeting

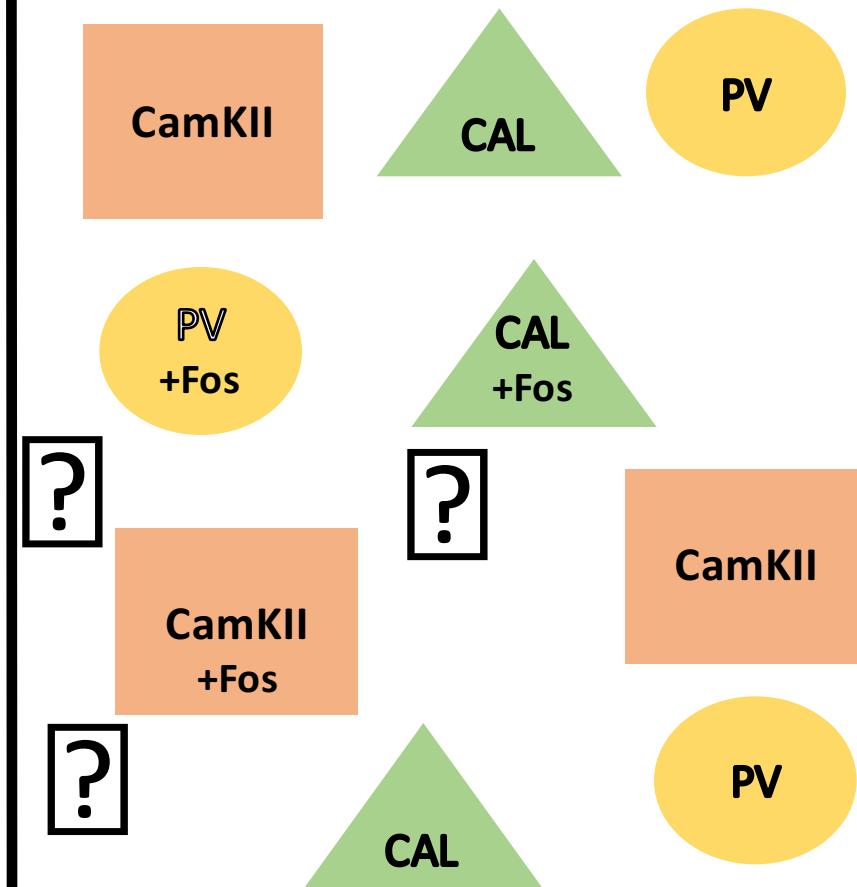
(e.g. molecular-dependent opsins/DREADDs)



Targets select cells irrespective
of activity in cognitive process

Activity-dependent targeting

(e.g., cFos driven opsins/DREADDs/**LacZ**)



Could allow access to cognition-specific activity irrespective of molecular phenotype

Adjusting amounts delay discounting (DD) task

Animals offered choice between two levers, associated with

Small immediate (SI) reward – 10µl of 10% sweetened condensed milk (SCM) delivered *without* delay, and

Large delayed (LD) reward – 20µl of 10% SCM delivered *with* delay

5 consecutive responses on same lever leads to reward adjustment. Preferred lever -**5µl SCM**, non-preferred lever **+5µl SCM**.

Reward adjustments stops when animal respond on both levers: **the indifference point**

E.g., a 25µl reward delivered with 9s has same subjective reward as 10µl reward delivered with 9s delay

$$V = \frac{bA}{1 + kD}$$

V = amount of SI reward at indifference point

D = delay associated with LD reward

A = amount of LD reward at indifference point

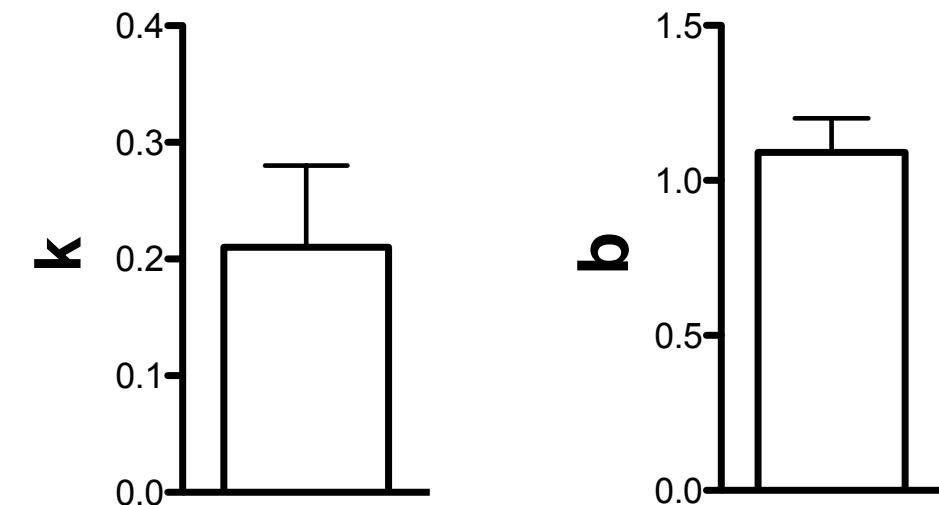
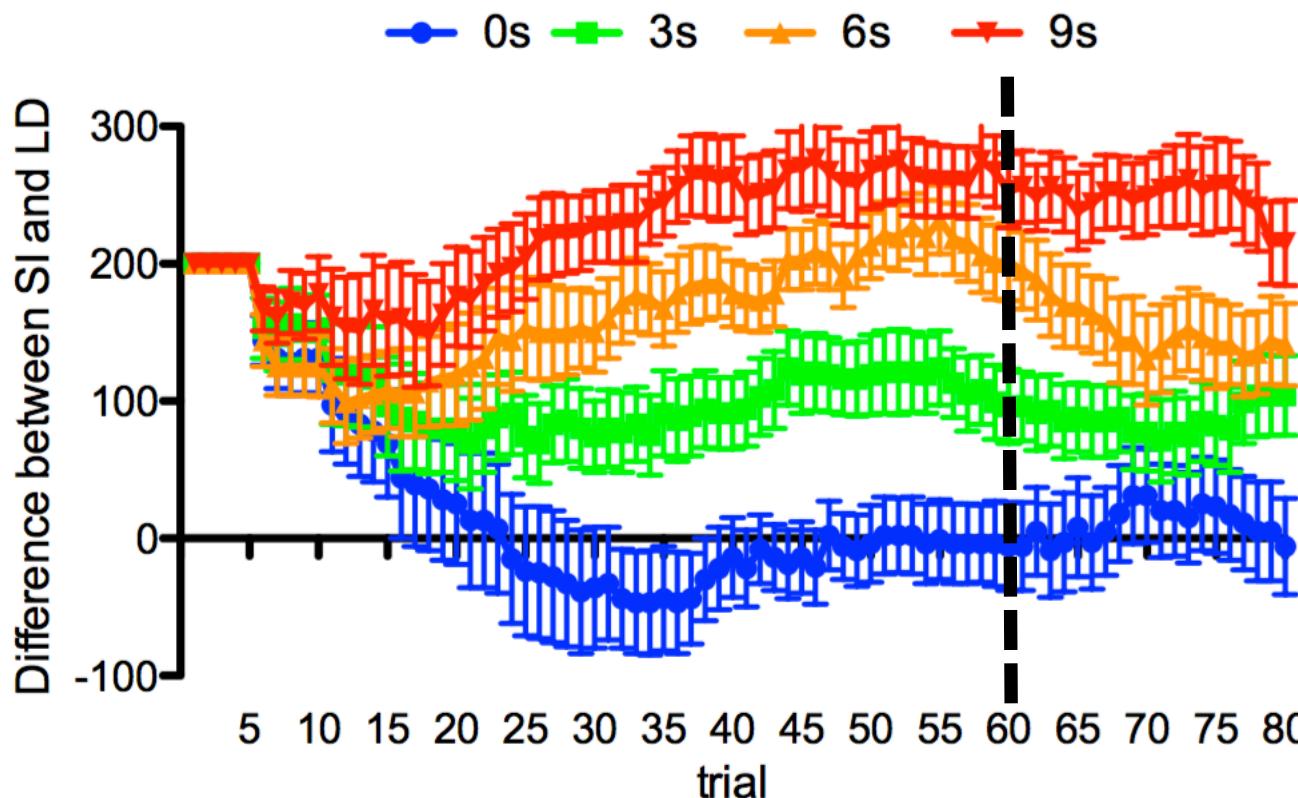
b = preference for LD at 0s-delay sessions (=1 no preference)

***k* = rate of reward devaluation produced by delay (higher values, more discounting)**

EXPERIMENTS

- Exp. 1 – Establish protocol for testing DD using WT C57 mice
- Exp. 2 – Localise bGal in eGFP/LacZ mice after DOX diet removal and DD testing
- Exp. 3 – Effect of excitotoxic OFC lesion on DD
- Exp. 4 – Effect of excitotoxic NAc lesion on DD
- Exp. 5 – Feasibility of lickometer system to assess alcohol intake

Exp 1: Adjusting-amounts DD parameters for WT C57's (N = 24)



Mice discriminate between 0-9s delays

Animals tested for 3 sessions at each delay, 80 trials per session

k and *b* calculated on mean SI/LD value difference over last 20 trials collapsed over 3 sessions.

Exp 2: eGFP/LacZ animals and DD

eGFP-/LacZ- (N=9), eGFP+/LacZ- (N=15), eGFP+/LacZ- (N=14), eGFP+/LacZ- (N=10), trained on DD to:

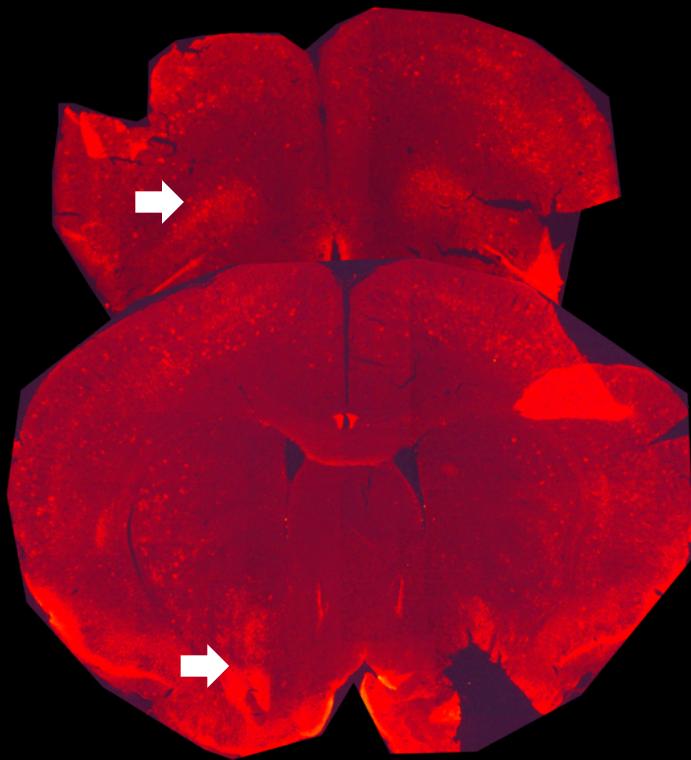
- Assess when DOX diet should be removed for bGal in eGFP+/LacZ+ mice , and no other groups, after DD
- Develop histology protocol and localize reliable bGal expression in eGFP+/LacZ+ mice after DD
- Test if possible genotype differences in DD at baseline without daun02

METHOD

- Animals tested on DD on DOX diet (no genotype differences observed)
- After final test day on DOX diet, the DOX diet was replaced by standard chow.
- Animals were re-tested on a single DD session (9s delay) either 1w, 2w, or 4w after DOX removal.
 - Animals were perfused 90min after single DD session

bGAL IHC in eGFP+/LacZ+ animals OFF dox diet after DD testing

1 week OFF Dox



2 weeks OFF Dox



4 weeks OFF Dox



IOFC and NAc, the two regions most often implicated DD, tend to show week-independent bGal expression in eGFP+/LacZ mice after testing

! 24h postfix in 4% PFA appear to quench protein activity and prevent xGal stain

● No operant/home cage controls, bGal expression could be related to general task performance

NMDA-induced OFC and NAc lesions and DD

Animals trained on DD, pair-matched for *k*, and infused with NMDA or saline vehicle in either:

OFC (NMDA = 17, sham N = 16)
(AP+2.8, ML ± 1.45, DV+2.8)

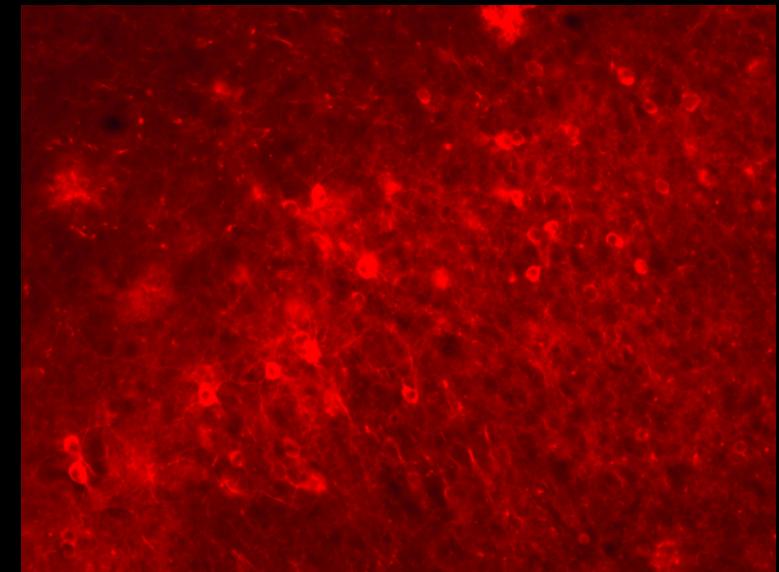
- 20mg/ml; 0.3μl;

NAc (NMDA N = 13, sham N = 12)
(AP+1.6, ML ± 0.9, DV+4.7)

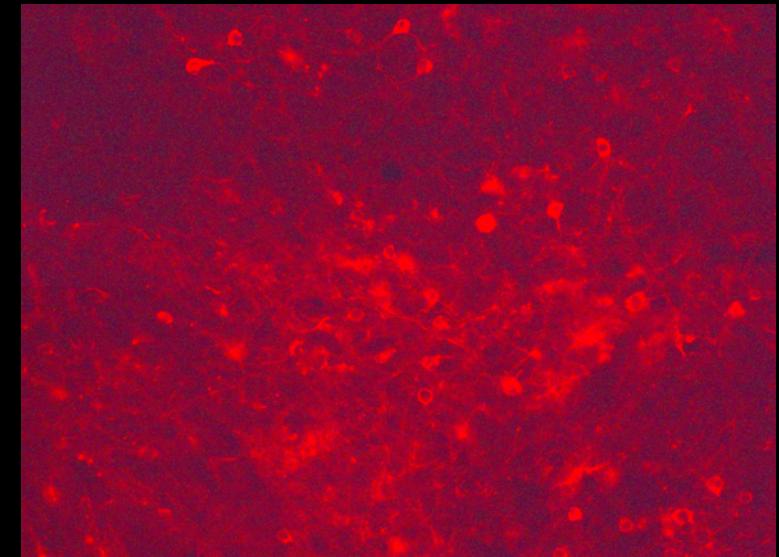
- 12.5mg/ml; 0.125μl;

Animals re-tested on DD 10 days post-surgery

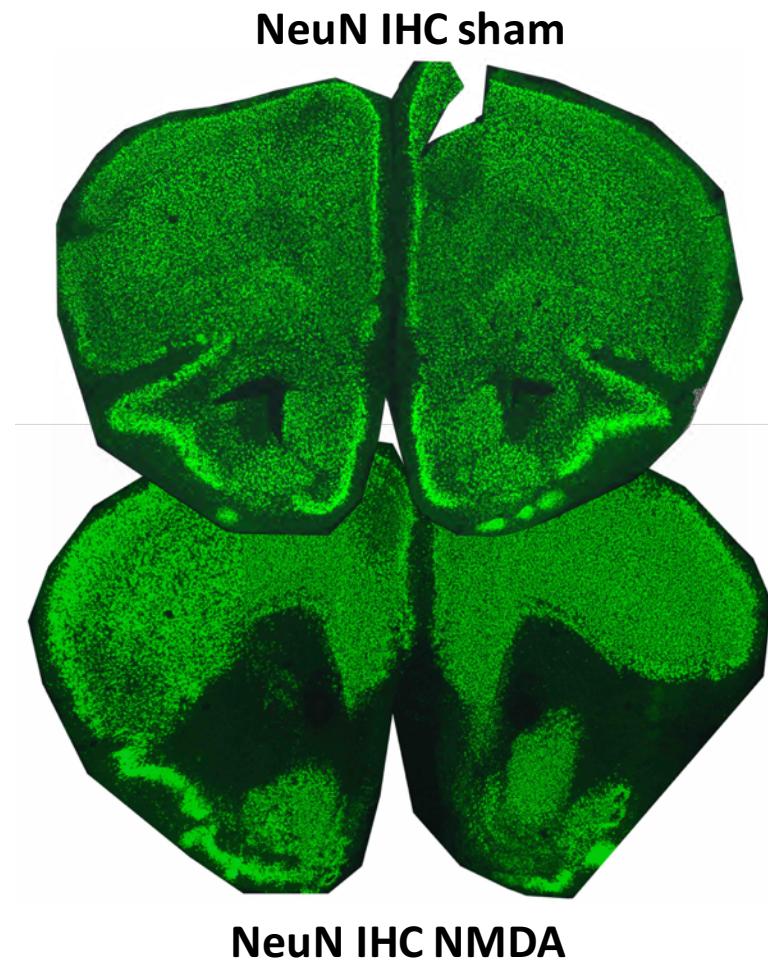
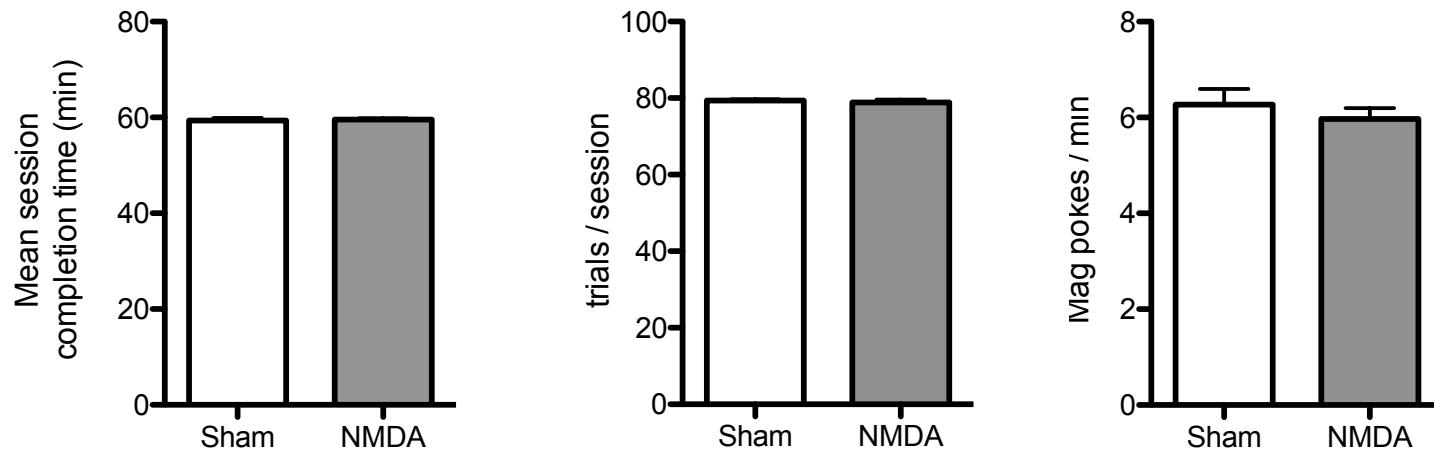
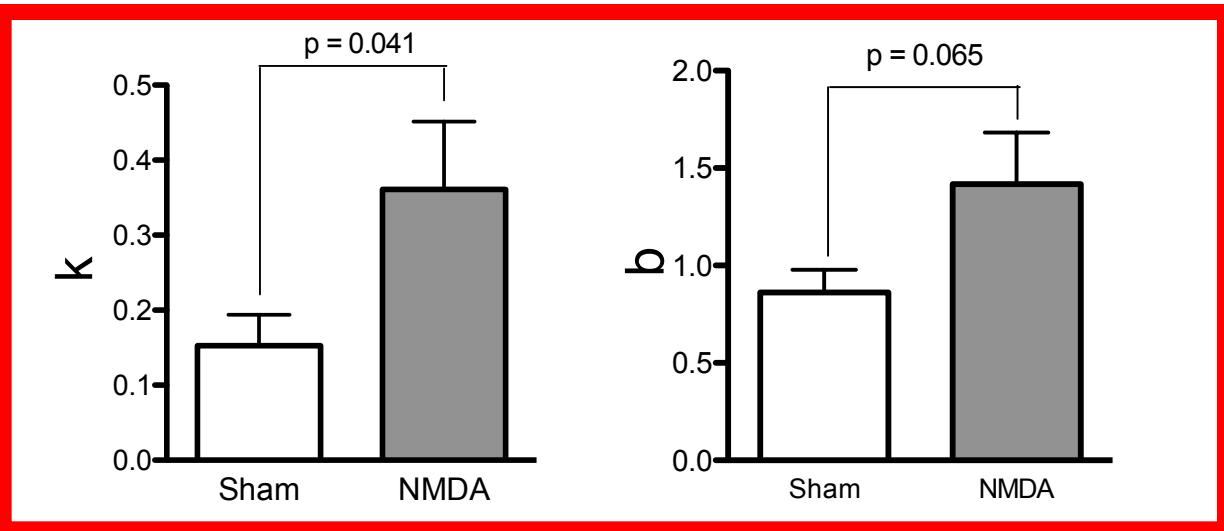
IHC bGal IOFC



IHC bGal NAc

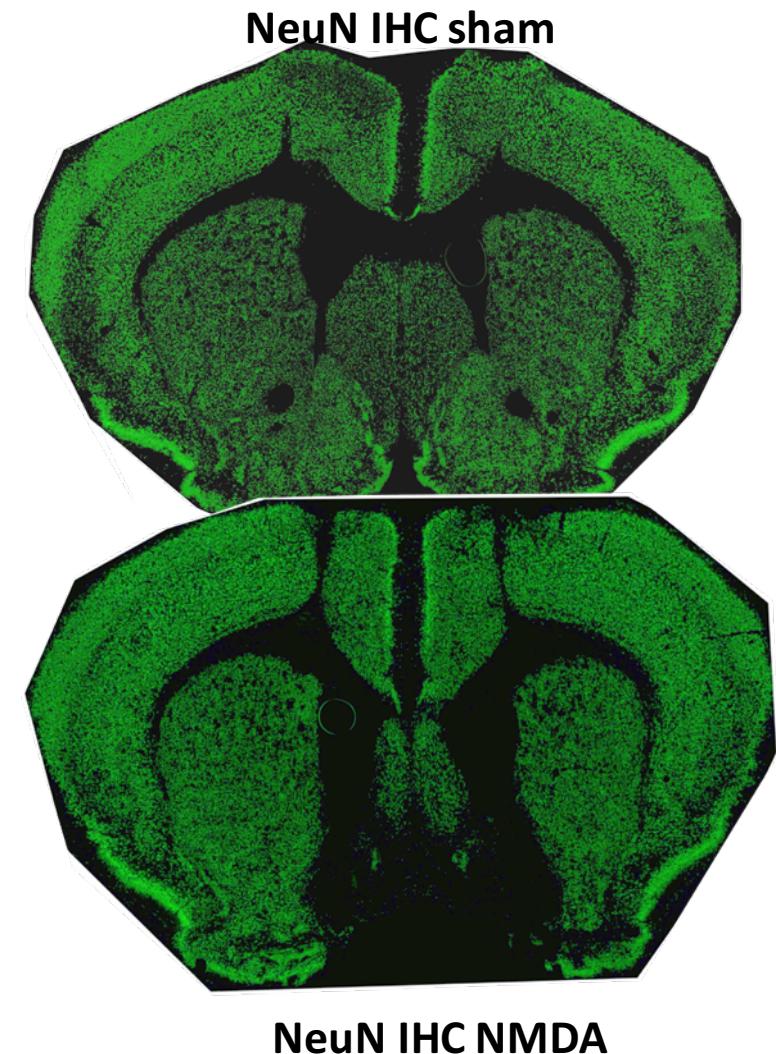
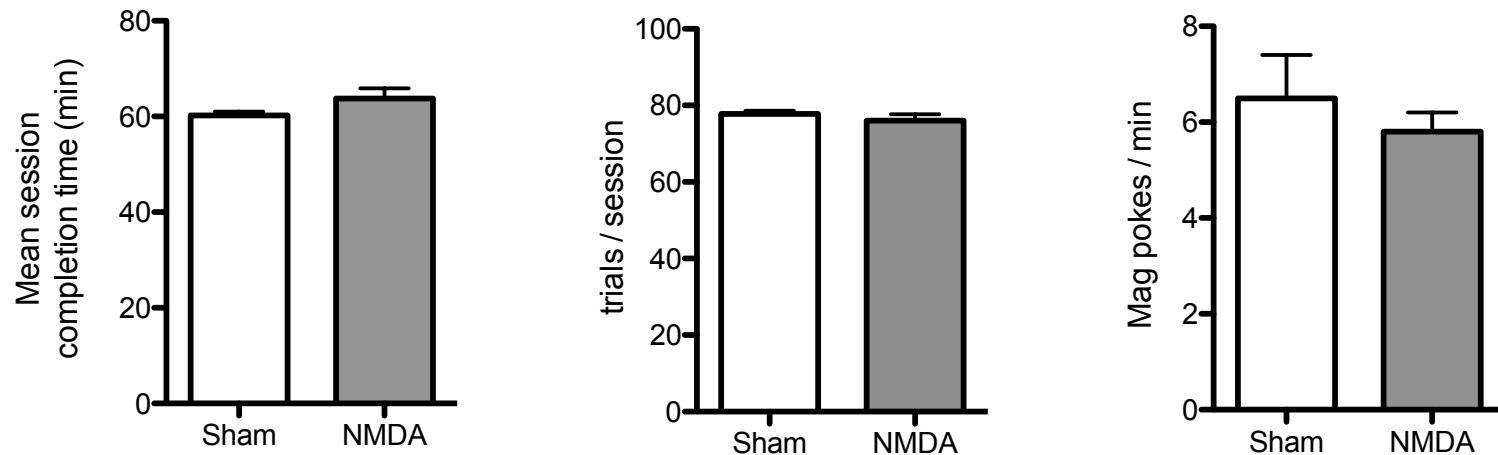
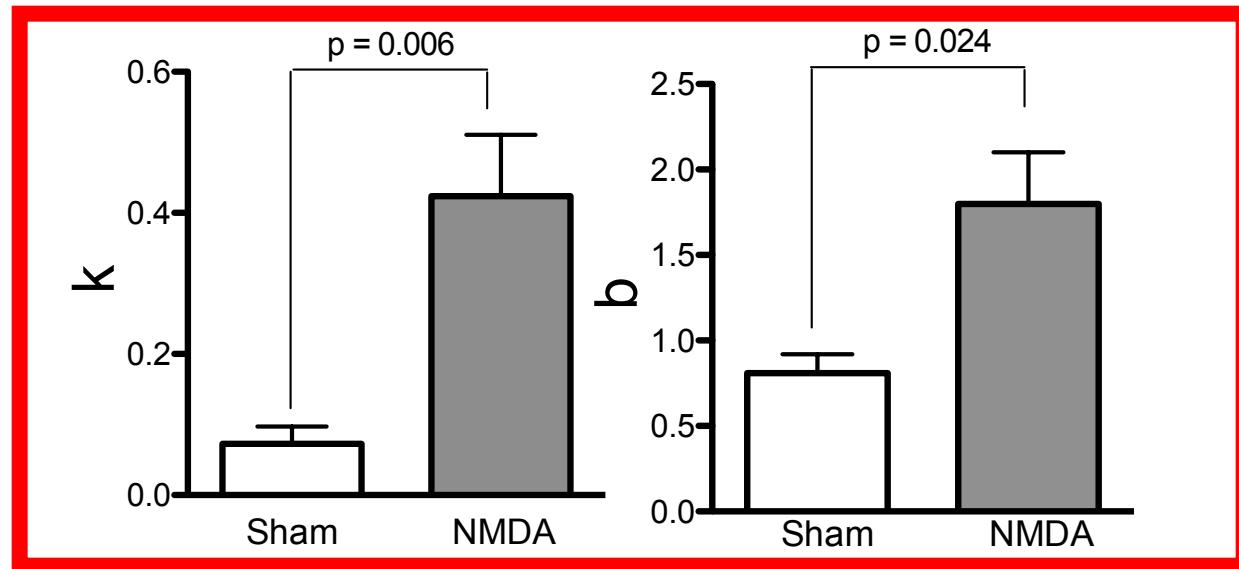


OFC lesions and DD



OFC lesioning increases delay discounting and tend to increase bias
No obvious non-specific effects from lesion

NAc lesions and DD



OFC lesioning increases delay discounting and tend to increase bias
No obvious non-specific effects from lesion

Lickometer chambers – 20% alcohol – 8pm-8am

N = 8 C57s with overnight 20% alcohol available – 6 days in home-cage followed by 6 days in lickometer boxes.



Pearson		MEAN_INTAKE_SCURRY	MEAN_INTAKE_HOME	MEAN_INTAKE_BW_SCURRY	MEAN_INTAKE_BW_HOME
MEAN_INTAKE_SCURRY	Pearson Correlation	1	.792*	0.696	0.523
	Sig. (2-tailed)		0.019	0.055	0.183
MEAN_INTAKE_HOME	Pearson Correlation	.792*	1	0.449	0.721*
	Sig. (2-tailed)	0.019		0.265	0.044
MEAN_INTAKE_BW_SCURRY	Pearson Correlation	0.696	0.449	1	.748*
	Sig. (2-tailed)	0.055	0.265		0.033
MEAN_INTAKE_BW_HOME	Pearson Correlation	0.523	.721*	.748*	1
	Sig. (2-tailed)	0.183	0.044	0.033	

Mean intake in home-cage correlate with mean licks in lickometer chambers
Suggests that lickometer boxes may be used for planned alcohol intake exps

On-going

- Establish efficacy of Daun02 and histological methods:
eGFP+/LacZ+ with OFC cannula to receive range of doses after 1day procedure likely to cause activation

- Effect of intra-OFC daun02 on DD in eGFP+/LacZ+ animals:
eGFP+/LacZ+ animals have been trained on DD task – intra-OFC cannulations planned
Will receive vehicle/daun02 and retested on DD

Planned

- If intra-OFC daun02 produce DD phenotype we assess alcohol intake in the same cohort
Some animals culled prior to alcohol study for histology

- If intra-OFC daun02 does not produce DD phenotype, potentially try alternative regions (e.g., NAc).