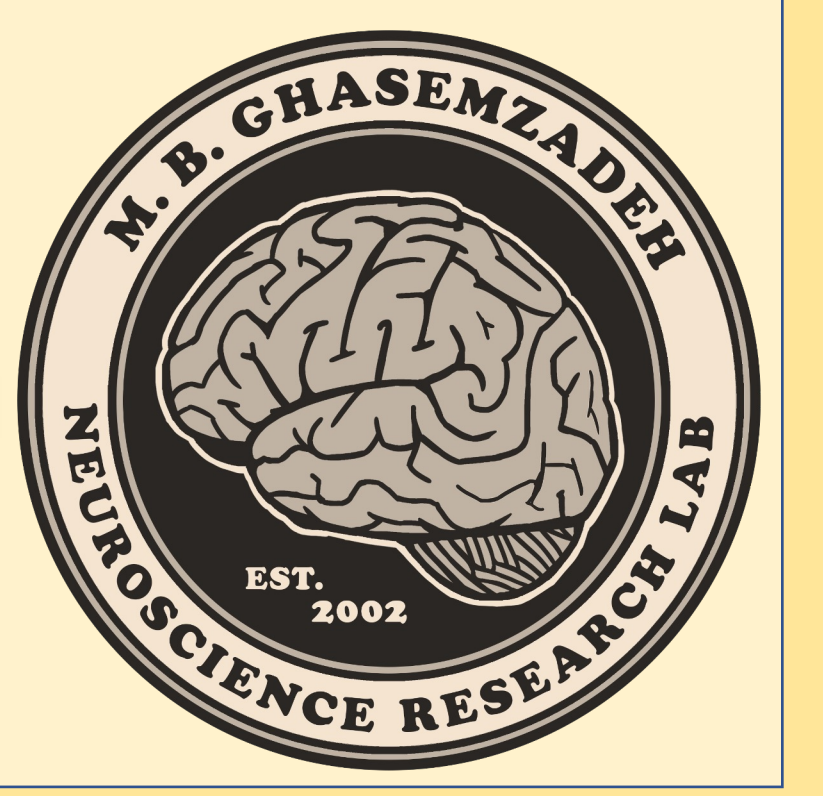# Regulation of drug seeking by nucleus accumbens mGlu5 receptor is both drug intake and withdrawal dependent

Divyank Sharma[1], Pouya Mirzaei[1], Oscar Burgos[1], M. Behnam Ghasemzadeh[1]

[1]Department of Biomedical Sciences, College of Health Sciences, Marquette University
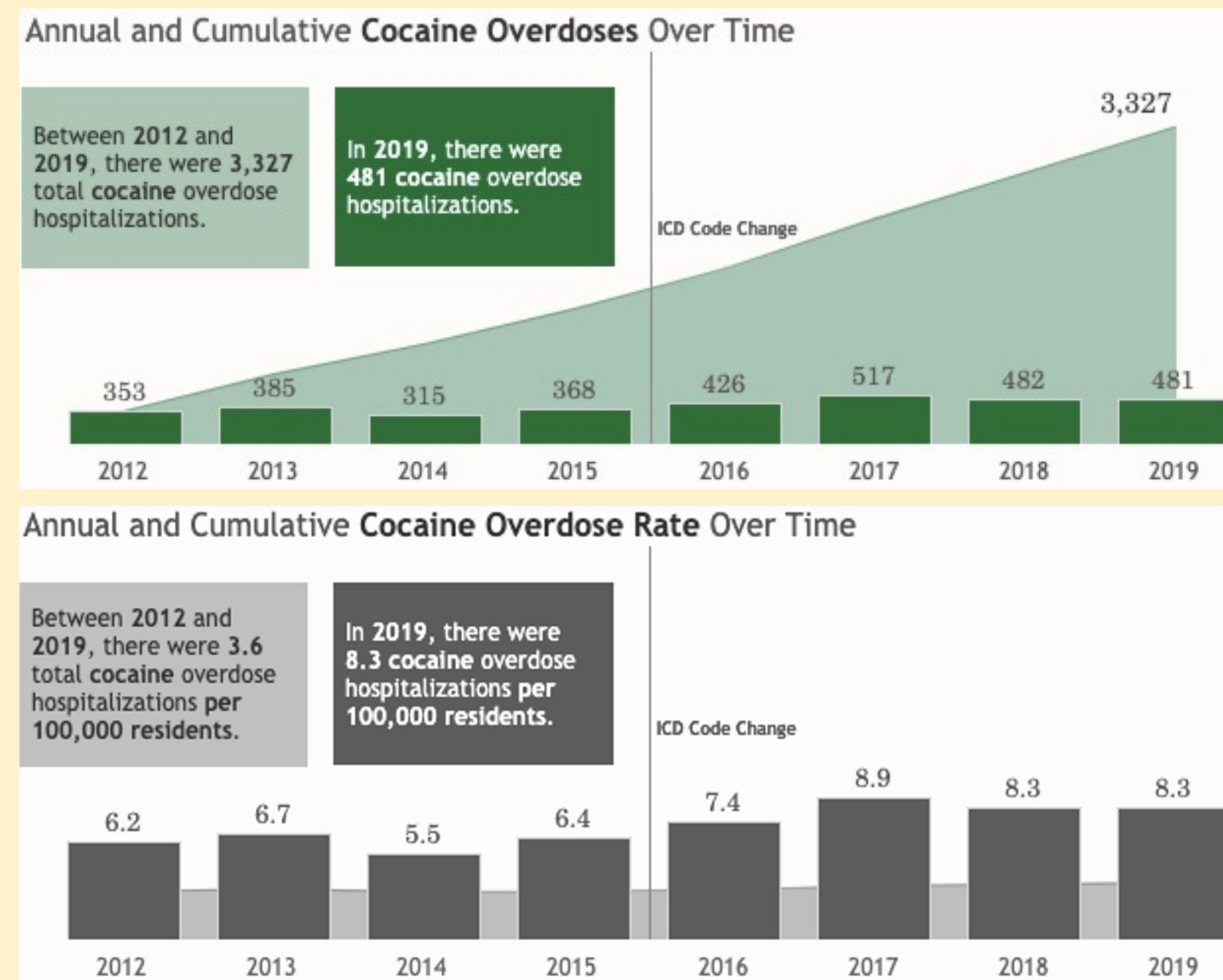
Introduction

Context

According to the National Institute of Drug Abuse, cocaine is an addictive stimulant that is often mixed with opioids such fentanyl which is a synthetic opioid[2]. The Wisconsin Department of Health Services indicates a a general increasing trend over the previous 7 years in cocaine related overdose hospitalizations[6].

- o Cocaine related overdose hospitalizations were 8.3 per 100,000 residents in 2019[6].
- o Southeastern Wisconsin had 2,433 total hospitalizations involving cocaine overdose hospitalizations[6].

Annual and Cumulative Cocaine Overdoses Over Time

Between 2012 and 2019, there were 3,327 total cocaine overdose hospitalizations. In 2019, there were 481 cocaine overdose hospitalizations. ICD Code Change

353 385 315 368 426 517 482 481
2012 2013 2014 2015 2016 2017 2018 2019

Annual and Cumulative Cocaine Overdose Rate Over Time

Between 2012 and 2019, there were 3.6 total cocaine overdose hospitalizations per 100,000 residents. In 2019, there were 8.3 cocaine overdose hospitalizations per 100,000 residents. ICD Code Change

6.2 6.7 5.5 6.4 7.4 8.9 8.3 8.3
2012 2013 2014 2015 2016 2017 2018 2019

Research Conducted

Previous studies have shown that group 1 metabotropic glutamate receptors (mGluR1 & mGluR5) have a critical role in regulation of both drug use and seeking behavior and withdrawal experienced after exposure to cocaine. Inhibition of mGluR5 receptor function results in reduced drug seeking behaviors in rats.

Neurobiology & Neuronal Levels LgA vs ShA

Impact

General exposure to cocaine results in increased levels of dopamine as a result of cocaine inhibition of dopamine reuptake in the synaptic cleft. Reinforcement of drug seeking behavior is, in part, a result of excess dopamine being left in the synapse due to inhibition of reuptake[5]. In addition, exposure to cocaine results in enduring alterations in the glutamatergic signaling in the reward circuit of the brain.
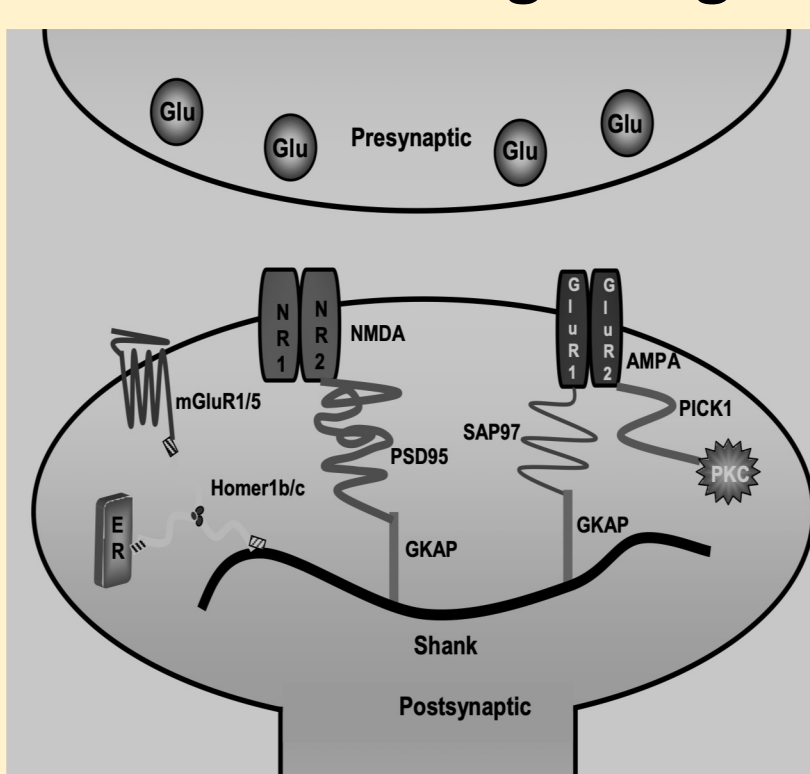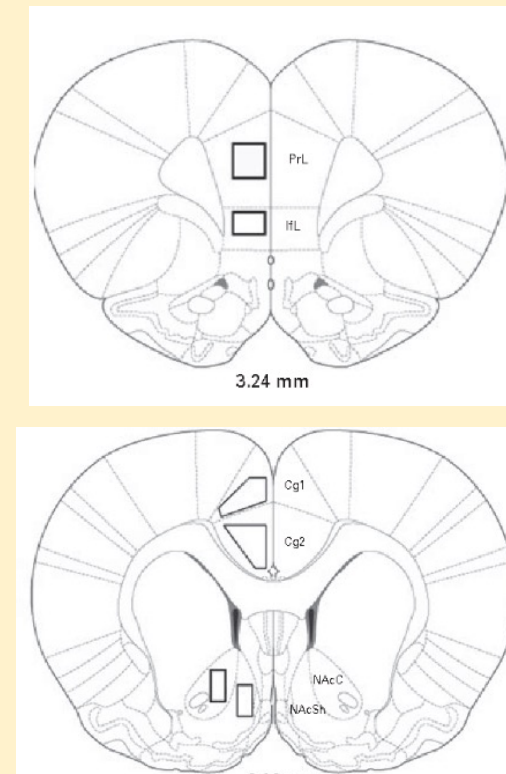
mGluR5 protein level decreases in NAshell in LgA rats
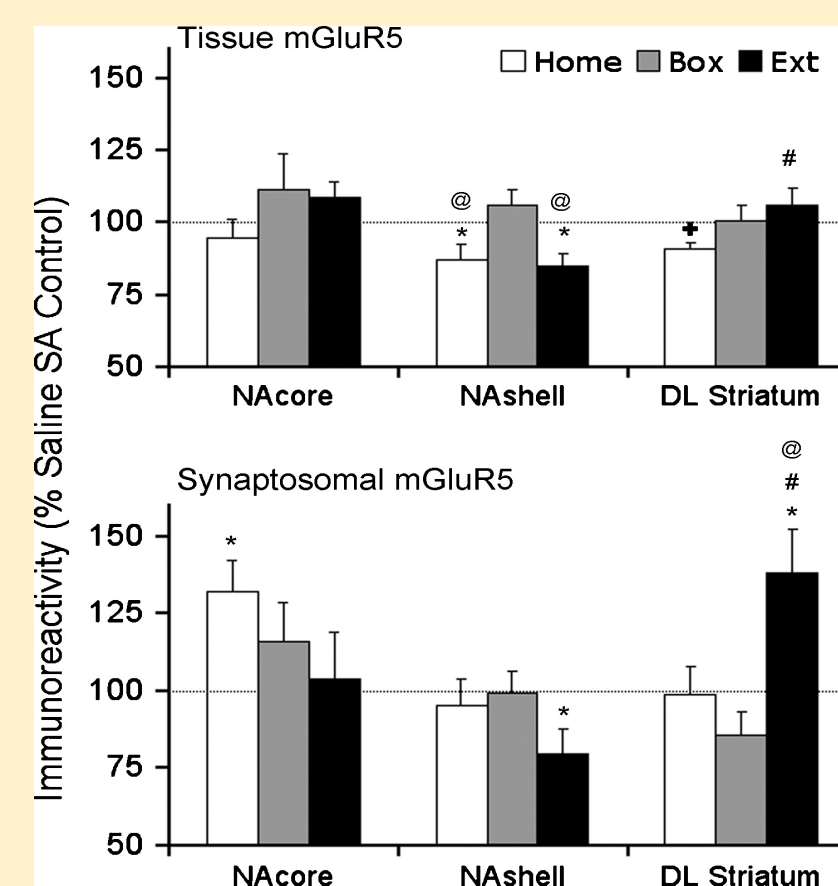
Drug intake (Self-Ad) in LgA vs ShA animal models
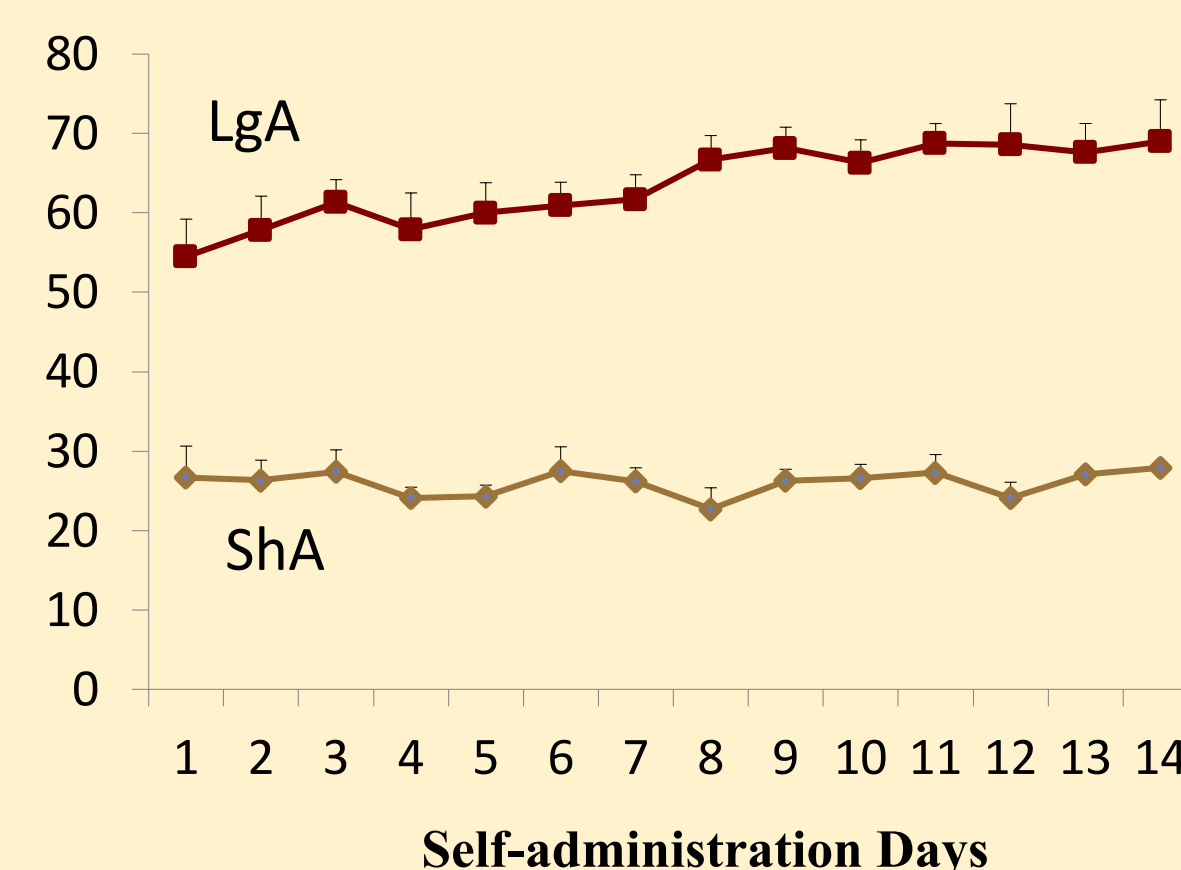
Glutamate Signaling

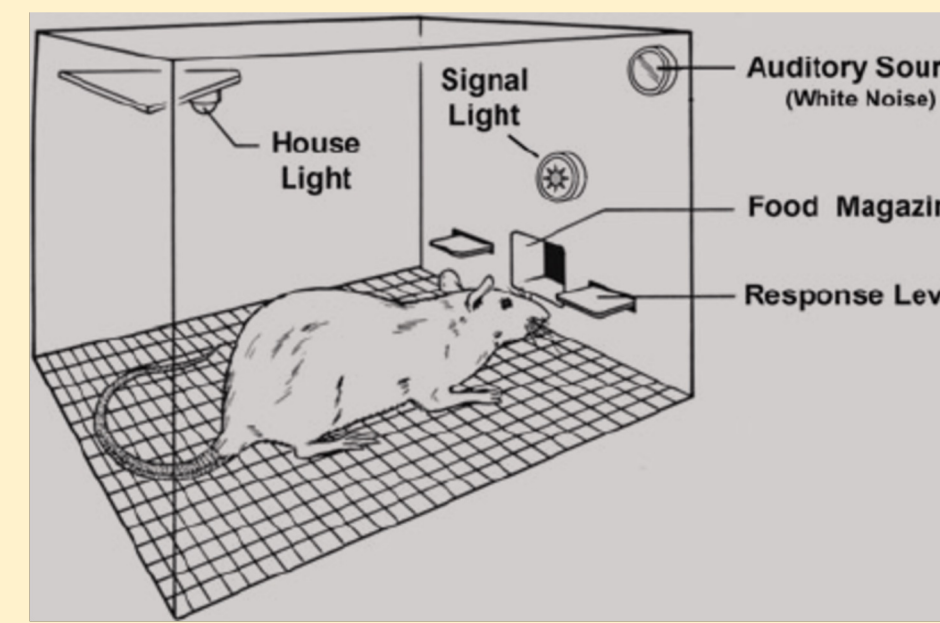**Regions of Focus**

The prelimbic and infralimbic cortices.

The nucleus accumbens core and shell regions.
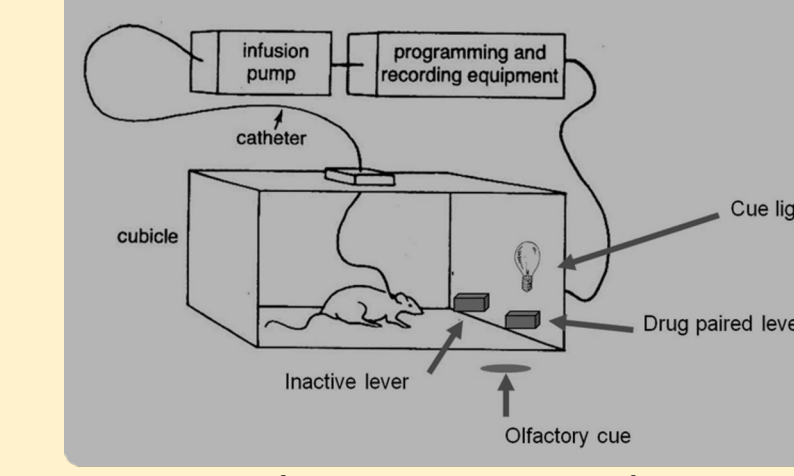
Methods

Short Access (ShA)/Long Access (LgA)

Food Training

Sprague-Dawley Rat (350-400 grams)

Duration = Until consistent presses are noticed
Criteria: 5%-10% food restricted
Received sugar pellets

Self Administration of Cocaine (Acquisition/Maintenance Phase)


ShA Duration/Dosage = 2 hrs;1 mg/kg;200 µl
LgA Duration/Dosage = 6 hrs;1 mg/kg;200 µl
Abstinence Phase = Colony Room
Experimental Groups Post Abstinence (drug seeking tests):

1. Microinject either 0.5 µL of saline or 3.0 µg MTEP (mGluR5 receptor blocker) in nucleus accumbens
2. 1 minute for injection and 5 min wait to allow liquid to disperse
3. Saline self-administered for 30-60 sec. after additional 5 min rest time

Results
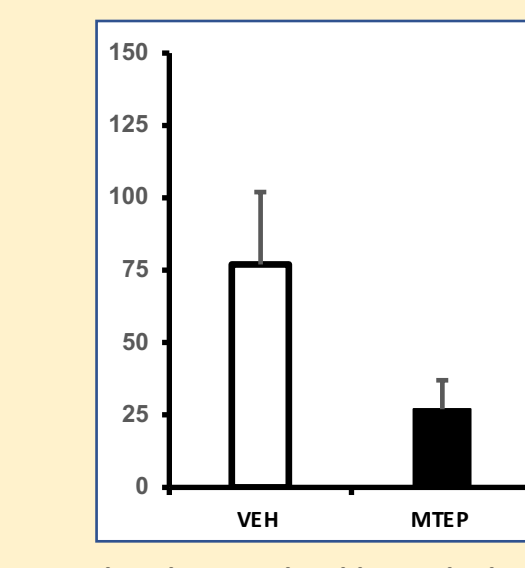
mGluR5 regulation of drug seeking in PFC and NA
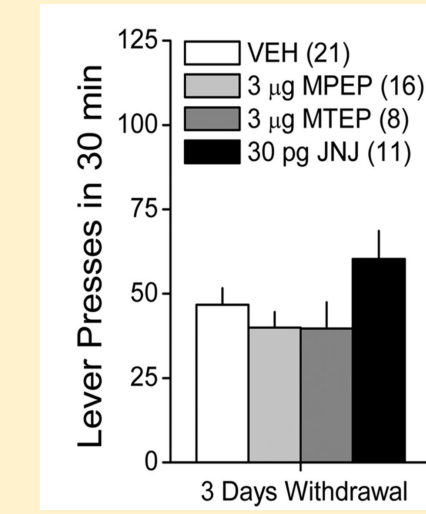
Osnat Ben-Shahar et al. J. Neurosci. 2013;33:495-506

Microinjection of mGluR blockers into vmPFC does not acutely influence cue-reinforced responding in LgA rats.
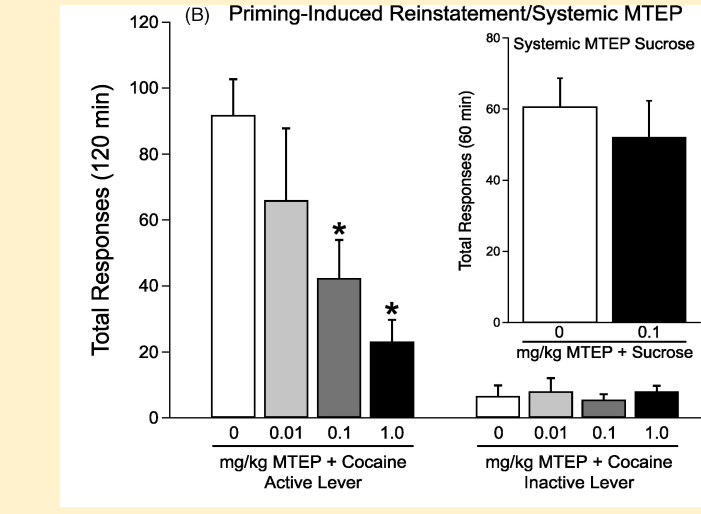
Kumaresan V et al. Behavioural brain research. 2009;202(2):238-244

Systemic blockade of mGluR5 receptor reduces reinstatement of drug seeking after extinction in ShA rats.
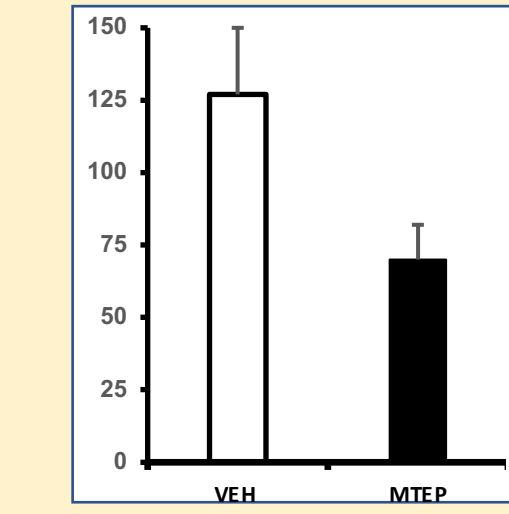
Knackstedt LA et al. Addiction biology. 2014;19(1):87-101

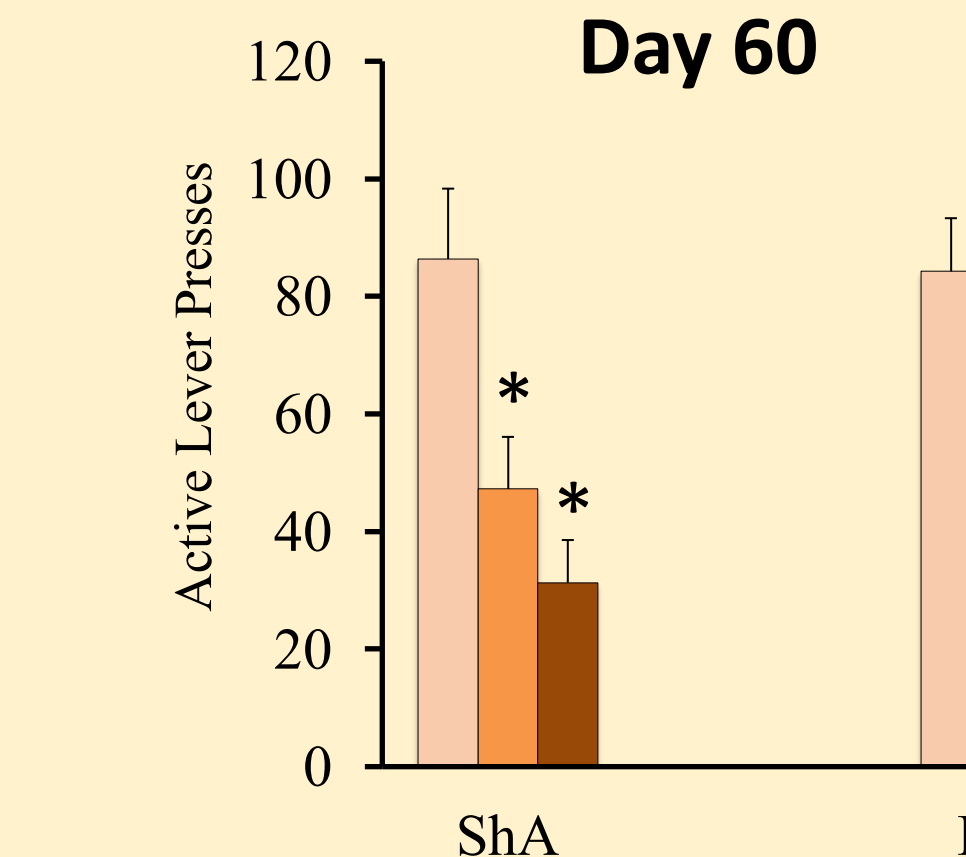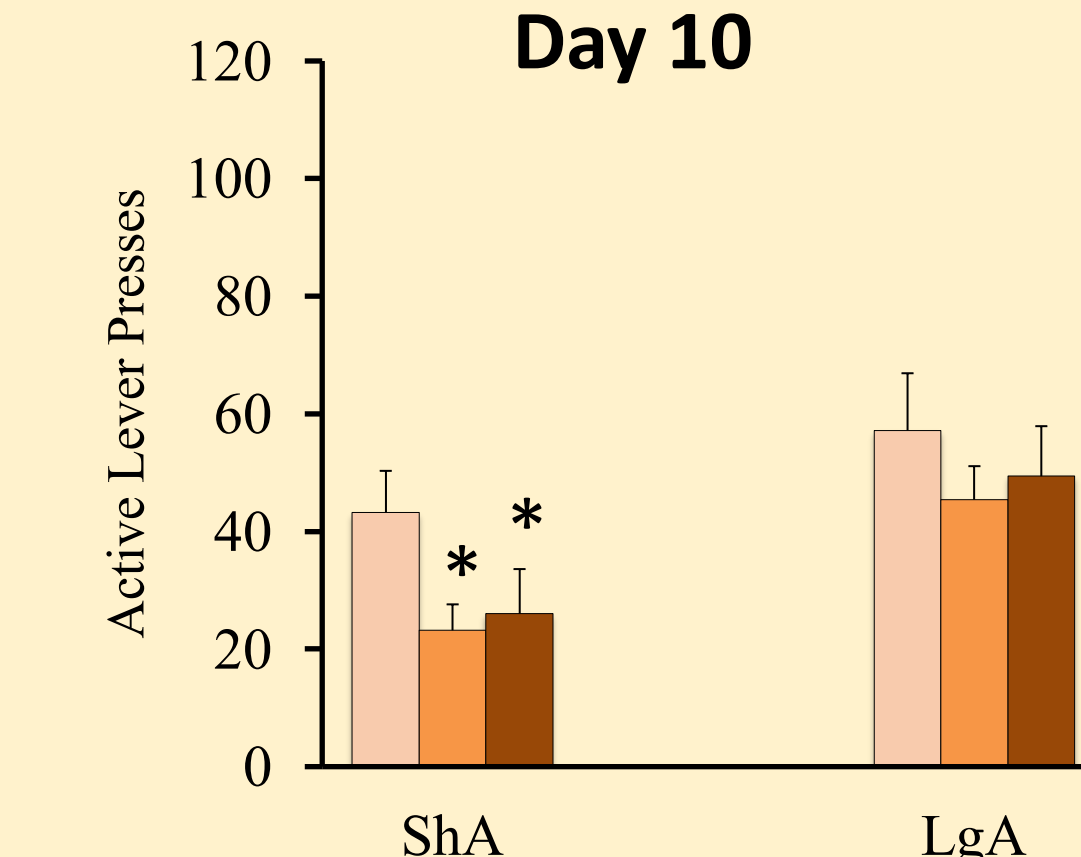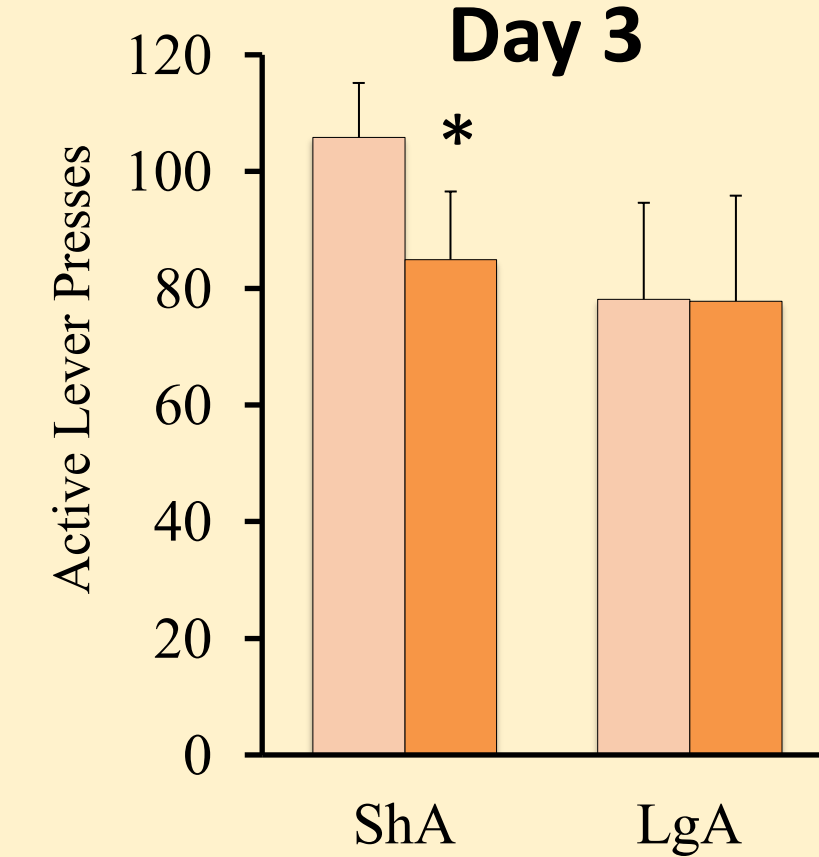Blockade of mGluR5 receptor in NA core reduces drug seeking after abstinence in ShA rats.

Knackstedt LA et al. Addiction biology. 2014;19(1):87-101

Blockade of mGluR5 receptor in NA core reduces cue-induced reinstatement of drug seeking after extinction in ShA rats.

**mGluR1 receptor regulates drug seeking in both ShA and LgA at all three abstinence periods**

Day 3 — Sal / JNJ 0.015 — ShA
Day 10 — ShA / LgA — Saline / JNJ 0.005 / JNJ 0.015
Day 60 — ShA / LgA
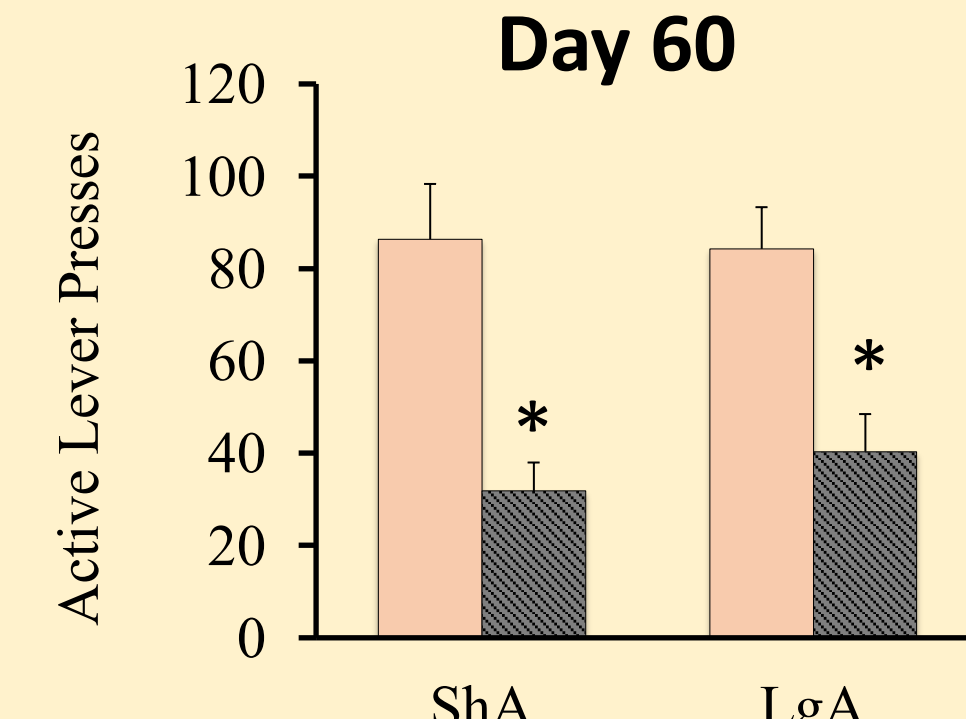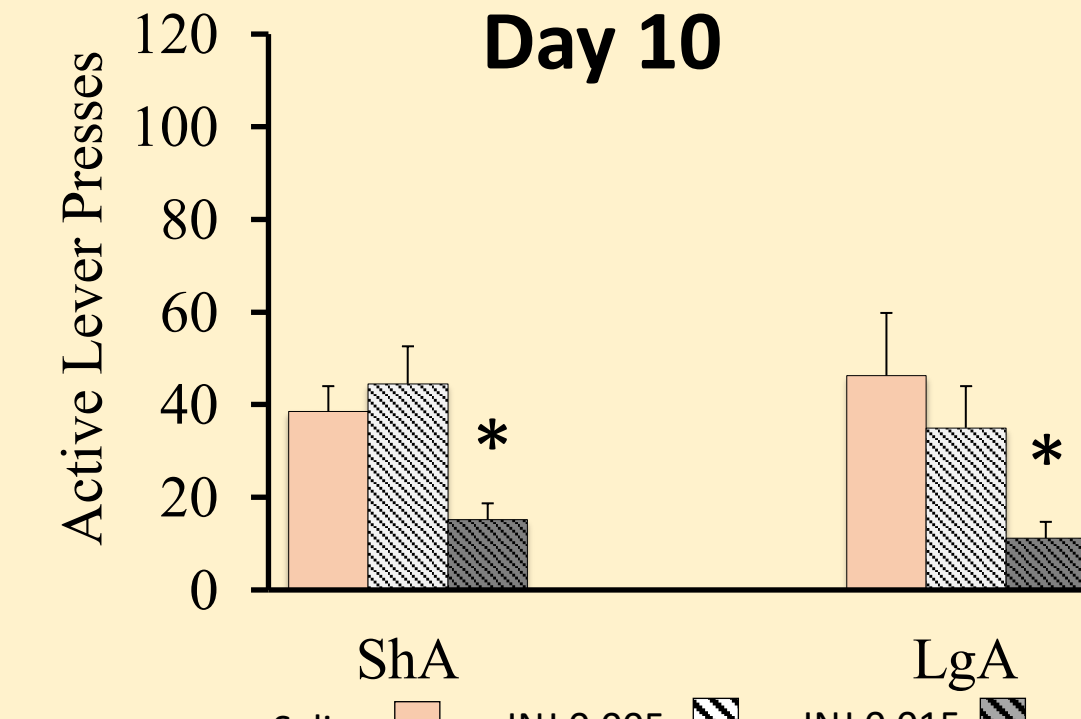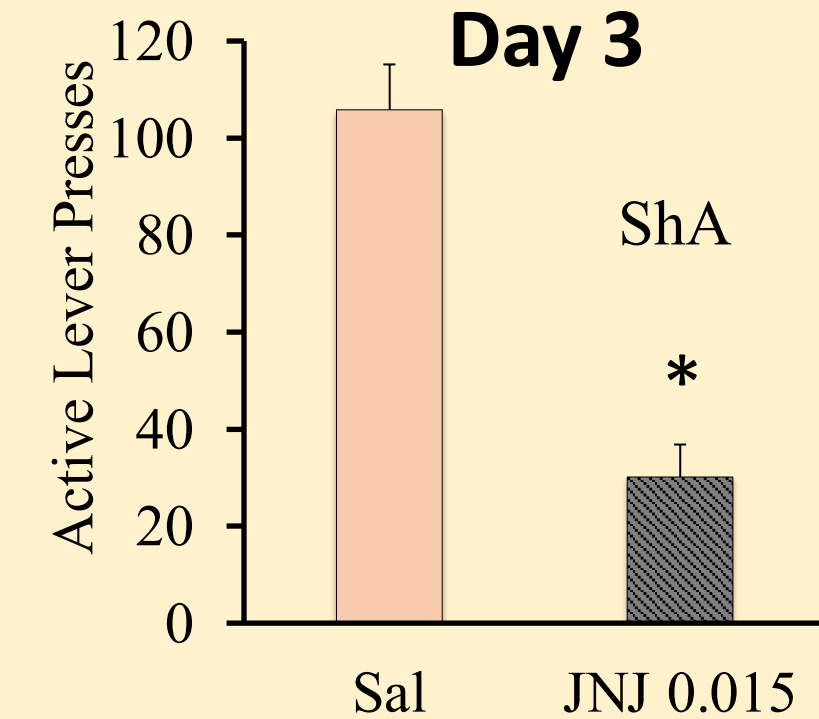
All drug administrations were systemic, mg/kg, sc.

mGluR5 receptor regulates drug seeking at all three abstinence periods in ShA but only after a long abstinence period in LgA
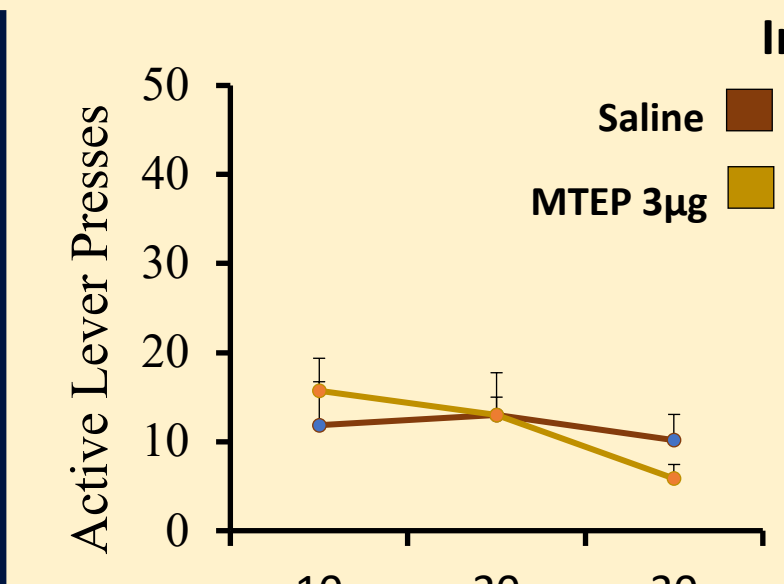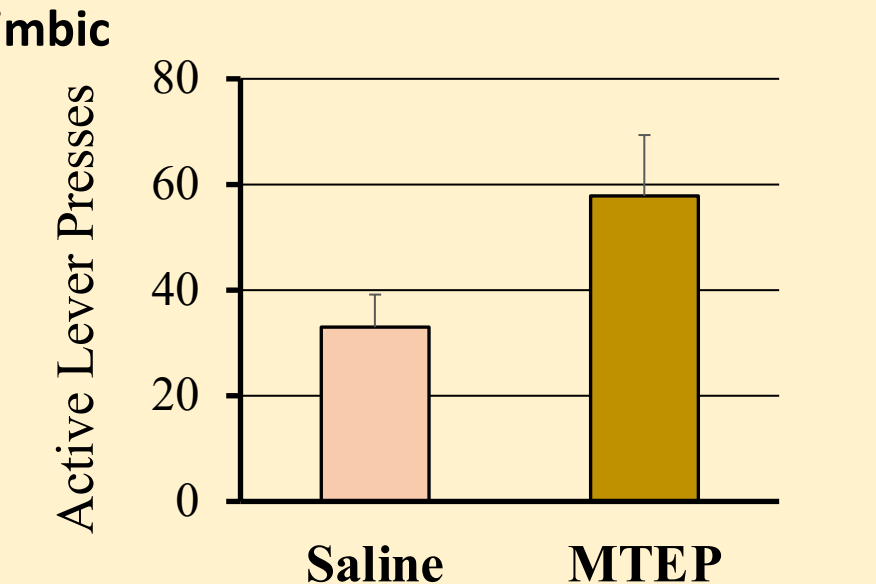

Day 3 — ShA / LgA
Day 10 — ShA / LgA
Day 60 — ShA / LgA

All drug administrations were systemic, mg/kg, ip.

**mGluR5 receptors in the Prefrontal cortex do not decrease drug seeking behavior in ShA model**

Prelimbic — Saline / MTEP 3µg
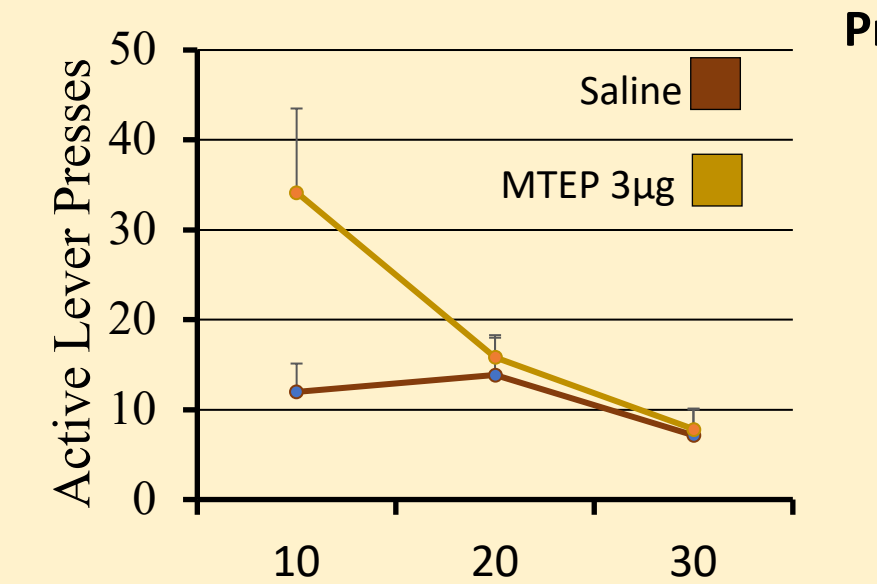
Blockade of mGluR5 receptor in prelimbic PFC increases drug seeking
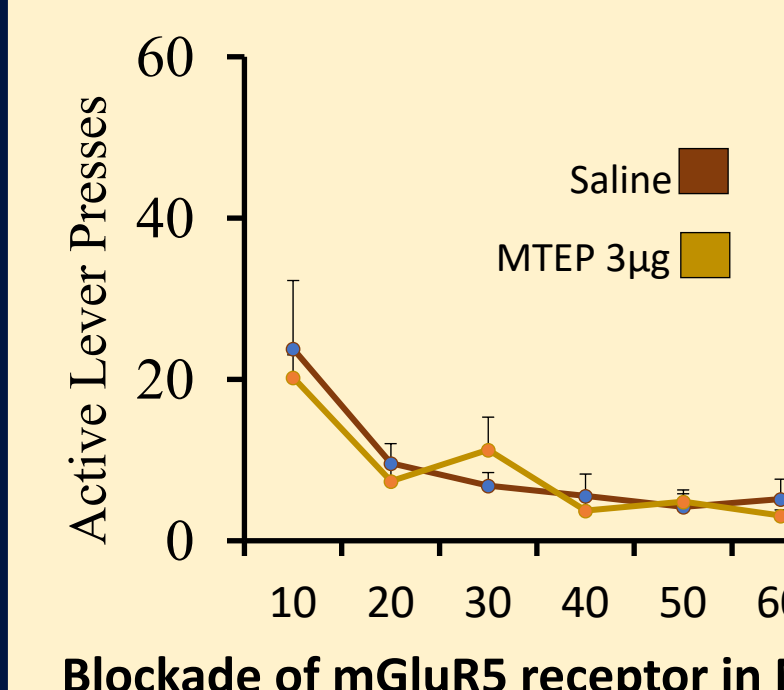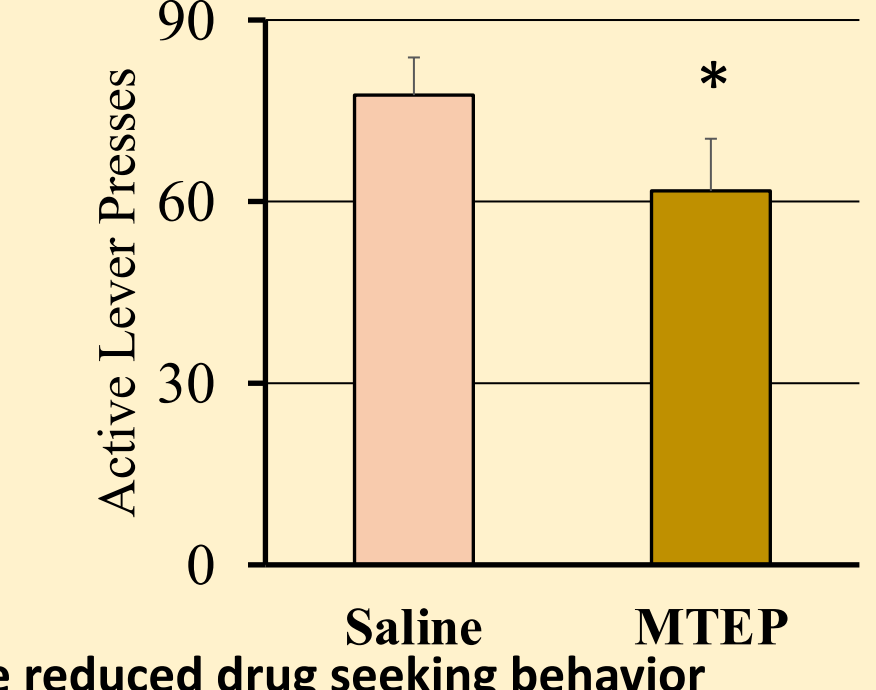
Infralimbic — Saline / MTEP 3µg

Blockade of mGluR5 receptor in infralimbic PFC does not affect drug seeking
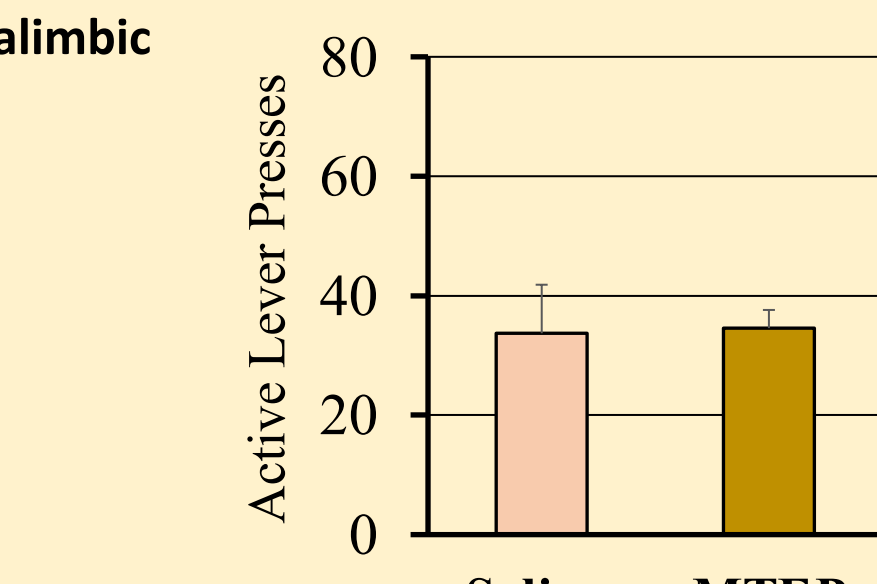
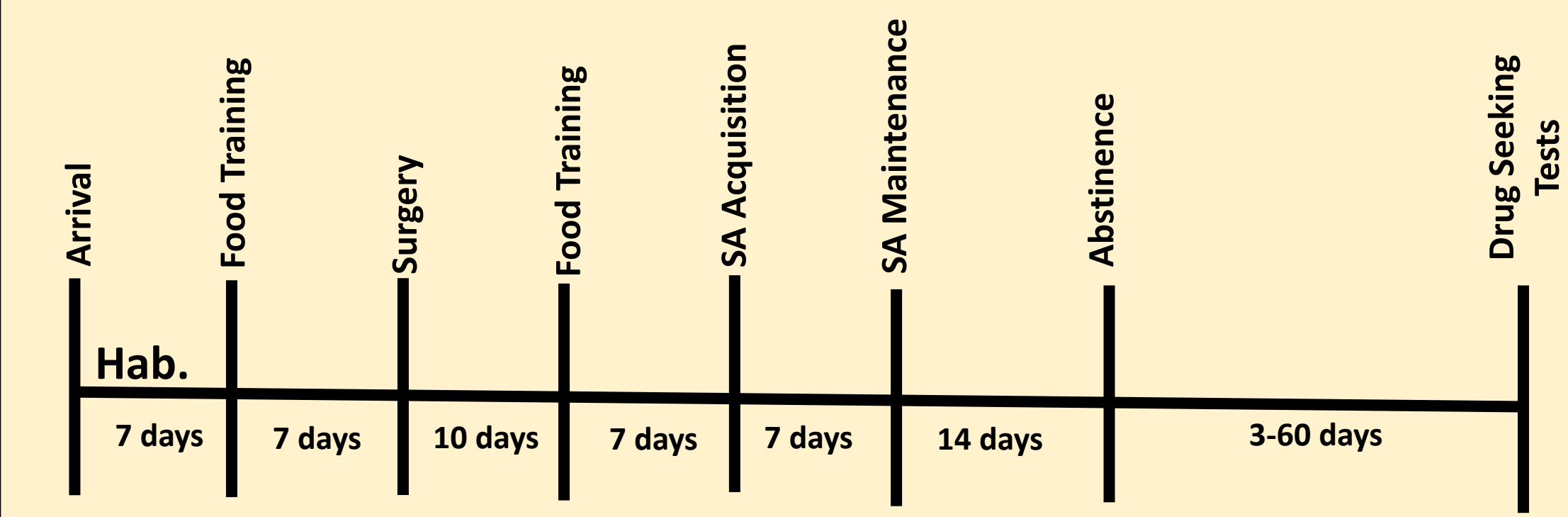**mGluR5 receptors in NAcore not shell regulate drug seeking after 10 days of abstinence in LgA model**

NAcore — Saline / MTEP 3µg
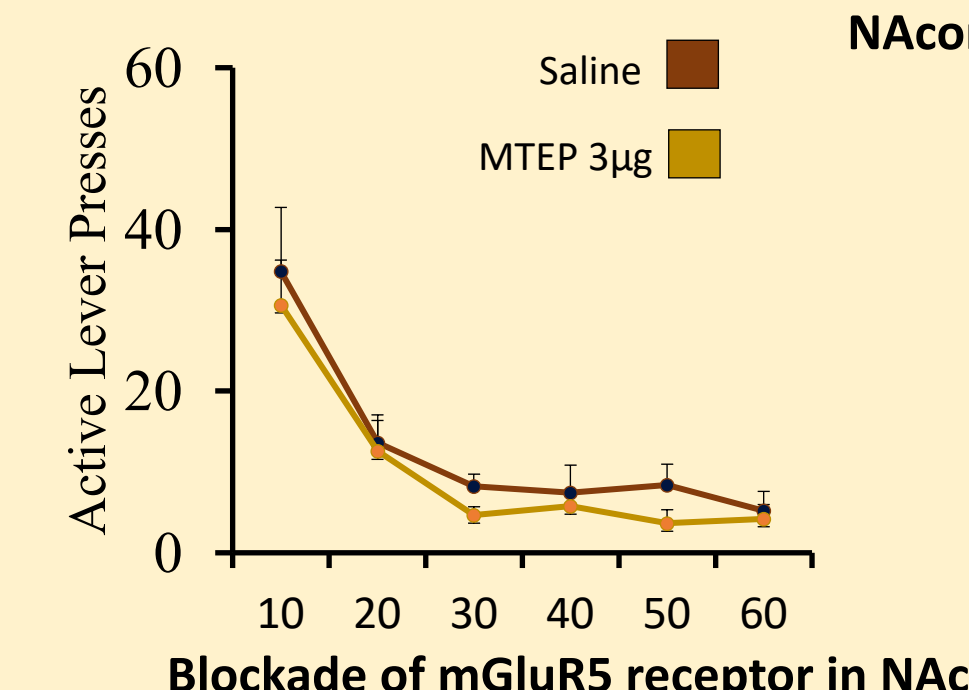
Blockade of mGluR5 receptor in NAcore reduced drug seeking behavior

NAshell — Saline / MTEP 3µg

Blockade of mGluR5 receptor in NAshell had no effect on drug seeking behavior

Experimental Timeline

Arrival — Food Training — Surgery — Food Training — SA Acquisition — SA Maintenance — Abstinence — Drug Seeking Tests

Hab.
7 days | 7 days | 10 days | 7 days | 7 days | 14 days | 3-60 days

Conclusions

- o Plasticity in glutamate signaling following cocaine exposure is brain region, drug intake, abstinence, and receptor specific.

- o Impact of cocaine self-administration varies for receptors mGluR1 & mGluR5.

- o Previous studies and collected data suggest mPFC mGluR5 receptors play minimal role in regulation of drug seeking for both ShA and LgA treated Sprague-Dawley rats.

- o Data presented suggests that impact of long access to cocaine (high intake) produces selective plasticity in mGluR5 signaling in the nucleus accumbens shell.

Future Direction

- o Analyzing molecular and cellular mechanisms mediating mGluR5 receptor plasticity: ShA vs. LgA.

- o Identifying the mechanism responsible for selective modulation of mGluR5 versus mGluR1 receptor by cocaine self-administration.

- o Investigate the role of mGluR5 receptor in the development of incubation of craving.

Acknowledgements

References:

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6. Stimulant outcomes: Hospitalizations. Wisconsin Department of Health Services. (2021, April 21). https://www.dhs.wisconsin.gov/aoda/stimulants-hospitalizations.htm.
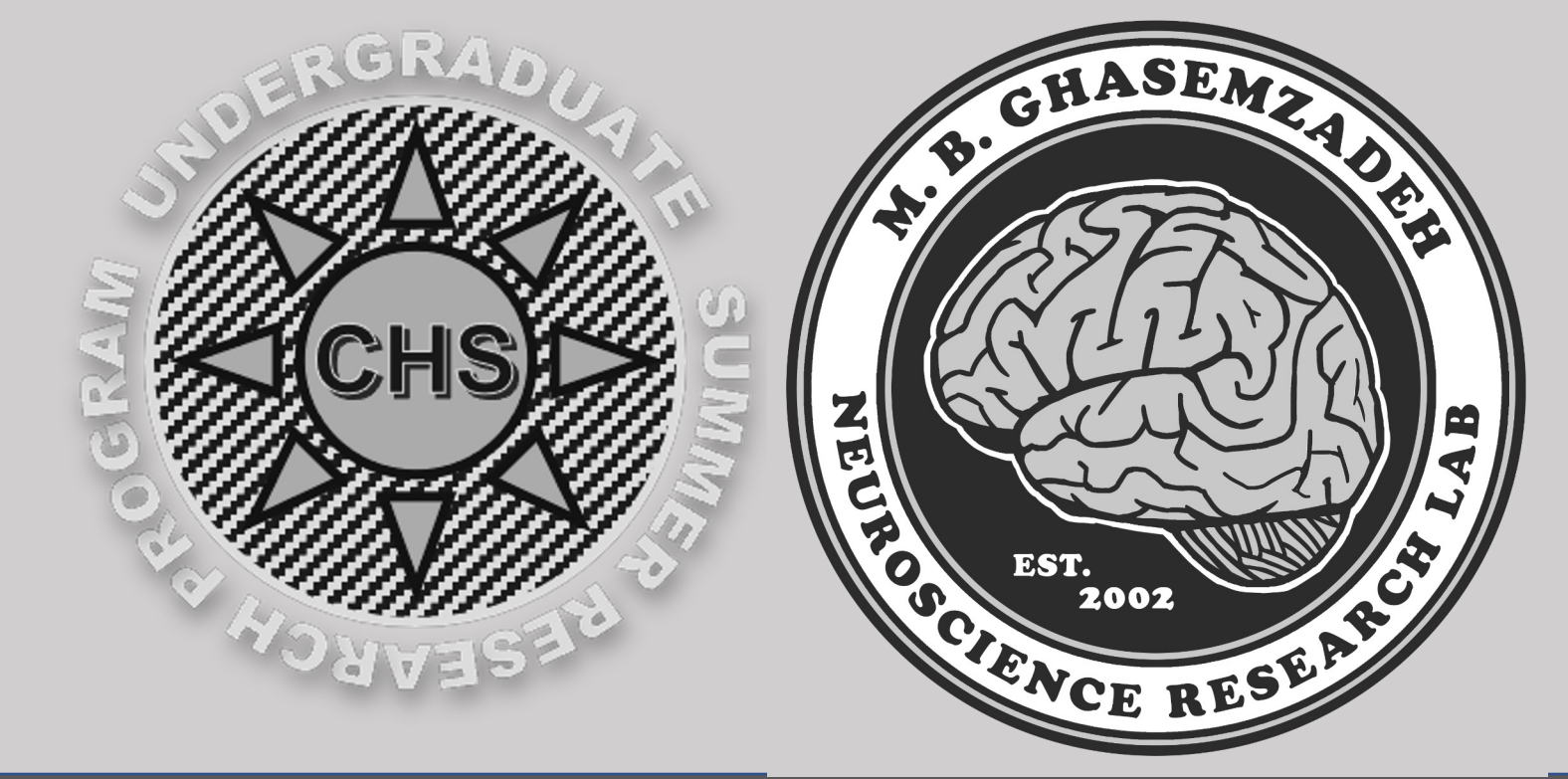
Regulation of Food Reward by mGluR5 Receptors
Optimizing DeepLabCut Artificial Intelligence Program for Behavioral Analysis

Divyank Sharma[1] & Abdallah Qasem[1], M. Behnam Ghasemzadeh[1]

[1]Department of Biomedical Sciences, College of Health Sciences, Marquette University

Introduction

Cocaine Addiction - MTEP

- According to the National Institute on Drug Abuse (NIDA) the United States has and continues to face an opioid epidemic. In the past four years funding for opioid research has increased from approximately 1.4 billion dollars to 1.9 billion dollars being allocated in hopes of developing a greater understanding of the science behind addiction, preventive measures that can be taken, treatment, and how cumulatively all can be implemented into society.[4]
  - Major obstacle faced is high relapse rates individuals face due to cues consistently perceived by their environment.
  - Prior research has determined that glutamate receptors play a crucial role in the release of dopamine and is responsible for regulation of the reward system located in the nucleus accumbens. Therefore, indicating the positive feedback motivating individuals to repeat and relapse on cocaine is based on intercellular signaling of glutamate.[1,2]
- Current research focuses on studying group 1 metabotropic glutamate receptors, specifically mGluR5 and its relationship to MTEP, a known mGluR5 blocker.

Schizophrenia – DeepLabCut AI Program

DeepLabCut was developed to assist with behavioral studies, primarily with rating locomotion and behavior in rodent studies, such as those that involve Sprague-Dawley rats during schizophrenia studies. The fundamental problem solved, and reason DeepLabCut is a favorable program is because it removes the extensive time investment and labor necessary to collect data from video recorded experiments such as elevated plus maze (EPM), a test used to assess anxiety within a rat. During this experiment, a rat is left on an elevated platform that consists of two open arms and two closed arms for 15 minutes. After the experiment is complete, videos recorded must be rated to measure amount of time the rat spent in each arm for later analysis.[3,5]
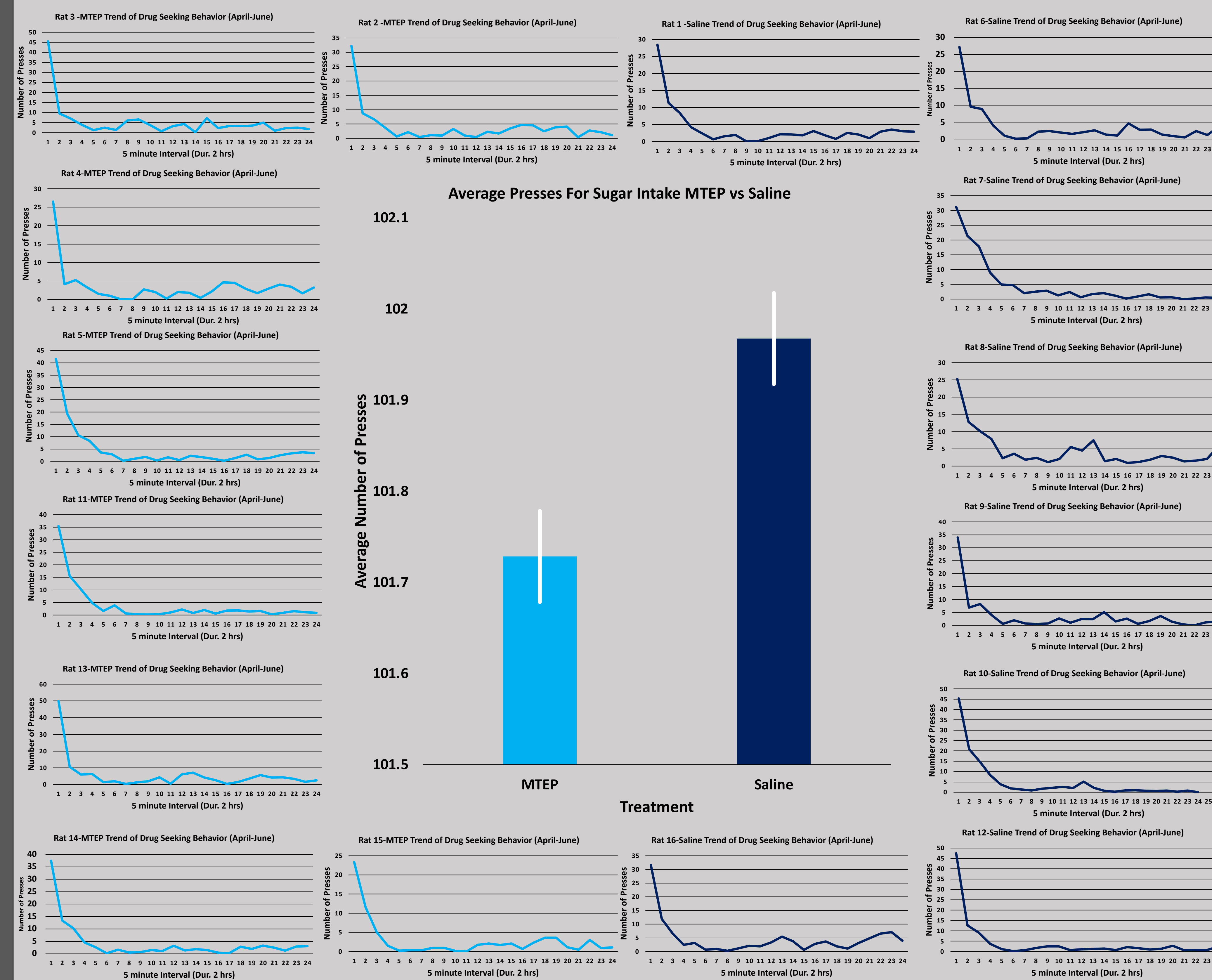
Methods

Cocaine Addiction - MTEP

- Sprague-Dawley rats arrived and were left in colony room to habituate for 5 -7 days post travel & consistently handled
- Rats were habituated to the operant chamber and food trained.
  - During habituation rats would be left in the operant chamber ranging from one to two hours to just sit.
  - Rats were then food trained for up to 7 days during which they received a sugar pellet for every time they pressed the right lever, each session lasting 2 hours.
- After rats had successfully and consistently been hitting for food (i.e. 60-70 lever presses in the two hour interval) rats were divided into two groups, control & experimental group
  - Control group received 0.5 μL of saline
  - Experimental group received 3.0 μg MTEP (mGluR5 receptor blocker)
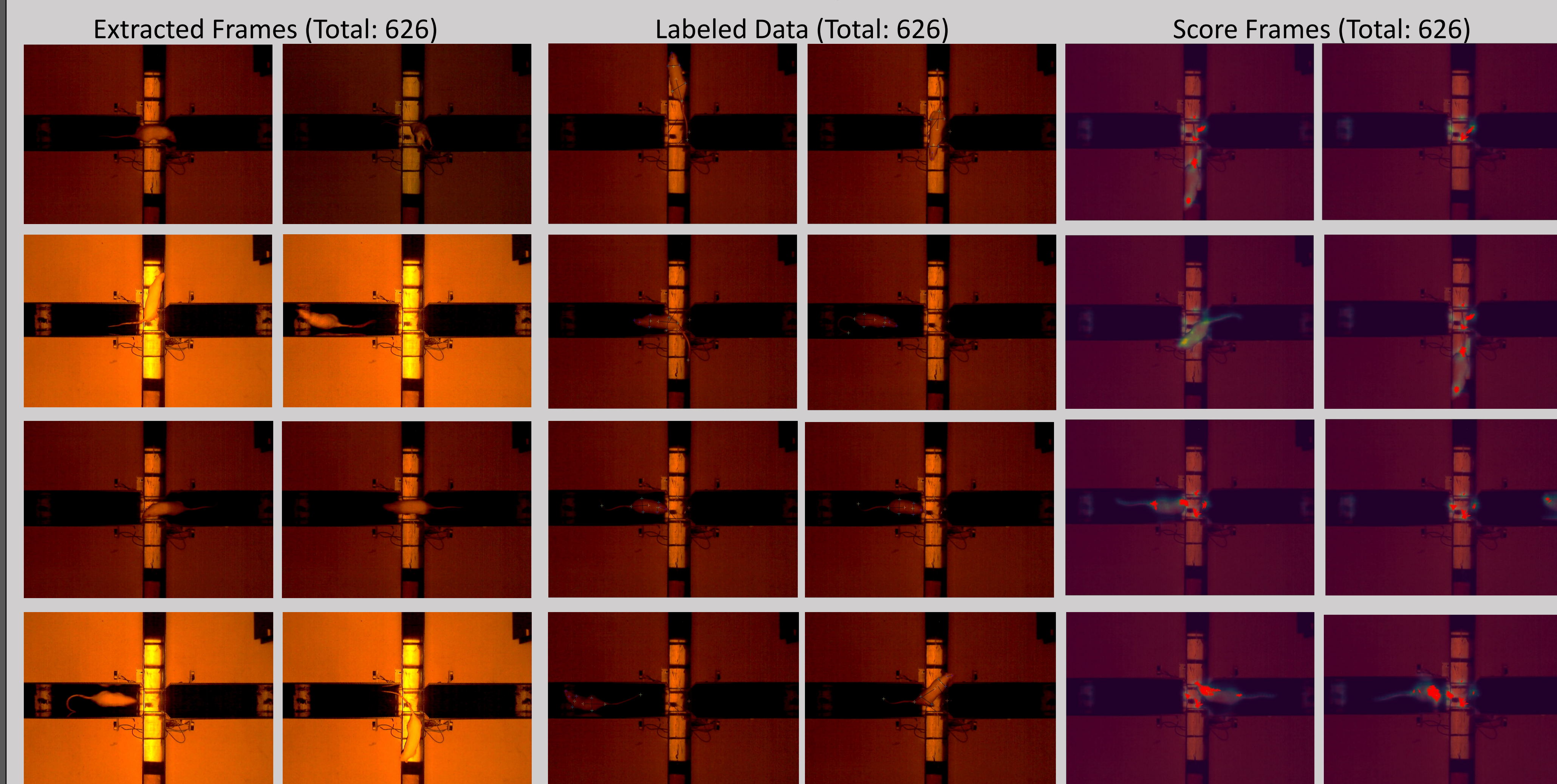- Number of presses between control & MTEP groups were compared

Schizophrenia – Programming DeepLab Cut

- EPM videos were uploaded to DeepLabCut and a configuration file was edited to set the parameters for what would be extracted from each video. A total of 626 frames were extracted from the videos, and were labelled with various markers. Markers indicated borders on the EPM maze and body parts of the rat. Labels were as follows:
  - Nose/Head/ Body/Centerbody
  - Rforepaw/Lforepaw/Rhindpaw/Lhindpaw
  - Tailbase/Tailend
  - Borders 1-4
- Markers placed were analyzed by the program at 95% confidence followed by a skeleton being built to teach the AI program what the shape of the rat is in comparison to the markers previously placed.
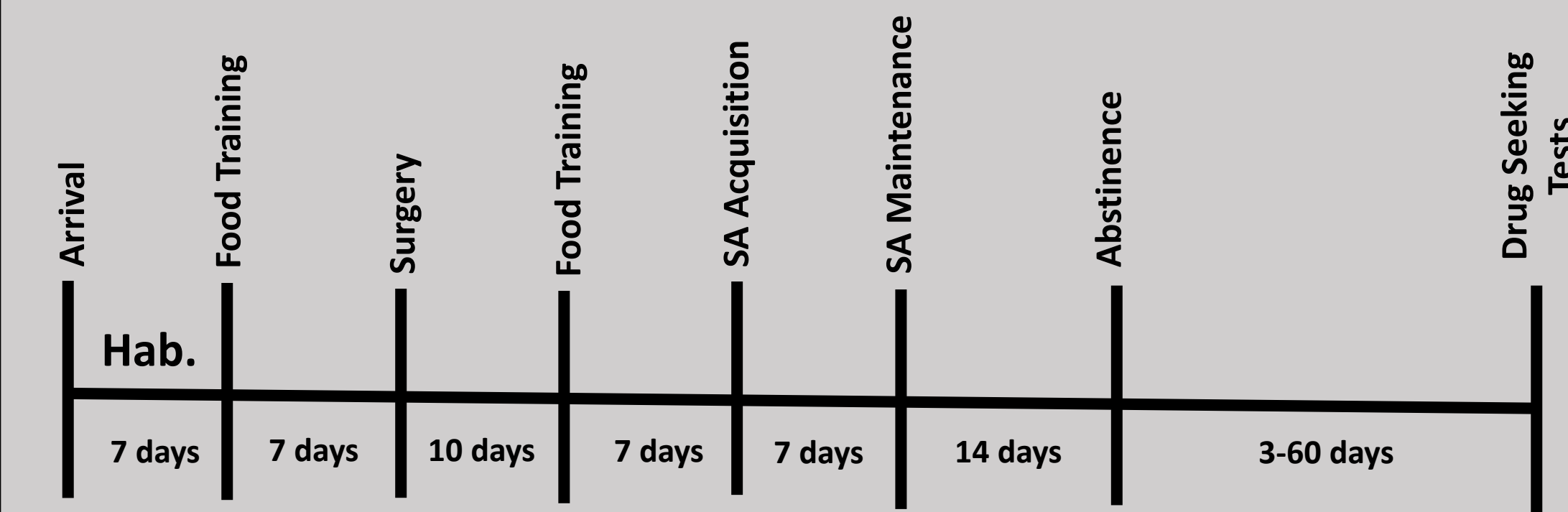
Results – MTEP

Average Presses For Sugar Intake MTEP vs Saline

Results – DeepLabCut AI

Extracted Frames (Total: 626) — Labeled Data (Total: 626) — Score Frames (Total: 626)

Experimental Timeline- Cocaine Addiction

Arrival — Food Training — Surgery — Food Training — SA Acquisition — SA Maintenance — Abstinence — Drug Seeking Tests

Hab. — 7 days — 7 days — 10 days — 7 days — 7 days — 14 days — 3-60 days

Conclusions

Cocaine Addiction - MTEP

- Plasticity in glutamate signaling following cocaine exposure is drug intake, abstinence, brain region, and receptor specific.
- MTEP, a blocker of receptor mGluR5, significantly decreased drug seaking behavior in Sprague-Dawley rats following food training.

Schizophrenia – DeepLabCut AI Program

- DeepLabCut will be an effective and efficient alternative to rating videos in comparison to manual rating.
- DeepLabCut will be as accurate with rating schizophrenia EPM videos, therefore validating the model of the program.

Future Direction

- Analyzing molecular and cellular mechanisms mediating mGluR5 receptor plasticity: ShA vs. LgA.
- Investigating and identifying the mechanism responsible for selective modulation of mGluR5 versus mGluR1 receptor by cocaine self-administration.
- Improve accuracy of DeepLabCut AI program for EPM videos by increasing sample size
- Expand use of DeepLabCut AI program to rate additional schizophrenia experiments such as social interaction and novel object recognition

Acknowledgements

References:

1. Ghasemzadeh, M. B., Vasudevan, P., Giles, C., Purgianto, A., Seubert, C., & Mantsch, J. R. (2011). Glutamatergic plasticity in medial prefrontal cortex and ventral tegmental area following extended-access cocaine self-administration. *Brain research*, 1413, 60–71. https://doi.org/10.1016/j.brainres.2011.06.041

1. Ghasemzadeh, M. B., Vasudevan, P., Mueller, C., Seubert, C., & Mantsch, J. R. (2009). Neuroadaptations in the cellular and postsynaptic group 1 metabotropic glutamate receptor mGluR5 and Homer proteins following extinction of cocaine self-administration. *Neuroscience letters*, 452(2), 167–171. https://doi.org/10.1016/j.neulet.2008.12.028

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