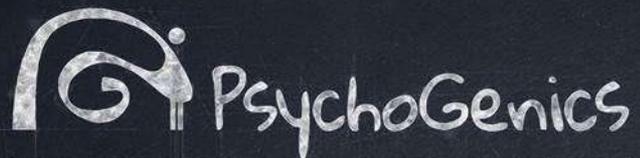


Redefining Drug Discovery Through Innovation

Preclinical Models of Schizophrenia

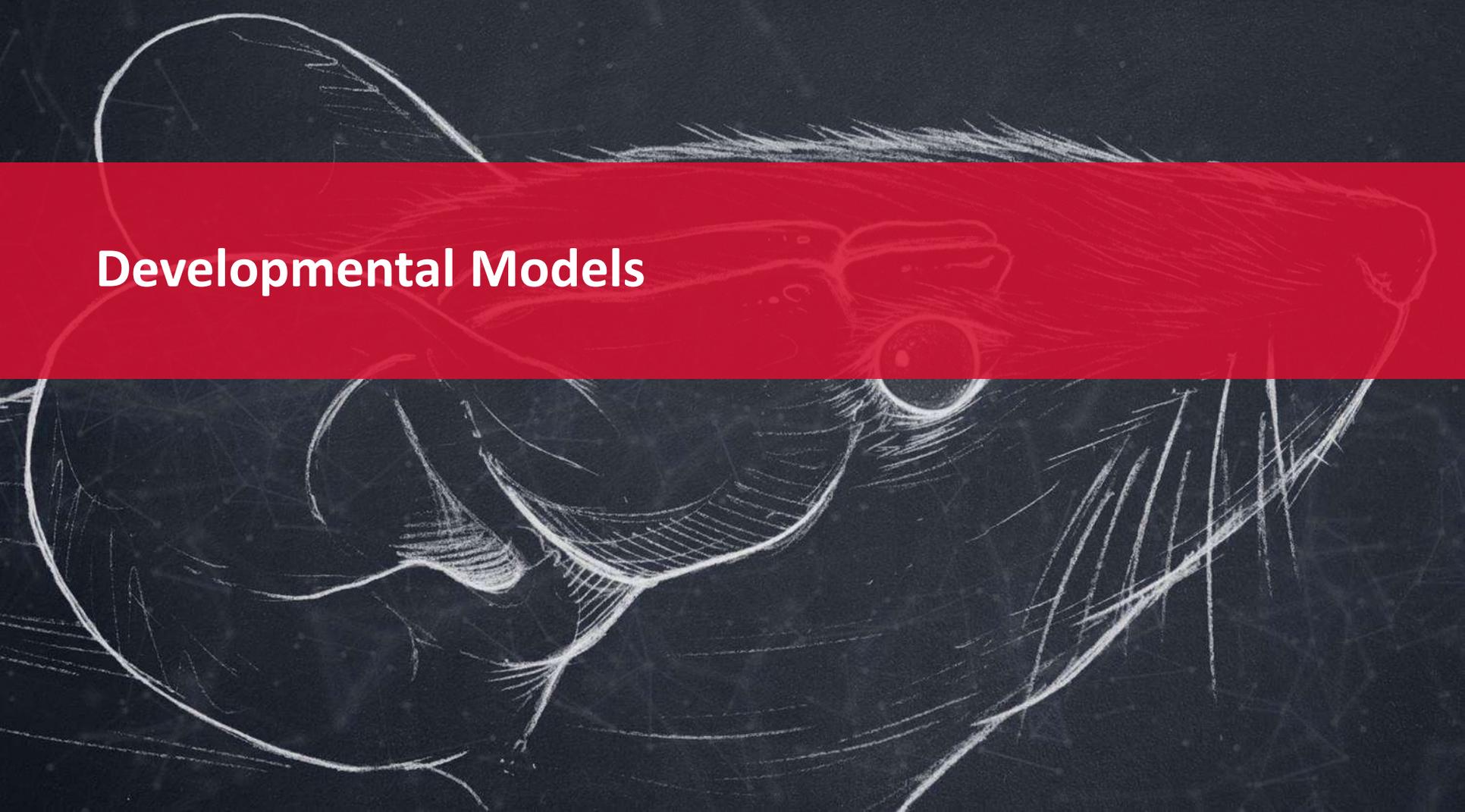
Preclinical Models

- PGI Offers several models that target positive, negative and cognitive deficits associated with schizophrenia
 - Simulant induced activity (locomotion, headshakes, rearing and climbing)
 - Prepulse Inhibition of Acoustic Startle
 - Conditioned Avoidance Response
 - PCP-induced deficits in Social Interaction
 - PCP-induced deficits in NOR
 - PCP-induced deficits in Social Recognition
 - MK-801 induced deficits in Reversal Learning
- Developmental Model: MAM
- SmartCube High throughput screening in genetic models including Neurexin, Neuregulin, DISC1 and Dysbindin
- Mismatched Negativity and LDAEP



Redefining Drug Discovery Through Innovation

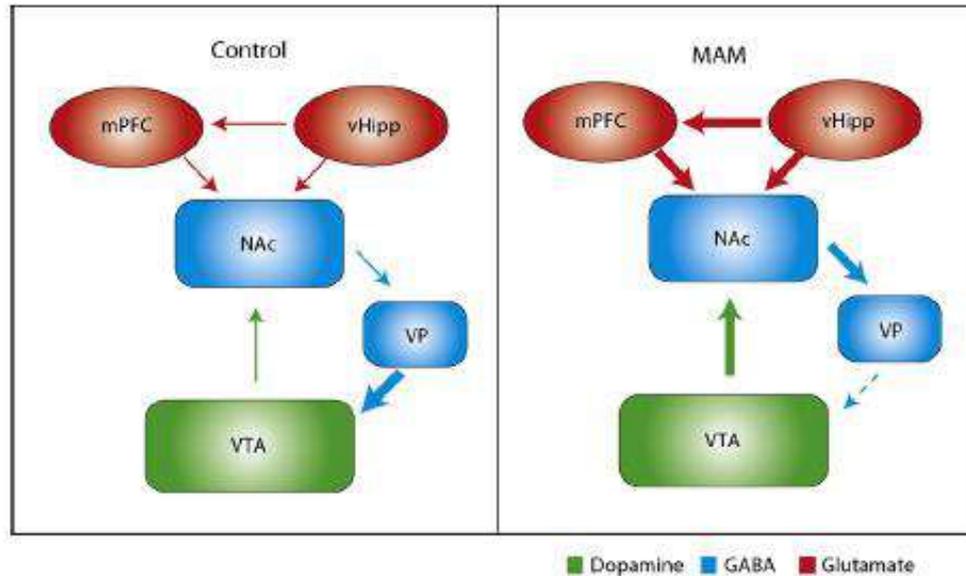
Developmental Models



Methylazoxymethanol (MAM) Model

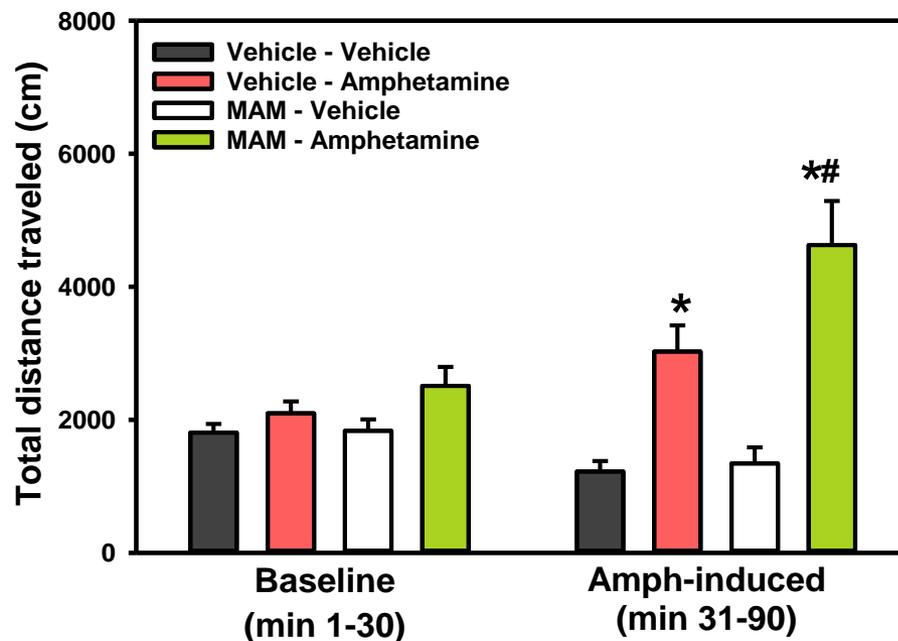
- The model is based on the notion that schizophrenia is a developmental disorder.
- Rats exposed to (MAM; 20-22 mg/kg) in utero on gestational day 17 have been proposed as a rat model of schizophrenia. Offspring of MAM-treated rats are reported to display
 - Decreased social interaction
 - Increased sensitivity to stimulant properties of amphetamine
 - Hyper-responsiveness to stress
 - Sensorimotor gating deficits
 - Increased orofacial dyskinesias
 - Impaired cognitive flexibility
 - Impaired episodic memory
 - Impaired latent inhibition
 - Defects in brain morphology
- Pathological changes in the brain are similar to those observed in human patients:
 - Decreased cortical thickness
 - Decreased medial temporal lobe and hippocampal volume
 - Increased neuronal density in mPFC
- Neuropathology in circuits implicated in schizophrenia:
 - Selective targeting of paralimbic, frontal and temporal cortices similar to schizophrenia patients

Modeling Pathology



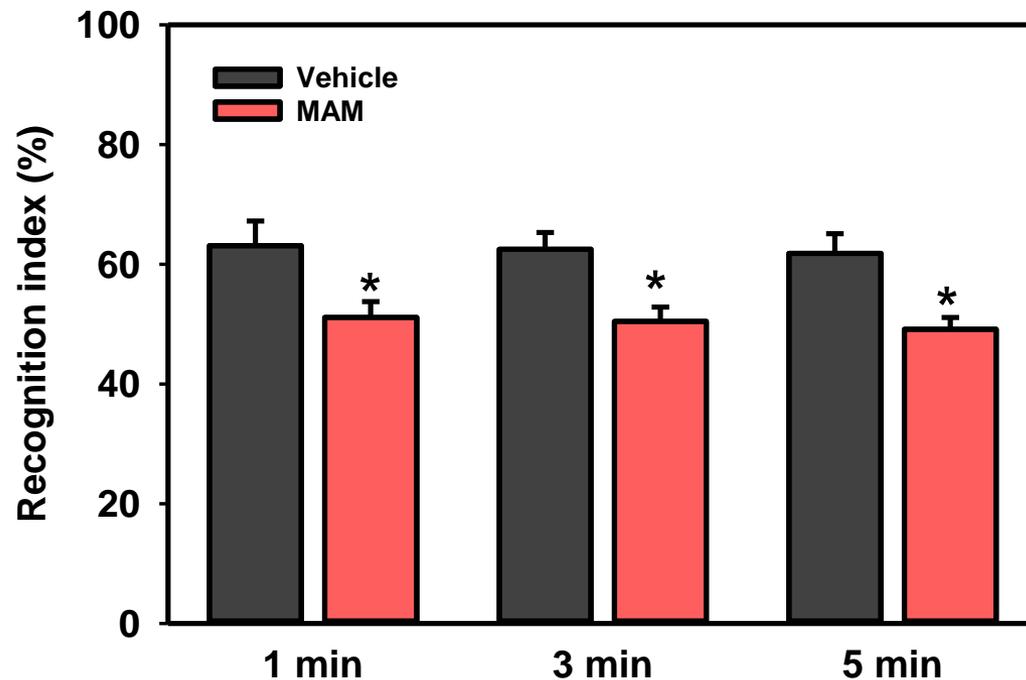
- Increased activation of Nucleus Accumbens (NAc) - Glutamate
- Disinhibition of VTA (via GABA output from VP) causes increased activation of mesolimbic DA system.
- Consistent with hyper-responsive DA neurotransmission in schizophrenia

Increased Sensitivity to Amphetamine



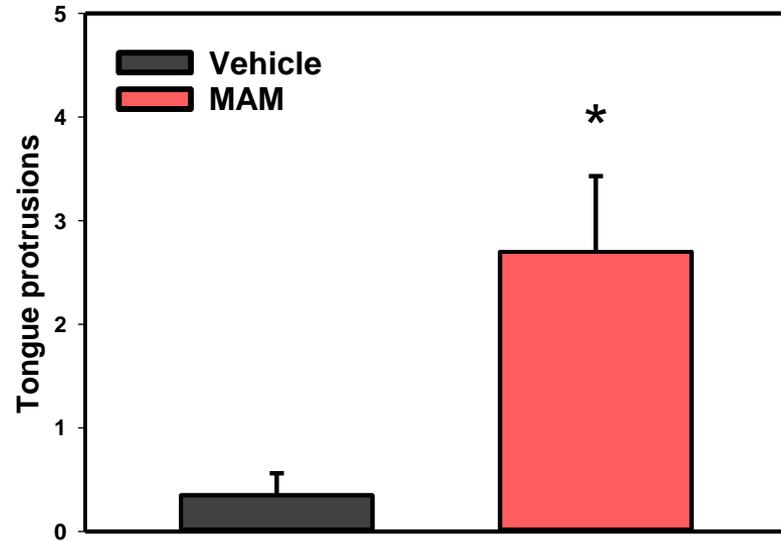
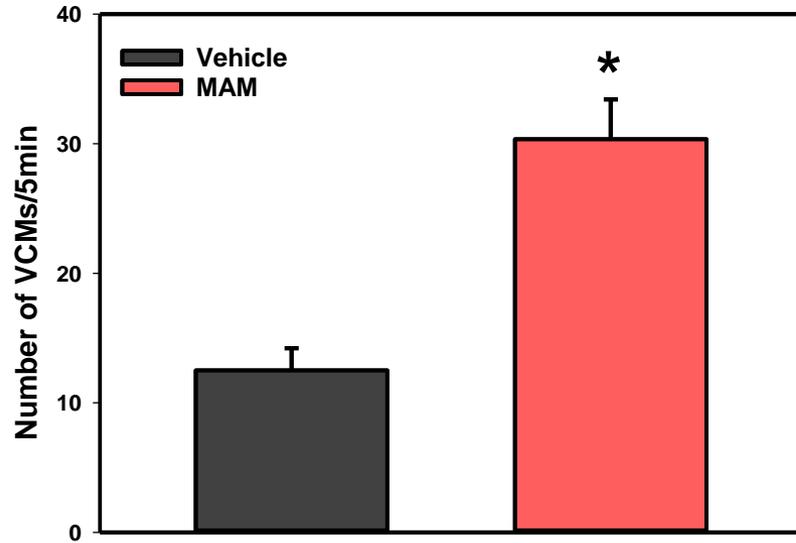
- MAM-treated offspring show increased locomotor activity in response to an acute injection of amphetamine (0.5 mg/kg) compared to vehicle-treated rats

Deficits in Novel Object Recognition Task



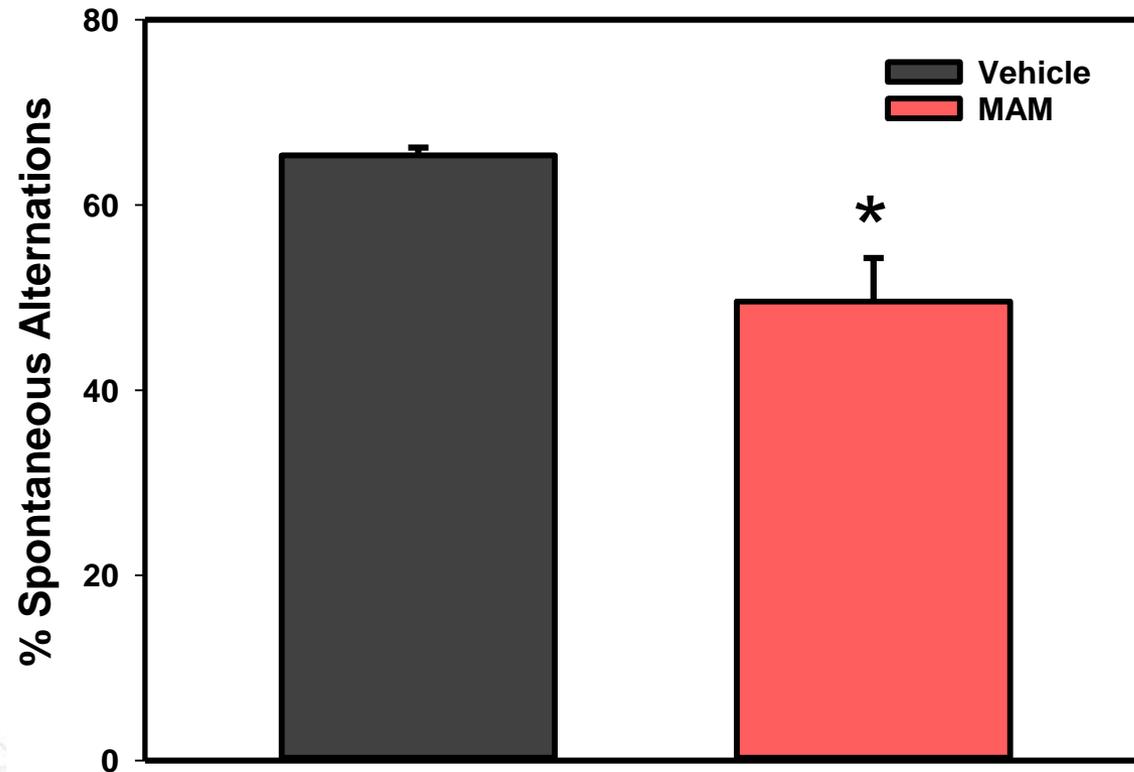
- Rats were allowed 3 days habituation to test chamber.
- Training was conducted in the pretense of 2 similar objects for 10 minutes.
- Testing was conducted for 5 minutes after 1 hour ITI, using one familiar and one novel object. Objects and placement (left/right) were counter-balanced across subjects

Increased Vacuous Chewing and Tongue Protrusions

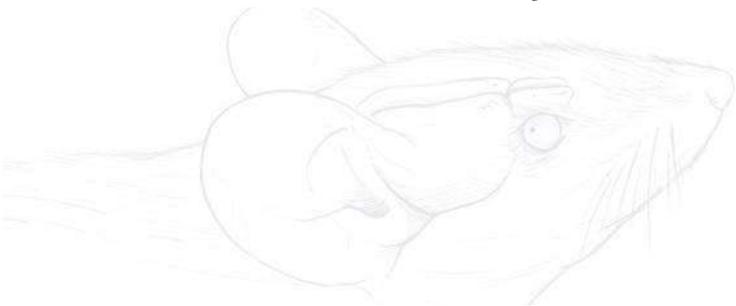
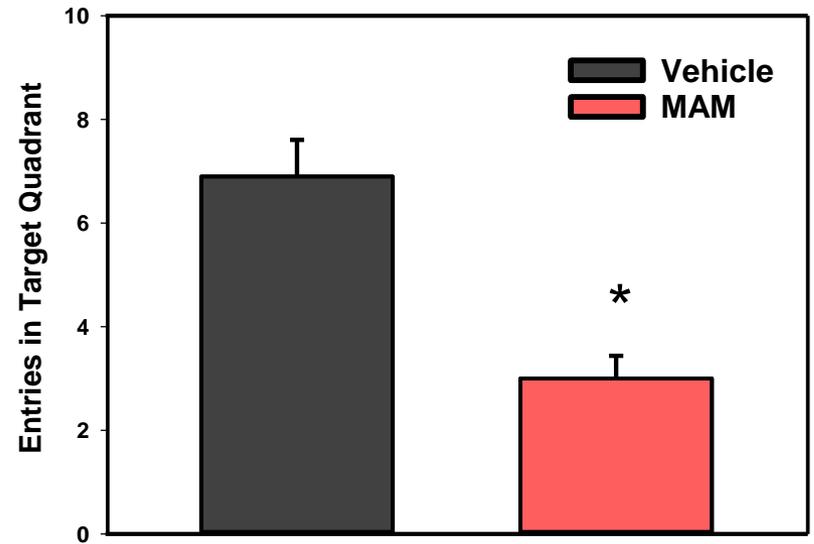
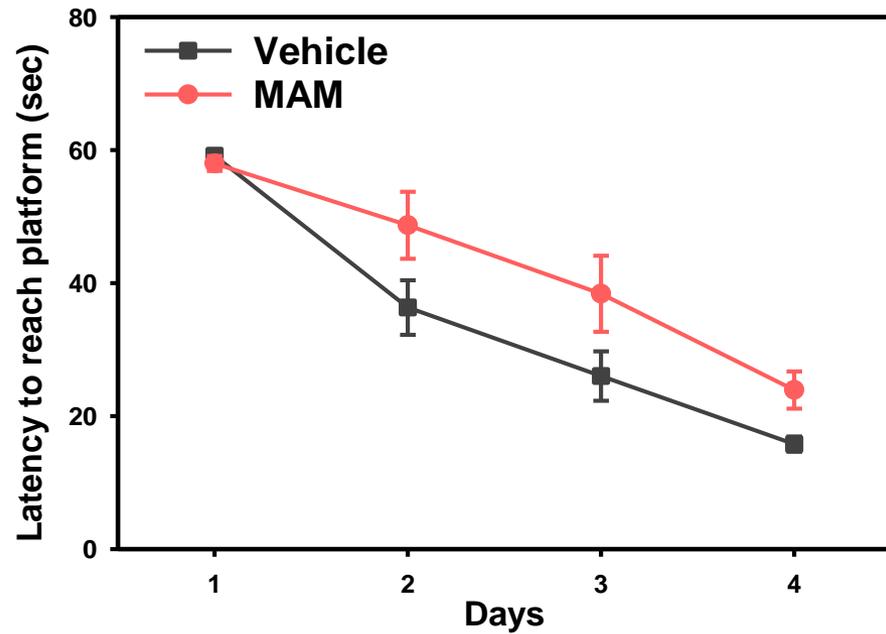


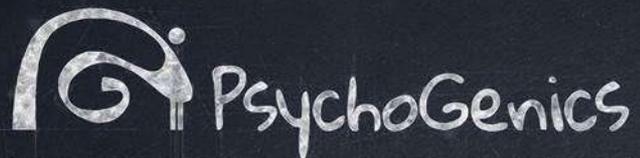
Adult rats from pregnant dams treated with either MAM or saline on GD17 were observed for 5 minutes and the number of vacuous chewing movements (VCMs: defined as single mouth openings not directed towards a physical material) and number of tongue protrusions were recorded. Offspring of MAM-treated rats showed a significant increase in VCM and tongue protrusions compared to offspring from saline-treated rats. Data are presented as mean \pm SEM. * $p < 0.05$ as compared to saline-treated controls.

Deficits in Spontaneous Alternations in Y maze



Deficits in MWM Test



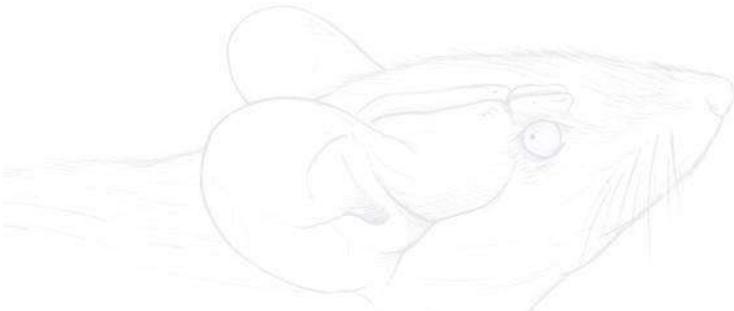
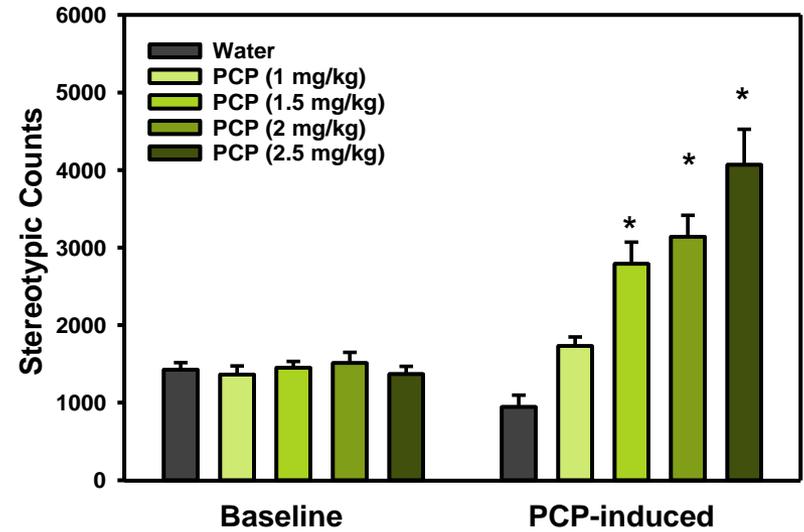
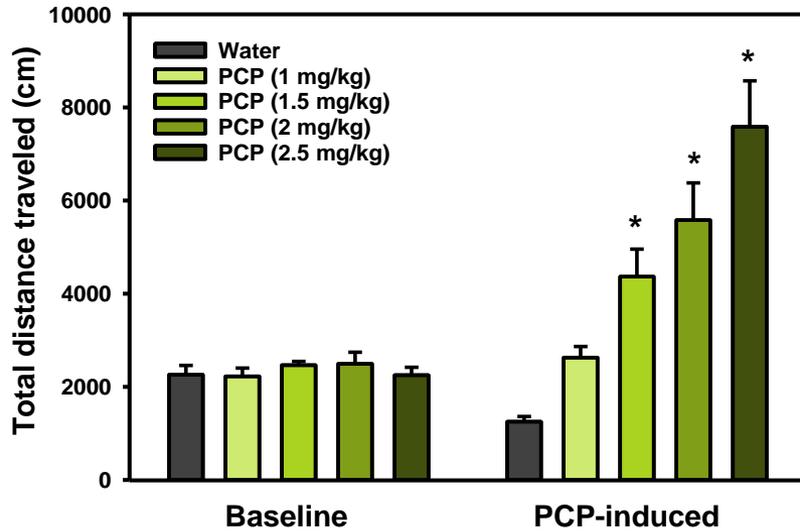


Redefining Drug Discovery Through Innovation

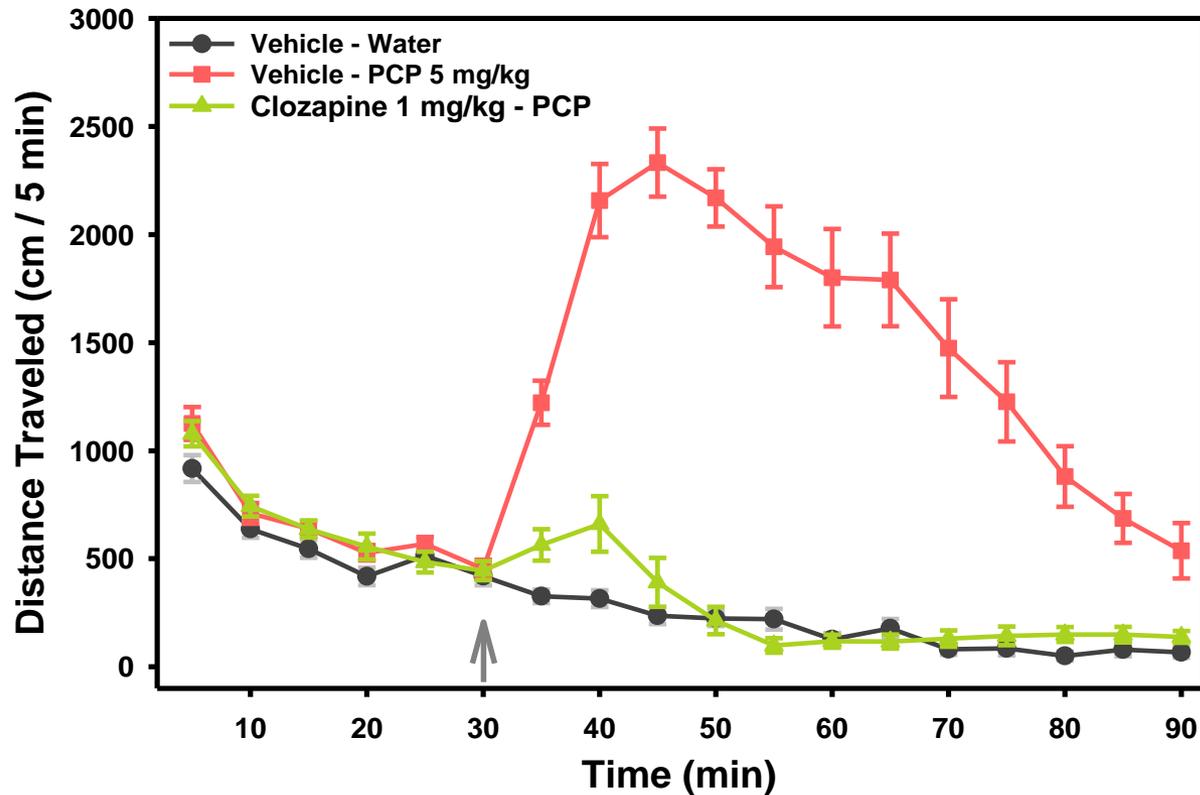
Stimulant-Induced Activity



PCP-Induced Activity in SD rats

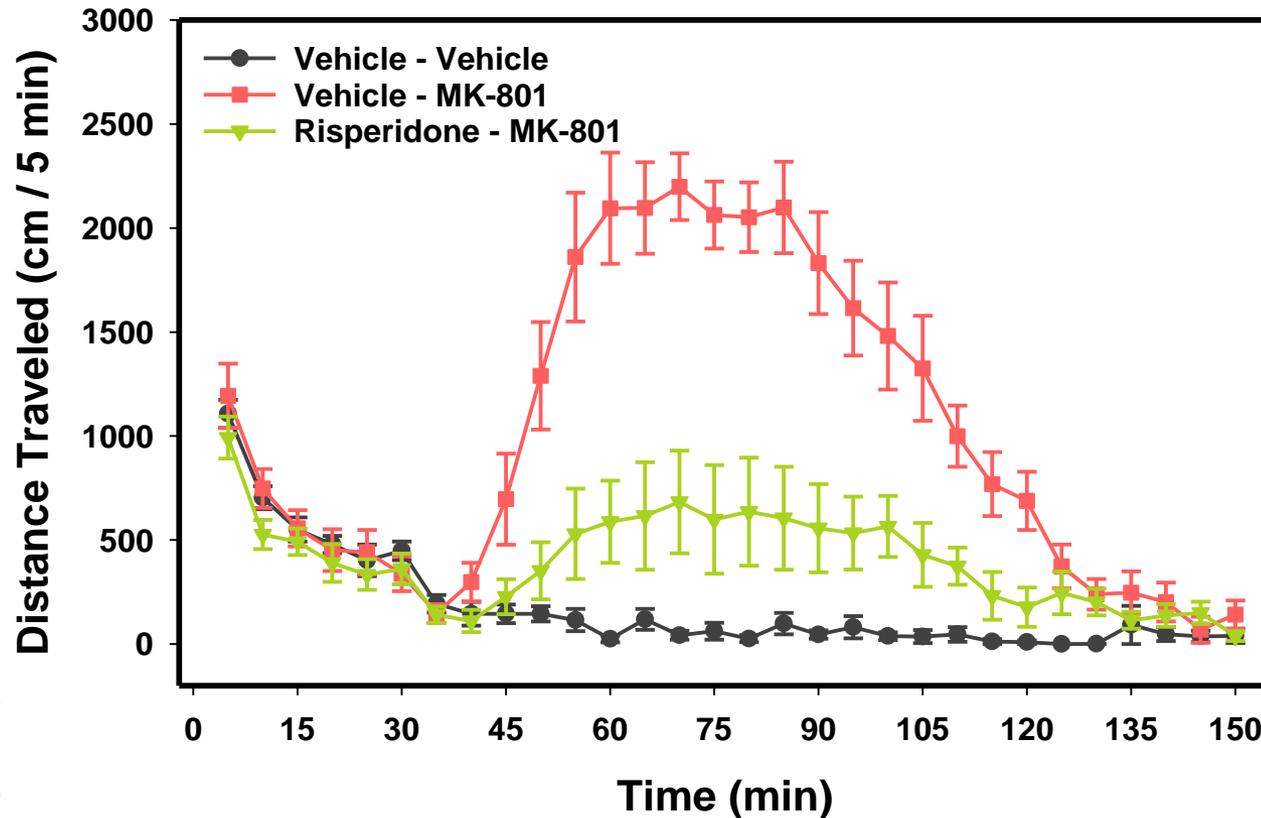


Clozapine Attenuates PCP-Induced Locomotion in Mice



Male C57BL6/J mice used. PCP injected IP at time 30 min. Clozapine injected IP at time 0 (30 min prior to PCP)

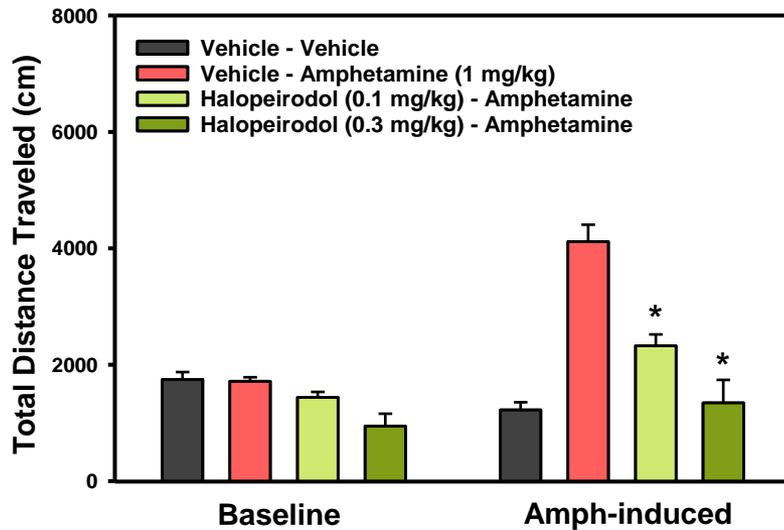
Risperidone Attenuates MK-801-Induced Locomotion in Mice



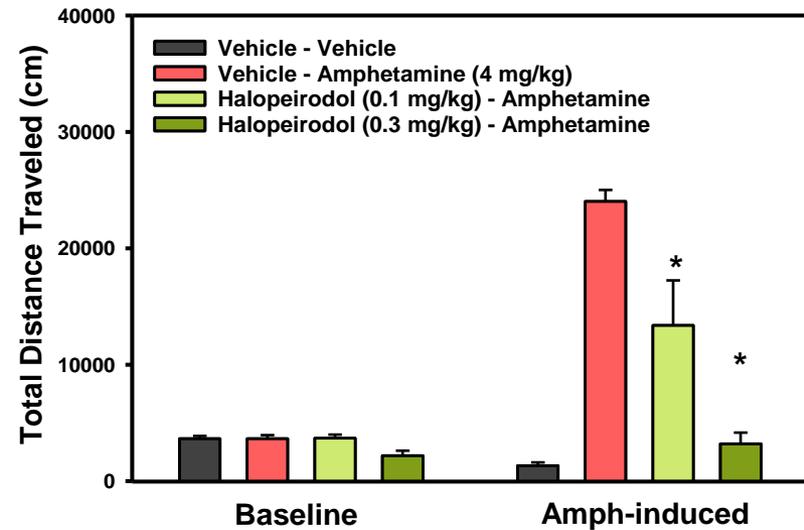
Male C57BL6/J mice used. MK-801 0.3 mg/kg injected IP at time 30 min. Risperidone 0.03 mg/kg injected IP at time 0

Haloperidol Attenuates Amphetamine-Induced Locomotion

SD Rats



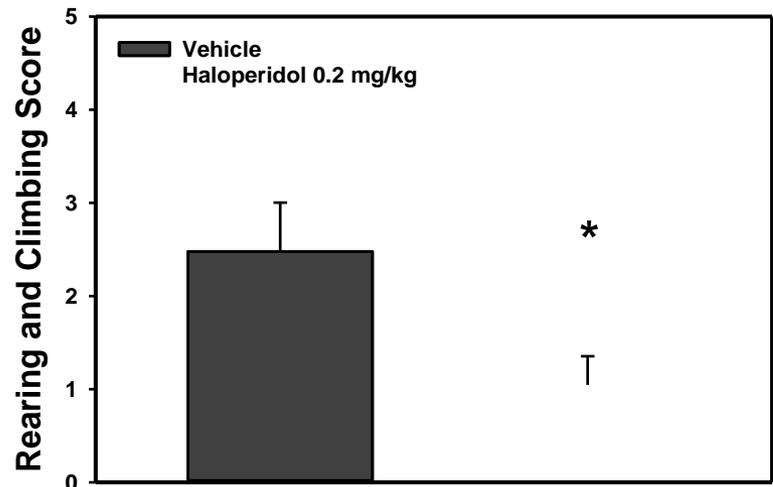
C57Bl6/J Mice



Apomorphine-Induced Rearing and Climbing

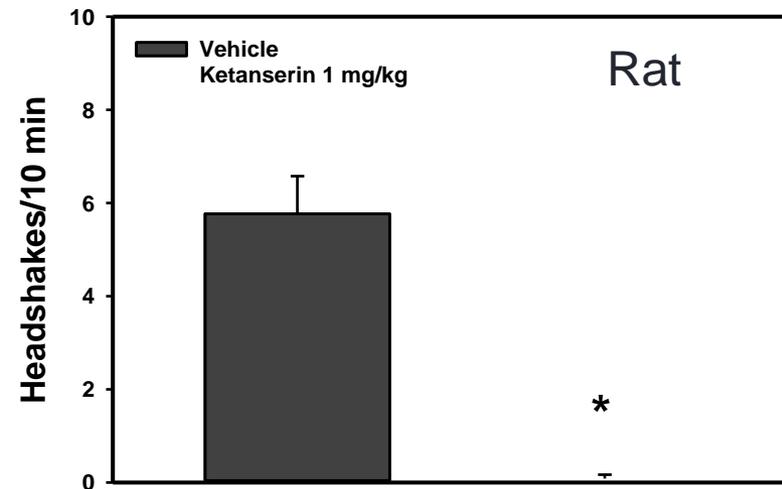
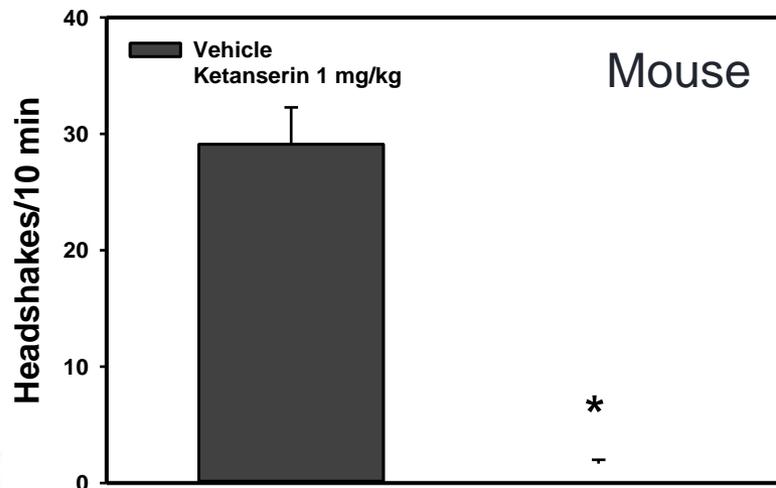
- Dysfunction of the dopaminergic system is thought to underlie some of the symptoms of schizophrenia.
- Apomorphine-induced rearing and climbing is used as a model for psychosis related to schizophrenia and other neurological disorders.
- Mice are observed for 10 – 15 sec every 2 min for 10 minutes after apomorphine (10 mg/kg; ip) injection and scored according to the following

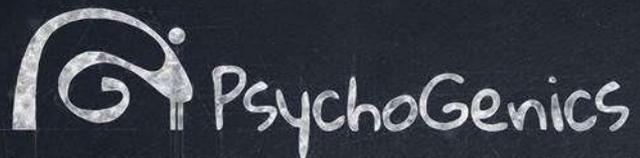
- 0 = all 4 paws on floor of cage
- 1 = 1 paw on cage wall
- 2 = 2 paws on cage wall while sitting on hind legs
- 3 = 2 paws on cage wall while standing on hind legs
- 4 = 3 paws on cage wall
- 5 = 4 paws on cage wall / intermittent climbing
- 6 = continuous climbing
- 7 = 2 paws holding top lid of climbing cage
- 8 = 4 paws holding cage lid / hanging from top



DOI-Induced Headshakes

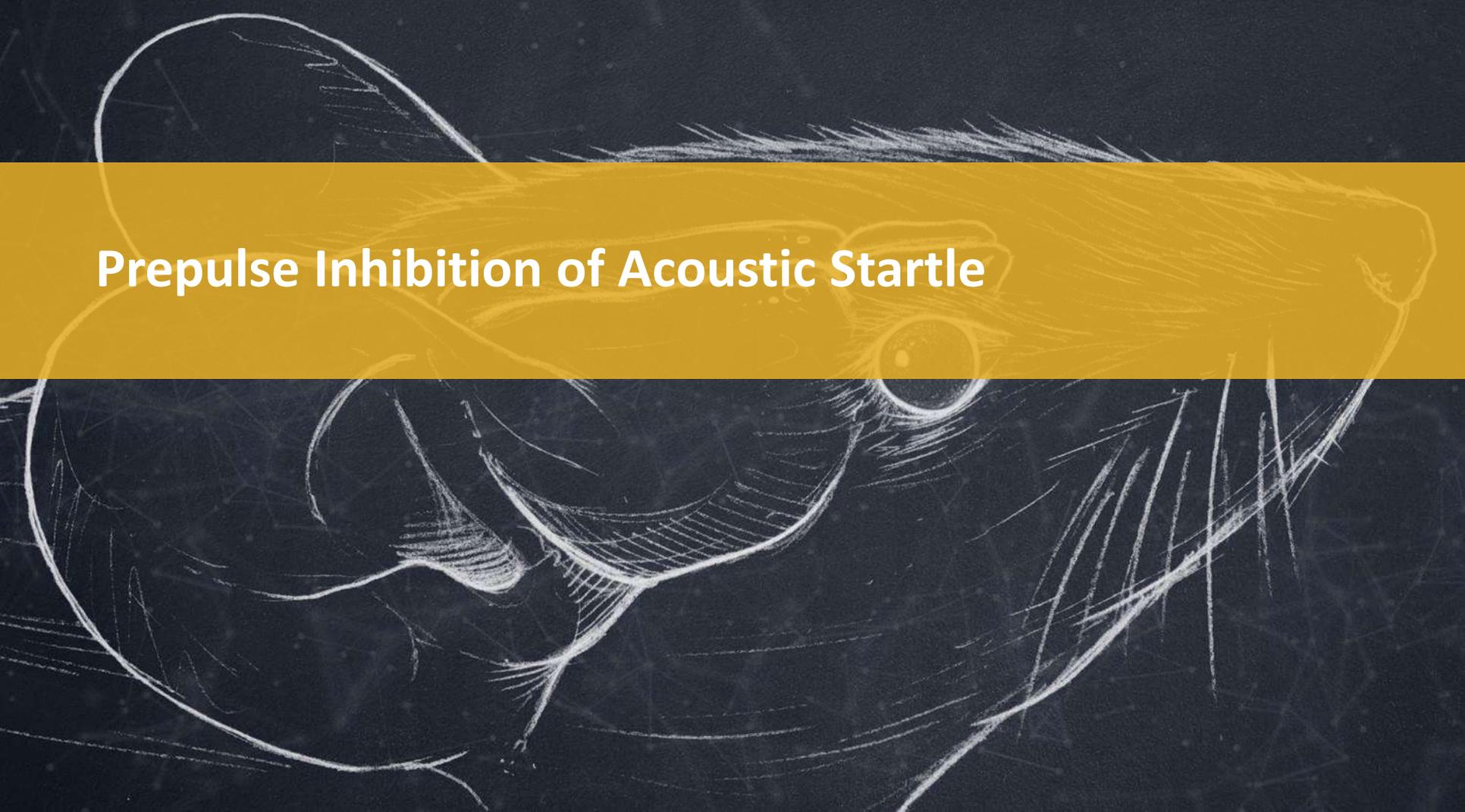
- Activation of 5-HT₂ receptors mediates behavioral effects of hallucinogenic drugs.
- DOI-induced headshakes in rodents is used to evaluate potential anti-psychotics and potential treatments for Tourette's Syndrome.
- DOI, a 5-HT_{2A/2C} agonist hallucinogen, induces head shakes seen as “wet dog” shakes in rodents.
- Rats are injected with DOI (2 mg/kg for mouse and 3 mg/kg for rat) and the number of headshakes or “wet dog” shakes are counted in a 10 minute period.





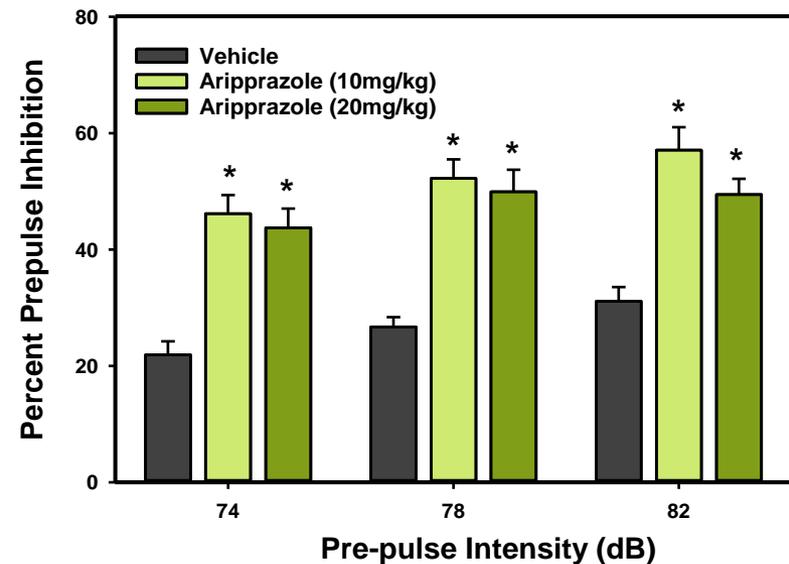
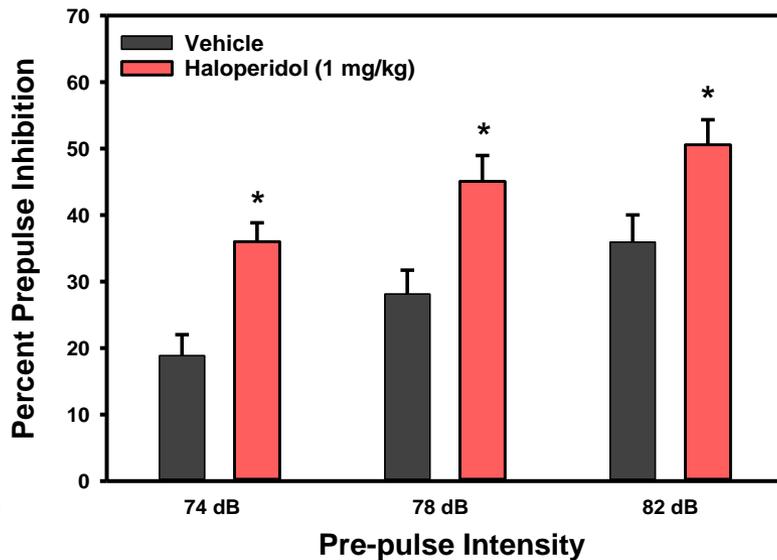
Redefining Drug Discovery Through Innovation

Prepulse Inhibition of Acoustic Startle



PPI

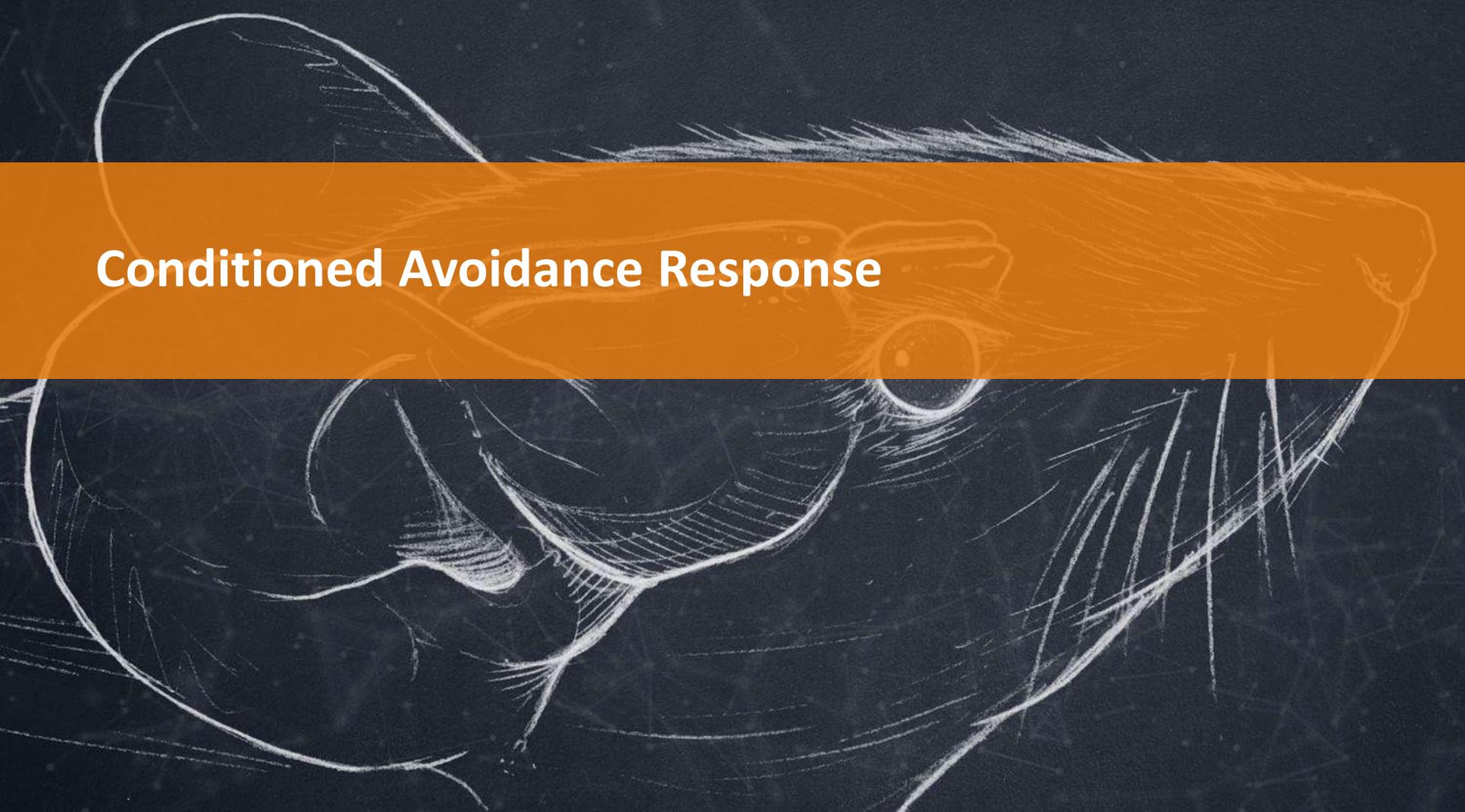
- The acoustic startle reflex is a very basic response to strong exteroceptive stimuli. A weak sound preceding the loud acoustic stimulus inhibits the startle reflex; this is called pre-pulse inhibition.
- It is widely used to assess sensorimotor reactivity (gating) in animals and humans, which is impaired in schizophrenia.
- In rodents, acute administration of PCP or MK-801 can disrupt PPI. Antipsychotic agents will increase the PPI response and also reverse the effects of disrupters.





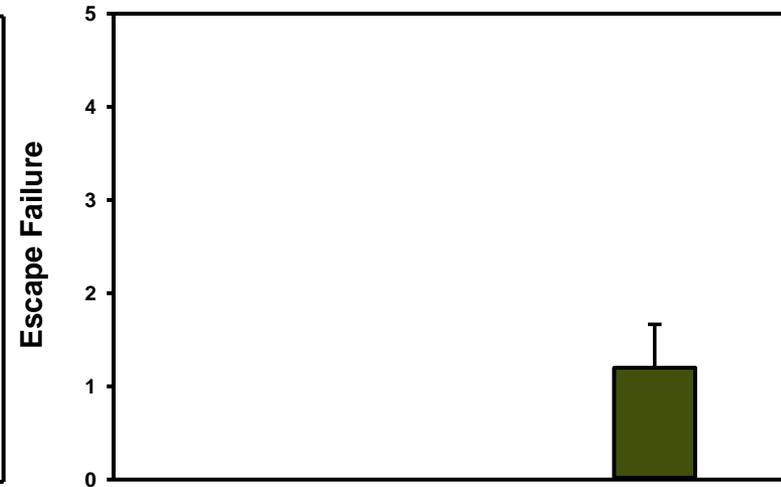
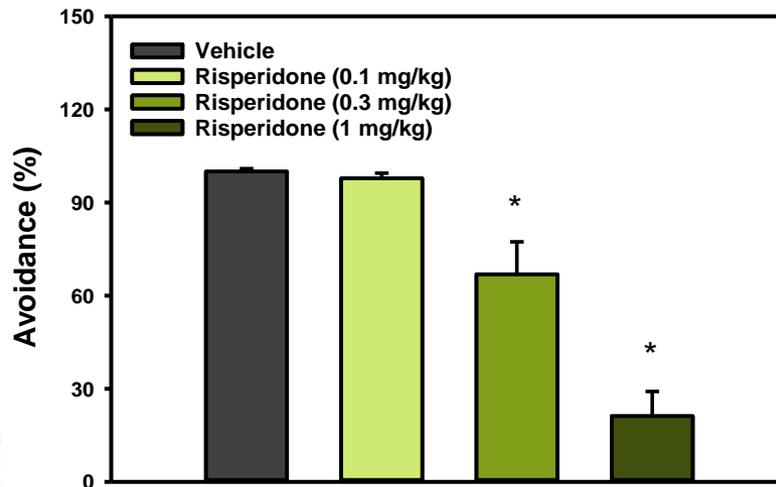
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Conditioned Avoidance Response

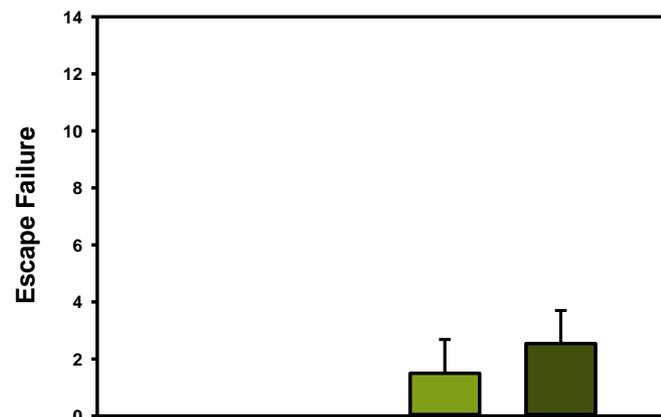
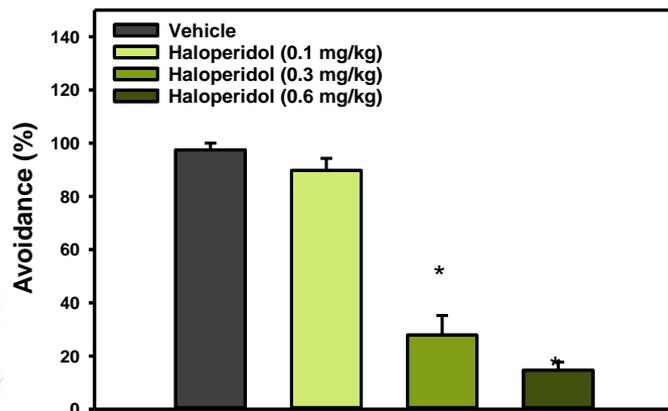
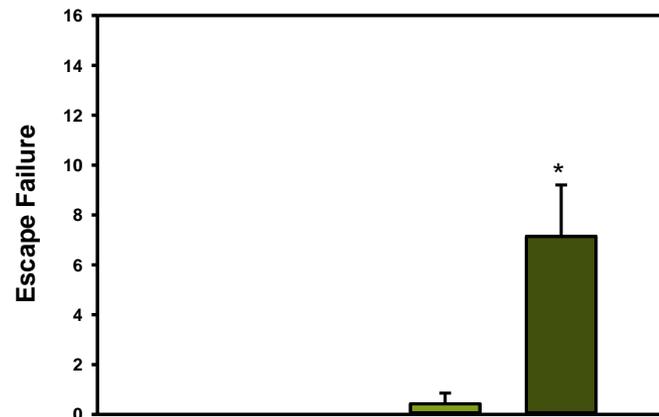
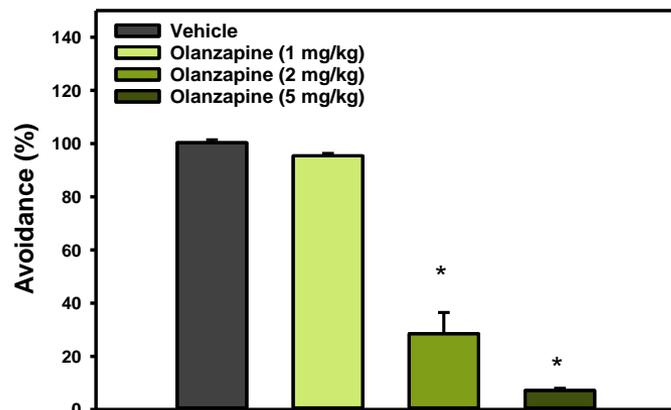


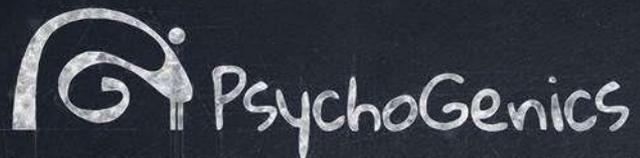
Risperidone Attenuates CAR

- In the CAR test, a foot-shock is delivered after presentation of a stimulus (e.g., light and tone). Subjects are trained in the task such that delivery of the mild foot-shock is avoided, rather than escaped after the start of shock delivery.
- Antipsychotics impair the ability of the test subject to avoid food-shock, but do not affect the escape response.
- By contrast, a compound that induces sedation (for example, a benzodiazepine) has no effect on avoidance responses.
- CAR is therefore predictive of antipsychotic efficacy and is frequently used to screen potential anti-psychotic medications



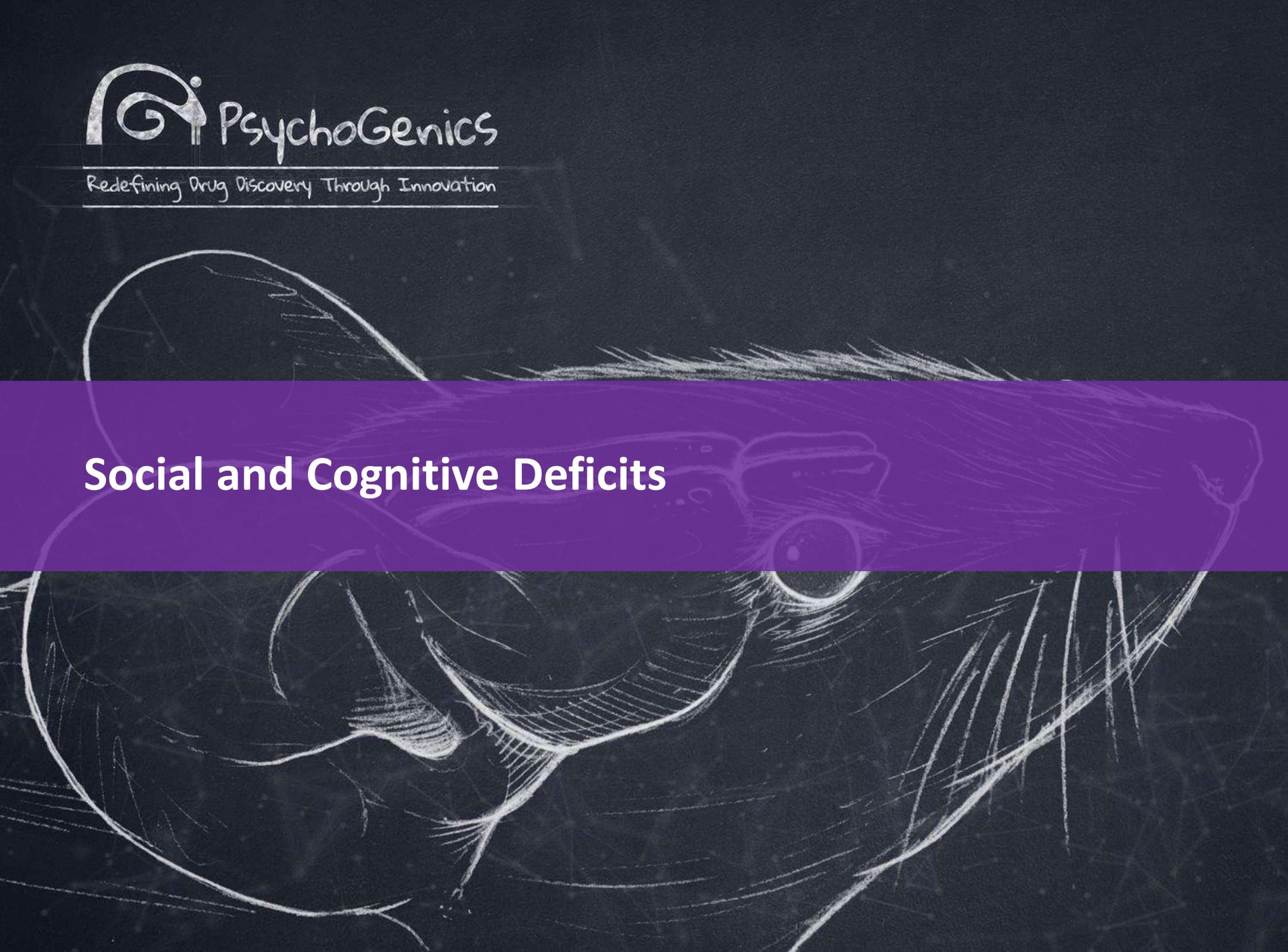
Olanzapine and Haloperidol attenuate CAR





PsychoGenics

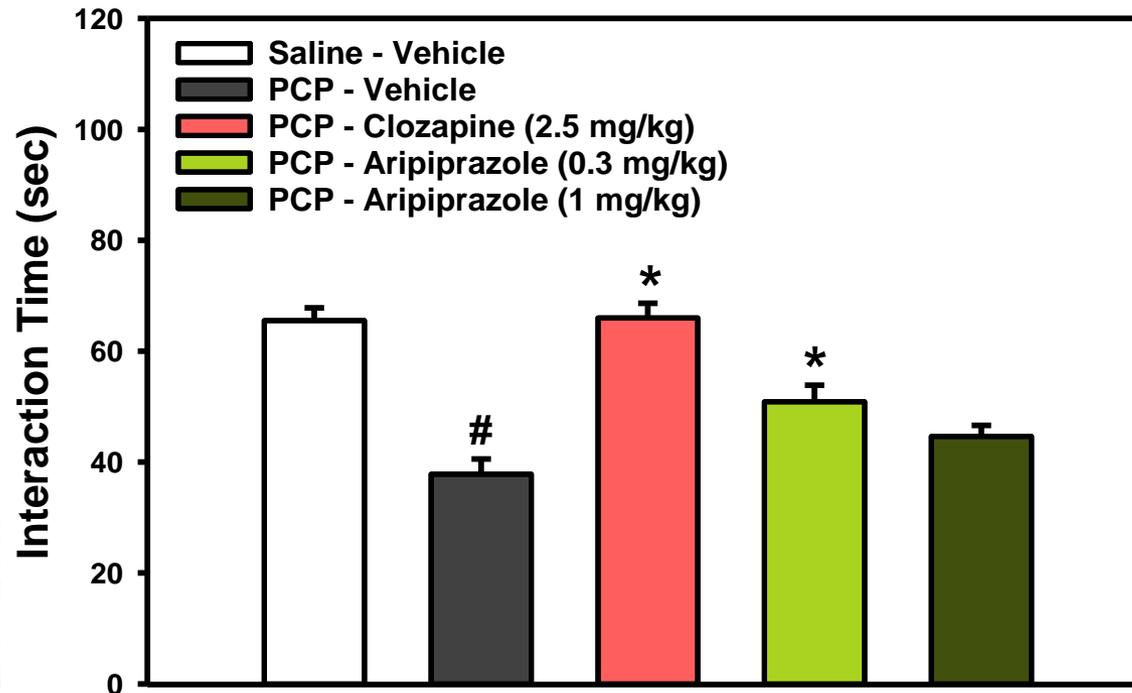
Redefining Drug Discovery Through Innovation

A white chalkboard-style drawing of a mouse's face is shown in profile, facing right. The drawing is composed of numerous fine, overlapping lines and shading, giving it a textured, hand-drawn appearance. A solid purple horizontal band is superimposed over the middle of the drawing, containing the text.

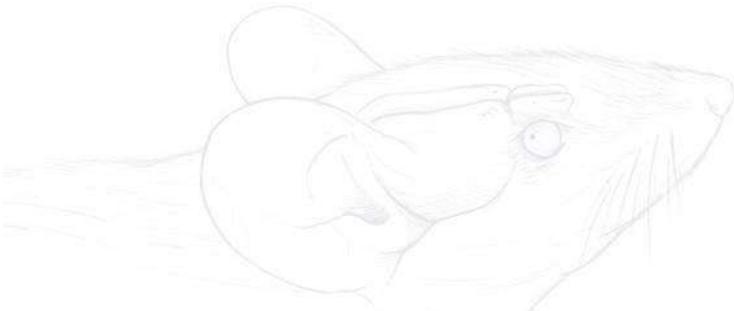
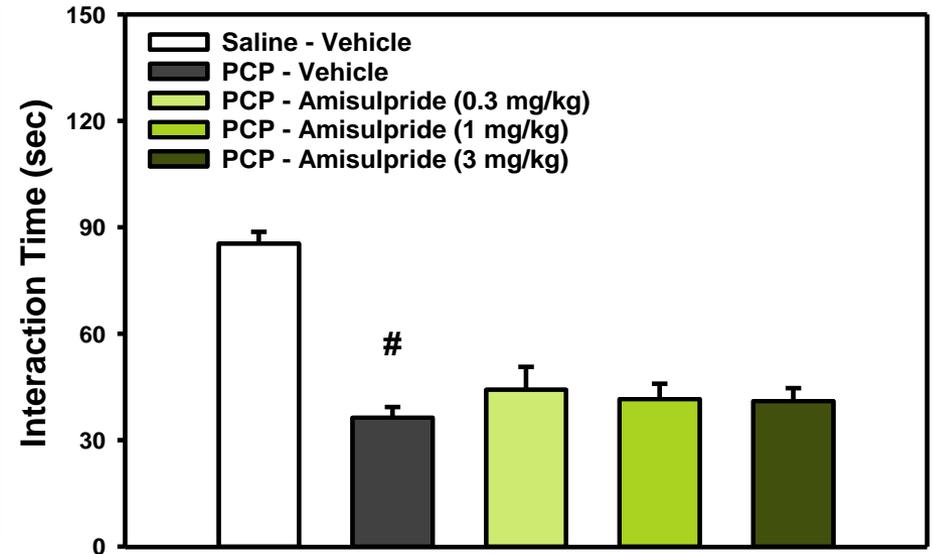
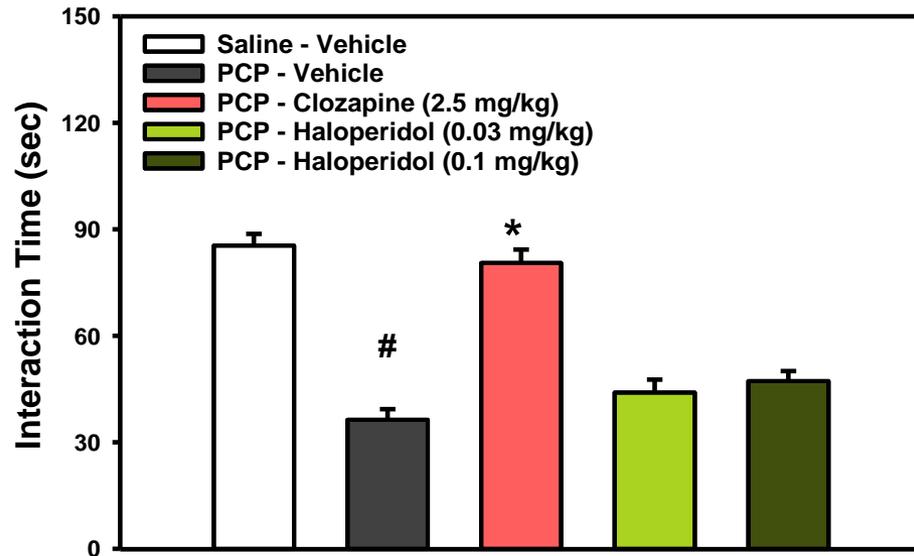
Social and Cognitive Deficits

Clozapine and Aripiprazole Attenuate Deficits in SI induced by PCP

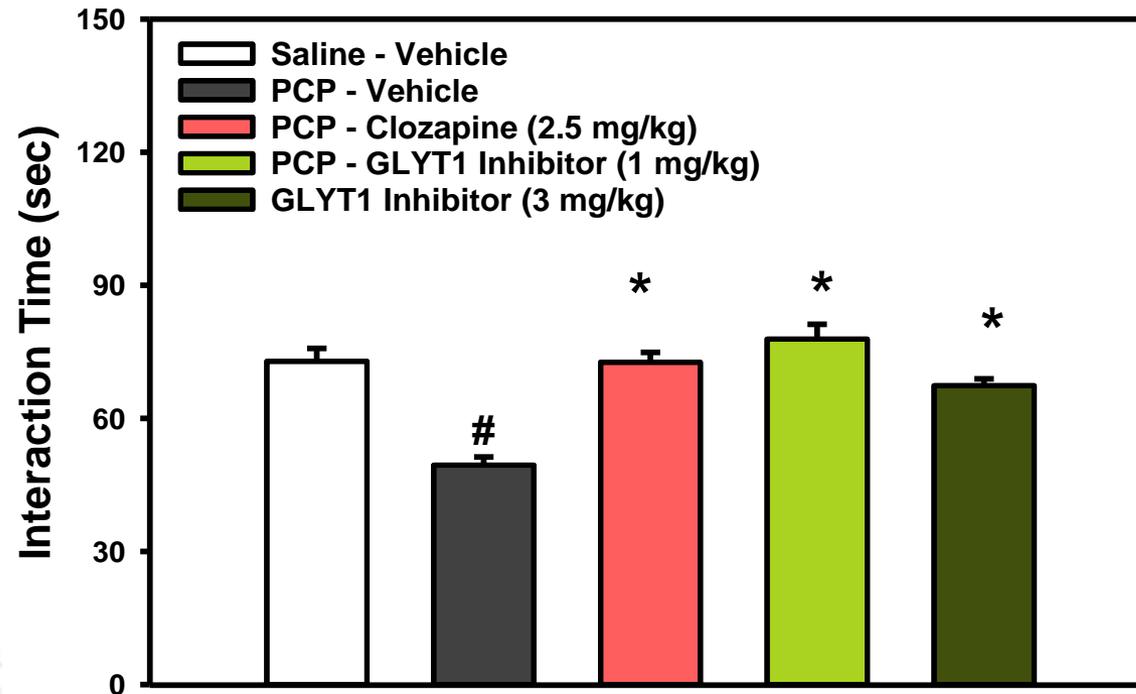
- Social isolation is part of the negative symptoms associated with schizophrenia.
- To date, atypical, but not typical, antipsychotics have been shown to be partially effective in treating the negative symptoms in schizophrenic patients.
- In rodents, 5 –day administration of PCP (2 mg/kg s.c.; BID) causes deficits in social interaction which can be reversed by treatment with clozapine.



Haloperidol and Amisulpride do not show efficacy in SI

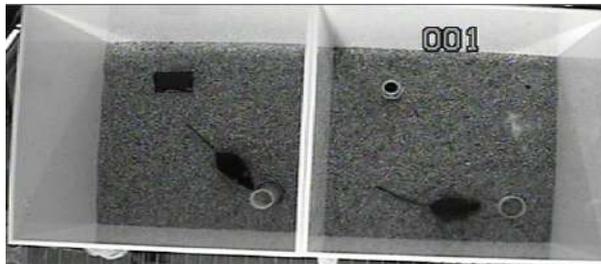


GLYT1 inhibitor attenuate PCP induced deficits in SI

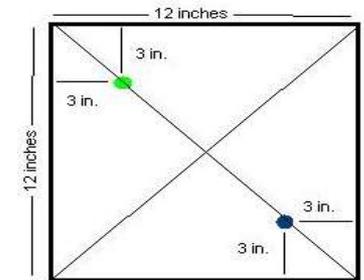


Novel Object Recognition

- Episodic/long-term memory; perirrhinal cortex and hippocampus
- Deficits: neurodevelopmental and pharmacological
- No appetitive/aversive stimuli: utilizes animals' natural exploratory drive
- Between-subjects design
- Measure: Recognition Index = novel object exploratory time / (novel + familiar objects total exploratory time) * 100



Rat



Habituation
10 min



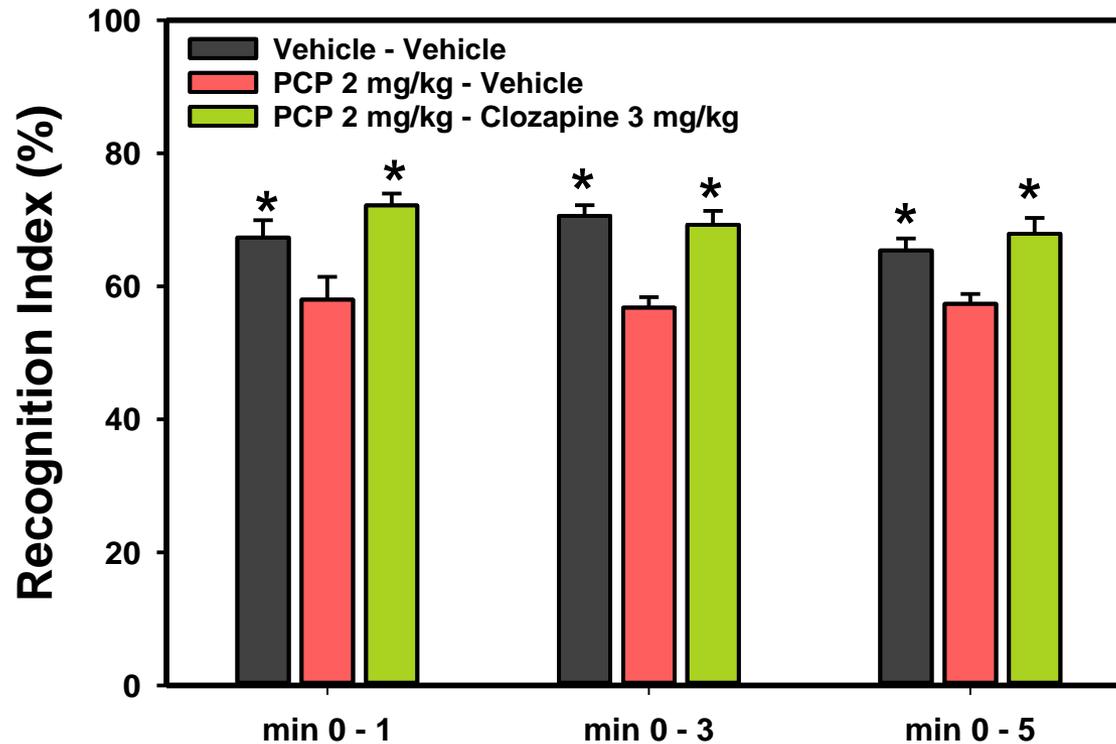
Training
3 min



Test 24/48 hrs
5 min

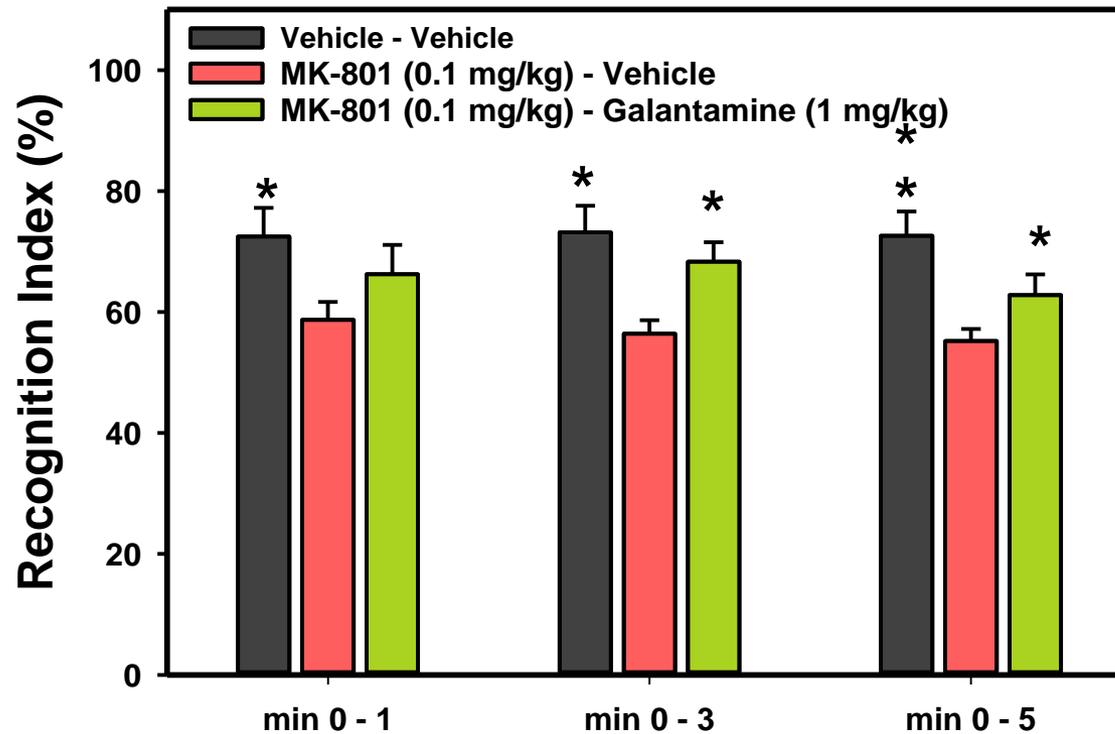


Clozapine Reverses PCP-induced Cognitive Deficits



- Male Long Evans rats are used
- PCP administered IP BID for 7 days. Test commences 5 days after last PCP injection.
- Clozapine administered i.p. 30 minutes prior to test

Galantamine Attenuates MK-801-Induced Cognitive Deficits

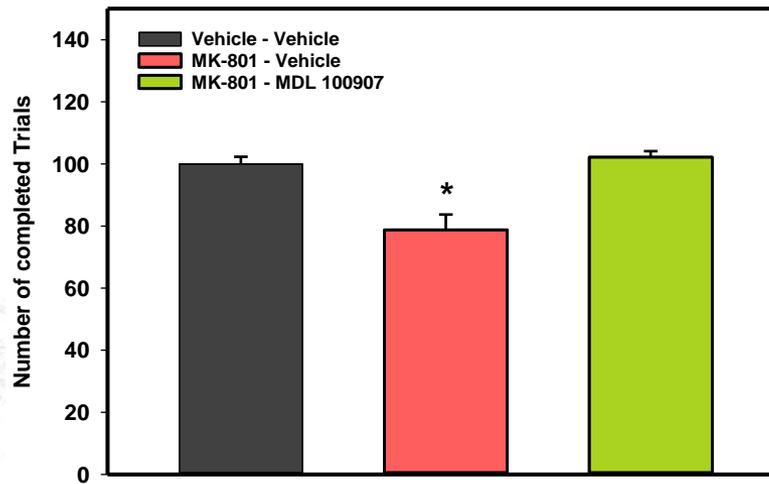
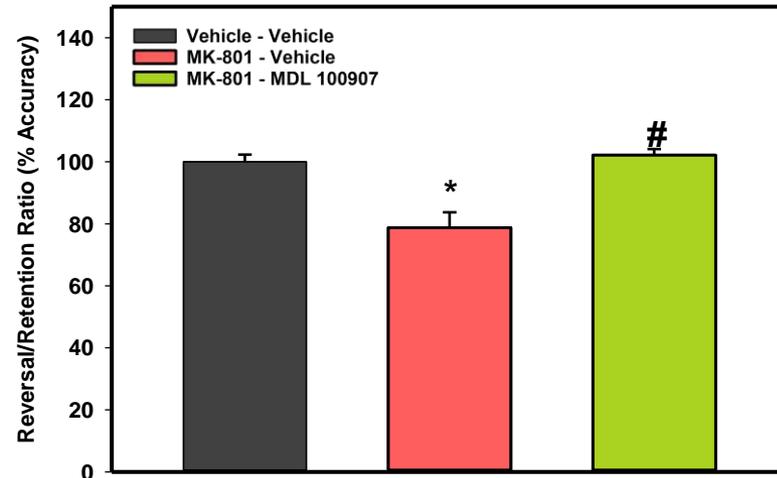
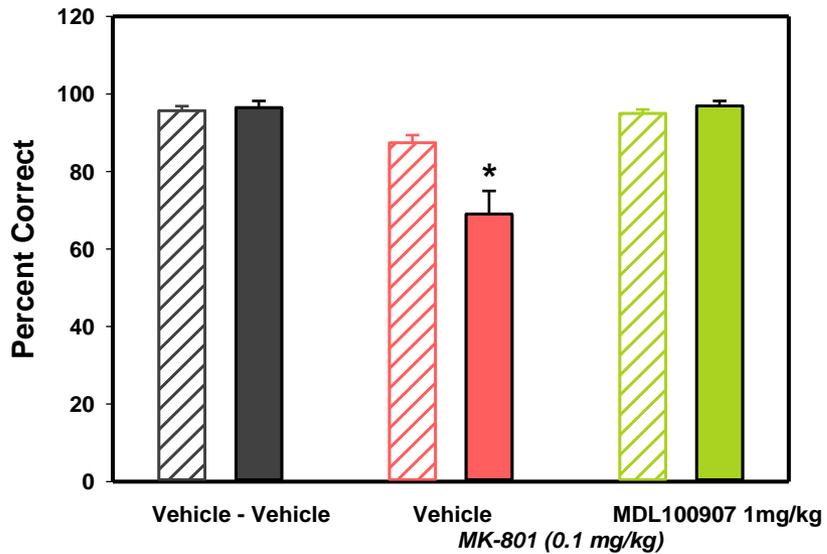


- Male Long Evans rats are used
- MK-801 (i.p) administered once 20 minute prior to training.
- Galantamine (i.p) administered once 20 minute prior to training.

Reversal Learning

- Serial Reversal Learning (SRL) is an operant conditioning learning task, in which the contingent relationship of a response (usually a specific lever) and an outcome (e.g., delivery of a food pellet) are unexpectedly reversed. Thus the animals have to alter their behavioral repertoire according to the changes of new contingencies, in order to obtain further reward.
- The deficit of flexibility in learning is evident in patients with schizophrenia and attention deficit hyperactivity disorder (ADHD) , probably due to their impaired orbito-perfrontal cortex function.
- **Methods**
 - Long-Evans rats (food restriction starting at 330g) are used in the studies. After 8-9 weeks of training, about 70% rats reach reversal test stage.
 - Reversal tests sessions consist of two phases, with the active lever contingency (side+cue) reversing between the first (retention) and second (reversal) phases.
 - Endpoint measures include
 - percent of correct, defined as the number of correct trials X 100, divided by the total completed trials. This parameter is calculated in both retention and reversal phases separately
 - Accuracy ratio, defined as percent correct in reversal phase X100, divided by percent correct in the retention phase.
 - Number of completed trials
 - A within-subject design is used in the studies. All rats went through all treatments across different weeks.

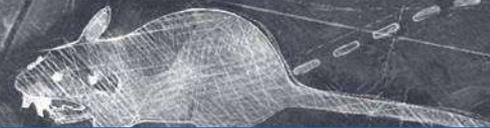
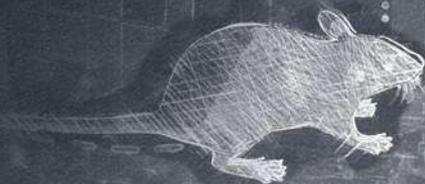
MDL 100907 Reverses MK-801 induced Deficits in RVL





Redefining Drug Discovery Through Innovation

1 Immobility



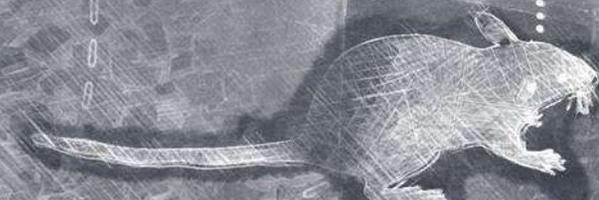
SmartCube Phenotyping of Mouse Models

7 Climbing

Immobility

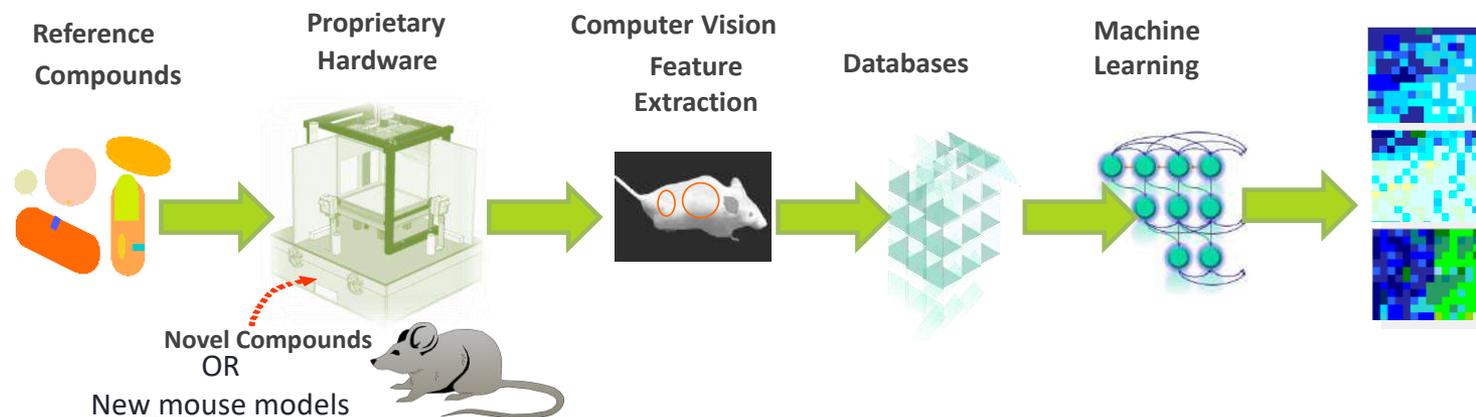


6 Visit



The SmartCube® System

- Mouse Based; high throughput
- Unbiased automated data collection and analysis
- Can be used for drug screening or phenotyping
- More than 2000 measured features
 - Activity, spatial patterns, spontaneous behavior, reactive behavior, gait..etc



The SmartCube® System



The SmartCube system consists of a white, cube-shaped enclosure with a transparent top. A mouse is visible inside the cube, standing on a bed of white bedding. The system is designed for automated behavioral analysis of small animals.

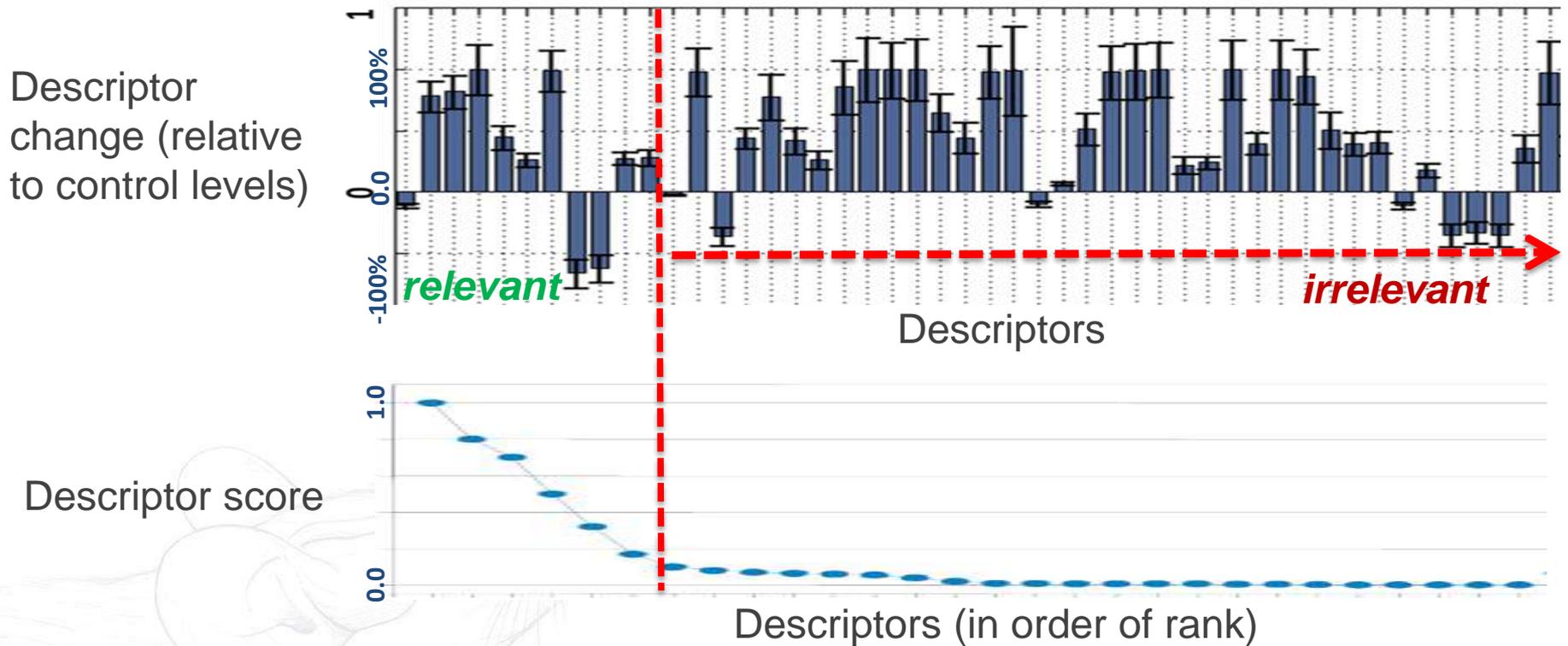
Observed Behavior:
Locomotion

Position X, Y (cm):
14.61, 16.91

Velocity X, Y (cm/s):
8.14, 3.39

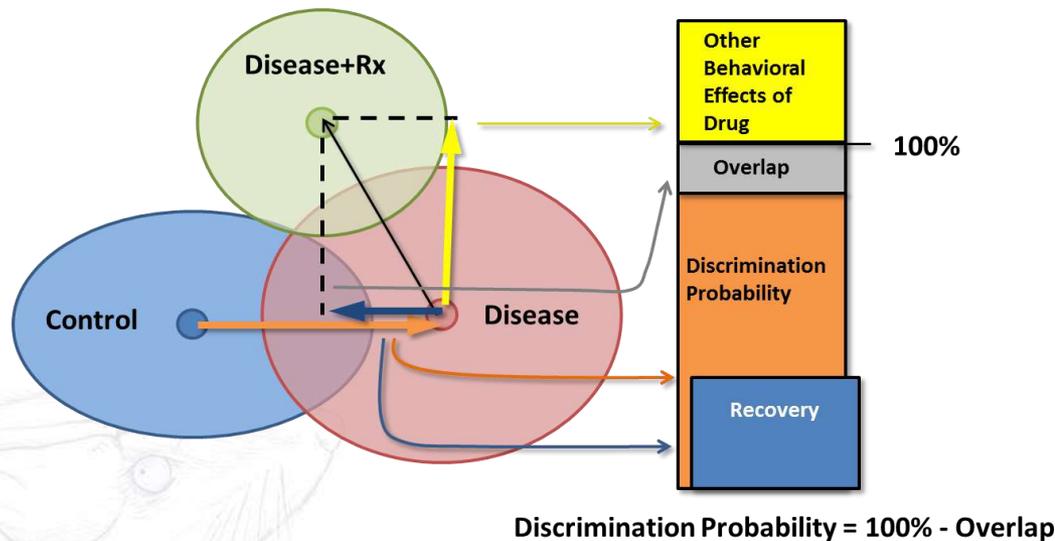
Descriptor Ranking Key to Model Building

- Rank all descriptors by influence score – how well can they predict the outcome compared to all other descriptors
- Apply weights to the highest ranked descriptors based on predictive power to build a model that can predict the outcome



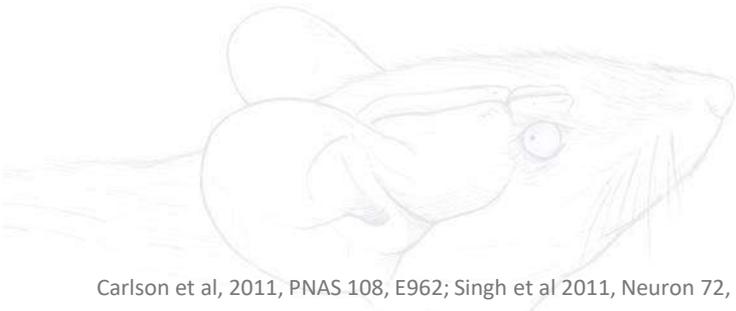
Feature Analysis

- Transform original feature set to the non-redundant de-correlated ranked features space
- Plot Control and Disease in the coordinate system formed by the two highest-ranked (best-discriminating between the two group's new features)
- Quality Measure of Disease Model = Overlap between the Control and Disease groups ($Discrimination\ Probability = 100\% - Overlap$)



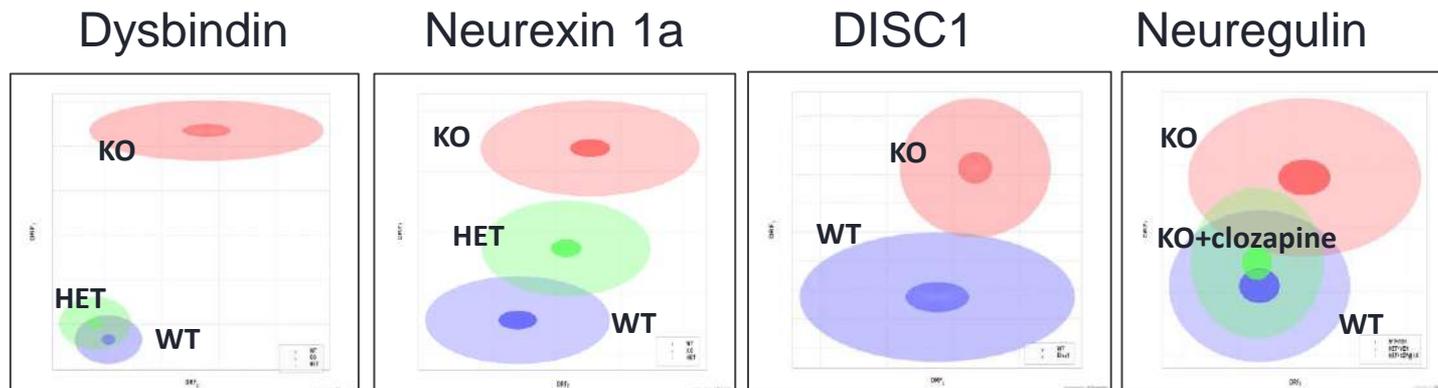
Outline of Mouse Models

- **Dysbindin 1**
 - Role in dopamine and glutamate signaling
- **Neuregulin1**
 - Important role in developing brain, NMDA and DA receptor expression/function
- **Neurexin1a**
 - genes encoding neurexins are implicated in autism and other cognitive diseases
- **Disc 1**
 - Molecular scaffold interacts with proteins required a.o. for neuronal migration



Carlson et al, 2011, PNAS 108, E962; Singh et al 2011, Neuron 72, 545; Sudhof, Nature 2008

Discrimination in SmartCube



**Discrimination
WT vs KO** **100%**

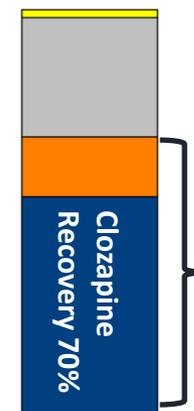
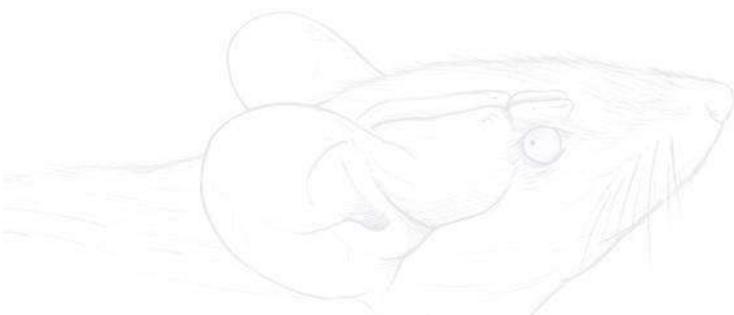
95%

78%

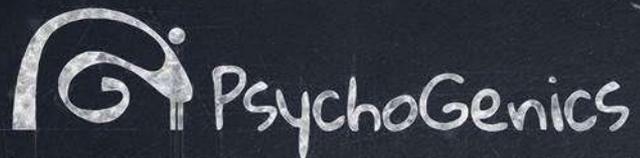
70%

**HET Proximity
to WT** **99%**

58%



**Discrimination
70%**

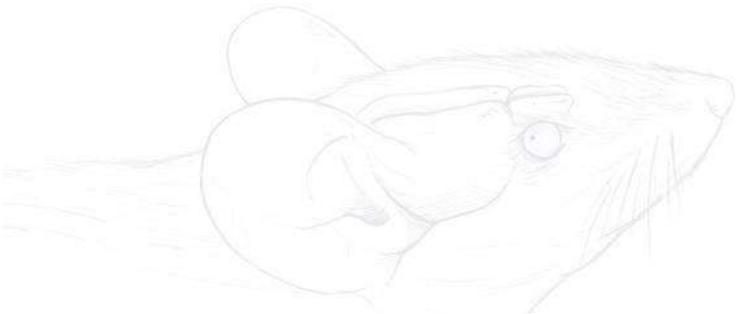


Redefining Drug Discovery Through Innovation

Mismatched Negativity

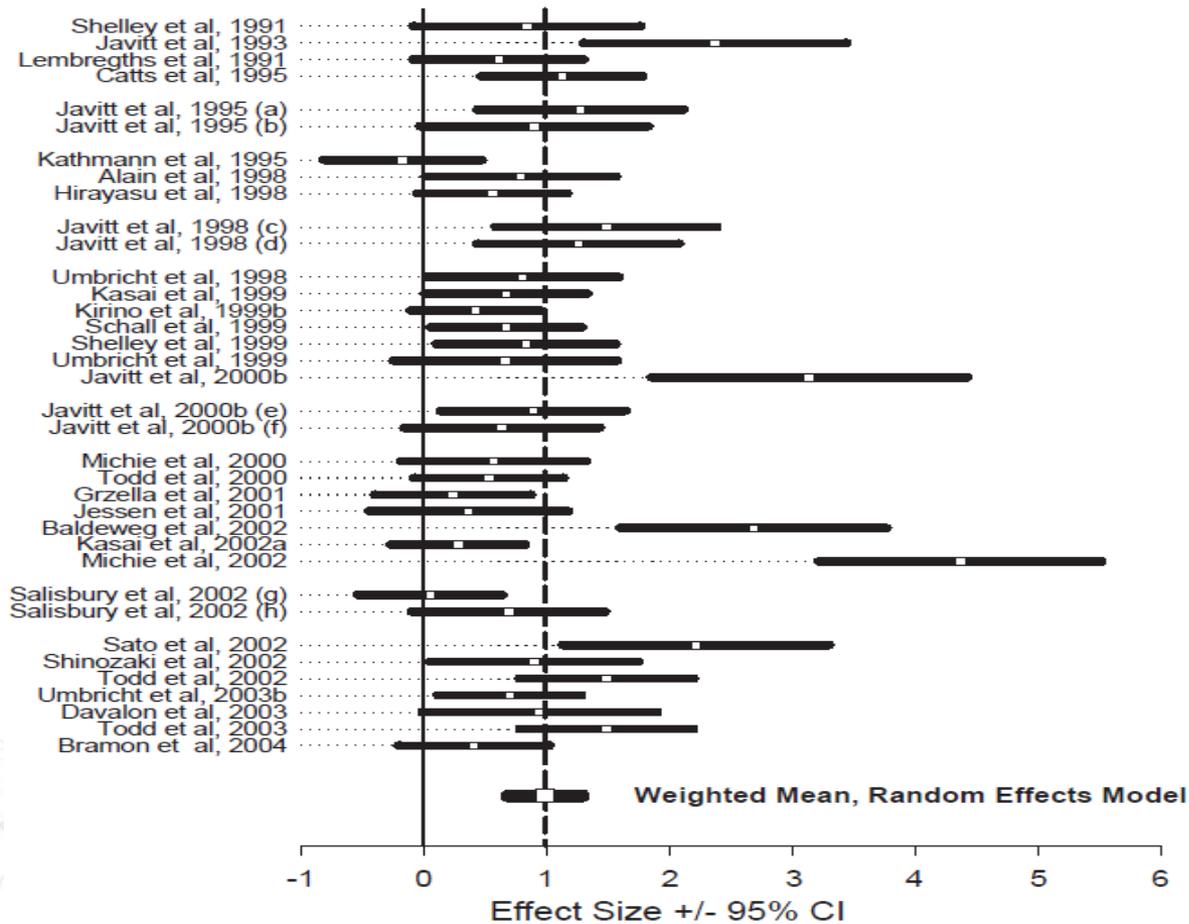
Mismatched Negativity (MMN)

- MMN is a validated, objective measure of central auditory processing and has become a key clinical biomarker in schizophrenia and holds utility in other neuropsychiatric disorders.
- MMN is an auditory evoked potential (AEP) elicited clinically by an auditory oddball paradigm in which a different, deviant ('oddball'; DEV) auditory (tonal) stimulus occurs infrequently and unexpectedly within a sequence of repetitive identical tonal stimuli ('standards', STD) and reflects pre-attentive processing dependent upon NMDA receptor function



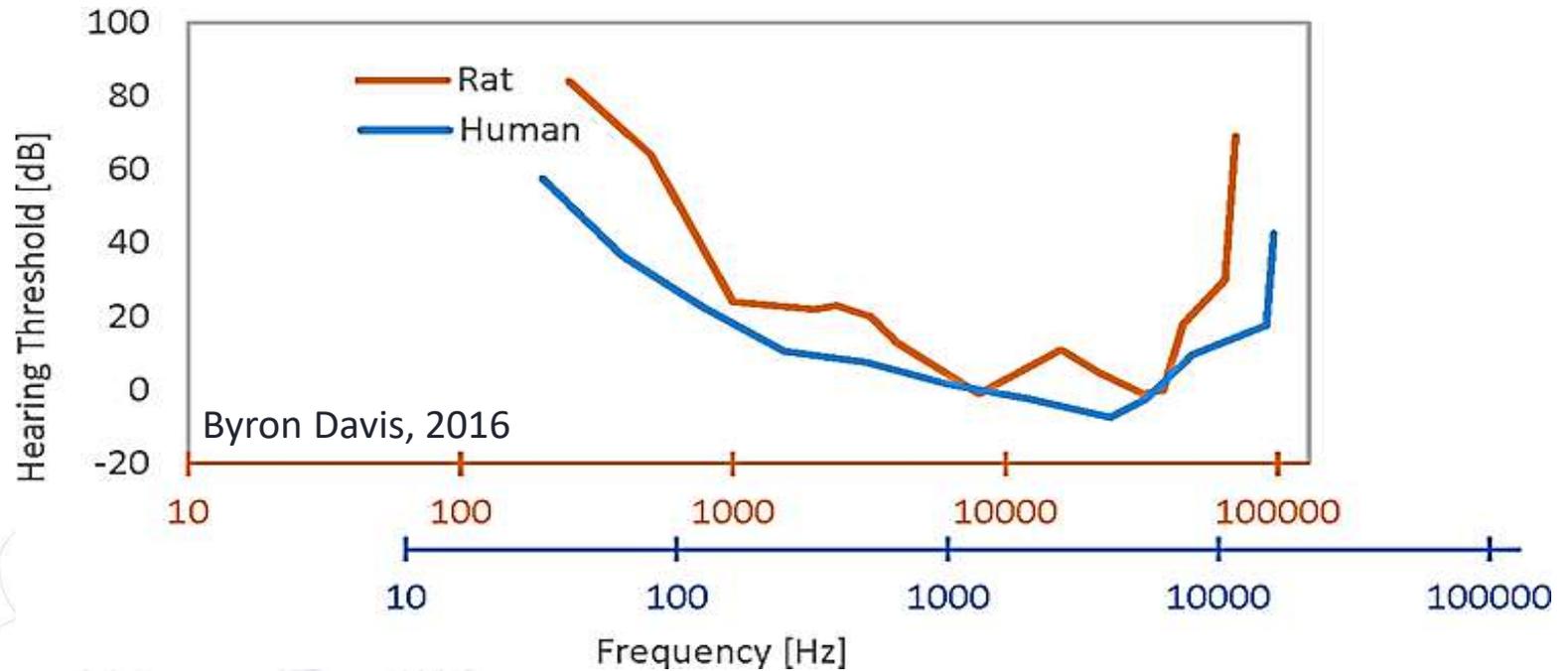
MMN deficits are a robust feature in chronic schizophrenia

D. Umbricht, S. Krljes / Schizophrenia Research 76 (2005) 1–23



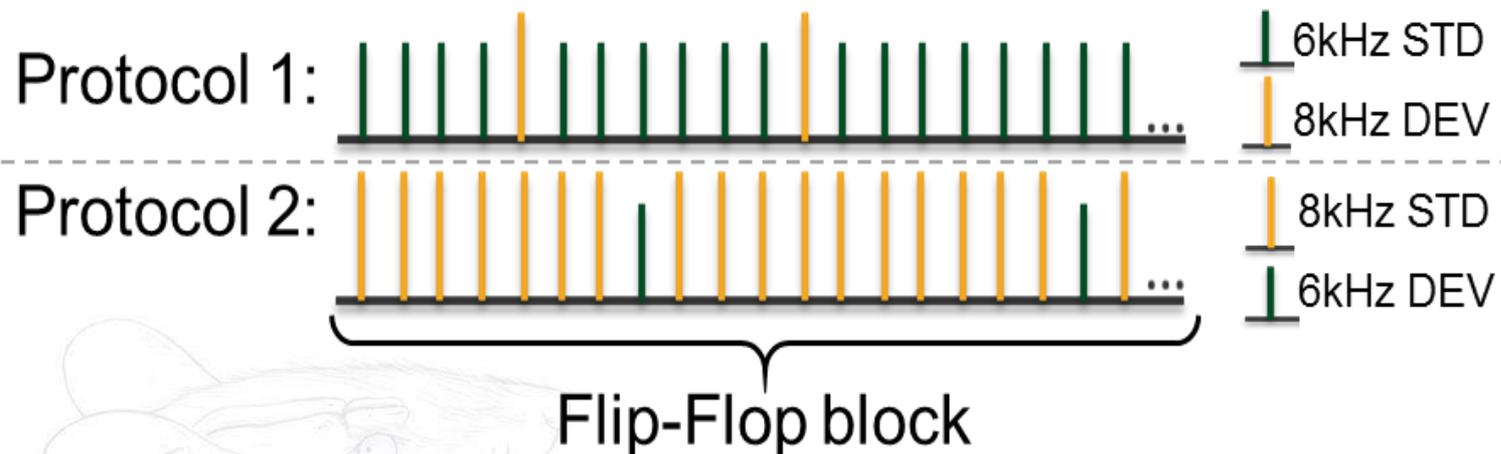
Optimized Oddball Protocol for Eliciting MMN in rats

- The auditory stimulation protocol to elicit MMN in rats was directly back-translated from clinical protocols with 2 exceptions
 - Higher frequency tones (6-8kHz) are used in the rat to compensate for different hearing ranges



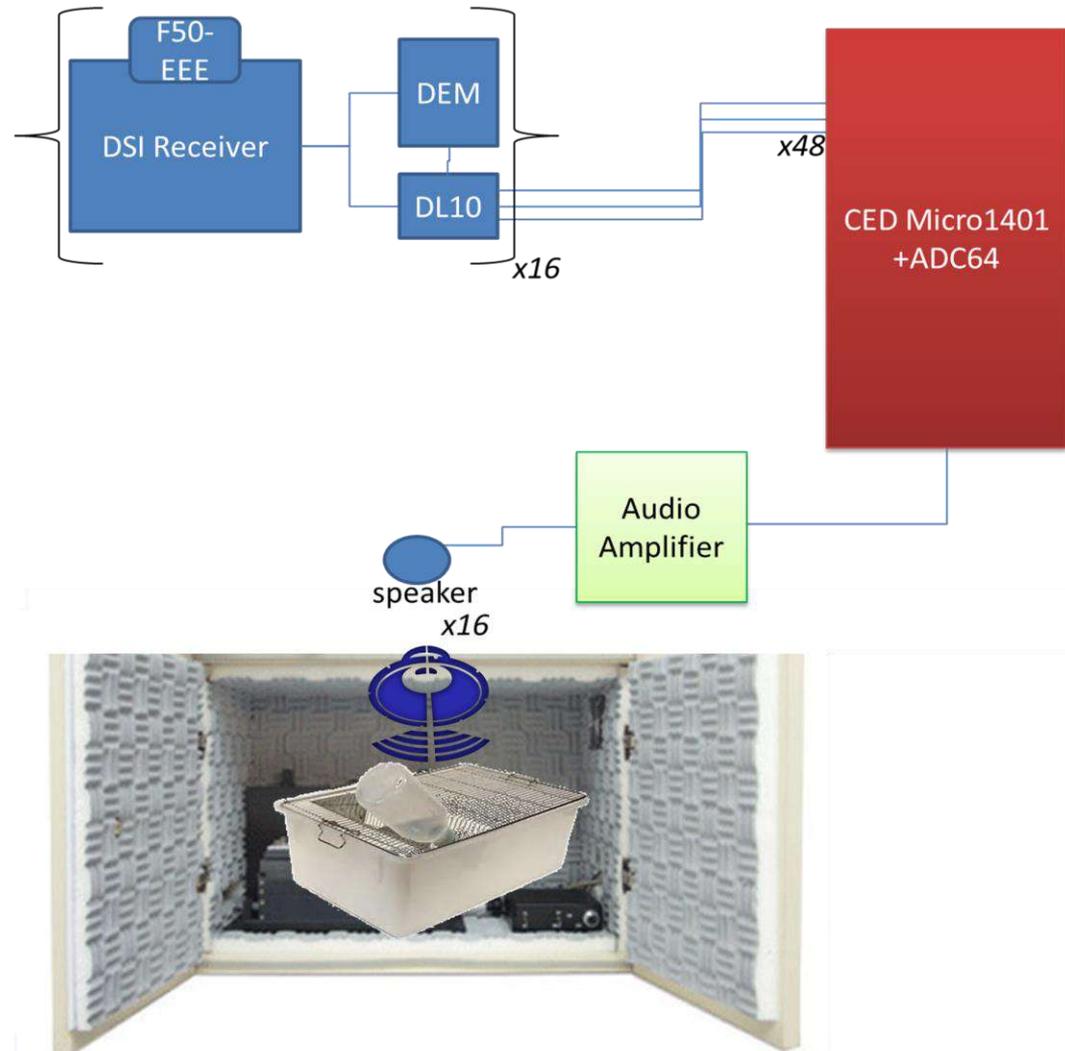
Optimized Oddball Protocol for eliciting MMN in rats (cnt'd)

- The auditory stimulation protocol to elicit MMN in rats was directly back-translated from clinical protocols with 2 exceptions
 - Since different tonal frequencies elicit different AEP amplitudes, a flip-flop paradigm is used and Difference Waves are calculated using tones of the same frequency for when that tone is presented as Standard (STD) or when its presented as Deviant (DEV)

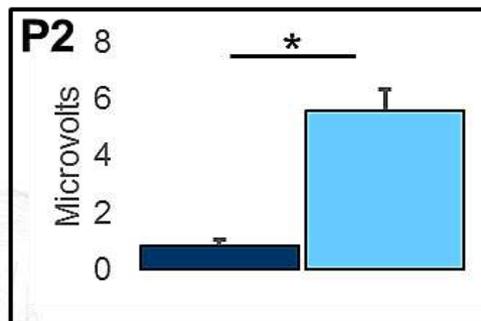
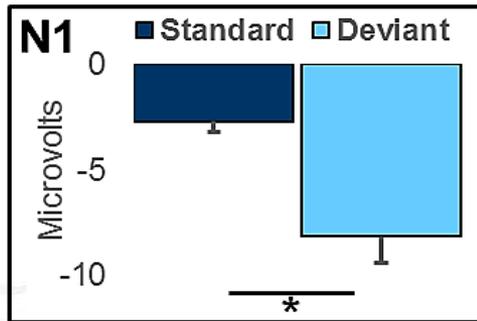
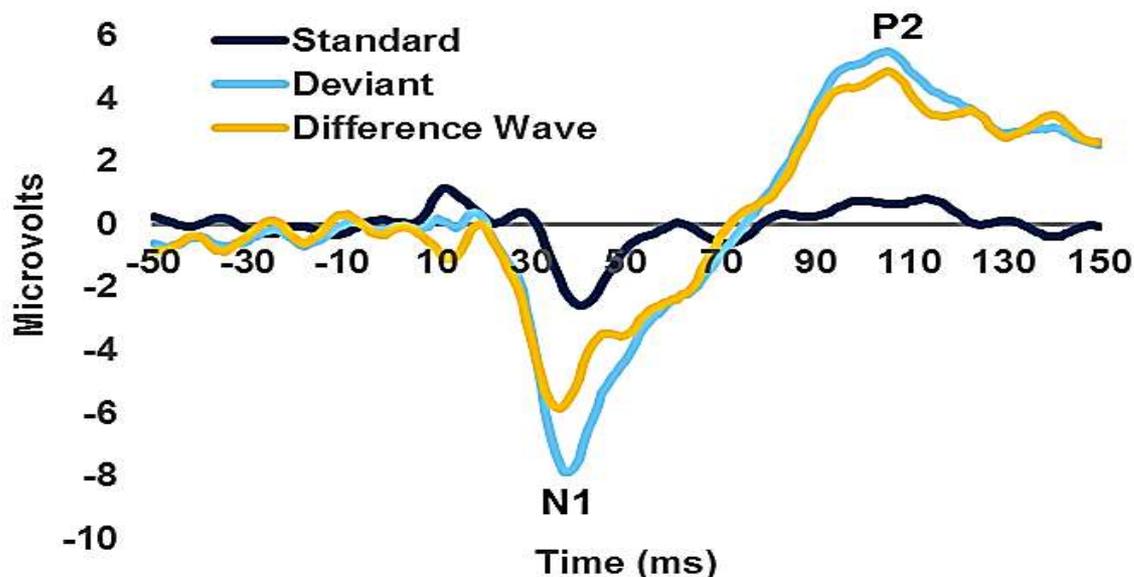


High-Throughput MMN for Use in Drug Discovery

Leveraging the high-throughput capability of **DSI's wireless EEG** with the temporal precision and data processing of **digitally-timestamped tonal stimuli of the CED system** provides a reliable turn-key solution to running **up to 16 animals simultaneously** in the MMN protocol.

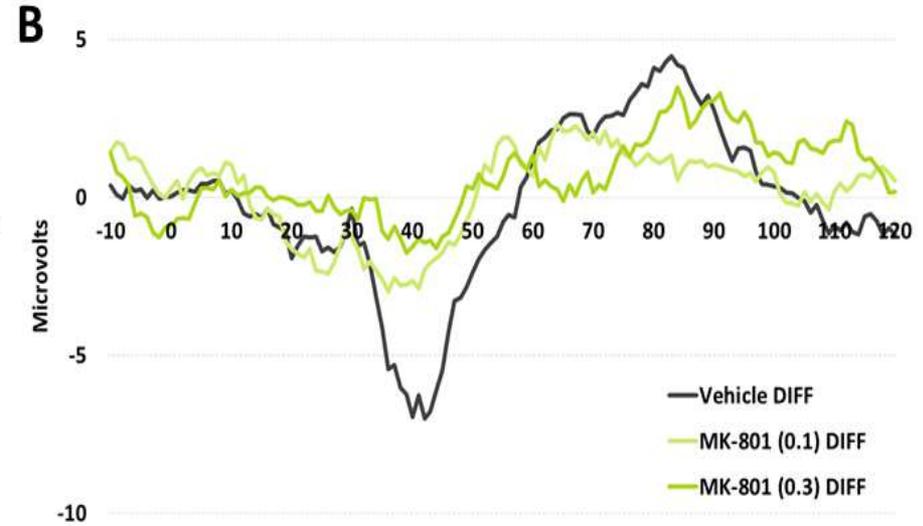
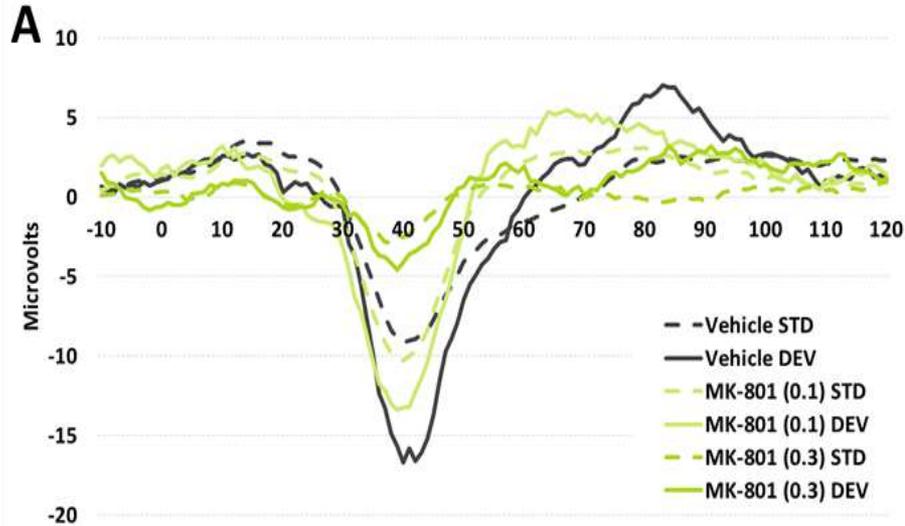


Auditory Evoked Potentials & MMN in Rats

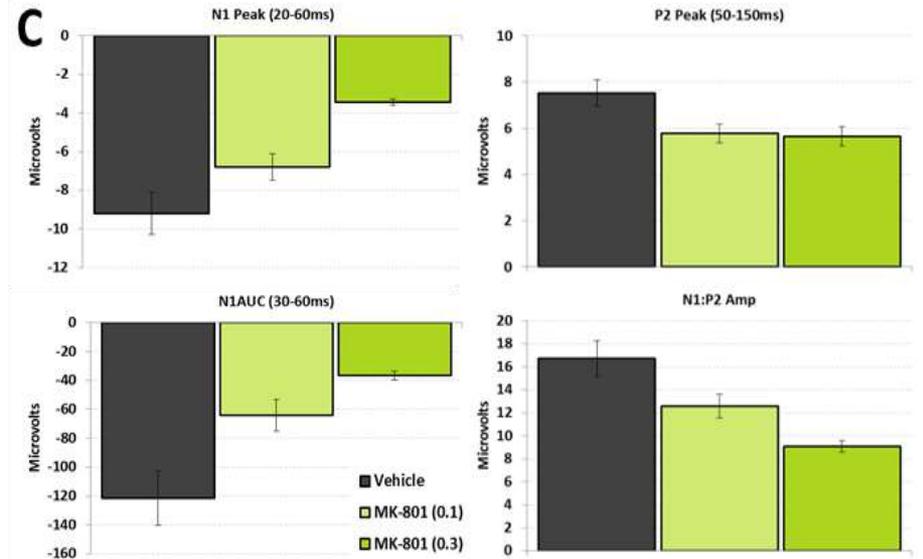


MMN is elicited clinically and preclinically using an auditory oddball paradigm in which randomly presented deviant tones are presented with 10% probability within a series of standard auditory tones (90%). The brain will adapt to and suppress response to the standard tones, however the “oddballs” inherently elicit a larger AEP. Subtracting the averaged EEG waveform in response to the standard (STD) tones from the averaged responses to deviant tones (DEV) reveals MMN, a component of the difference potential waveform.

NMDA Involvement in MMN in Rats



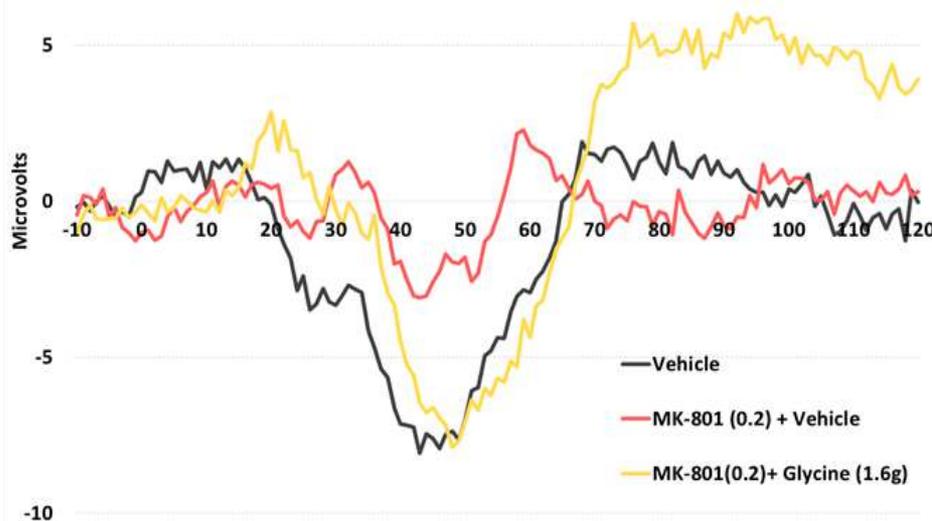
The NMDA-receptor antagonist MK-801 dose-dependently suppresses Mismatch Negativity (MMN). MK-801 (0.1 & 0.3 mg/kg, i.p.) suppresses (A) Standard (STD) and Deviant (DEV) and (B) Difference (DIFF) waveforms 40-60min post-dose in SD rats. The DIFF waveform components (C) are used as a metric of Mismatch Negativity. Data are presented as mean \pm SEM (n=8-9).



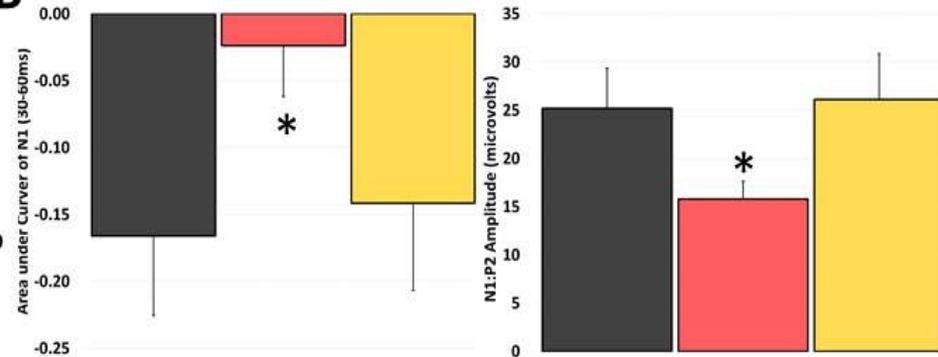
NMDA Involvement in MMN in Rats

*MK-801 (0.2mg/kg)-impaired MMN can be restored by Glycine (1.6g/kg) as seen in the Difference waveforms (A) and its waveform components (B). Data are presented as mean \pm SEM (n=13-16). * p <0.05, t-test vs. vehicle. MK-801 (0.2) and Glycine were given 60 and 30 minutes prior to MMN respectively.*

A



B

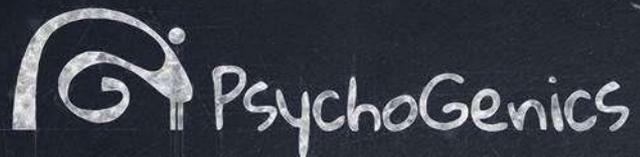


Similar to clinical observations:

- Acute high-dose Glycine attenuates mismatch negativity (MMN) in healthy human controls (Leung et al., 2007).
- Increased duration MMN after acute administration of Glycine in Schizophrenia patients (Greenwood, et al., 2018)

MMN in rats

- We have validated the presence of MMN in rats using frequency oddball protocols similar to those used clinically.
- Further we have developed a platform by which MMN can be run on multiple animals simultaneously thereby accommodating pharmacological studies (i.e. in a cross-over design).
- We have also shown that the MMN is impaired by NMDA antagonism (MK-801). Specifically, MK-801 at doses of 0.2, 0.3, and 0.5 mg/kg, i.p. induce robust impairment of MMN. Selecting the lowest dose of MK-801 is critical in testing compounds' effects on reversing the induced deficit. Likewise it is important to consider the time course of MK-801's lasting effects.
- MMN is an index of cognitive decline and disturbed central auditory processing in ageing and many neurological and neuropsychiatric disorders and thus presents as a highly attractive biomarker in drug discovery



Redefining Drug Discovery Through Innovation

Loudness Dependence of Auditory Evoked Potentials

Loudness Dependence of Auditory Evoked Potentials (LDAEP)

- LDAEP has been characterized as an *in vivo* marker of central 5-HT activity, which tonically modulates auditory cortical processing. LDAEP has been used to assess serotonergic modulation of auditory cortical processing and has been correlated to negative symptoms in schizophrenia.

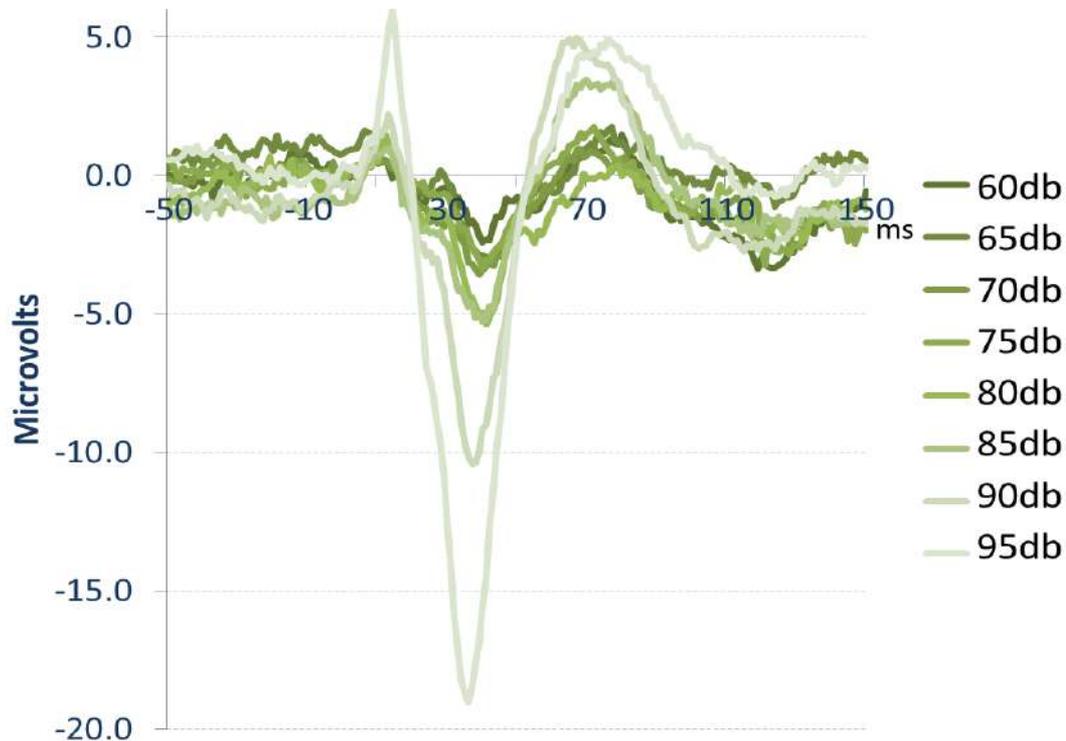


Figure: Increasing sound intensities (decibels, db) produce in a normal brain increased AEP amplitudes. Characteristic changes to the P1, N1 and P2 are observed with increased sound intensity. Data are presented as mean \pm SEM ($n=8$).