# Mouse Models of Autism to Test Hypotheses and Develop Treatments


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***NIH, Bethesda, MD***


# Causes of Autism(s) are unknown: Strongest evidence is genetic

- 4:1 frequency ratio of boys:girls
- Concordance is 60-92% for monozygotic twins
- Concordance is 0-10% in dizygotic twins, 5-10% in siblings; population frequency is ~ 0.6%
- Linkage analyses indicate many genes underlying this complex disease, including linkages at chromosomal loci 15q11-q13, 16p11.2, 22q13, X-linked
- Copy number variants; epigenetic factors
- Multiple putative candidate genes include *GABA-β3*, *5-HTT*, *MET, PTEN*, *En2*, *UBE3a*, *CNTNAP2*, neurexins, neuroligins, shanks, and genes for comorbid neurodevelopmental disorders including *FMR1, MECP2, TSC*

***Crawley, 2010***

# Mouse Models with Mutations in Candidate Genes

*Adapted from Abrahams and Geschwind, Nature Reviews Genetics 2008*

| Human Gene | Co-morbid Syndrome | Mutant Mouse |
|---|---|---|
| *AVPR1a* | | Yes |
| *CACNA1C* | Timothy | Yes |
| *CADPS2* | | No |
| *CNTNAP2* | | Yes |
| *DHCR7* | Smith-Lemli-Opitz | Yes |
| *EN2* | | Yes |
| *FMR1* | Fragile X | Yes |
| *GABARβ2* | | Yes |
| *ITBG3* | | Yes |
| *MECP2* | Rett | Yes |
| *MET* | (tumors) | Yes |
| *NRXN1* | | Yes |
| *NLGN3* | | Yes |
| *NLGN4* | | Yes |
| *OXTR* | | Yes |
| *PTEN* | (cancers) | Yes |
| *RELN* | | Yes |
| *SHANK3* | 22q13 deletion Phelan-McDermid | Yes |
| *SLC6A4* | | Yes |
| *TSC1, TSc2* | Tuberous sclerosis | Yes |
| *UBE3A* | Angelman | Yes |

*Crawley, 2010*

# Why are Mouse Models Useful?

- **Mice and humans share 99% of their genes**
  (*Thomas Bourgeron, neuroligin4 and shank3 mice, IMFAR 2008*)
- **Similar brain anatomy**
- **Similar biochemistry, neurotransmitters, receptors**
  (*Mark Bear, mGluR5 receptor upregulated in Fragile X mice, IMFAR 2009)*
- **Similar physiology, brain electrophysiology**
- **Analogous behavioral endophenotypes**
  *Mice are a social species*
- **Mouse models of neuropsychiatric disorders provide research tools to test hypotheses about causes**
- **Mouse models of neuropsychiatric disorders provide translational tools to develop treatments**

*Crawley, 2009*

# Don’t anthropomorphize!

## There will never be an “autistic mouse.”

- Rather, we are generating mouse behavioral assays with analogies to the symptoms of autism (face validity)
- Mouse models are used to test hypotheses, e.g. genes linked to symptoms of autism, brain development, neuroanatomical abnormalities, immune dysfunctions, or environmental toxins (construct validity)
- Robust mouse models offer translational tools to evaluate treatment efficacy (predictive validity)

***Crawley, 2008***

# How would you model the behavioral symptoms of autism in mice?

- ***Which of the human behavioral symptoms may have reasonable endophenotype analogies in mice?***
- ***Which of the human symptoms are the most essential to include in an animal model?***

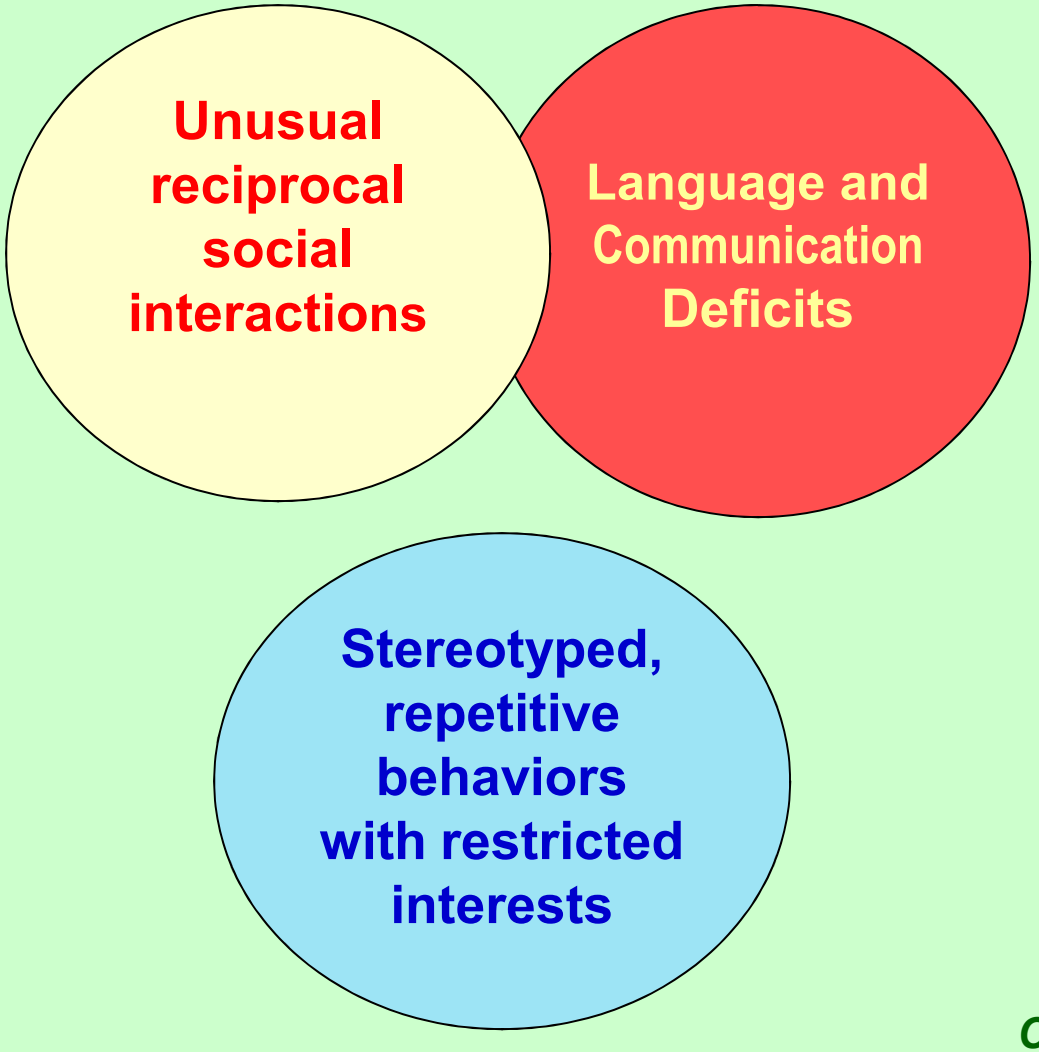
***Crawley, 2008***

**Autism is a neurodevelopmental disorder of unknown causes**

**Diagnosis currently requires evidence in three categories of defined behavioral symptoms:**

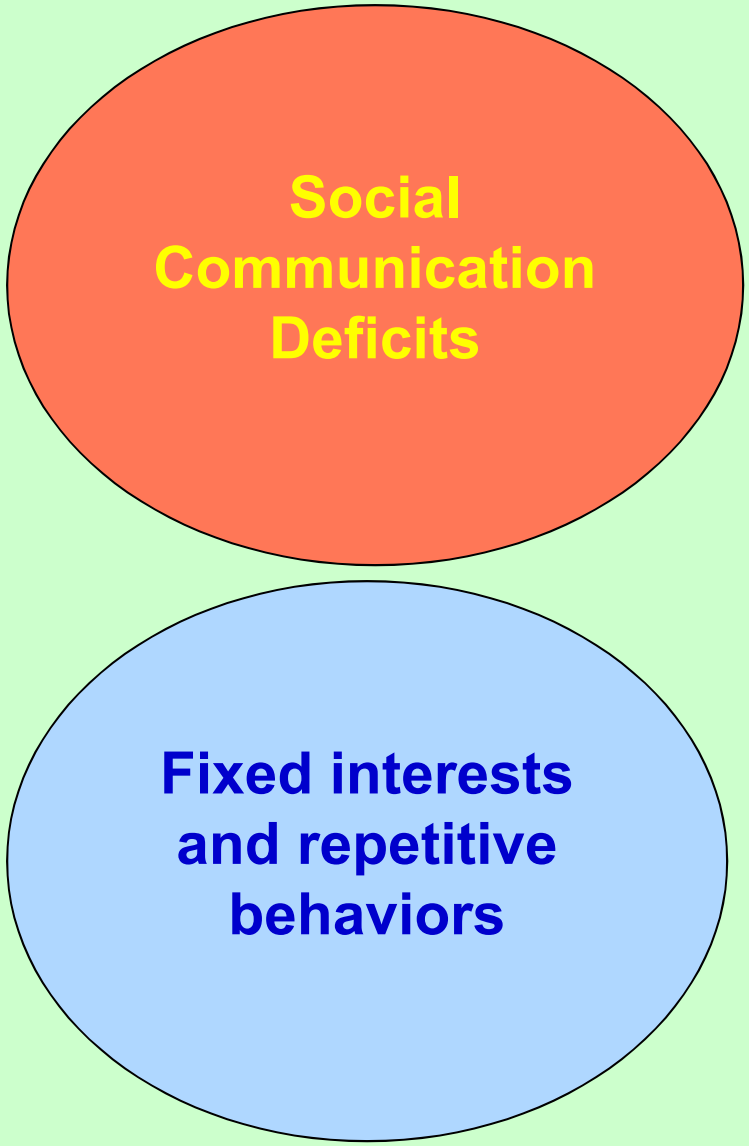
***Crawley, 2008***

# DSM-IV-TR Diagnostic Symptoms of Autism




*Crawley, 2008*

# Proposed DSM-V Diagnostic Criteria for Autism




*Crawley, 2008*

# Some of the Associated Symptoms of Autism

- Seizures
- Anxiety
- Low IQ
- Sleep disruption
- Hyperreactivity and hyporeactivity to sensory stimuli

*Crawley, 2008*

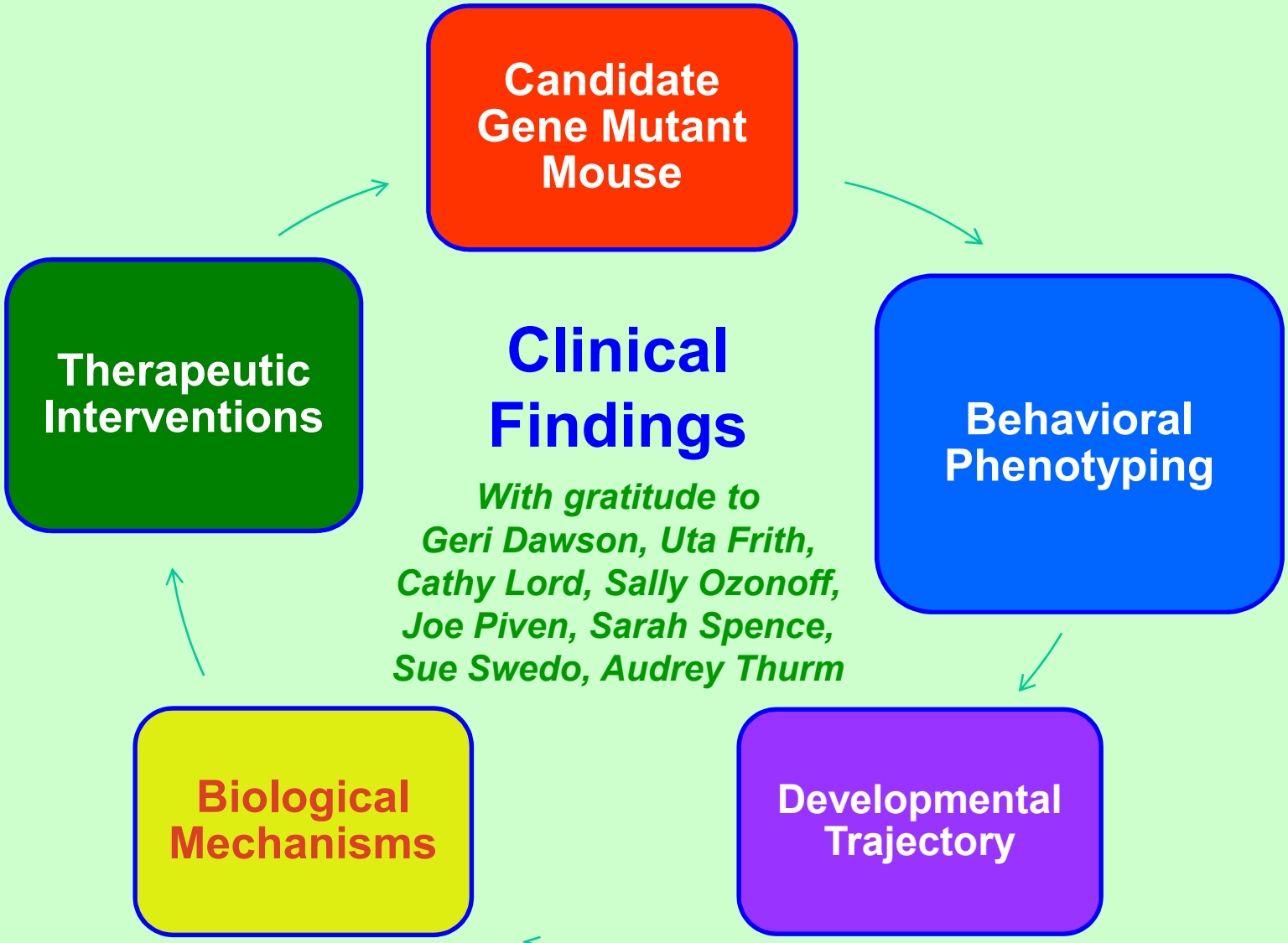
# No biological markers

## *Tentative biological correlates:*

- **Abnormalities in white matter connectivity**
- **Macroencephaly during early development**
- **Platelet hyperserotonemia**
- **Fewer Purkinje cells in the cerebellum**
- **Reduced activation of the fusiform gyrus and amygdala while engaged in social tasks during fMRI imaging**
- **Co-morbid with symptoms of other neurodevelopmental disorders**

***Crawley, 2008***

## LBN STRATEGY
## for testing genetic hypotheses about the causes of autism




*Crawley, 2009*

# Mouse Models of Autism
# BEHAVIORAL PHENOTYPING TEAM 2007-2008

*Jacqueline Crawley, Chief, Laboratory of Behavioral Neuroscience*
*National Institute of Mental Health, IRP, NIH*
*Bethesda, Maryland, USA*

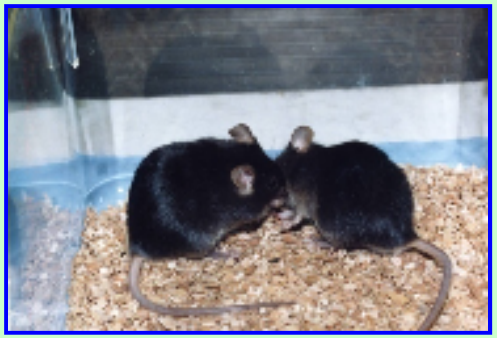
## Postdoctoral fellows and visiting scientists 2006-2010

**Florence Roullet**
**Jill Silverman**
**Mu Yang**
Kathryn Chadman
Hewlet McFarlane
Bryce Ryan
Maria Luisa Scattoni
Markus Wöhr

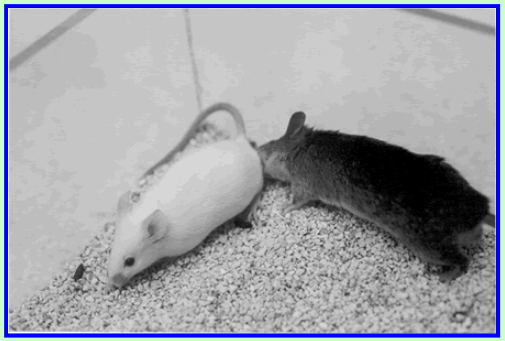
# Animal Models of Autism
# Core Symptom #1:

Impaired social interaction

# Some social behaviors of *Mus musculus*

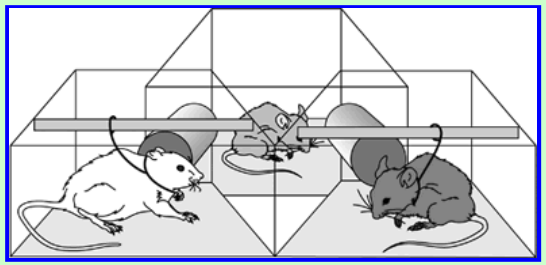


nose-to-nose sniff

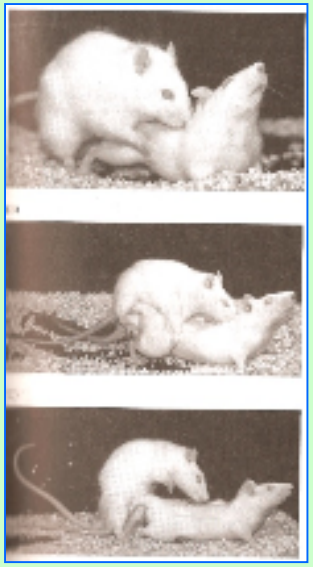


anogenital exploration

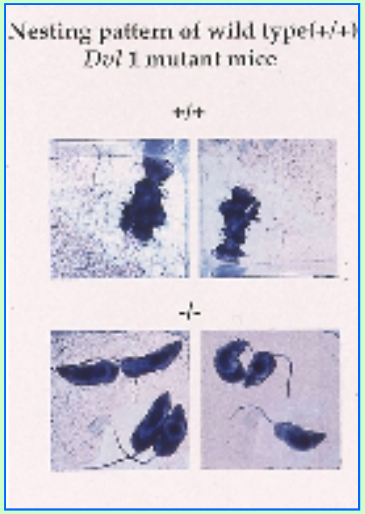
fighting



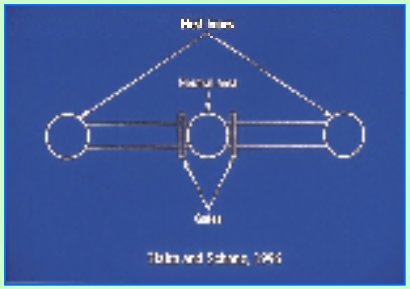
social memory



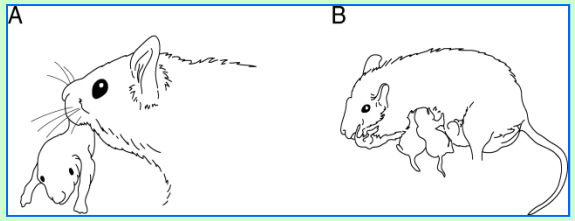
mating



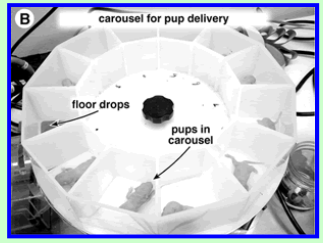

nesting



dominance




parenting




motivation

*Crawley, 2008*

# Modeling the Aloof, Passive Subtype

*Leo Kanner, Johns Hopkins University*
*Autistic disturbances of affective contact*
*Nervous Child 2:217-250, 1943*

**"The children of our group have all shown their extreme aloneness from the very beginning of life, not responding to anything that comes to them from the outside world."**

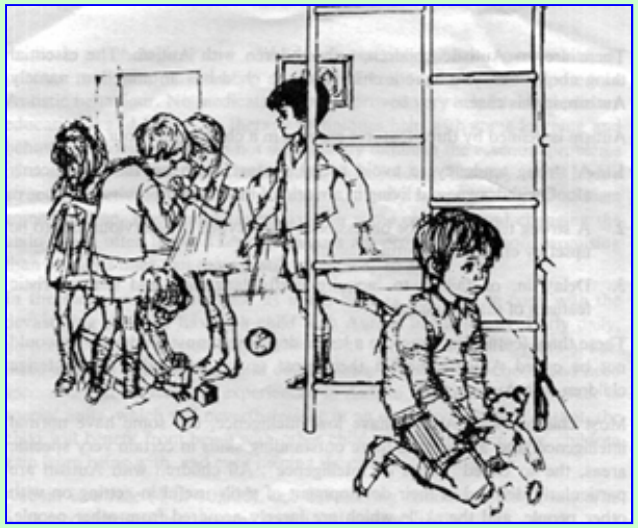
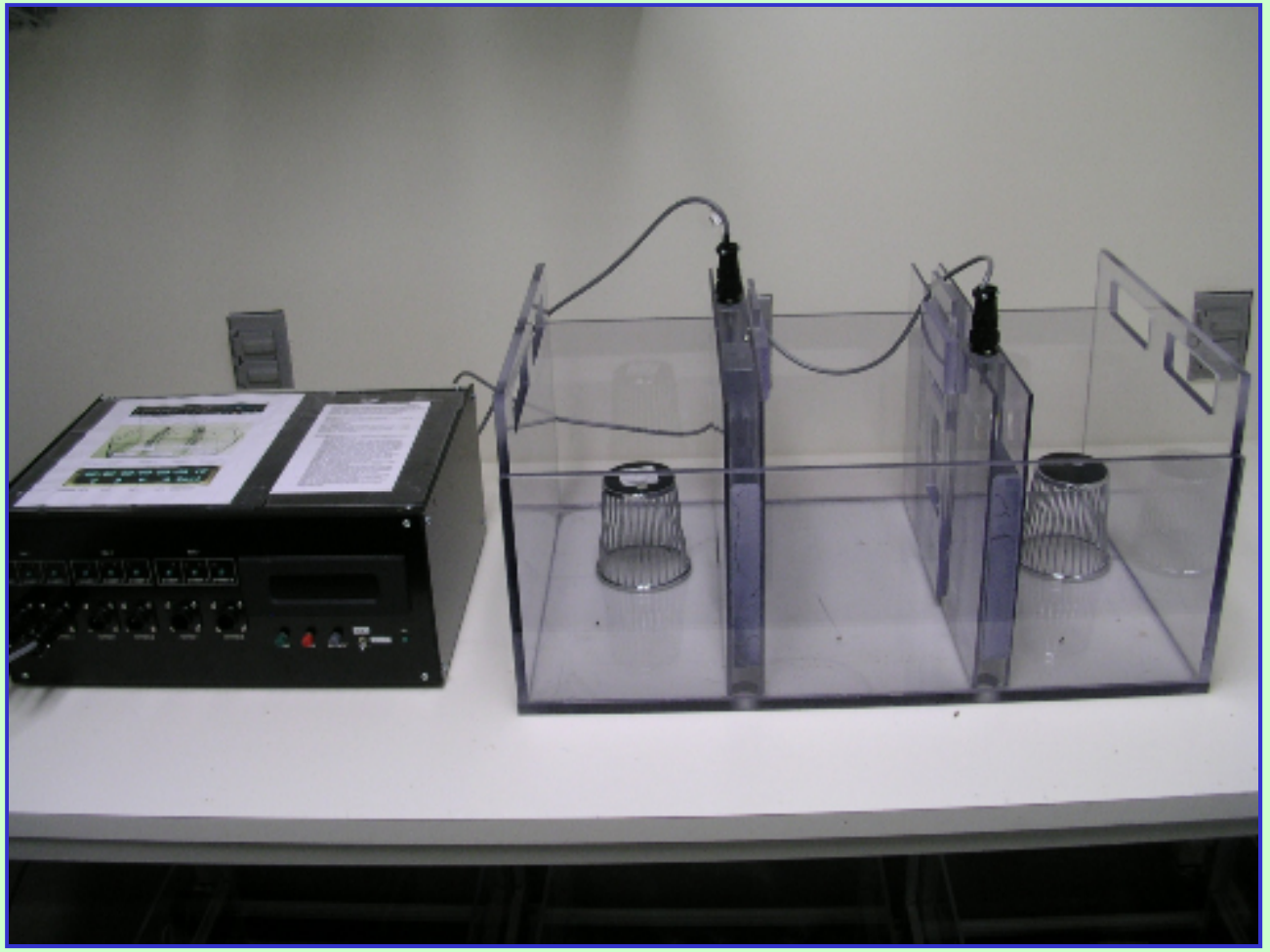


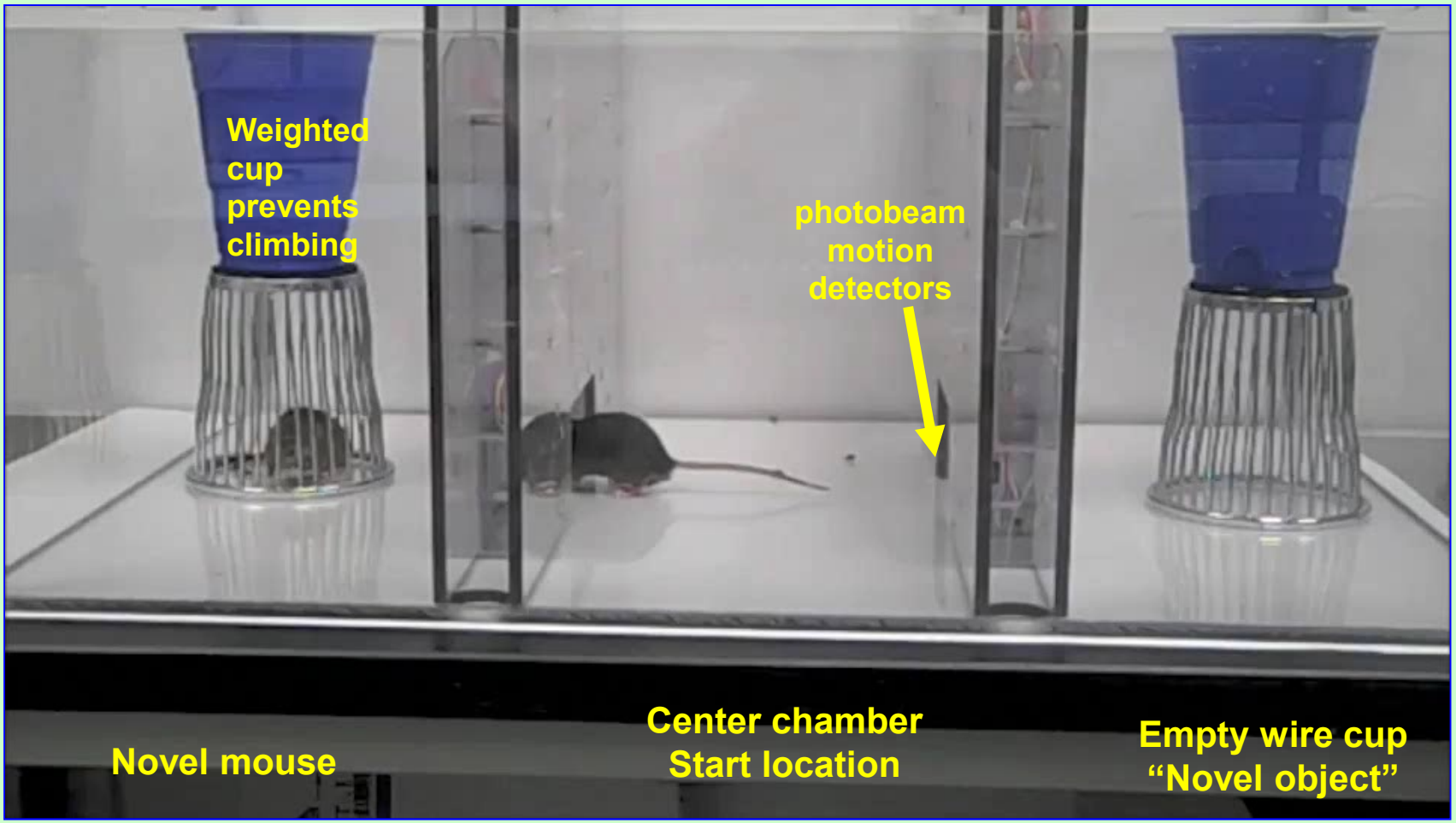
**Illustration from Hutt and Hutt, www.autismuk.com, 1970**

# Automated Social Approach Apparatus



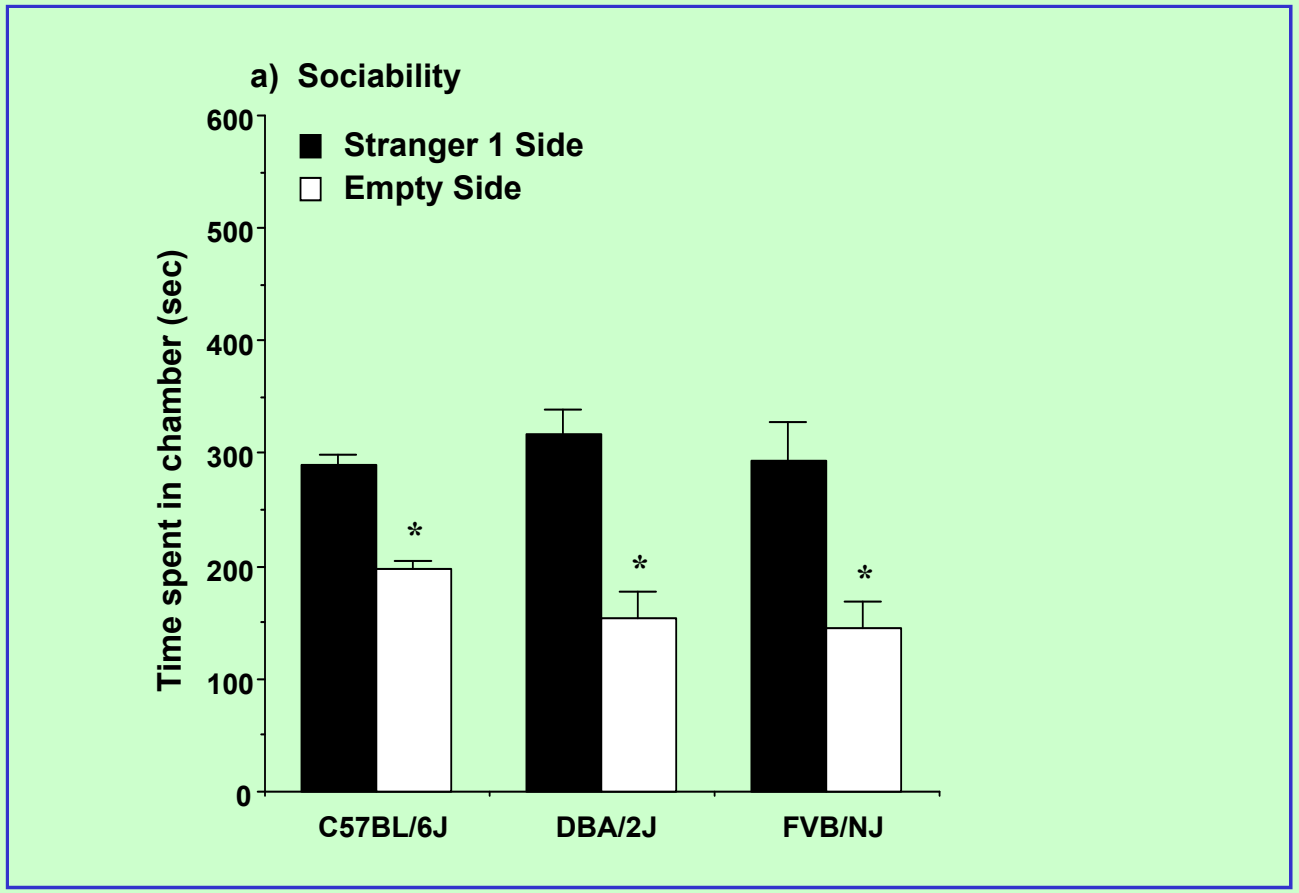
**Designed and fabricated by Jacqueline Crawley, NIMH, Sheryl Moy, UNC, George Dold and coworkers,**
**NIMH/NINDS Research Services Branch, Bethesda, MD**

# Sociability in Adult C57BL/6J (B6) Mice



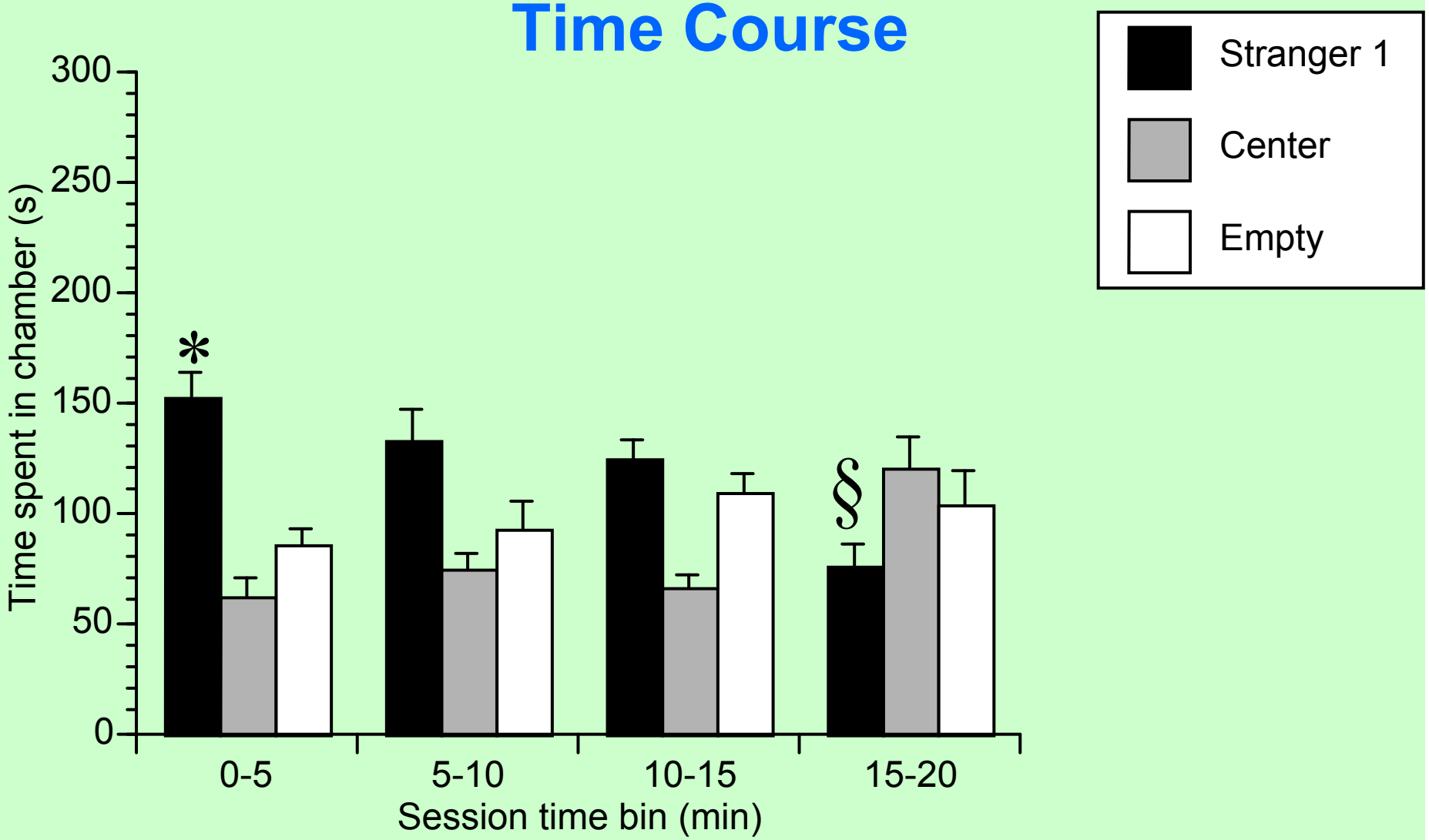

*Video by Mu Yang and Adam Katz, LBN, NIMH 2009*

# Significant Sociability Scores in Three Inbred Strains of Mice



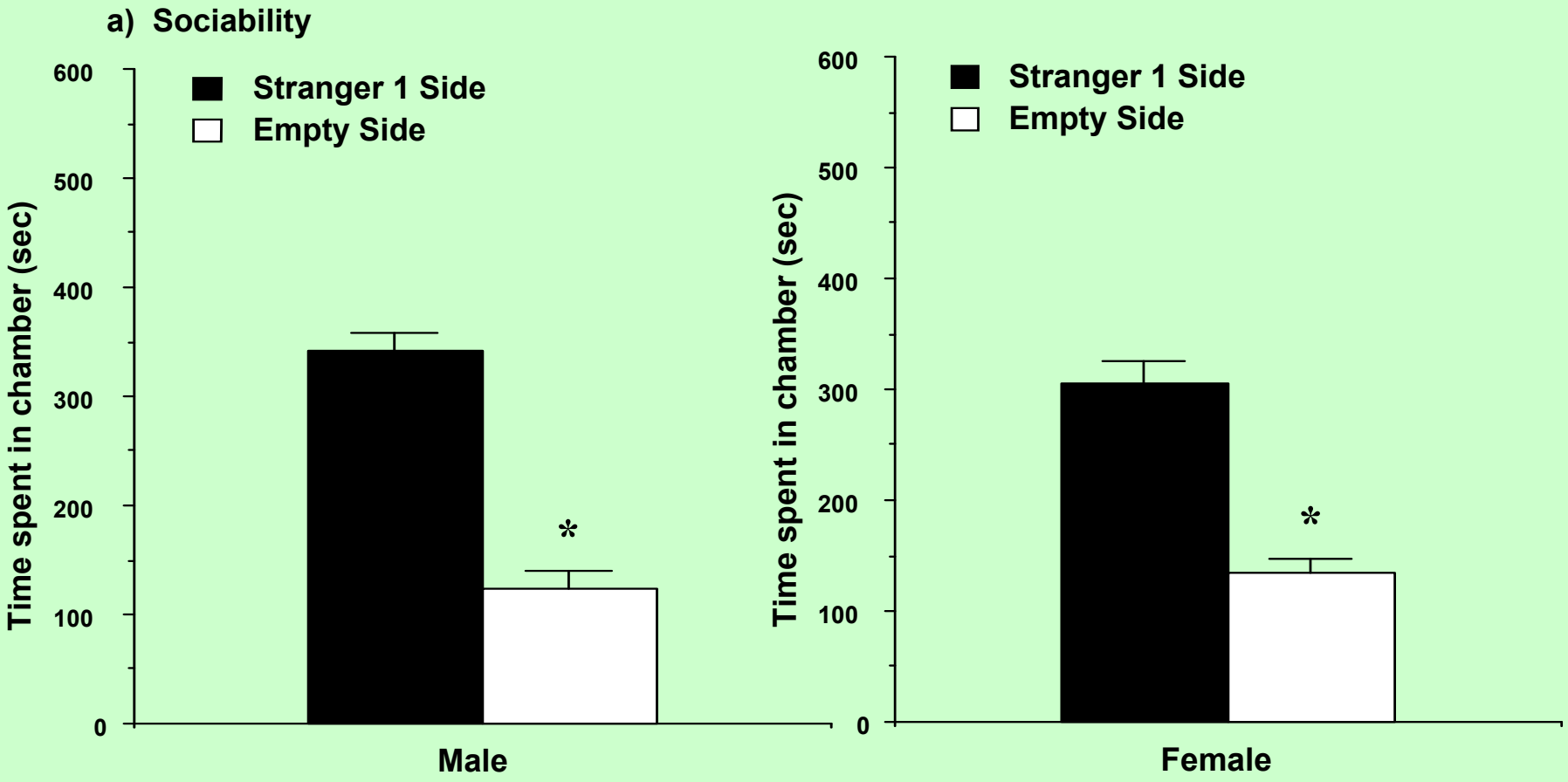

*Moy et al., Genes, Brain and Behavior, 2004*

# Time Course



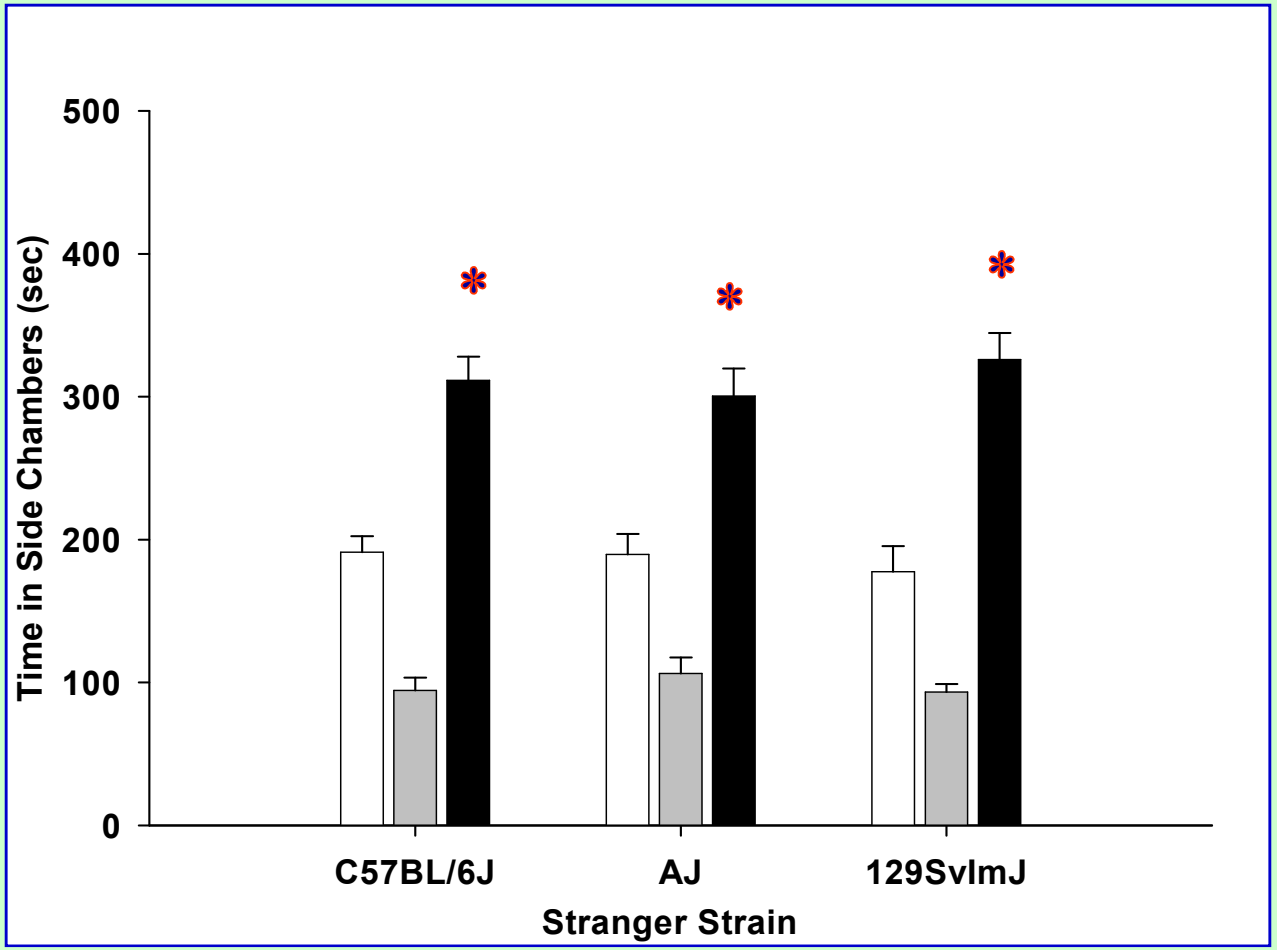

*Nadler et al., Genes, Brain and Behavior 2004*

# Similar Sociability Scores in Male and Female C57BL/6J



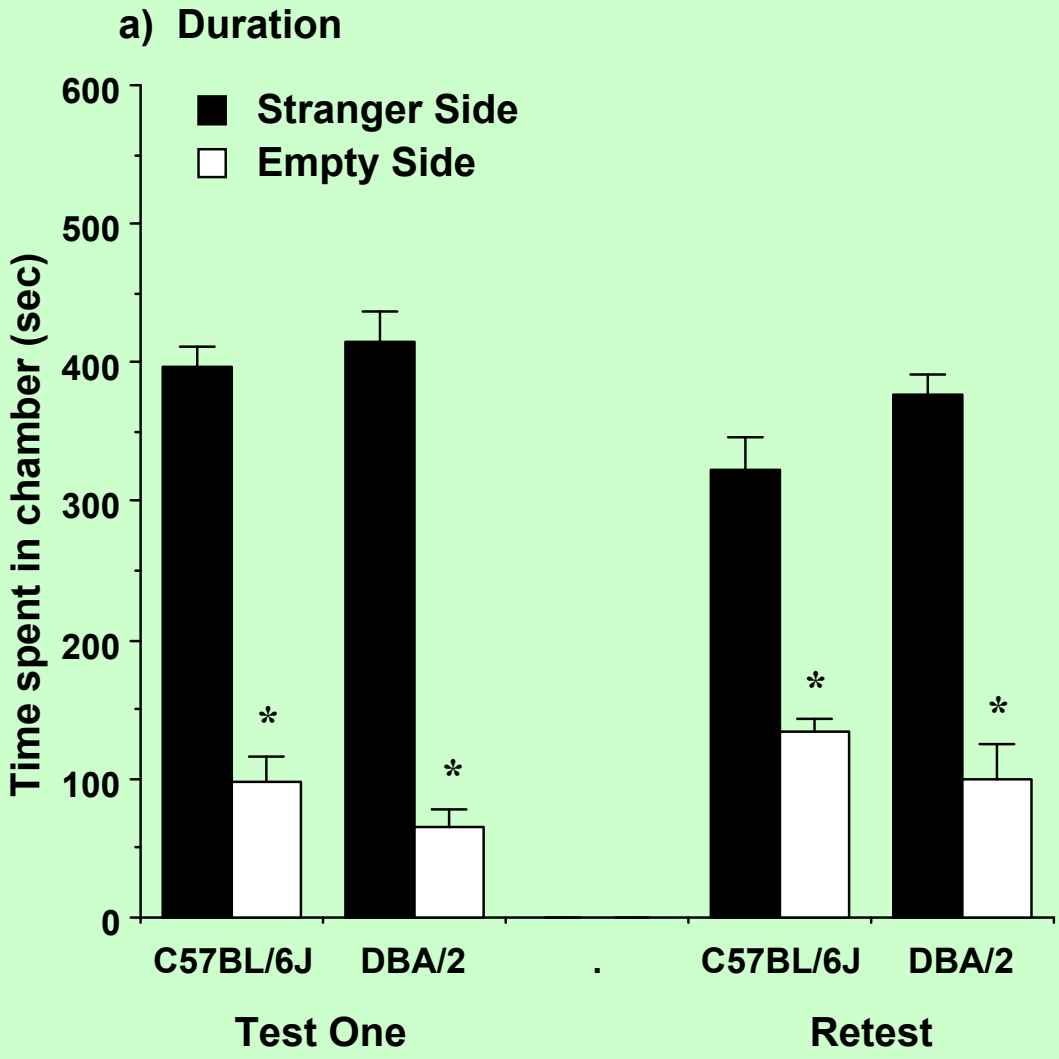

*Moy et al., 2004*

# Similar sociability scores when the subject mouse is the same or different strain as the novel mouse



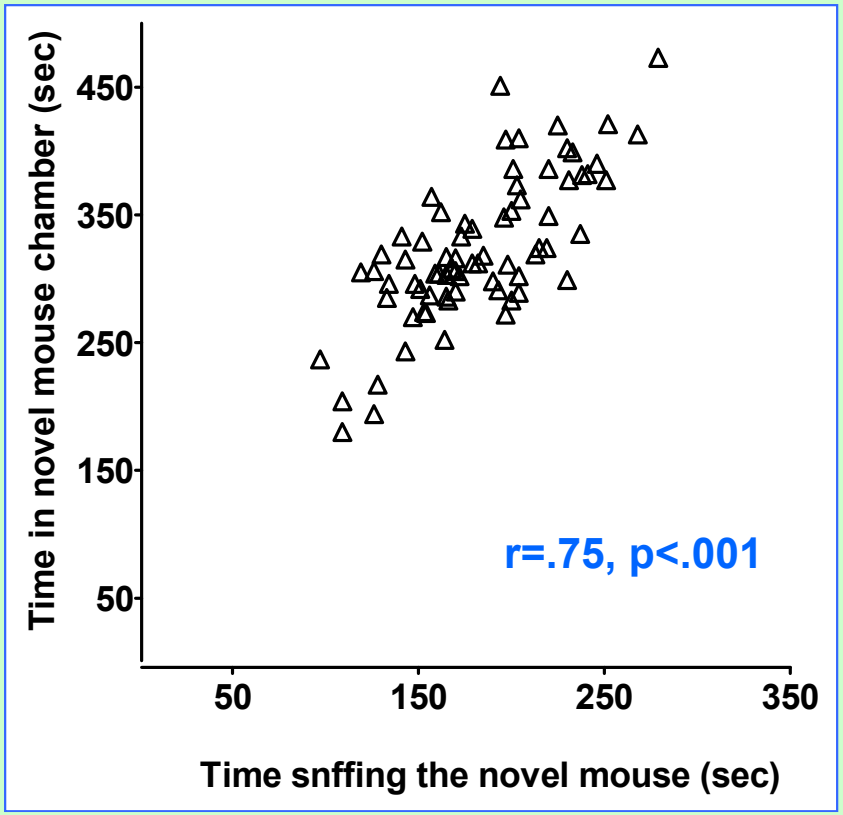

*Yang and Crawley, NIMH 2007*

# Similar sociability scores in repeated testing of the same individuals as juveniles adults and as adults



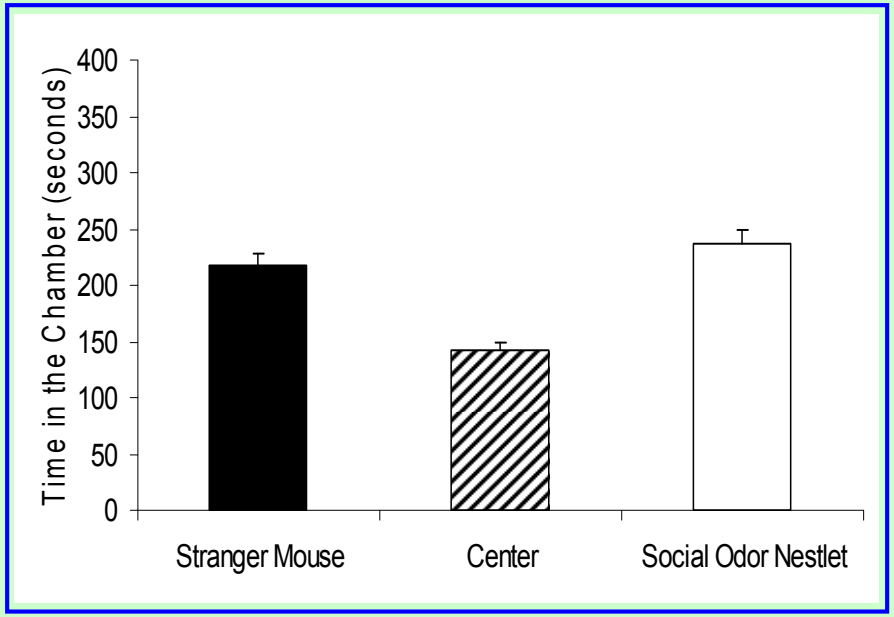

*Moy et al., 2004*

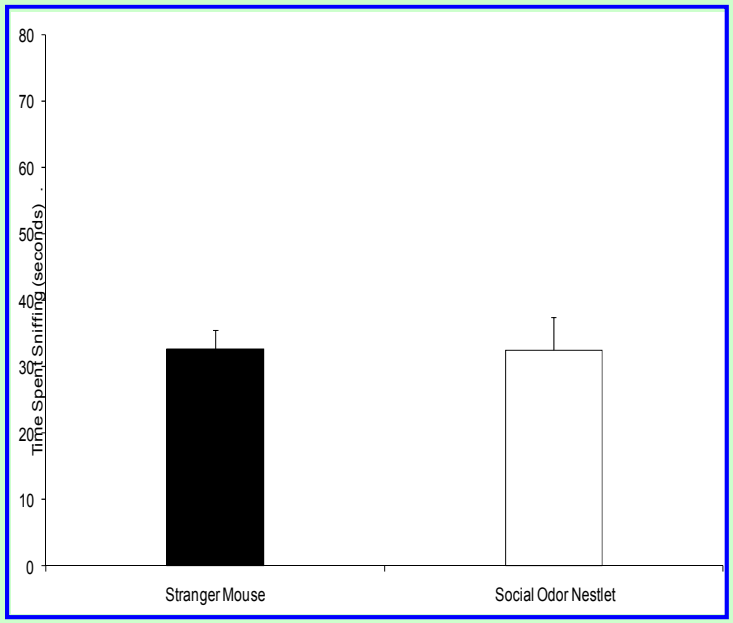
# Mouse Social Approach Task
## Social sniffing correlates with time in chamber




*Mu Yang, LBN, NIMH, 2009*

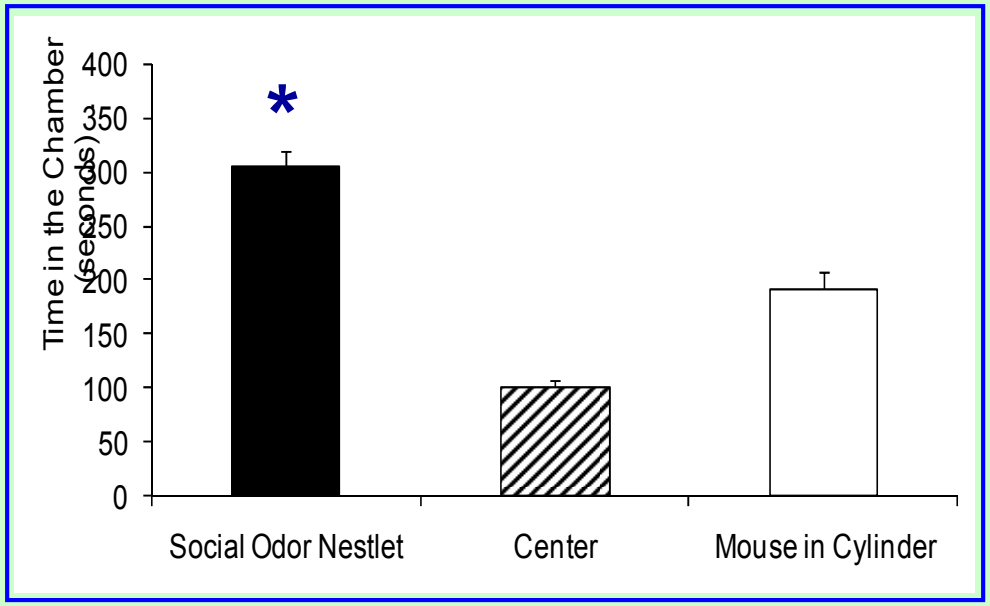
# B6 adult male mice display similar interest in exploring a novel mouse and a novel social odor on an inanimate Nestlet square

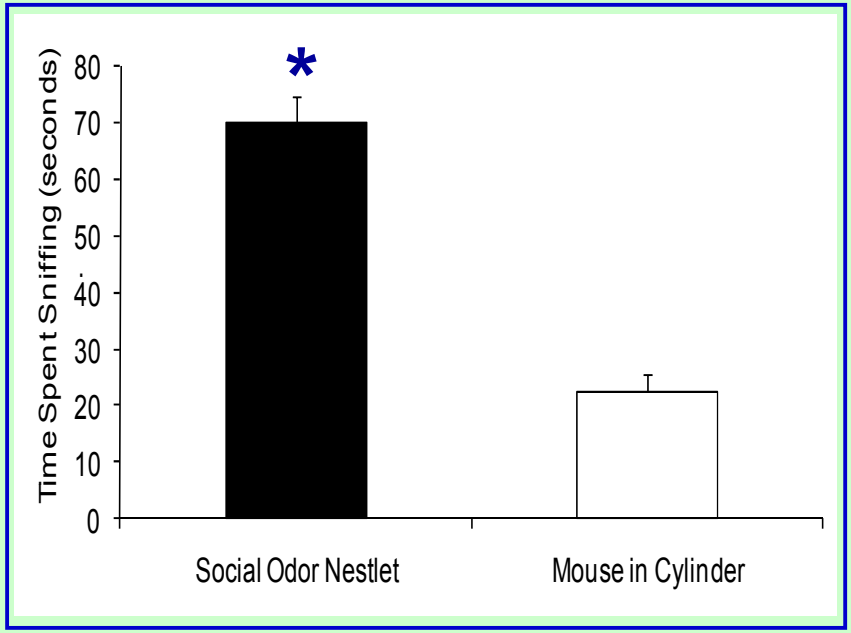



***Ryan et al., Behavioural Brain Research 2008***

# B6 adult male mice more time exploring a novel social odor on an inanimate Nestlet square than exploring a novel mouse enclosed in a Plexiglas cylinder



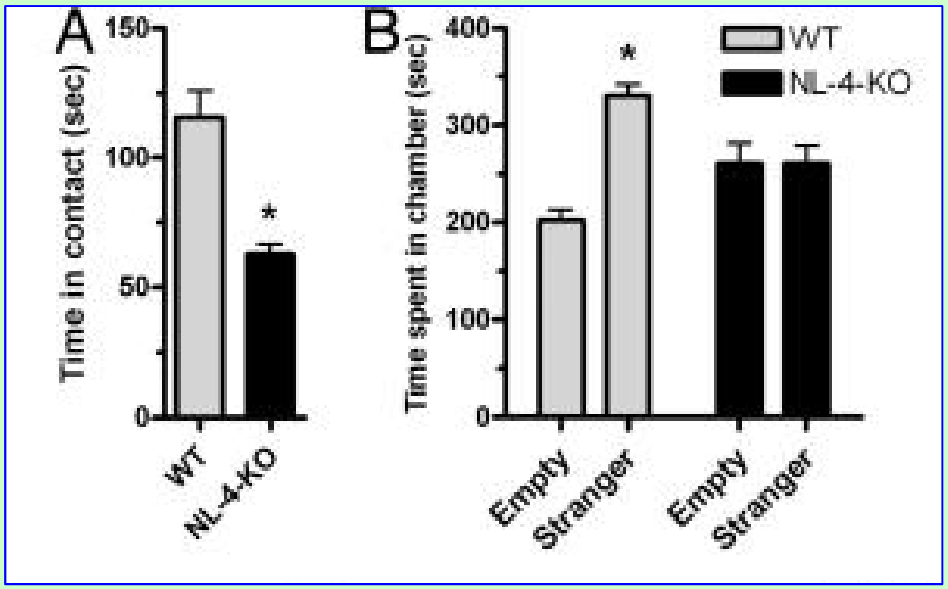

***Ryan et al., Behavioural Brain Research 2008***

## 1. REVERSE GENETICS: Mutant lines tested on adult social approach LBN 2004-2010

| Mutation | Collaborator/Source | Phenotype | Publication |
|---|---|---|---|
| Oxytocin (line1) | Scott Young, NIMH | Normal sociability | Crawley et al. 2007 |
| Oxytocin (line2) | Larry Young, Emory | Normal sociability | Crawley et al., 2007 |
| Vasopressin Avpr1b | Scott Young, NIMH | Normal sociability | Yang et al., 2007 |
| VIP | Jim Waschek, UCLA | Absence of sociability in offspring of VIP -/- dams | Stack et al., 2008 |
| BDNF-tg | JAX (Huang, Tonegawa) | Normal sociability | Silverman et al., in prep |
| Serotonin transporter | Dennis Murphy, NIMH | Reduced sociability<br>Reduced pref soc novelty | Moy et al., 2009 |
| Neuroligin-2<br>Neuroligin-3<br>Neuroligin-4 | Nils Brose, Max Planck<br>Nat Heintz, Rockefeller<br>Nils Brose, Max Planck | In progress<br>Normal sociability<br>In progress | Chadman et al., 2008 |
| Shank1<br><br>Shank 3 | Morgan Sheng, MIT<br><br>J Buxbaum, Mt Sinai | Normal sociability<br>Fewer pup vocalizations<br>In progress | Silverman et al., in prep<br>Wöhr, in preparation |
| Disc1 | M Pletnikov, JHopkinsU | Normal sociability<br>Reduced pref soc novelty | |
| Fmr1 | Bill Greenough, U Illinois | Reduced sociability on FVB background<br>Normal sociability on B6 | Moy et al., 2009 |
| Engrailed-2 | Karl Herrup, Case U<br>Manny DiCicco-Bloom, NJ | Inactivity confound<br>In progress | Moy et al., 2009<br>Silverman, in progress |
| Smith Lemli Opitz | Kathy Sulik, UNC<br>Denny Porter, NICHD | Inactivity confound<br>Inactivity confound | Moy et al., 2009 |

## Jamain et al., 2008
## Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism

# Neuroligin-4 knockout mice

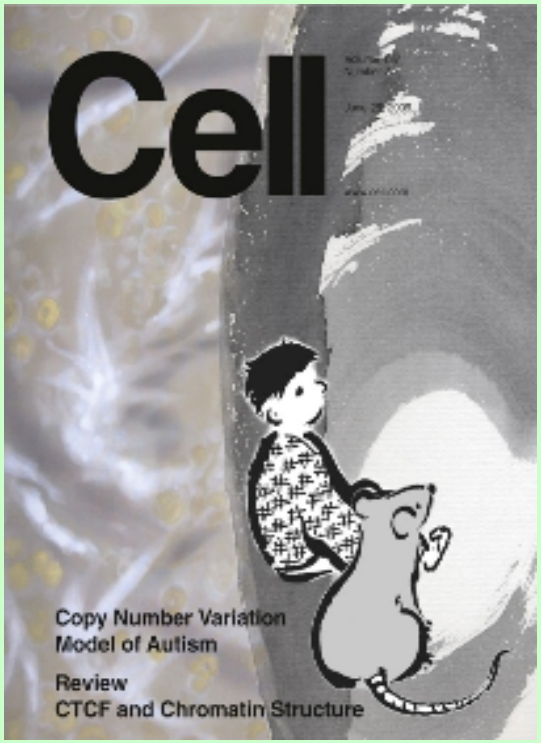


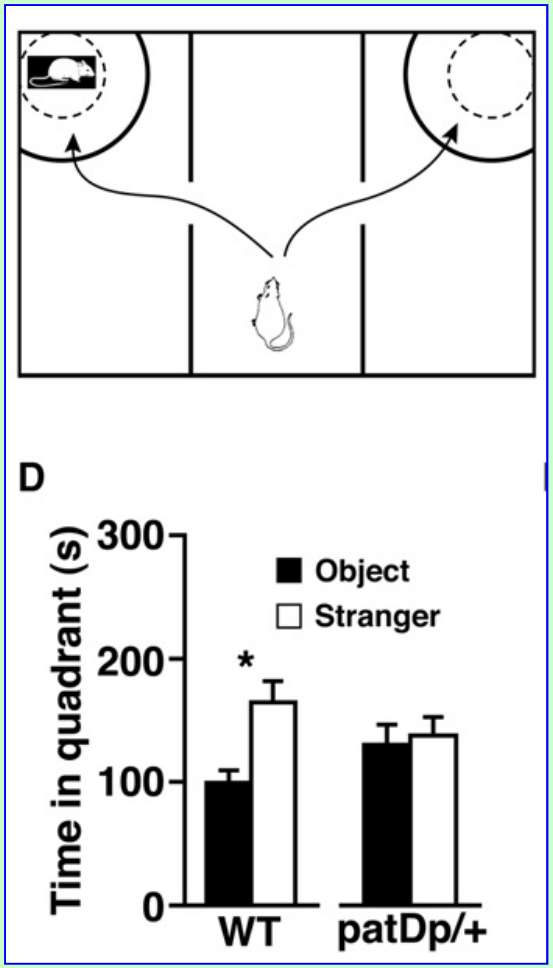

A) Less reciprocal social interactions in NL-4 than WT, between pairs of male mice of the same genotype

B) Absence of sociability in NL-4 in the 3-chambered social approach task

*Crawley 2010*

## Nakatani et al., 2009
## Abnormal behavior in a chromosome-engineered mouse model for human 15q11-13 duplication seen in autism



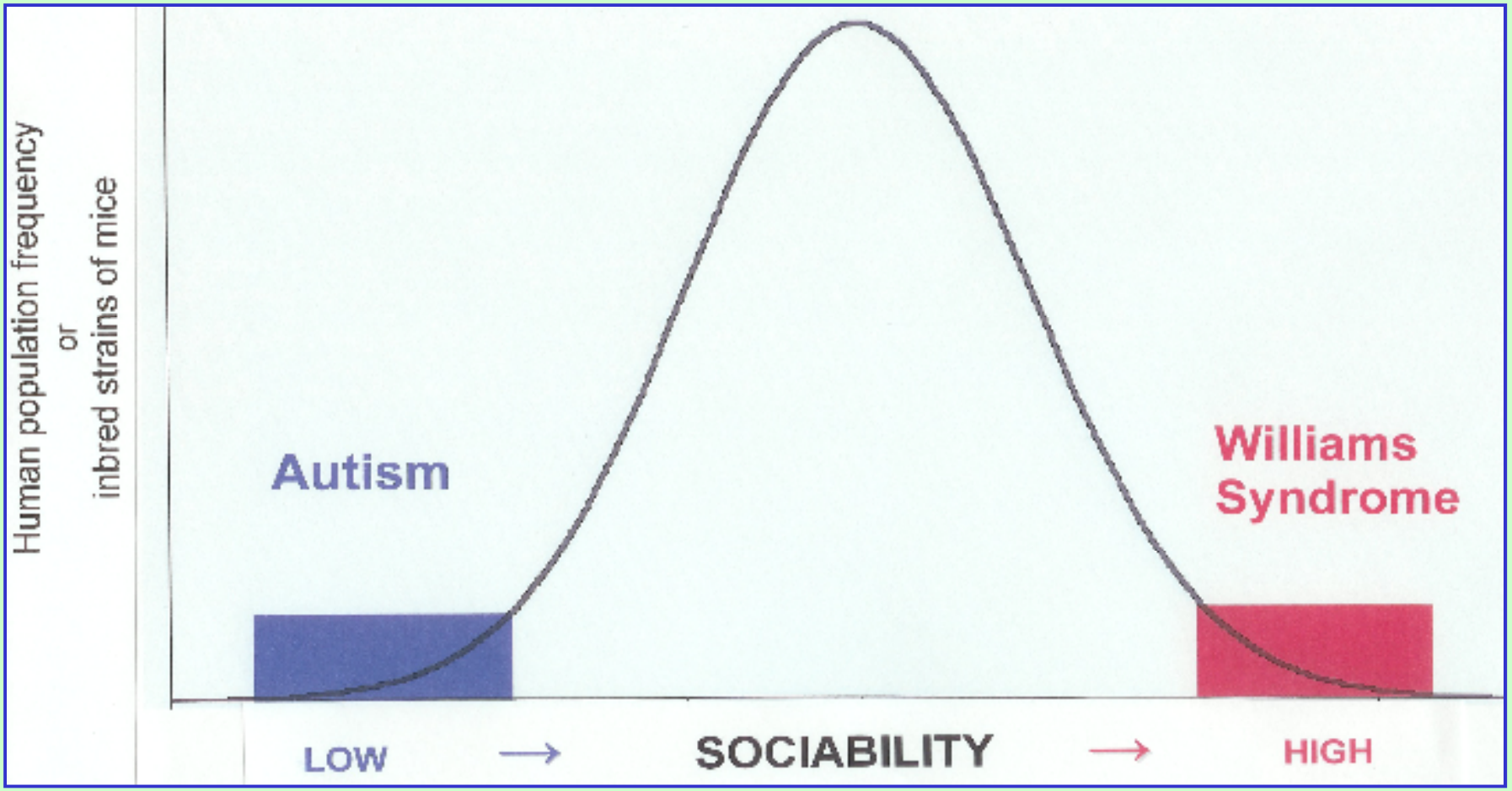




*Crawley 2010*

*Tsuyoshi Miyakawa,*
*Toru Takumi*

## 2. Forward Genetics

*Hypothesis: Behavioral traits of autism are at the extremes of natural variation in the population distribution, caused by rare polymorphisms in multiple genes*




***Crawley, 2005***

## 2. Forward Genetics: UNC STAART project focused on inbred strains from the International Mouse Phenome Project

*A) Behavioral traits*
*B) DNA microarray expression*
*C) Cluster analysis, gene ontology*

**C57-related**
C57BL/6J
C57L/J
C58/J

**Swiss**
FVB/NJ
SJL/J
NOD/LtJ
SWR/J

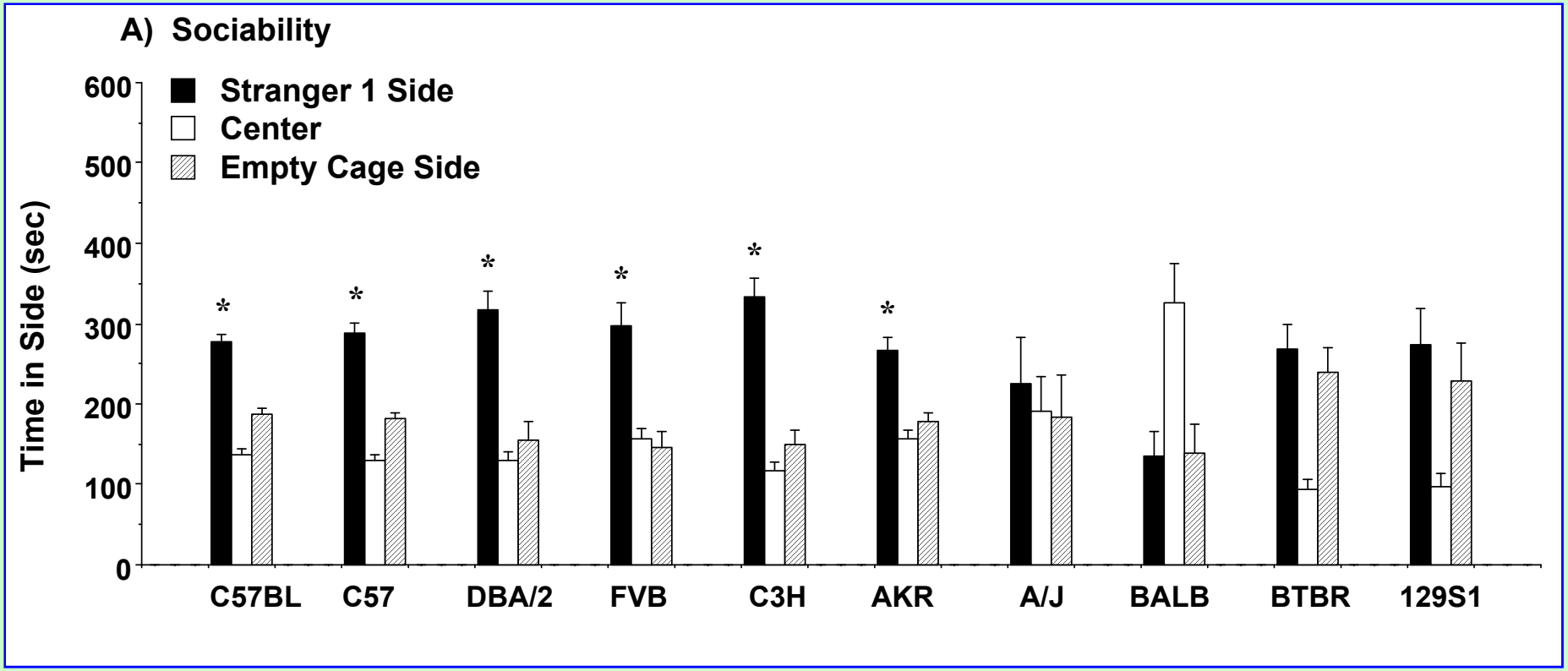
**Castle's**
129S1/SvImg
A/J
BALB/cByJ
C3H/HeJ
DBA/2J
AKR/J
NZB/B2NJ
SM/J
BTBR T+tf/tf

**Wild-derived**
PERA/Ei
MOLF/Ei
CAST/Ei
SPRET/Ei

**Other**
PL/J

*Crawley, 2006*

# University of North Carolina STAART Project 4
## Sociability in first 10 inbred strains

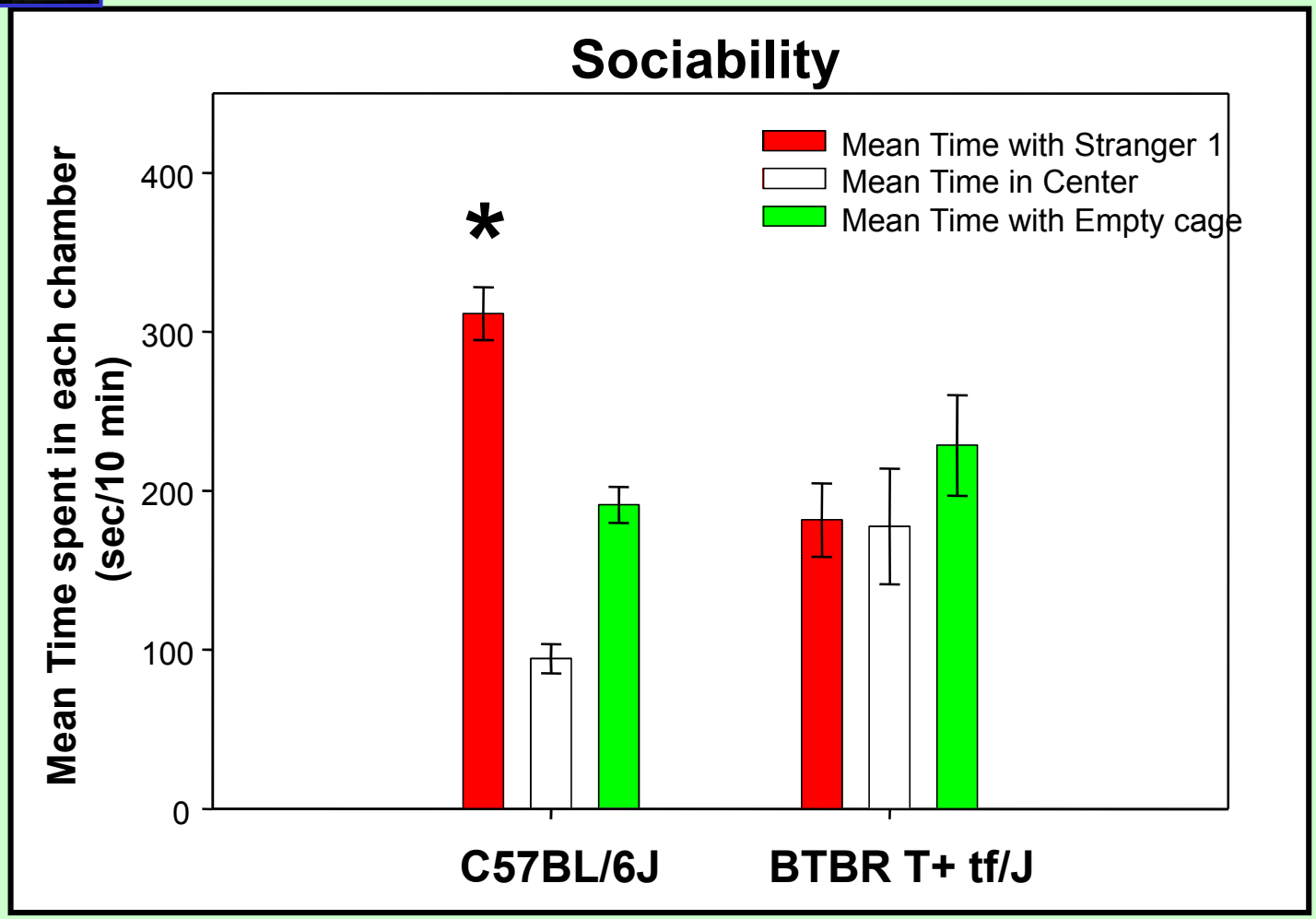



Moy et al., *Behavioural Brain Research, 2007*

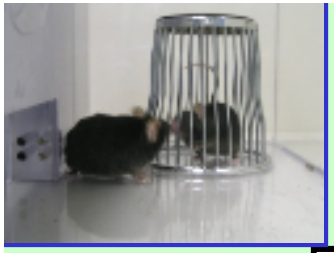
## At LBN NIMH Bethesda

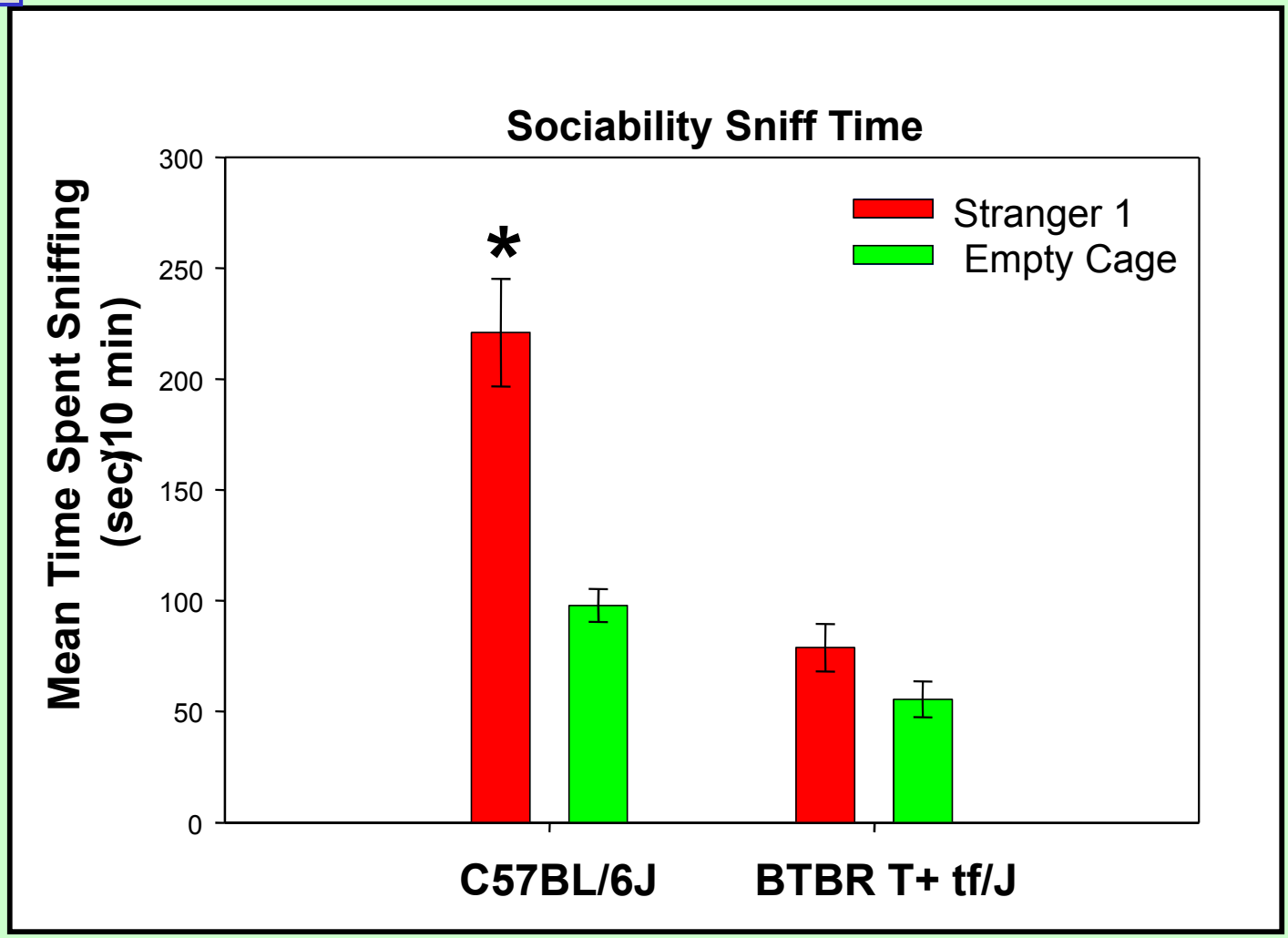
## Replication of BTBR social approach deficit

## During dark phase of circadian cycle



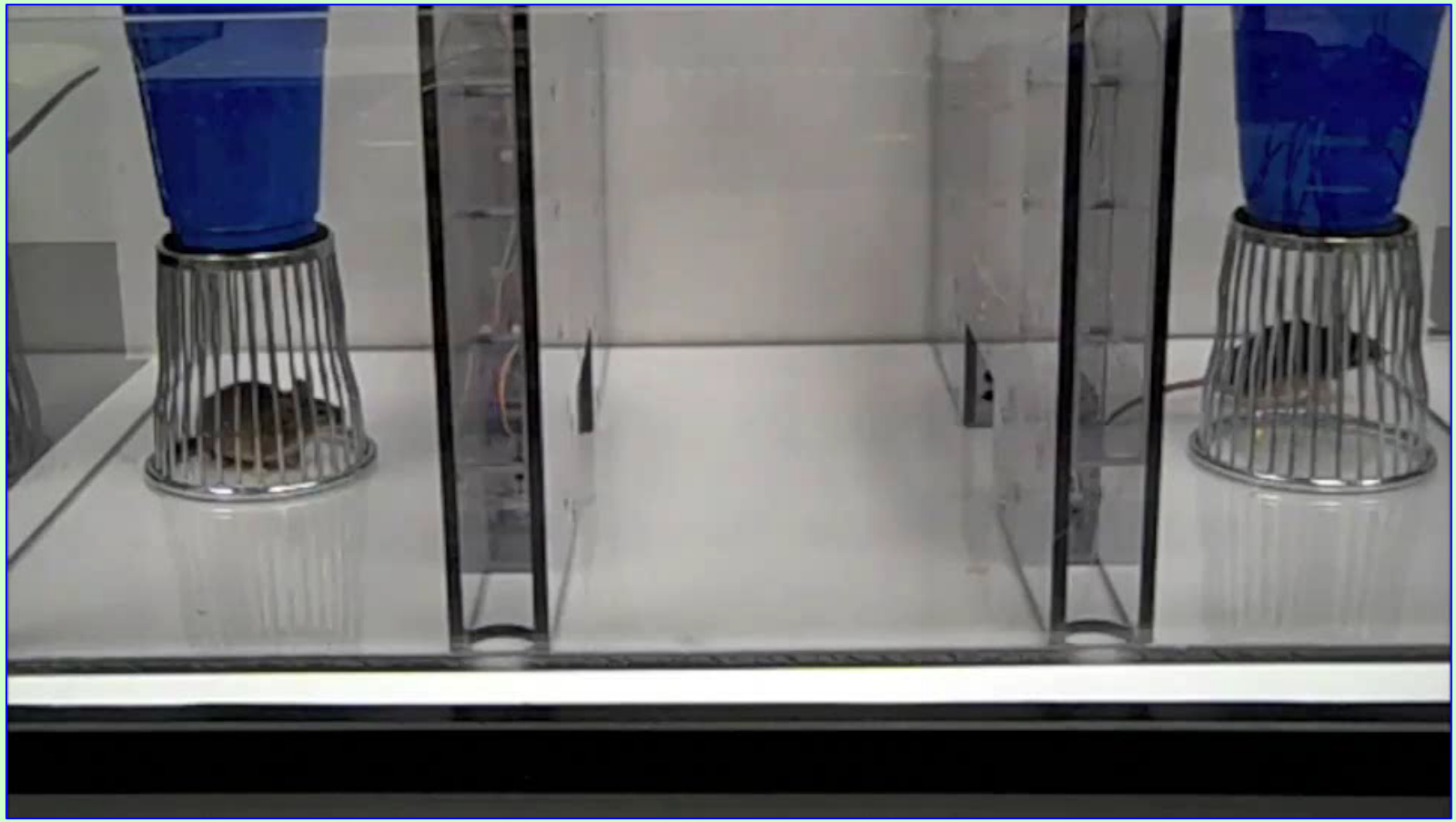

*McFarlane et al., Genes, Brain and Behavior, 2007*






*McFarlane et al., 2007*

# Absence of Sociability in Adult BTBR T+tf/J (BTBR)



*Video by Mu Yang and Adam Katz, LBN, NIMH 2009*

# BTBR is a useful inbred strain

- To design tasks relevant to the diagnostic symptoms of autism
- To develop treatment protocols
- Genetically homogenous, commercially available from JAX, easy to breed

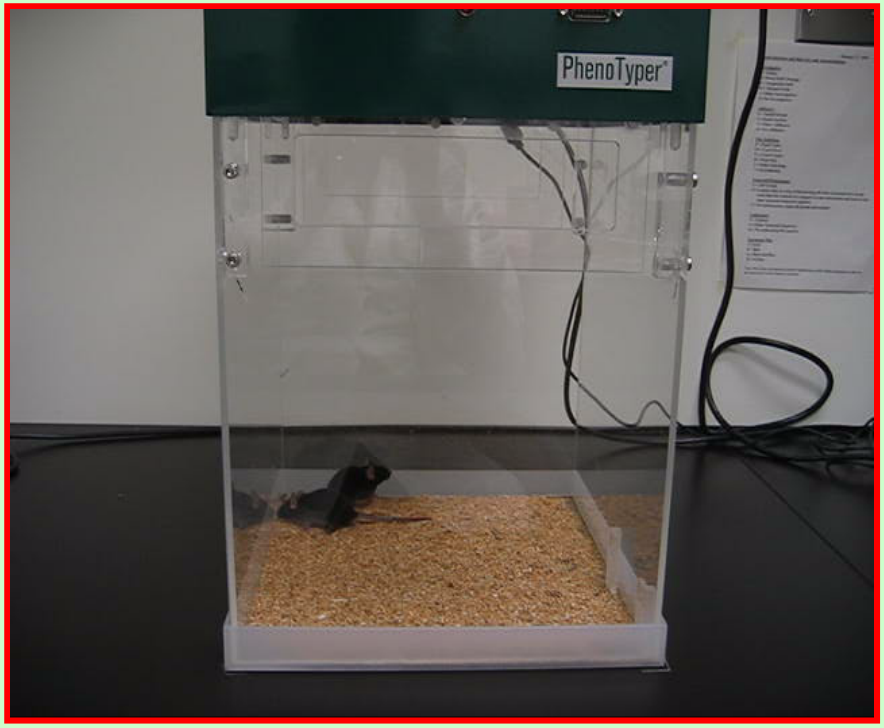
# Reciprocal Social Interactions

Pairs of 21 day old juveniles
Pairs of adults
Same or different strains
Same or different genotypes
Same or different sexes
From different home cages

10 – 30 minute test session

Events scored include social approach, social grooming, following, crawling over/under, etc.

Control measures of non-social grooming, exploration, and activity are simultaneously scored.

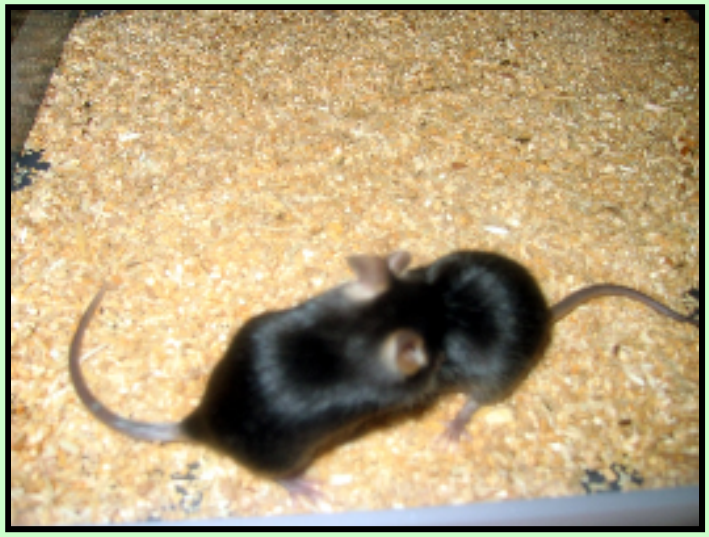


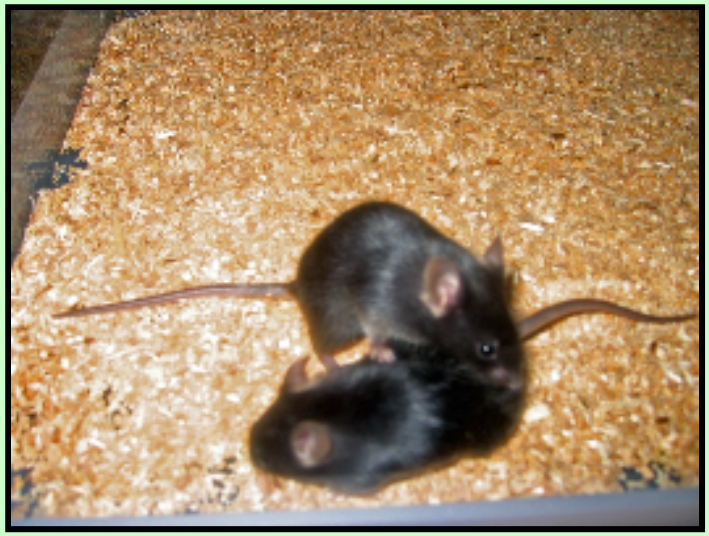

*Noldus Observer Phenotyper*
*with digital videocamera, keypad event recorder,*
*and frame-by-frame data analysis software*

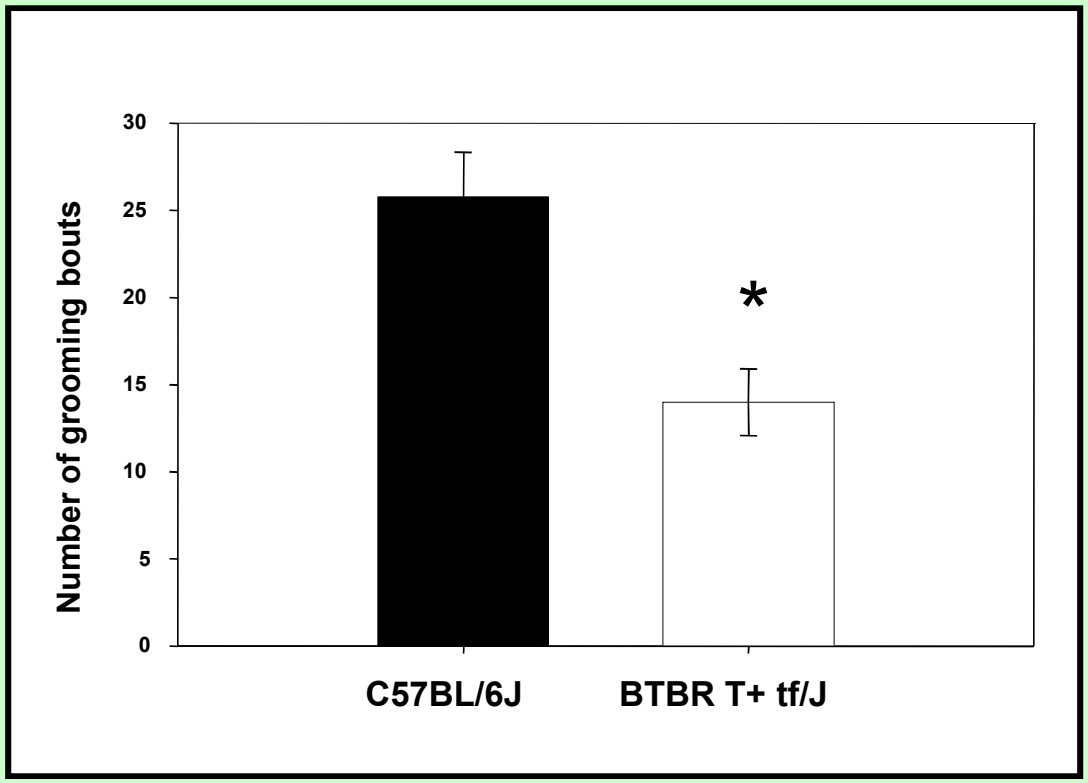
*McFarlane and Crawley, NIMH 2006*

# Social Grooming

## One mouse grooms the other



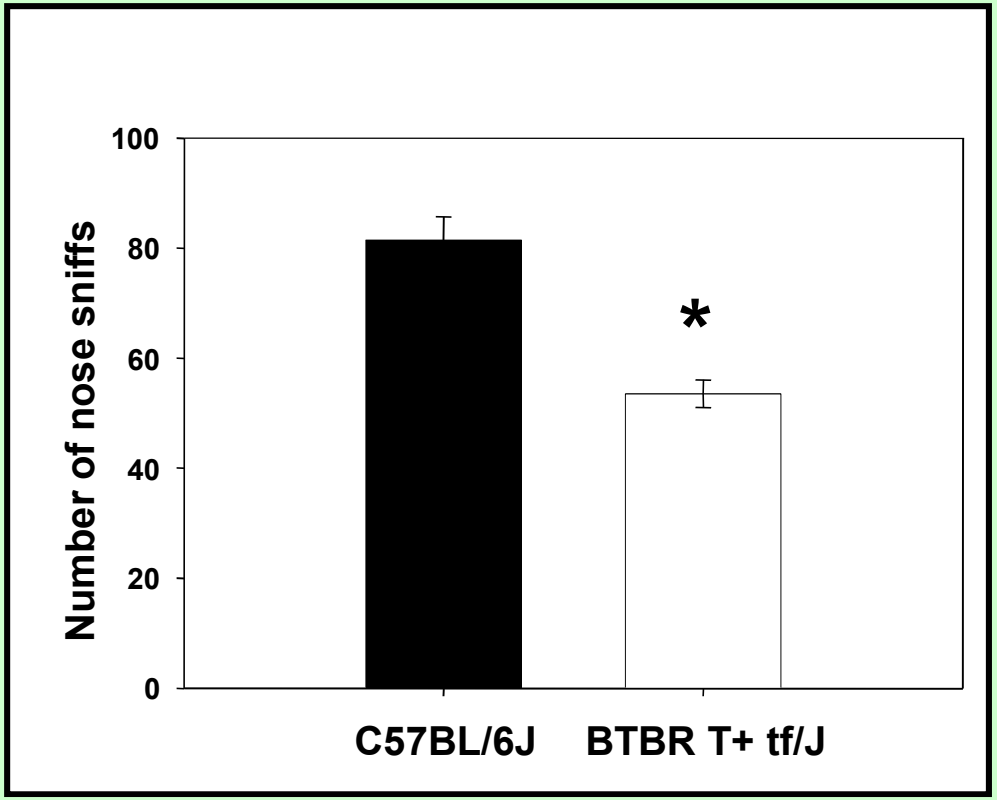





*McFarlane et al., 2007*

# Nose-to-Nose Sniffing

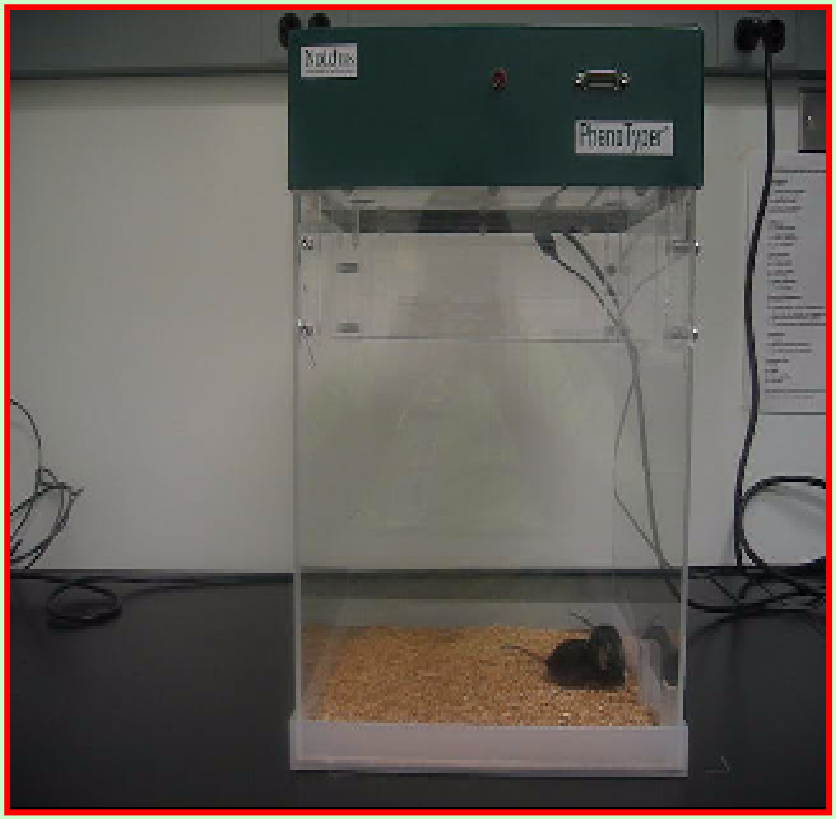
## One mouse sniffs the snout region of the other

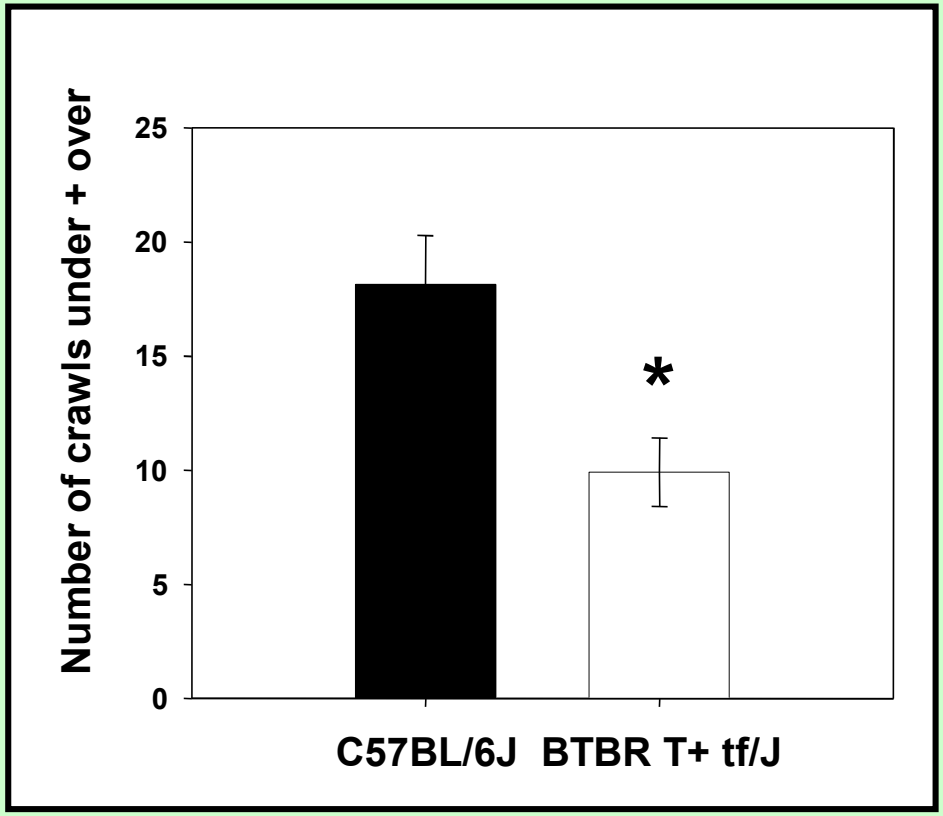



*McFarlane et al., 2007*

# Crawl under or over

## One mouse pushes under or walks across the other

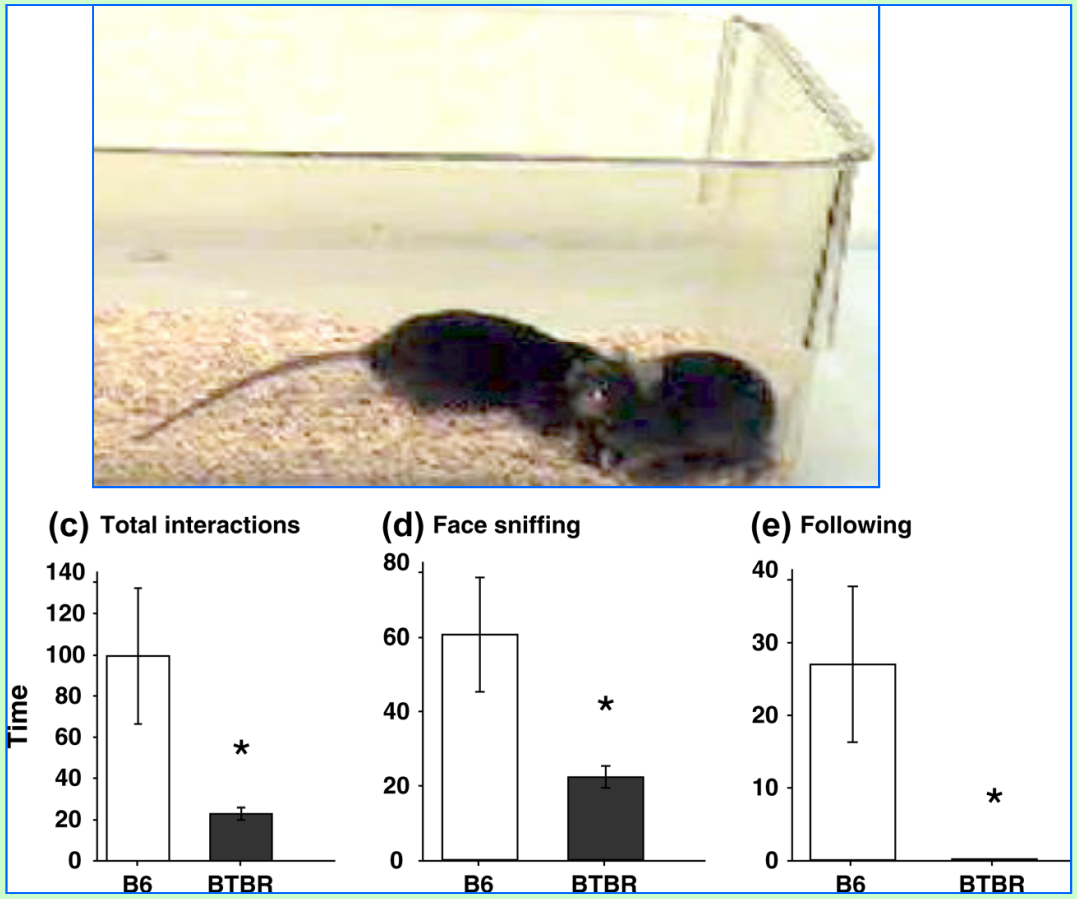



*McFarlane et al., 2007*

# Corroboration across three laboratories

## *Reciprocal social interaction deficits in adult BTBR T+tf/J pairs versus B6 pairs, tested in an empty cage*




*Valerie Bolivar, Wadsworth Institute, University of Albany, NY*

# Neurodevelopmental Milestones

## Postnatal days 2-14

Body weight
Body length
Eye opening
Incisor eruption
Pinna detachment
Forelimb placing
Bar holding

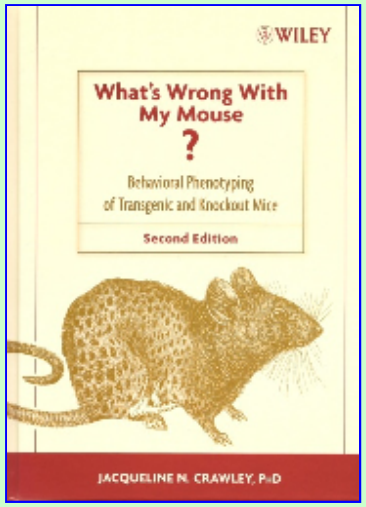
Homing test
Cliff aversion
Negative geotaxis
Righting reflex
Screen climbing
Auditory startle
Separation vocalizations

***Scattoni et al, 2008***

# Control Measures

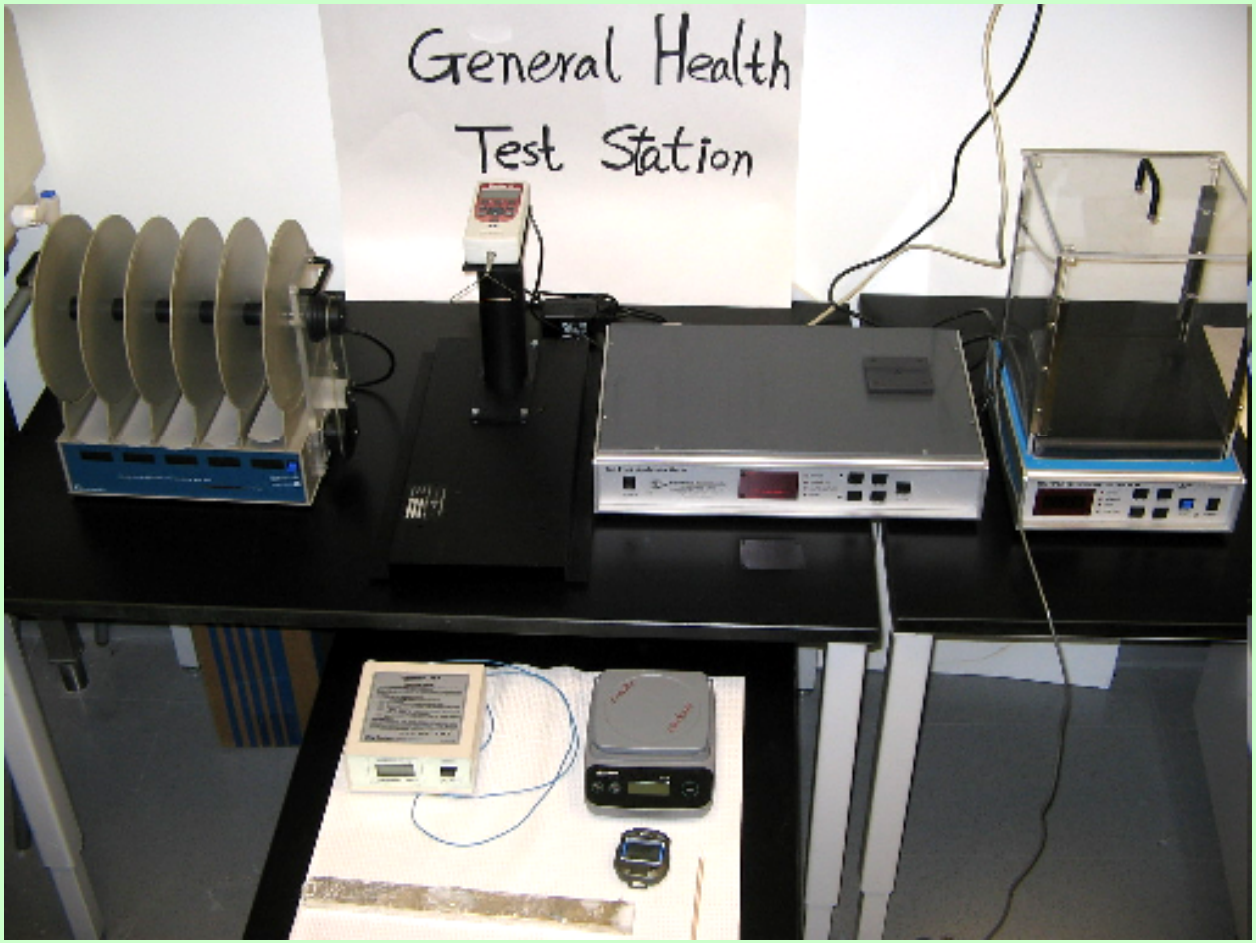
## For physical health and procedural abilities

- General Health (body weight, appearance of fur, home cage behaviors nesting patterns)
- Neurological reflexes (startle, righting)
- Sensory abilities (hearing, vision, olfaction)
- Motor abilities (locomotion, coordination and balance, muscle strength)




***Crawley, 2009***

# General Health, Neurological Reflexes, Sensory Abilities, Motor Functions




*Mu Yang, LBN, NIMH, 2009*

# General Health and Neurological Reflexes

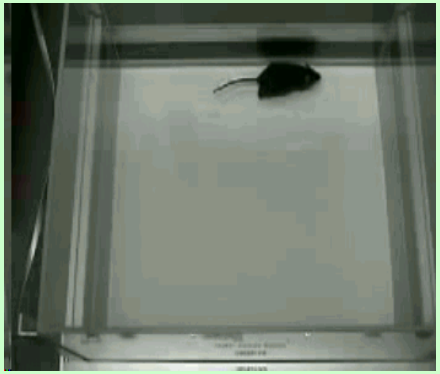
## *Shank1*

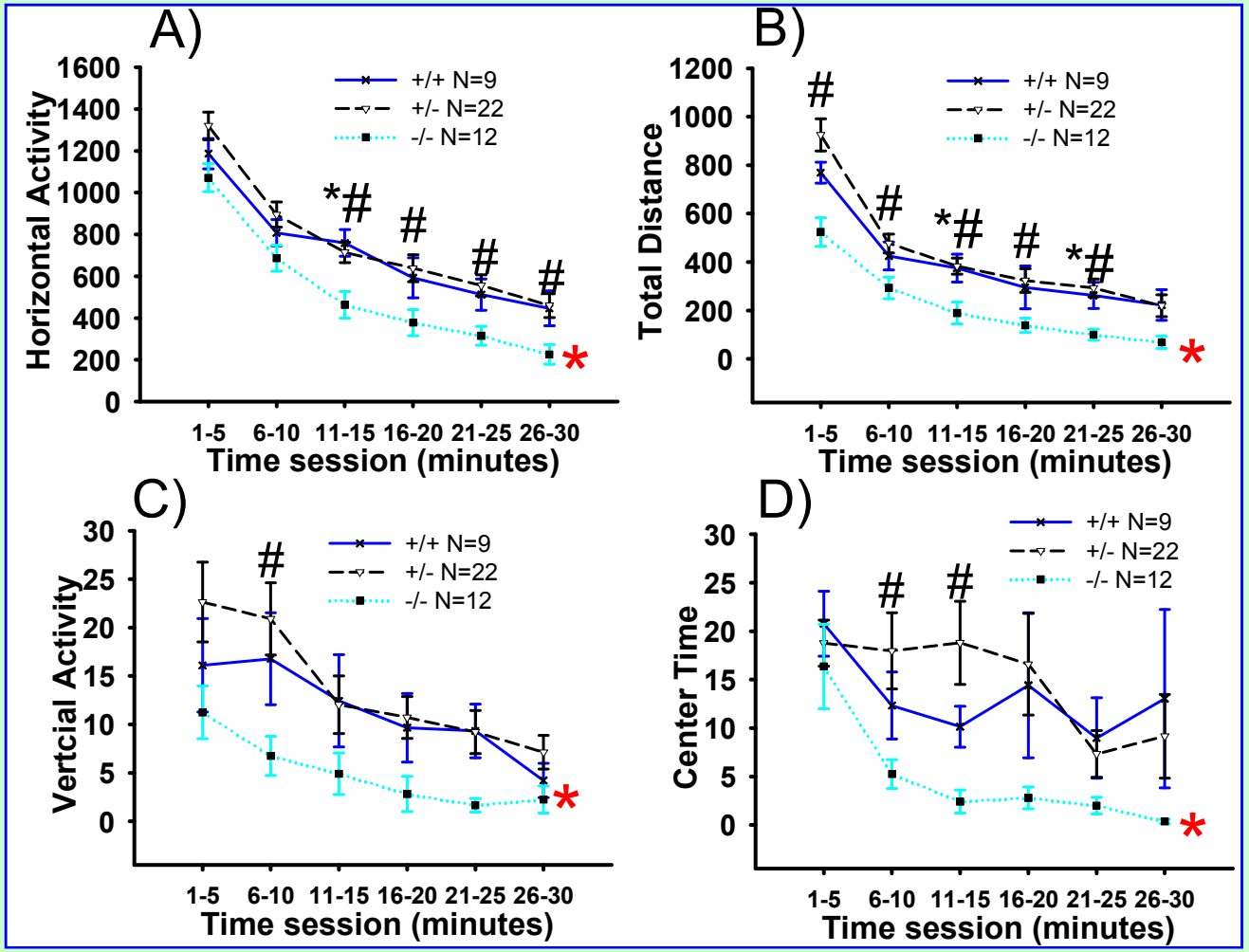
| Genotypes | +/+ | +/- | -/- | p |
|---|---|---|---|---|
| Body temperature | 36.7±.28 | 36.3±.45 | 37.7±.27 | .03 * |
| Body weight | 25.1±1.5 | 23.0±1.7 | 25.1±1.6 | .57 |
| Fur condition (3 pt scale) | 2 | 2 | 2 | NA |
| Bald patches (%) | 6% | 8% | 0% | .63 |
| Missing whiskers (%) | 20% | 16% | 9% | .74 |
| Piloerection | 0% | 0% | 0% | NA |
| Body tone (3 pt scale) | 100% | 100% | 100% | NA |
| Limb tone (3 pt scale) | 100% | 100% | 100% | NA |
| Wild running (%) | 0% | 0% | 0% | NA |
| Sterotypies (%) | 0% | 0% | 0% | NA |
| Exploration (3 pt scale) | 2 | 1.9 | 1.9 | .64 |
| Trunk curl (%) | 86% | 83% | 63% | .32 |
| Wire hang (latency sec) | 55.9±2.8 | 60.0 | 44.2±8.0 | .05 * |
| Righting reflex (%) | 100% | 100% | 100% | NA |
| Corneal reflex(%) | 90% | 100% | 93% | .59 |
| Auditory startle (%) | 100% | 100% | 100% | NA |
| Dowel biting (3 pt scale) | .26 | 41 | .36 | .70 |

***Silverman, Turner, Barkan, Tolu, Hung, Sheng Crawley, SFN 2009***

# Open Field Exploratory Locomotion

## *Shank1*

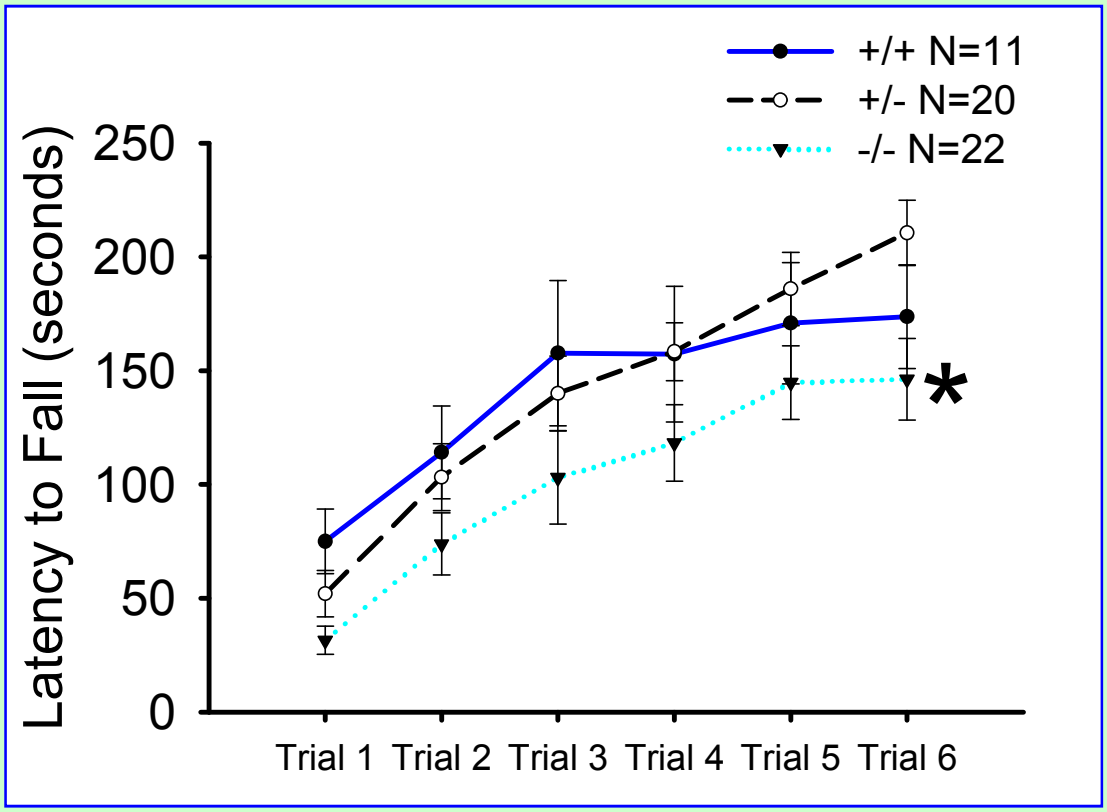





*Silverman et al., SFN 2009; Replicates Hung et al., 2008*

# Rotarod Motor Coordination and Balance

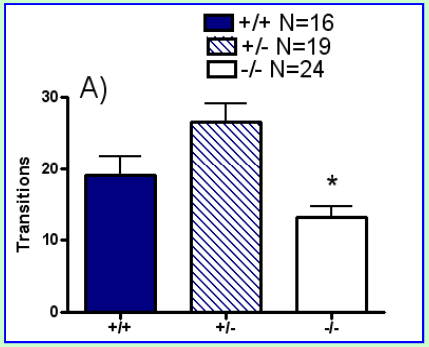
## *Shank1*

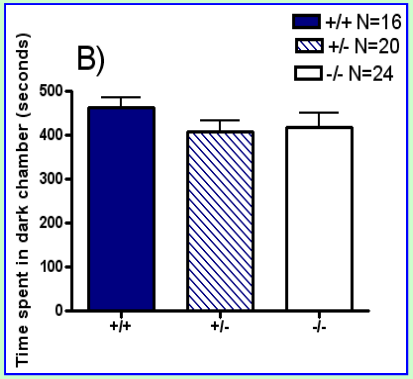



***Silverman, Diagne, Turner, Barkan, Tolu, Hung, Sheng Crawley, SFN 2009;***
***Replicates Hung et al., 2008***

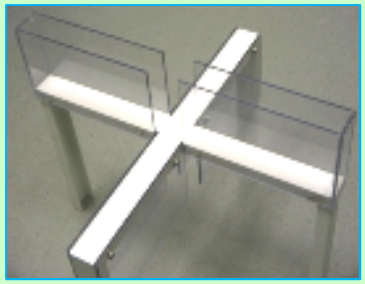
# *Shank1* Anxiety-related behaviors

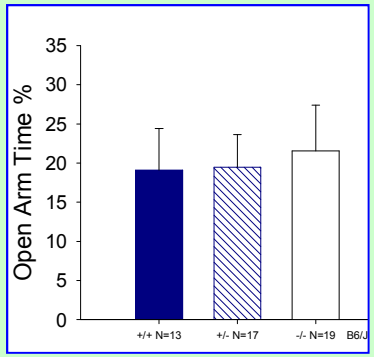
## Light ↔ Dark Transitions

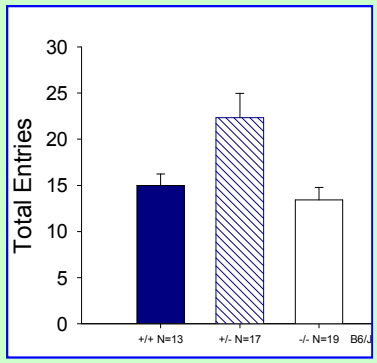



## Elevated Plus-Maze





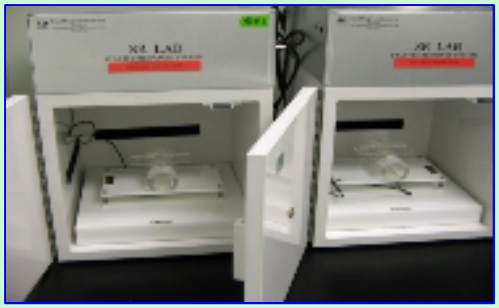




***Silverman, et al., SFN 2009; Replicates Hung et al., 2008***

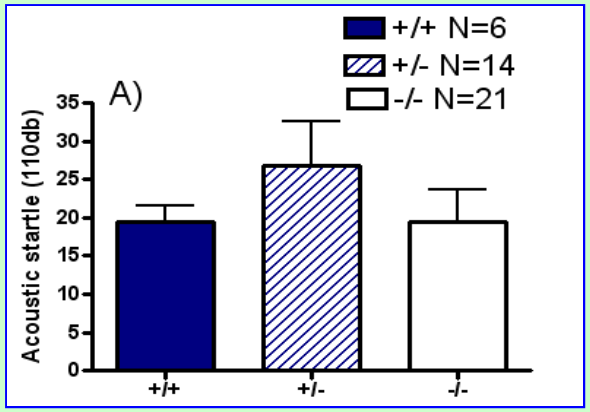
# Auditory and Sensorimotor Gating

## Acoustic Startle and Prepulse Inhibiton

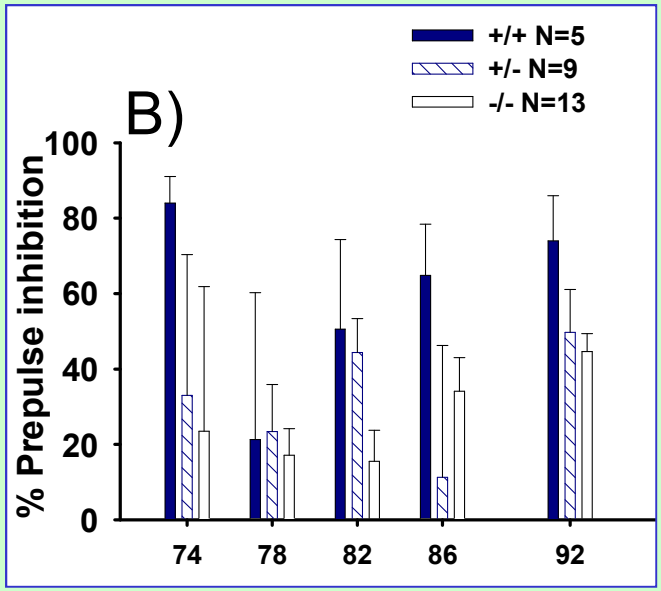
### *Shank1*



## Acoustic Startle




## Prepulse Inhibition of Acoustic Startle

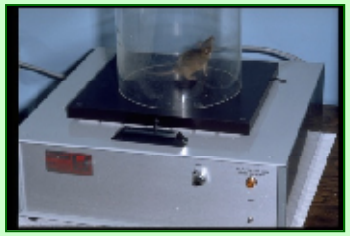


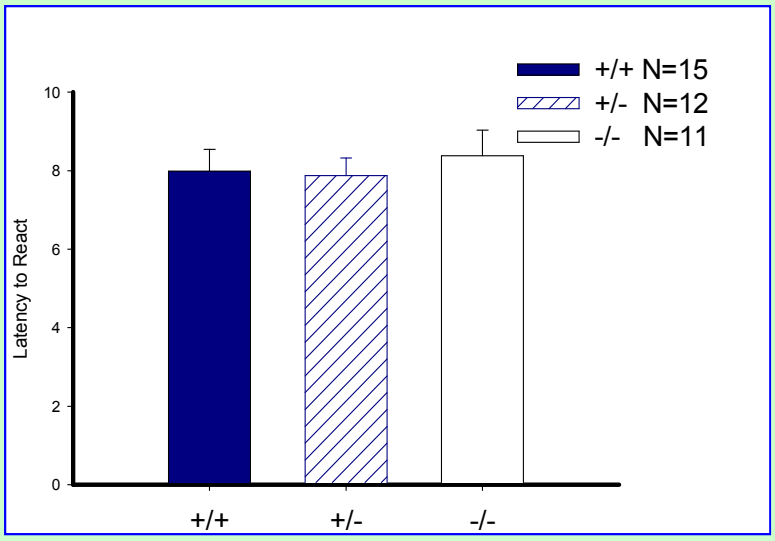

*Silverman, Turner, Barkan, Tolu, Hung, Sheng Crawley, SFN 2009*

# Pain Sensitivity

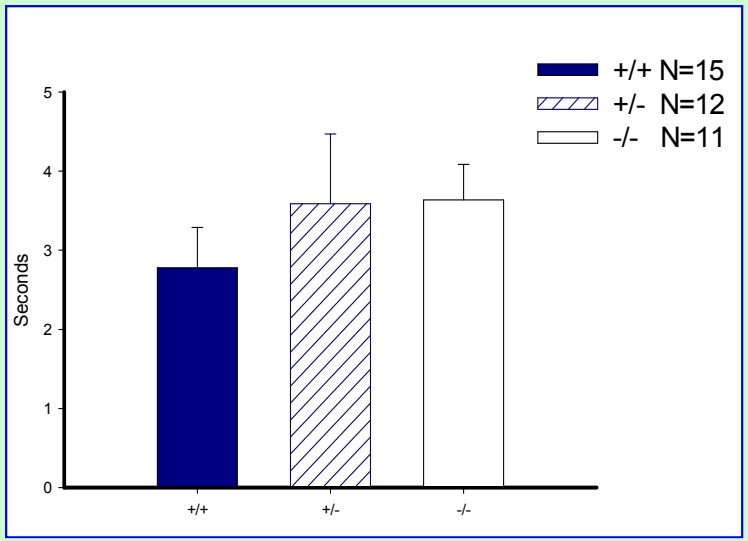
## *Shank1*

### Hot Plate






### Tail Flick




*Silverman, Turner, Barkan, Tolu, Hung, Sheng Crawley, SFN 2009*

Table 1. Physical characteristics, vocalizations, home cage behavior, sensory reflexes, olfactory ability, and a motor test. Data shown are means ± SEM for weight and latency measures, percent of cages for home cage measures, and percent of mice for other measures.

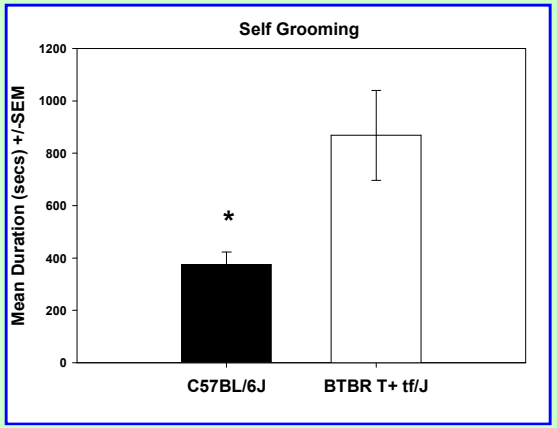
| | C57BL/6J | C57L/J | DBA/2J | FVB/NJ | C3H/HeJ | AKR/J | A/J | BALB/cByJ | BTBR | 129S1 |
|---|---|---|---|---|---|---|---|---|---|---|
| Physical Characteristics | | | | | | | | | | |
| Body weight (g) | 20.1 ± 0.5 | 15 ± 0.3 | 18.8 ± 0.4 | 19.6 ± 0.3 | 19.8±0.3 | 19.9 ± 0.5 | 18.4±0.5 | 20.9 ± 0.3 | 20±0.8 | 19.5 ± 0.3 |
| Poor coat condition | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 8% | 0% |
| Piloerection | 0% | 0% | 6% | 35% | 5% | 0% | 0% | 15% | 4% | 0% |
| Vocalization during handling or reflex test | 15% | 26% | 67% | 0% | 0% | 90% | 0% | 30% | 1% | 10% |
| Home cage (% cages) | | | | | | | | | | |
| Nest building | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Huddling | 100% | 100% | 100% | 80% | 100% | 100% | 100% | 100% | 100% | 80% |
| Aberrant responses | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Reflexes (% of mice normal) | | | | | | | | | | |
| Corneal | 85% | 100% | 78% | 100% | 100% | 100% | 100% | 90% | 80% | 100% |
| Visual placing | 100% | 100% | 100% | 100% | 100% | 80% | 80% | 100% | 100% | 100% |
| Vibrissae orienting | 100% | 100% | 83% | 100% | 100% | 100% | 100% | 95% | 100% | 100% |
| Preyer reflex | 90% | 90% | 100% | 100% | 100% | 100% | 90% | 80% | 92% | 100% |
| Olfaction test | | | | | | | | | | |
| Uncovered buried food (% mice) | 85%* | 100%* | 100%* | 100% | 60% | 80%* | 70% | 100%* | 83%* | 80%* |
| Latency to find (sec) | 282±67 | 167±32 | 158±34 | 198±46 | 639±72 | 409±70 | 423±81 | 84±15 | 379±89 | 337±72 |
| Motor coordination | | | | | | | | | | |
| Rotarod latency (sec) | 118±16 | 98±11 | 127±7 | 129±16 | 51±9 | 195±19 | 102±9 | 117±10 | 69±15 | 177±19 |
| Inverting on rotarod | | | | | | | | | | |

Moy et al., *Behavioural Brain Research, 2007*

# Animal Models of Autism
# Core Symptom #3:

Stereotypies, repetitive behaviors,
resistance to change in habit,
restricted interests

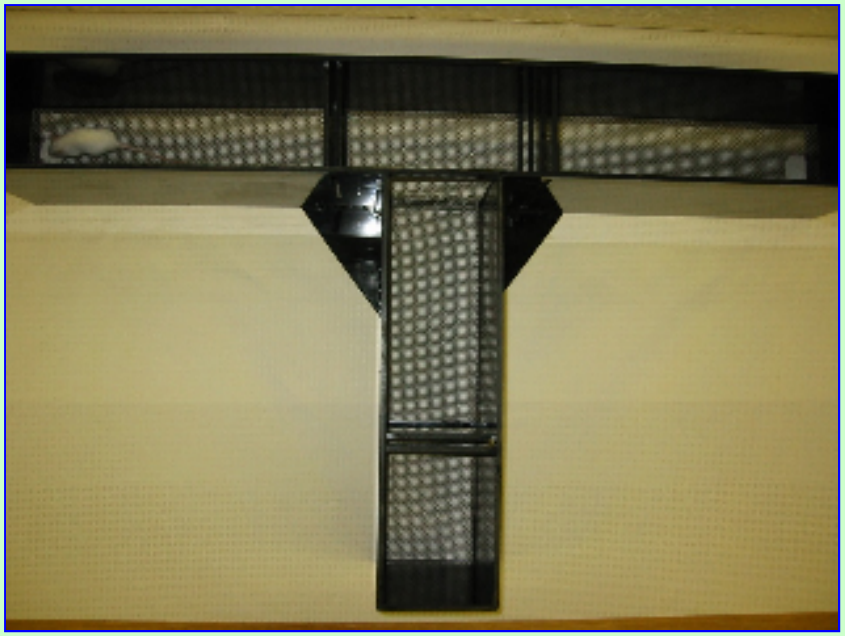
# REPETITIVE SELF-GROOMING IN BTBR T+tf/J





*Yang, McFarlane and Crawley, NIMH, 2007*

## Resistance to change in habit, perseveration:
## Reversal of a position habit

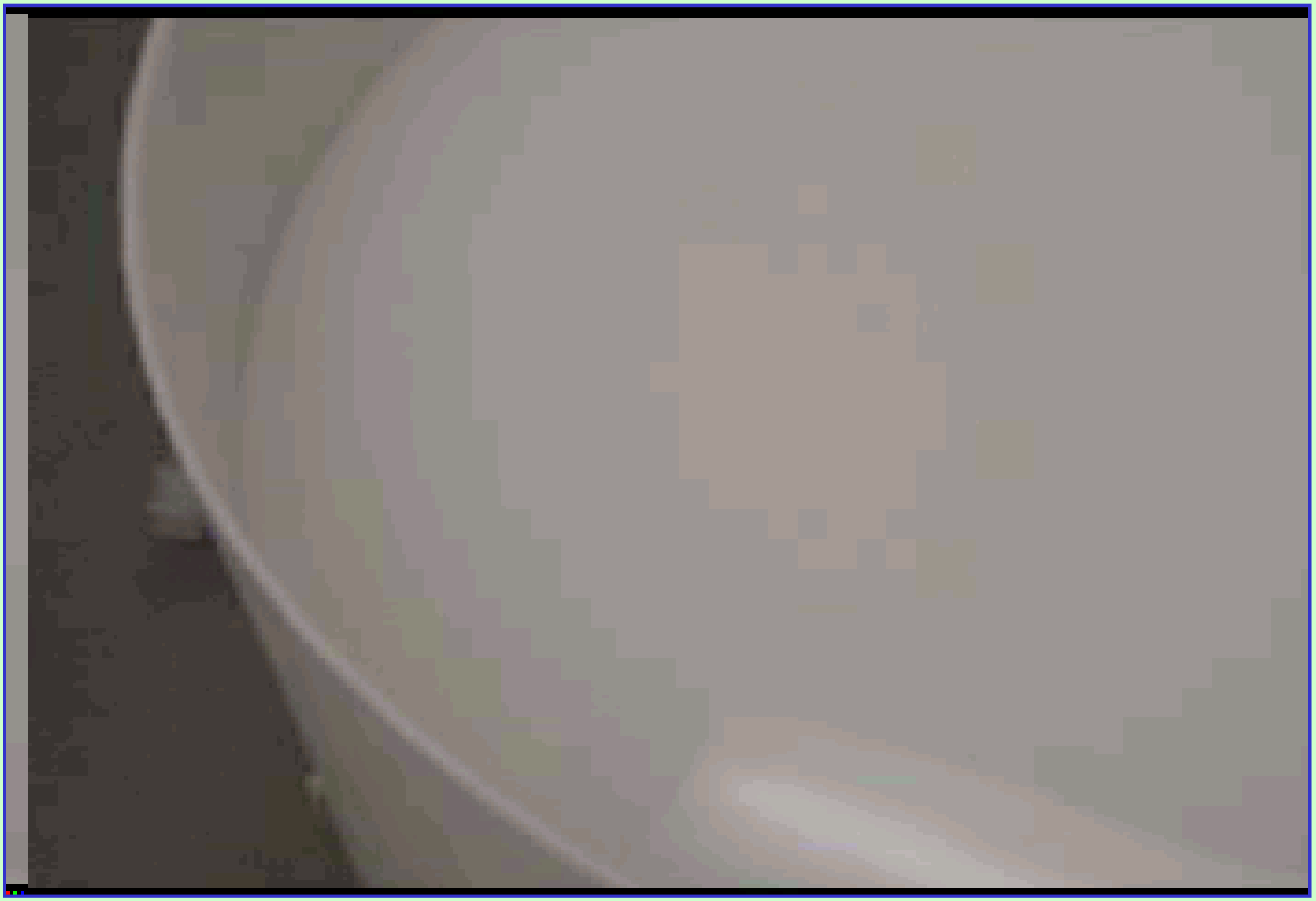


T-maze

Morris water maze

*Crawley, 2008*

# Morris Water Task Hidden Platform

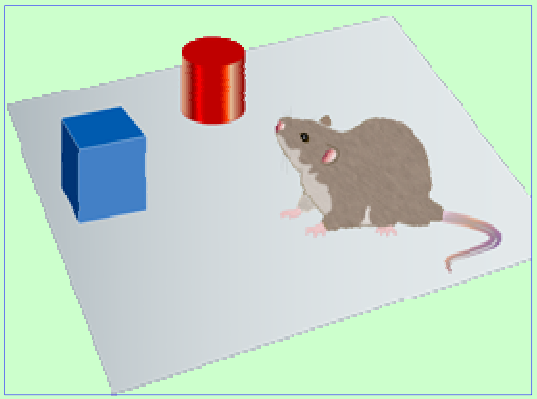


*Andrew Holmes, NIMH, 2001*

# Morris Water Maze Reversal

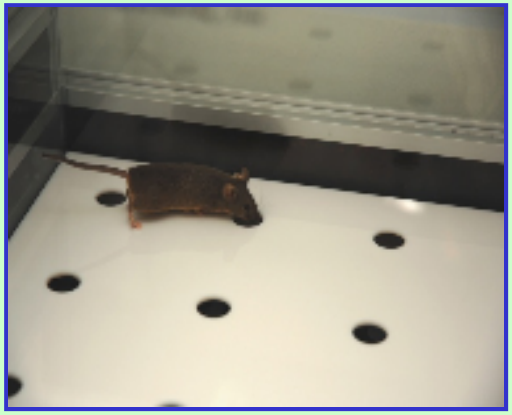
*Andrew Holmes, NIMH, 2001*

# Restricted Interests



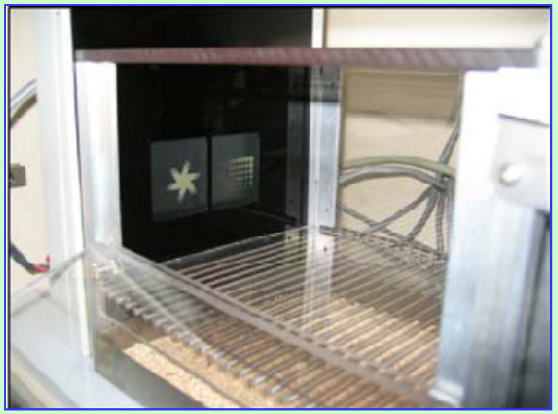
## Novel Object Exploration

*Photograph from Down Syndrome.org, Buckley and Sacks, 2007*



## Accuscan Holeboard

*Photograph by Jacqueline Crawley, LBN, NIMH, and Janet Stephens, NIH Macrophotography, 2007*



## Touch Screen

*Photograph from Andrew Holmes, NIAAA, 2006*

*Crawley, 2010*

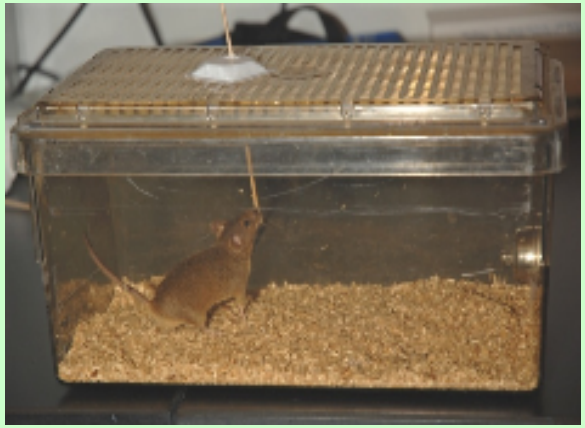
# Animal Models of Autism
# Core Symptom #2:

## Communication

Olfactory and auditory signals
and responses:

***Interactive and intentional***

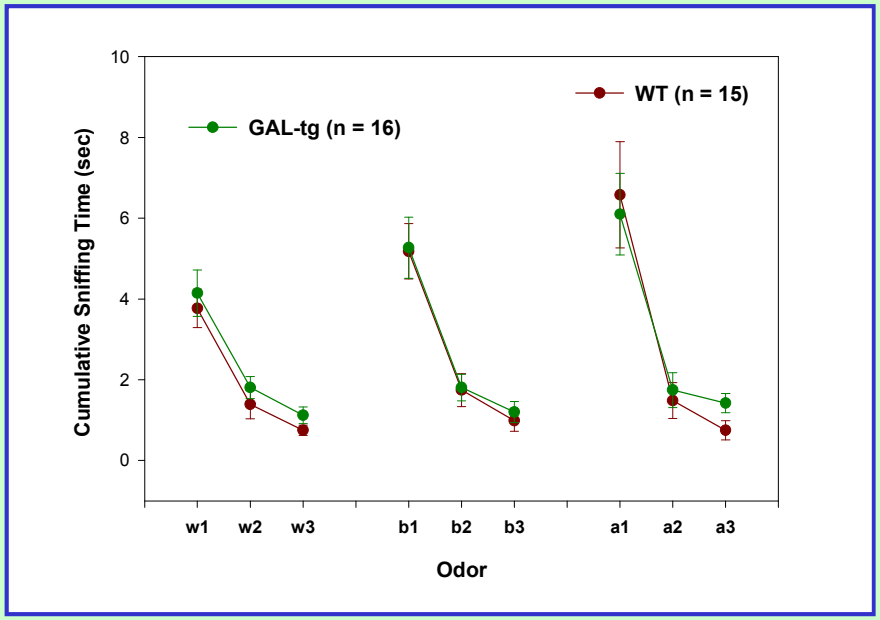
# Olfactory Habituation/Dishabituation

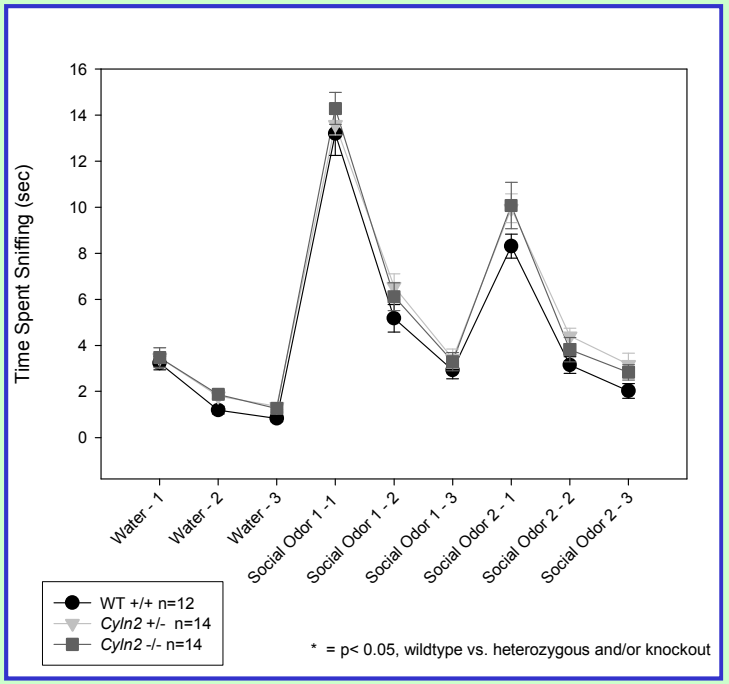


**Mu Yang and Adam Katz, LBN, NIMH, 2010**

***Crawley, 2007***

# Olfactory Habituation/Dishabituation

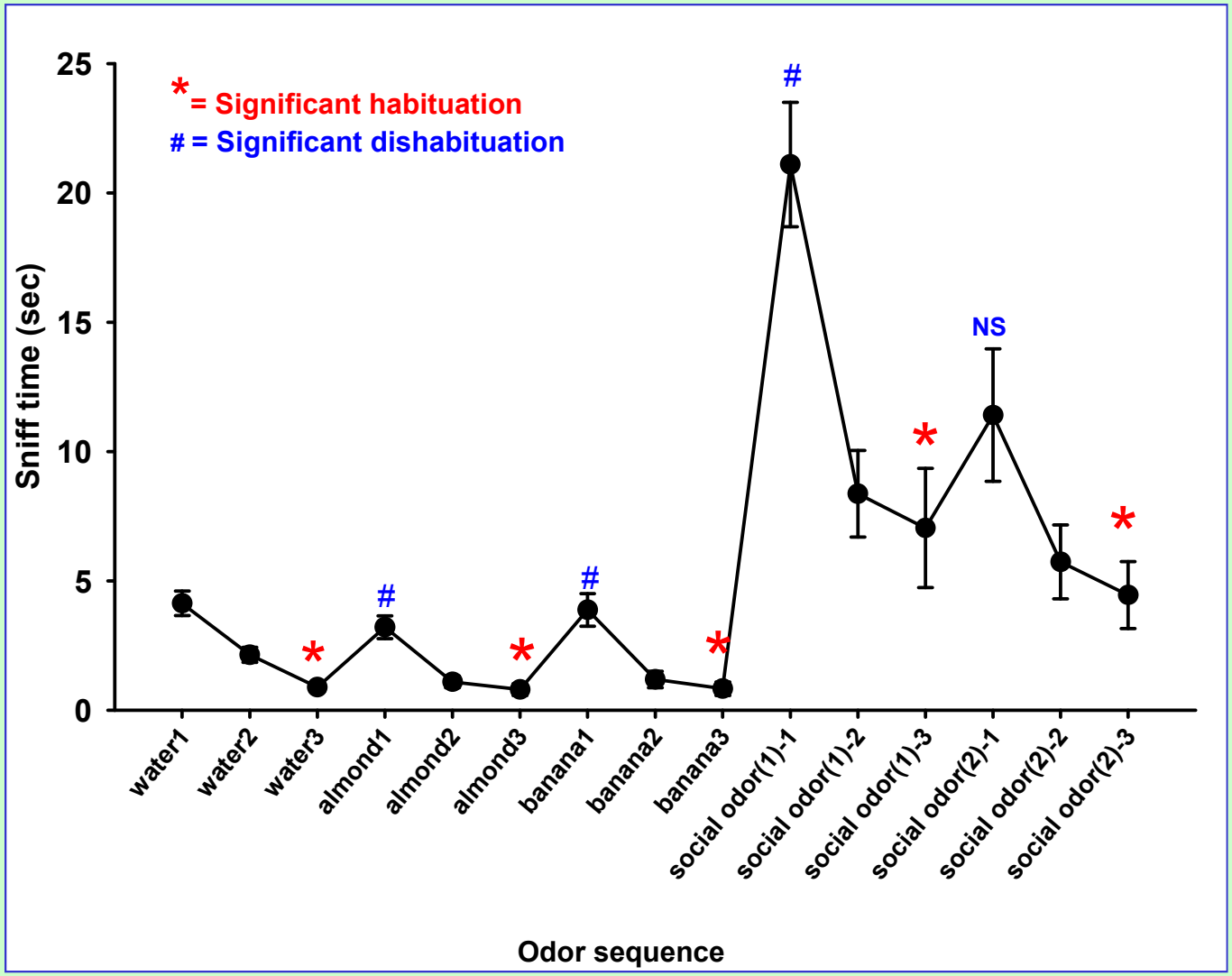



**Non-social odors**
**Galanin overexpressing transgenic mice**
**Wrenn et al., 2003**

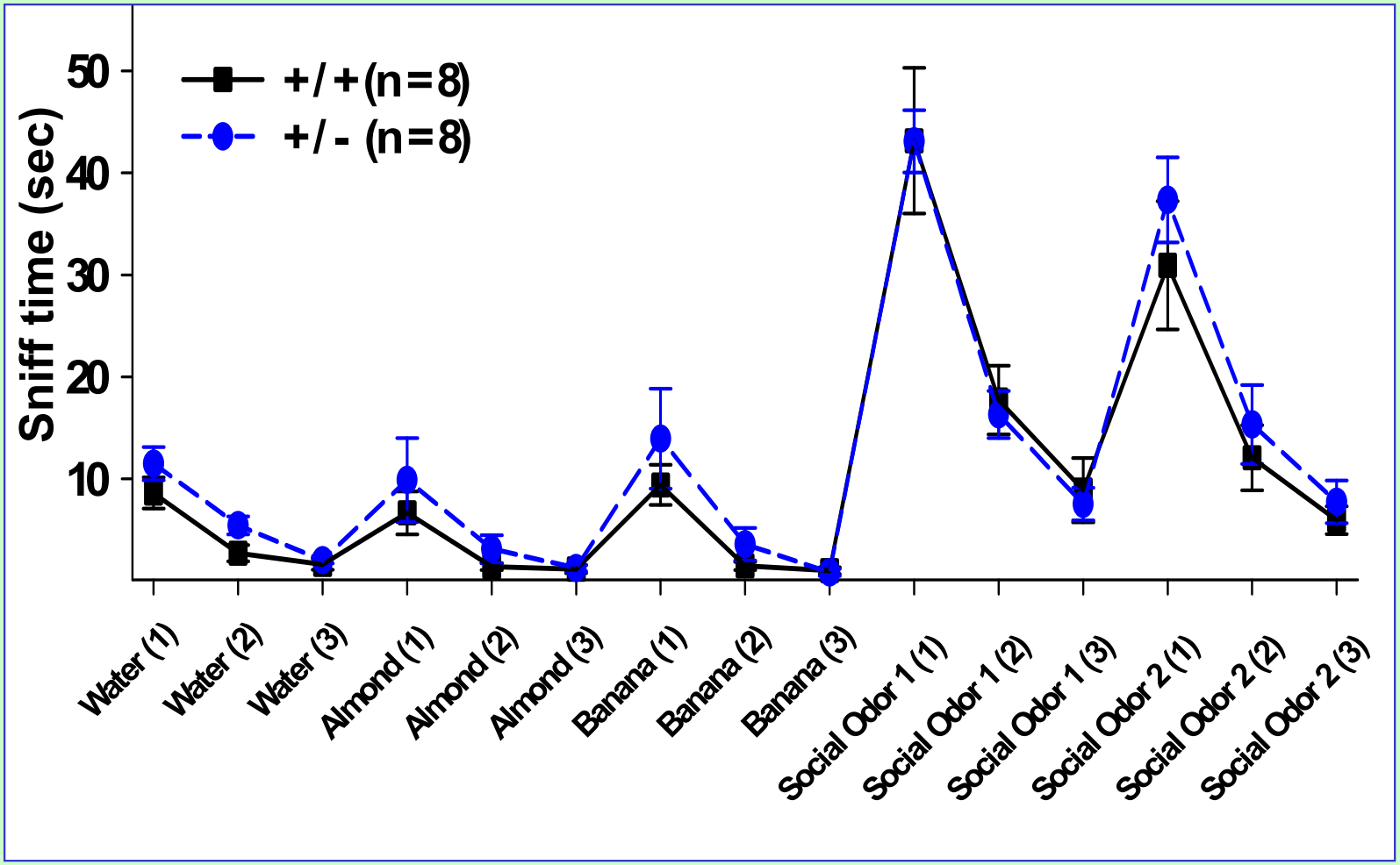
**Social odors**
***Cyln2* Williams model mice**
**Cuasay, Cohen, Hill, Crawley 2005**

# BTBR Olfactory Habituation/Dishabituation



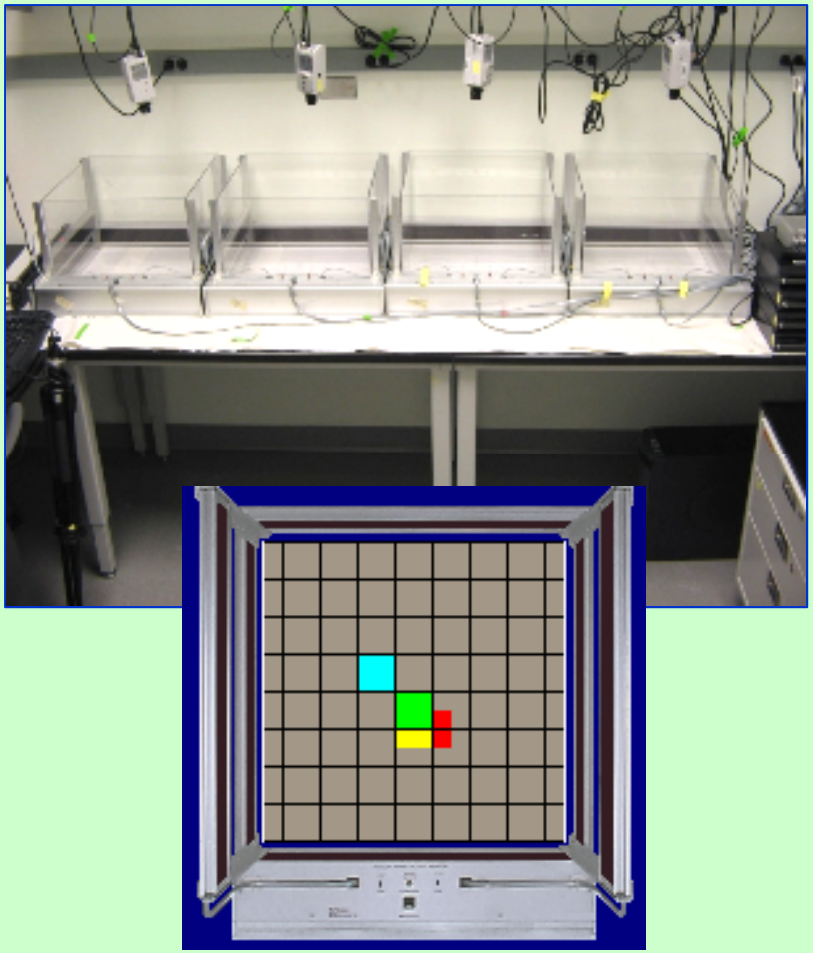

Yang, Clarke, Katz, Crawley NIMH 2010

# *Shank3* Olfactory Habituation/Dishabituation

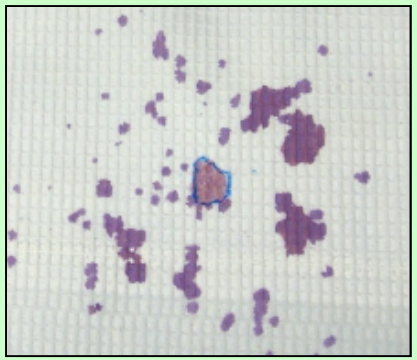



Saxena and Crawley, NIMH, Buxbaum Mt. Sinai, 2010

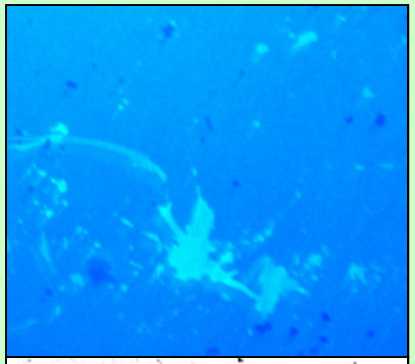
# Olfactory Scent Marking and Countermarking



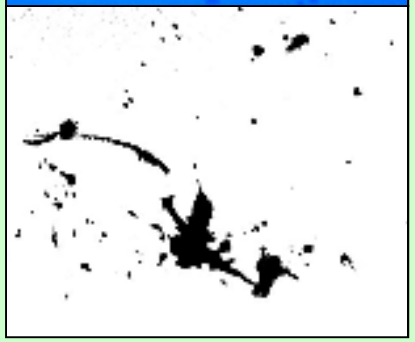
Florence Roullet, LBN, 2009



Ninhydrin spray + pen outline

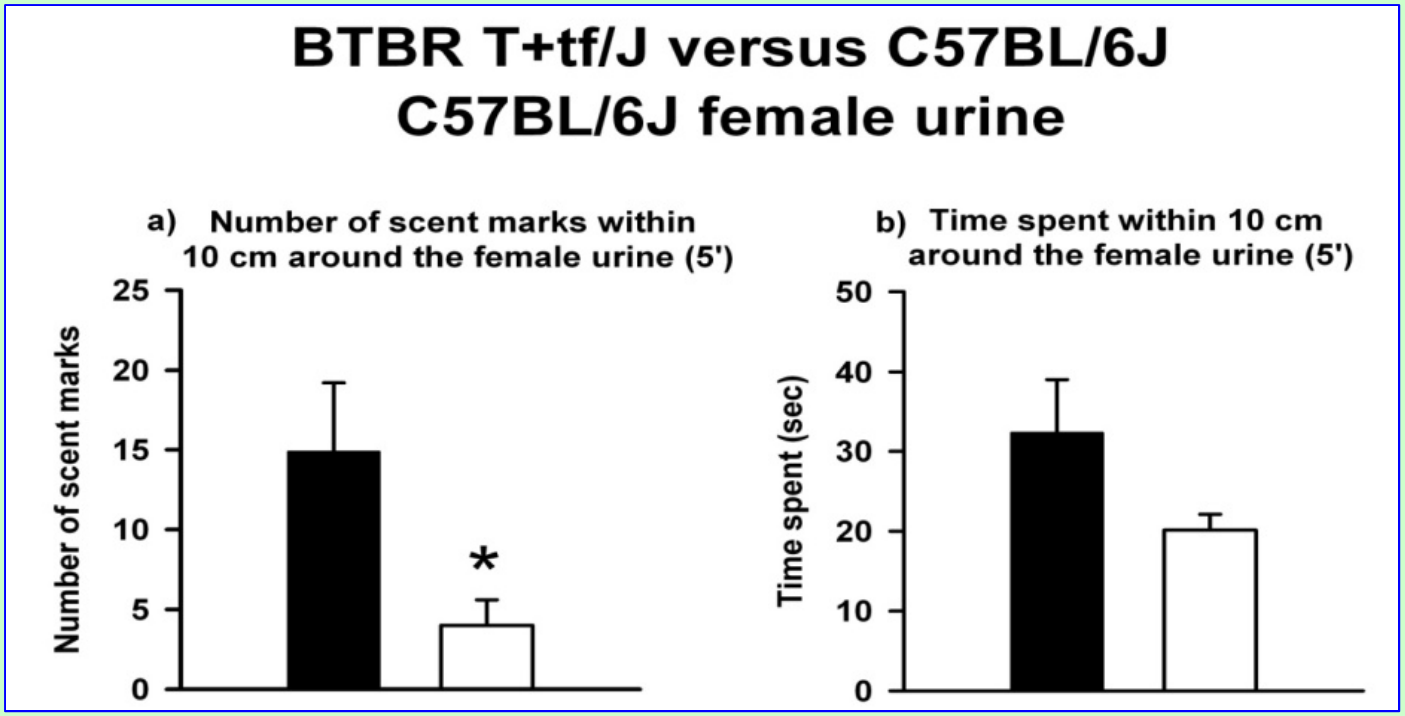


Photograph under ultraviolet lamp



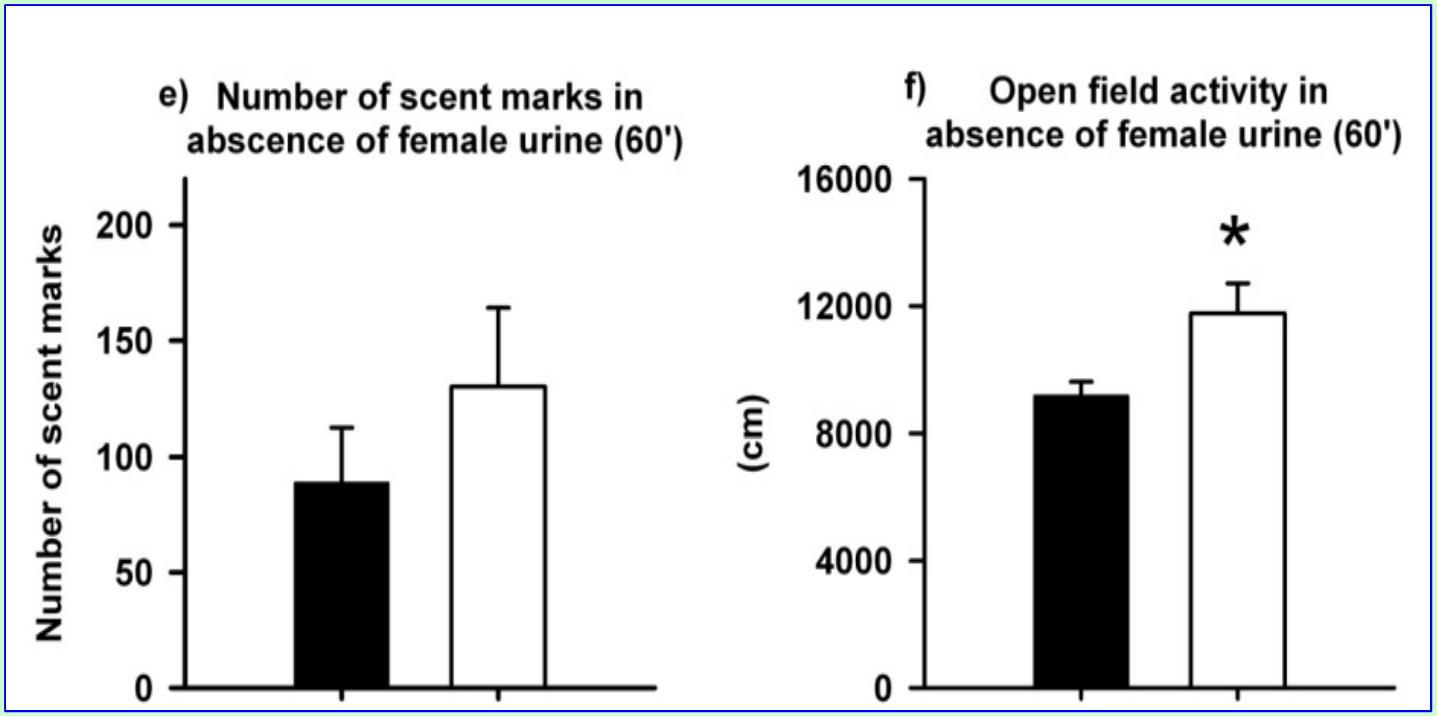
Picassa + NIH Image software

# Scent marking to female urine




Florence Roullet, NIMH, 2009

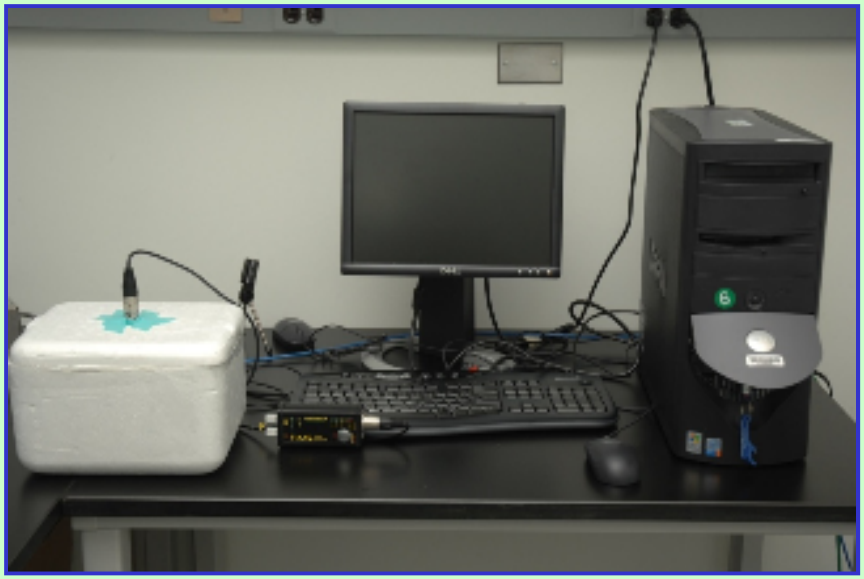
# Scent marking in a clean open field



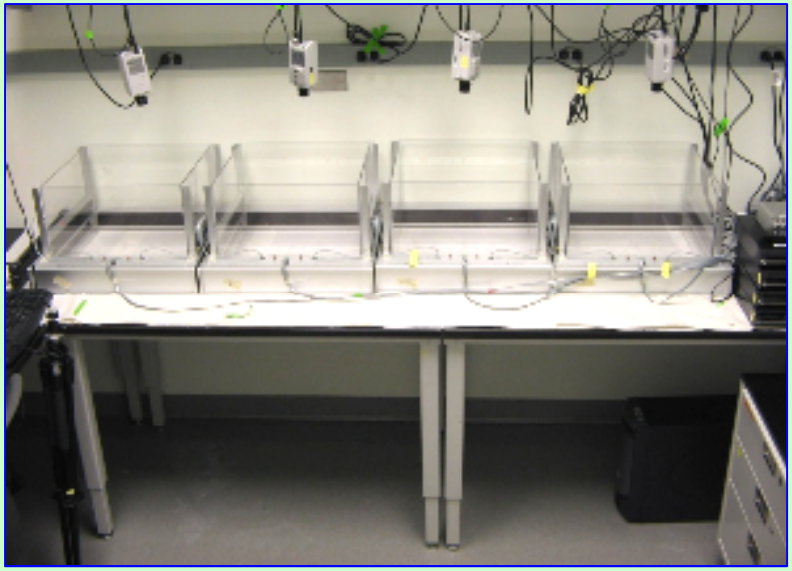

Florence Roullet, NIMH, 2009

# Ultrasonic Vocalizations

## Avisoft Recording and Analysis System



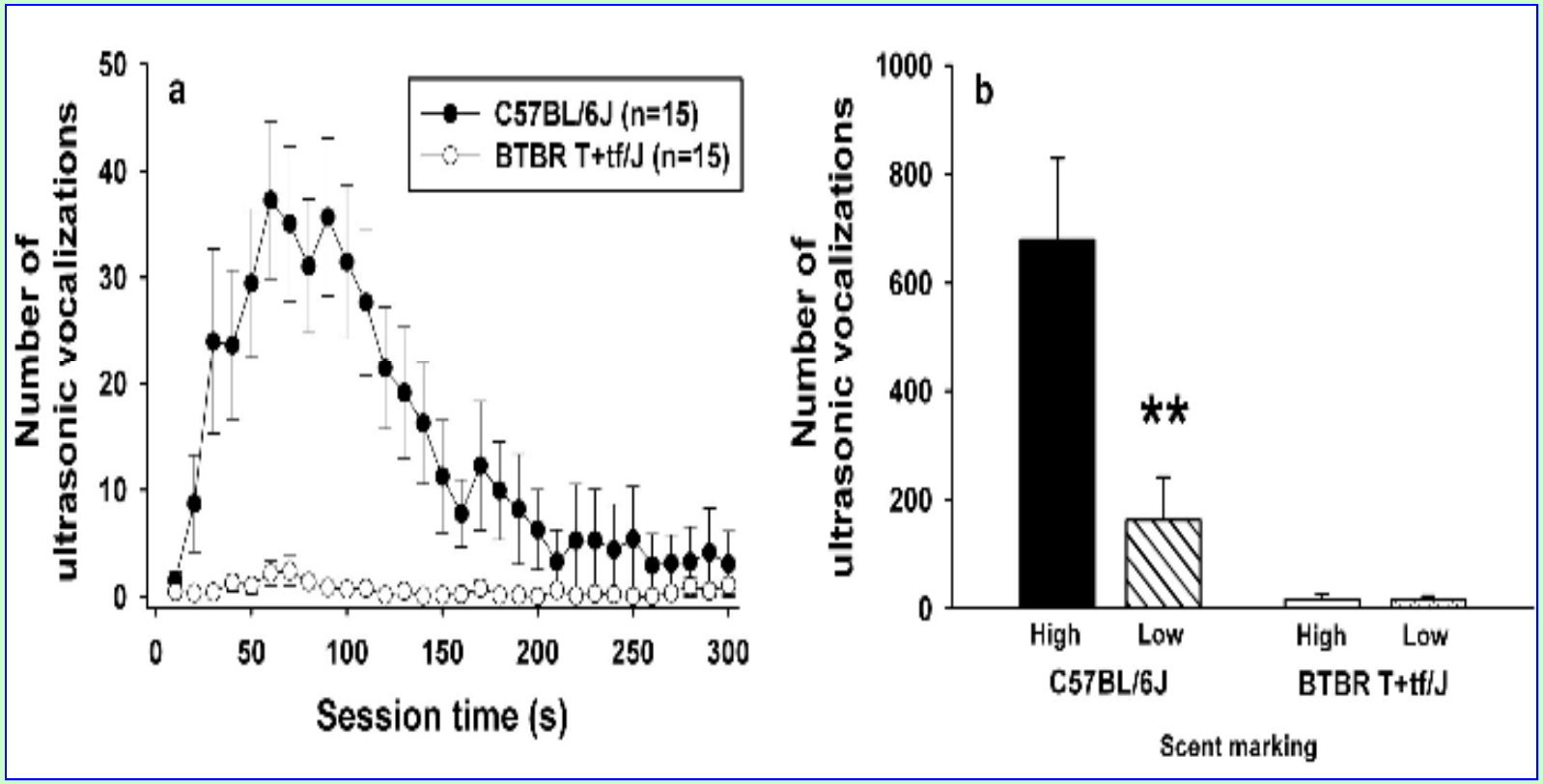
**Separated Pups**



**Adult Social Settings**

*Photographs by Maria Luisa Scattoni and Mu Yang, LBN, NIMH, and Janet Stephens, NIH Macrophotography, 2007*

# Ultrasonic vocalizations during scent marking to B6 female urine

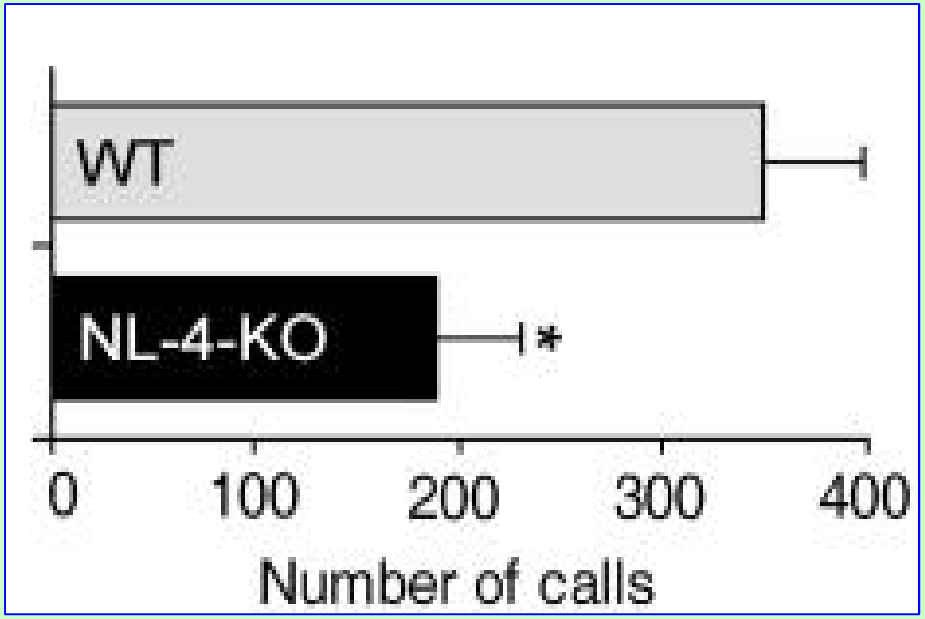



Markus Wöhr, Florence Roullet, and Crawley, *Genes Brain and Behavior*, in press

Jamain et al., 2008

## Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism

# Neuroligin-4 knockout mice



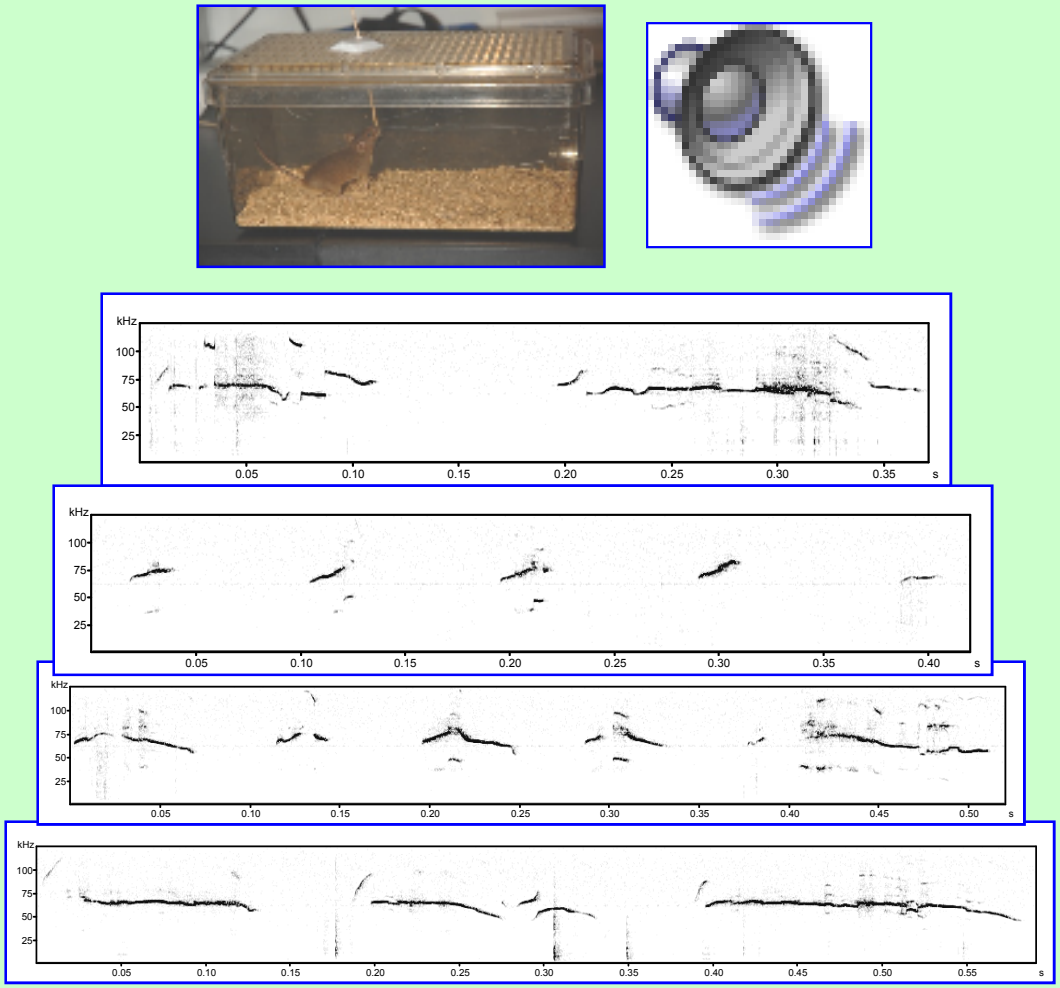

**Fewer ultrasonic vocalizations by NL-4 than WT male mouse interacting with estrus female mouse**

***Sylvie Granon and Thomas Bourgeron***

***Crawley 2010***

# One mouse sniffs the urine of another mouse

## *Ultrasonic vocalizations recorded with Avisoft system*



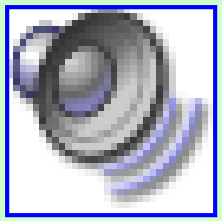

*Maria Luisa Scattoni and Jacqueline Crawley, LBN, NIMH, 2007*

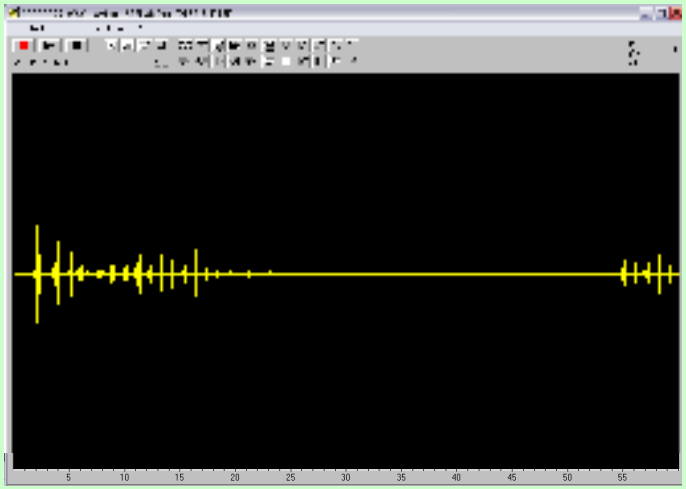
# Ultrasonic Distress Calls from Separated Mouse Pups

*Photograph by Maria Luisa Scattoni, LBN, NIMH,*
*and Janet Stephens, NIH Macrophotography, 2007*

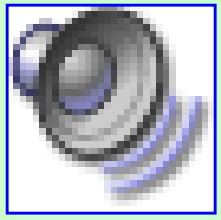
# C57BL6/J and BTBR T+tf/J
# separated pup ultrasonic vocalizations

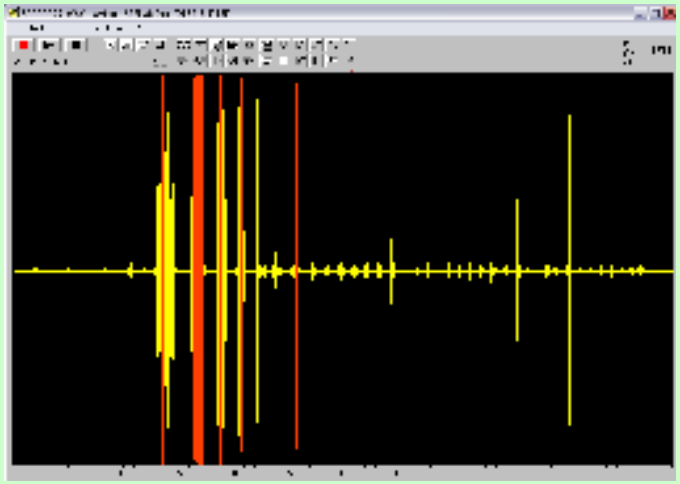
**B6**



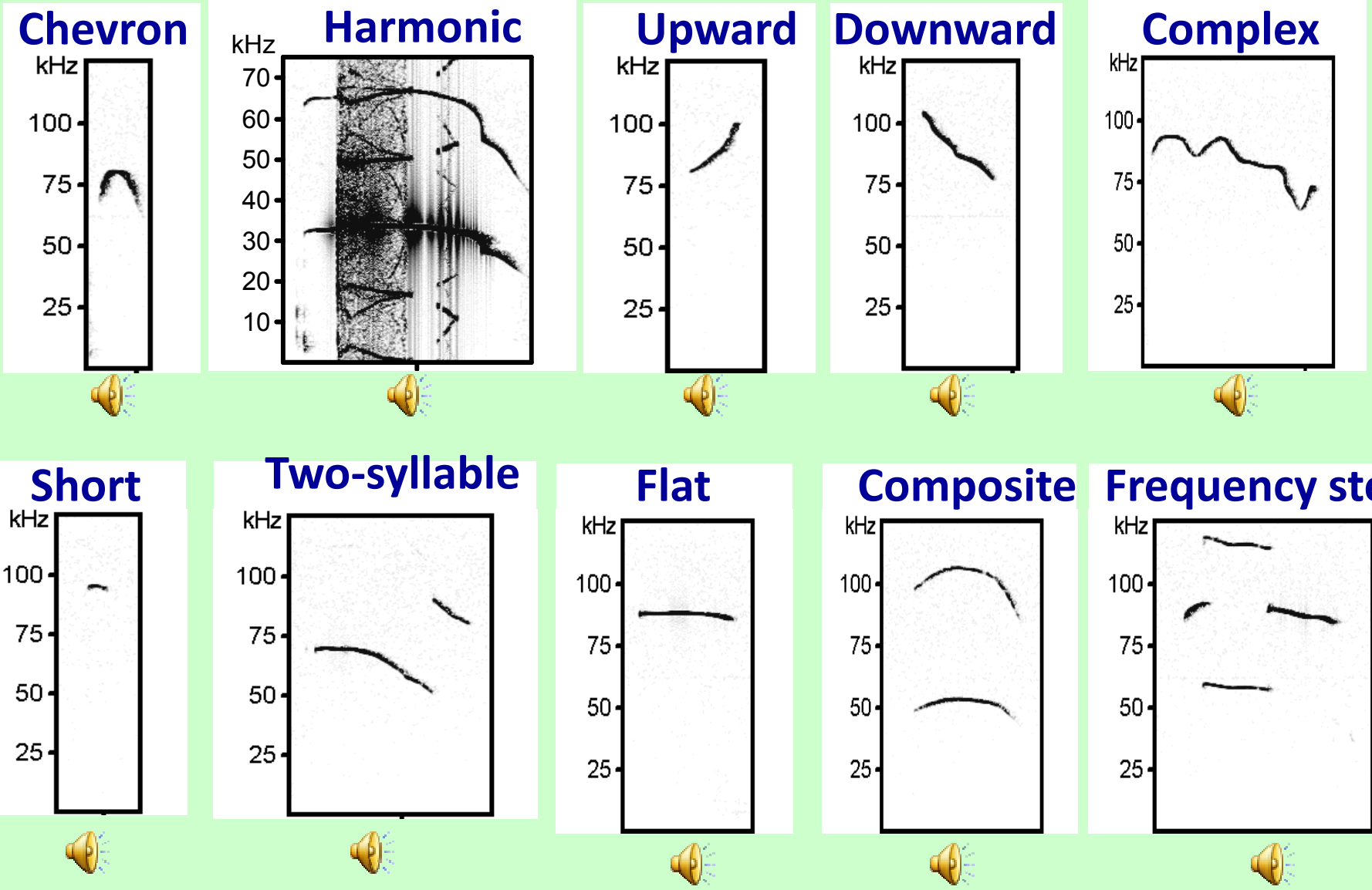


**BTBR**





*Scattoni,*
*NIMH 2007*

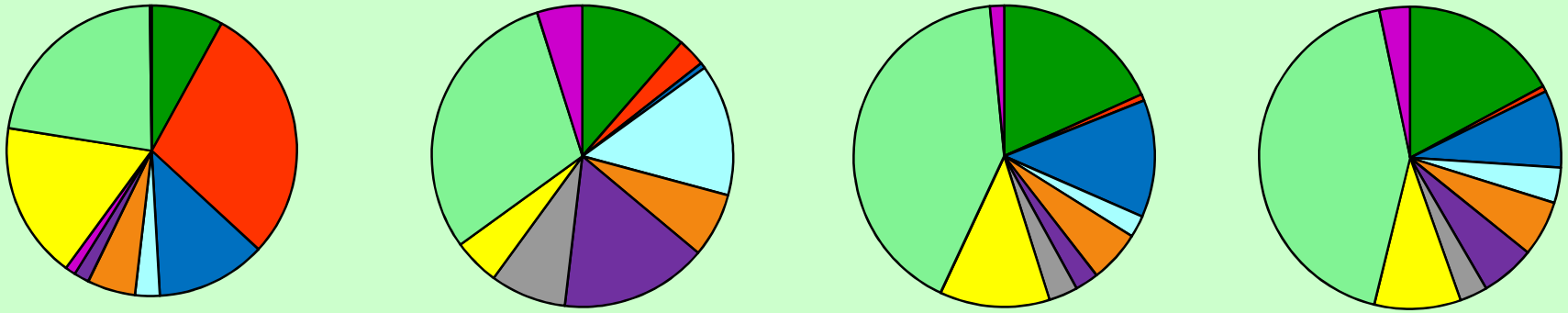



# 10 Categories of Pup Separation Vocalizations

*Scattoni, Gandhy, Ricceri, and Crawley, PLoS One, 2008*

# Pup Call Distributions: 4 Inbred Strains

BTBR C57BL/6J 129X1 FVB/NJ



Chevron
Harmonics
Short
Complex
Composite
Downward
Upward
Flat
Frequency steps
Two-syllables

*Scattoni et al., PLoS One, 2008*

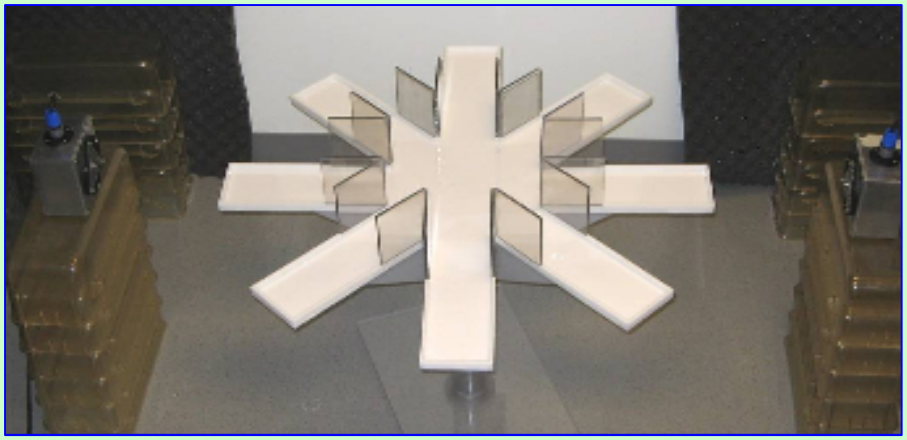
# Mouse Ultrasonic Vocalizations

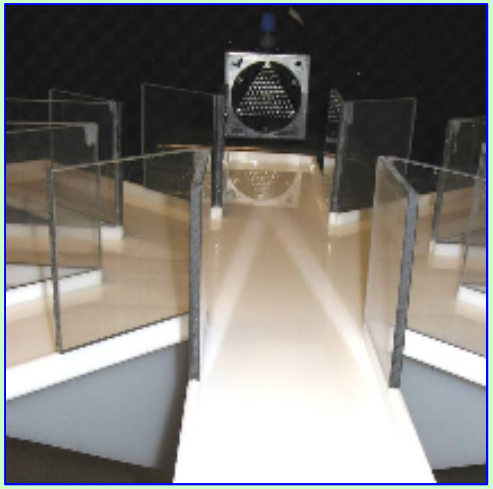
Is it communication?

**Play back recordings, score responses**

***Crawley, 2009***

# Playback of calls through an ultrasonic speaker at the end of one arm of a radial maze





**Markus Wöhr, 2009**

***Photo by Mu Yang***

# Mouse Ultrasonic Vocalizations

Are calls quantitative and replicable enough to use as an assay?

**Record calls in various social situations**

***Crawley, 2009***

## Multiple social settings?

# Male-Male Resident Intruder

**Maria Luisa Scattoni, Mu Yang and Adam Katz, LBN, NIMH, 2010**

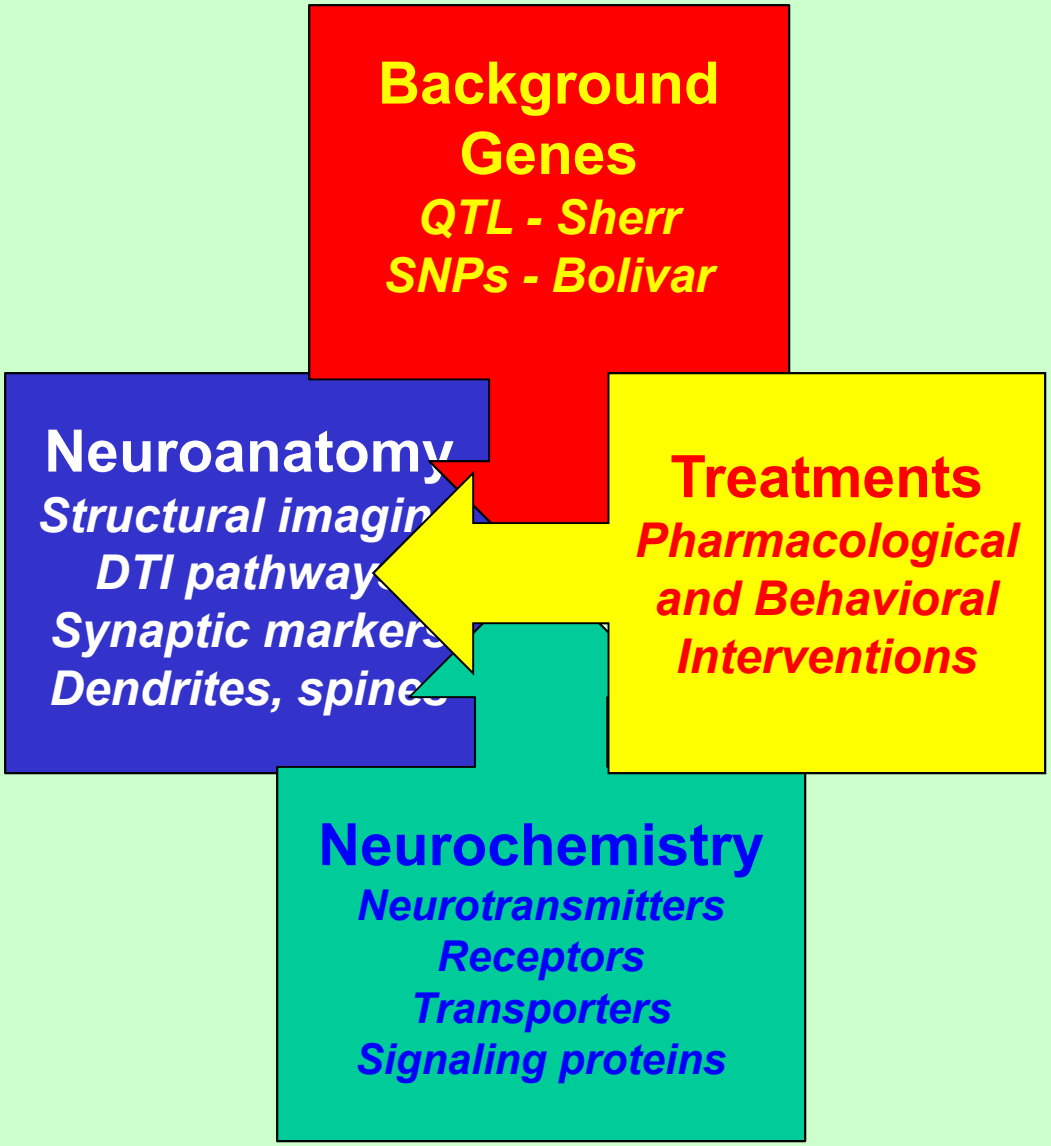
***Crawley 2010***

## Autism-Relevant Behavioral Phenotypes of the BTBR T+tf/J Inbred Strain

- Juveniles display low reciprocal social interaction
- Adults fail to display sociability in the 3-chambered automated social approach task and display low reciprocal social interactions
- Pups and adults emit unusual patterns and numbers of ultrasonic vocalizations, low olfactory scent marking
- Adults and juveniles display high repetitive self-grooming
- No artifactual confounds, normal on measures of general health, sensory abilities, motor functions, anxiety-related behaviors

*Crawley, 2010*

# Investigating Biological Mechanisms



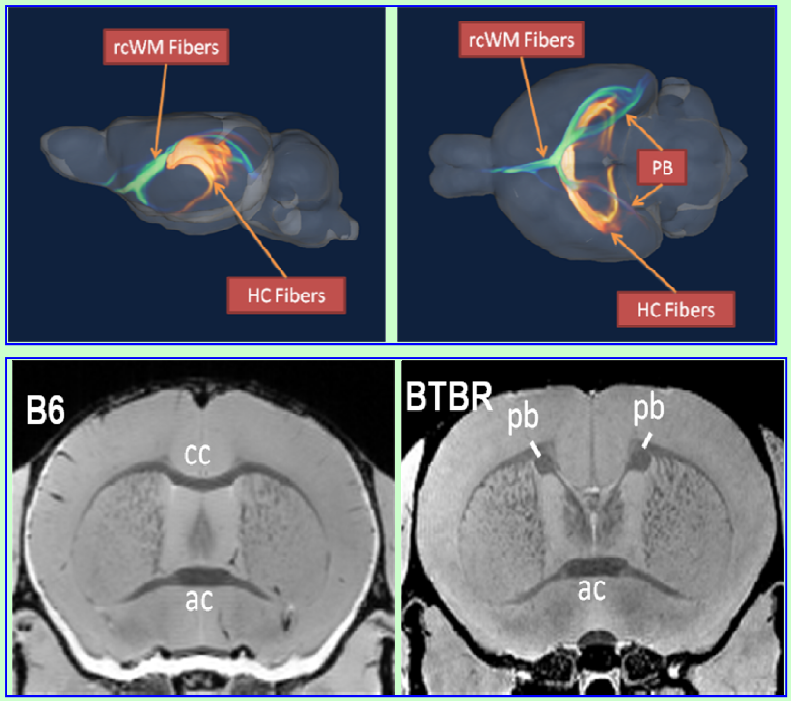

*Crawley, 2010*

*Douglas Wahlsten, Pamela Metten, John Crabbe*

# Survey of 21 inbred mouse strains in two laboratories reveals that BTBR T/1 tf/tf has severely reduced hippocampal commissure and absent corpus callosum

*Brain Research 971 (2003) 47–54*

# Morphometric imaging of BTBR versus B6 to investigate abnormalities in neuroanatomical structures and white matter connectivity

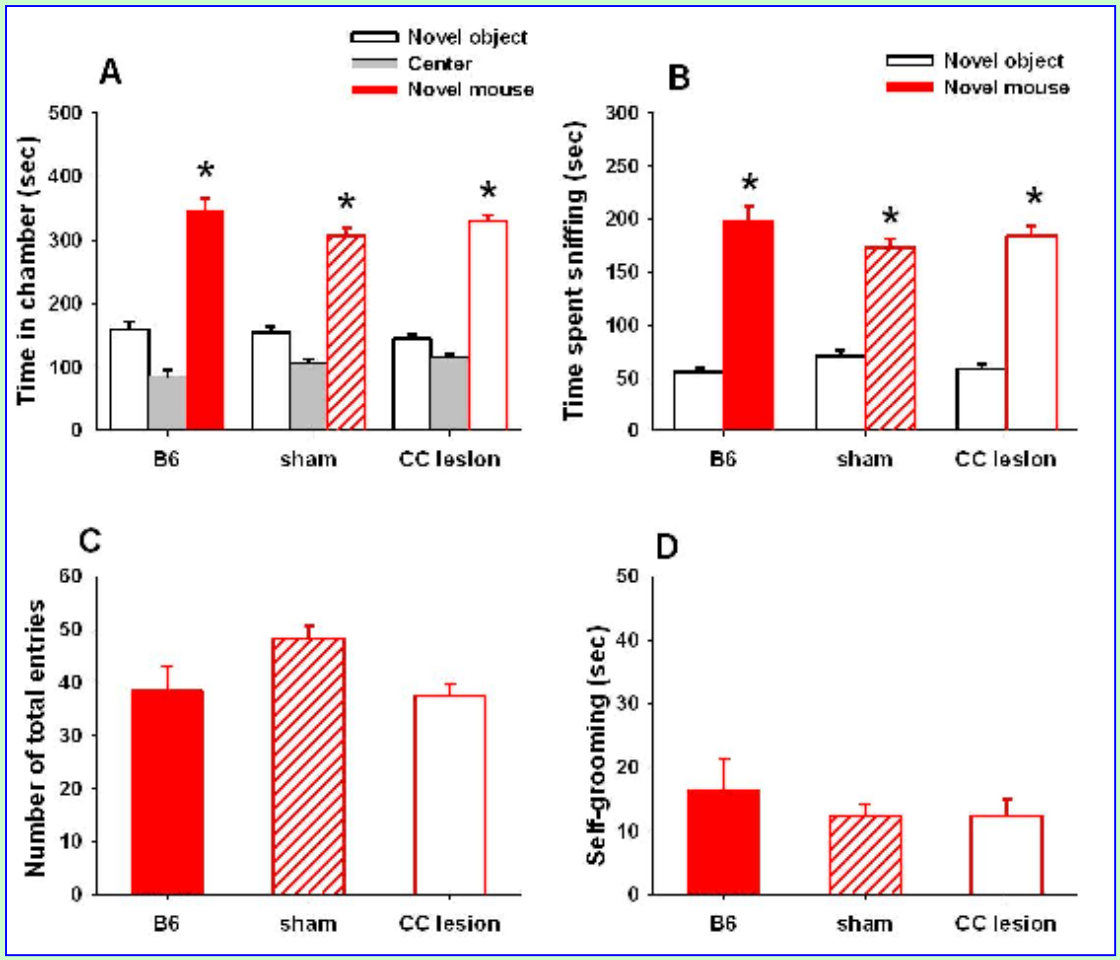



**Visualization of corpus callosum and hippocampal commissure in B6 and BTBR**

**J. Michael Tyszka and Ralph Adolphs, Caltech, 2009**

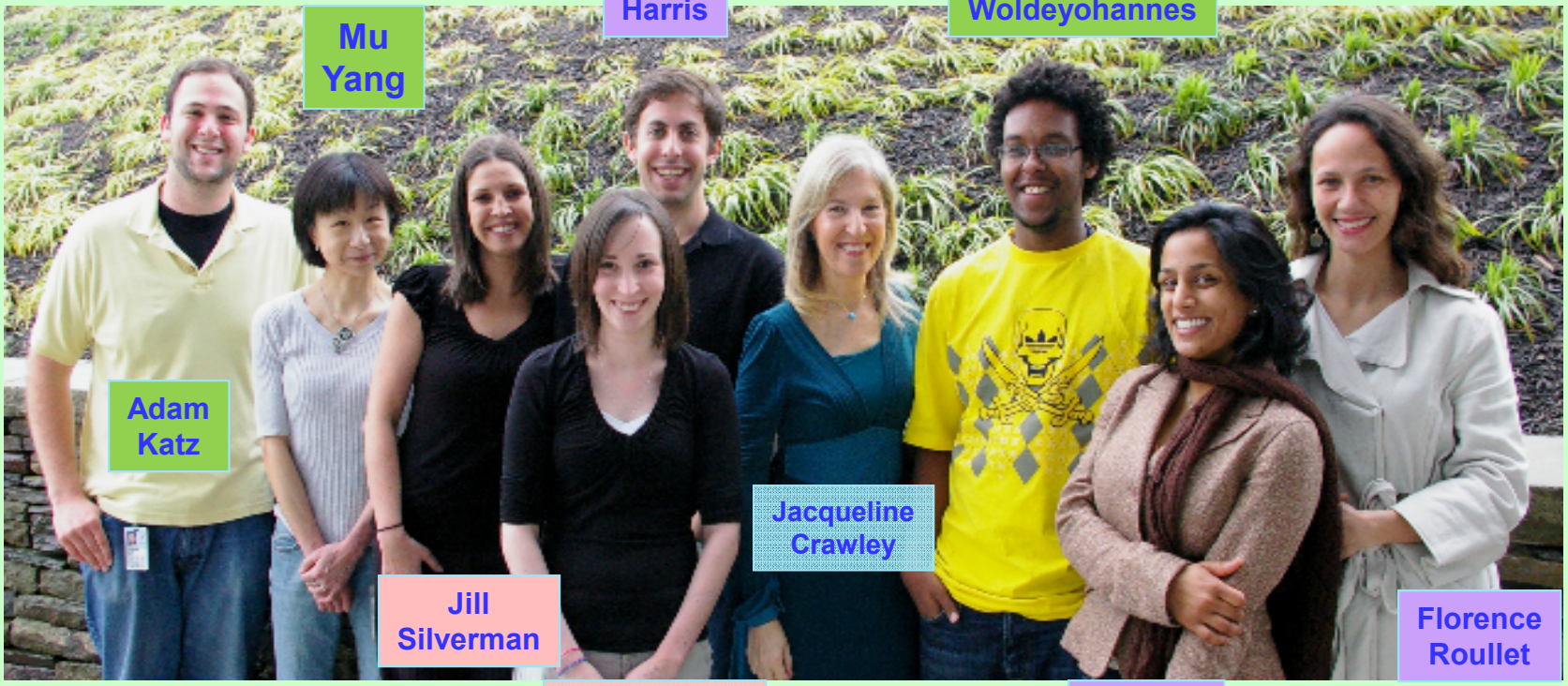
# Corpus callosum lesions at postnatal day 7 do not impair adult social behaviors in C57BL/6J mice:

## Not corpus callosum per se, but other white matter connectivity abnormalities in BTBR?




Yang, et al., *Eur J Neurosci* 2009

# Behavioral Interventions and Drug Treatments




LBN, NIMH, April 2010

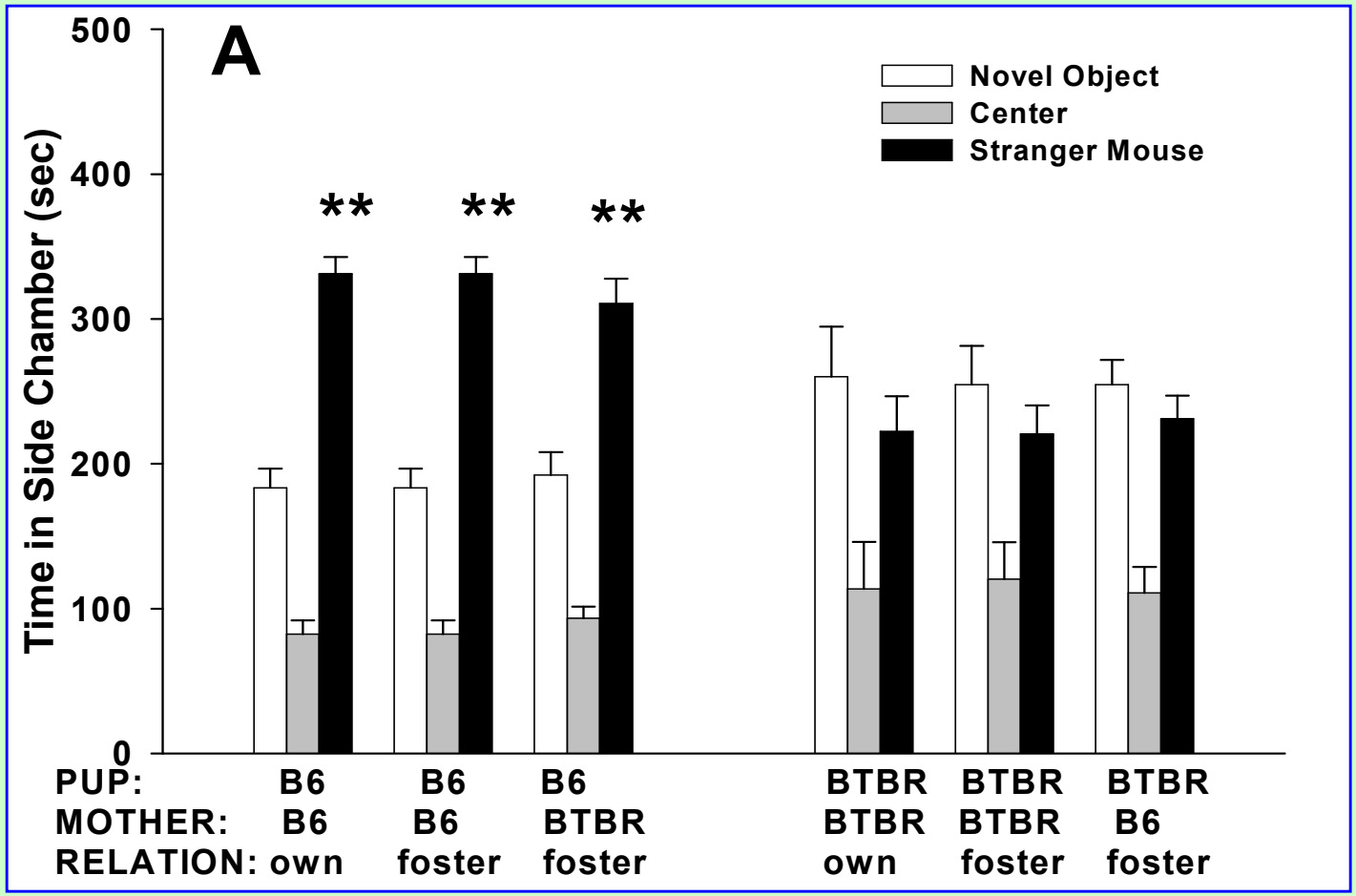
Translational Applications of
Autism-Relevant Phenotypes
for Treatment Discovery

# Behavioral Interventions:

***Currently the most effective treatment for symptoms of autism***

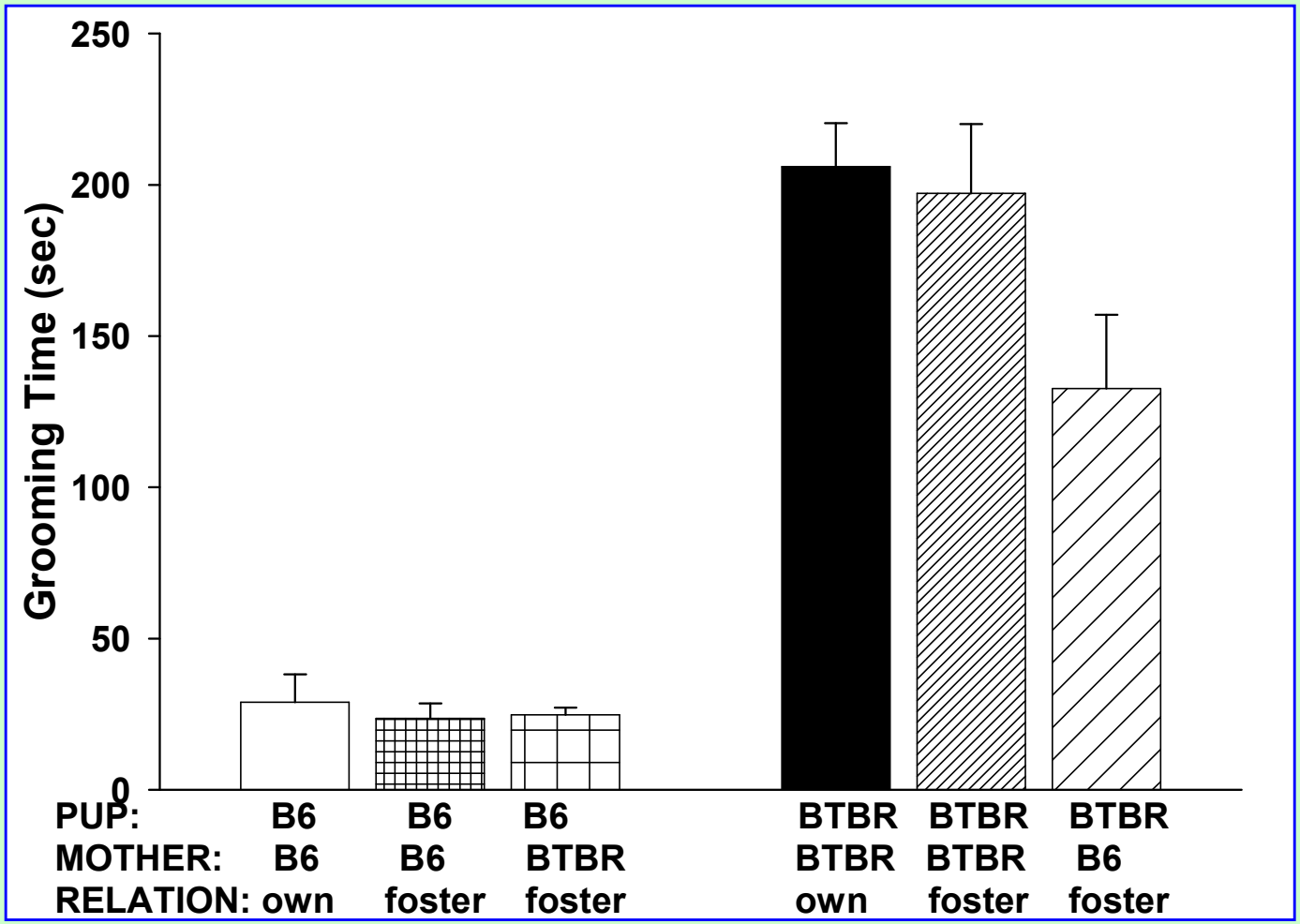
***Crawley, 2010***

# Social deficits in BTBR T+tf/J were unchanged by cross-fostering with B6 mothers



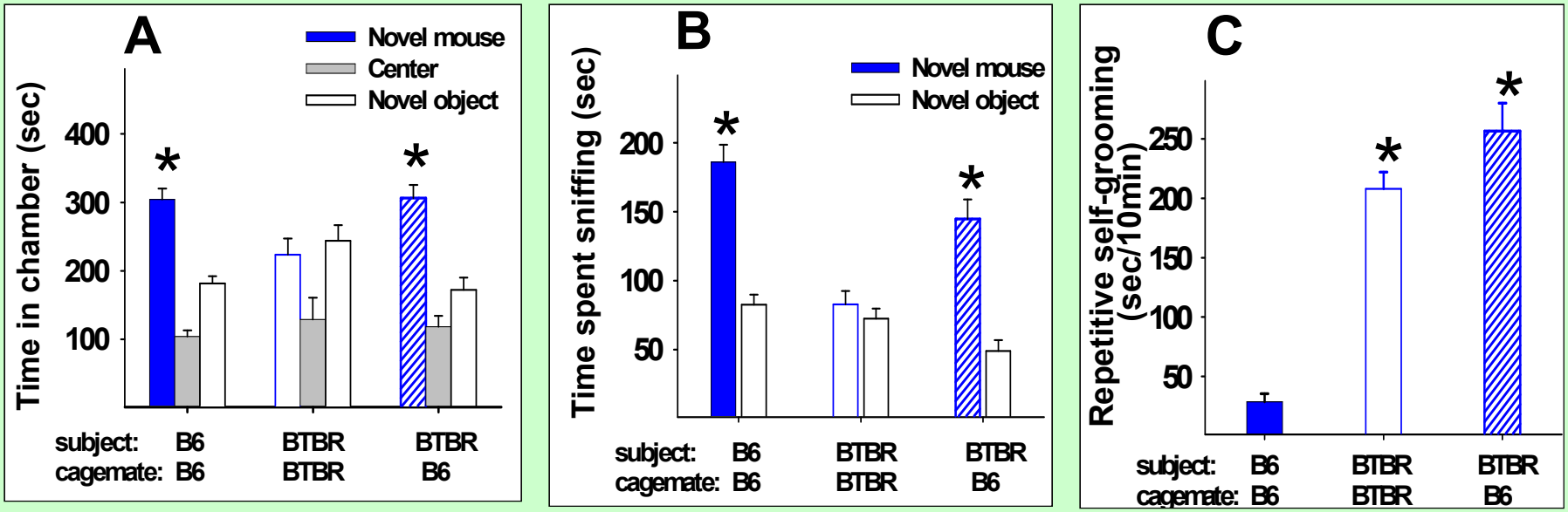

Yang et al., *Int J Dev Neurosci*, 2007

# Excessive grooming in BTBR T+tf/J was unchanged by cross-fostering with B6 mothers




Yang et al., *Int J Dev Neurosci*, 2007

# Housing BTBR with B6 as Juveniles Rescued Adult Sociability




*Yang, Perry, Weber and Crawley, 2009*

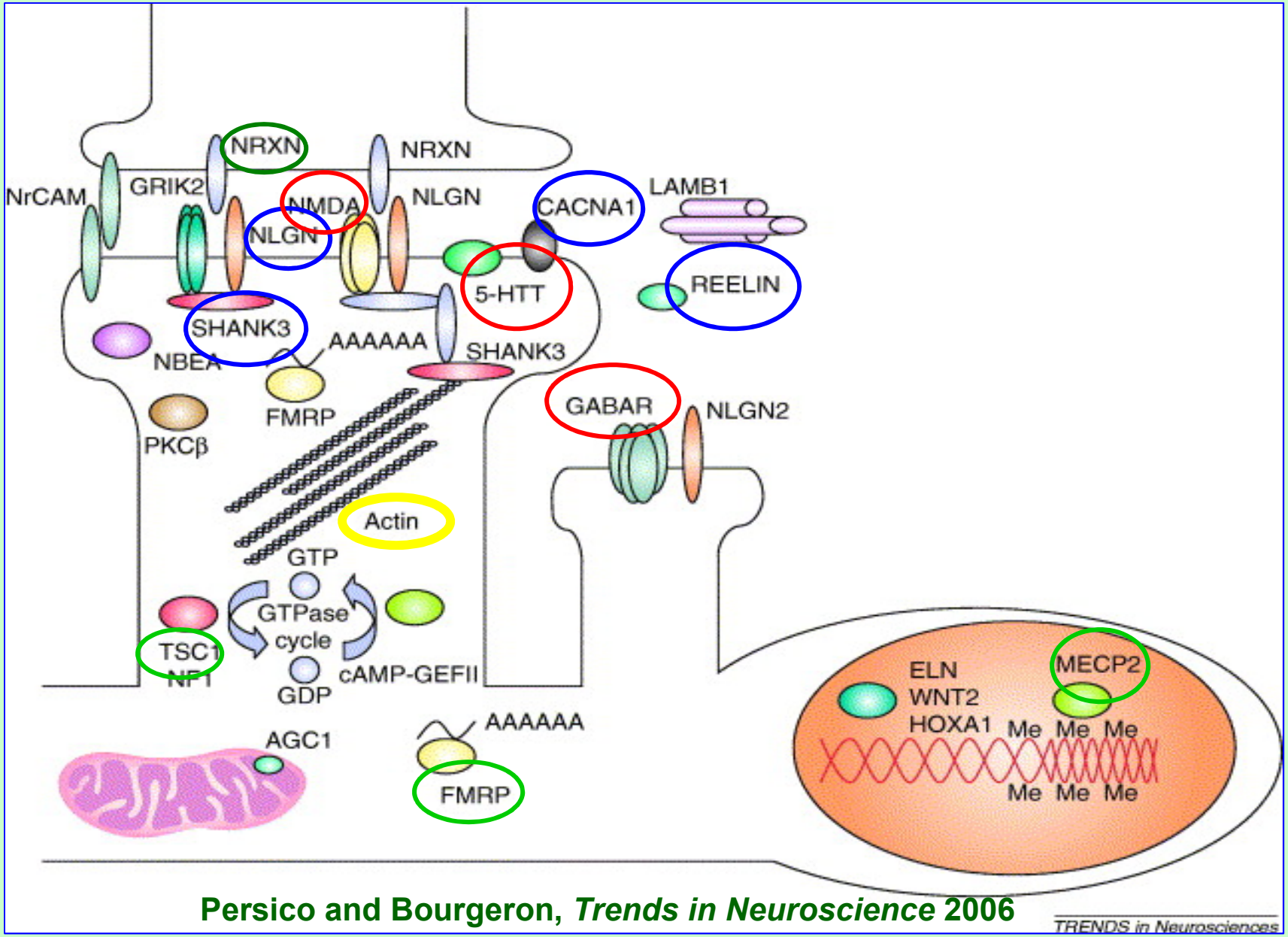
Translational Applications of
Autism-Relevant Phenotypes
for Treatment Discovery

# Pharmacological Interventions:

## *Not too late to change synapses?*

***Crawley, 2010***

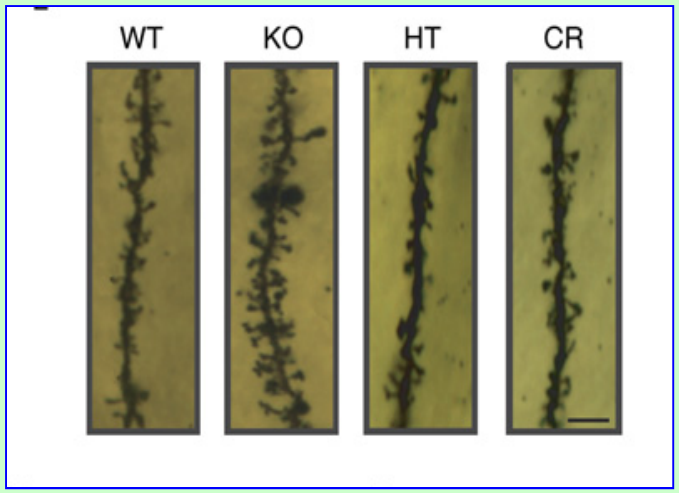
# Synaptic Gene Mutations in Autism: Dynamic Mechanisms




**Persico and Bourgeron, *Trends in Neuroscience* 2006**

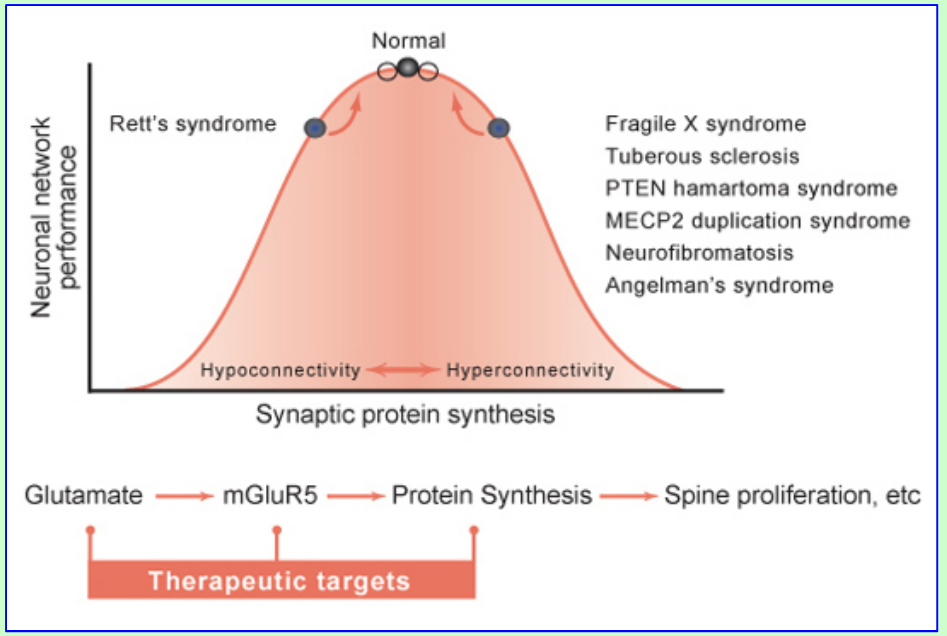
***Crawley, 2010***

## Dölen et al., *Neuron* 2007
## Correction of Fragile X syndrome in mice




**High density of dendritic spines in visual cortex of *Frmr1* knockout mice (KO), Normal spine densities in WT and *mGluR5* knockout mice (HT), Rescue by breeding KO x HT (double knockout cross, CR)**

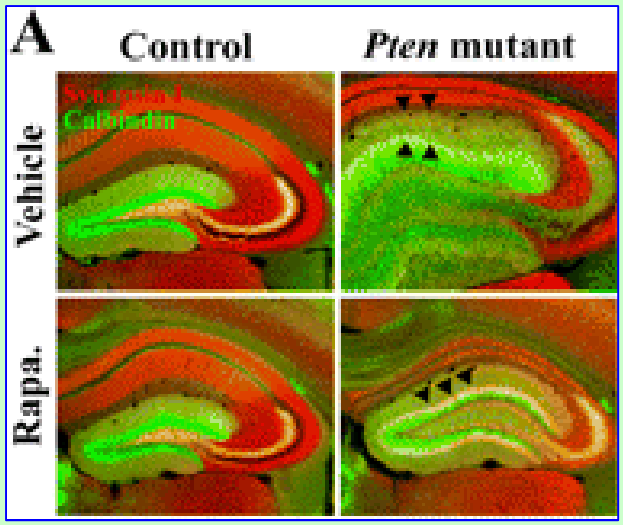
## mGluR5 Treatment Strategy

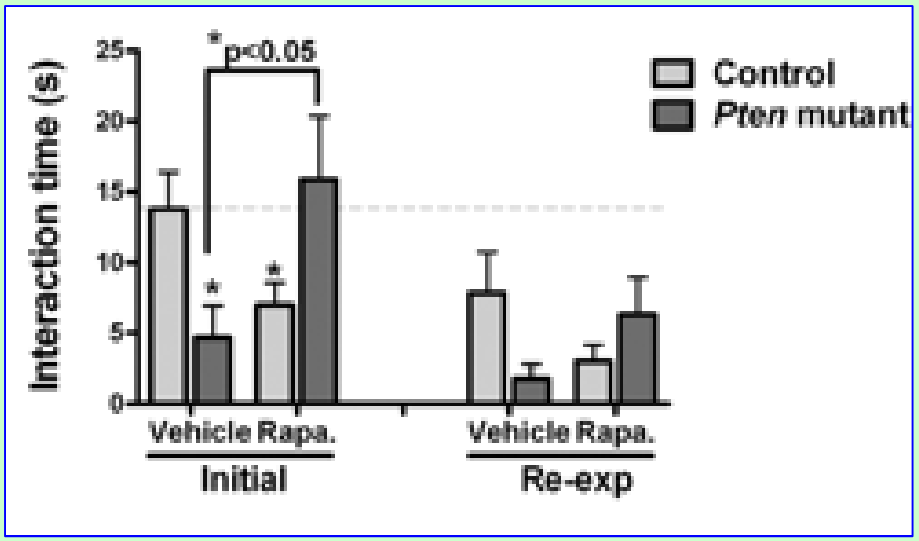



**From Kelleher and Bear, *Cell* 2008**
**www.seasidetherapeutics.com**

***Crawley 2010***

## Zhou et al., *J Neurosci* 2009
# Pharmacological inhibition of mTORC1 suppresses anatomical, cellular, and behavioral abnormalities in neural-specific *Pten* knock-out mice




**Rapamycin treatment reduces the larger head size and enlarged hippocampal dentate gyrus in *Pten* mutant mice**

**Rapamycin treatment increases reciprocal social interactions in *Pten* mutant mice**

***Luis Parada, University of Texas Southwestern***

**PTEN (phosphatase and tensin homolog deleted on chromosome ten) is a major negative regulator of the phosphatidylinositol-3 kinase (PI3K)/AKT/Tsc/mTOR pathway. Rapamycin is an mTOR1 inhibitor**

***Crawley 2010***

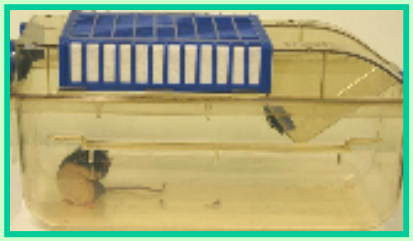
# Translational Applications of Autism-Relevant Phenotypes for Drug Treatment Discovery: Experimental Design

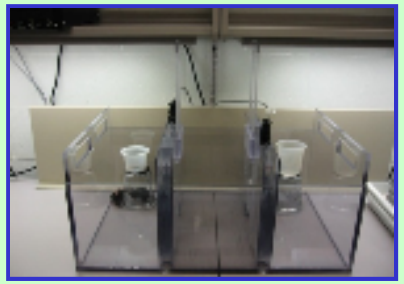
- Inject 3 drug doses or vehicle i.p.,10 ml/kg volume
- N=10 per dose per strain per behavioral task
- Compare BTBR versus B6

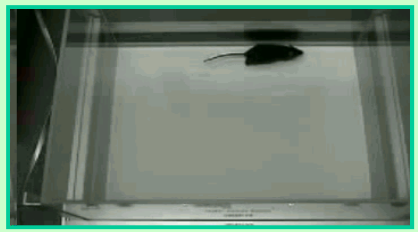
1. Self-Grooming Assessment
   - 5 minute habituation
   - 10 minute test session

2. Social Approach
   - 20 minute habituation
   - 10 minute sociability session

3. Open Field Locomotion
   - 30 minutes in Accuscan automated open field



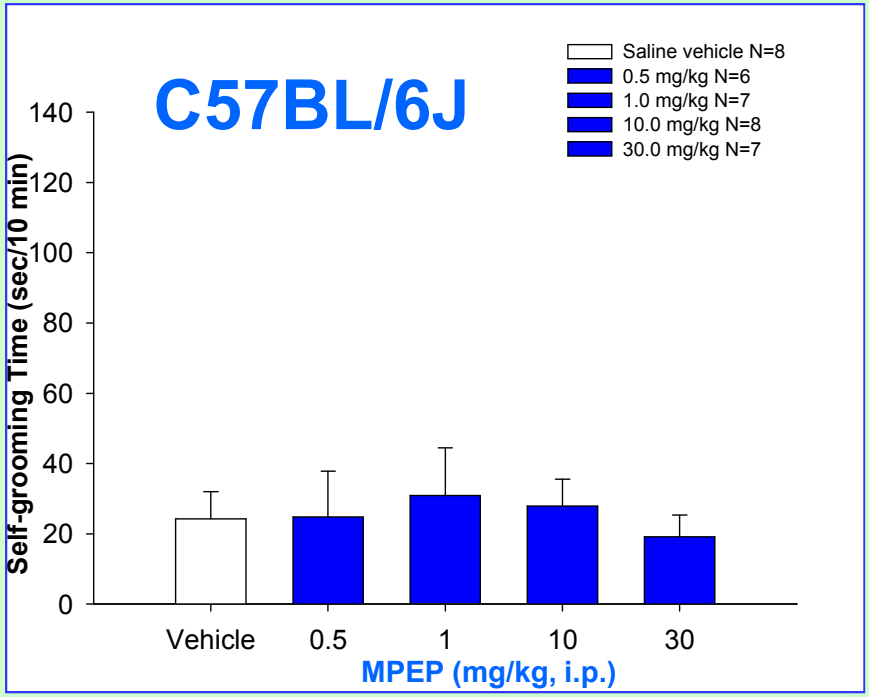


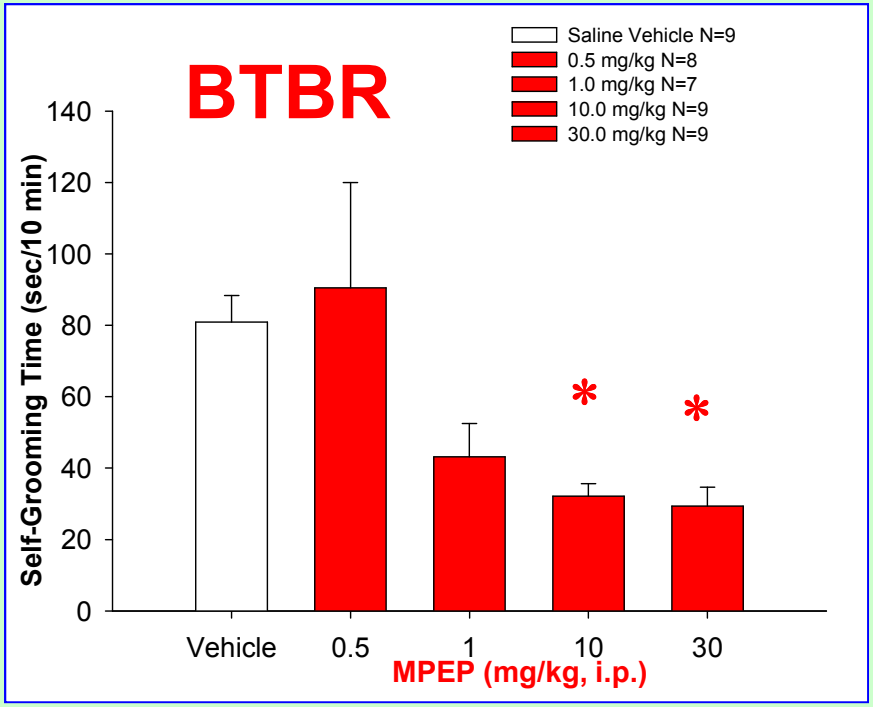


***Silverman and Crawley, 2009***

# MPEP, is an mGluR5 antagonist, a drug class that blocks seizures and reverses dendritic abnormalities in Fragile X mice

## Time in Chamber

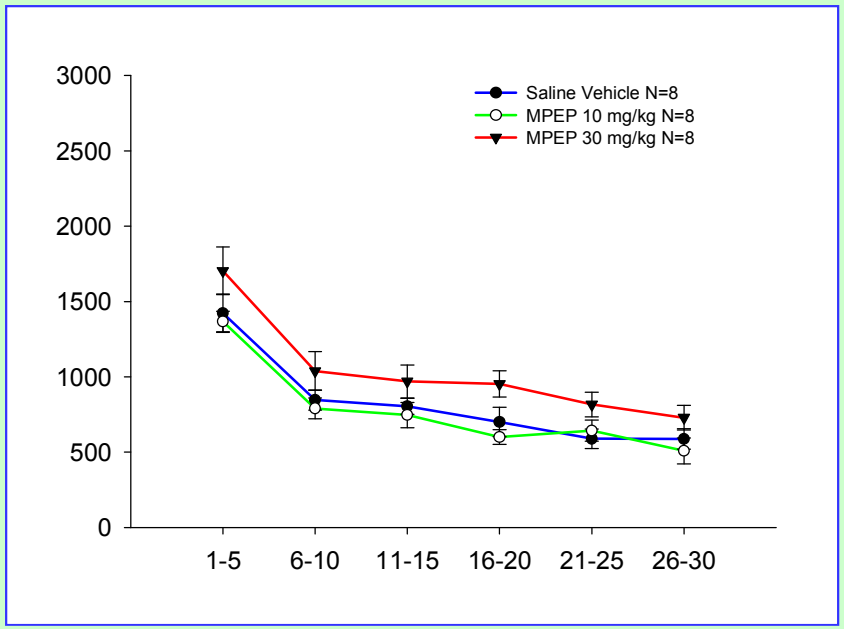



Silverman et al., *Neuropsychopharmacology* 2010

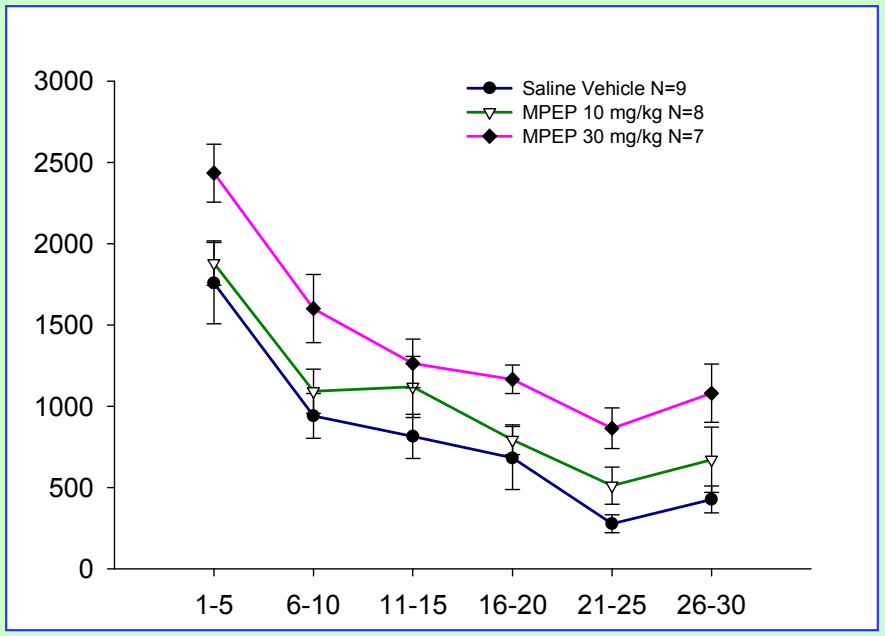
## MPEP is not sedative
## at doses than reduce repetitive self-grooming

### C57BL/6J




Open field locomotion (min)

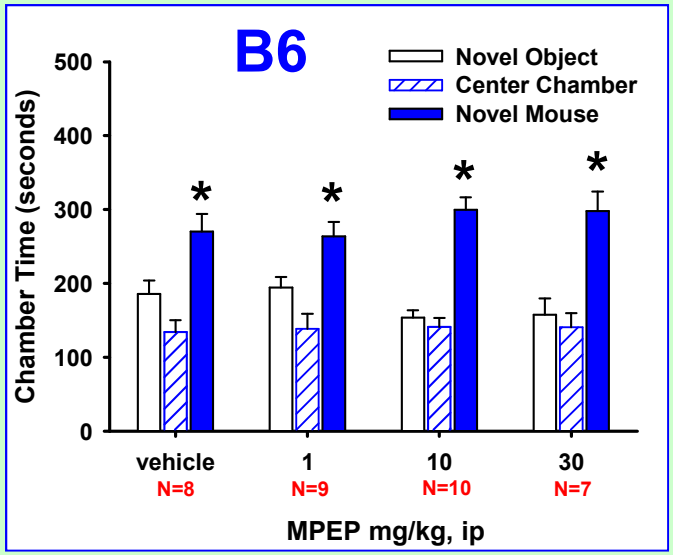
### BTBR T+tf/J

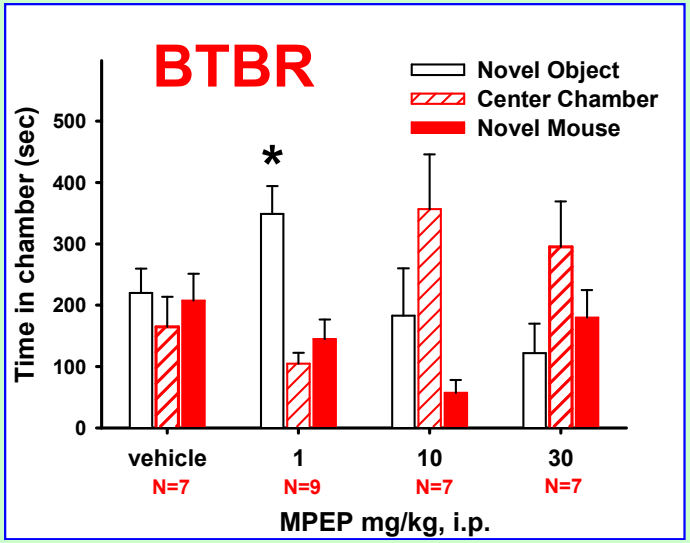


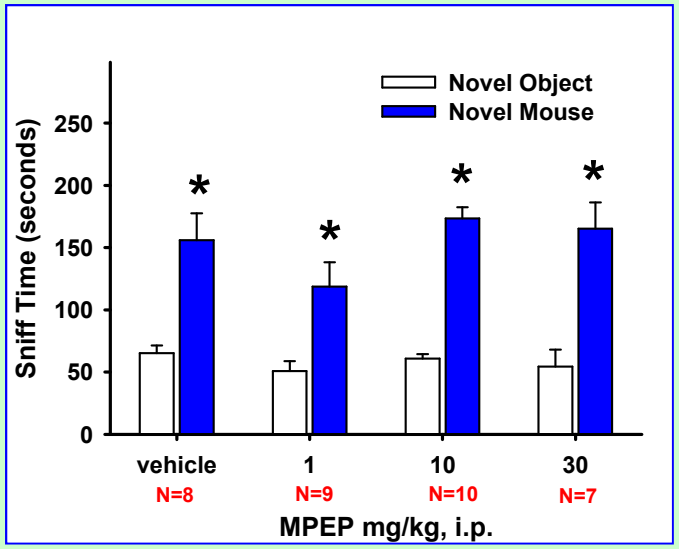

Open field locomotion (min)

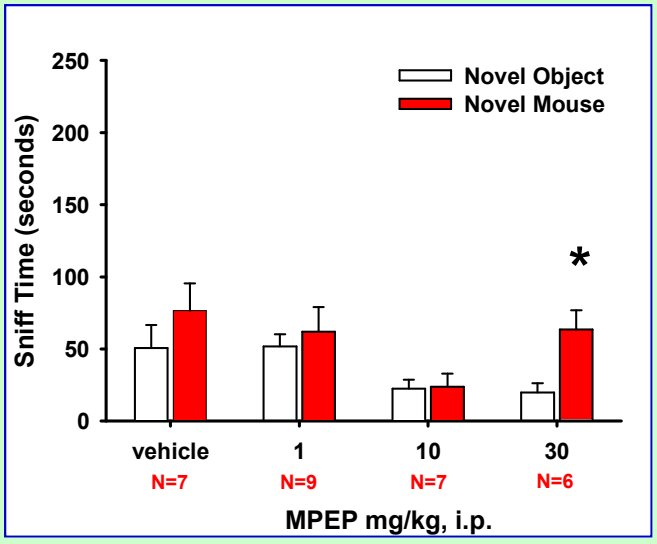
Silverman et al., *Neuropsychopharmacology* 2010

# MPEP did not reverse the sociability deficit in BTBR




Silverman et al., *Neuropsychopharmacology* 2010

## Translational Applications of Mouse Models of Autism

### Preclinical drug testing in progress in our laboratory:

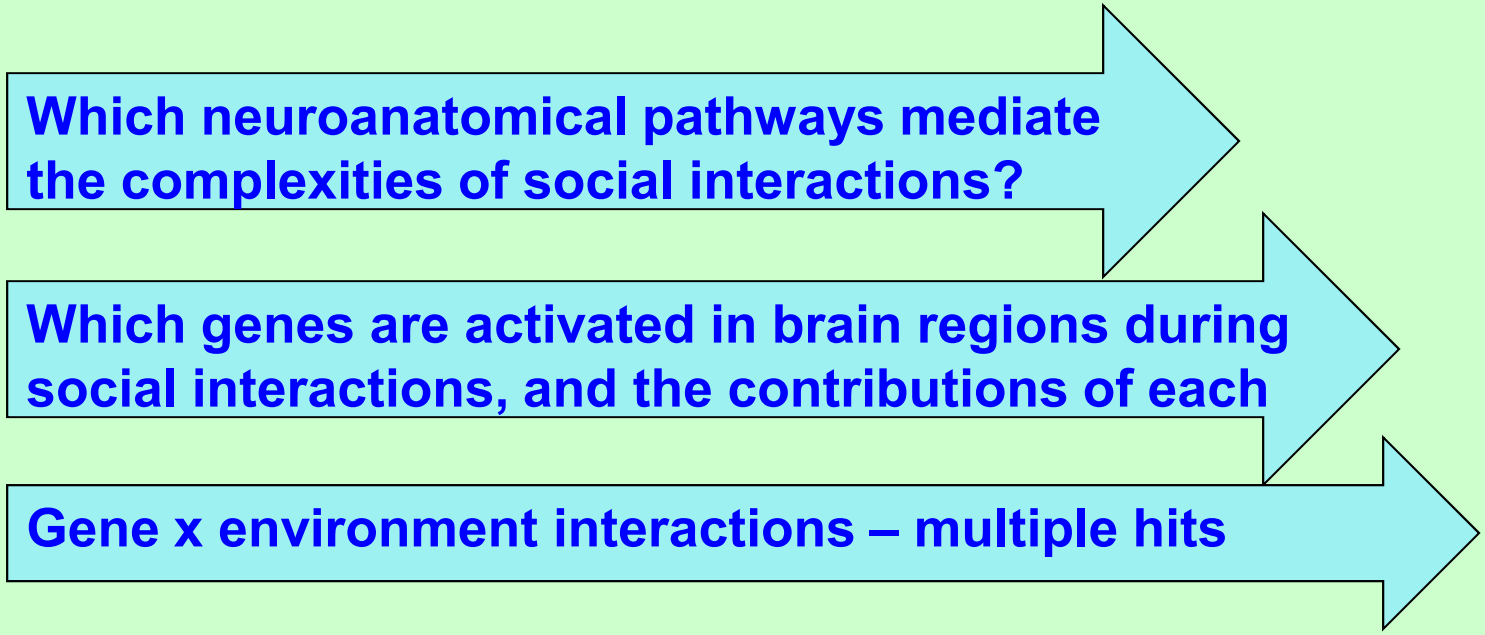
- **MTEP (mGluR5 receptor antagonist)**
- **Risperidone (antipsychotic approved by FDA for irritability symptoms of autism)**
- **CX546 (AMPA receptor modulator)**
- **Your suggestions?**

***Crawley, 2010***

# Where is the field now?

**We are still in the early stages of choosing and optimizing the phenotyping assays for mouse models of autism**

**What we need to know:**




*Crawley, 2010*

## Where is the field now?

Ongoing discussions between
autism clinical researchers and
basic research mouse modelers will
contribute to the iterative,
convergent process
of designing analogous diagnostics,
discovering the causes of autism,
and developing effective treatments.

*Crawley, 2010*