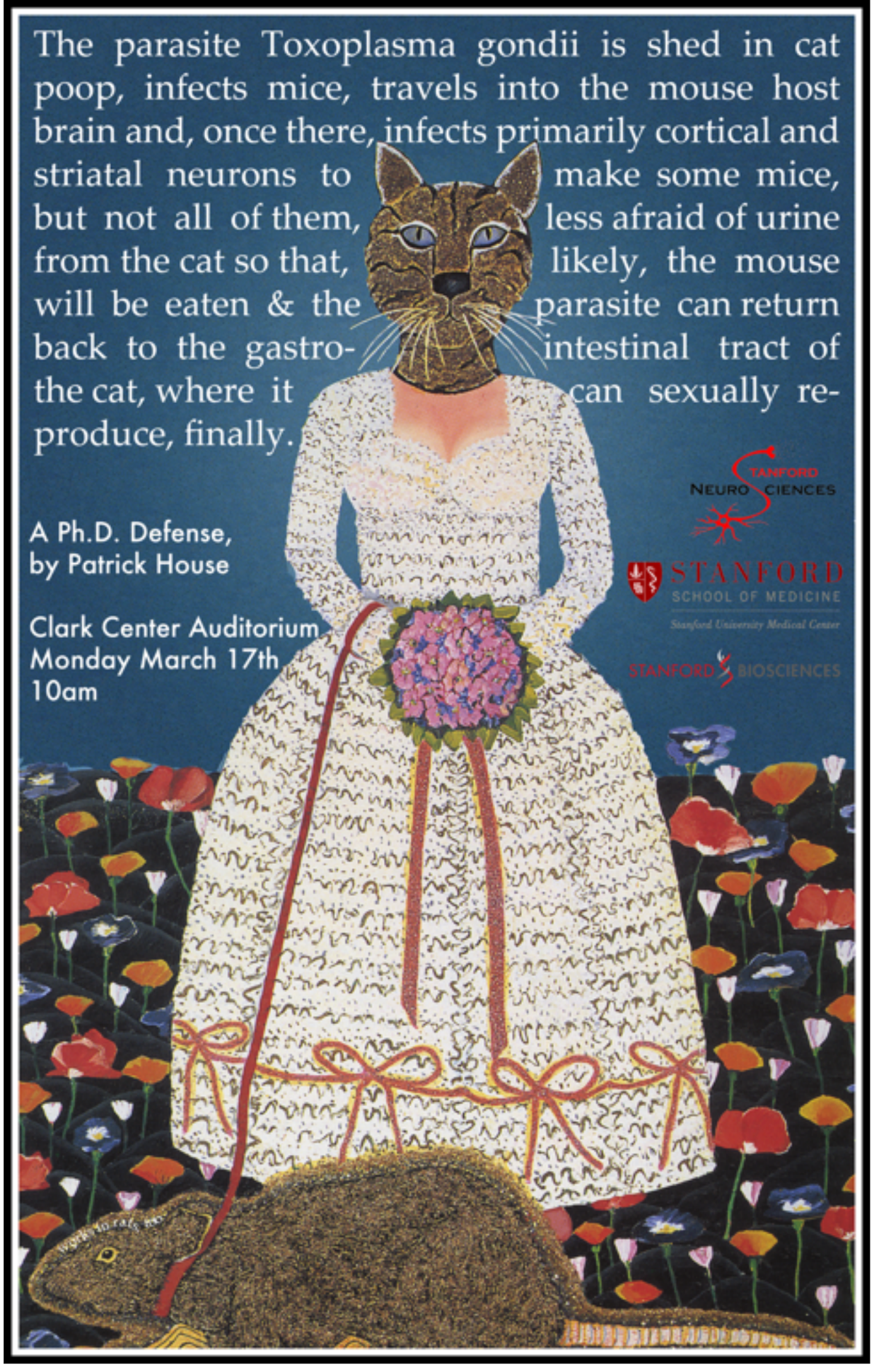
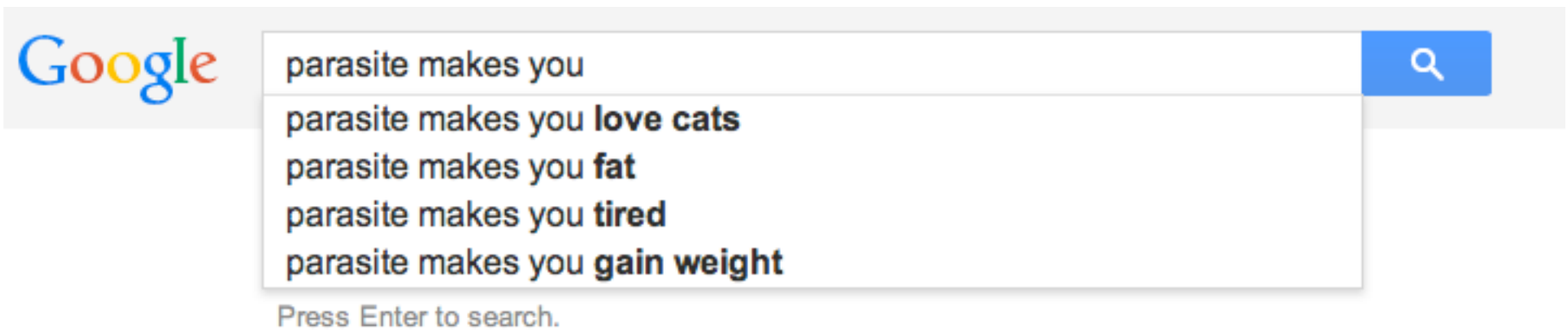
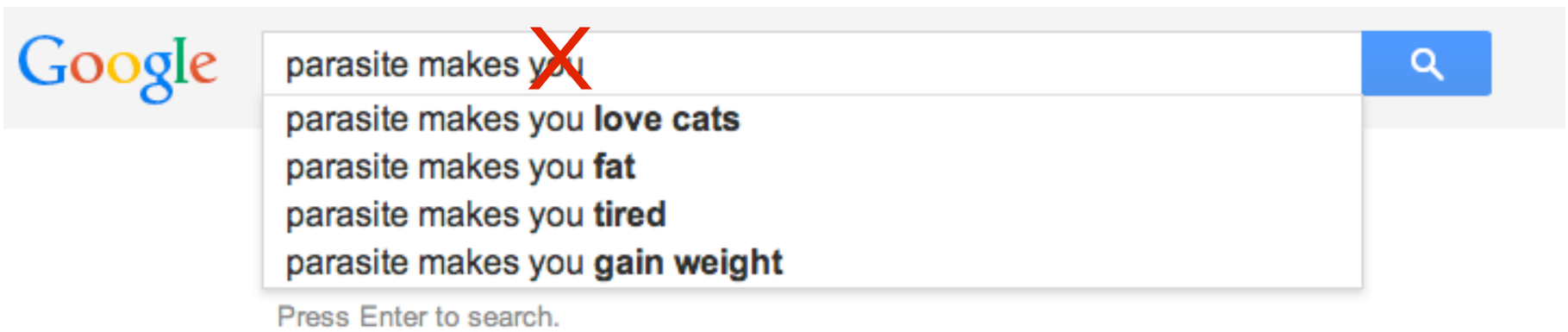

The parasite Toxoplasma gondii is shed in cat poop, infects mice, travels into the mouse host brain and, once there, infects primarily cortical and striatal neurons to make some mice, but not all of them, less afraid of urine from the cat so that, likely, the mouse will be eaten & the parasite can return back to the gastro- intestinal tract of the cat, where it can sexually re- produce, finally.
A Ph.D. Defense, by Patrick House
Clark Center Auditorium
Monday March 17th
10am
STANFORD NEUROSCIENCES
STANFORD SCHOOL OF MEDICINE
Stanford University Medical Center
STANFORD BIOSCIENCES

# The Single-celled Parasite *Toxoplasma gondii* Alters Innate Fear in Mice by Infecting Primarily Cortical and Striatal Neurons



# The Single-celled Parasite *Toxoplasma gondii* Alters Innate Fear in Mice by Infecting Primarily Cortical and Striatal Neurons

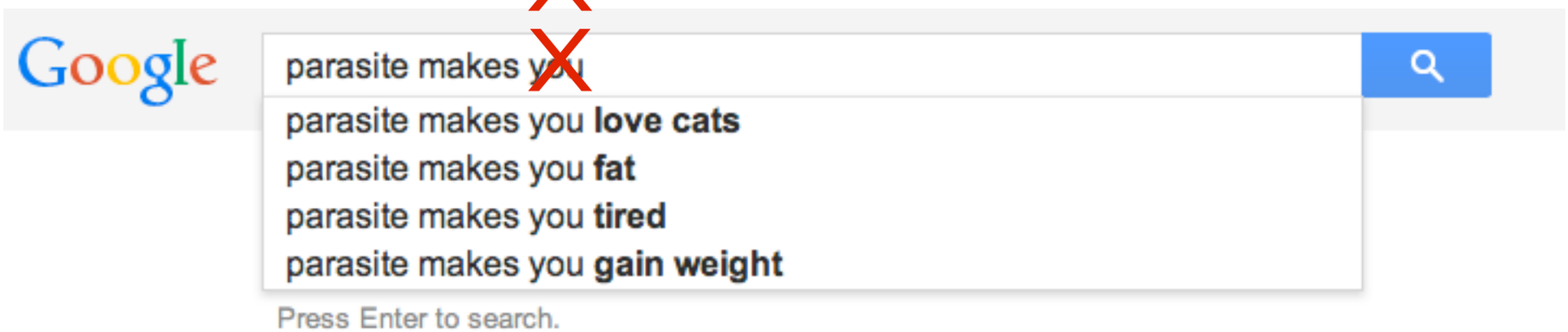
mice



# The Single-celled Parasite *Toxoplasma gondii* Alters Innate Fear in Mice by Infecting Primarily Cortical and Striatal Neurons

some mice

~~mice~~



THE PARASITE

IN RATS

IN MICE

IN PEOPLE

THE PARASITE

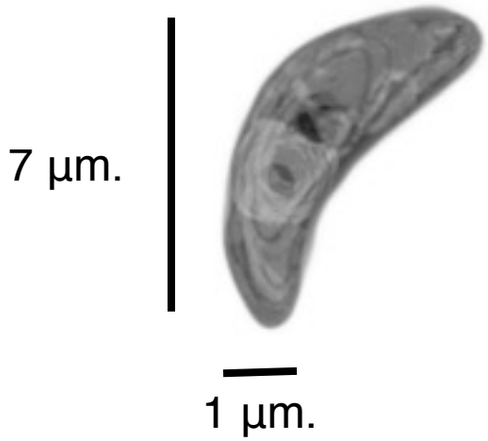
IN RATS

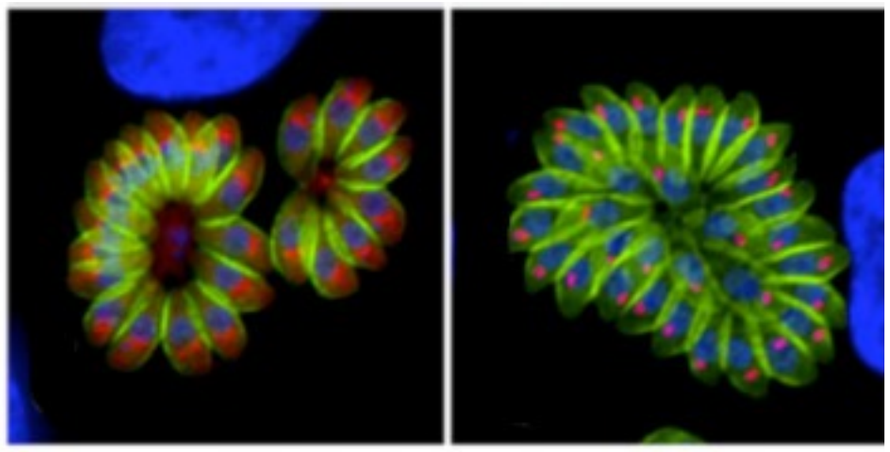
IN MICE

IN PEOPLE

# THE PARASITE

*Toxoplasma gondii* (‘Gandhi’ - ‘eye’ )



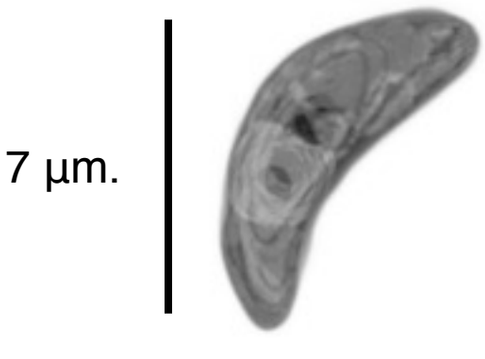
Carruthers Lab, U Michigan

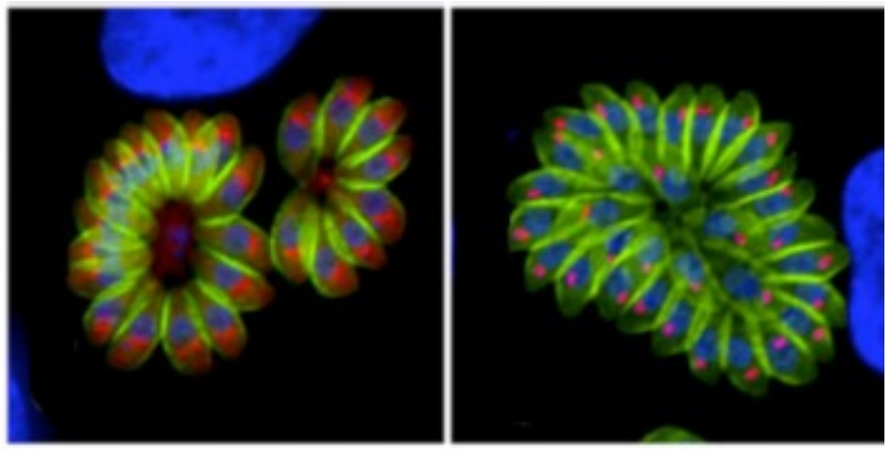
- single-celled protozoan;
- single parasite, tachyzoite stage;
- clusters of parasite, bradyzoite stage;
- encyst chronically for life of host in muscle or brain tissue;
- can infect all vertebrates

Carruthers Lab, U Michigan

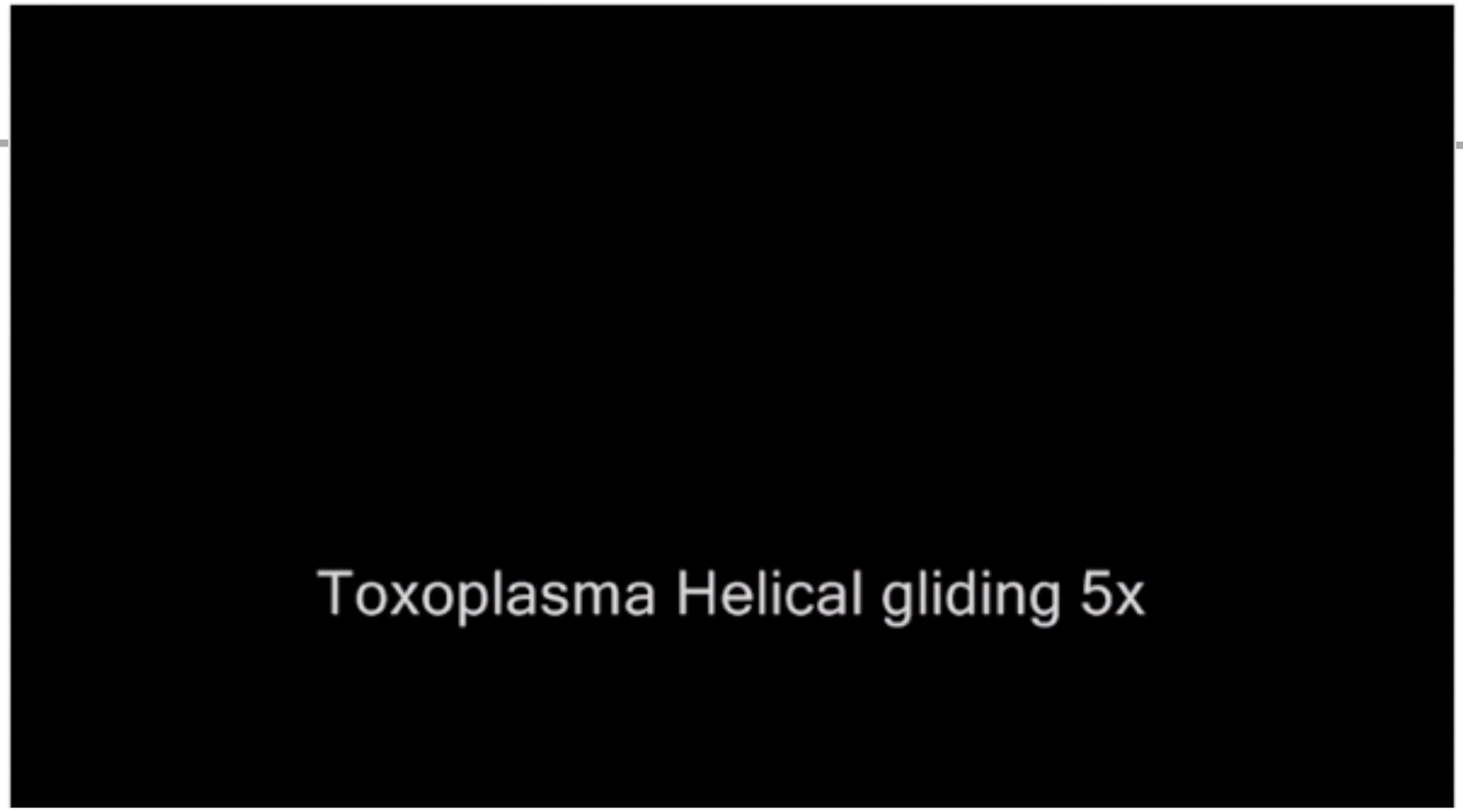
# THE PARASITE

*Toxoplasma gondii* (‘Gandhi’ - ‘eye’ )



- single-celled protozoan;
- single parasite, tachyzoite stage;
- clusters of parasite, bradyzoite stage;
- encyst chronically for life of host in muscle or brain tissue;
- can infect all vertebrates



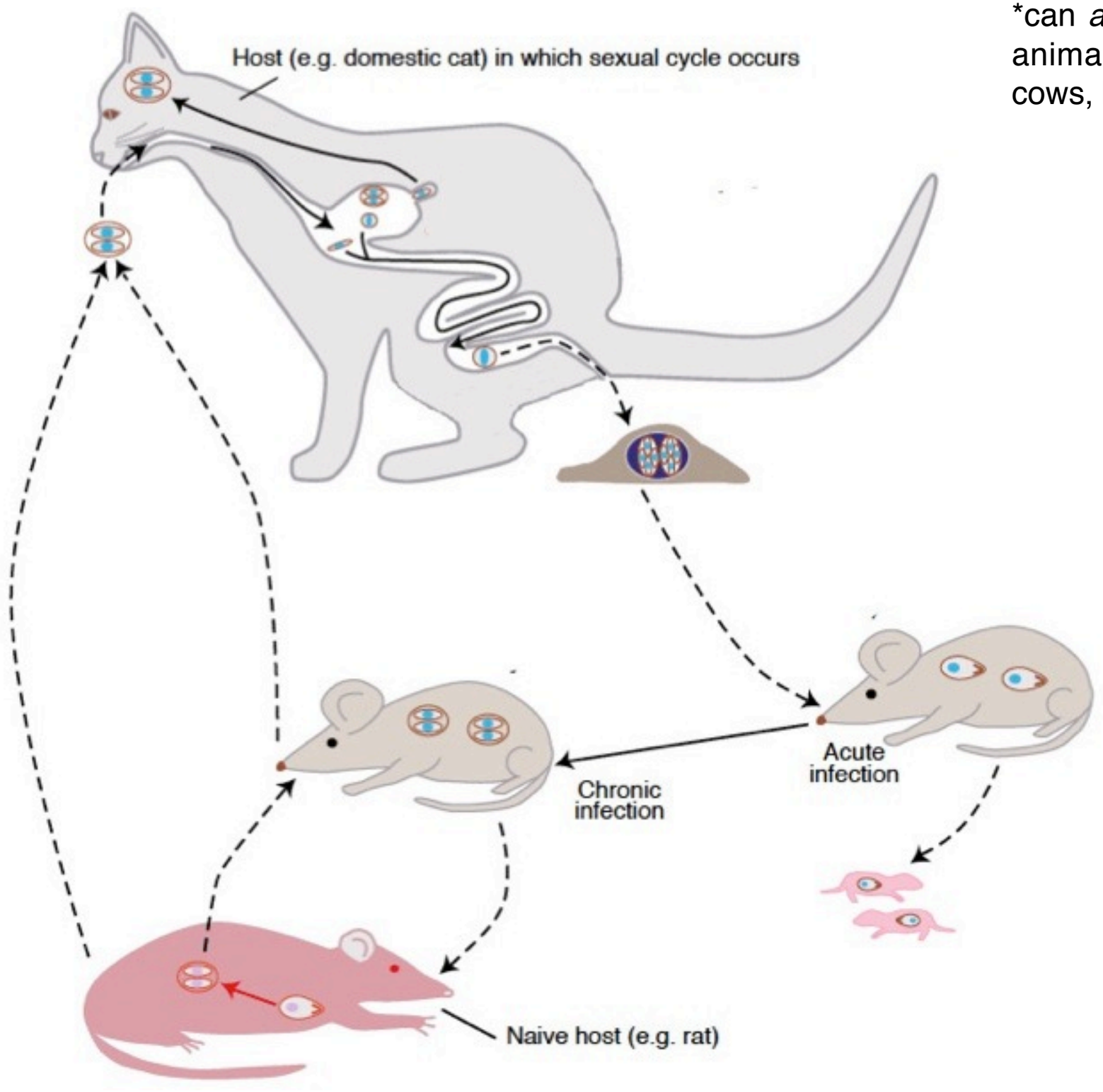

Carruthers Lab, U Michigan




Carruthers Lab, U Michigan

# THE PARASITE

## Toxo Can Only Sexually* Reproduce in Cats




*can *asexually* reproduce in other animals, e.g. pandas, whales, cows, humans

# THE PARASITE

## 1 - 4 Billion People Worldwide Test Positive for Toxo





International Journal for Parasitology

Volume 32, Issue 9, August 2002, Pages 1193–1199

Prevalence of *Toxoplasma gondii* in commercial meat products as monitored by polymerase chain reaction – food for thought?

Tanya V. Aspinall, Damian Marlee, John E. Hyde, Paul F.G. Sims

THERAPEUTICS-DIAGNOSTICS

### HIGH PREVALENCE OF VIABLE *TOXOPLASMA GONDII* INFECTION IN MARKET WEIGHT PIGS FROM A FARM IN MASSACHUSETTS

**J. P. Dubey , H. R. Gamble *, D. Hill , C. Sreekumar , S. Romand †, and P. Thulliez †**

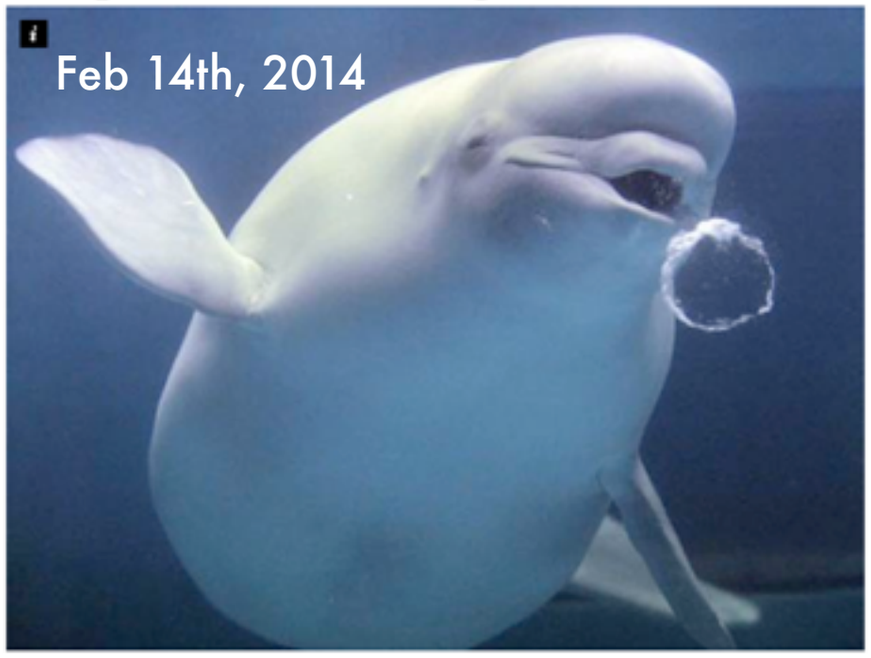
Parasite Biology, Epidemiology and Systematics Laboratory, Building 1001, Animal and Natural Resources Institute, Agricultural Research Service, United States Department of Agriculture, Beltsville Agricultural Research Center-East, Beltsville, Maryland 20705.
jdubey@anri.barc.usda.gov

# THE PARASITE

## Toxo Can (& Does) Infect Any Vertebrate

News > Science

### Killer cat parasite spreads to Arctic: Toxoplasma found in beluga whales




Concern for Native Inuits over potentially dangerous parasite as whale meat is an important part of their diet

### Cat parasite 'is killing otters'

By Paul Rincon
BBC News website science reporter, St Louis

Feb 19th, 2006

**A parasite carried by cats is killing off sea otters, a veterinary specialist has told a major US science conference.**

The Californian researcher has called for owners to keep their cats indoors.


Sea otters were hunted heavily for their fur in the 1800s

Cat faeces carrying *Toxoplasma* parasites wash into US waterways and then into the sea where they can infect otters, causing brain disease.

SCIENCE NEWS

HOME / SCIENCE NEWS / BACTERIA, TOXOPLASMA INFECTIONS BLAMED FOR GIANT PANDA'S DEATH

### Bacteria, toxoplasma infections blamed for giant panda's death

March. 14, 2014 at 1:02 PM | 0 Comments (Leave a comment)

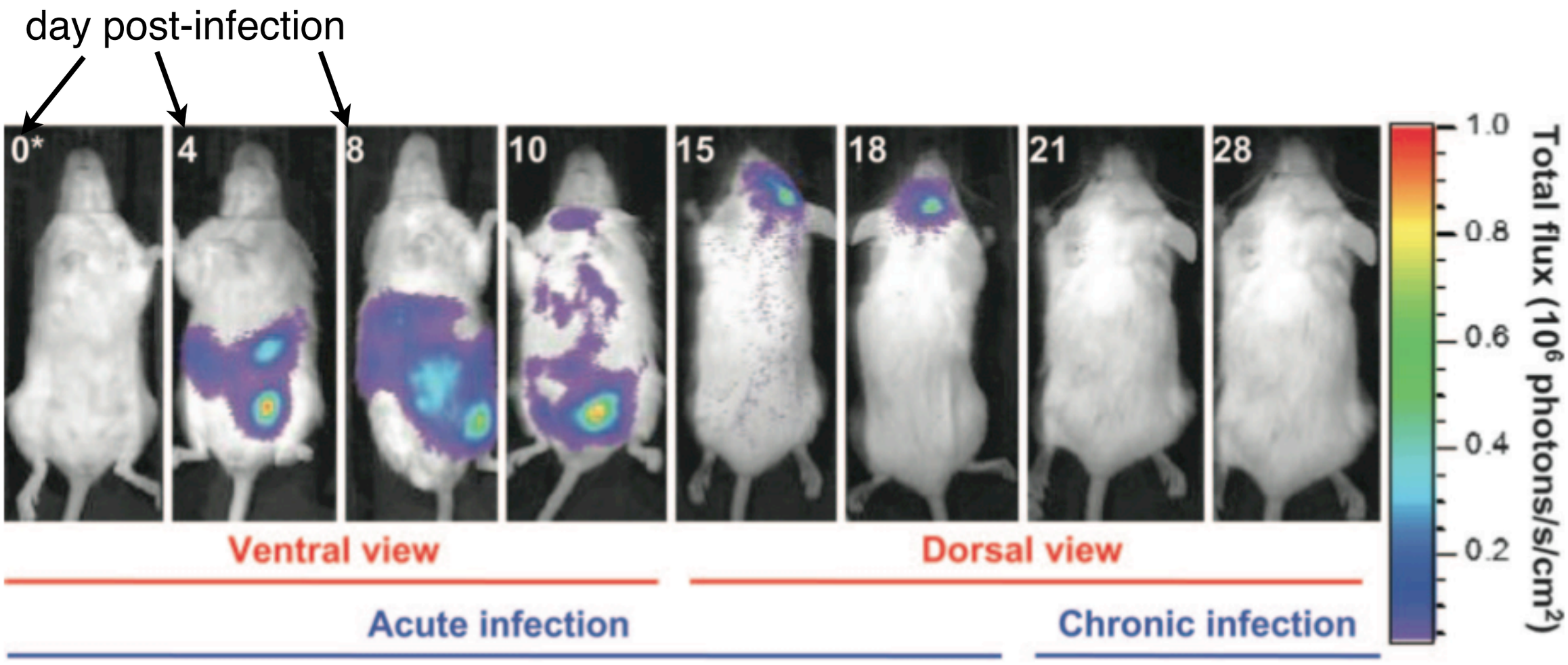



Bao Bao, the giant panda cub, is seen during her media preview at the Giant Panda House at the Smithsonian National Zoological Park in Washington, D.C., January 6, 2014. The cub, who was born on August 23, 2013, made its public debut on Jan 18. UPI/Kevin Dietsch | License Photo

"This is also the first time we found Toxoplasma in a giant panda, so we know very little about the infection and its pathology as it relates to this animal,"

-Head of Wolong Giant Panda Reserve

# Toxo Gets Into the Brain of Its Host a Few Weeks After Infection

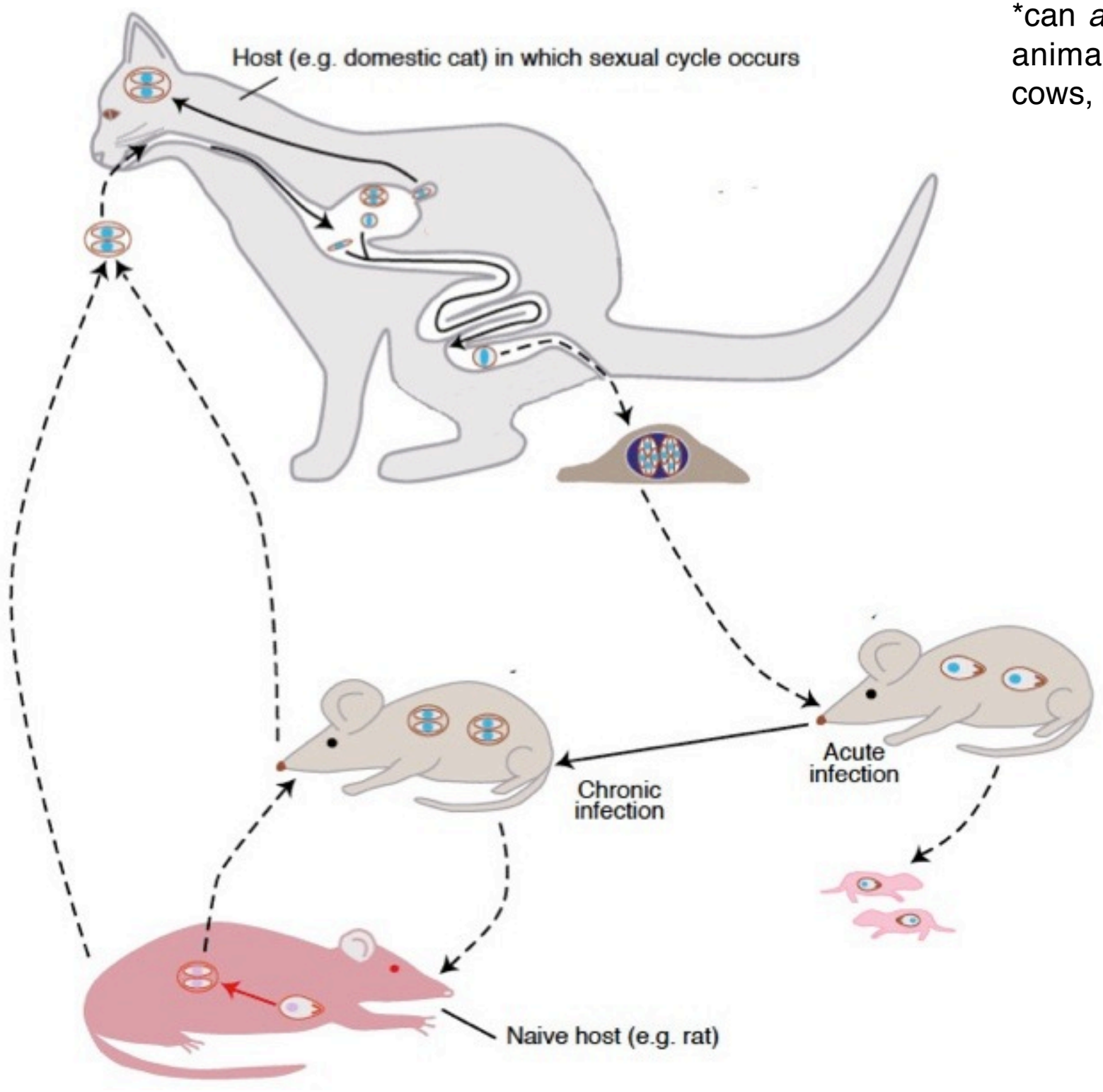



(Vyas et al. 2007 *PNAS*)

- Above, the Toxo parasite is genetically modified to bioluminesce. Toxo enters chronic central nervous system stage around day 21 and persists in the brain. It gets into the brain by hijacking immune system cells which have privileged access to the central nervous tissue.

# THE PARASITE

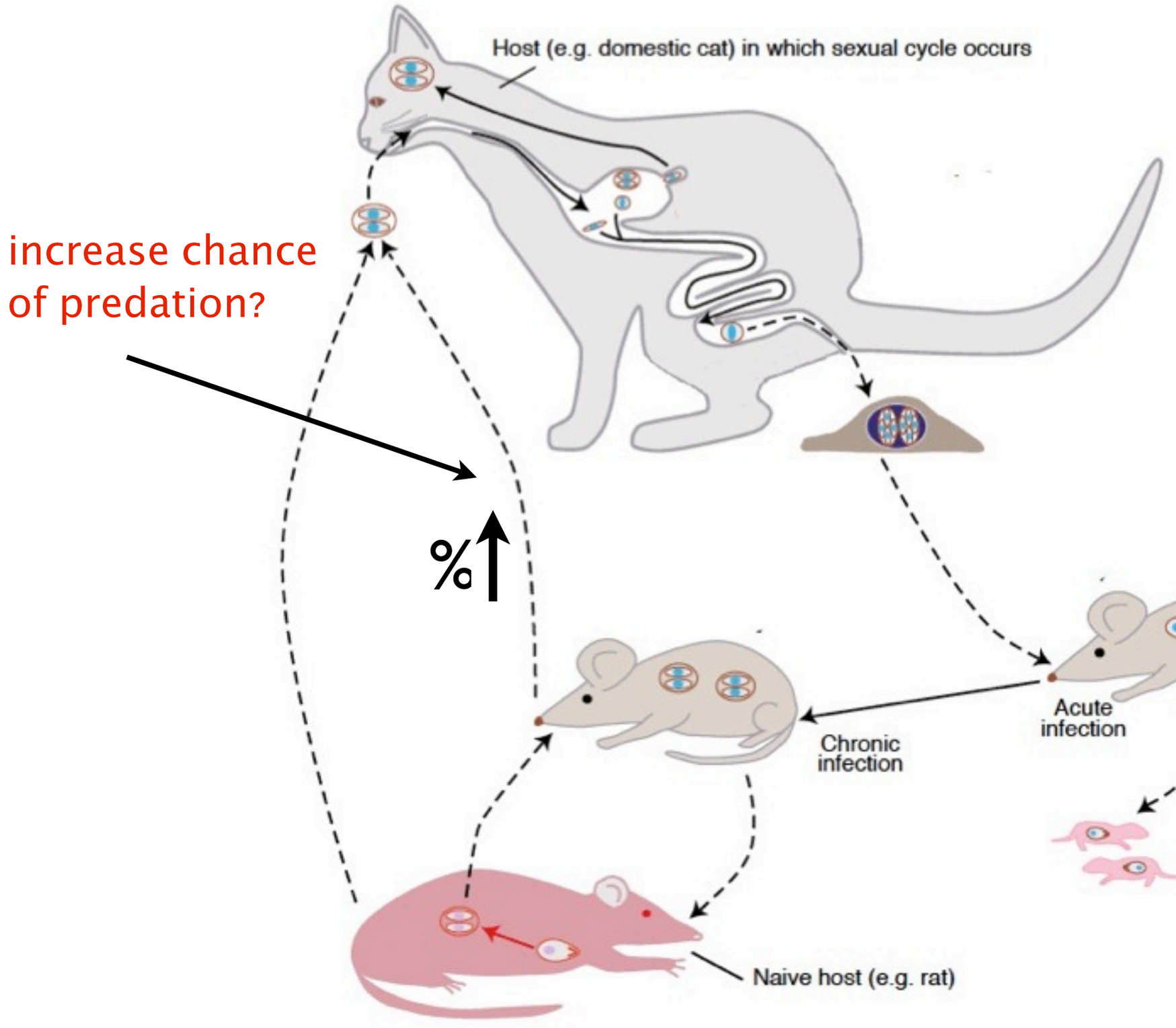
## Toxo Can Only Sexually* Reproduce in Cats




*can *asexually* reproduce in other animals, e.g. pandas, whales, cows, humans

# THE PARASITE

## Toxo Can Only Sexually* Reproduce in Cats




*can *asexually* reproduce in other animals, e.g. pandas, whales, cows, humans

## Toxoplasma Behavioral Studies in **Mice**

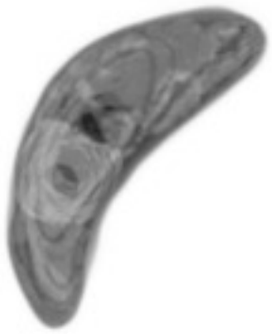
| Name et al | Yr | Mouse | Toxo | Sex | Route | Week p.i. | Test | Finding |
|---|---|---|---|---|---|---|---|---|
| *Wang* | 13 | BALB/c | PRU | Both | Maternal | 10 w | Memory, etc | learning & memory deficits |
| *Gatkowska* | 13 | C3H/HeJ | ME49 | Both | IP (20 cysts) | 3 & 6w | | increased monoamines |
| *Costa* | 12 | B6 | ME49 | Female | IP (10^4 tachys) | 9w | Open field, etc | increased exploration |
| *Goodwin* | 12 | CD-1 | VEG | Both | Maternal | 4 & 8 w | Barnes maze | short-term memory deficit |
| *Jung* | 12 | Tg2567 | Me49 | | IP (15 cysts) | 24w | Y, Water, etc | reverses memory deficits in AZ |
| *Haroon* | 12 | BALB/c | DX | F | Oral (4 cysts) | 4 & 8w | Open field | loss-of-aversion |
| *Yolken* | 12 | BALB/c | PRU | F | IP (400) | 8-16w | 3 chamber | loss-of-aversion |
| *Gatkovska* | 12 | B6 | Me49 | M | IP (20 cysts) | 3 & 6w | Open field, etc | reduced exploration |
| *Gulinello* | 10 | B6 | Me49 | M | IP (1000) | 7w | Open field, etc | motor coordination / sensory |
| *Hermes* | 08 | Swiss | Me49 | F | IP (100 cysts) | 48w | Open field, etc | reduced exploration, motor |
| *Hodkova* | 07 | B* | HIF | | Oral (10) | 10w | 8 arm, etc | memory / learning deficit |
| *Vyas* | 07 | BALB/c | PRU | F | IP (400) | 4-5w | Circle | loss-of-aversion |
| *Chao* | 92 | | | | | | | |
| *Arnott* | 90 | | | | | | | |
| *Arnott* | 90 | | | | | | | |
| *Graham* | 84 | | | | | | | |
| *Hutchinson* | 80 | | | | | | | |

## Toxoplasma Behavioral Studies in **Rats**

| Name et al | Yr | Rat | Toxo | Sex | Route | Week p.i. | Test | Finding |
|---|---|---|---|---|---|---|---|---|
| *Evans* | 14 | Long-Evans | PRU | Male | IP (10^6 tachys) | varied | Open field, etc. | loss-of-aversion |
| *House* | 11 | Long-Evans | PRU | Male | IP (10^7 tachys) | 6w | Forced exposure | altered neural activity |
| *Webster* | 08 | Lister | ME49 | | Oral (20 cysts) | 4w | Y | loss-of-aversion |
| *Vyas* | 07 | Long-Evans | PRU | Male | IP (10^7) | 7w - 8w | Forced choice | loss-of-aversion |
| *Vyas* | 07 | Long-Evans | PRU | Male | IP (10^6 tachys) | 4w - 5w | Anxiety, fear, etc | loss-of-aversion |
| *Gonzalez* | 07 | Wistar | RH | | IP (1500 tachys) | 4w - 7w | Social, EPM | decreased anxiety |
| *Wesbter* | 06 | Lister | ME49 | | Oral (20 cysts) | 4 w | Open field | loss-of-aversion; HAL rescue |
| *Webster* | 00 | Lister | RRA | | Oral (20 cysts) | | Open field | loss-of-aversion |
| *Webster* | 95 | norvegicus | natural | Both | natural | | Social, exploration | lowered neophobia |
| *Webster* | 94 | wild brown | natural | | Oral (20 cysts) | | Novel object | lowered neophobia |
| *Grzywinski* | 73 | | | | | | | |

# THE PARASITE

## Is Toxo Altering Dopamine Levels in its host?



Tyrosine

TYROSINE HYDROXYLASE

L-Dopa

DOPA DECARBOXYLASE

Dopamine

- Toxoplasma has a gene for making Tyrosine Hydroxylase (Gaskell 2009)
- Toxoplasma increases Dopamine levels (~15%) in the brains of infected mice (Stibbs 1985)

# Questions: Known, Unknown and Known Unknowns

**Behavioral**

Are Toxo-infected animals less afraid of cats, or more attracted to cats?
Does Toxo make all rodents less afraid of cats, or just some of them?

**Neurobiological**

How much Toxo gets in the brain after infection?
Does Toxo preferentially infect a certain neural subtype?
Does Toxo exhibit preference for some brain regions over others?
What does Toxo do to neurons it infects? Does it kill them? Alter their signaling?
Does Toxo infection increase dopamine levels in an individual neuron?

**Ecological**

Why can Toxo only sexually reproduce in the cat?

**Human & Medical**

How do we kill Toxo once it encysts in the brain?
How do we vaccinate against Toxo?
How many people worldwide have Toxo? 1 - 4 billion
Does it influence human behavior?

# Questions: Known, Unknown and Known Unknowns

**Behavioral**

Are Toxo-infected animals less afraid of cats, or more attracted to cats?

THE PARASITE

IN RATS

IN MICE

IN PEOPLE

# IN RATS

## Toxo Increases Activity in Brain Regions of Sexual Attraction after Cat Urine Exposure in Male Rats

odor

↓

olfactory bulb

↓

amygdala

↓

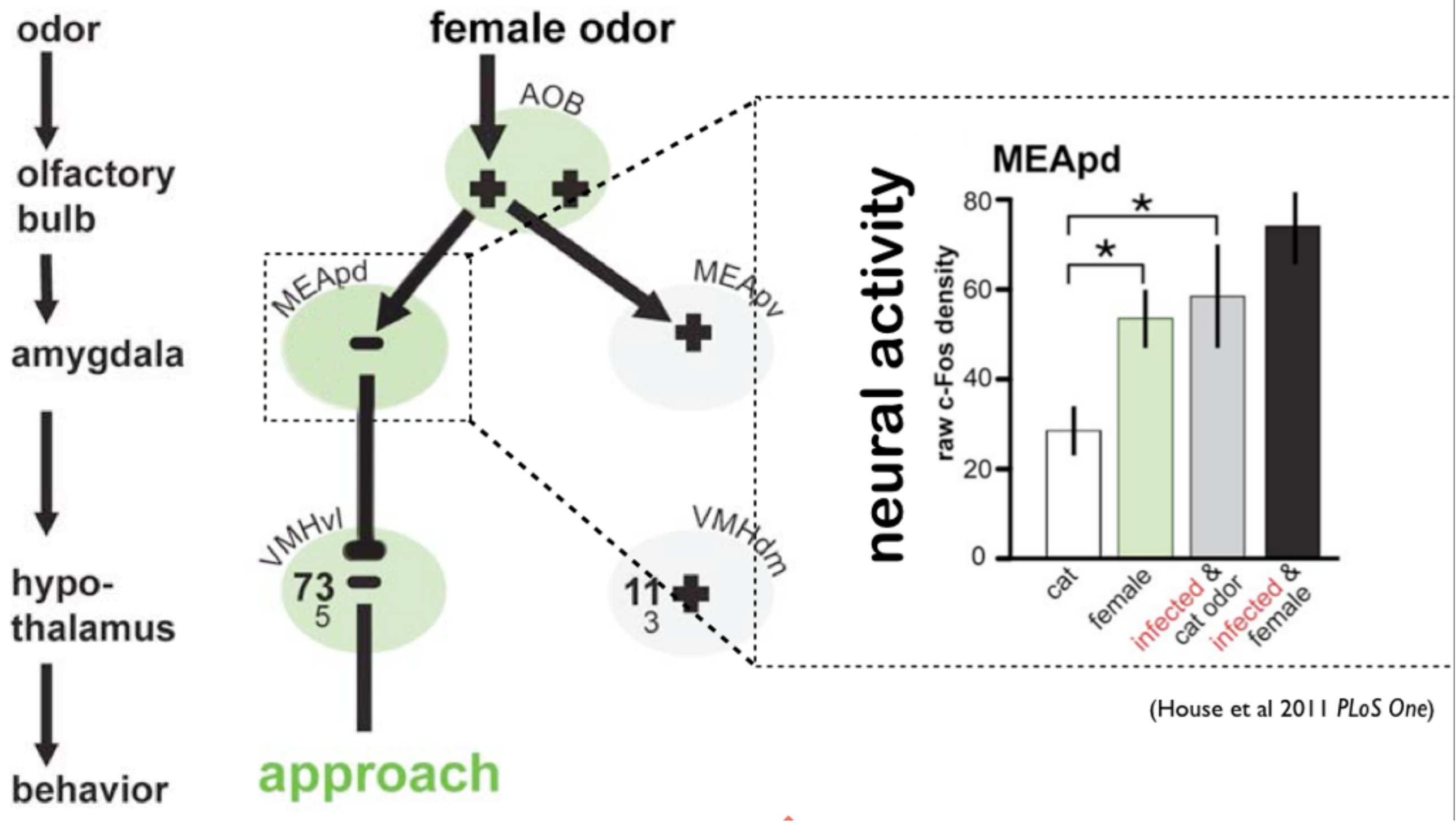
hypo-thalamus

↓

behavior

# IN RATS

## Toxo Increases Activity in Brain Regions of Sexual Attraction after Cat Urine Exposure in Male Rats




(House et al 2011 *PLoS One*)

THE PARASITE

IN RATS

IN MICE

IN PEOPLE

# How to Make a Toxo Disease Model in the Mouse

1. Establish a reproducible phenotype;



2. Pretend we know nothing about Toxo;



3. Screen drugs against phenotype (anti-psychotics, SSRIs);



4. Infer from drugs that interact with behavior a plausible mechanism of action for Toxo;



5. Join the ranks of other disease models

# How to Make a Toxo Disease Model in the Mouse

1. Establish a reproducible phenotype;

2. Pretend we know nothing about Toxo other than the behavioral phenotype

3. Screen drugs against phenotype (anti-psychotics, SSRIs);

4. Infer from drugs that interact with behavior a plausible mechanism of action for Toxo

5. Join the ranks of other disease models

IN MICE

# High Variability in Toxo Mouse Models

## Toxoplasma Behavioral Studies in **Mice**

| Name et al | Yr | Mouse | Toxo | Sex | Route | Week p.i. | Test | Finding |
|---|---|---|---|---|---|---|---|---|
| *Wang* | 13 | BALB/c | PRU | Both | Maternal | 10 w | Memory, etc | learning & memory deficits |
| *Gatkowska* | 13 | C3H/HeJ | ME49 | Both | IP (20 cysts) | 3 & 6w | | increased monoamines |
| *Costa* | 12 | B6 | ME49 | Female | IP (10^4 tachys) | 9w | Open field, etc | increased exploration |
| *Goodwin* | 12 | CD-1 | VEG | Both | Maternal | 4 & 8 w | Barnes maze | short-term memory deficit |
| *Jung* | 12 | Tg2567 | Me49 | | IP (15 cysts) | 24w | Y, Water, etc | reverses memory deficits in AZ |
| *Haroon* | 12 | BALB/c | DX | F | Oral (4 cysts) | 4 & 8w | Open field | loss-of-aversion |
| *Yolken* | 12 | BALB/c | PRU | F | IP (400) | 8-16w | 3 chamber | loss-of-aversion |
| *Gatkovska* | 12 | B6 | Me49 | M | IP (20 cysts) | 3 & 6w | Open field, etc | reduced exploration |
| *Gulinello* | 10 | B6 | Me49 | M | IP (1000) | 7w | Open field, etc | motor coordination / sensory |
| *Hermes* | 08 | Swiss | Me49 | F | IP (100 cysts) | 48w | Open field, etc | reduced exploration, motor |
| *Hodkova* | 07 | B* | HIF | | Oral (10) | 10w | 8 arm, etc | memory / learning deficit |
| *Vyas* | 07 | BALB/c | PRU | F | IP (400) | 4-5w | Circle | loss-of-aversion |
| *Chao* | 92 | | | | | | | |
| *Arnott* | 90 | | | | | | | |
| *Arnott* | 90 | | | | | | | |
| *Graham* | 84 | | | | | | | |
| *Hutchinson* | 80 | | | | | | | |

IN MICE

# High Variability in Toxo Mouse Models

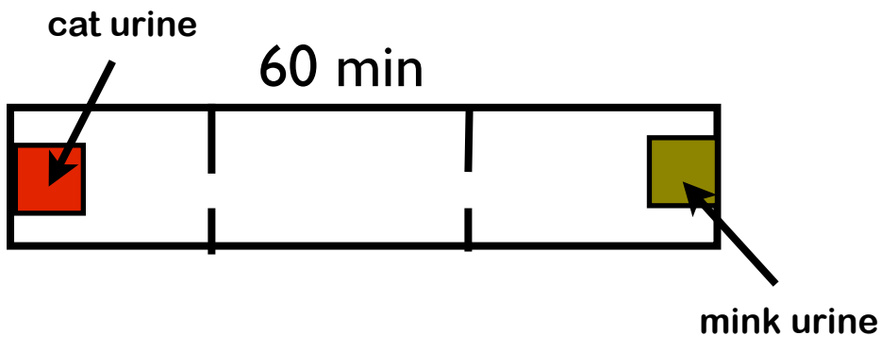
## Toxoplasma Behavioral Studies in **Mice**

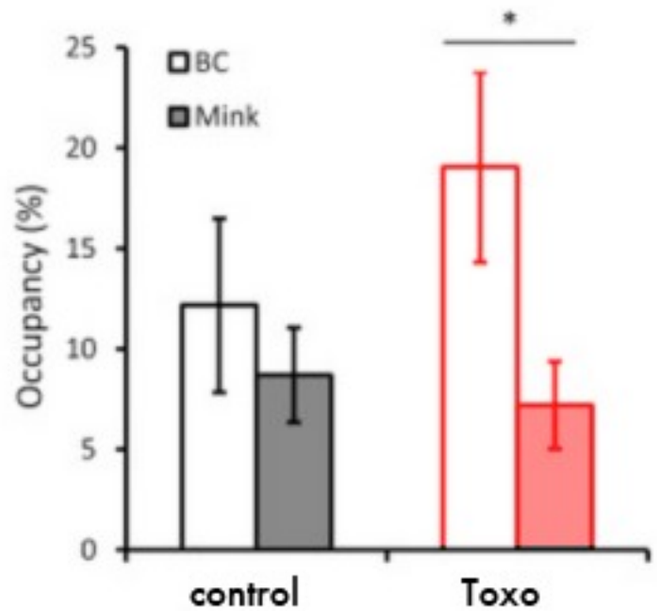
| Name et al | Yr | Mouse | Toxo | Sex | Route | Week p.i. | Test | Finding |
|---|---|---|---|---|---|---|---|---|
| *Wang* | 13 | BALB/c | PRU | Both | Maternal | 10 w | Memory, etc | learning & memory deficits |
| *Gatkowska* | 13 | C3H/HeJ | ME49 | Both | IP (20 cysts) | 3 & 6w | | increased monoamines |
| *Costa* | 12 | B6 | ME49 | Female | IP (10^4 tachys) | 9w | Open field, etc | increased exploration |
| *Goodwin* | 12 | CD-1 | VEG | Both | Maternal | 4 & 8 w | Barnes maze | short-term memory deficit |
| *Jung* | 12 | Tg2567 | Me49 | | IP (15 cysts) | 24w | Y, Water, etc | reverses memory deficits in AZ |
| *Haroon* | 12 | BALB/c | DX | F | Oral (4 cysts) | 4 & 8w | Open field | loss-of-aversion |
| *Yolken* | 12 | BALB/c | PRU | F | IP (400) | 8-16w | 3 chamber | loss-of-aversion |
| *Gatkovska* | 12 | B6 | Me49 | M | IP (20 cysts) | 3 & 6w | Open field, etc | reduced exploration |
| *Gulinello* | 10 | B6 | Me49 | M | IP (1000) | 7w | Open field, etc | motor coordination / sensory |
| *Hermes* | 08 | Swiss | Me49 | F | IP (100 cysts) | 48w | Open field, etc | reduced exploration, motor |
| *Hodkova* | 07 | B* | HIF | | Oral (10) | 10w | 8 arm, etc | memory / learning deficit |
| *Vyas* | 07 | BALB/c | PRU | F | IP (400) | 4-5w | Circle | loss-of-aversion |
| *Chao* | 92 | | | | | | | |
| *Arnott* | 90 | | | | | | | |
| *Arnott* | 90 | | | | | | | |
| *Graham* | 84 | | | | | | | |
| *Hutchinson* | 80 | | | | | | | |

- Not one consistent, established, reproducible behavior

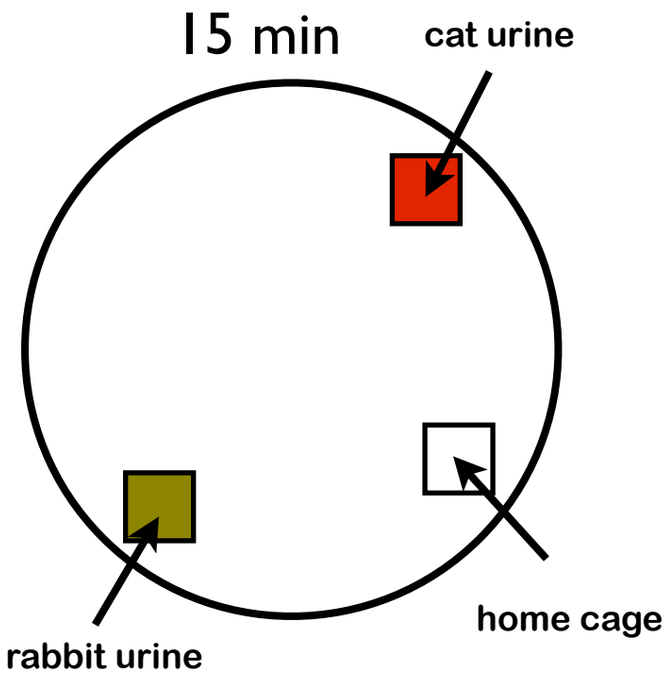
# IN MICE Different Arenas, Mice, Toxo Strain, Measures, etc

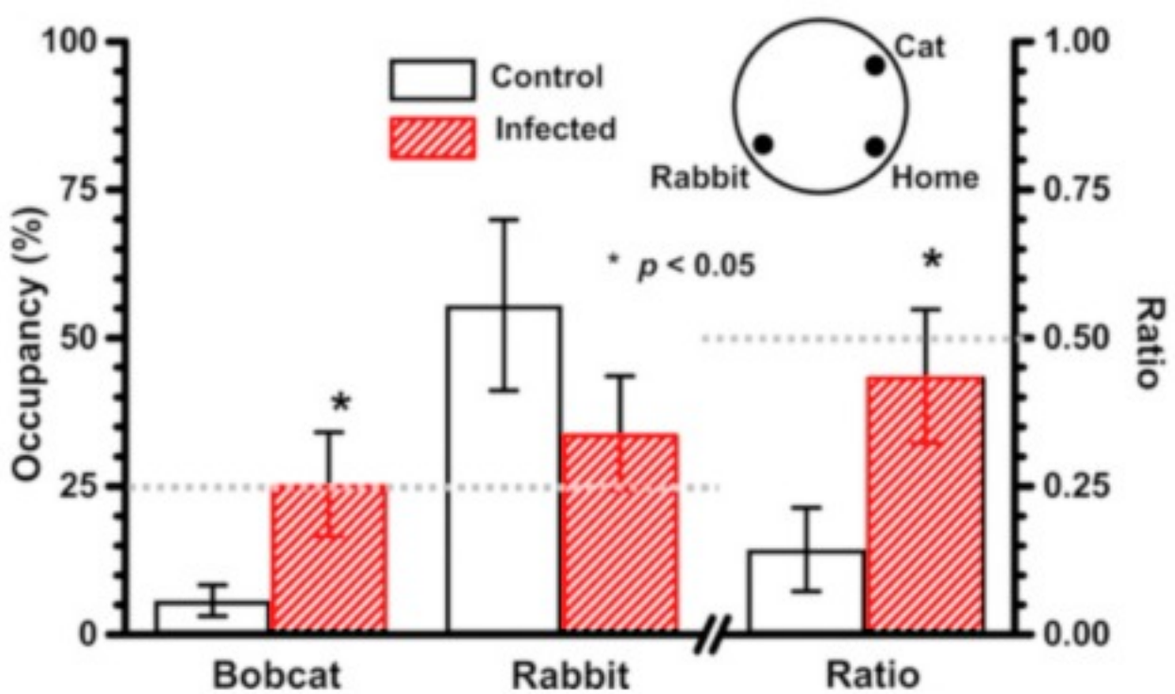
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Yolken* | 12 | BALB/c | PRU | Both | IP (400) | 8-16w | 3 chamber |




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Vyas* | 07 | BALB/c | PRU | F | IP (400) | 4-5w | Circle |



# (Why cat urine as opposed to cat?)

‘direct‘

cat

cat is here

fear, freezing

no Toxo effect

‘indirect’

cat urine
(www.bobcatpee.com)

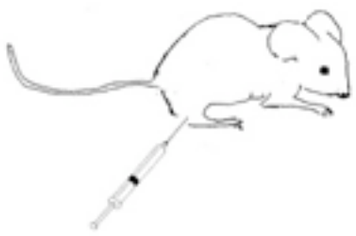
cat was here

anxiety, exploration, some freezing

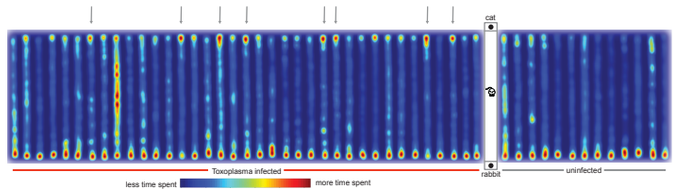
**Toxo effect**

# IN MICE

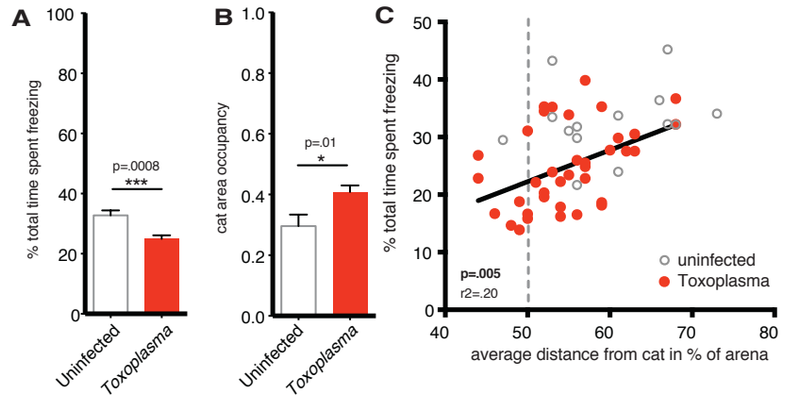
Goal: 1. Are there reproducible changes in mouse behavior from Toxo?



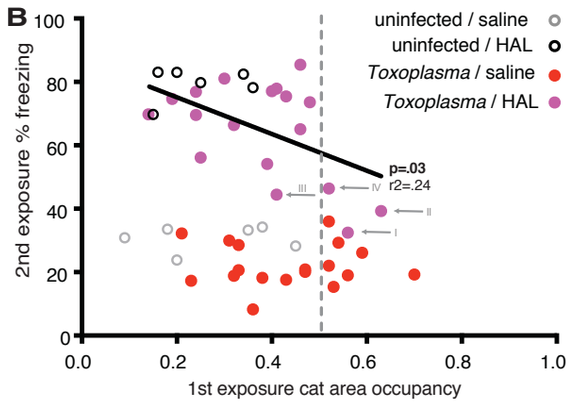
infect i.p. 400
Toxo tachyzoites



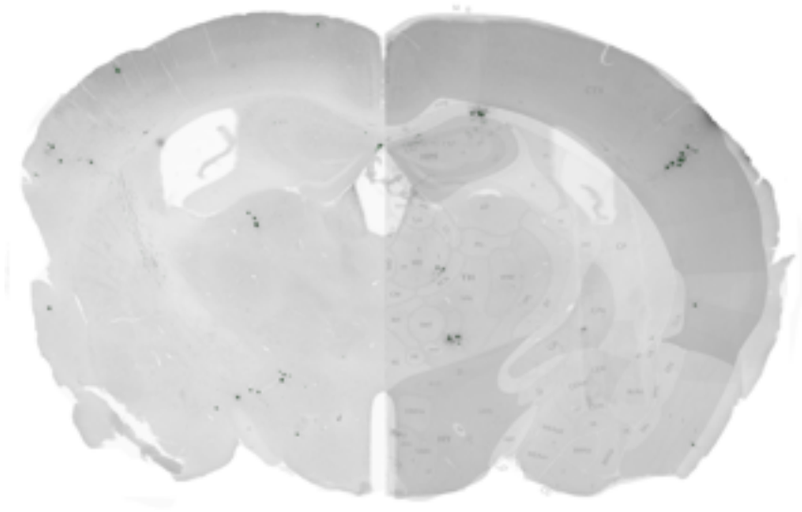

assess behavior




analyze behavior

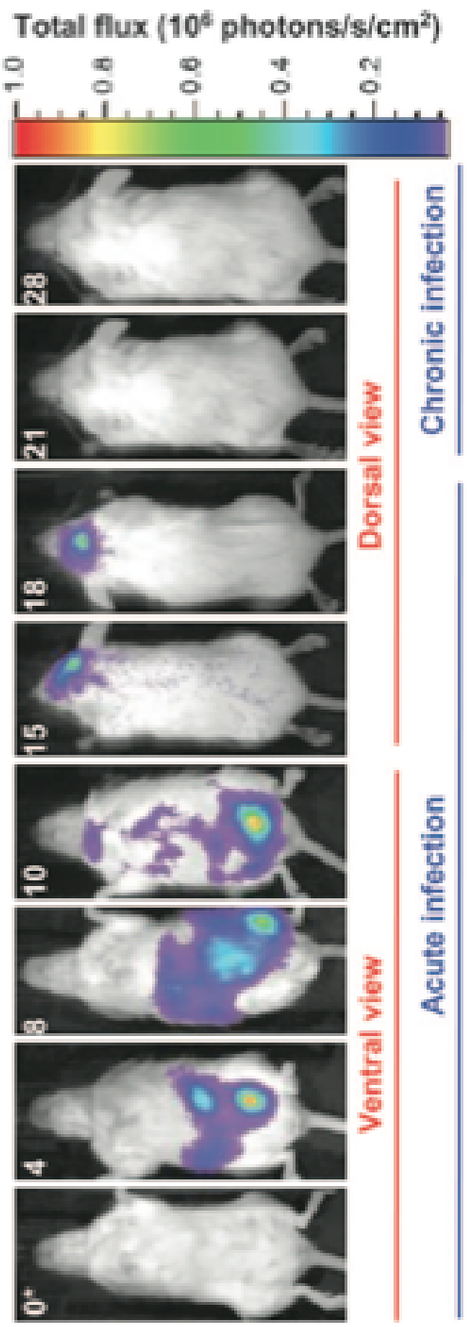


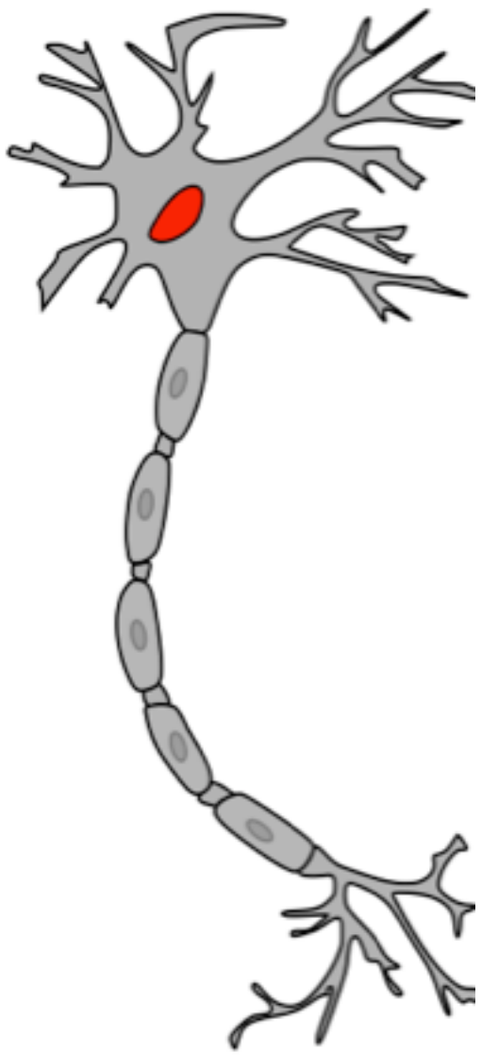

correlate location of Toxoplasma - infected neurons to behavioral measures



count all Toxoplasma-infected neurons, by region, in entire brain

# Tracking Toxo: Modify Toxo Itself to Bioluminesce



**Finds Toxo**

(Vyas et al. 2007 *PNAS*)

# THE PARASITE

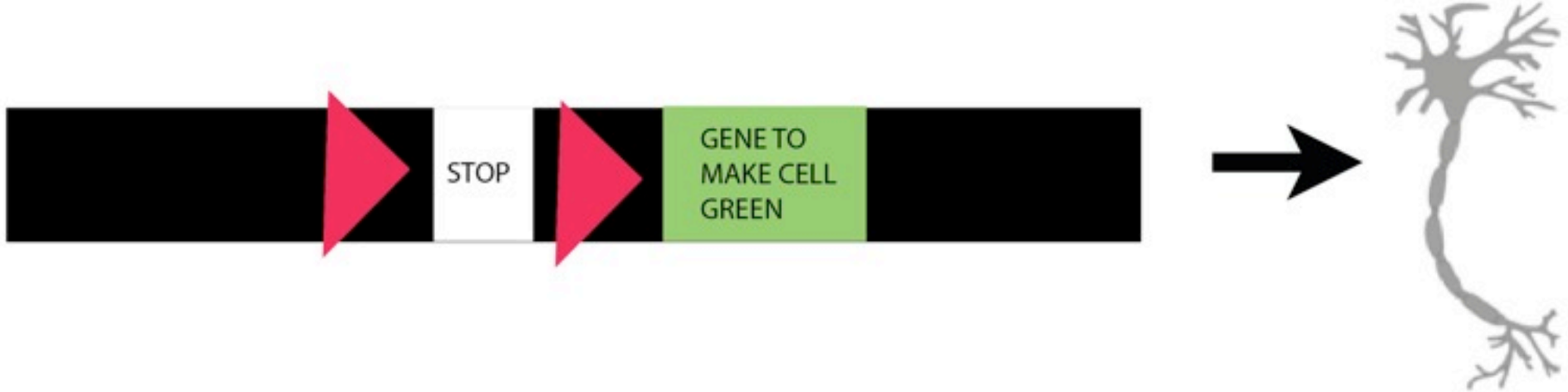
## A Genetically Modified Parasite Lets us Track Toxo to Single Neurons

BRIEF COMMUNICATION ABSTRACT

*Nature Methods* **7**, 307 - 309 (2010)
Published online: 7 March 2010 | doi:10.1038/nmeth.1438

### *Toxoplasma* secreting Cre recombinase for analysis of host-parasite interactions

Anita A Koshy[1,2], Ashley E Fouts[1,4], Melissa B Lodoen[1,4], Ozan Alkan[1,3,4], Helen M Blau[1,3] & John C Boothroyd[1]



# THE PARASITE

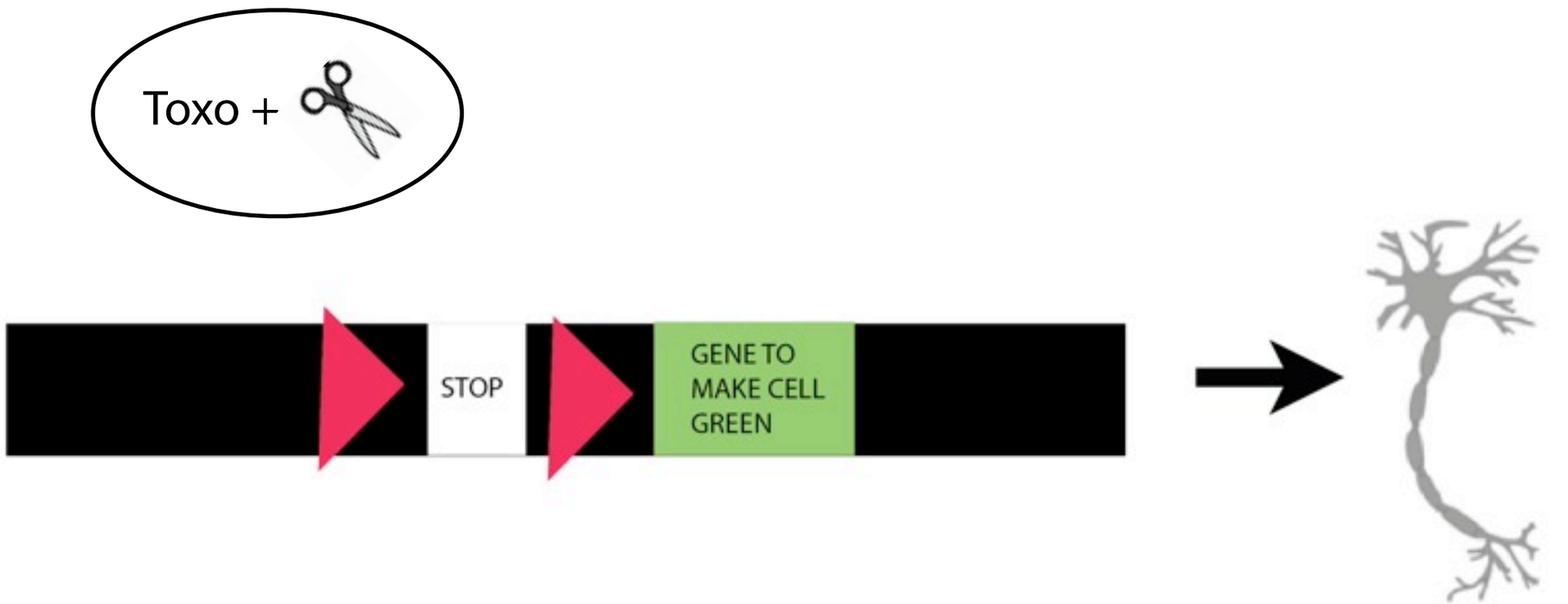
## A Genetically Modified Parasite Lets us Track Toxo to Single Neurons

**BRIEF COMMUNICATION ABSTRACT**

*Nature Methods* **7**, 307 - 309 (2010)
Published online: 7 March 2010 | doi:10.1038/nmeth.1438

### *Toxoplasma* secreting Cre recombinase for analysis of host-parasite interactions

Anita A Koshy[1,2], Ashley E Fouts[1,4], Melissa B Lodoen[1,4], Ozan Alkan[1,3,4], Helen M Blau[1,3] & John C Boothroyd[1]



# THE PARASITE

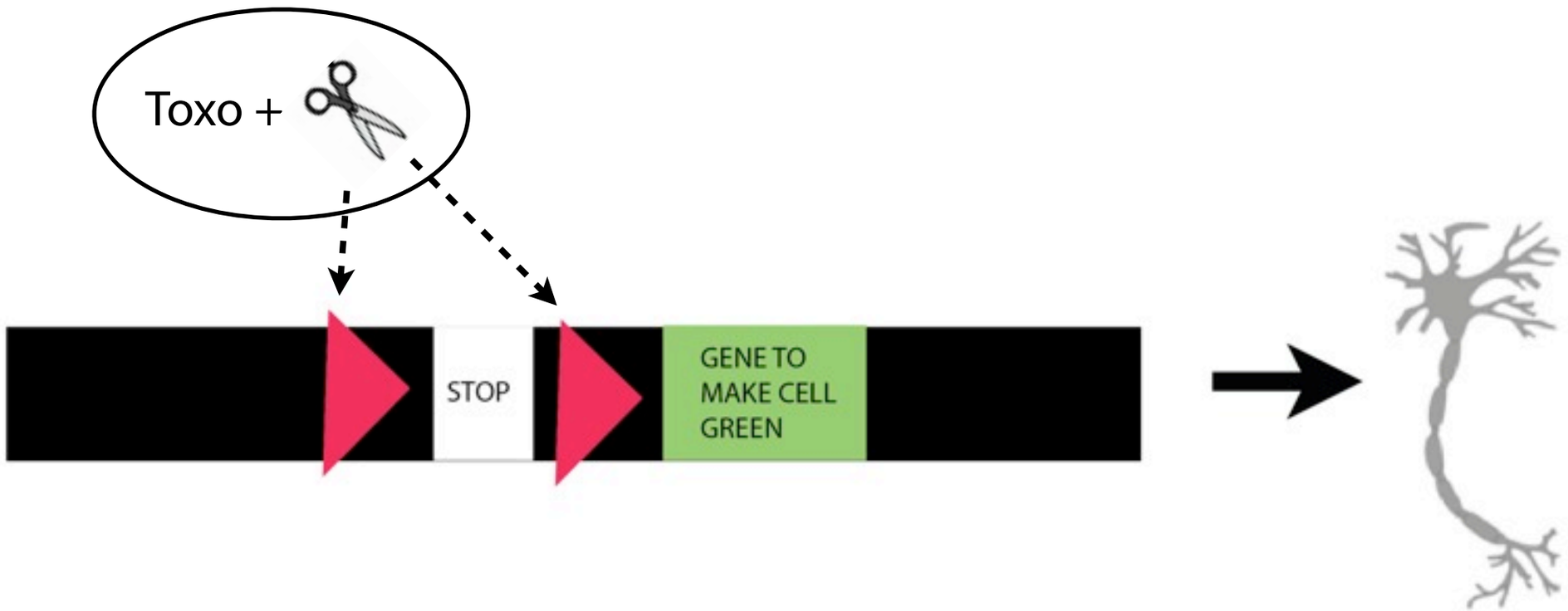
## A Genetically Modified Parasite Lets us Track Toxo to Single Neurons

BRIEF COMMUNICATION ABSTRACT

*Nature Methods* **7**, 307 - 309 (2010)
Published online: 7 March 2010 | doi:10.1038/nmeth.1438

### *Toxoplasma* secreting Cre recombinase for analysis of host-parasite interactions

Anita A Koshy[1,2], Ashley E Fouts[1,4], Melissa B Lodoen[1,4], Ozan Alkan[1,3,4], Helen M Blau[1,3] & John C Boothroyd[1]



# THE PARASITE

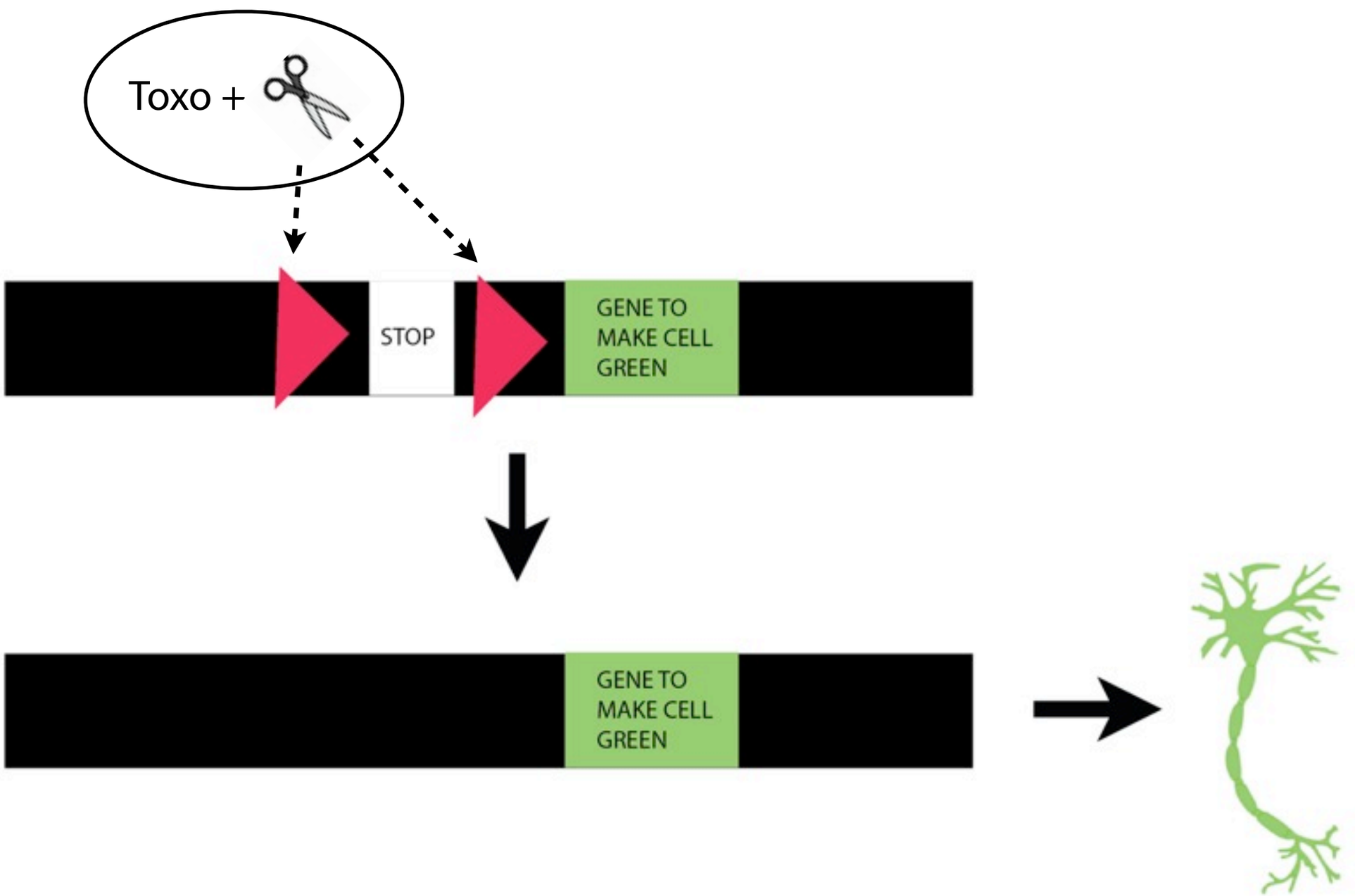
## A Genetically Modified Parasite Lets us Track Toxo to Single Neurons

BRIEF COMMUNICATION ABSTRACT

*Nature Methods* **7**, 307 - 309 (2010)
Published online: 7 March 2010 | doi:10.1038/nmeth.1438

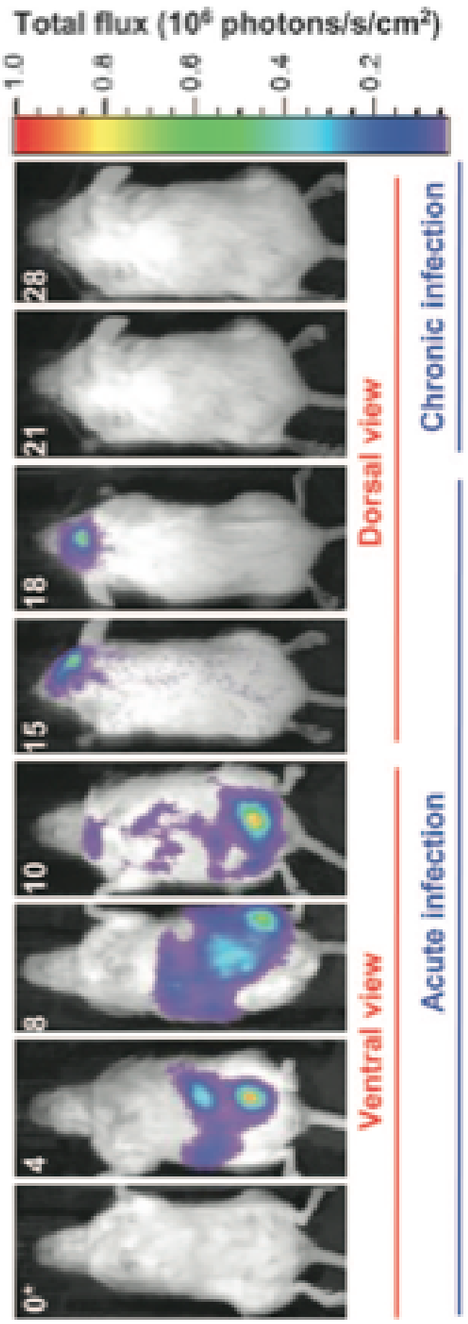
### *Toxoplasma* secreting Cre recombinase for analysis of host-parasite interactions

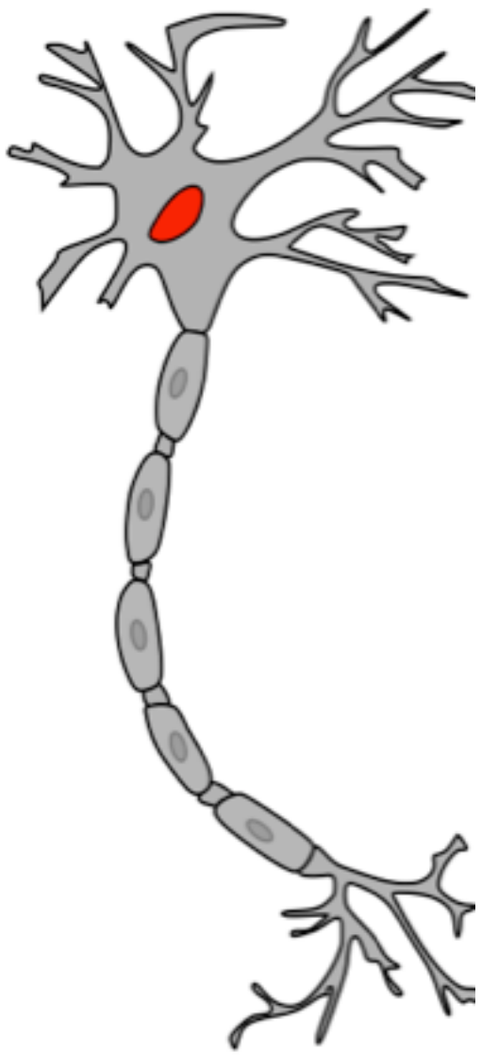
Anita A Koshy[1,2], Ashley E Fouts[1,4], Melissa B Lodoen[1,4], Ozan Alkan[1,3,4], Helen M Blau[1,3] & John C Boothroyd[1]



# THE PARASITE

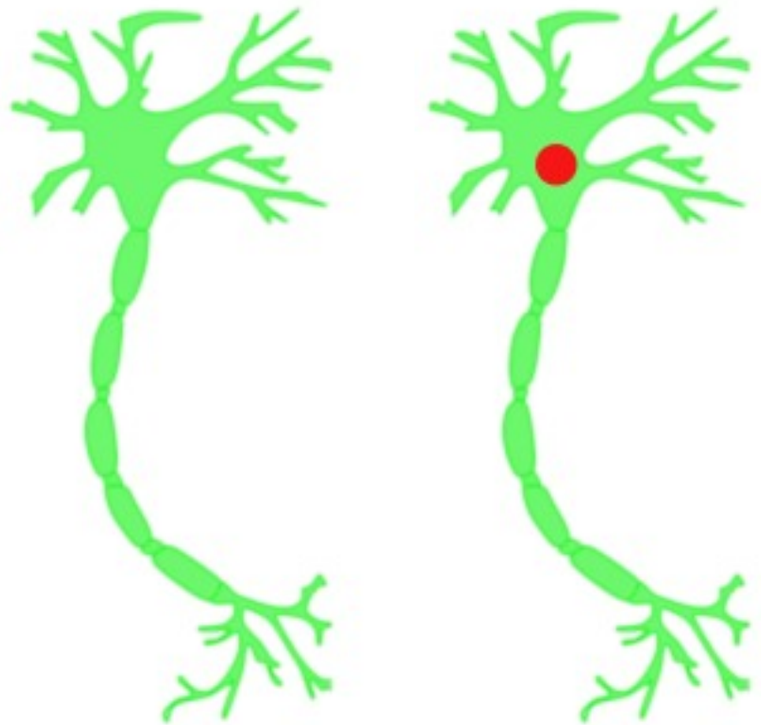
## A Genetically Modified Parasite Lets us Track Toxo to Single Neurons



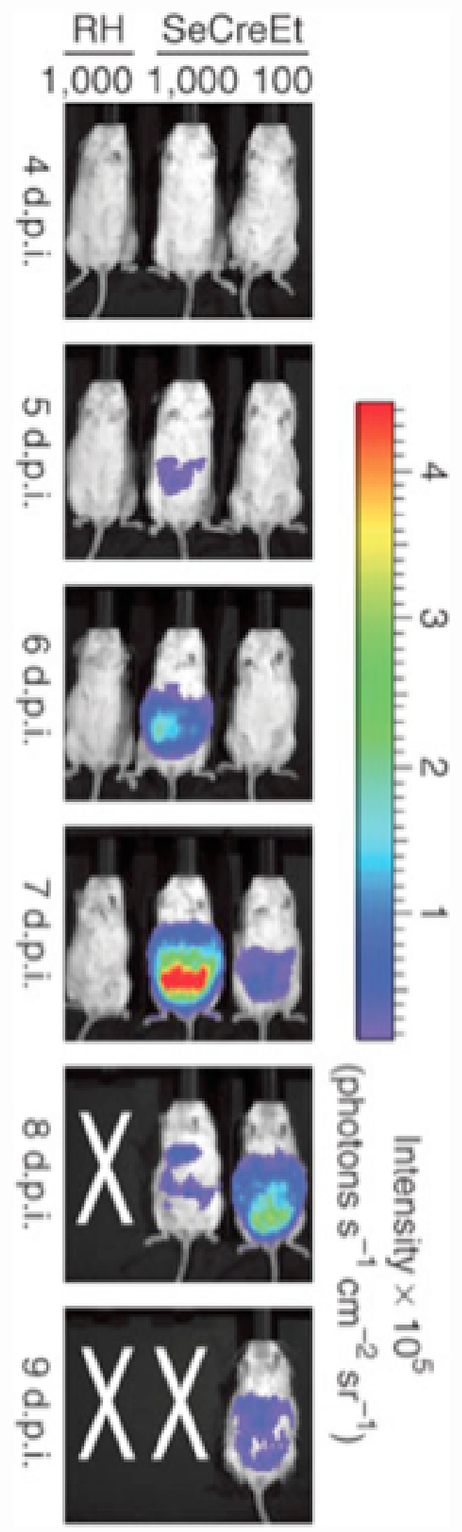



**Finds Toxo**

(Vyas et al. 2007 *PNAS*)



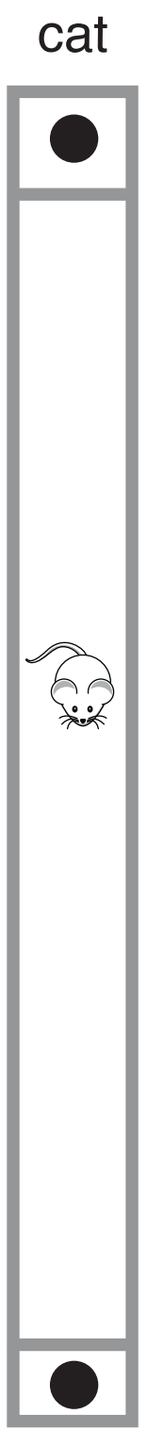
**Finds what Toxo infects**




(Koshy et al. 2010 *Nature Methods*)

# IN MICE

Goal: 1. Are there reproducible changes in mouse behavior from Toxo?




## Infection Details

| | |
|---|---|
| Mouse: | B6 (ROSA26) |
| Strain: | TypeII PcA |
| Route: | IP (400 tachys) |
| Sex: | M |
| Week p.i.: | 9 w |
| Test: | Cat Urine Aversion |

## Behavior Details

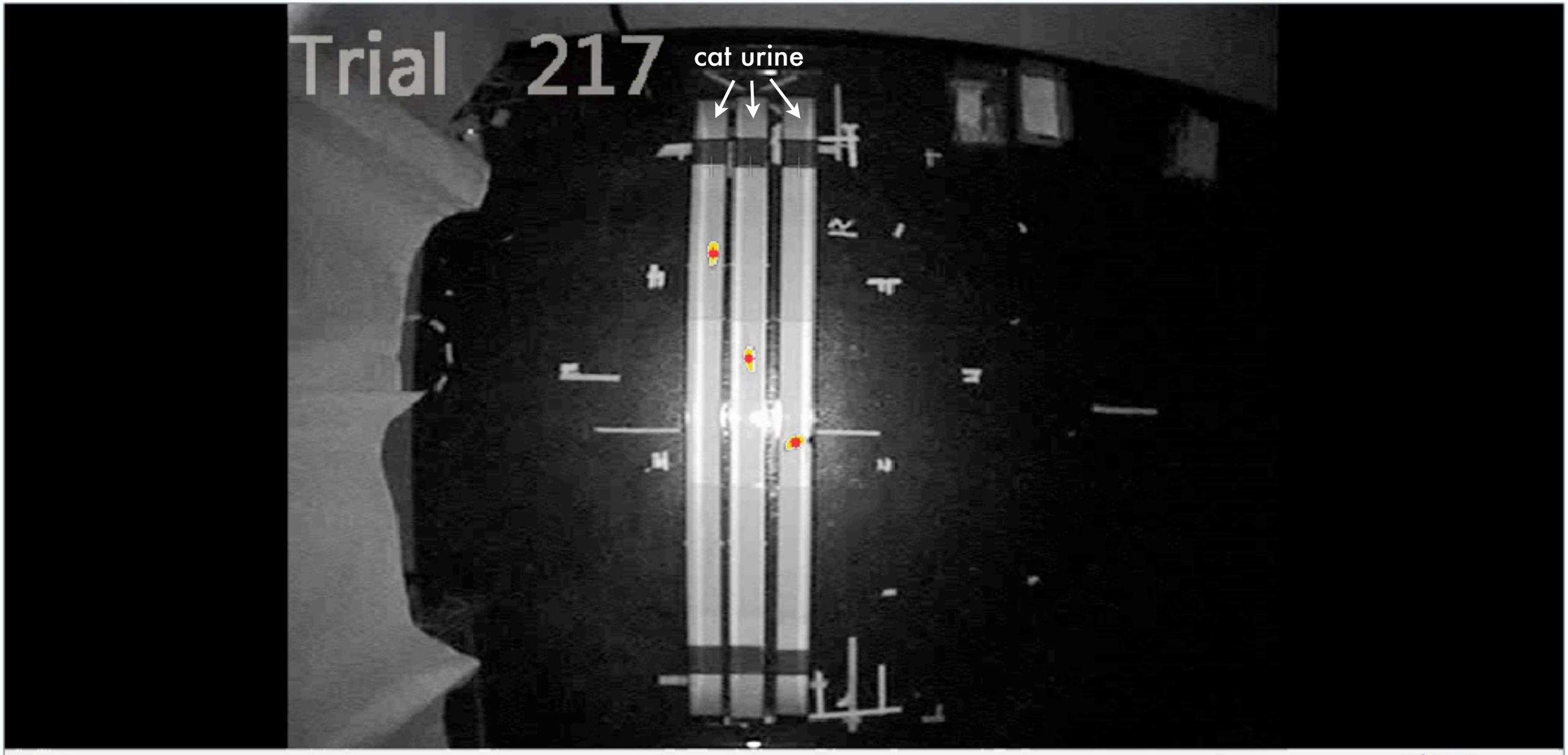
**DISTANCE** = total distance traveled in cm;

| | | |
|---|---|---|
| *Graham* | 84 | increased movement in Toxo mice |
| *Hutchinson* | 80 | increased movement in Toxo mice |
| *Yolken* | 12 | increased movement in Toxo mice |

**FREEZING** = % of total time spent immobile; more freezing –> more afraid

**CAT OCCUPANCY** = time spent in $\frac{\text{cat quadrant}}{(\text{cat quadrant} + \text{rabbit quadrant})}$

PRED DAY 3 Zs 21 1 2 3 _OPF01_OPF02_OPF03_OPF04_0001.avi




Position:

Video Time
0:00:00.000

DISTANCE

FREEZING

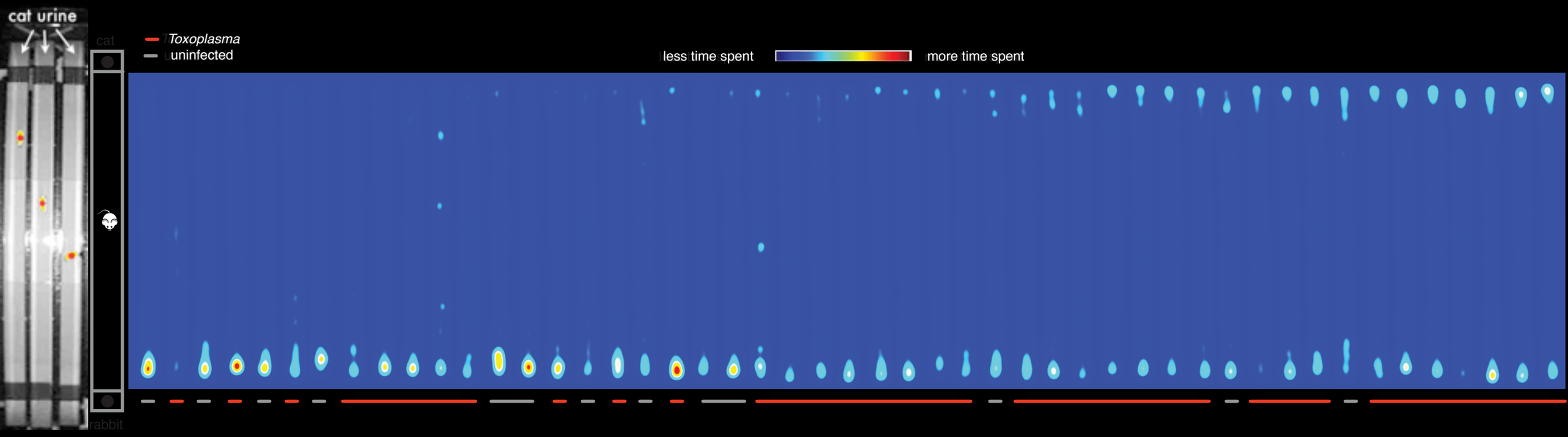
CAT OCCUPANCY

Trial Status | Independent Variables | Dependent Variables | Manu

| Trial | Arena | Velocity | Distance moved | Mobility state | | Distance to point | | |
|---|---|---|---|---|---|---|---|---|
| | | ... | ... | ... | | ... | | |
| | | Center-point | Center-point | Mobile | Immobile | L Cat / Center-point | M Cat / Center-point | R Cat / Center-point |
| | | Current | Total | Current | Current | Mean | Mean | Mean |
| | | cm/s | cm | | | cm | cm | cm |
| Trial 217 | Arena 1 | - | - | - | - | - | - | - |
| | Arena 2 | - | - | - | - | - | - | - |
| | Arena 3 | - | - | - | - | - | - | - |

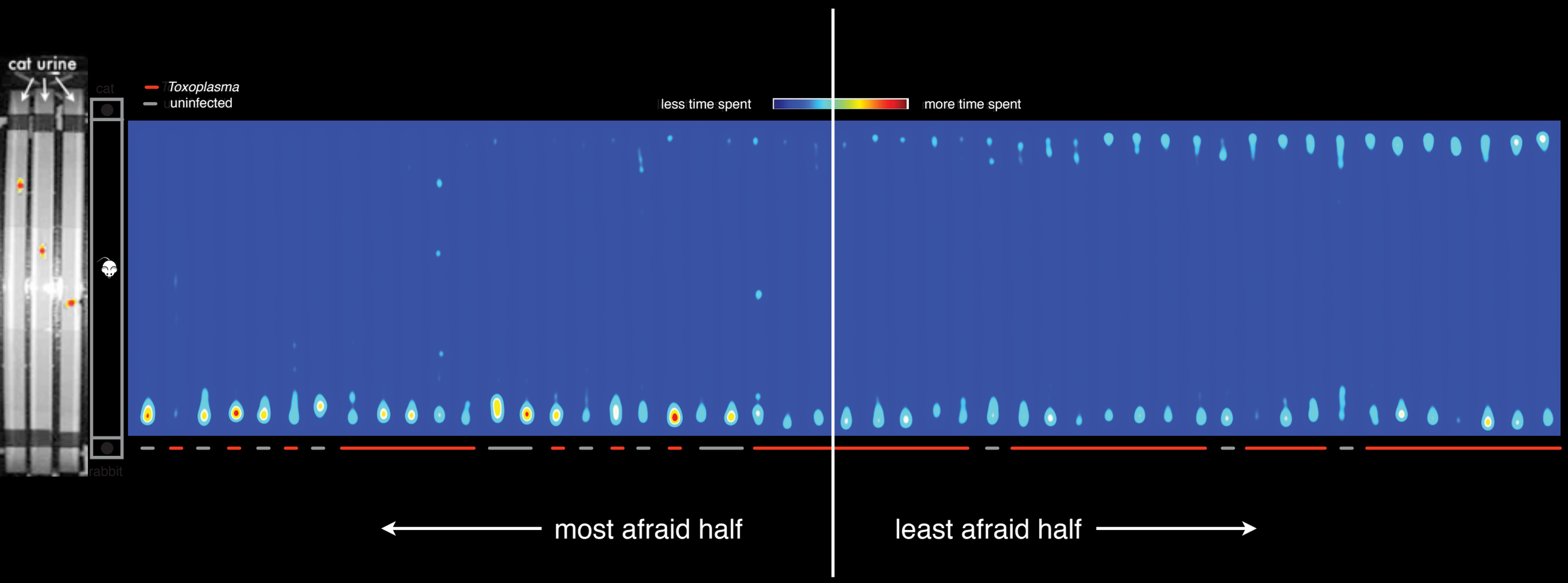
# IN MICE

## Infected mice spend more time closer to cat urine; high individual variability



# IN MICE

## Infected mice spend more time closer to cat urine; high individual variability

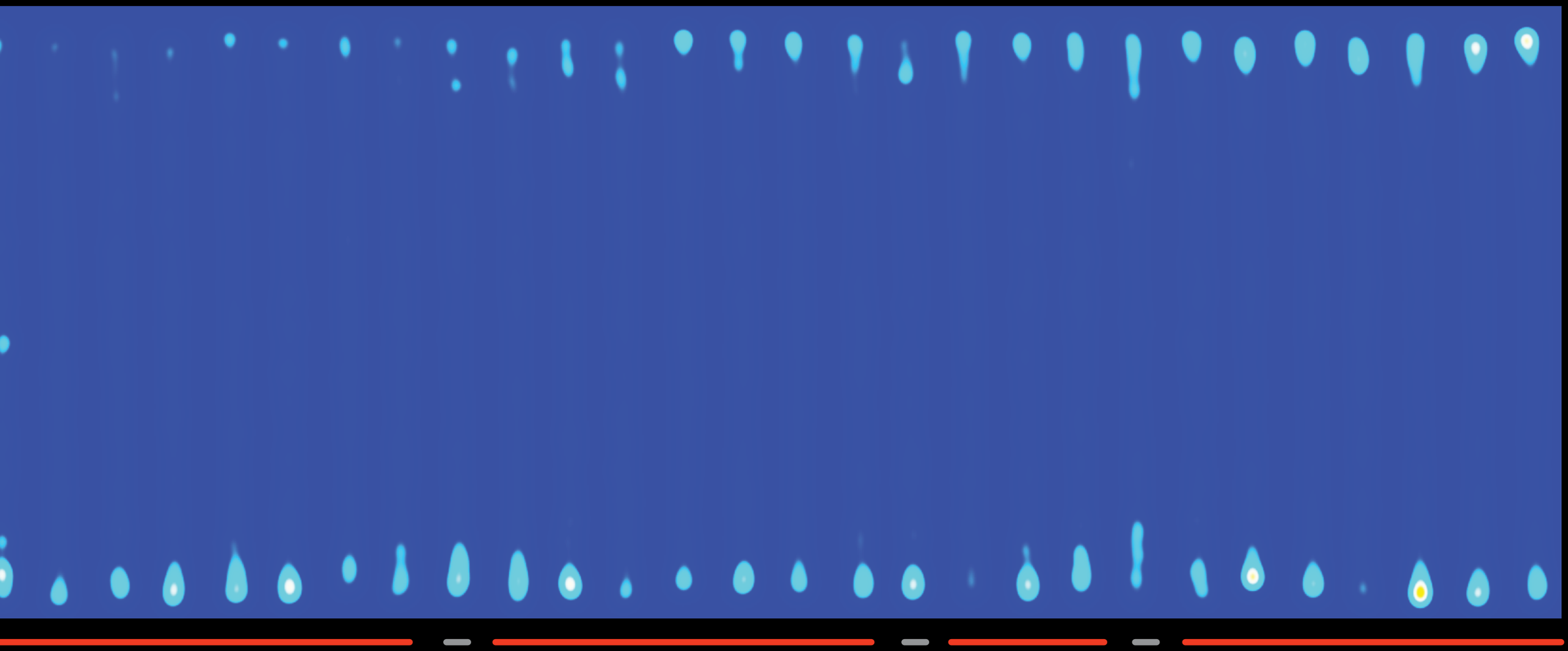


# IN MICE

Infected mice spend more time closer to cat urine; high individual variability

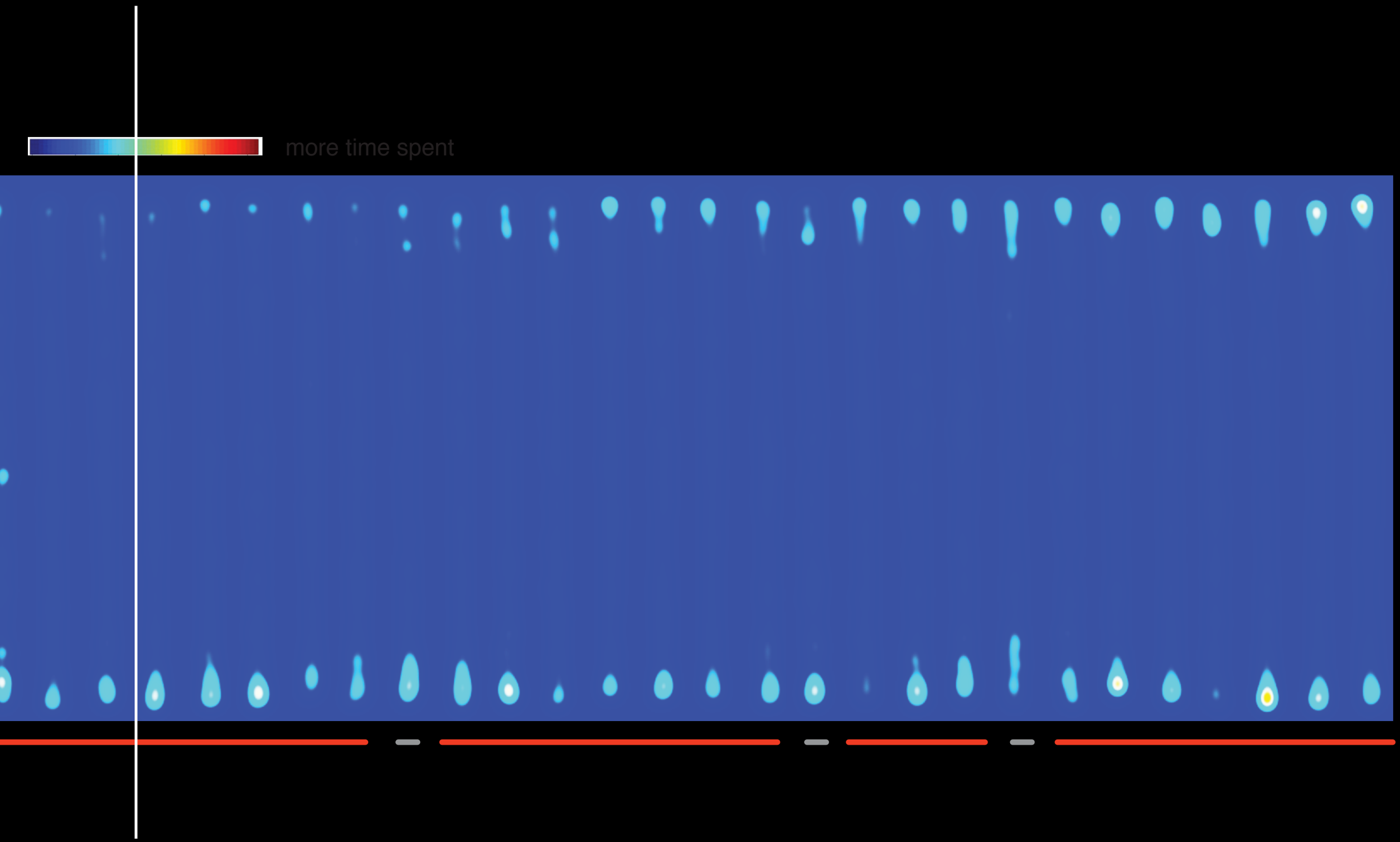


more time spent



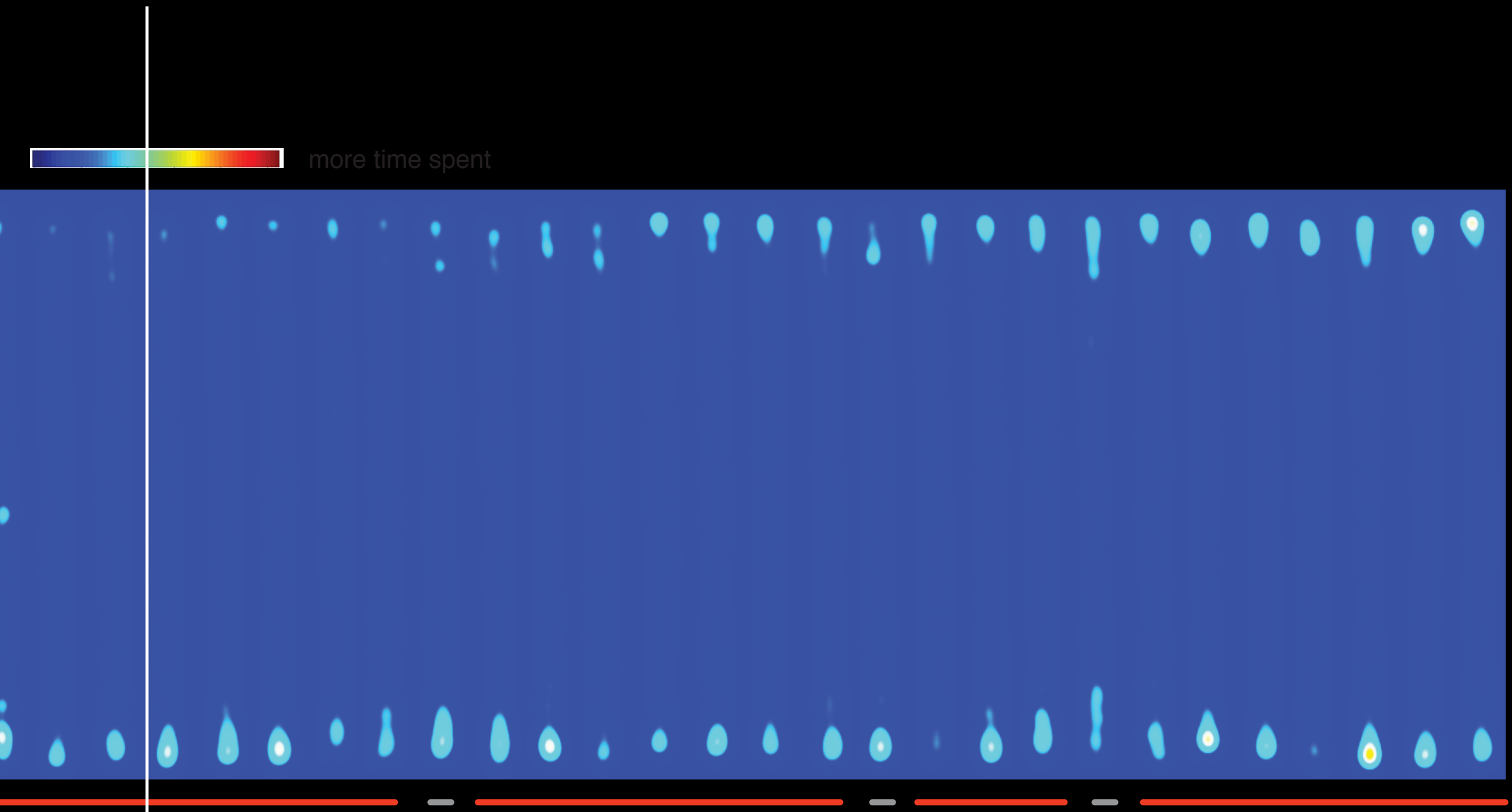
# IN MICE

Infected mice spend more time closer to cat urine; high individual variability



# IN MICE

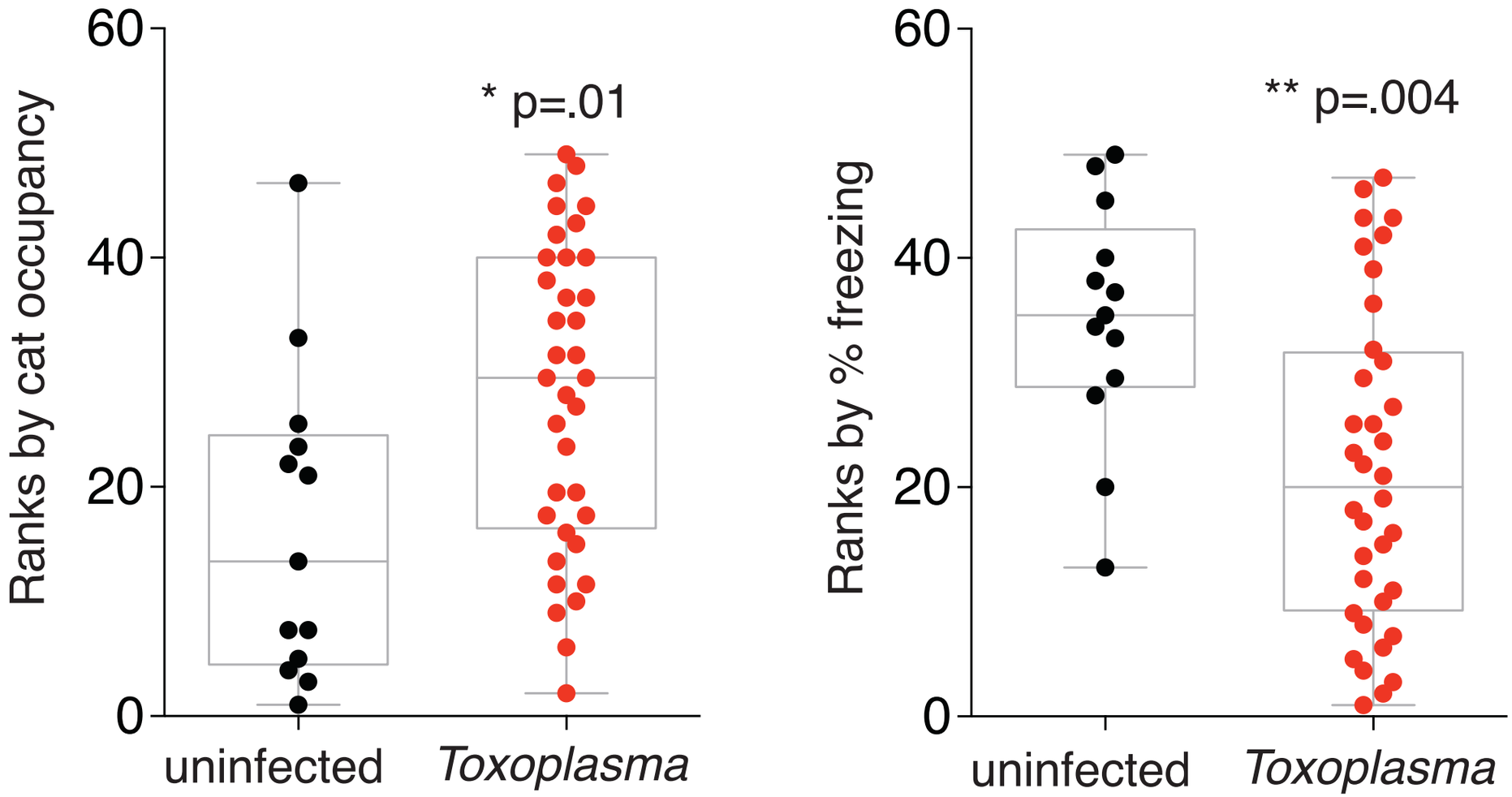
Infected mice spend more time closer to cat urine; high individual variability




- 88% of the top half of least afraid mice are Toxoplasma-infected
- if random, this number would be approximately 50%

# IN MICE

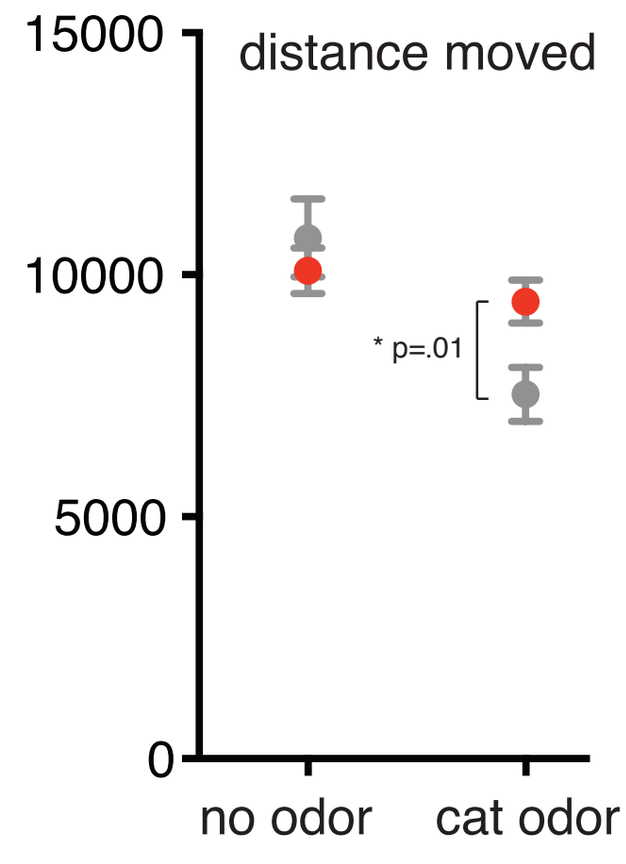
Infected mice spend more time closer to cat urine; high individual variability

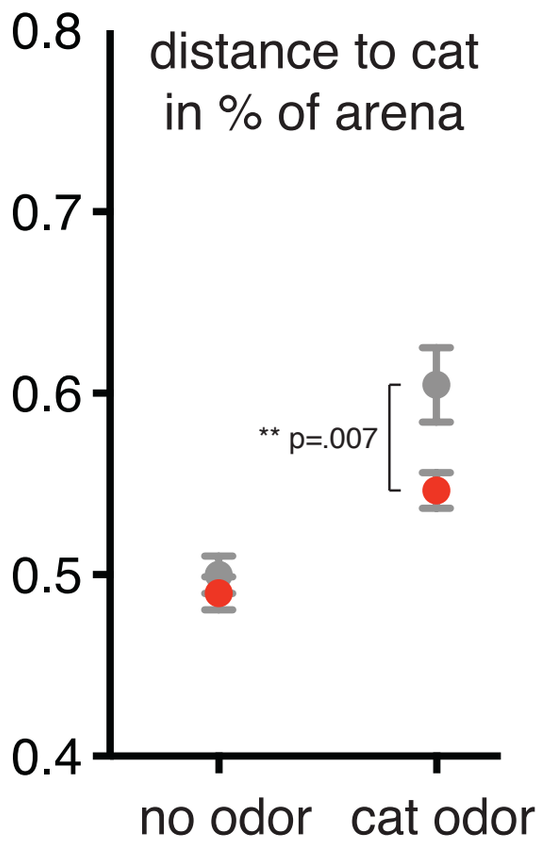


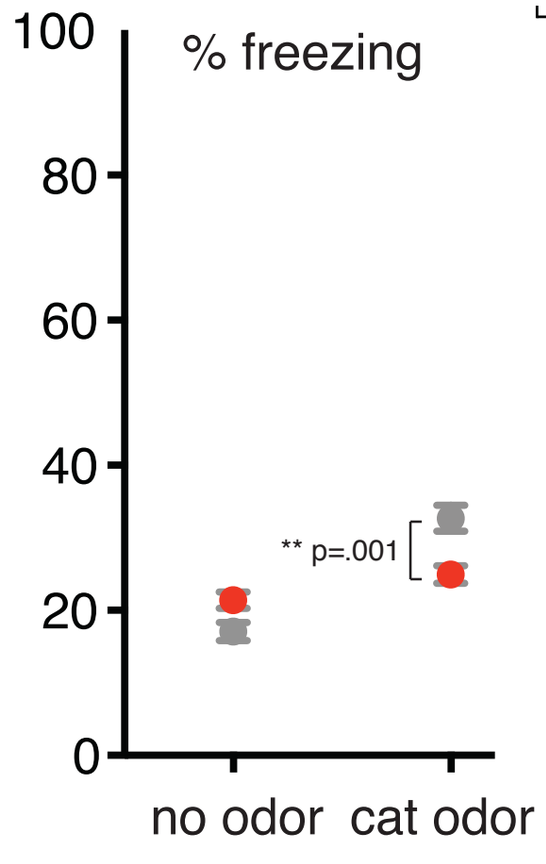

* p<.05, ** p<.01; Mann-Whitney U

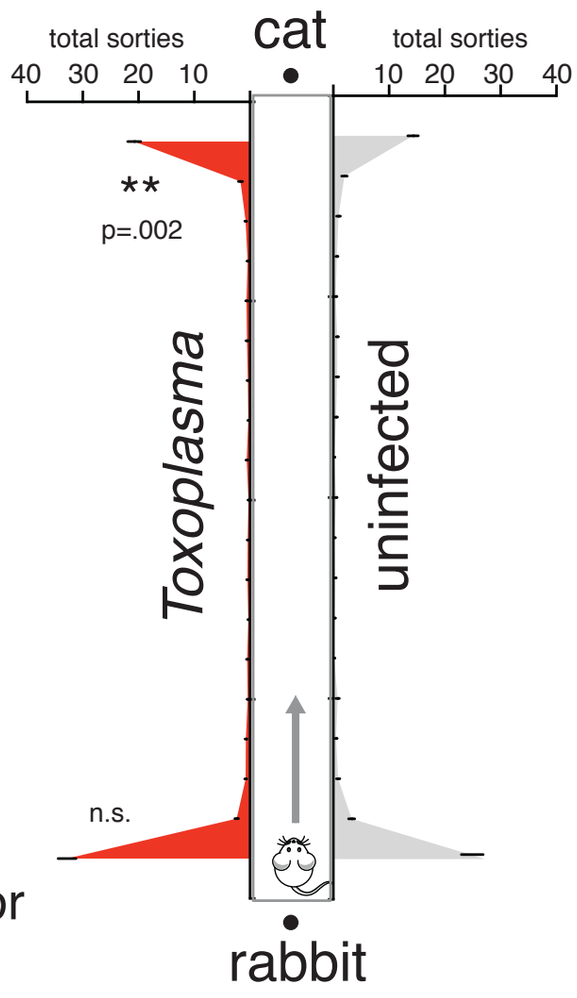
# IN MICE

## Goal: 1. Are there reproducible changes in mouse behavior from Toxo?




In the presence of cat urine, *Toxoplasma*-infected mice:

- move more; *, p=.01
- spend more time closer to the cat urine; **, p=.007
- freeze less; **, p=.001
- make more exploratory bouts to cat urine; **,p=.002

# How to Make a Toxo Disease Model

1. Establish a reproducible phenotype;



2. Pretend we know nothing about Toxo other than the behavioral phenotype



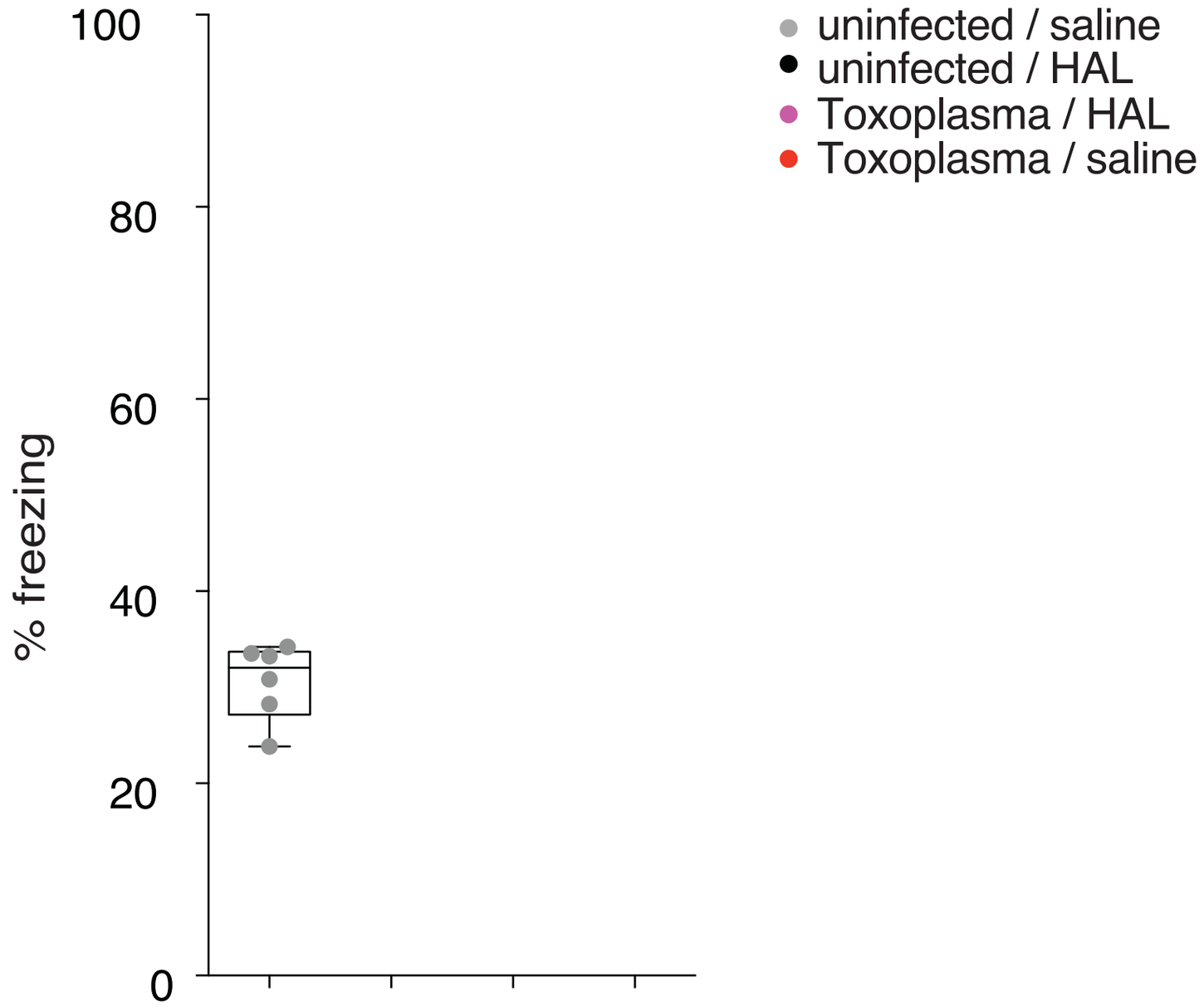
3. Screen drugs against phenotype (anti-psychotics, SSRIs);

4. Infer from drugs that interact with behavior a plausible mechanism of action for Toxo

5. Join the ranks of other disease models

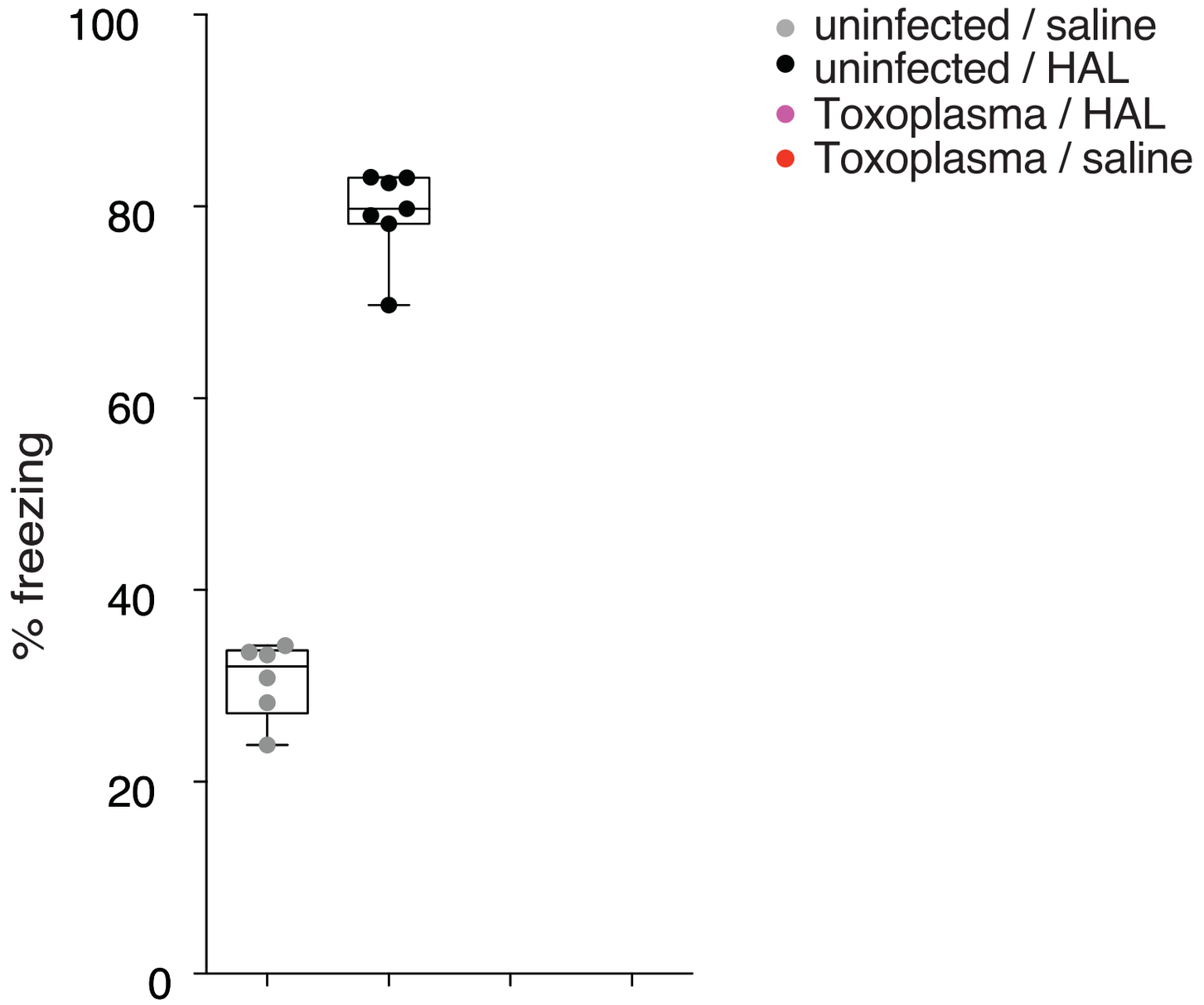
# IN MICE

## Blocking dopamine (via Haloperidol) Increases Freezing in Mice



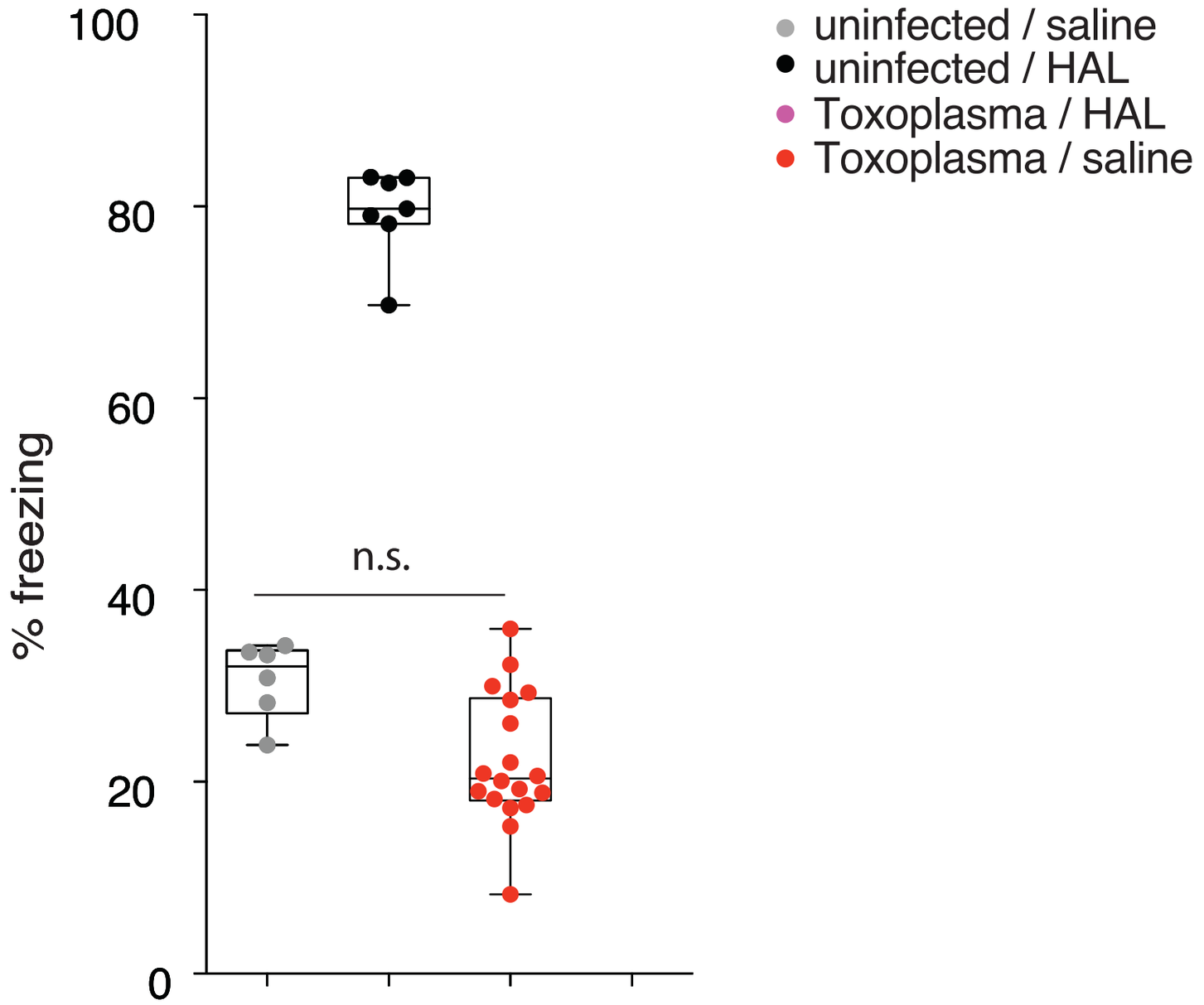
# IN MICE

## Blocking dopamine (via Haloperidol) Increases Freezing in Mice



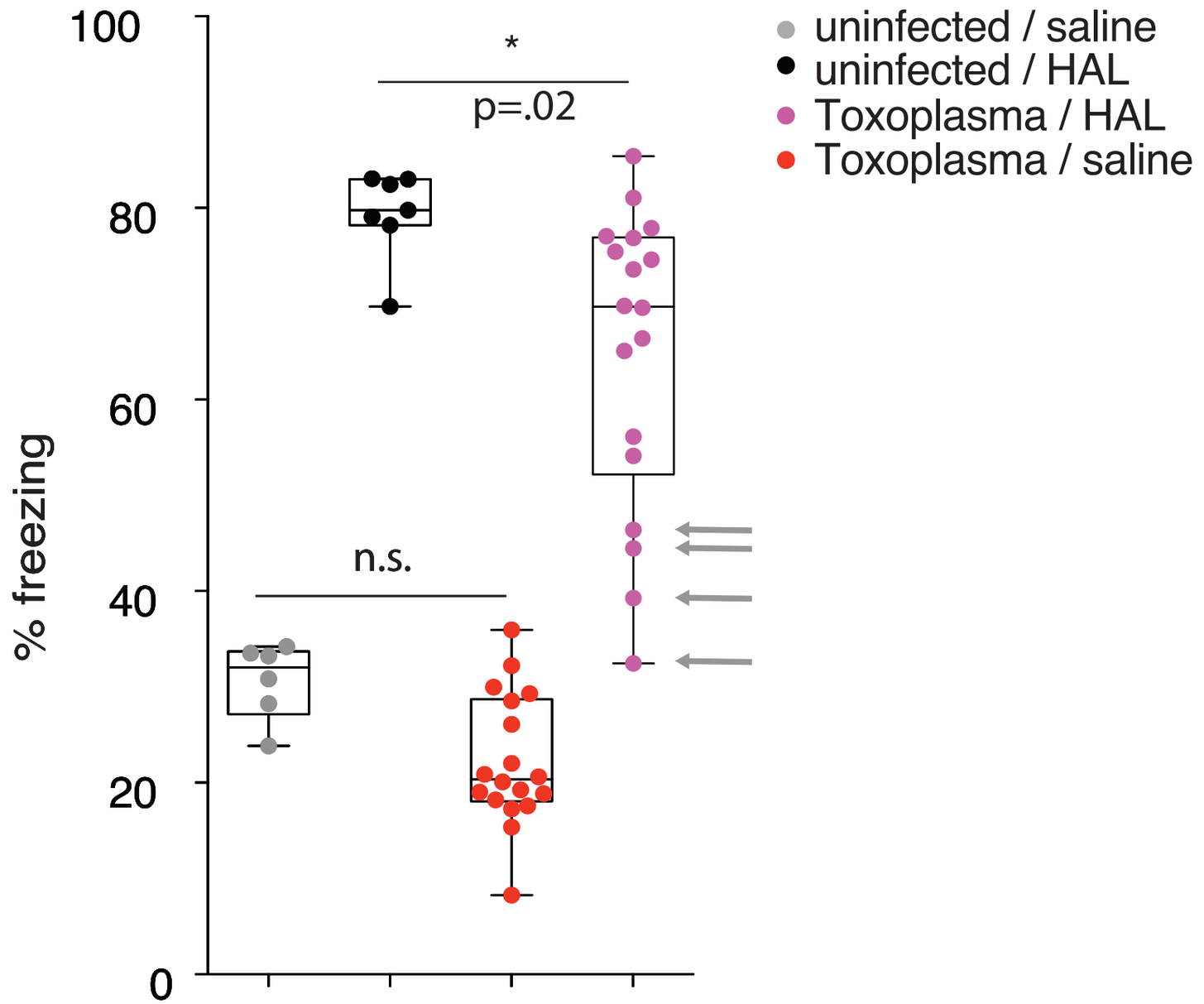
# IN MICE

## Blocking dopamine (via Haloperidol) Increases Freezing in Mice

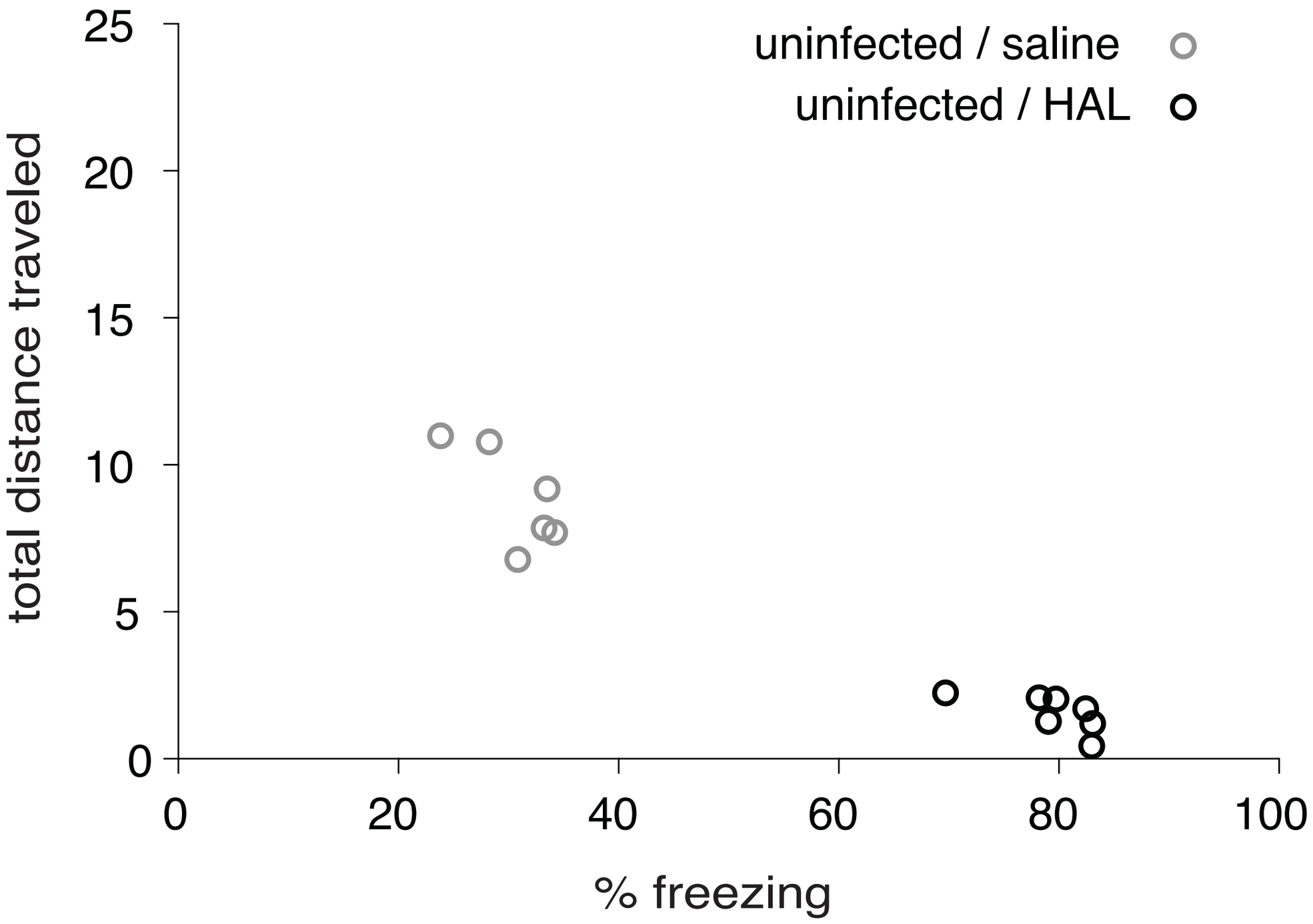


# IN MICE

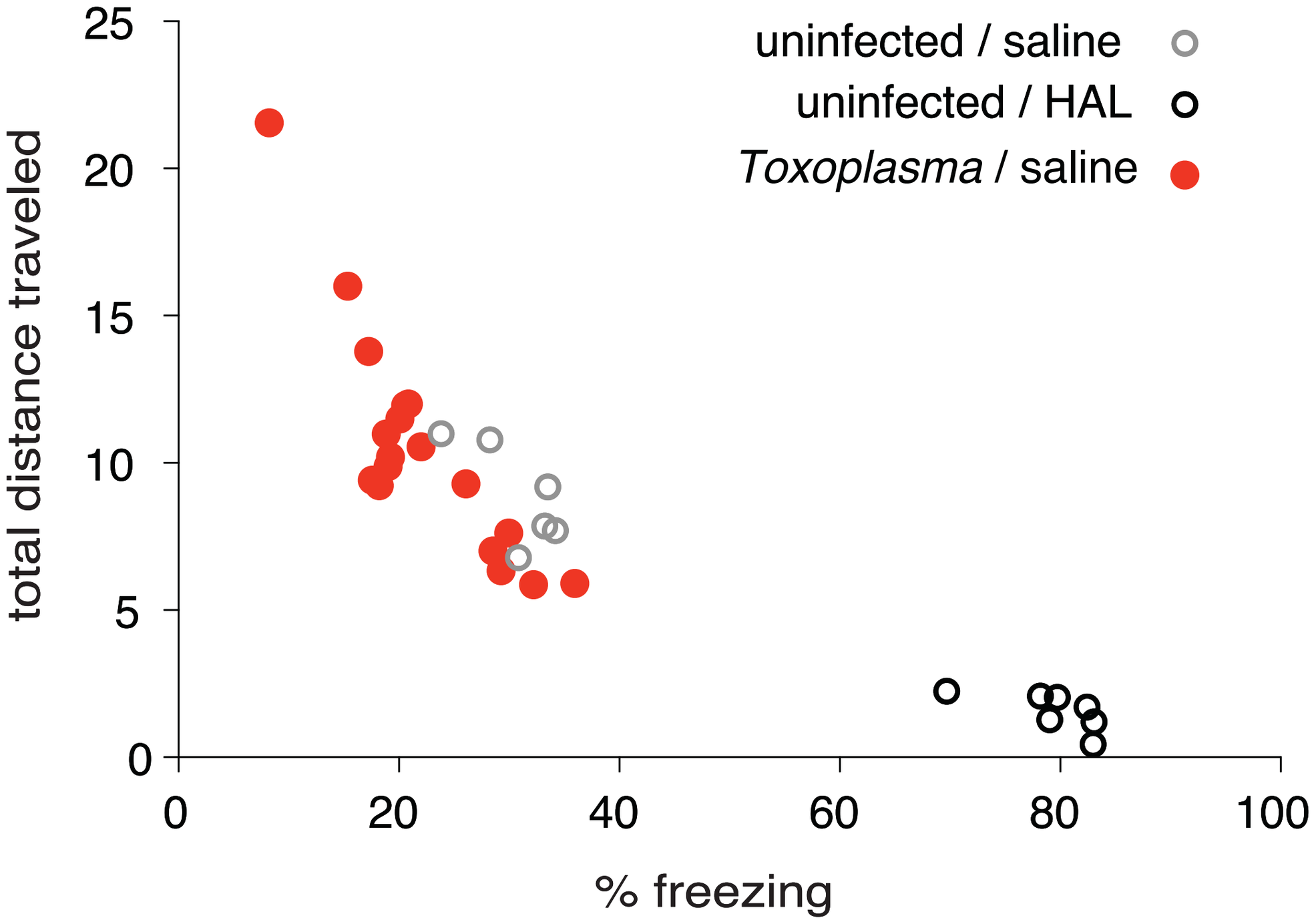
## Blocking dopamine (via Haloperidol) Increases Freezing in Mice



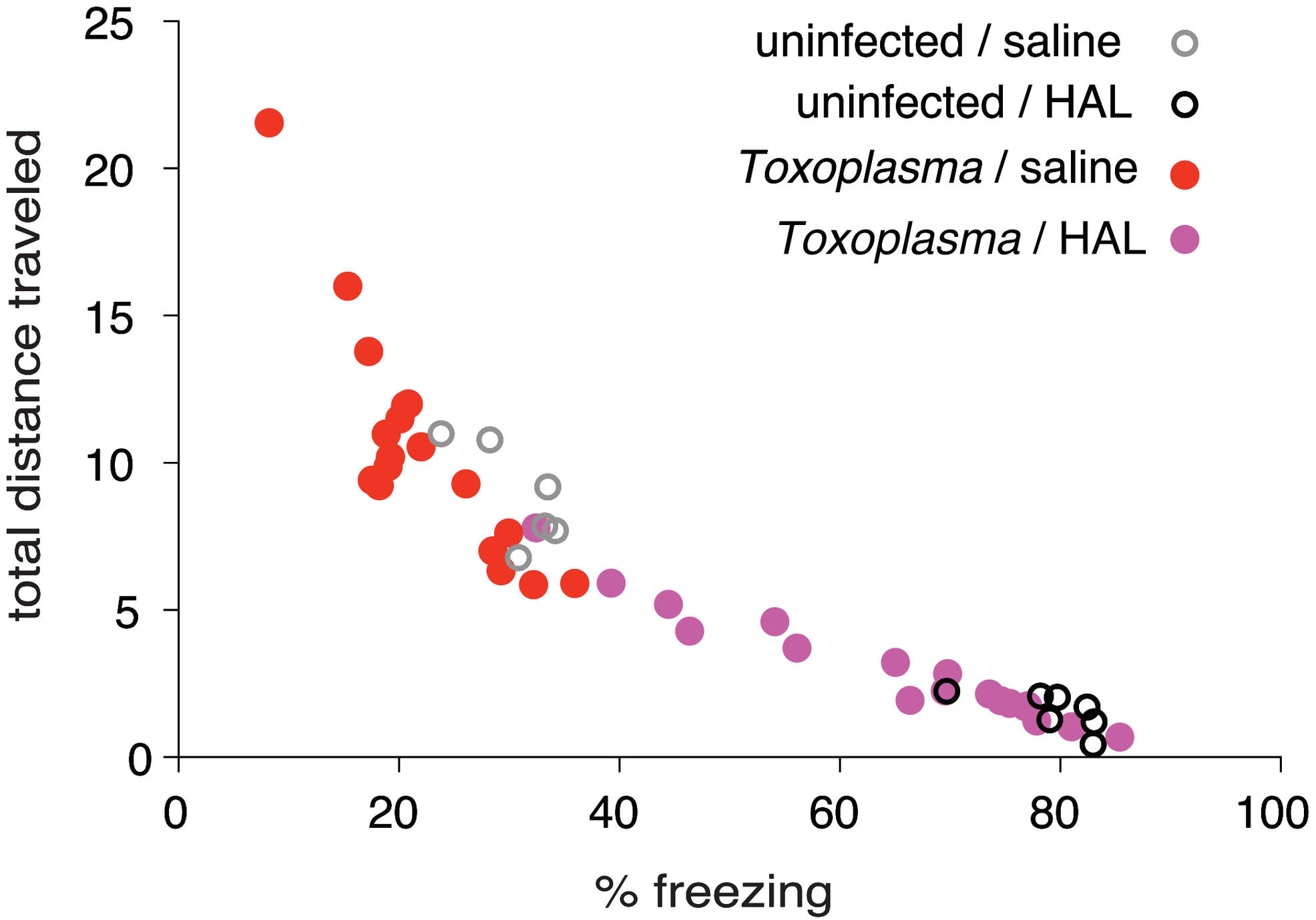
# IN MICE Toxo Infection Protects Against Dopamine Blocker (HAL) **in Some Mice**



# IN MICE Toxo Infection Protects Against Dopamine Blocker (HAL) **in Some Mice**

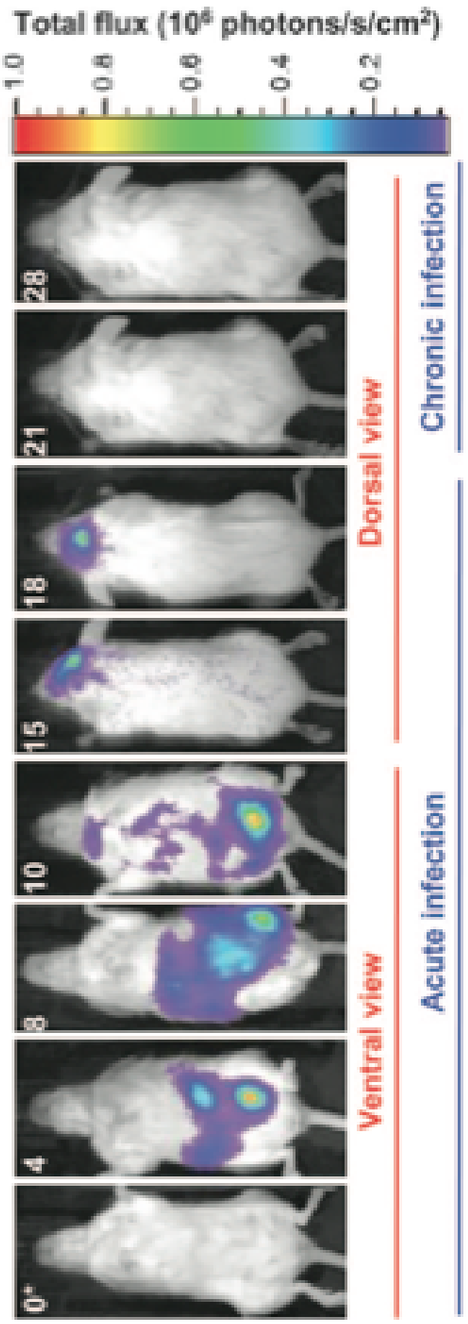


# IN MICE Toxo Infection Protects Against Dopamine Blocker (HAL) **in Some Mice**

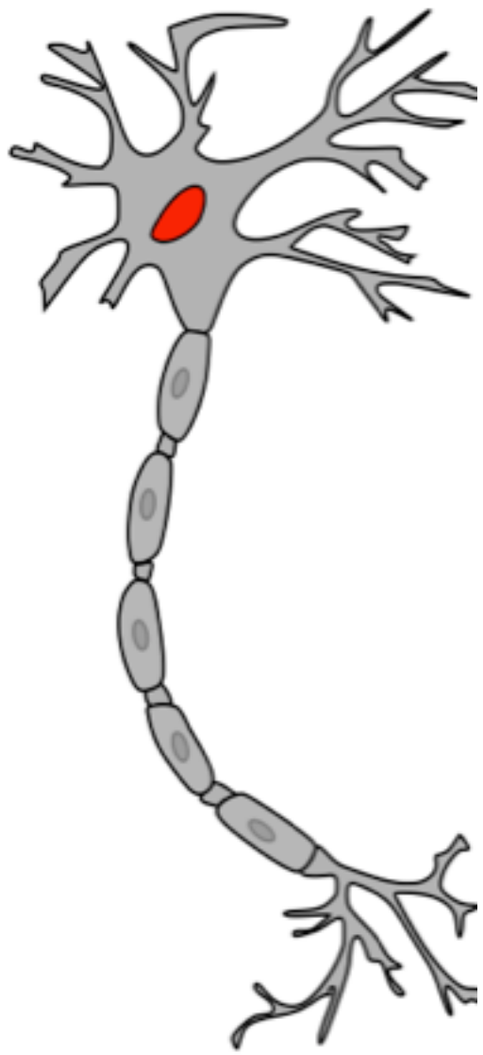


# THE PARASITE

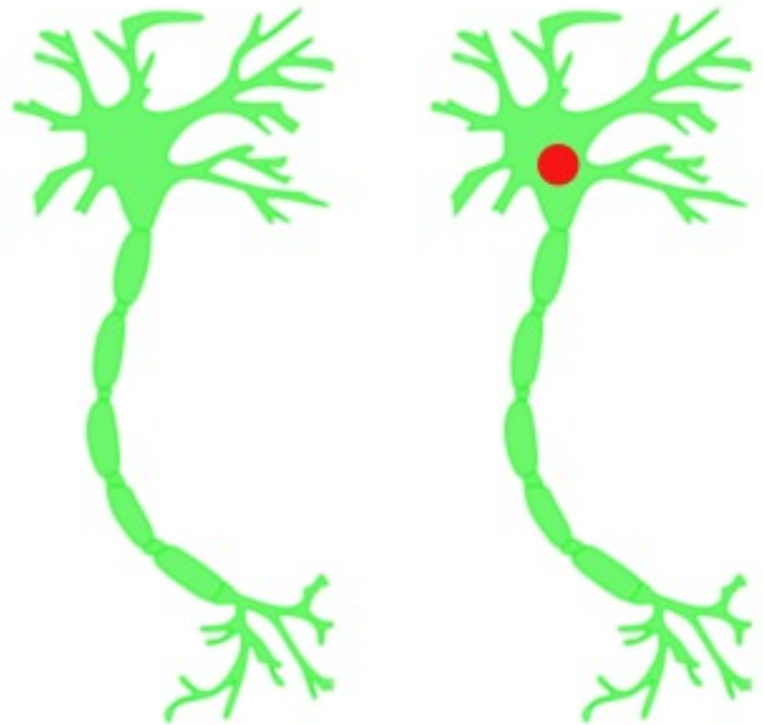
## Reminder: Genetically Modified Parasite Makes Toxo Trackable



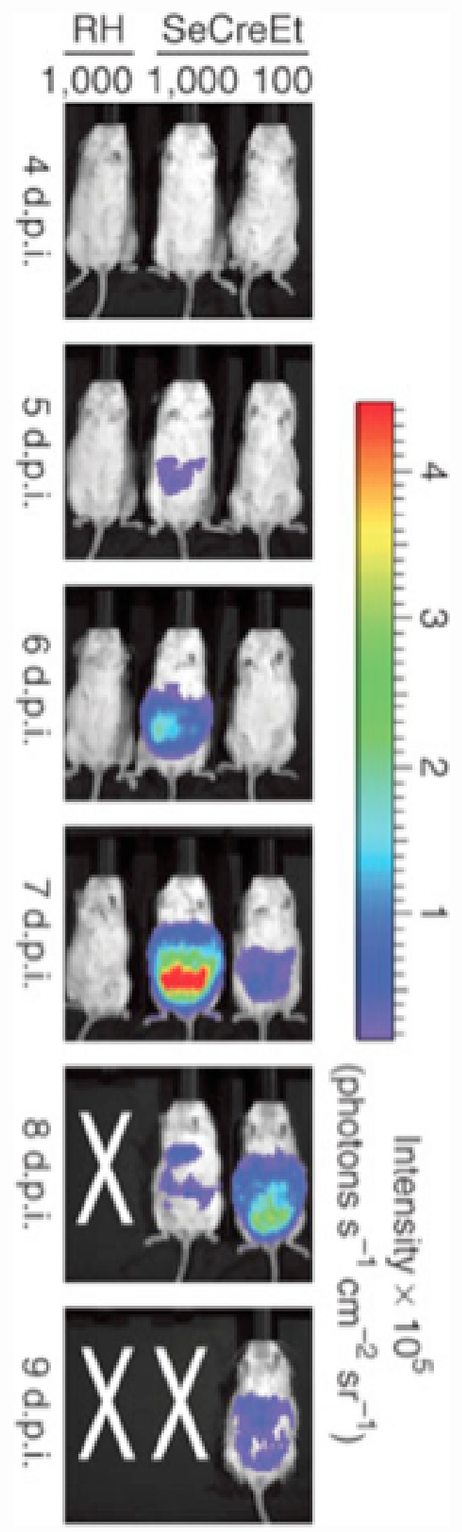

(Vyas 2007 *PNAS*)



**Finds Toxo**

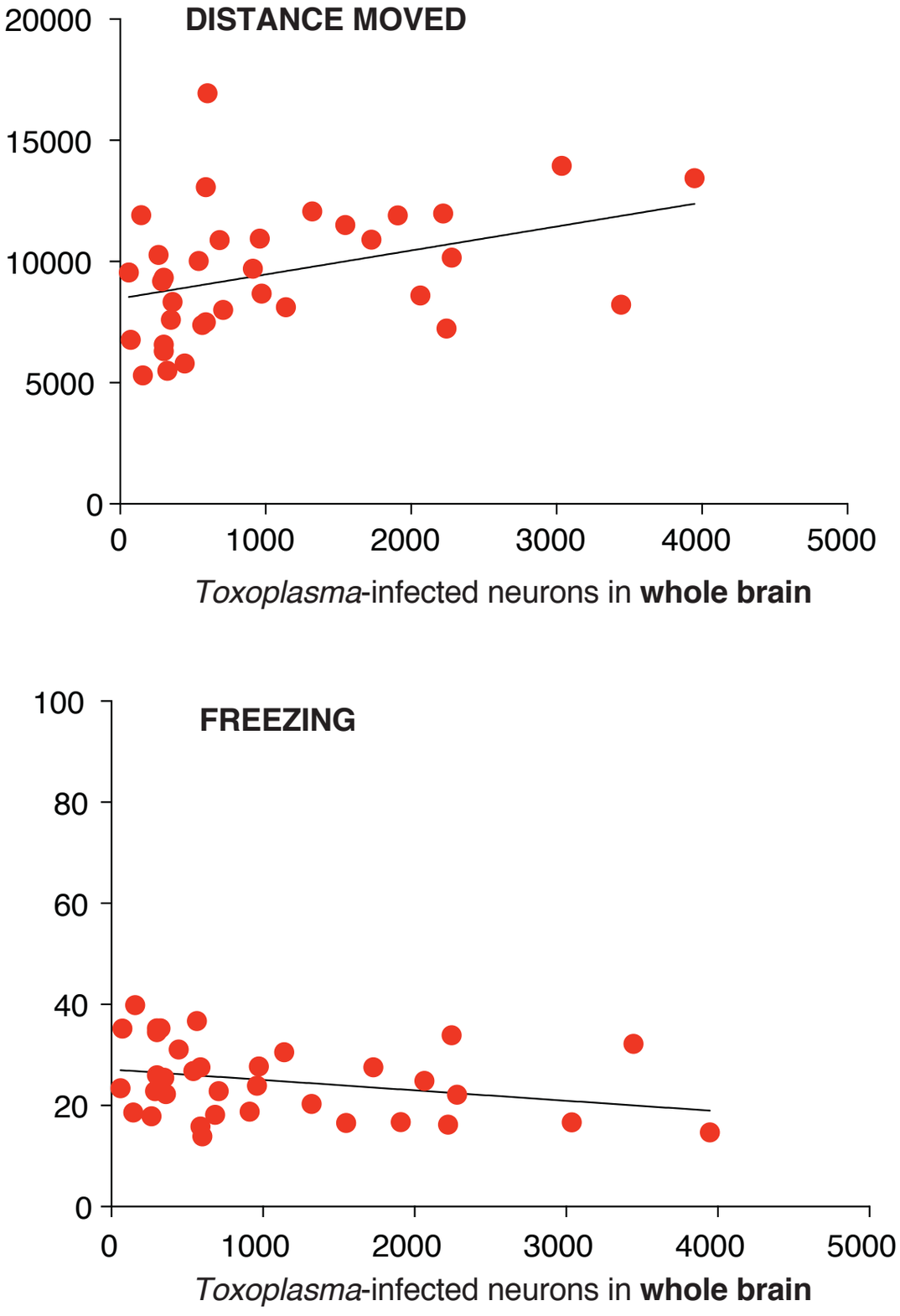


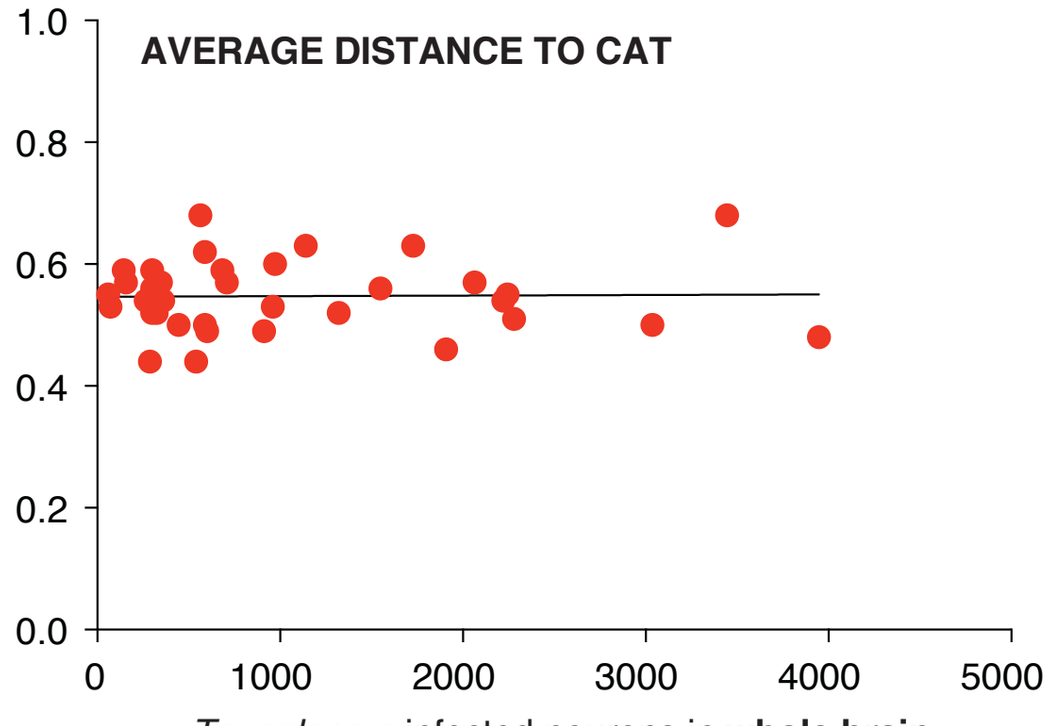
**Finds what Toxo infects**




(Koshy 2009 *Nature Methods*)

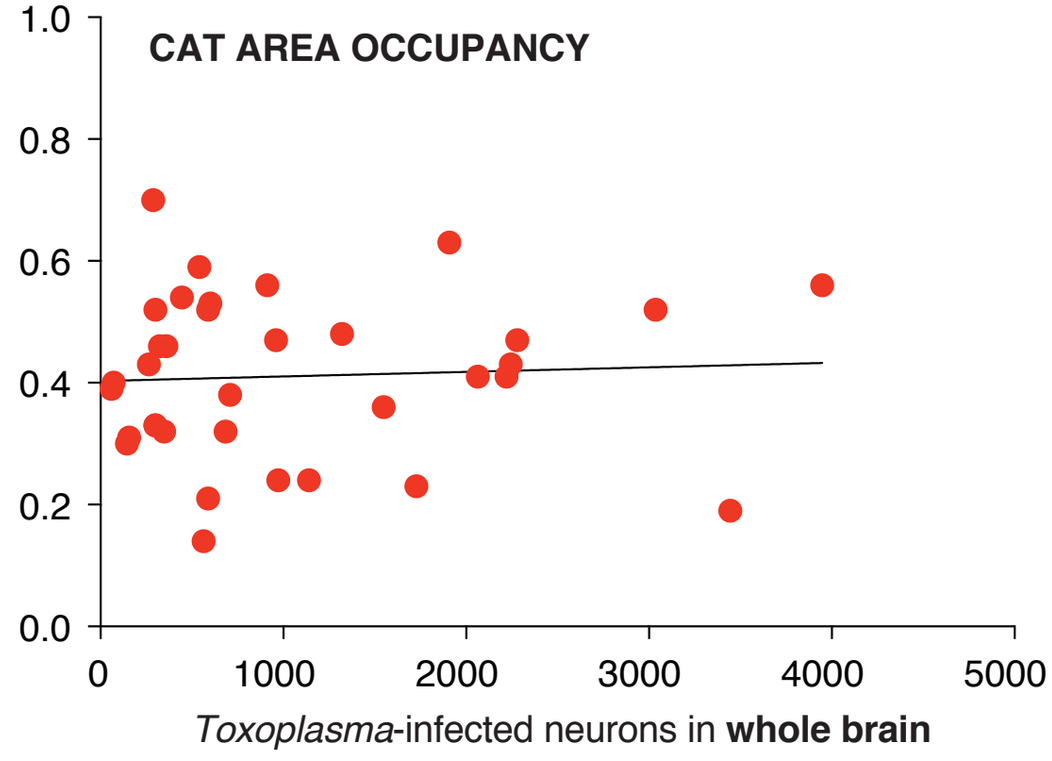
# IN MICE Total number of Toxoplasma-infected Neurons Do Not Correlate with any Behaviors.



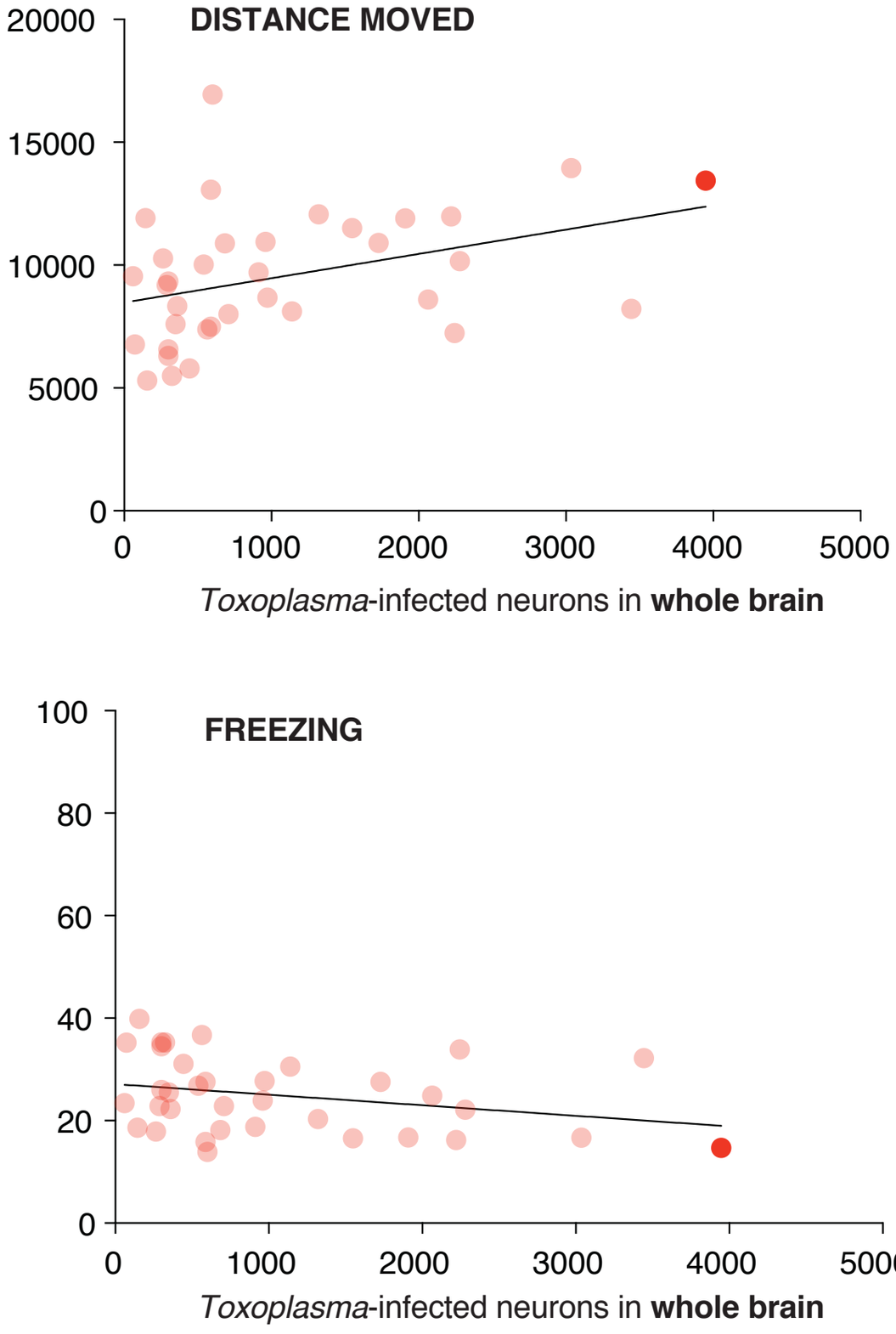


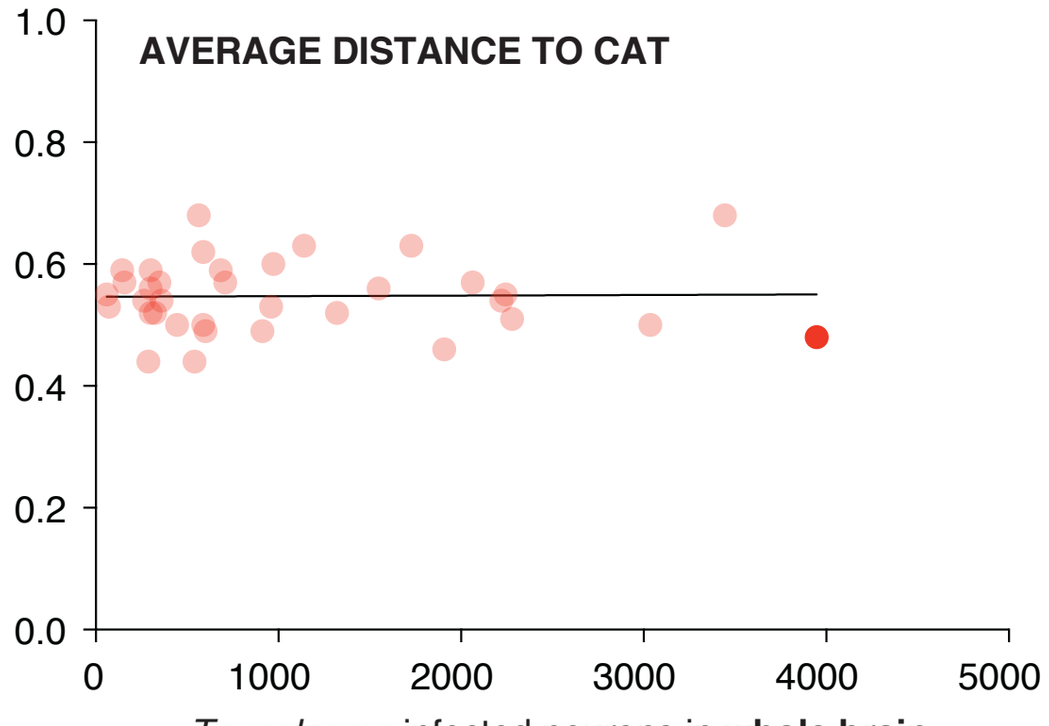




# IN MICE

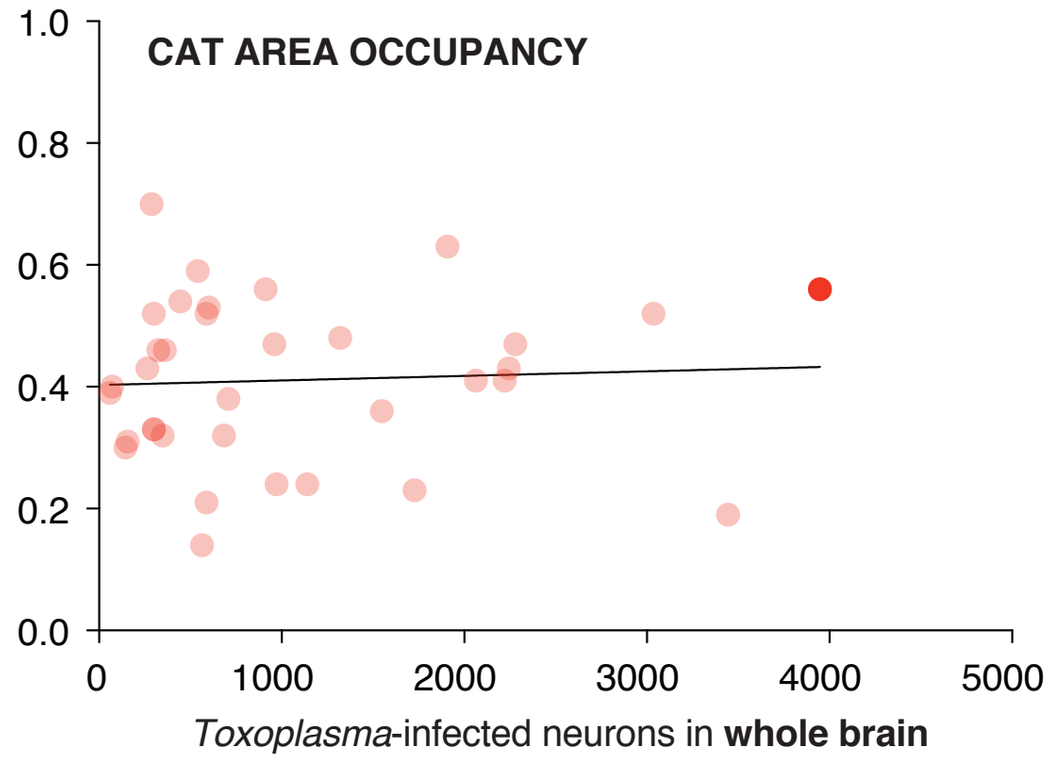
Total number of Toxoplasma-infected neurons do not correlate with any behaviors.



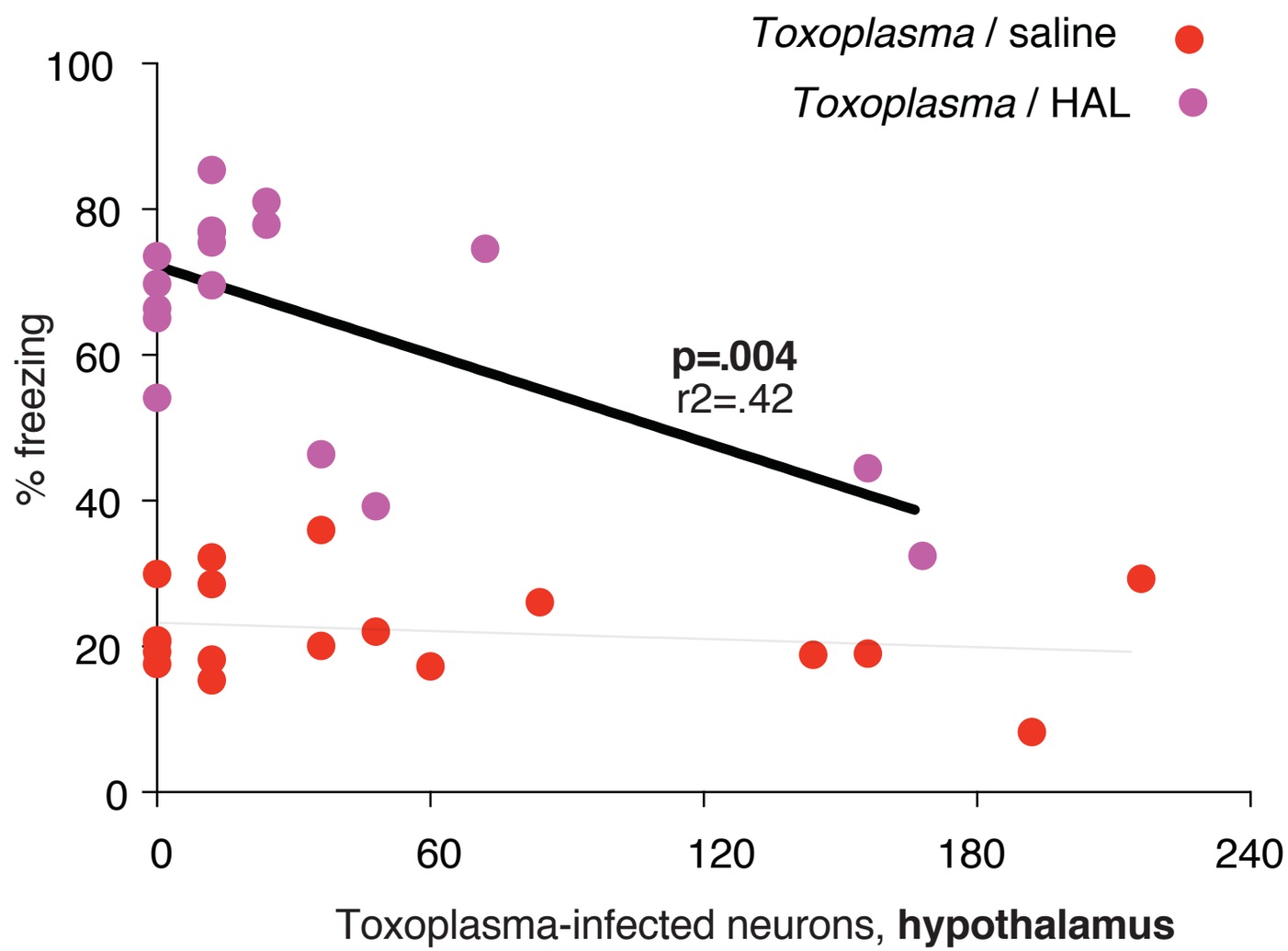






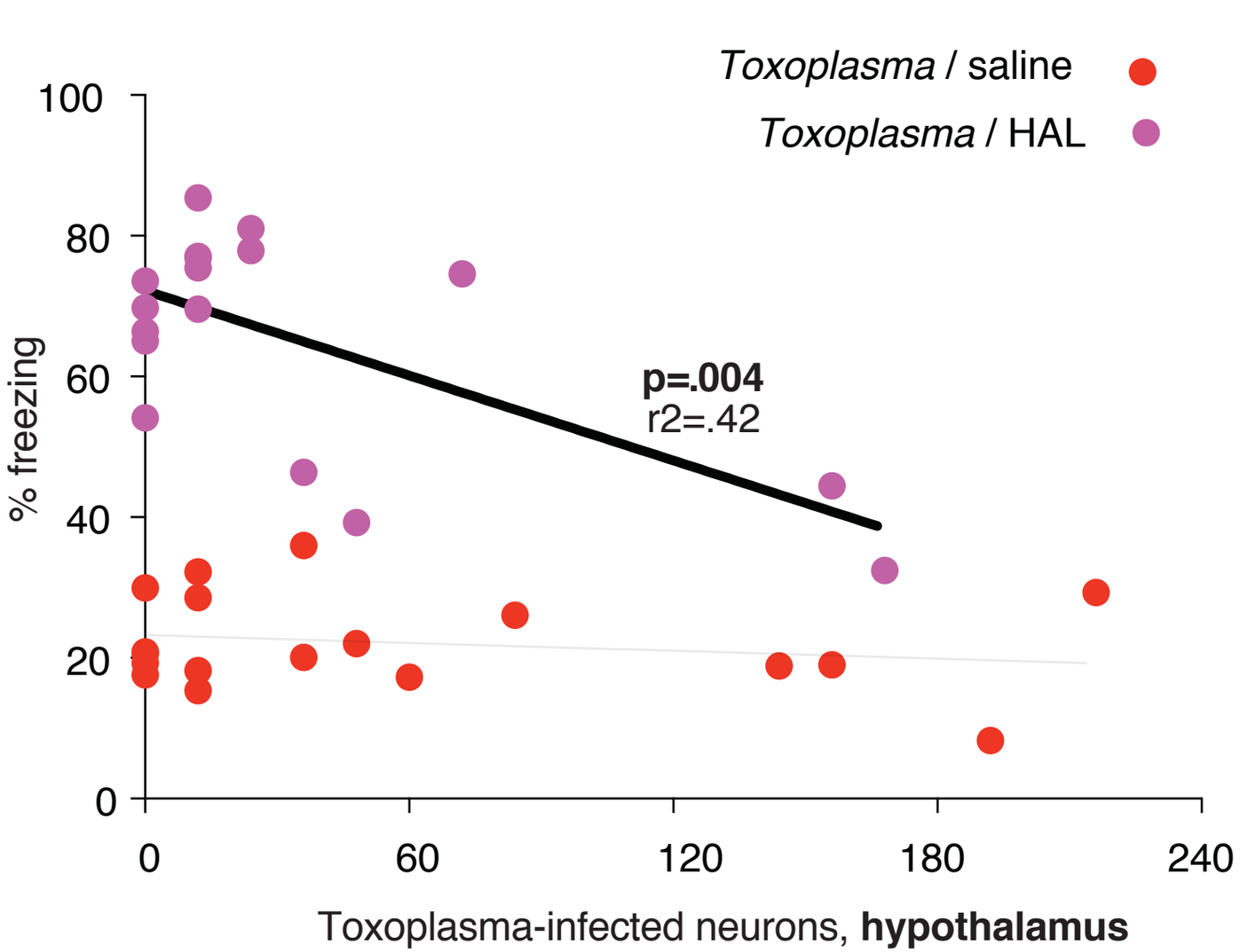
IN MICE

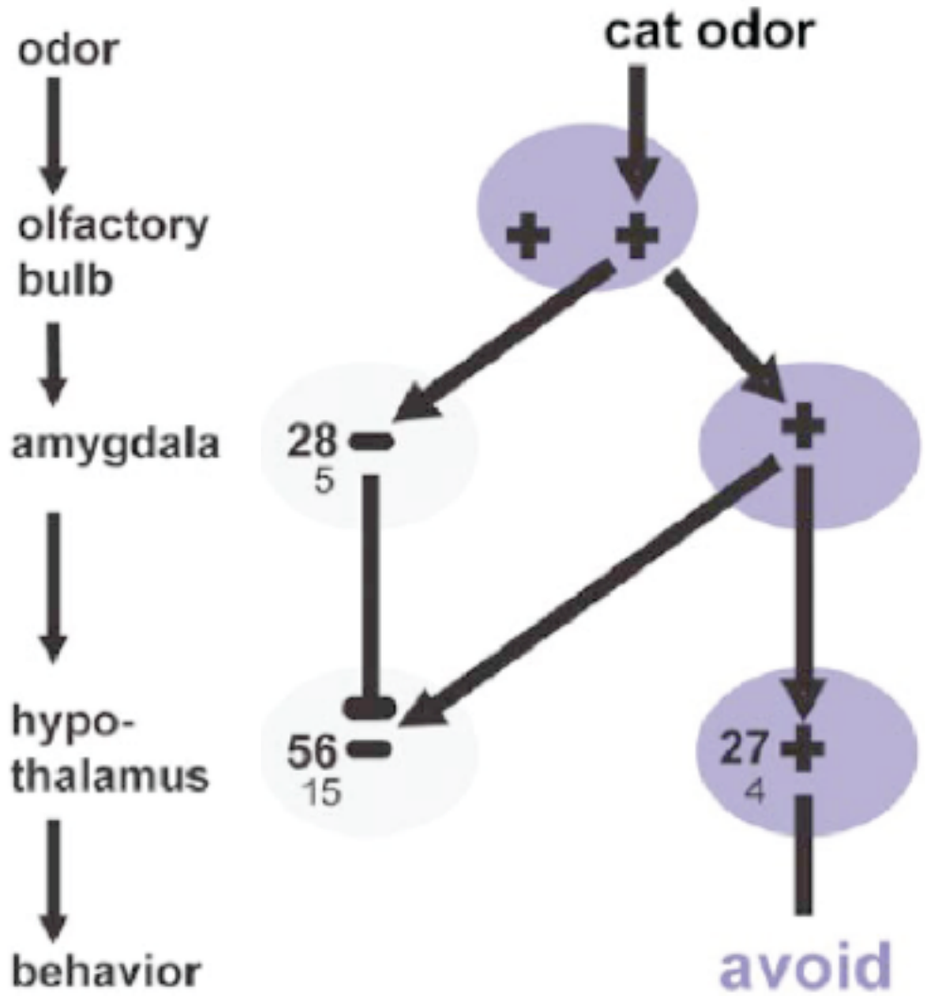
# Toxo in the Hypothalamus Buffers Against Blocking of Dopamine



# IN MICE

## Toxo in the Hypothalamus Buffers Against Blocking of Dopamine

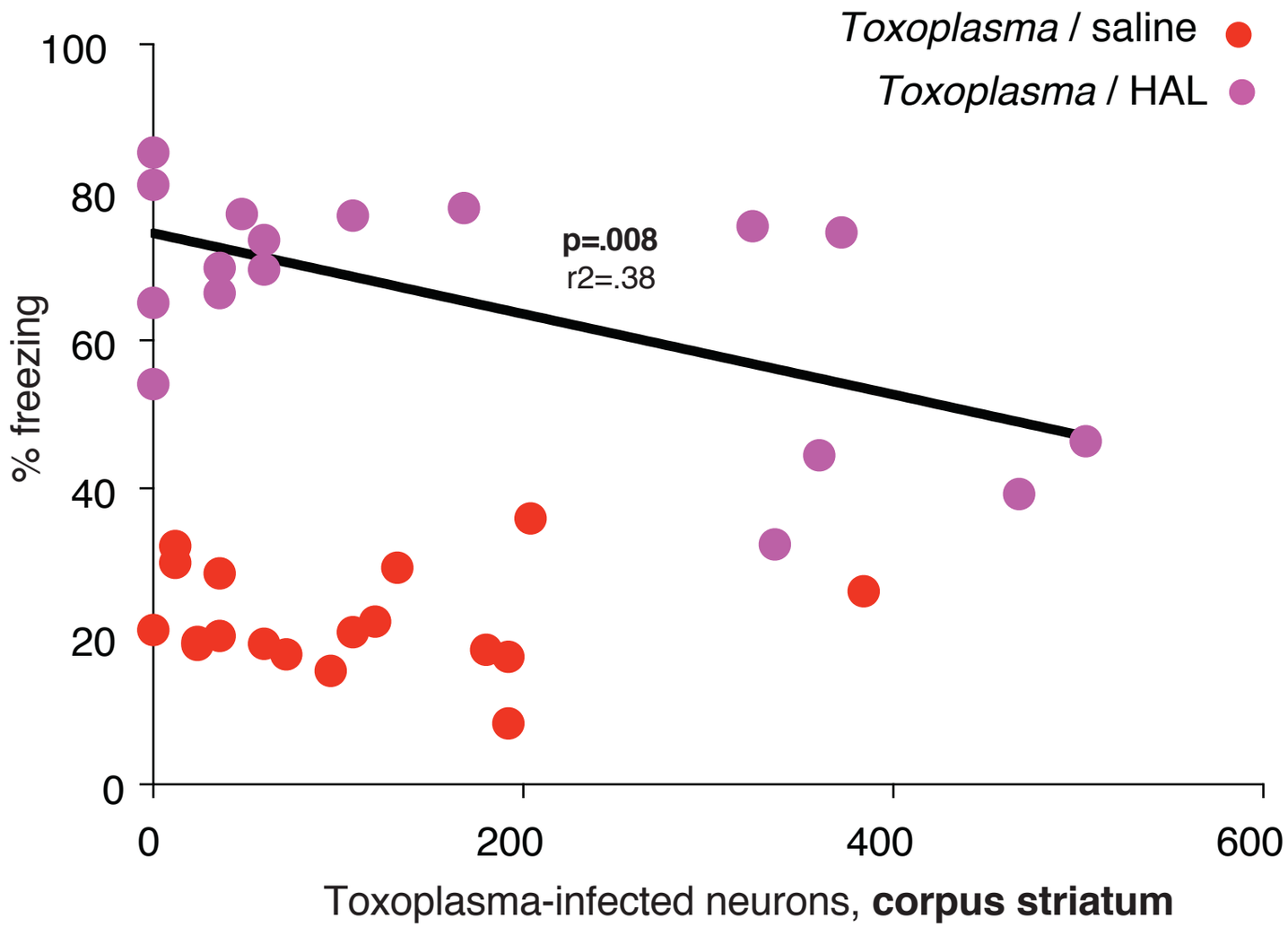



(House et al 2011 *PLoS One*)

# IN MICE

## Toxo in the Striatum Buffers Against Blocking of Dopamine



# Dopamine is released into the Striatum

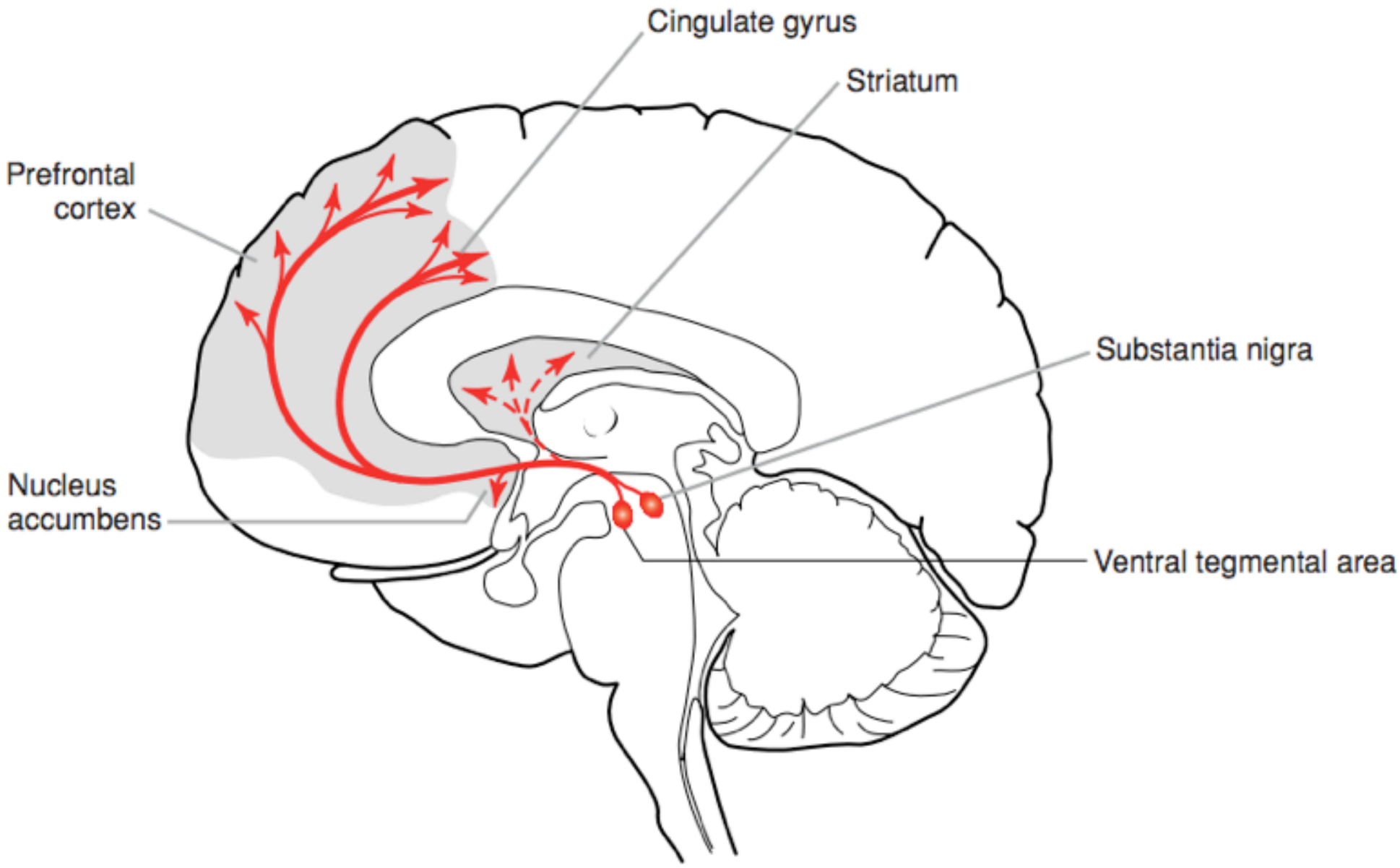



**Figure 2**

Dopamine projections to the forebrain. Illustrated are projections from the ventral tegmental area to the nucleus accumbens, and prefrontal cerebral cortex, and projections from the substantia nigra to the dorsal striatum (caudate and putamen and related structures).

"The most prominent dopaminergic cell group resides in the ventral part of midbrain, which contains approximately 90% of the total number of brain dopaminergic cells." (Anderson 2004)

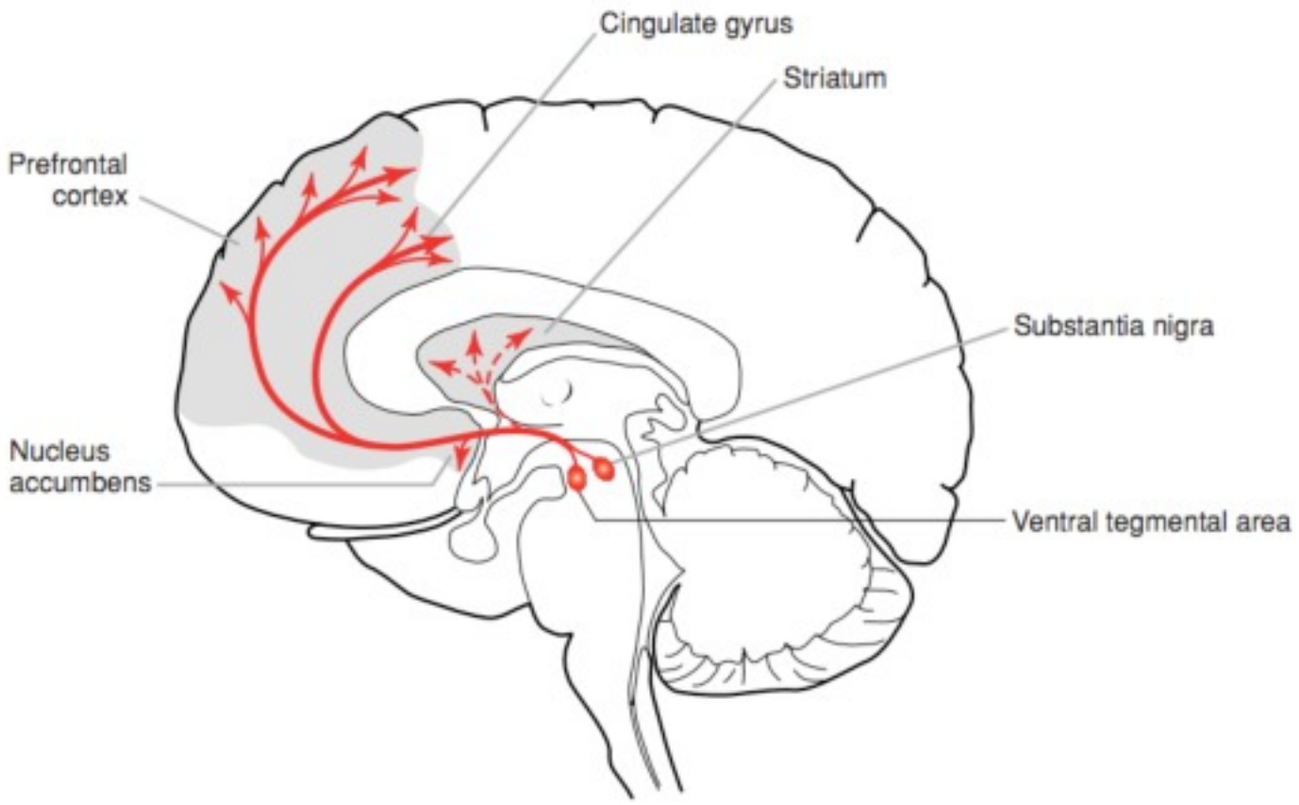
"Considerable differences exist in the numbers of midbrain dopaminergic cell bodies in various mammals ranging from about [**25,000 in the mouse**], 45,000 in the rat, 165,000 in the macaca monkey, to 590,000 in human beings" (Anderson 2004)

# The Striatum Has the Highest Density of Dopamine Receptors in the Brain

1. **Striatum** is part of the basal ganglia, a group of nuclei that function as single unit for output of voluntary motor control

2. **Striatum** has highest density of dopamine (D1, D2) receptors in the brain

3. HAL reduces dopamine transmission by blocking the D2 receptor in the **striatum**, leading to increased freezing.

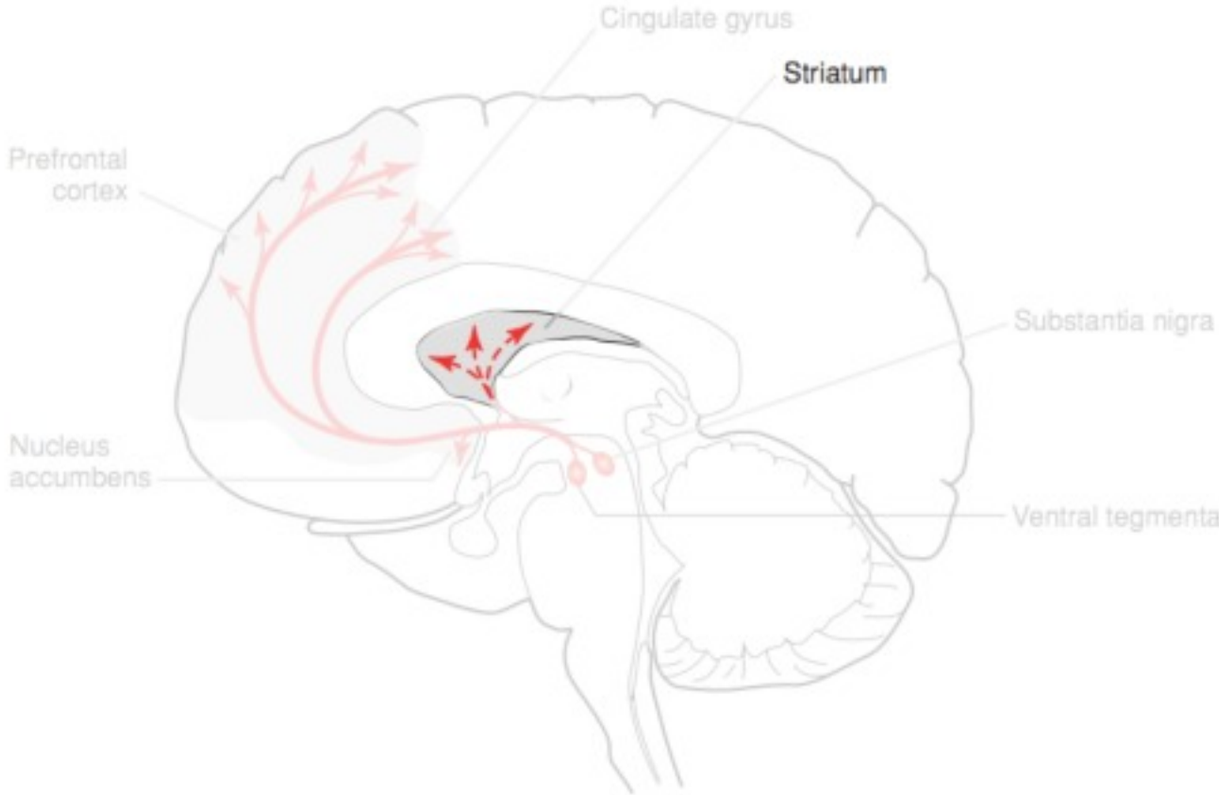


# The Striatum Has the Highest Density of Dopamine Receptors in the Brain

1. **Striatum** is part of the basal ganglia, a group of nuclei that function as single unit for output of voluntary motor control

2. **Striatum** has highest density of dopamine (D1, D2) receptors in the brain

3. HAL reduces dopamine transmission by blocking the D2 receptor in the **striatum**, leading to increased freezing.

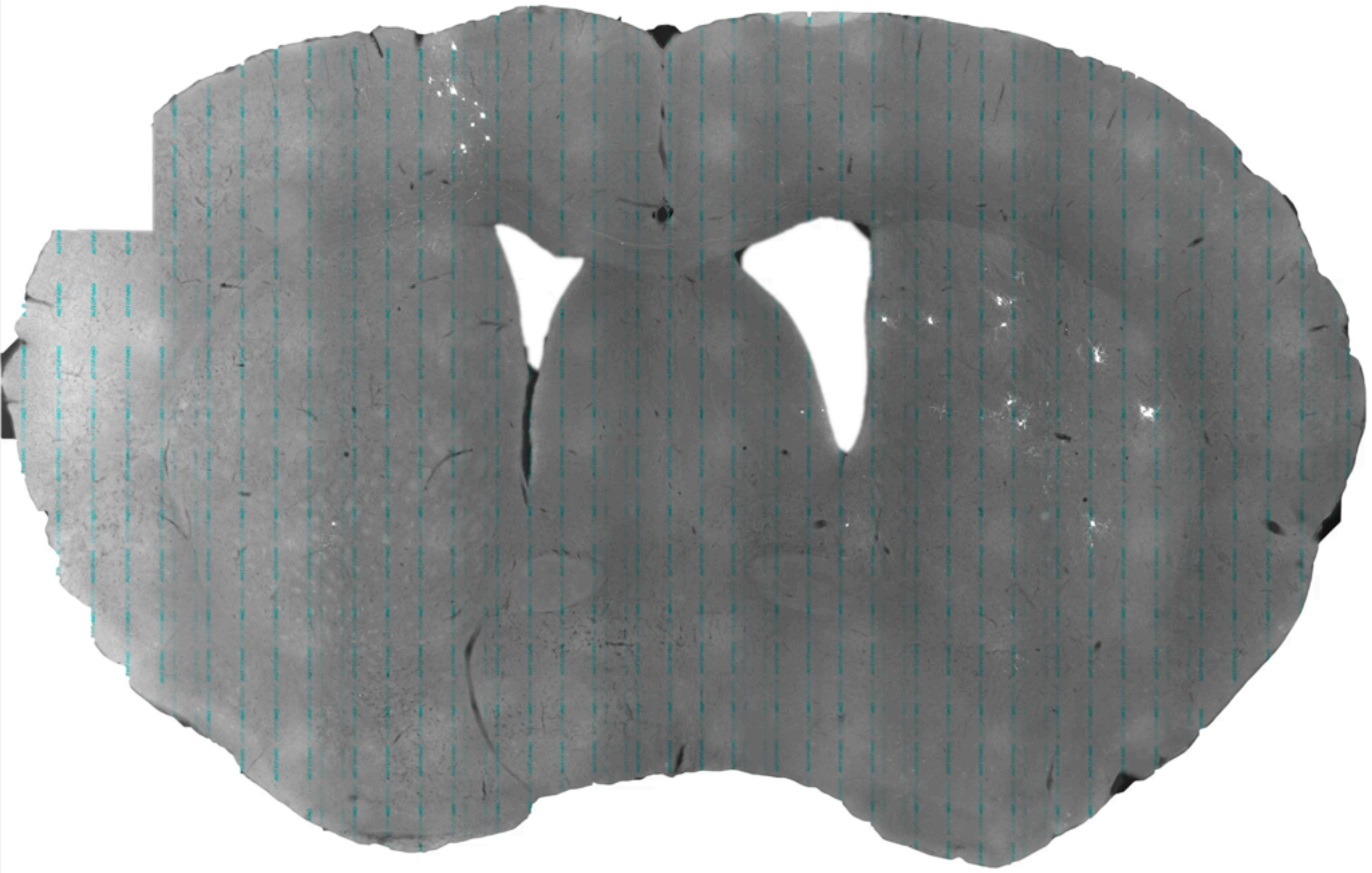


IN MICE

# The Striatum Has the Highest Density of Dopamine Receptors in the Brain

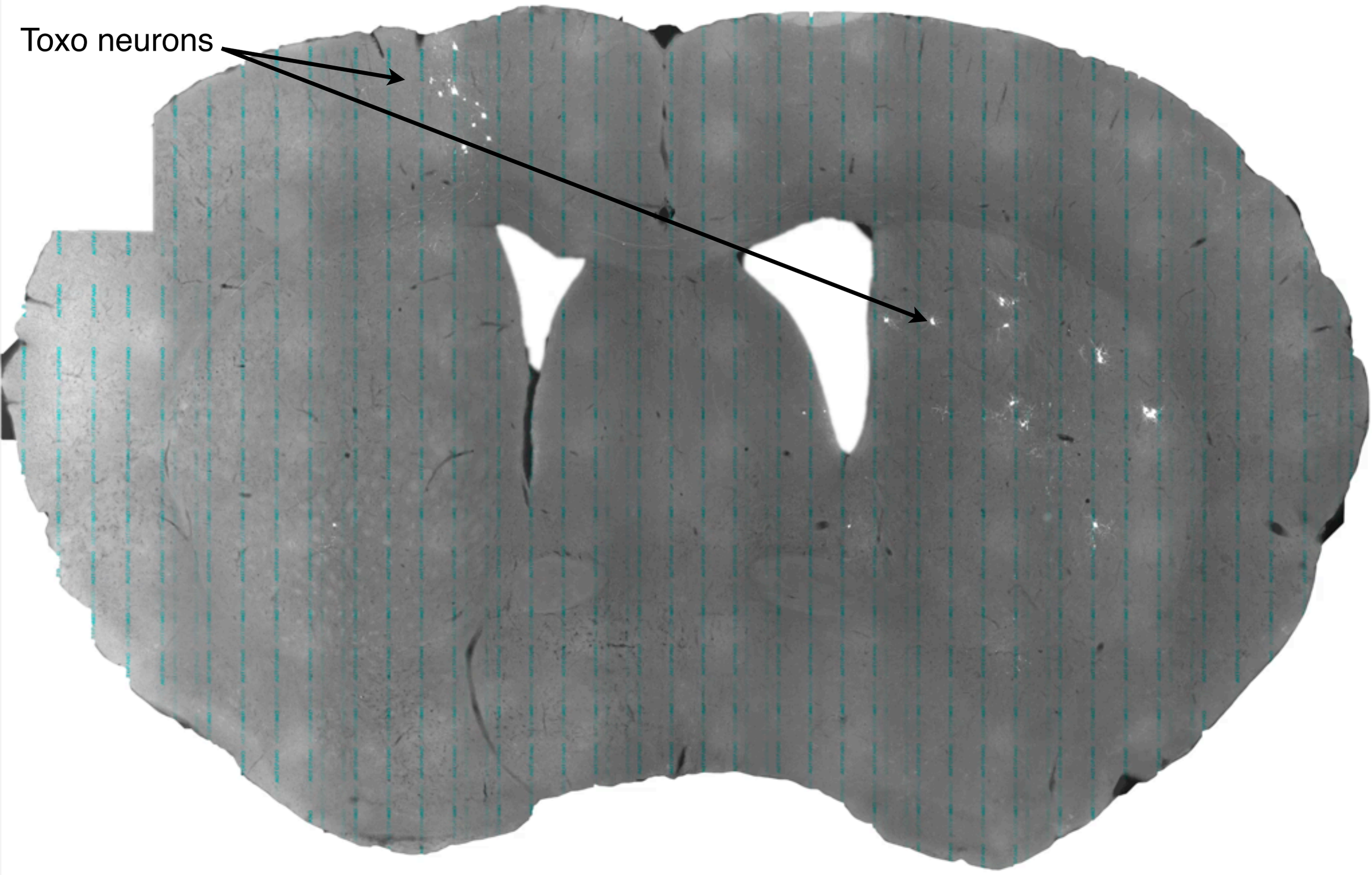
IN MICE

# The Striatum Has the Highest Density of Dopamine Receptors in the Brain



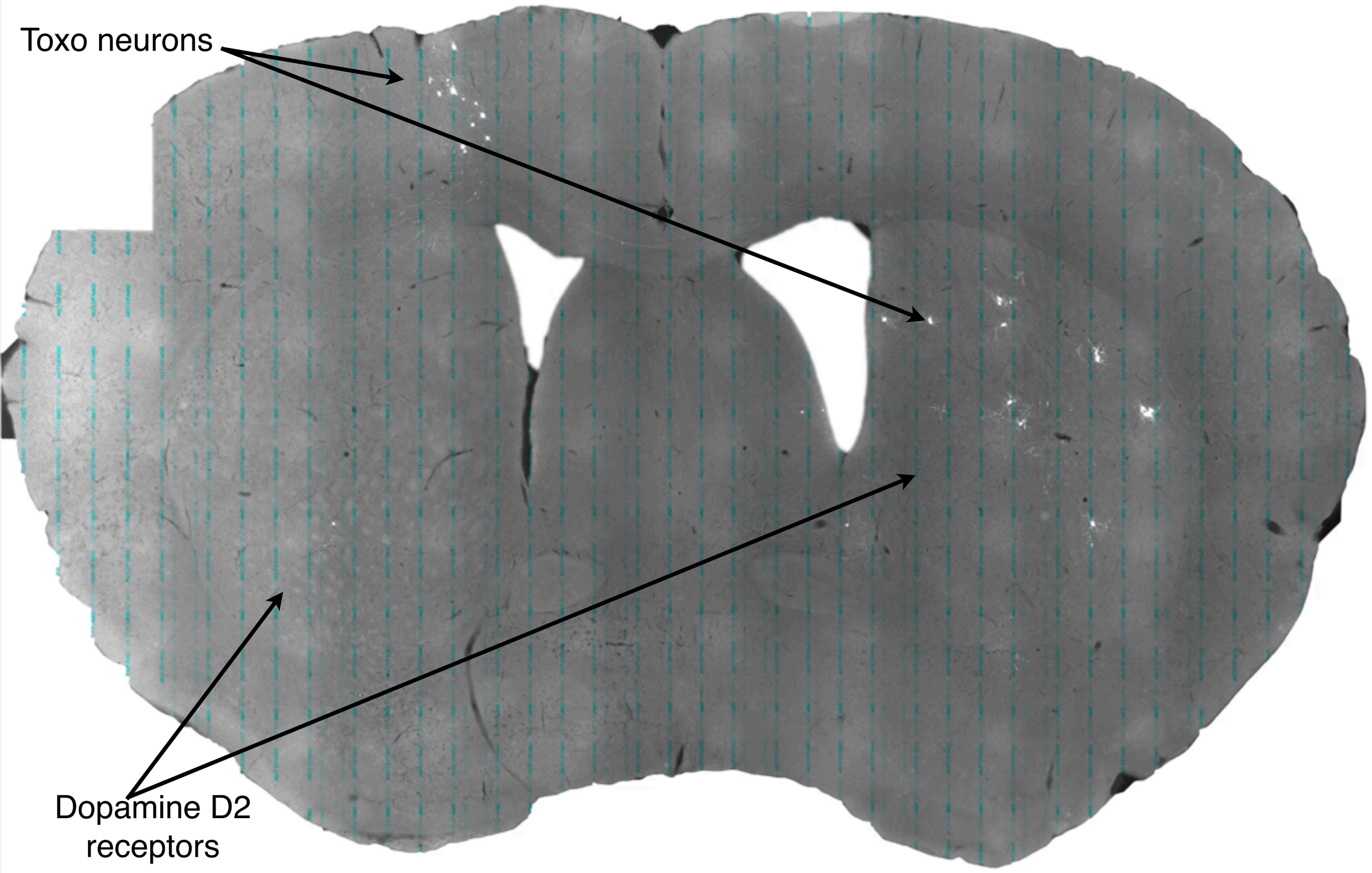
IN MICE

# The Striatum Has the Highest Density of Dopamine Receptors in the Brain



# IN MICE

## The Striatum Has the Highest Density of Dopamine Receptors in the Brain

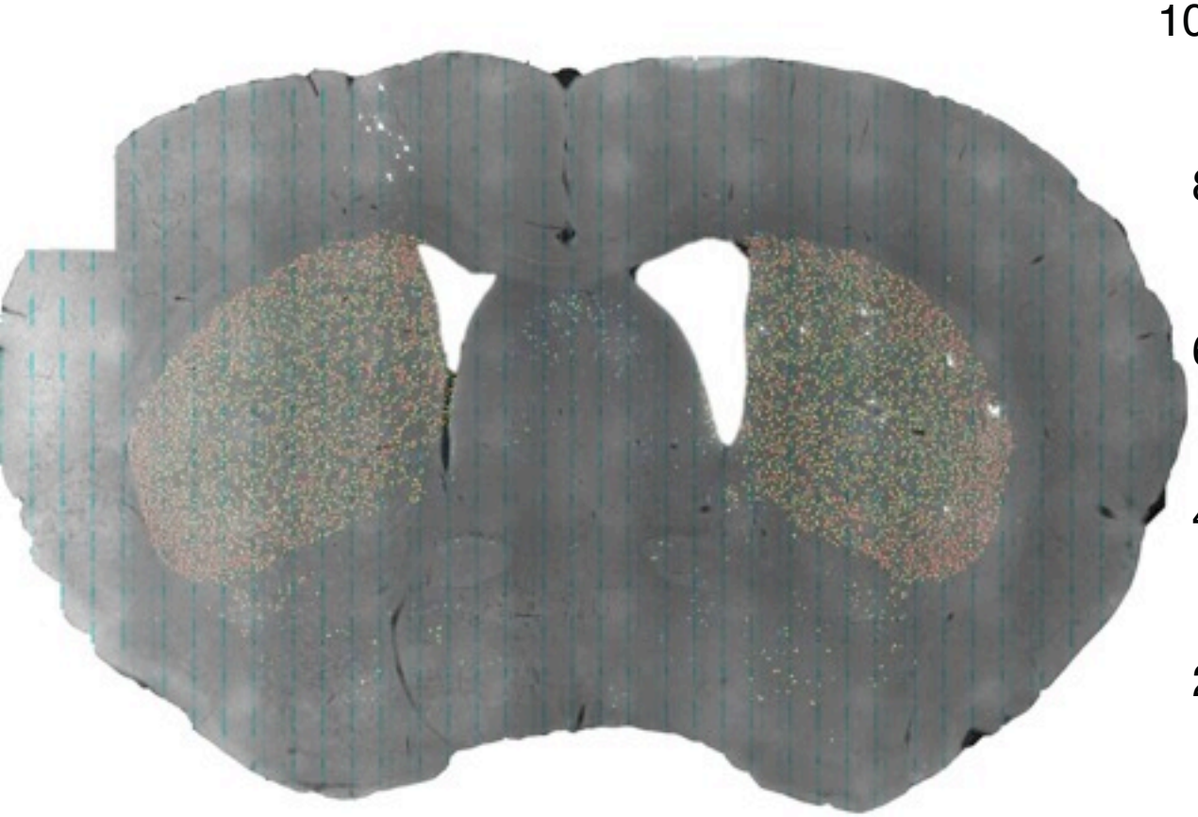


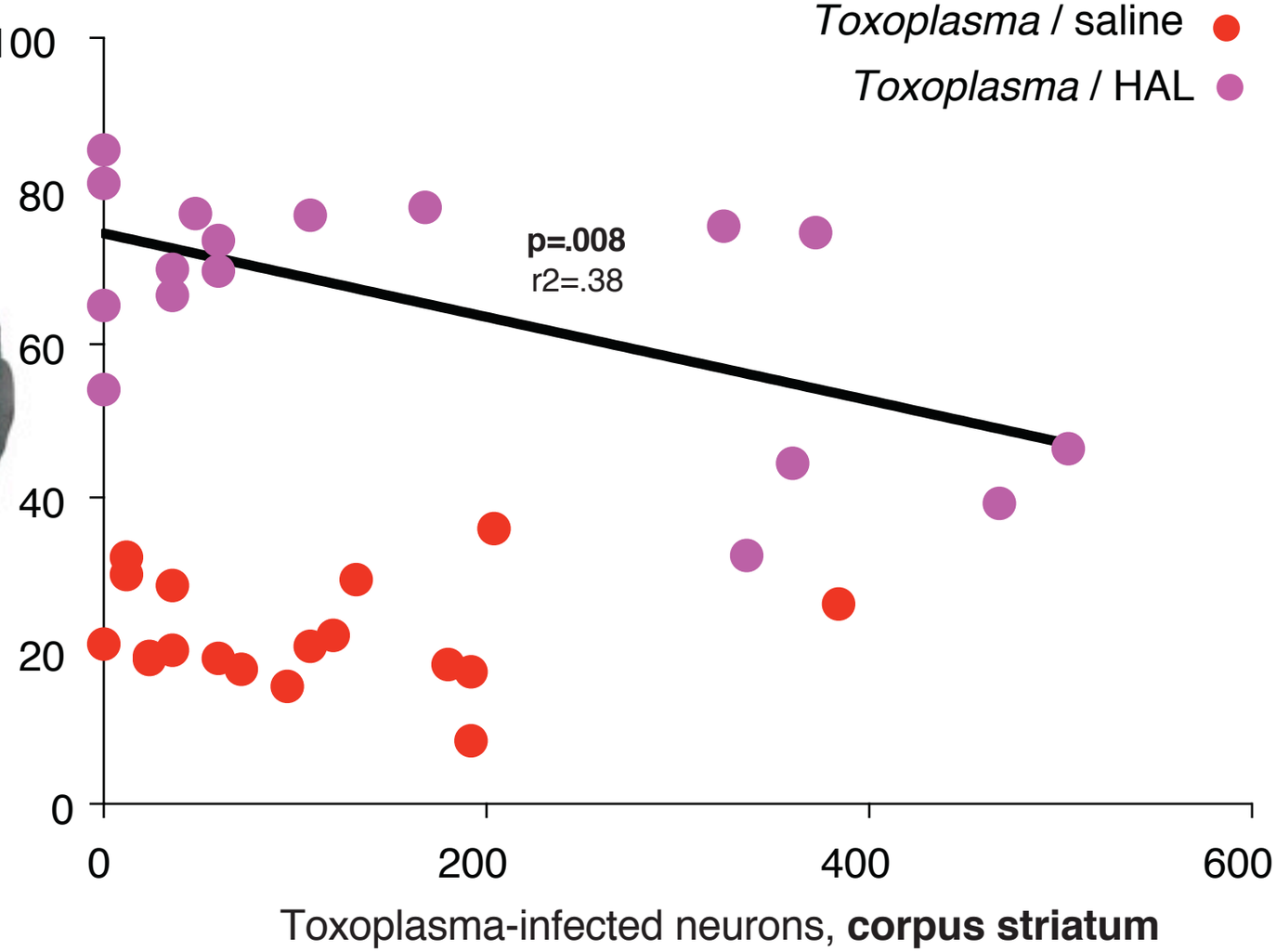
IN MICE

# Are Toxo and HAL Interacting at the Striatum?

**Finding:** Toxo infection in striatum predicts response to dopamine blocking drug.

**Potential Model:**
I. Toxo increases dopamine during course of infection;
II. Drug comes in, tries to block dopamine;
III. Drug has reduced effect because of interaction with Toxo.





# Mechanism: Can Toxo increase dopamine levels?



Tyrosine

TYROSINE HYDROXYLASE

L-Dopa

DOPA DECARBOXYLASE

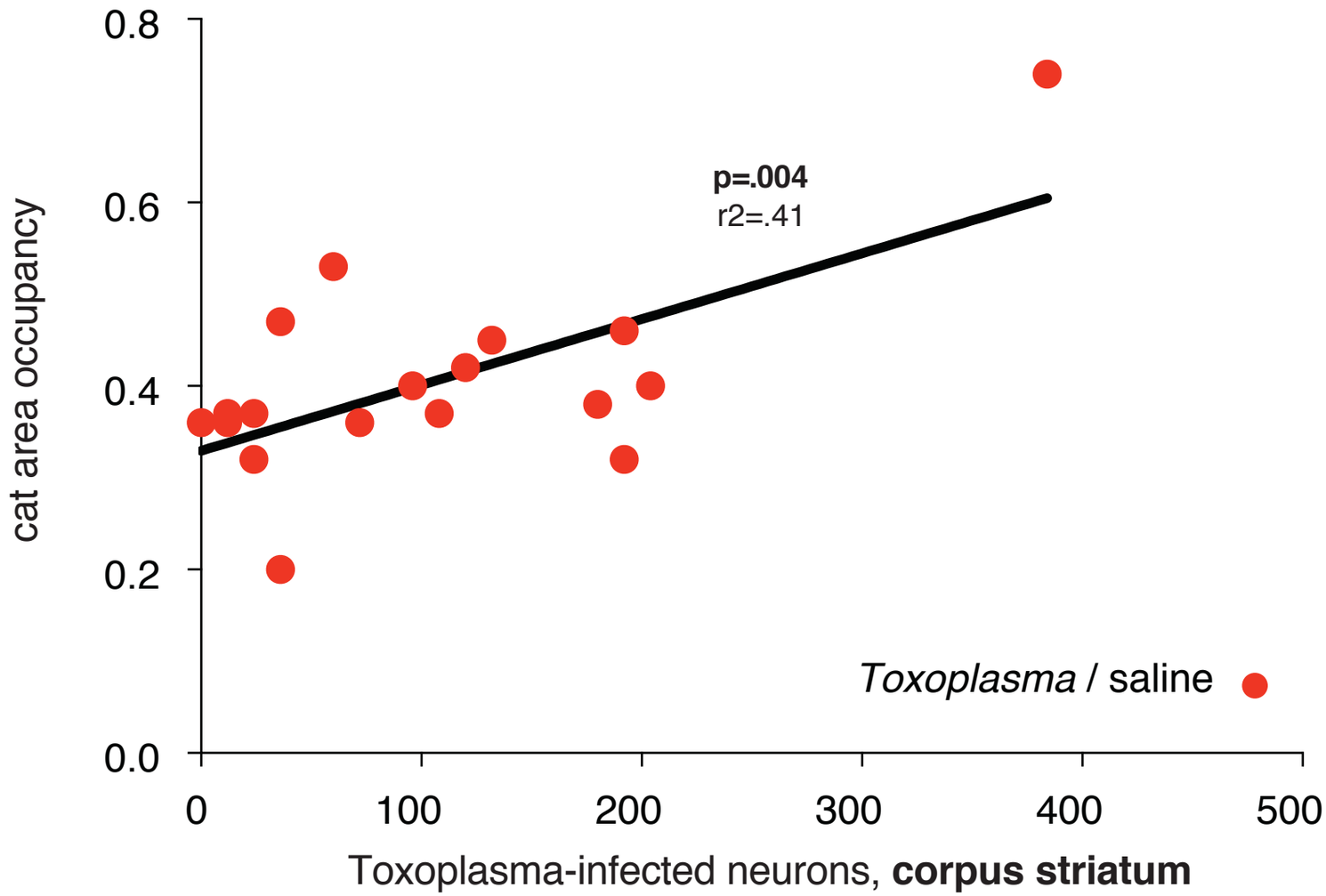
Dopamine

- Toxoplasma increases Dopamine levels (~15%) in the brains of infected mice (Stibbs 1985)
- Toxoplasma has a gene for making Tyrosine Hydroxylase (Gaskell 2009)
- Toxoplasma cysts are full of dopamine (Prandovszky 2011)
- Dopamine antagonist (& antipsychotic) Haloperidol reverses Toxoplasma behavior in rats (Webster 2006)
- Toxoplasma infection in the striatum protects against Haloperidol-induced changes in mouse behavior (House et al, in prep)

Okay, having Toxo in the striatum protects against dopamine blocking, but what about those animals that didn't get the drug? What does Toxo in the striatum do to them?

IN MICE

# Toxoplasma in the Striatum Also Predicts Loss-of-aversion to Cat Urine



# SUMMARY

## Toxoplasma Behavioral Studies in **Mice**

| Name et al | Yr | Mouse | Toxo | Sex | Route | Week p.i. | Test | Finding |
|---|---|---|---|---|---|---|---|---|
| ***House*** | **14** | **B6** | **PRU** | **Male** | **IP (400 tachys)** | **9w** | **Forced choice** | **loss-of-aversion*** |
| *Wang* | 13 | BALB/c | PRU | Both | Maternal | 10 w | Memory, etc | learning & memory deficits |
| *Gatkowska* | 13 | C3H/HeJ | ME49 | Both | IP (20 cysts) | 3 & 6w | | increased monoamines |
| *Costa* | 12 | B6 | ME49 | Female | IP (10^4 tachys) | 9w | Open field, etc | increased exploration |
| *Goodwin* | 12 | CD-1 | VEG | Both | Maternal | 4 & 8 w | Barnes maze | short-term memory deficit |
| *Jung* | 12 | Tg2567 | Me49 | | IP (15 cysts) | 24w | Y, Water, etc | reverses memory deficits in AZ |
| *Haroon* | 12 | BALB/c | DX | F | Oral (4 cysts) | 4 & 8w | Open field | loss-of-aversion |
| *Yolken* | 12 | BALB/c | PRU | F | IP (400) | 8-16w | 3 chamber | loss-of-aversion |
| *Gatkovska* | 12 | B6 | Me49 | M | IP (20 cysts) | 3 & 6w | Open field, etc | reduced exploration |
| *Gulinello* | 10 | B6 | Me49 | M | IP (1000) | 7w | Open field, etc | motor coordination / sensory |
| *Hermes* | 08 | Swiss | Me49 | F | IP (100 cysts) | 48w | Open field, etc | reduced exploration, motor |
| *Hodkova* | 07 | B* | HIF | | Oral (10) | 10w | 8 arm, etc | memory / learning deficit |
| *Vyas* | 07 | BALB/c | PRU | F | IP (400) | 4-5w | Circle | loss-of-aversion |
| *Chao* | 92 | | | | | | | |
| *Arnott* | 90 | | | | | | | |
| *Arnott* | 90 | | | | | | | |
| *Graham* | 84 | | | | | | | |
| *Hutchinson* | 80 | | | | | | | |

THE PARASITE

IN RATS

IN MICE

IN PEOPLE

# IN PEOPLE Toxo is the Third Highest non-genetic Risk Factor for Schizophrenia

Odds Ratio / Relative Risk

>5

having an schizophrenic mother (RR 9.31; 95% CI 7.24–11.96)
father (RR 7.20; 95% CI 5.10–10.16)
sibling (RR 6.99; 95% CI 5.38–9.08)

genes

4

being the offspring of immigrants from selected countries (RR 4.5; 95% CI 1.5–13.1)

3

being raised in an urban area (RR 2.75; 95% CI 2.31–3.28)

having antibodies for *Toxoplasma gondii* (OR 2.73; 95% CI 2.10–3.60)

being born in an urban area (RR 2.24; 95% CI 1.92–2.61)

cannabis use (OR 2.10–2.93; 95% CI 1.08–6.13)

having minor physical anomalies (OR 2.23; 95% CI 1.42–3.58)

having a father 55 or older (OR 2.21–5.92; 95% CI 1.46-17.02)

2

traumatic brain injury (OR 1.65; 95% CI 1.17–2.32)

sex abuse in childhood (OR 1.46; 95% CI 0.84–2.52)

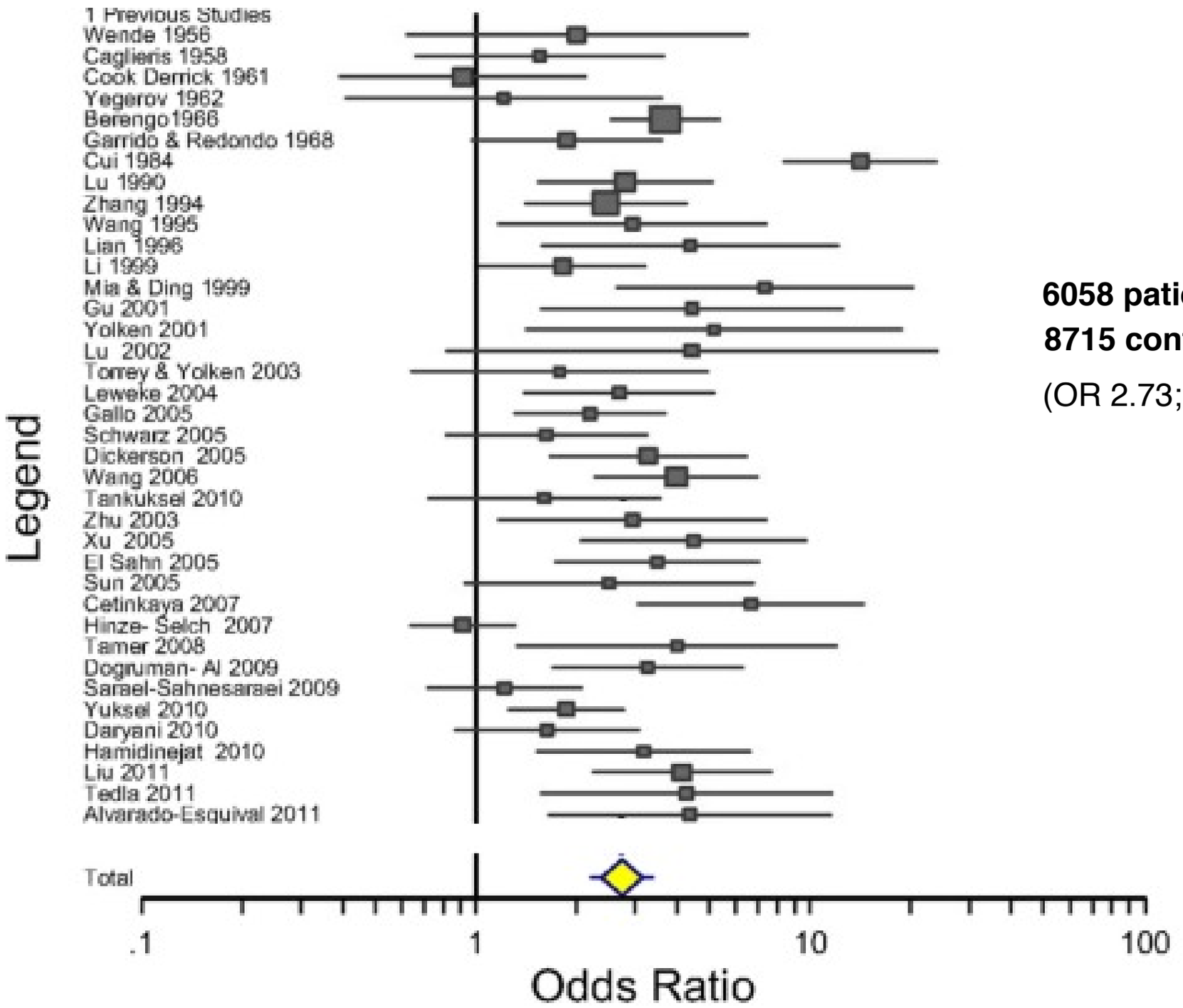
having a father 45 or older (OR 1.21–1.66; 95% CI 1.09–2.01)

1

environment

IN PEOPLE

# Meta-analysis of link to Schizophrenia




**6058 patients**

**8715 controls**

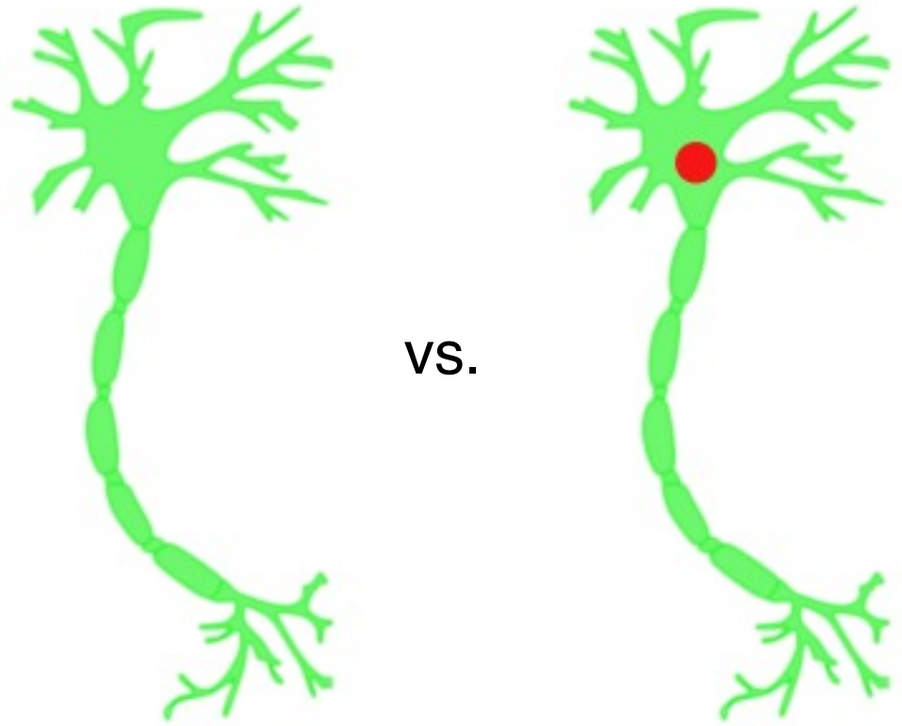
(OR 2.73; 95% CI 2.10–3.60)

# SUMMARY in a paragraph

*Toxoplasma* makes mice move more, freeze less, and spend more time exploring cat urine. But not all infected mice show these changes—some mice show no change at all. Plausibly, the variation in behavior has something to do with which neurons in the mouse brain are infected. Using a genetically engineered *Toxoplasma* parasite, we were able to detect all neurons infected with *Toxoplasma.* *Toxoplasma* infection is sparse throughout the brain and infection burden ranges from a few hundred to four thousand neurons in the mouse brain. After analyzing the entire brain and counting the number and location of all infected neurons, two brain regions, the hypothalamus and striatum, were correlated with variations in behavior. Mice given the dopamine blocking drug Haloperidol were buffered against the effects of the drug proportional to the amount of infection they had in the striatum. *Toxoplasma* infection in the striatum also correlated with loss-of-aversion to cat urine in mice not given Haloperidol.

# FUTURE DIRECTIONS

- What percentage of neurons does Toxo alter vs. infect?
- Does Toxo preferentially infect a certain neuronal subtype?
- Does Toxo exhibit preference for some brain regions over others?
- What does Toxo do to neurons it infects? Does it kill them? Alter their signaling?
- Is Toxo altering dopamine receptor density in the striatum? In all regions it infects?




- Genetically engineered Cre-secreting parasite can be used with any Cre-dependent mouse.
  e.g. Cre-dependent apoptosis: Could kill all Toxo-infected neurons.
  e.g. Cre-dependent optogenetics: Could turn on or off only Toxo-infected neurons.

# ACKNOWLEDGEMENTS

# Thank You


Sapolsky Lab
* = Team Toxo

**Sam Bowers ***
**Andrew Evans ***
**Zuri Evans ***
**Doruk Golcu ***
**I-ping Lee ***
**Nathan Manley**
**Rupshi Mitra**
**Shawn Sorrells**
**Ajai Vyas ***
**Melissa Works**
et al.

Committee
**John Boothroyd** Microbiology & Immunology
**Russ Fernald** Biology
**Hank Greely** (chair) Law
**Shaul Hestrin** Comparative Medicine
**Josef Parvizi** Neurology

Awesome
**Ross & Katie**

Collaborators
**Anita Koshy**
**Boothroyd Lab**

Funding
Stanley Medical Research Institute
NIH R01