Behavioral Neuroscience

Lecture 3: The new era in the study of brain mechanisms underlying social behavior in animal models

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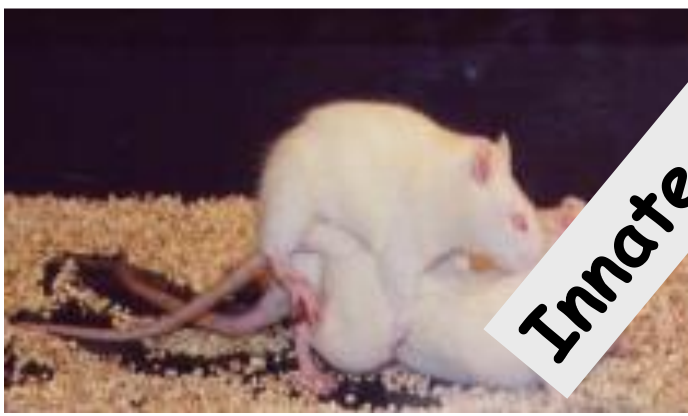
Social behavior in mammalian species

- Most striking categories of sexually dimorphic behaviors
- Essential for survival and reproduction of all species
- Complex behavior controlled by multiple genes and environmental factors
- Controlled by simple sensory signals (*e.g.* pheromones)
- Innate (genetically-predetermined) behaviors

Sexually dimorphic social and sexual behaviors in rodents are all regulated by hormones

Innate Behavioral Repertoires

Aggressive behavior



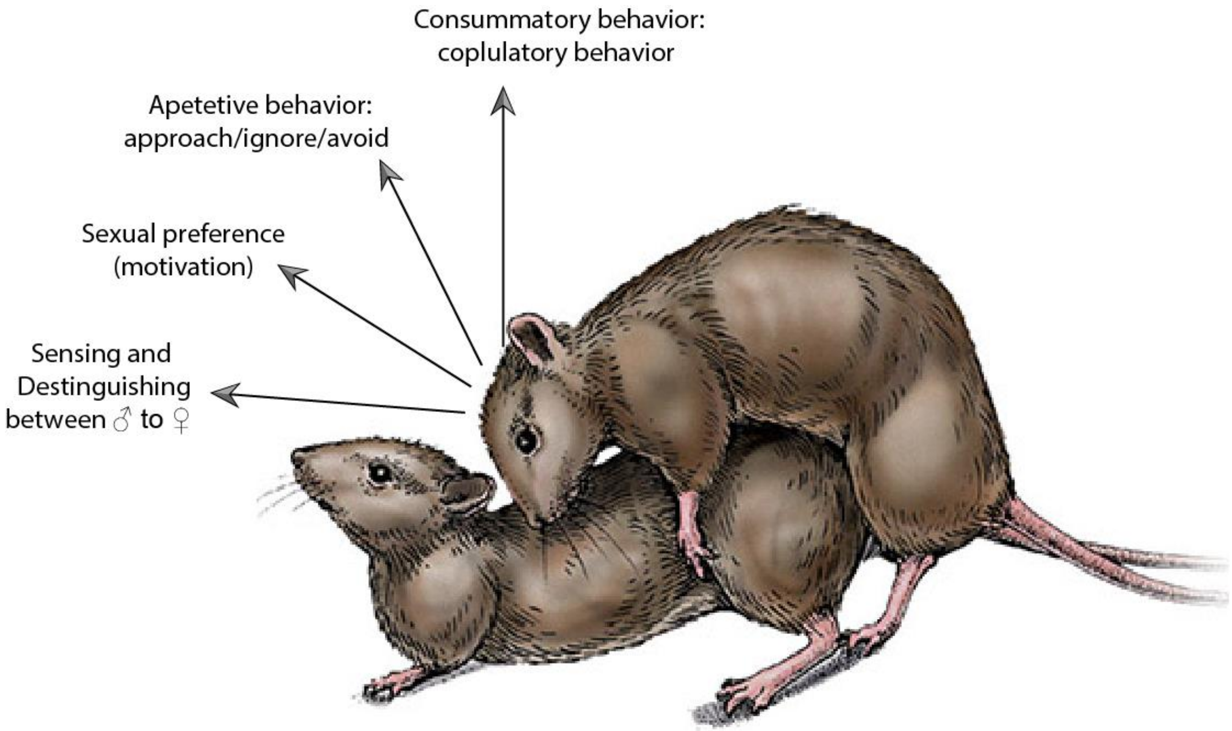
Sexual behavior

Maternal behavior

Mating behavior in male mice requires the activation of a complex set of behavioral displays

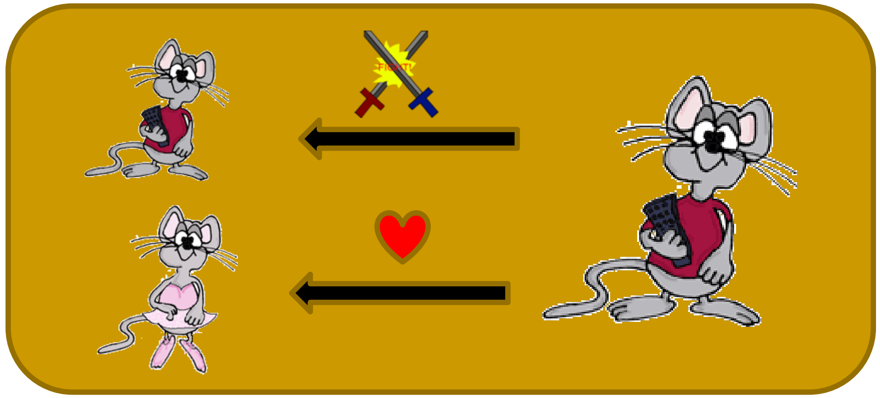
Regulated by:

- **Gonadal hormones** (testosterone, estradiol)
- **Neuropeptides** (oxytocin)
- **Neurotransmitters** (dopamine, serotonin)
- **Sensory signals** (olfactory information)



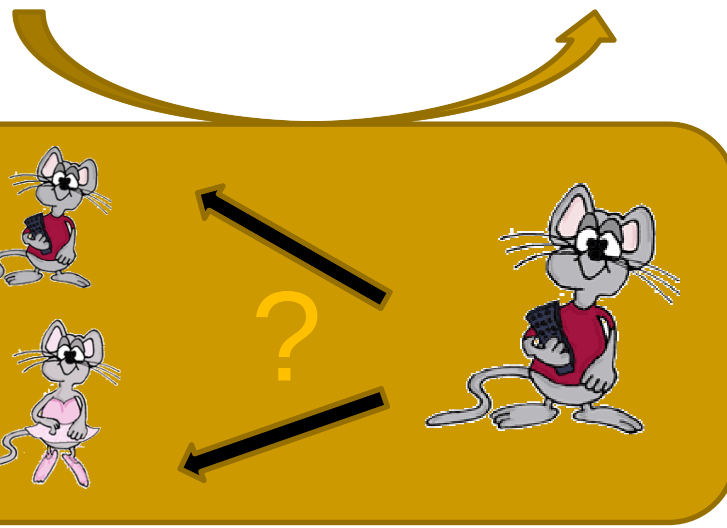
- **Innate stereotypic behaviors that do not require prior learning**
- **Controlled by genetically pre-programmed hard-wired circuits**

The influence of past environmental stimuli on male sexual preference



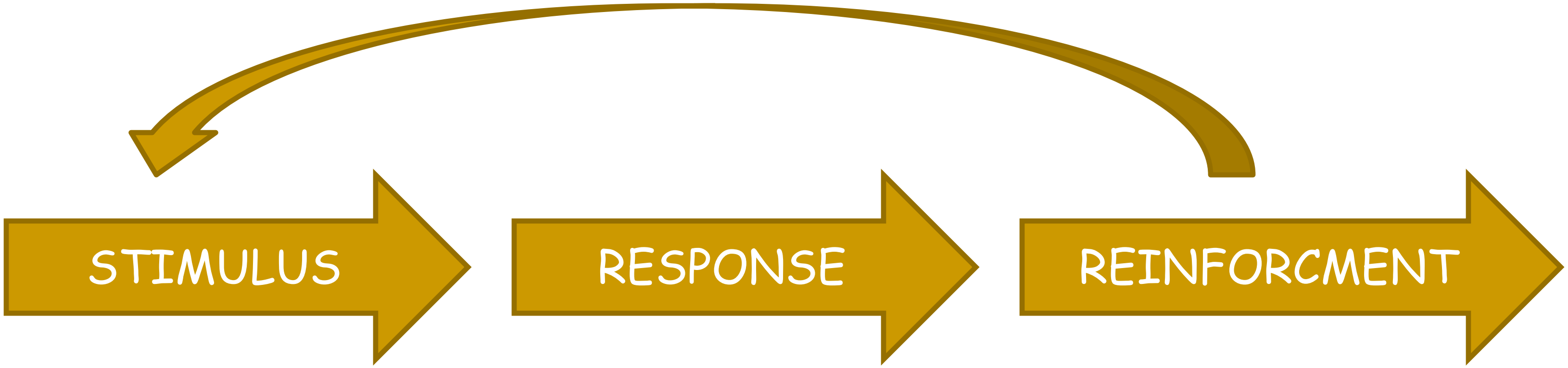
Conditional sex-specific pheromonal aversion

Associating female odor to a mild stomach ache /nausea



Beny and Kimchi 2016, Gene Brain and Behav

Classical conditioning



If your behavior is followed by a positive consequence, you are more likely to repeat the act in the future;

If it is followed by a negative consequence, you are less likely to repeat it.

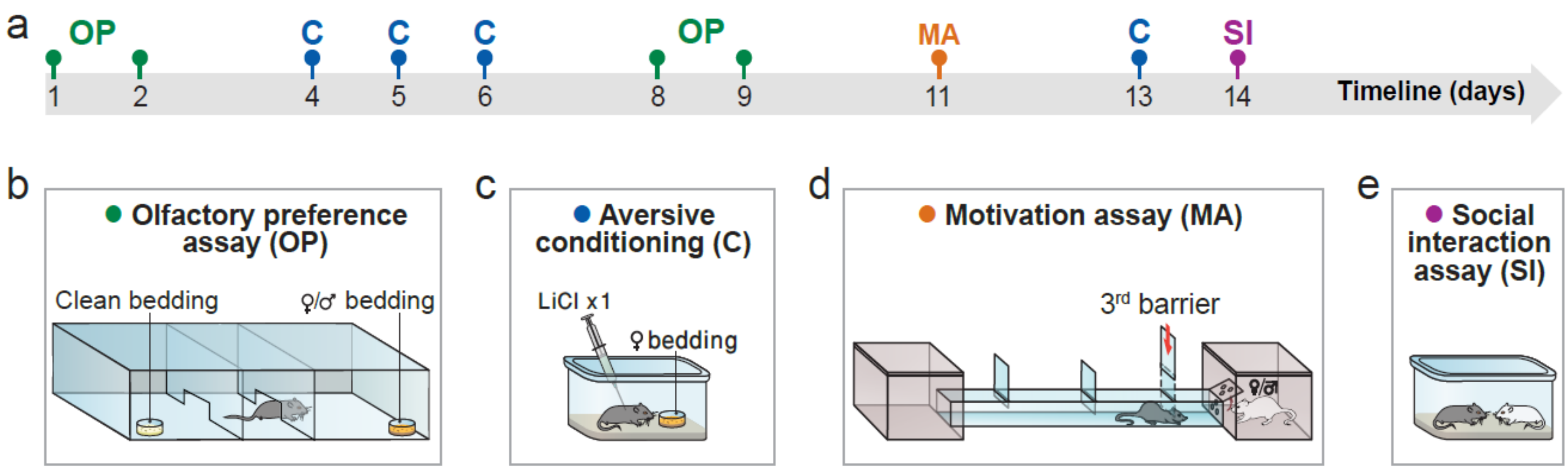
Methods

Conditioned Odor Aversion

LiCl injection

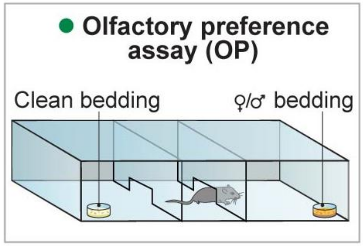
Female or Male soiled bedding

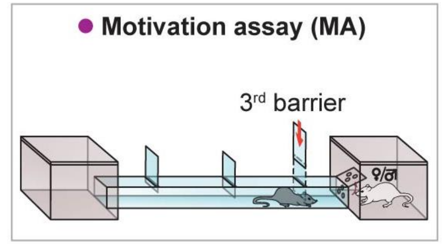
Method: working scheme

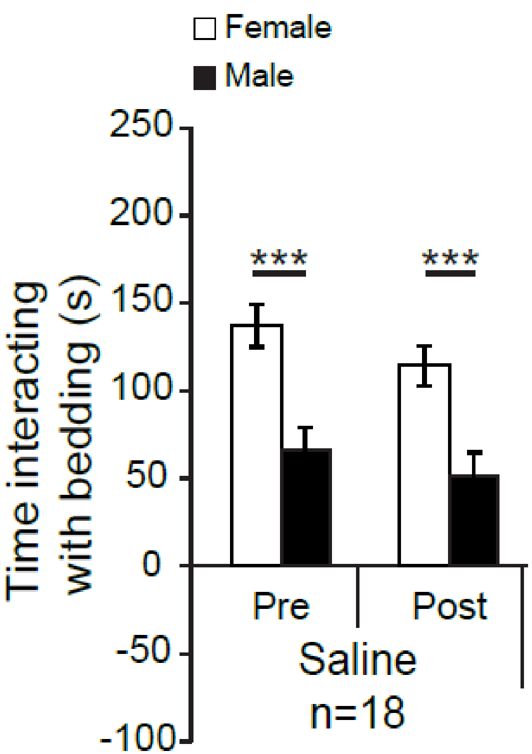


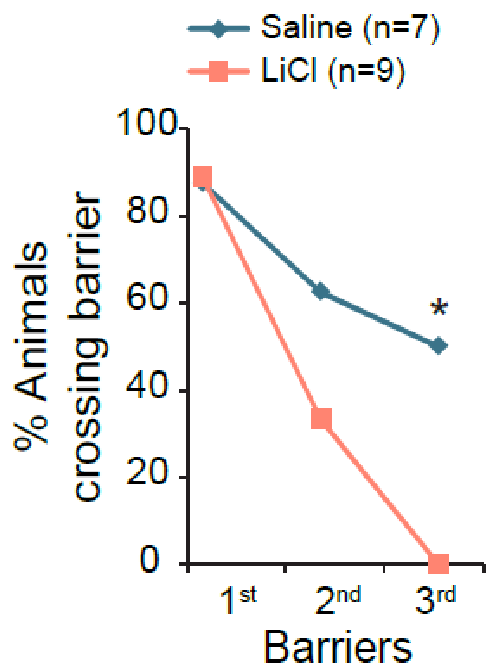
Beny and Kimchi 2016, Gene Brain and Behav

Female-specific negative conditioning impairs sexual preference and motivation in WT males



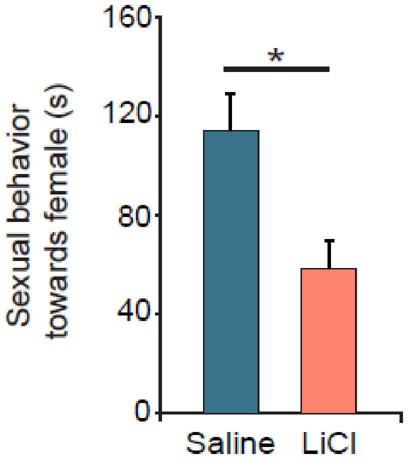


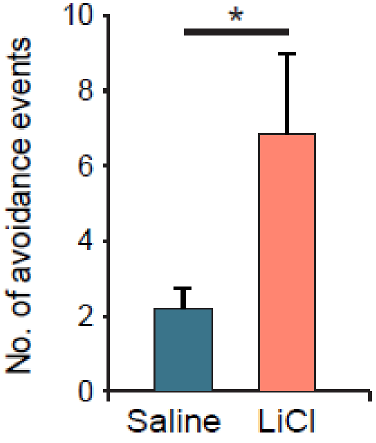




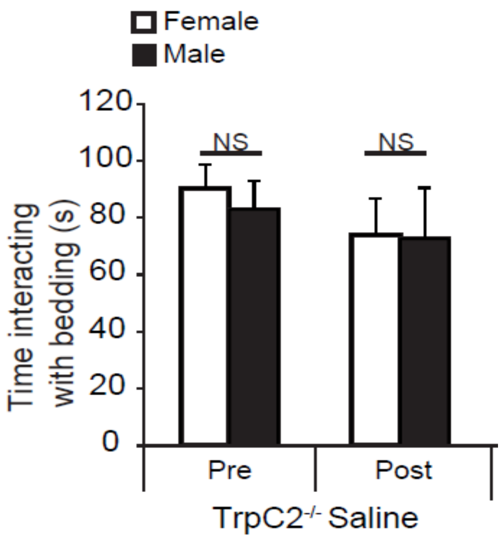
Beny and Kimchi 2016, Gene Brain and Behav

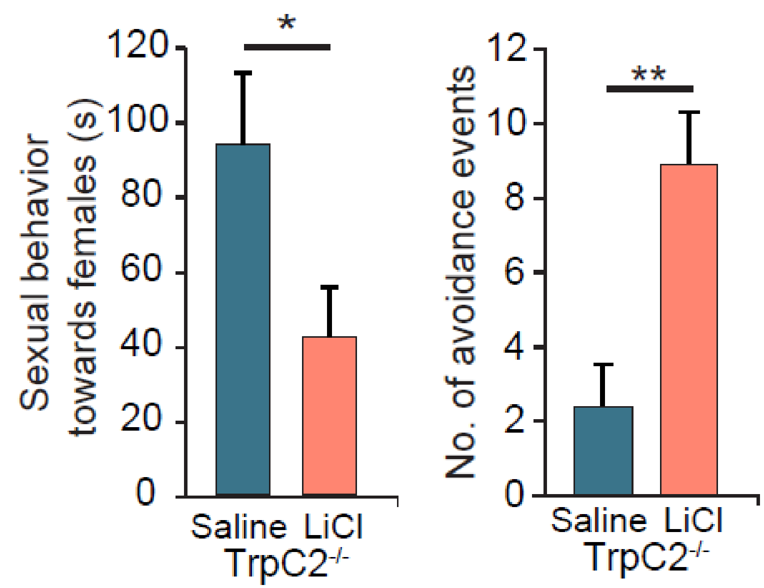
Female-specific negative conditioning induces social anxiety in males





Beny and Kimchi 2016, Gene Brain and Behav

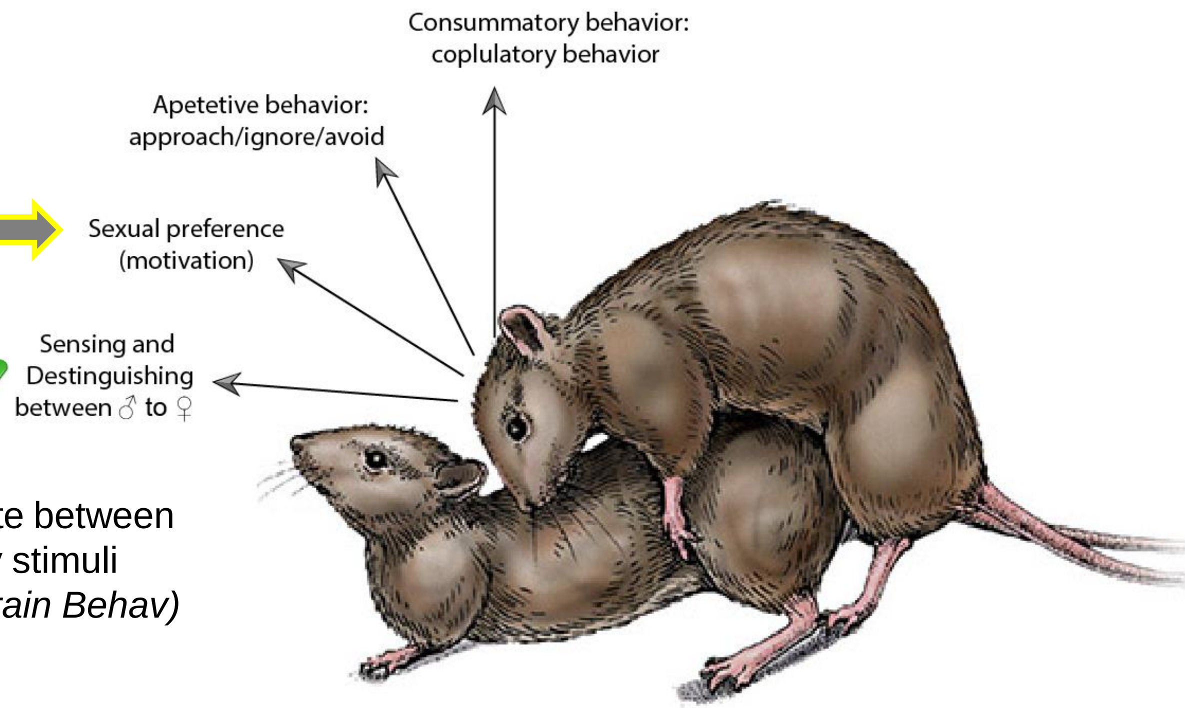




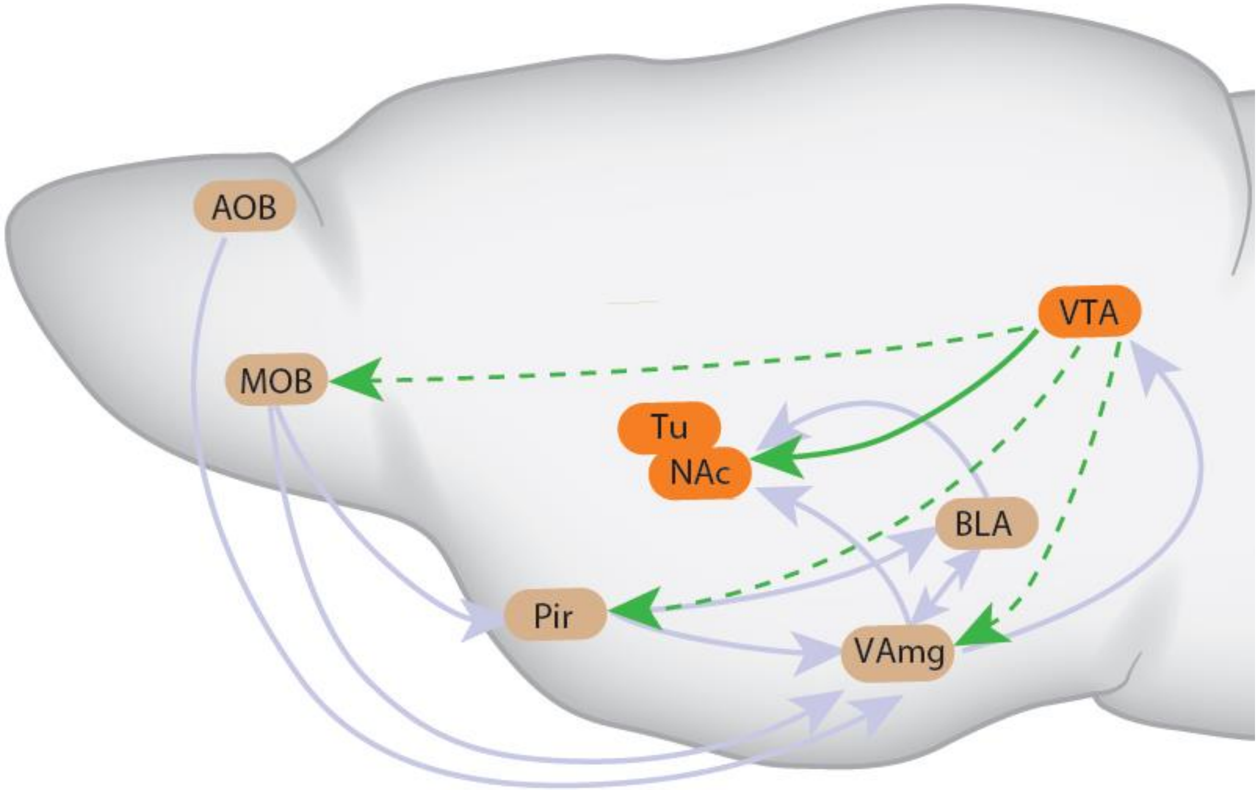
Beny and Kimchi 2016, Gene Brain and Behav

Mating behavior in male mouse requires the activation of a complex set of behavioral displays

Decision–making process via the dopaminergic reward system?



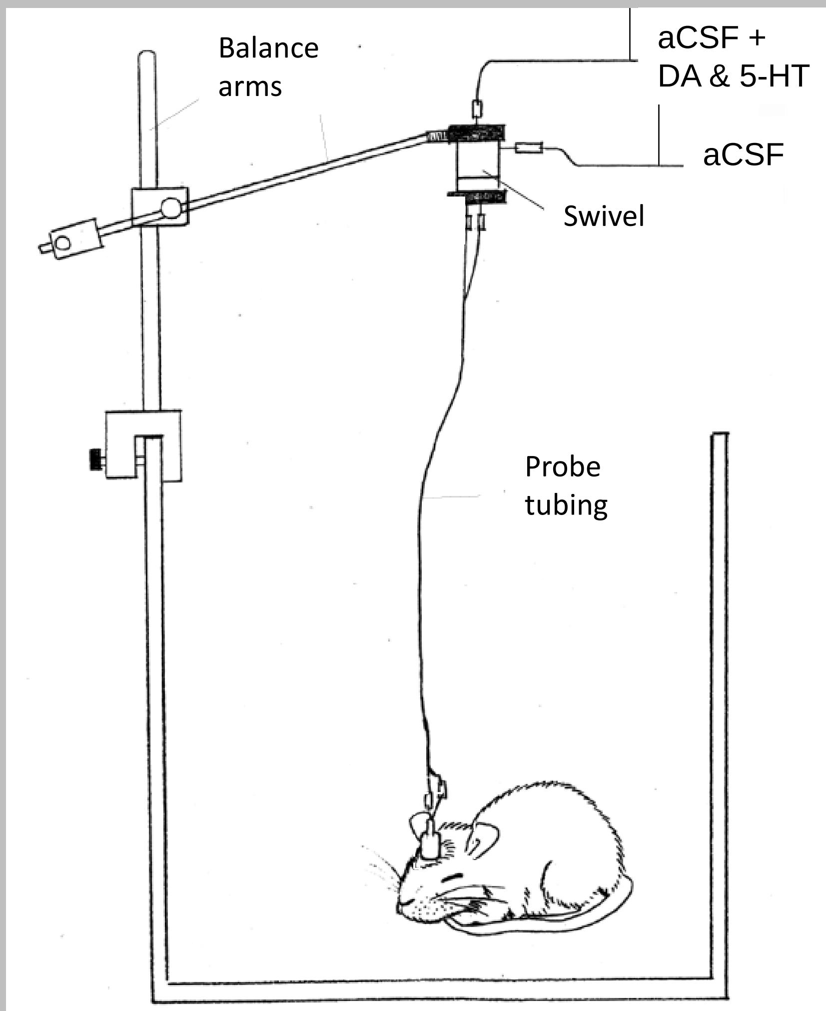
TrpC2-KO can discriminate between male and female olfactory stimuli (Beny et al 2016, *Gene brain Behav)*



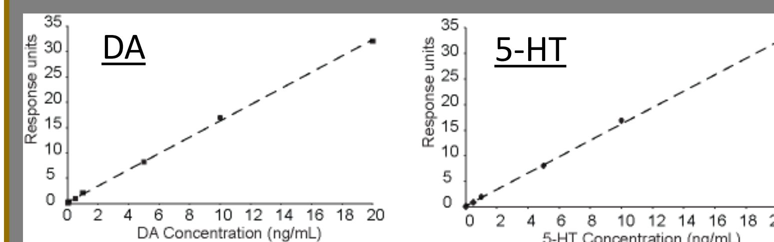
Reward areas
Olfactory areas

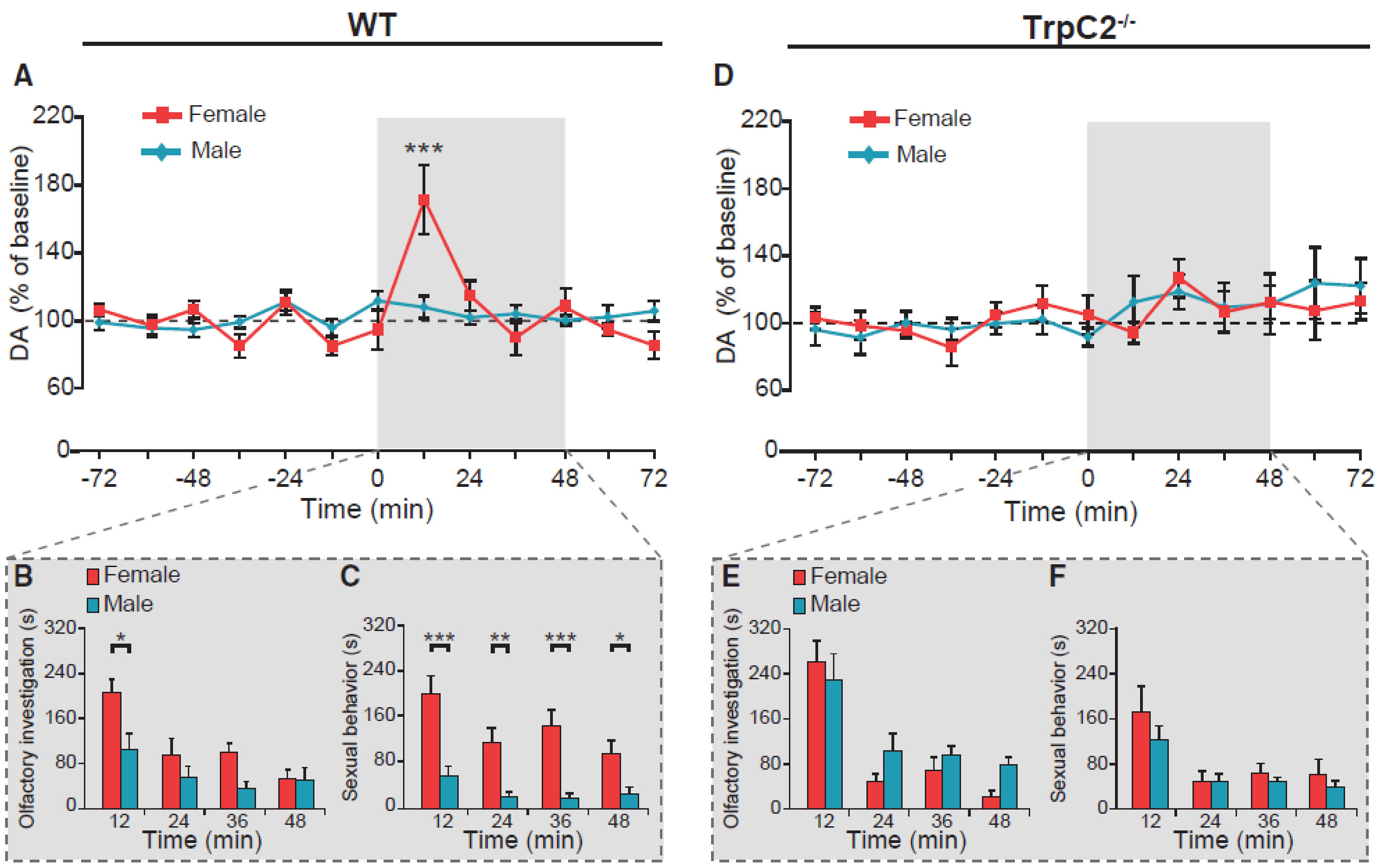
Measuring changes in dopamine (DA) and serotonin (5-HT) released in the NAc during social behavior in male mice

<u>Sampling the cerebrospinal fluid (CSF):</u>
in vivo microdialysis system



<u>Quantification of DA and 5-HT in CSF samples:</u>
UPLC-MS/MS system

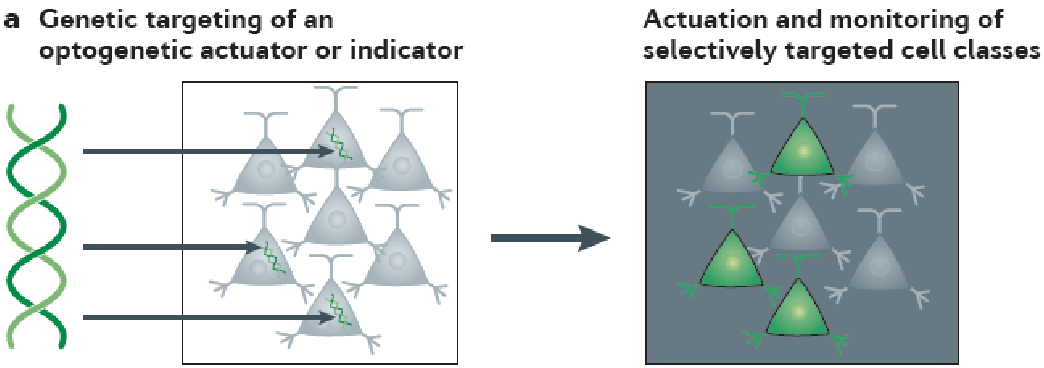


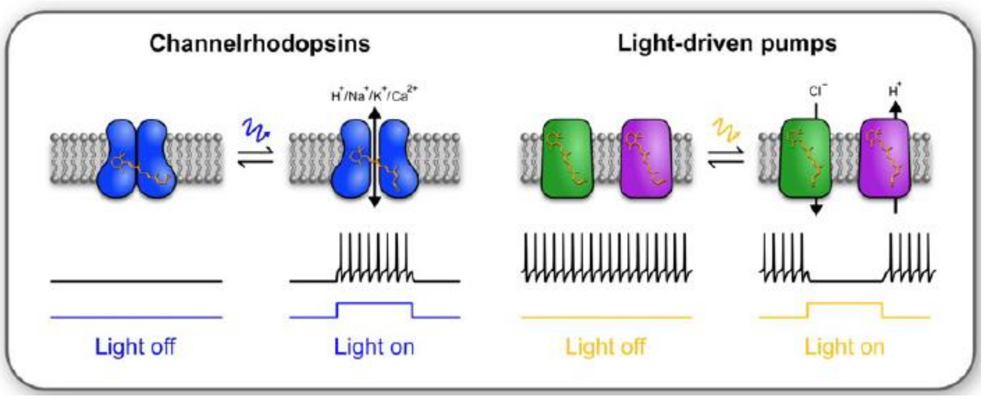


Shefer et al, 2017, Cell Reports

Exploring the direct role of VTA-NAc dopaminergic pathway in mediating sexual preference using optogenetics

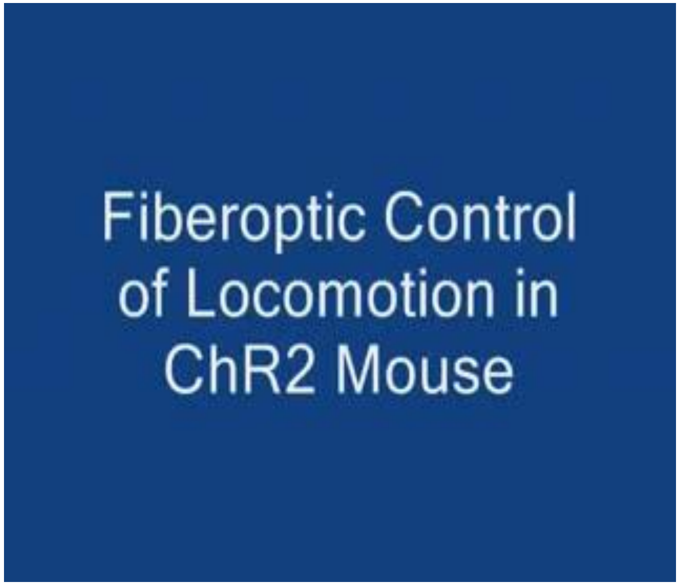
Optogenetics: Genetic and viral tools to conditionally alter neuronal activity in distinct brain regions and neuronal populations



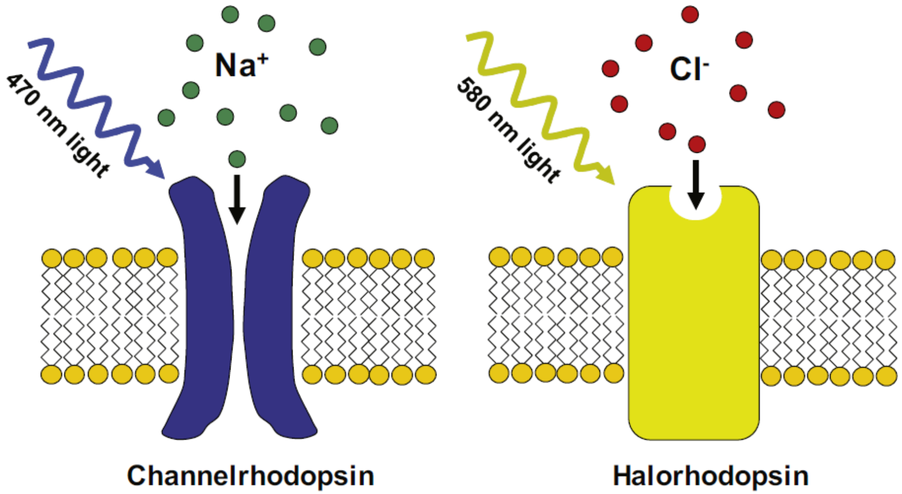


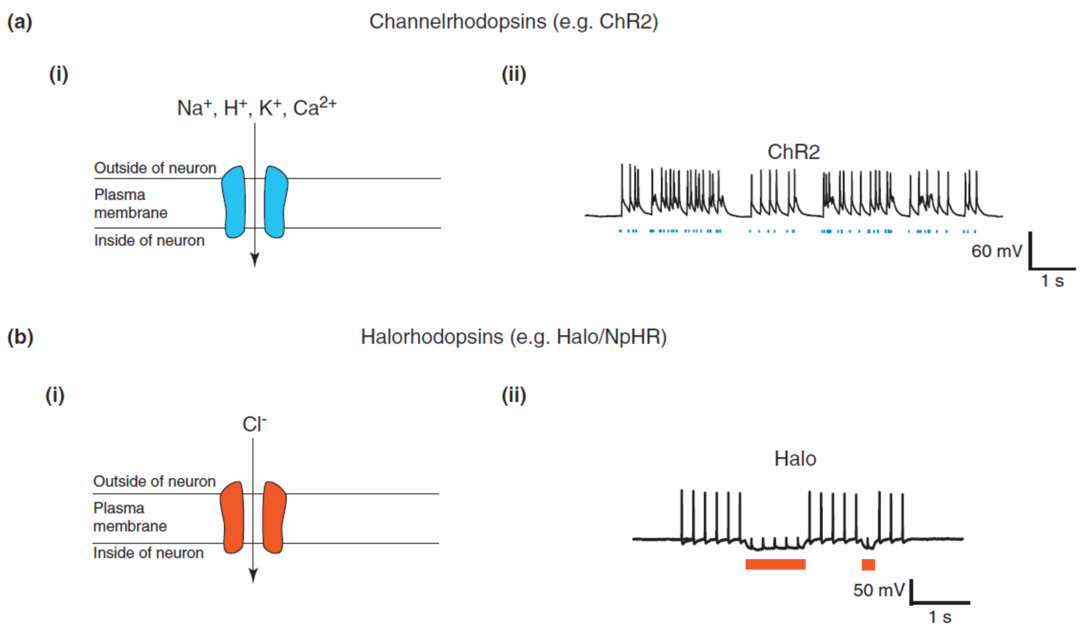
Neuronal activation

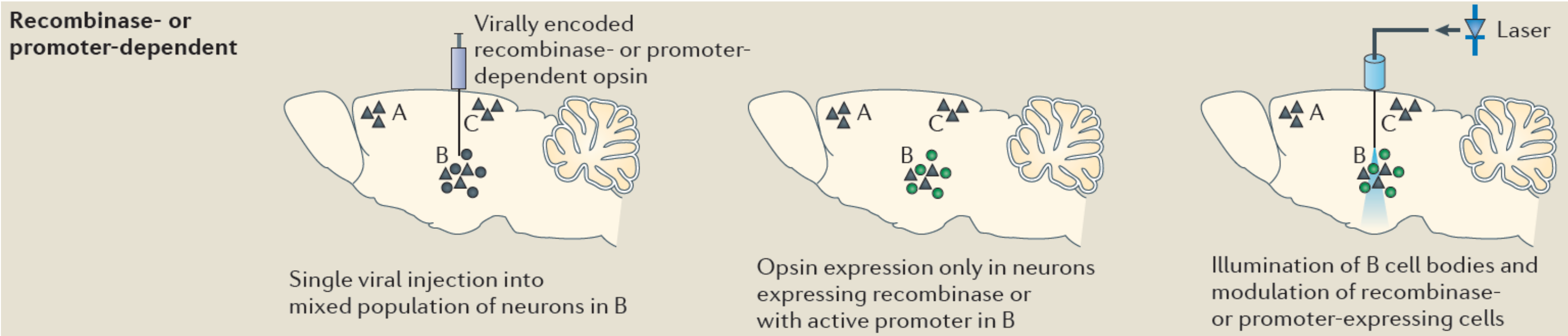
Neuronal inhibition

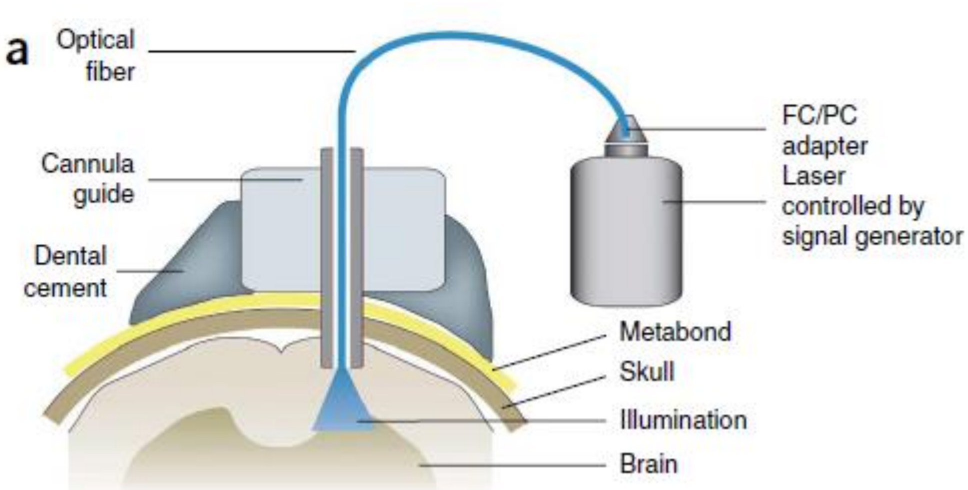


Neuronal manipulation using Optogenetic



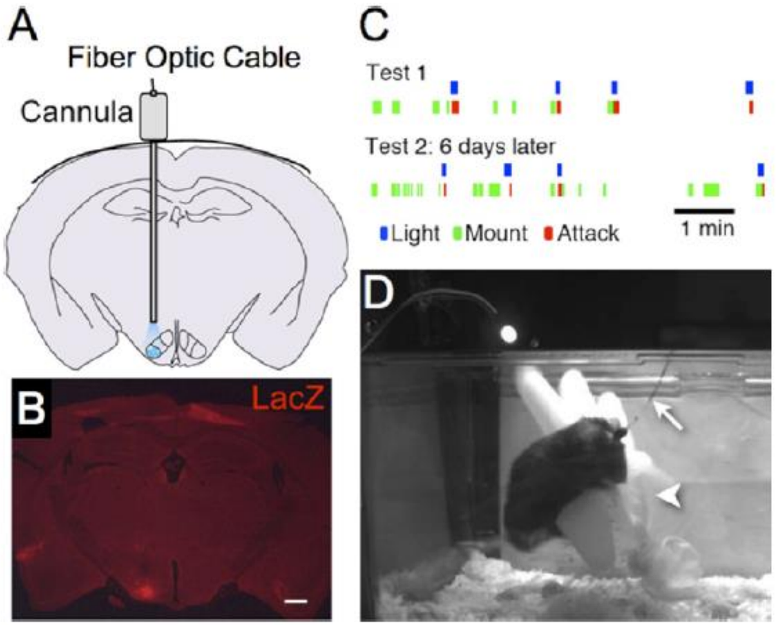


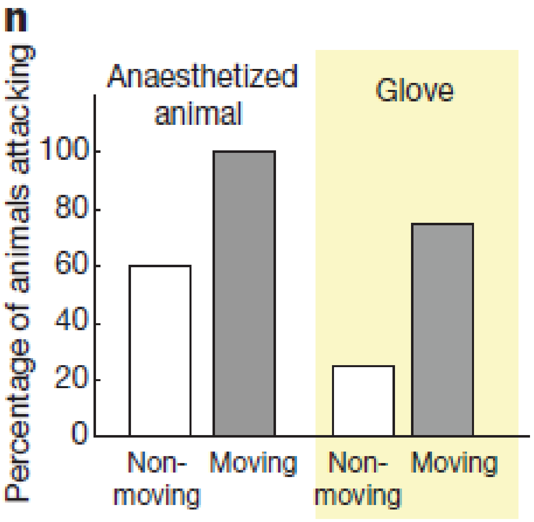




Yizharar et al. 2011, Neuron

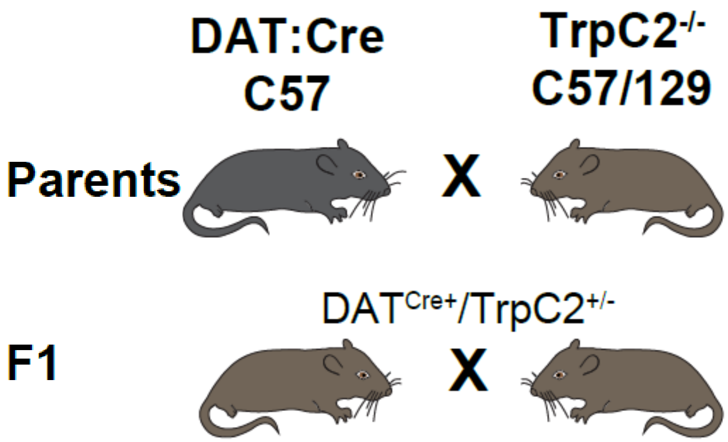
Activation of aggressive behavior using optogenetics in the VMH

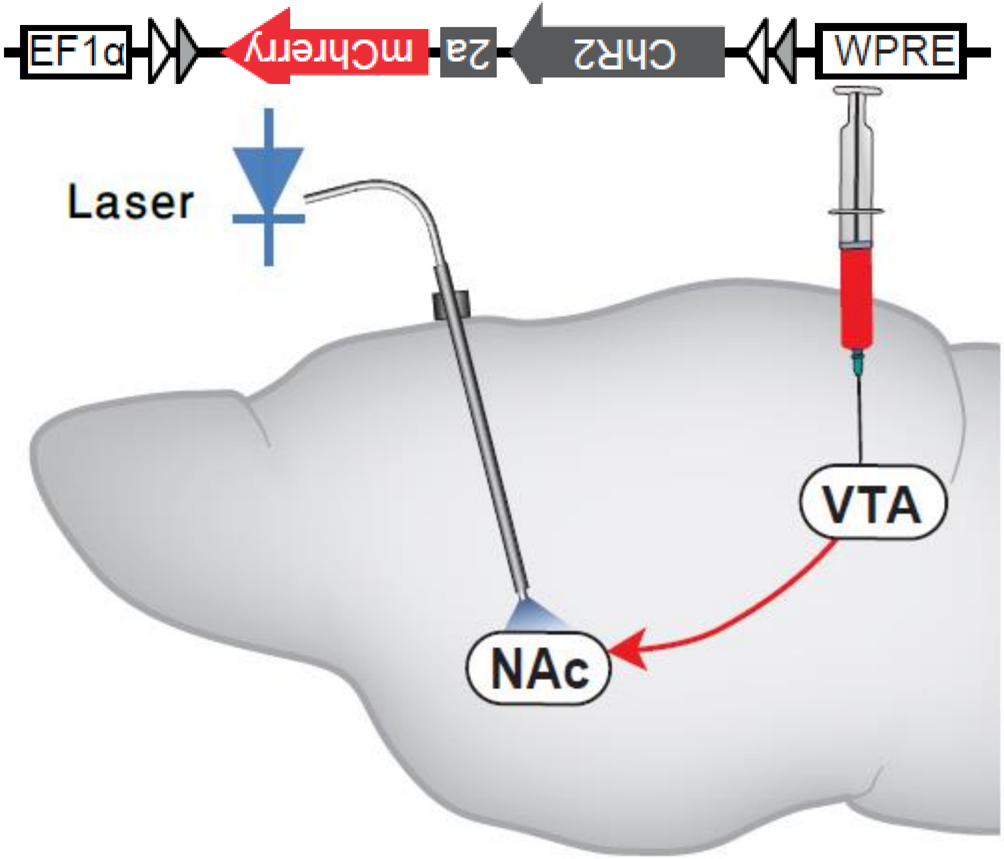




Lin et al, 2011, Nature

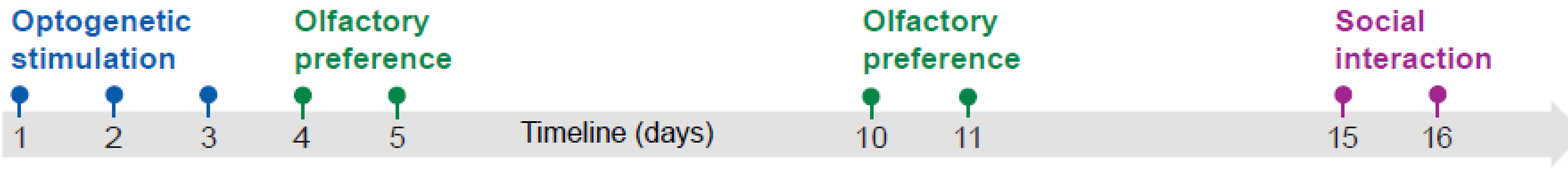
Optogenetic activation of VTA-NAc dopaminergic neurons





Shefer et al, 2017, Cell Reports

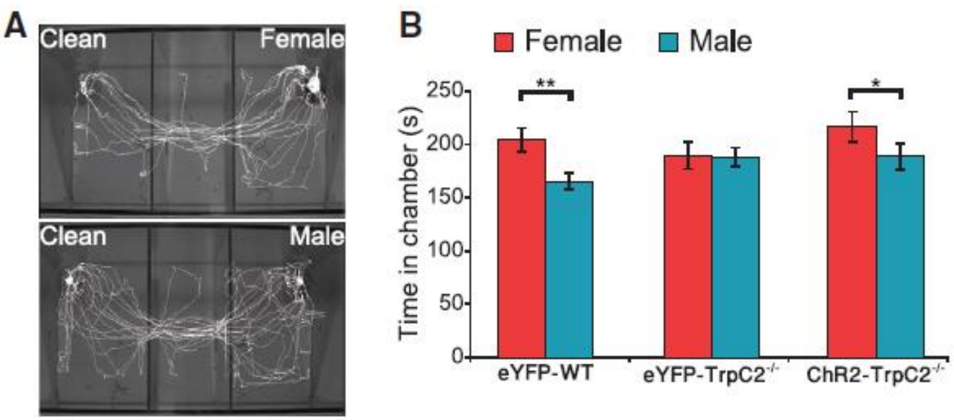
Optogenetic activation protocol

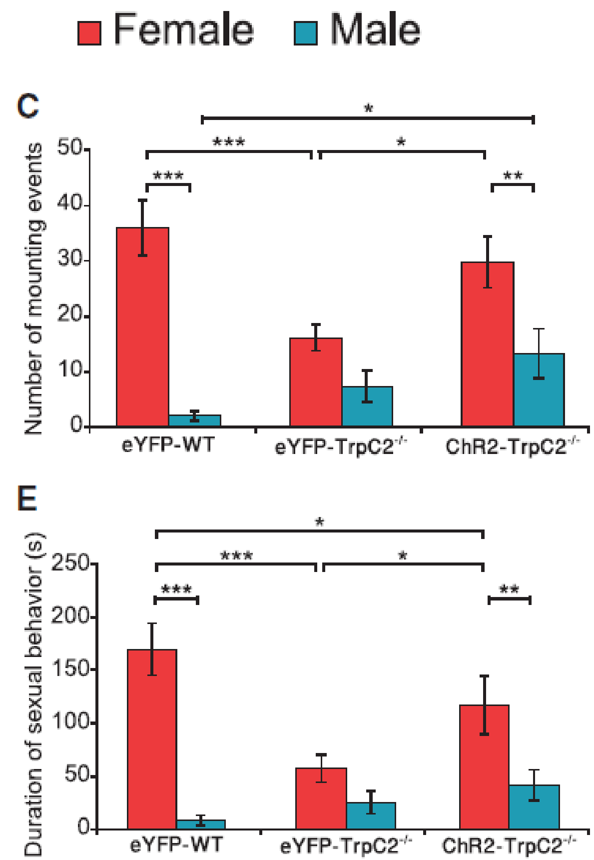


Shefer et al, 2017, Cell Reports

Sexual behavior

Olfactory preference





Shefer et al, 2017, Cell Reports

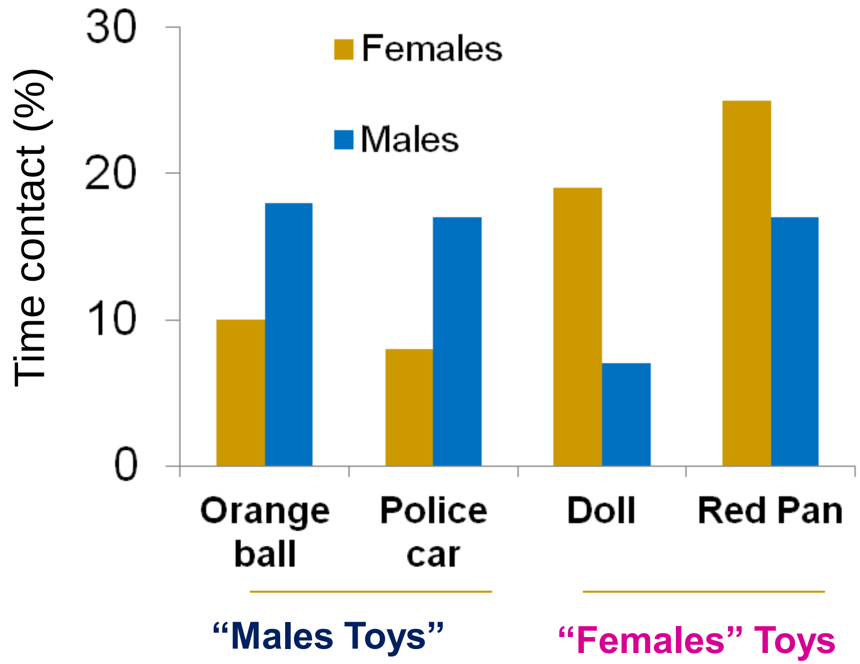
summary

Selective activation of dopaminergic neuronal projections in the NAc of TrpC2$^{-/-}$ males:

- Restores the lost olfactory preference
- Improves sexual behavior performance towards females
- Had no effect on aggressive behavior towards male intruders

Sexual dimorphism in behavior: Nature versus Nurture

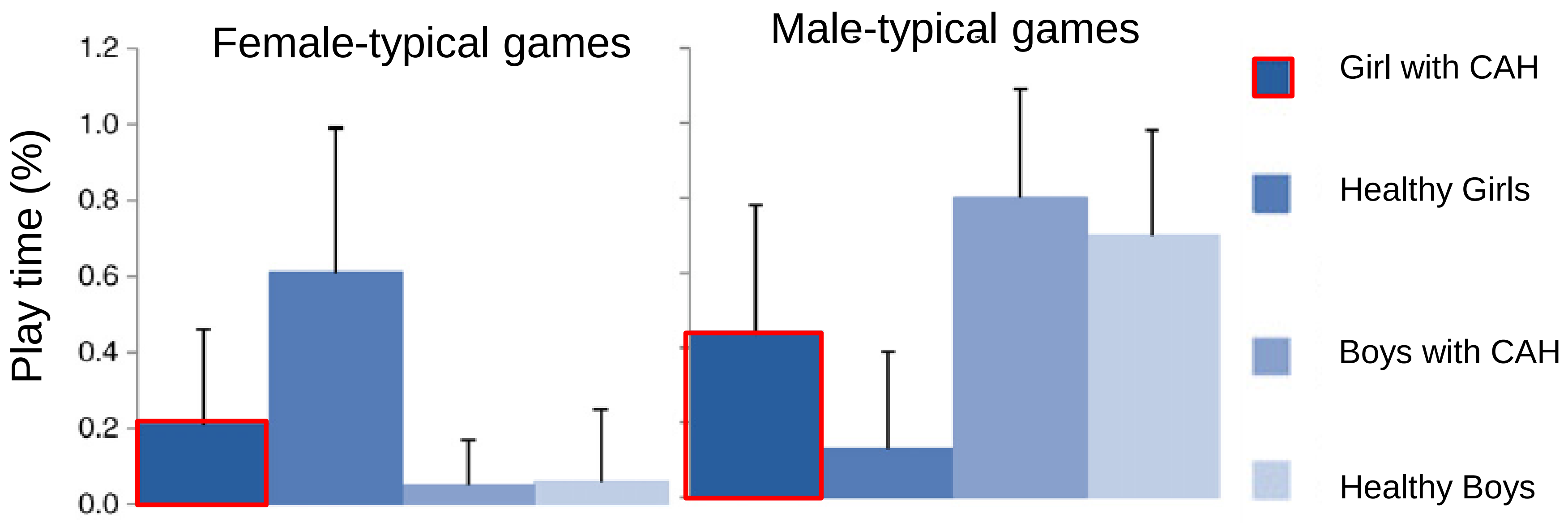
Sexual dimorphism in human behavior: Nature versus Nurture



Alexander and Hines, 2002, Evol Hum Behav

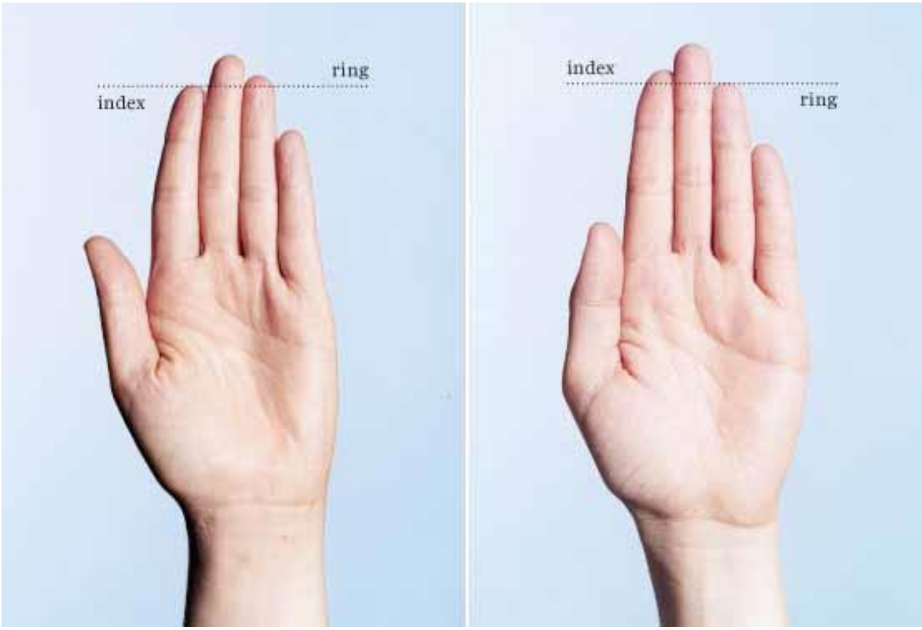
Congenital Adrenal Hyperplasia (CAH)- Genetic disease

Elevated exposure to testosterone during development



Pasterski et al 2005, Child Develop

Sex difference in digit ratio 2D:4D



Male-typical ratio   Female-typical ratio

- The index fingers of most men are shorter than their ring fingers, and for most women they are the same length or longer.
- Right-hand 2D:4D might be a better indicator of prenatal androgenisation than left-hand 2D:4D.
- 2D:4D ratios may provide a useful retrospective marker for early androgen exposure, making it possible to correlate such exposure with human behavior.

Williams, T. J. et al (2000) Nature

Table 4 Correlations between left and right hand digit ratios and scores on the personality scales

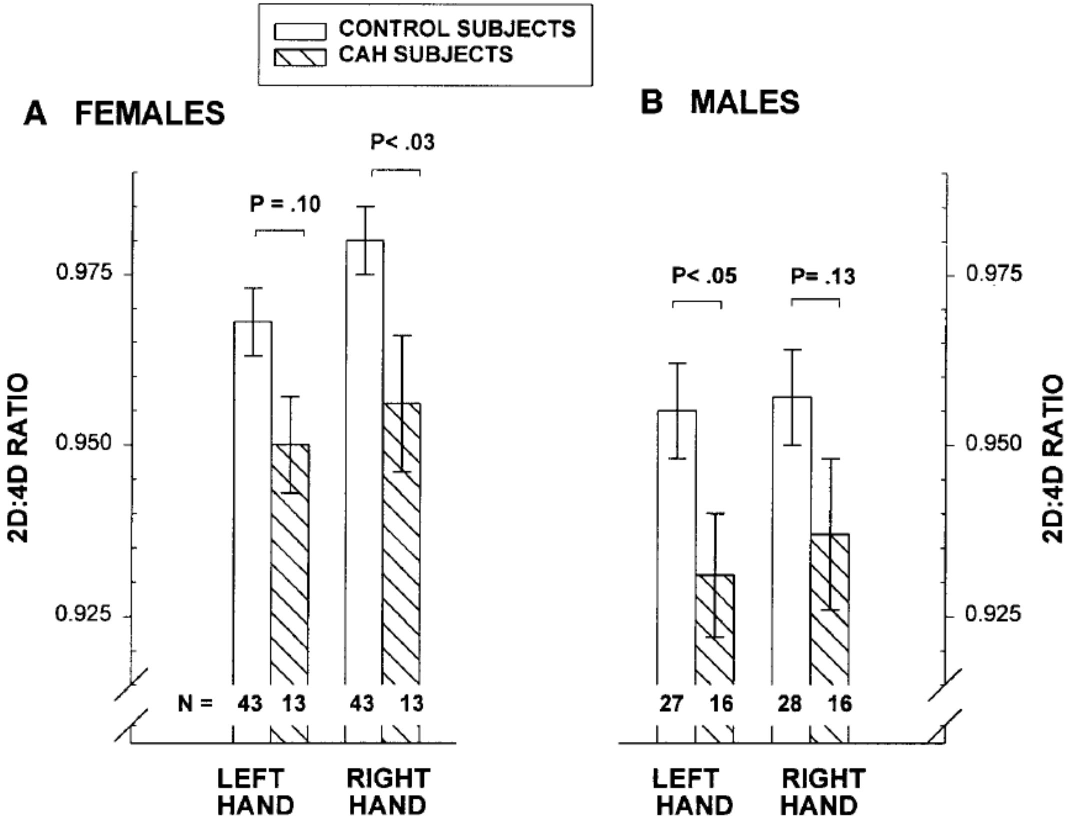
Scale	Men (n = 87)[a]		Women (n = 75)		Combined (n = 162)	
	Left r	Right r	Left r	Right r	Left r	Right r
Assertiveness	–.01	–.01	–.15	–.14	–.08	–.08
Emotional empathy	–.05	–.15	–.08	.11	.21***	.23***
PAQ masculinity	–.02	–.06	–.20*	.04	–.19**	–.11
PAQ femininity	–.15	–.05	–.09	–.21*	–.24***	–.22***
Nurturance	.14	–.01	–.17	–.12	.19**	.11
Sensation seeking	–.22**	–.10	–.10	–.23**	–.22***	–.22***
Boredom	–.19*	–.07	–.14	–.16	–.20***	–.14*
Disinhibition	–.12	–.12	–.04	–.13	–.12	–.14*
Experience seeking	–.13	.03	.05	–.14	–.05	–.05
Thrill/adventure	–.17	–.20*	–.16	–.25**	–.22***	–.25***
Aggression total	–.13	–.13	–.08	–.34***	–.15*	–.28****
Verbal	–.22**	–.22**	–.10	–.20*	–.20***	–.25***
Hostility	–.01	–.11	–.02	–.29**	–.03	–.20***
Physical	–.04	–.01	–.06	–.31***	–.14*	–.23***
Anger	–.17	–.10	–.11	–.29**	–.11	–.18**

****$p < .001$, ***$p < .01$, **$p < .05$, *$p < .10$

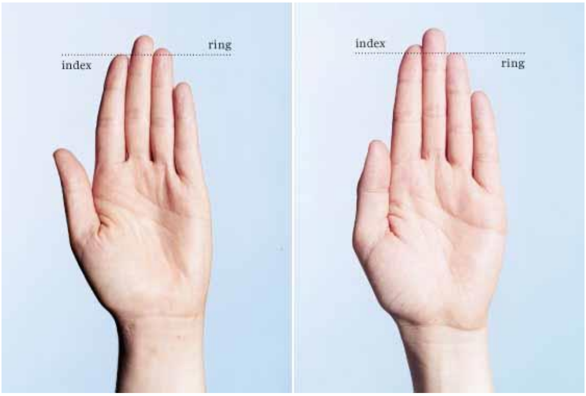
[a] Ns reduced on some scales due to missed items

Hampson et al 2008, Arch Sex Behav

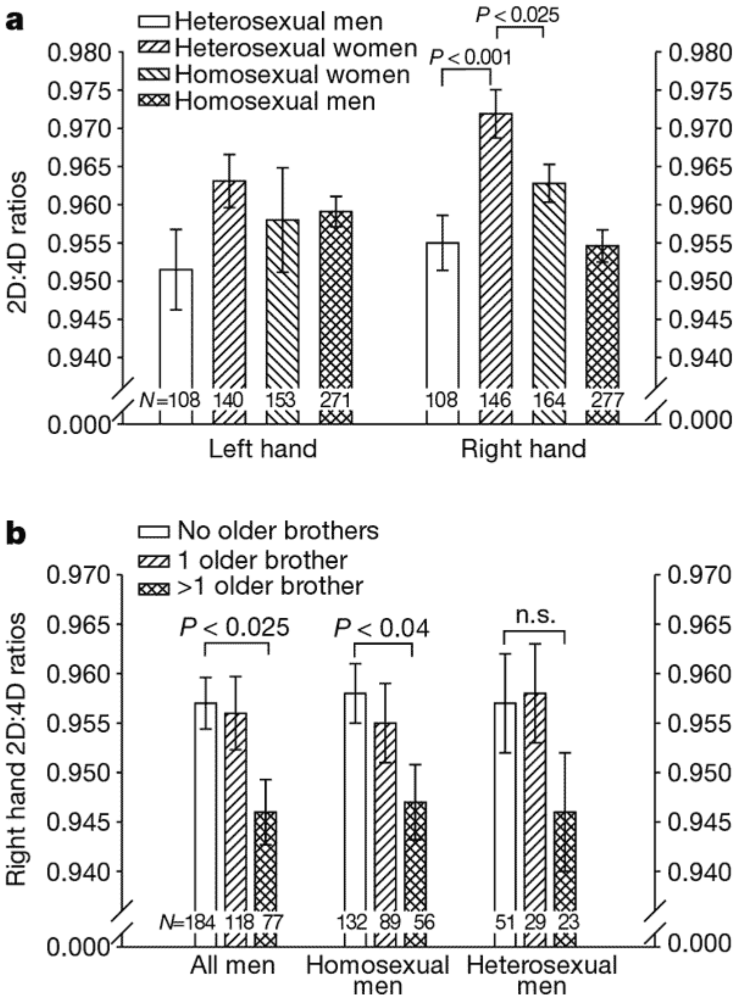
Masculinized Finger Length Patterns in Human Males and Females with Congenital Adrenal Hyperplasia



Brown et al 2002, Horm and Behav



Male-typical ratio Female-typical ratio



- Gay men and women <u>tend</u> to have reversed ratios.

Williams, T. J. et al (2000) Nature

The boy who was raised as a girl

Twins Bruce and Brian Reimer were born in Canada as two perfectly normal boys

Bruce's penis was damaged
During an unsuccessfully surgery for
urinary problems

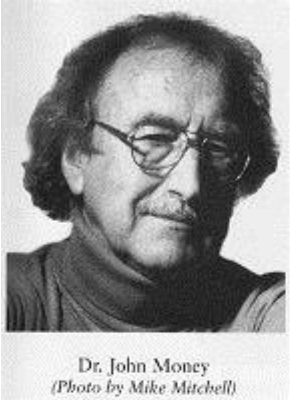
Dr John Money was a psychologist specializing in sex changes

Dr. John Money
(Photo by Mike Mitchell)

Suggested the "ideal" sex change experiment

The boy who was raised as a girl

Suggested the "ideal" sex change treatment



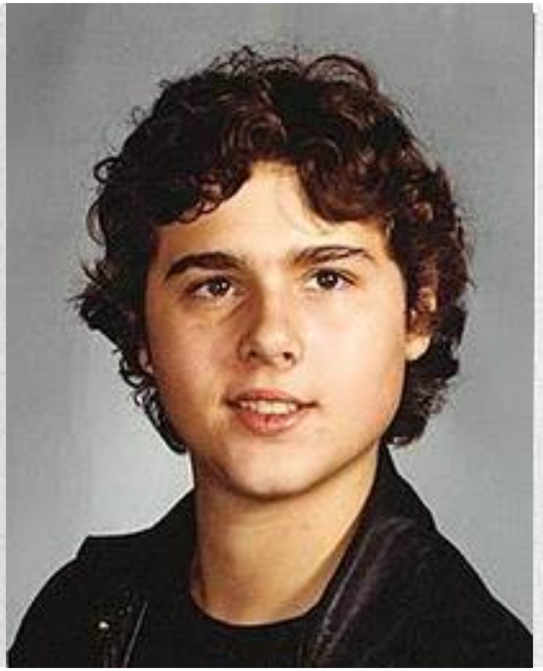
Dr Money genuinely believed that Bruce had a better chance of living a happy life as a woman than as a man without a penis

At the age of ~2 years old Bruce is castrated and treated with female sex hormones

Bruce raised as Brenda

The boy who was raised as a girl

David got married but later became depressed



At the age of 38 David committed suicide (2 years after his brother died from a drug overdose)

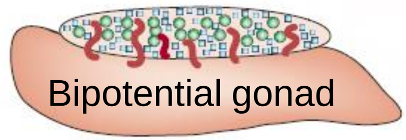
At the age of 15 Brenda switch again
To a male named David

http://youtube/MUTcwqR4Q4Y

http://www.bbc.co.uk/news/health-11814300

Dimorphism of the brain: differentiation and activation

XY ♂

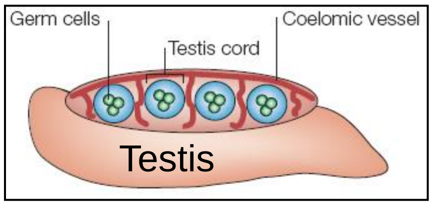


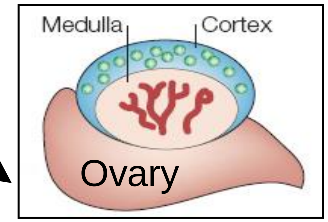
♀ XX

SRY gene

+

-

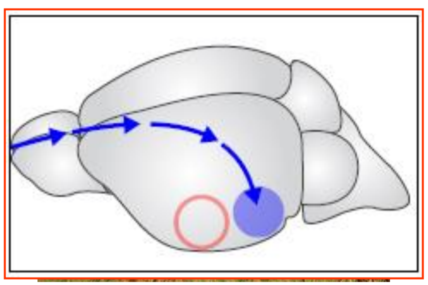


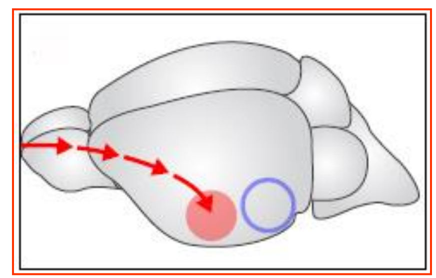


Testosterone &
Estradiol

Organization
(permanent changes)

Estradiol &
Progesterone





Testosterone &
Estradiol

Activation

Estradiol &
Progesterone

Sex-specific behaviors

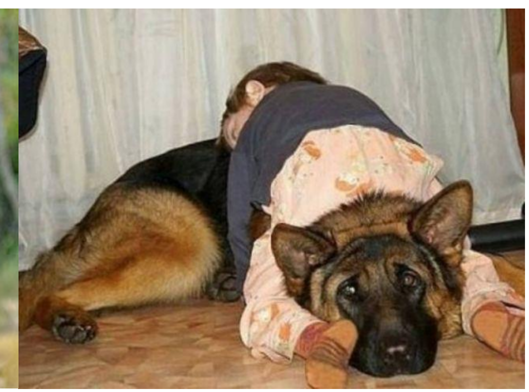
How are sexually dimorphic reproductive behaviors encoded by the male and female brain?

Dimorphic brain functions/structures ⇨ Dimorphic social behaviors ?

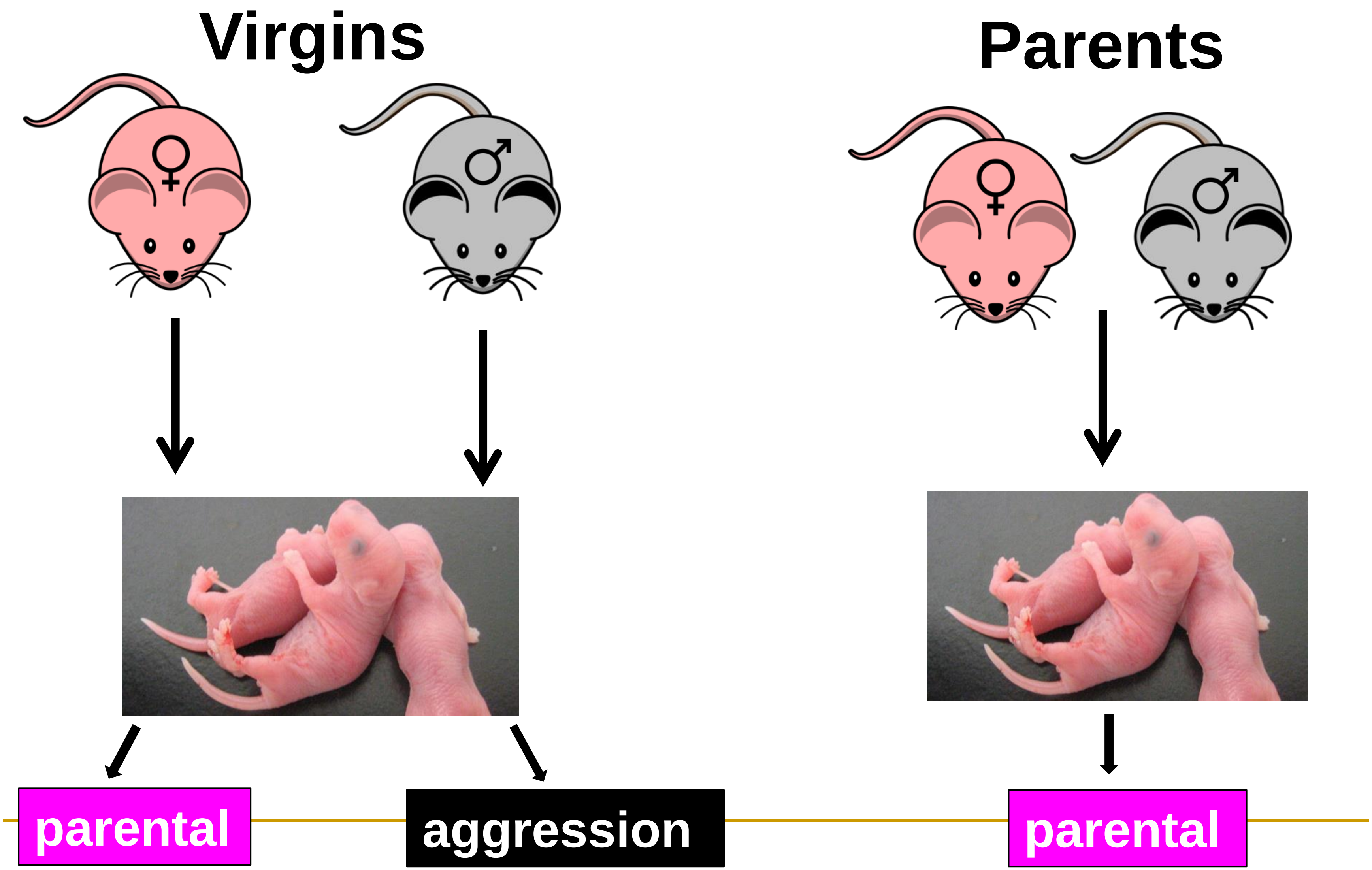
Parental care- evolutionary conserved behavior



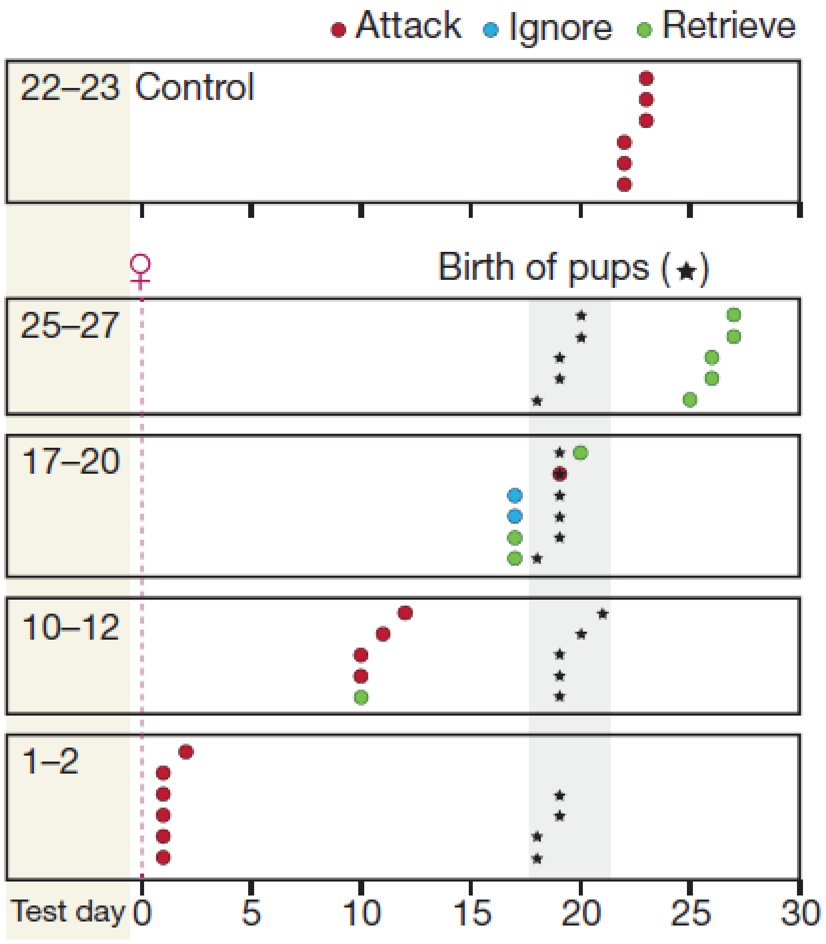




Sexual dimorphism in pup-directed behaviors

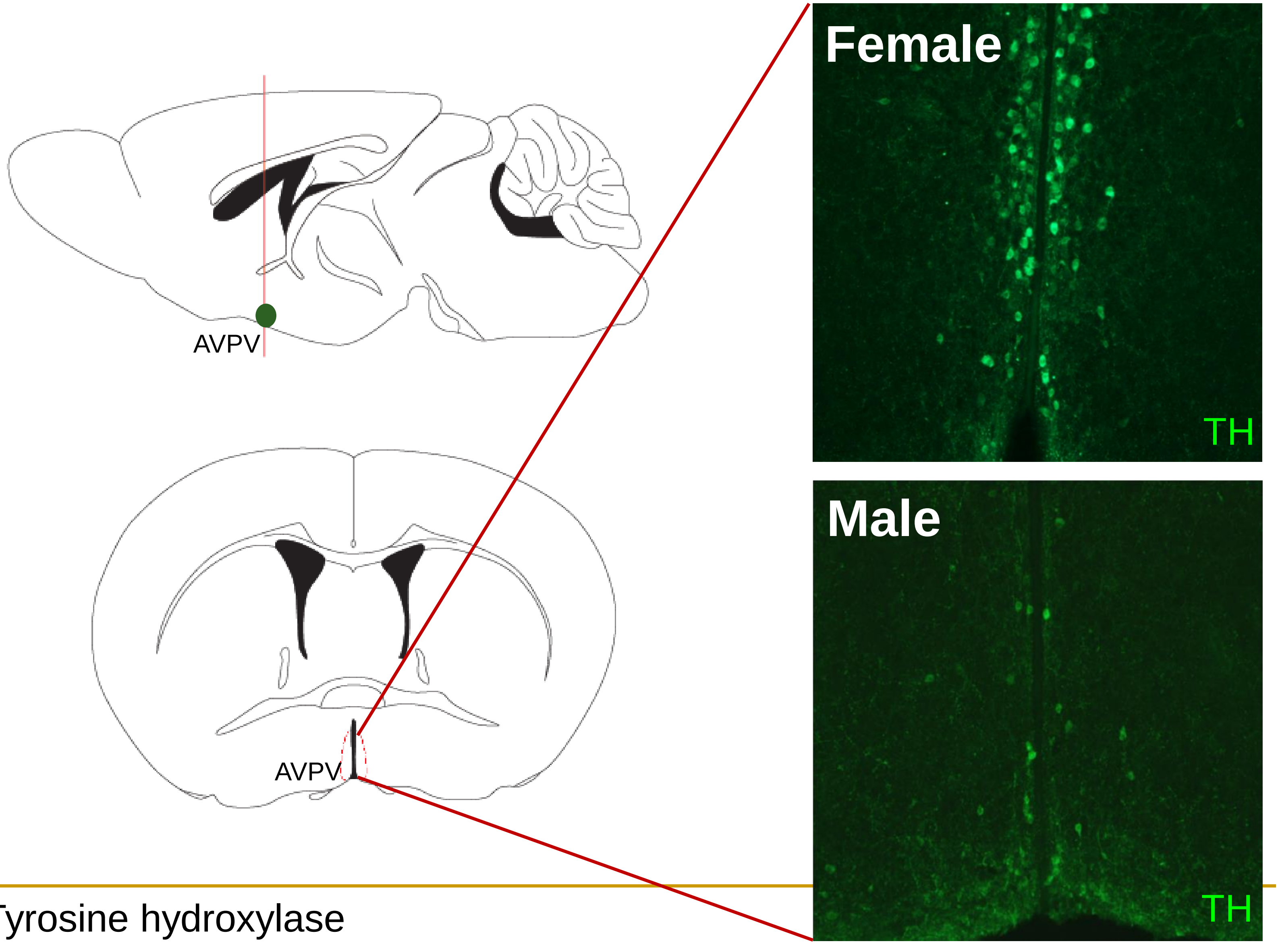


Paternal care following cohabitation with females subsequent to mating



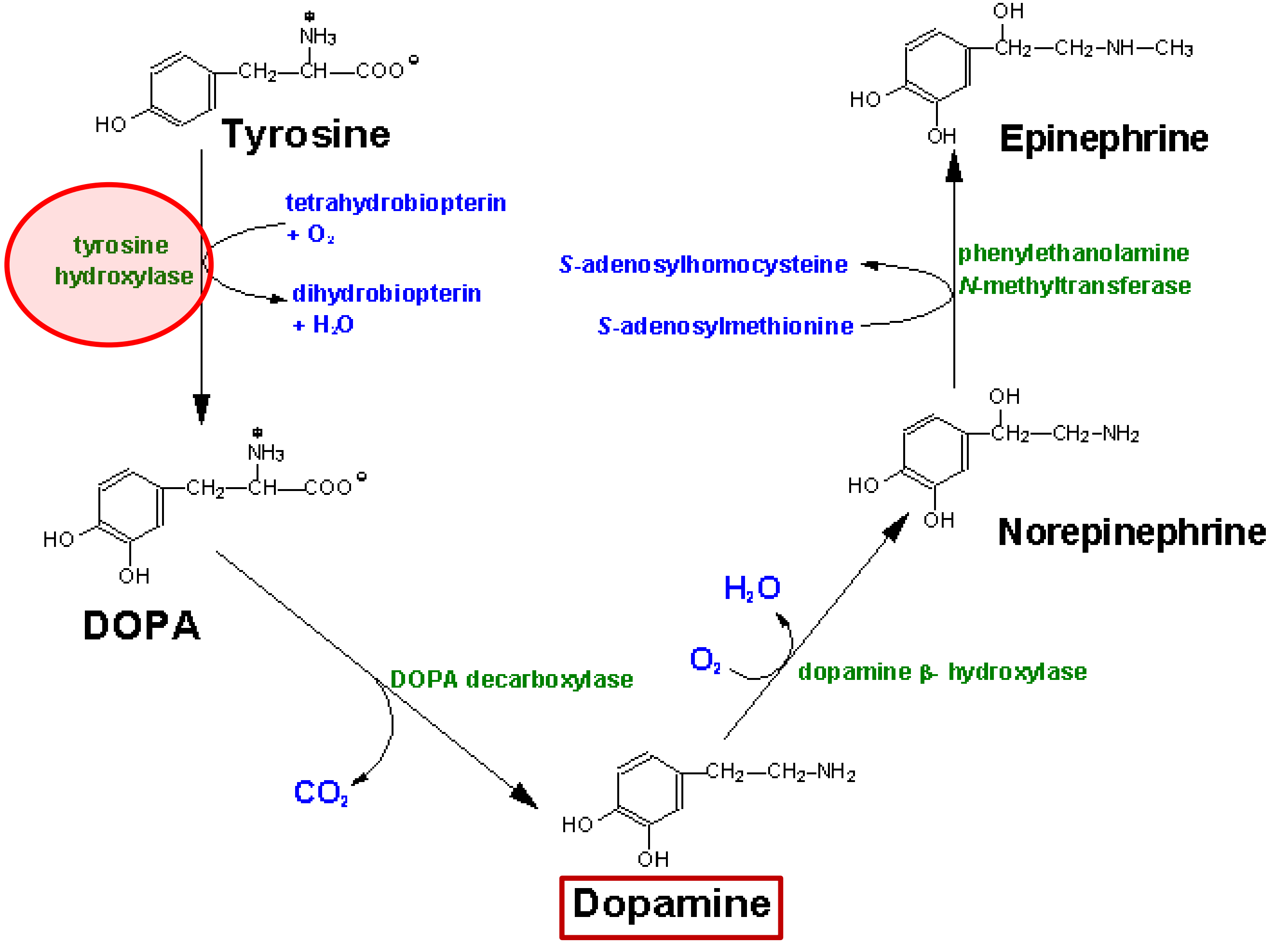
Wu et al 2015; *Nature*

Sexual dimorphism in tyrosine hydroxylase-positive neurons in the Anteroventral Periventricular Nucleus (AVPV)

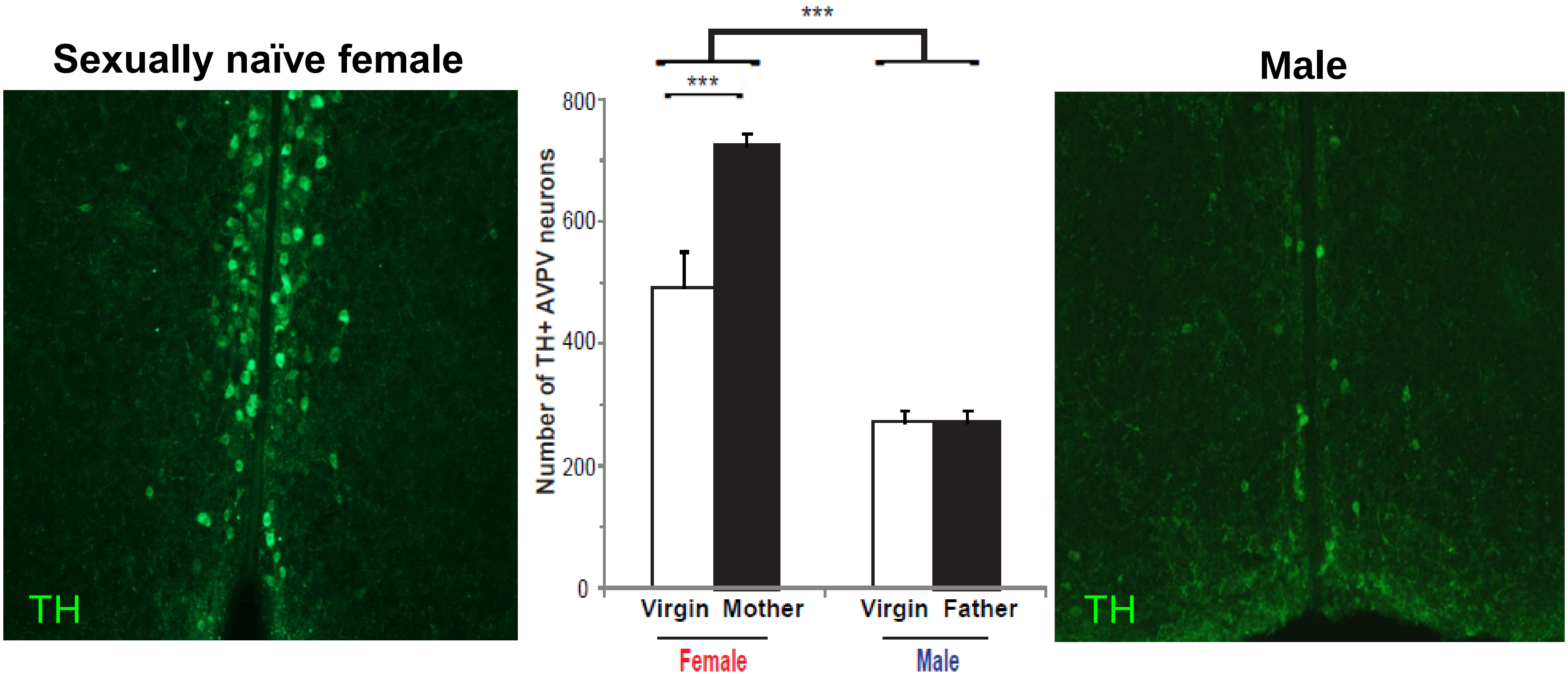


TH= Tyrosine hydroxylase

TH-expressing neurons in the AVPV can produce dopamine



More dopamine (TH+) secreting neurons in females than in males in the AVPV brain region



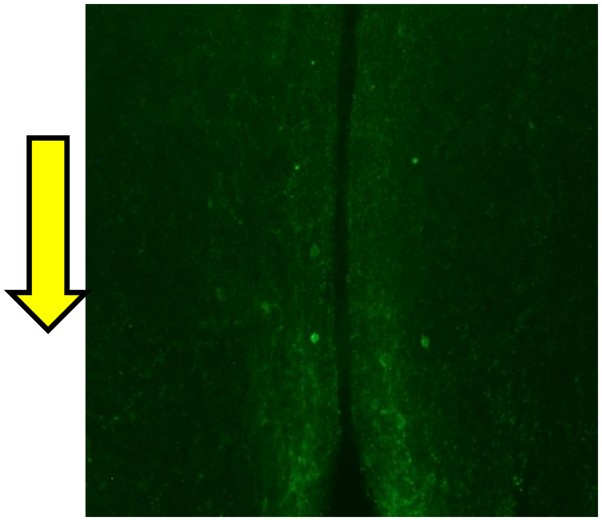
Green = anti-TH ab

TH= Tyrosine Hydroxylase
AVPV= Anteroventral hypothalamic area

Scott et al. 2015; *Nature*

Selective manipulations of TH+ AVPV neurons in adult males and females

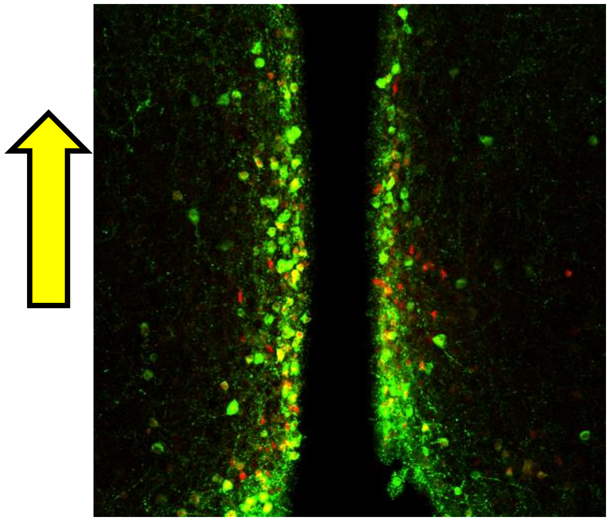
Neuronal ablation



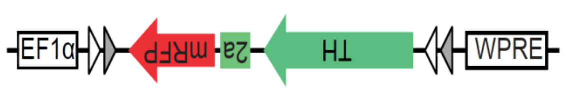
TH-ablation

6-OHDA

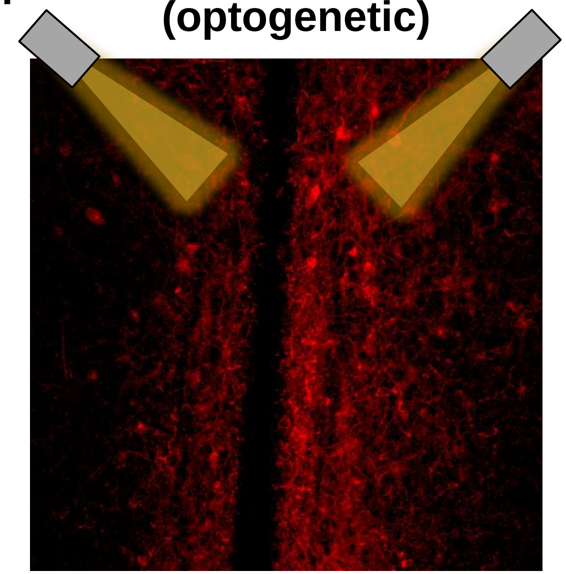
Neuronal over-expression



TH-overexpression



Neuronal activation using light stimulation (optogenetic)



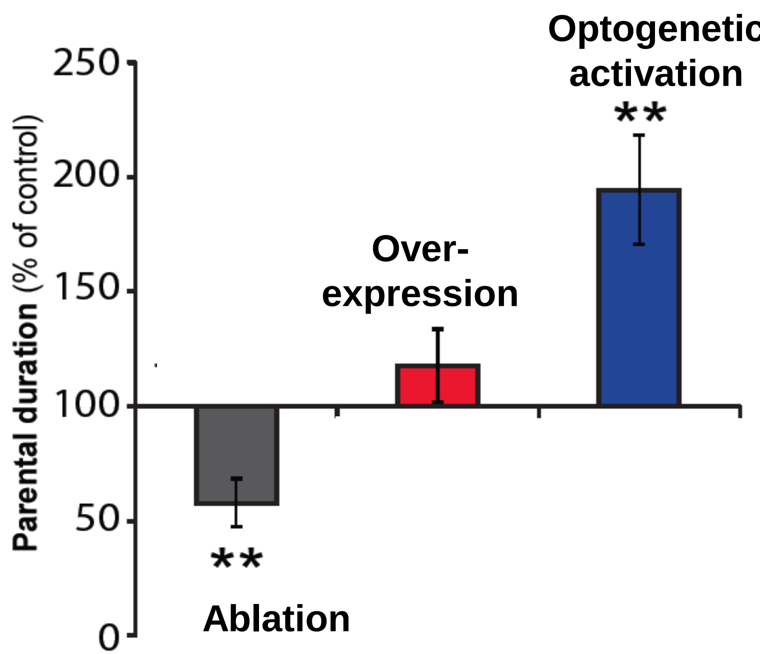
TH-ChR2

In females, hypothalamic dopaminergic (TH+ AVPV) neurons promote maternal care

♀

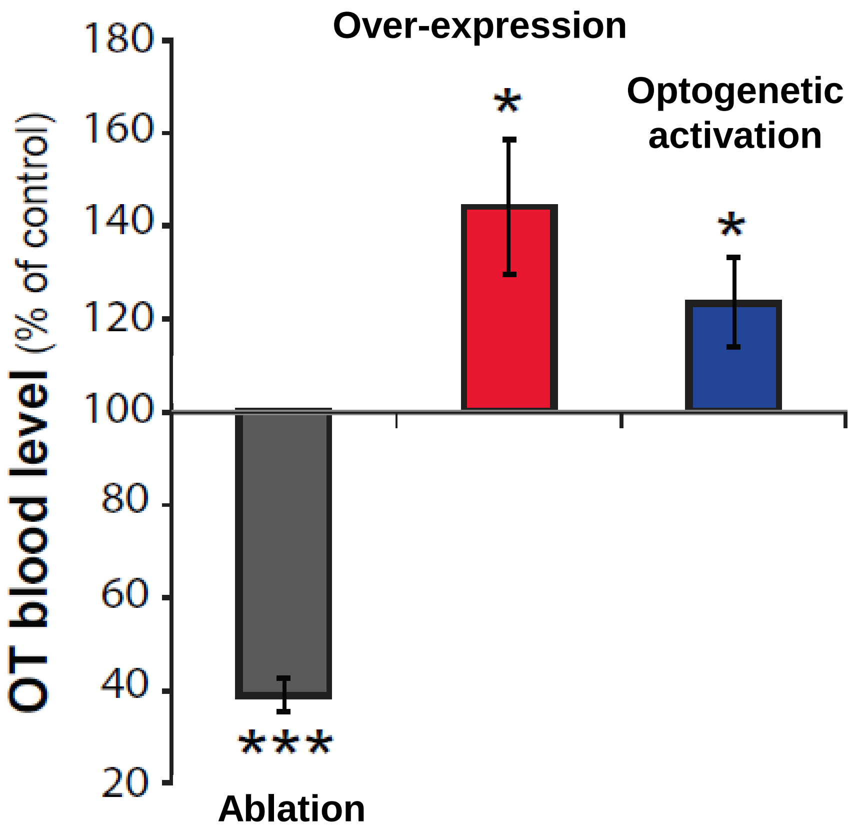
Crouching over the pups

Pup retrieval back to the nest

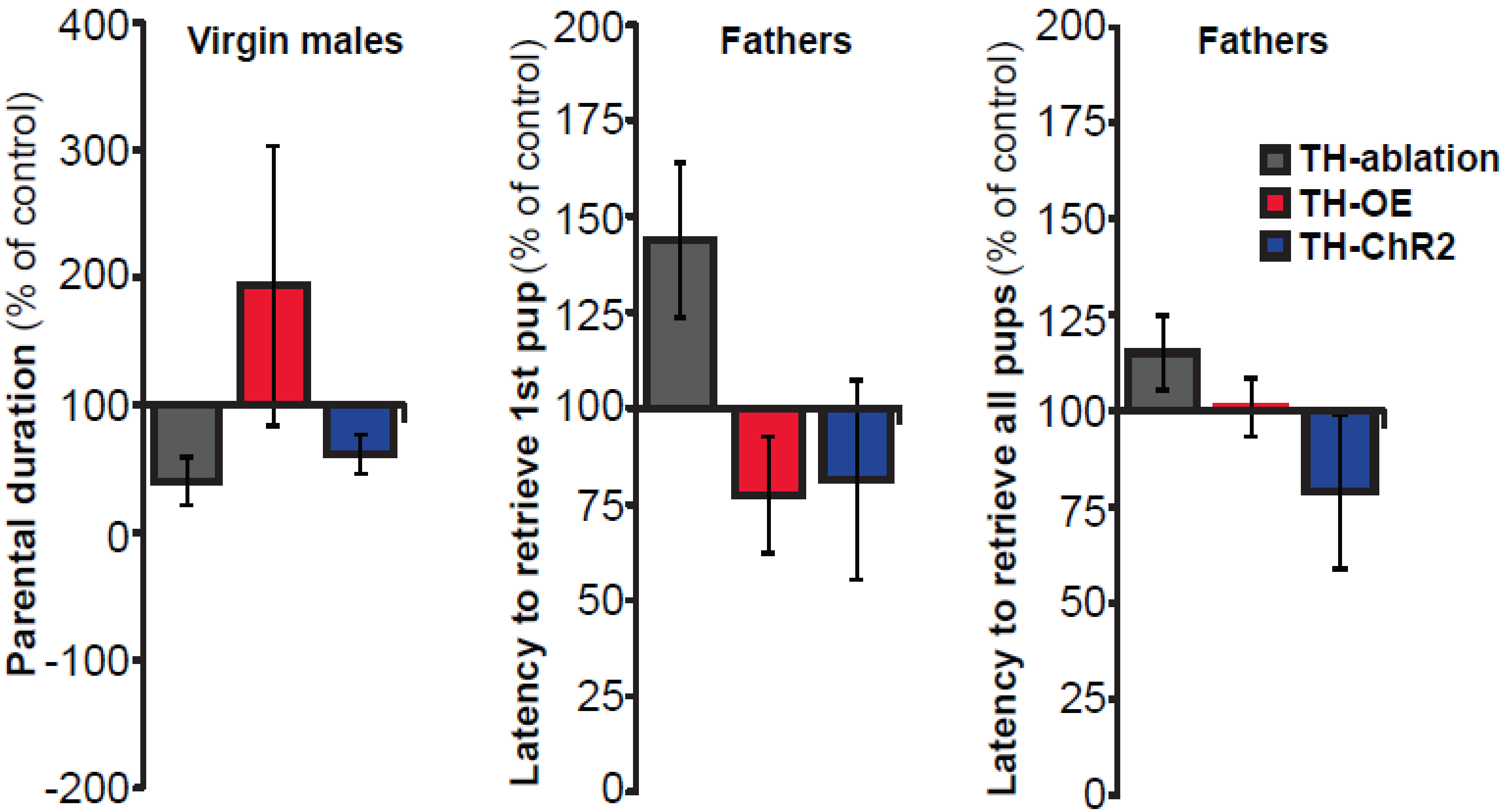


In females, TH+ AVPV neurons trigger oxytocin (OT) secretion

♀

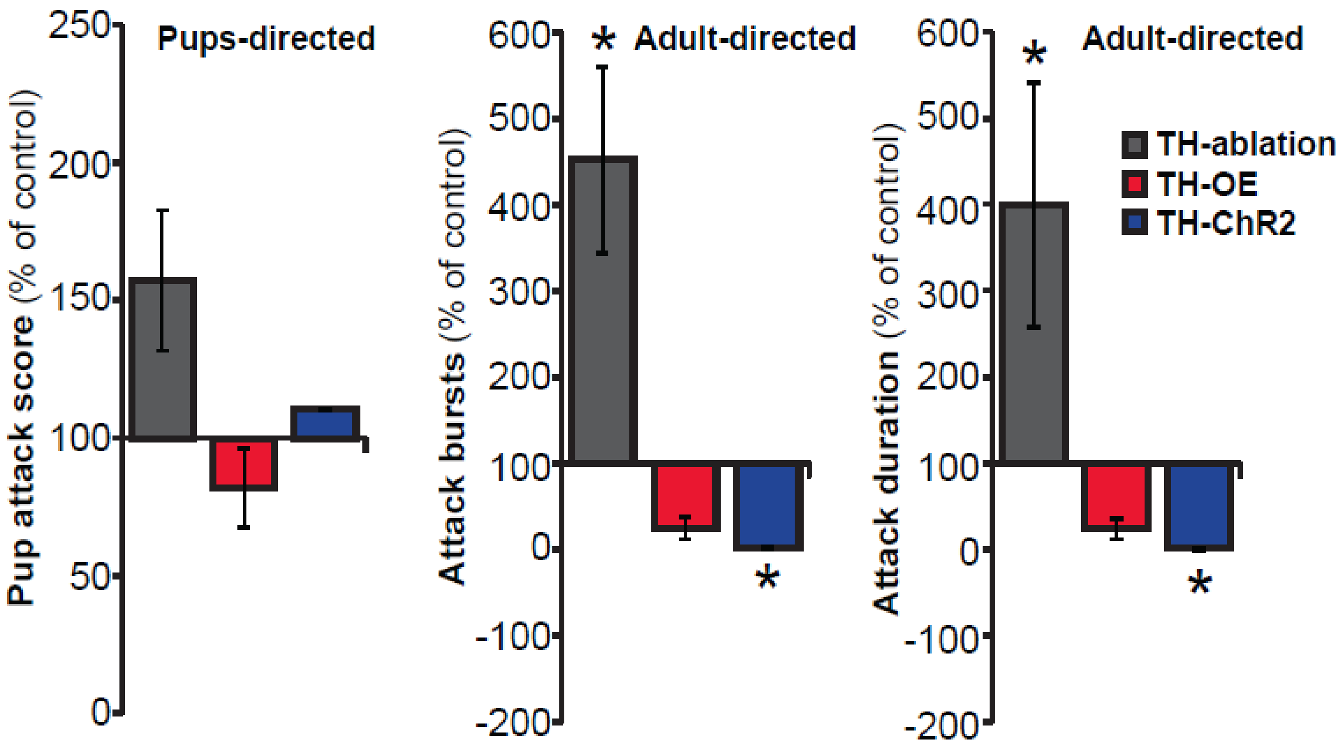


TH+ AVPV neurons are not involved in the regulation of parental behavior in males



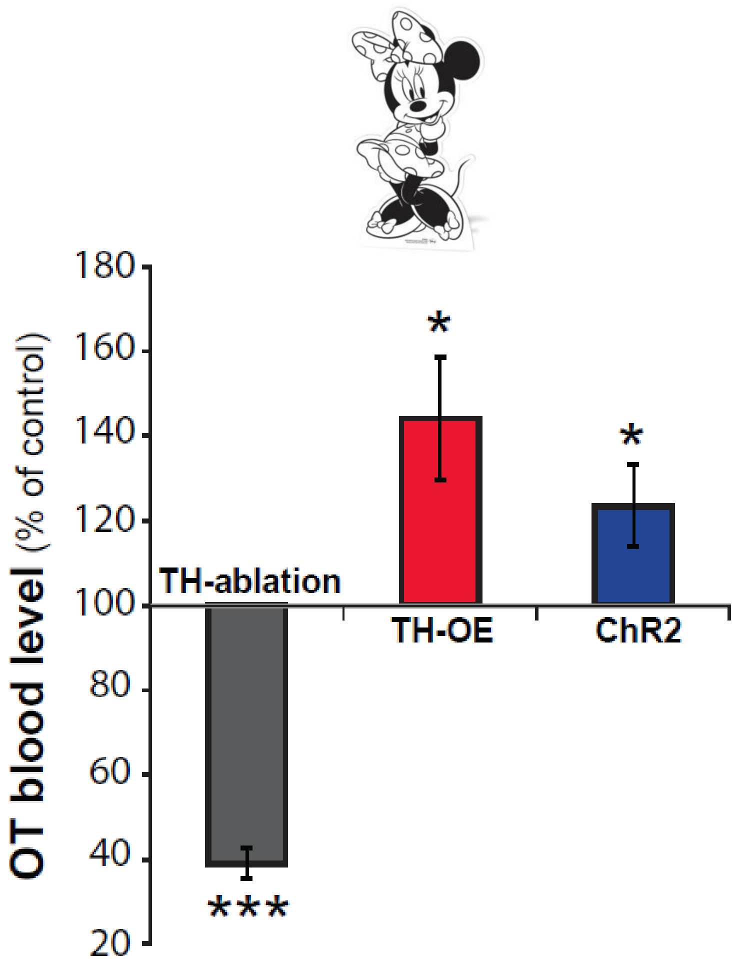
Scott et al. 2015; *Nature*

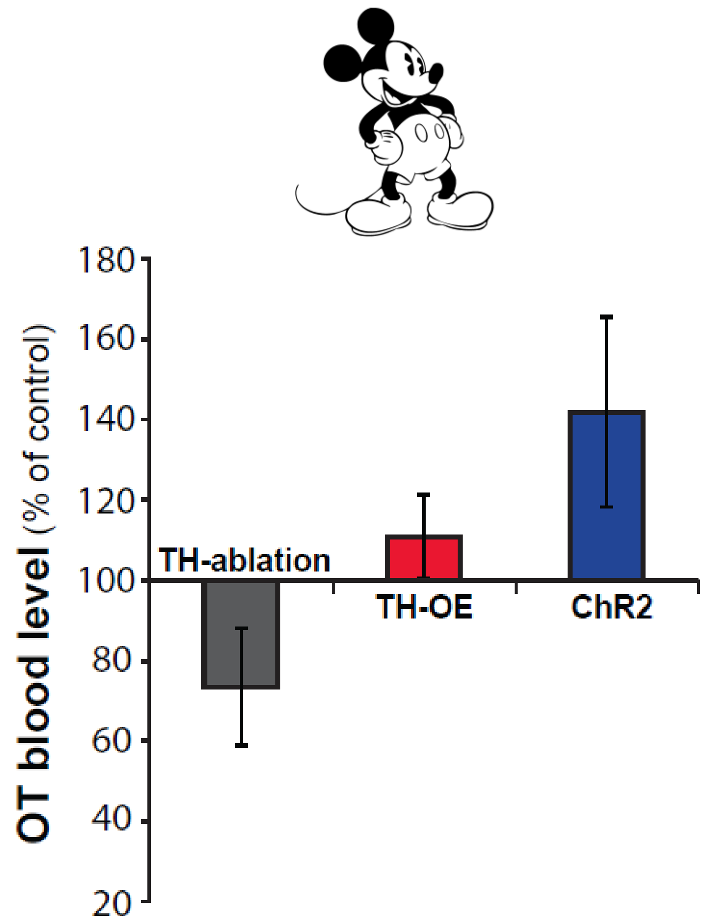
TH+ AVPV neurons are involved in suppression of conspecific aggressive behaviors



Scott et al. 2015; *Nature*

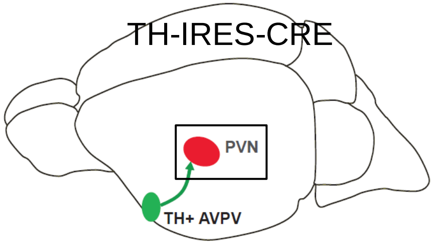
TH+ AVPV neurons are not involved in the regulation of oxytocin release in males

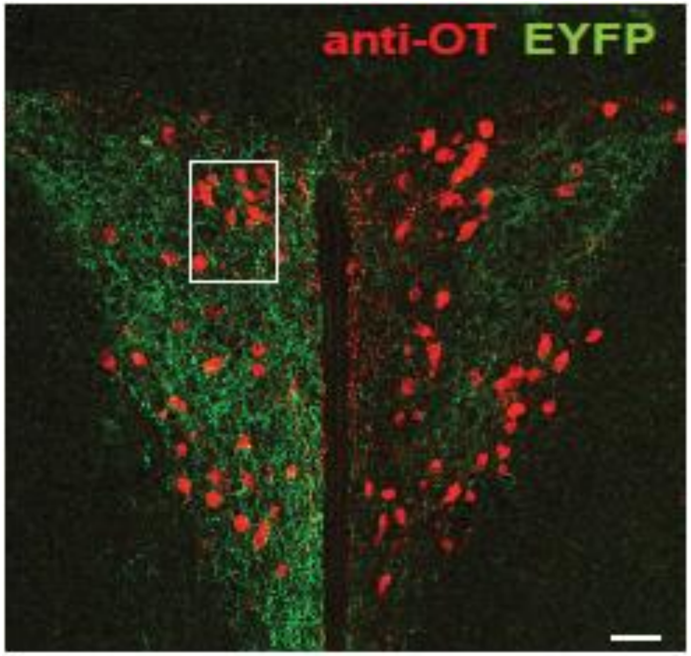


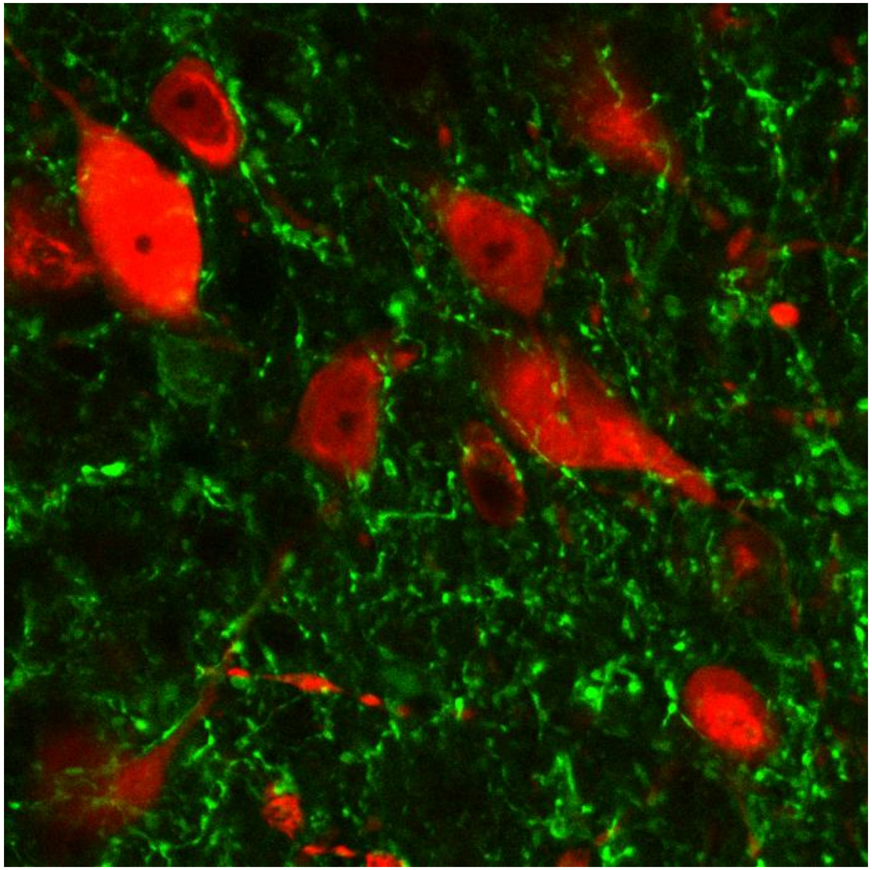


Scott et al. 2015; *Nature*

TH+ AVPV labeled fibers in close proximity to OT+ PVN neurons





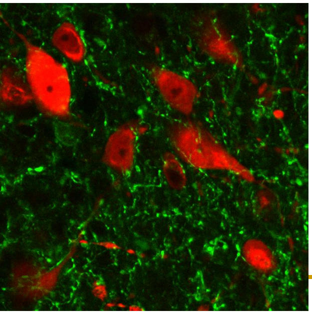


Scott et al. 2015; *Nature*

Main findings

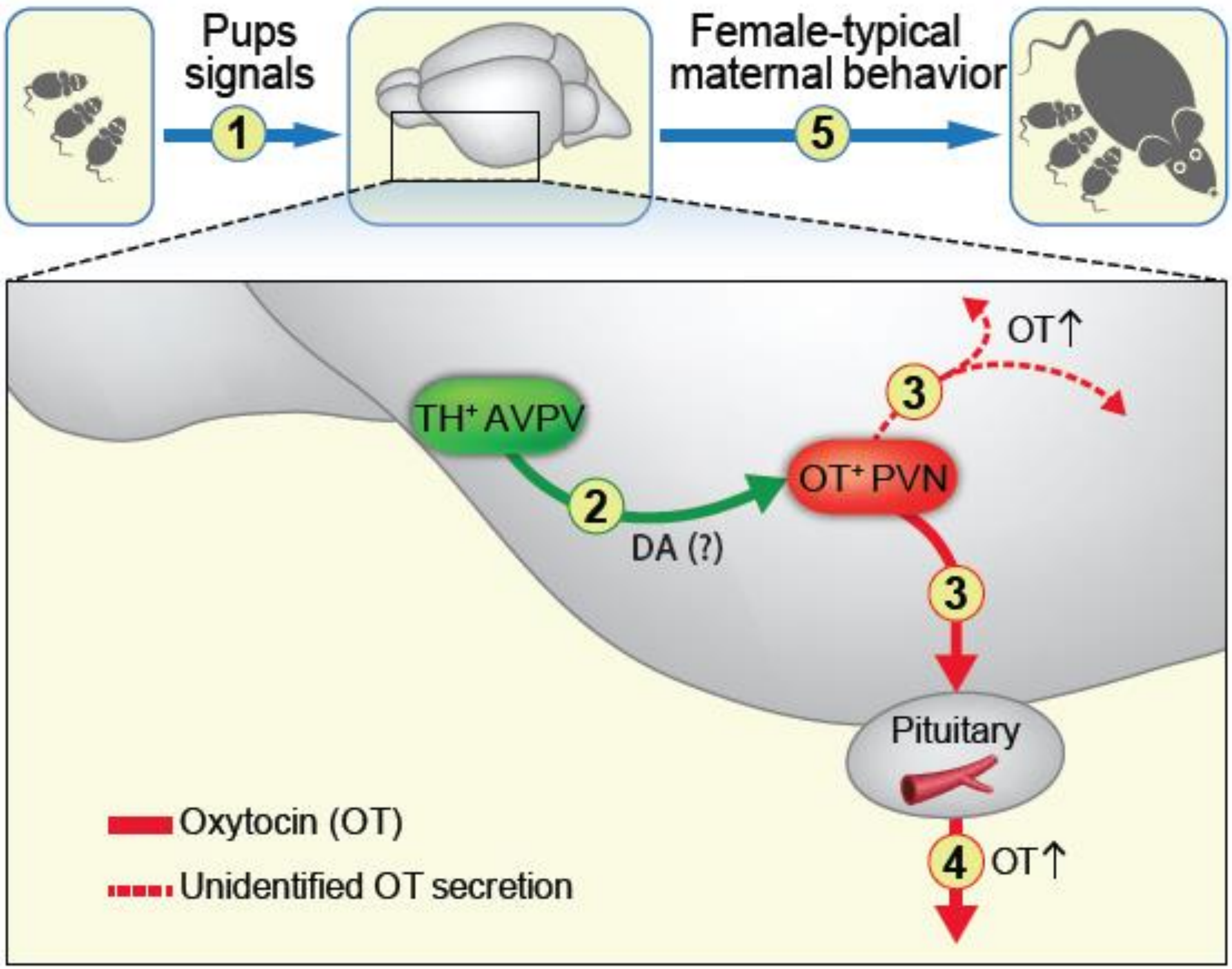
• In females, TH+ AVPV neurons promote parental behavior and are involved in the regulation of OT secretion to the blood circulation

•In males, TH+ AVPV neurons repress conspecific aggression and appear not to be required for the control of OT secretion or parental care



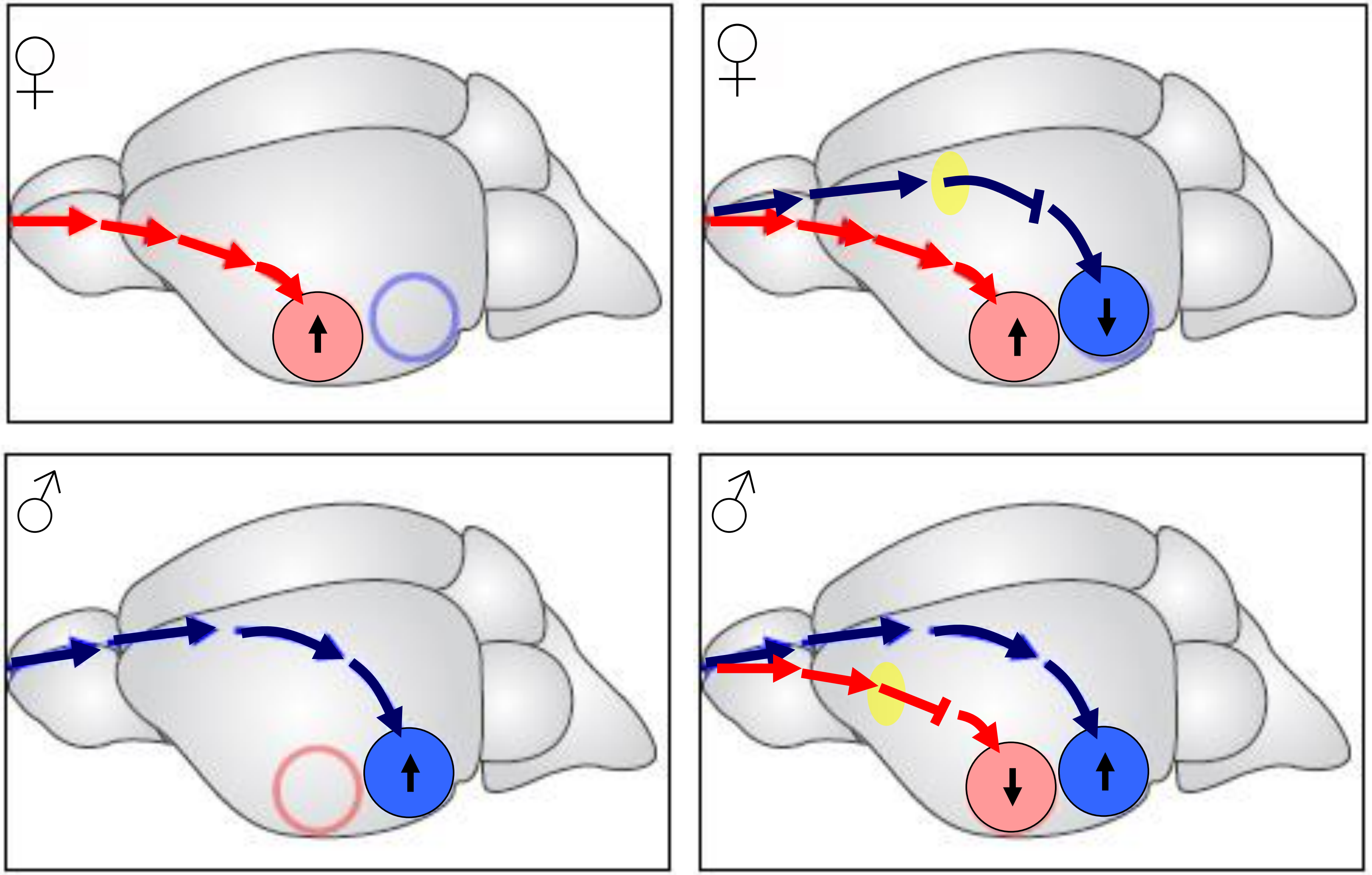
• TH+ AVPV neurons are monosynaptically connected to OT+ PVN neurons and are involved in OT release

The mechanisms underlying maternal behavior – Suggested model



Old model

New model



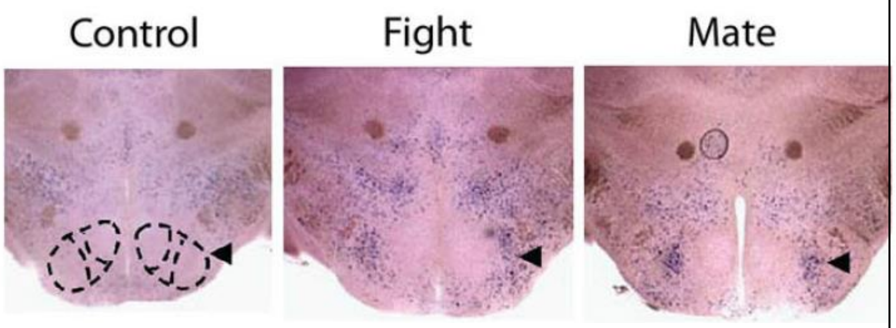
Neuronal coding of sex-specific social behaviors in mice models

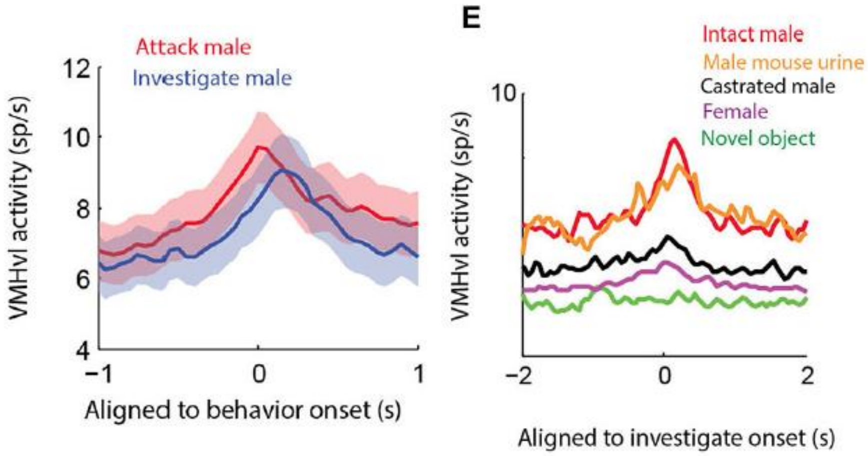
Examples:

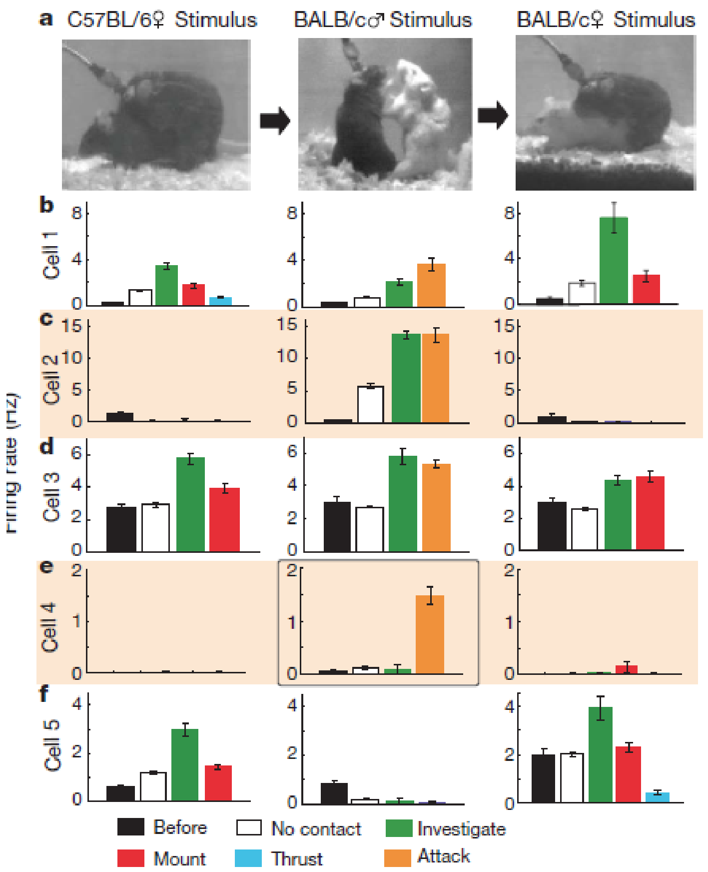
1. Aggressive behavior in the VMH
2. Sex-specific pheromone cues in the AOB and MeA
3. Sexual preference in the MeA

Functional identification of an aggression locus in the mouse hypothalamus (VMH)

Immediate early gene (FOS) induction during fighting and mating in the VMH



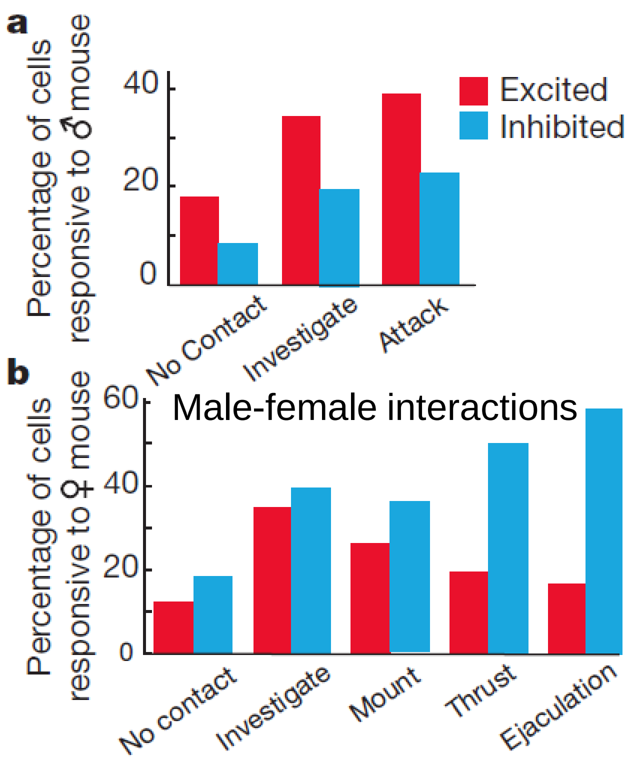


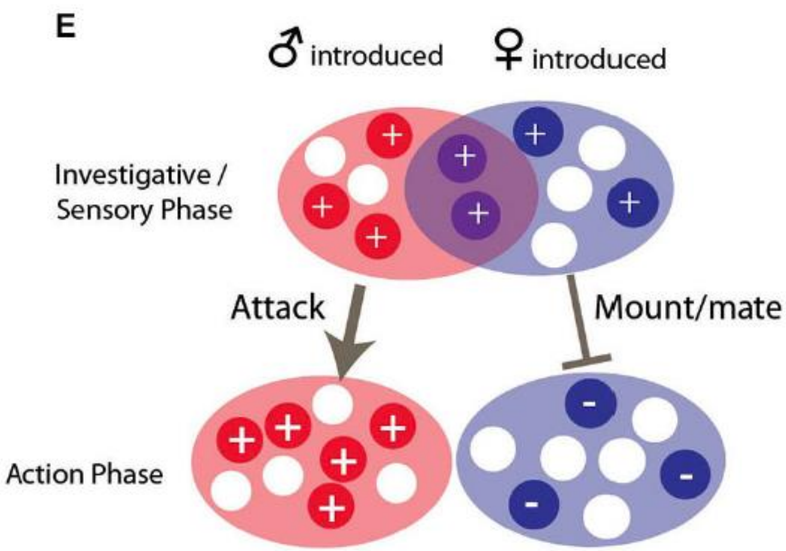


Lin et al 2011, Nature
Falkner and Lin 2014, Front Sys Neuros

Cell responses in VMH during mating and fighting

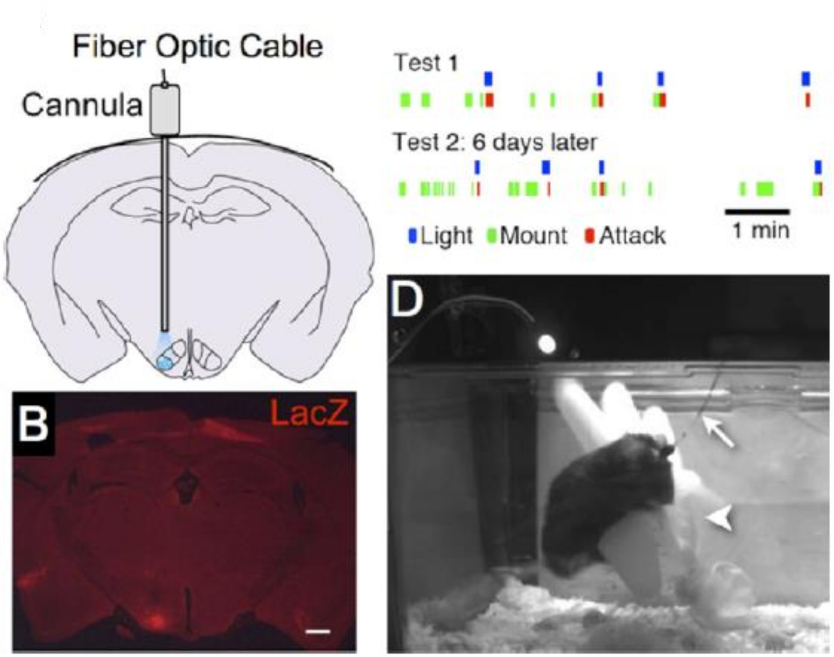
Male-male interactions

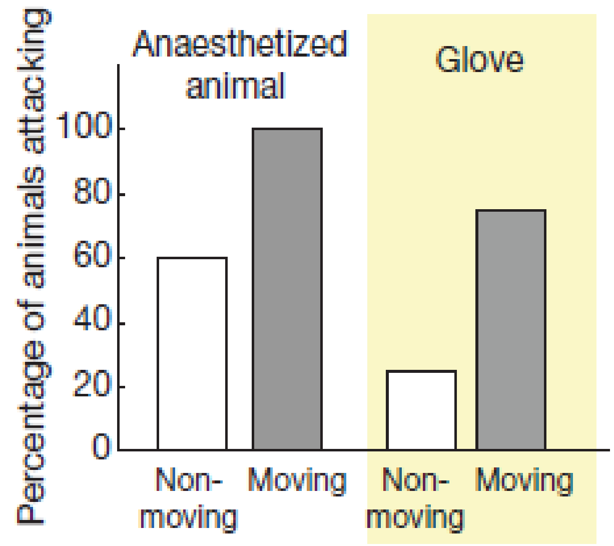


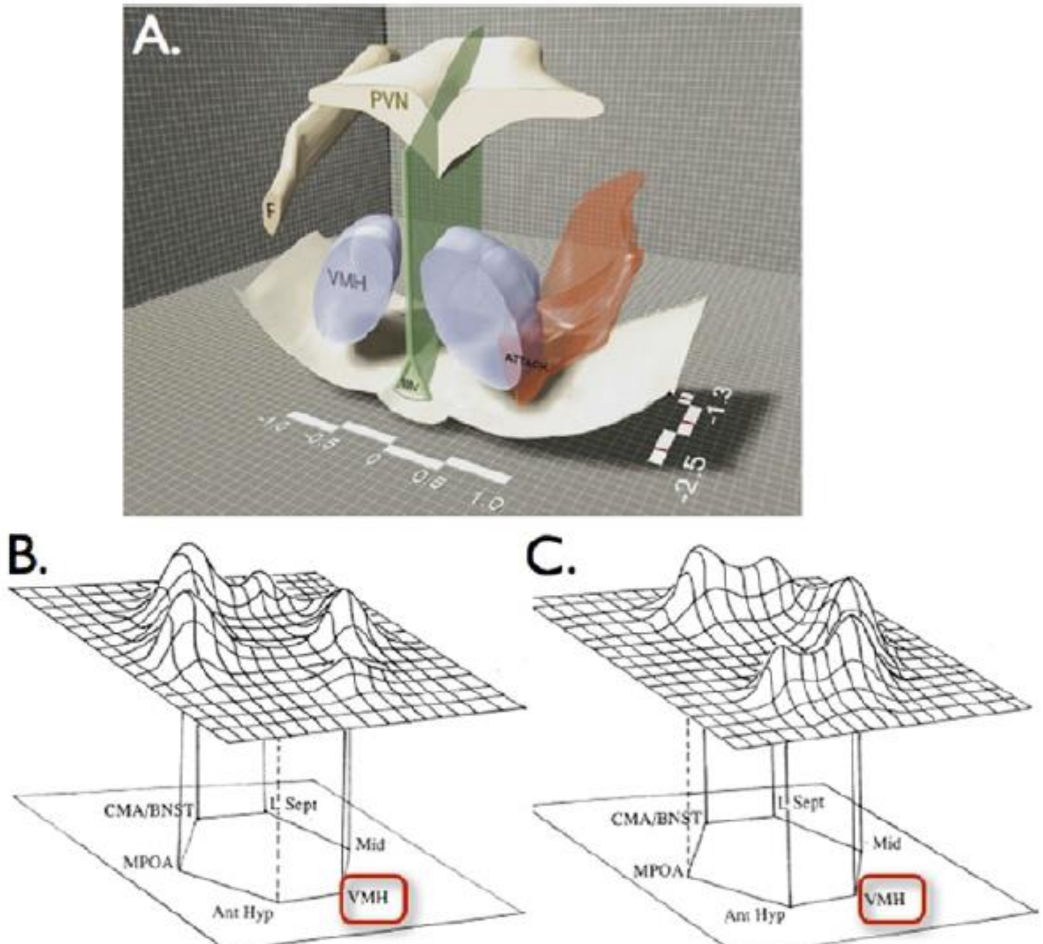


Lin et al 2011, Nature
Falkner and Lin 2014, Front Sys Neuros

Activation of aggressive behavior using optogenetics in the VMH



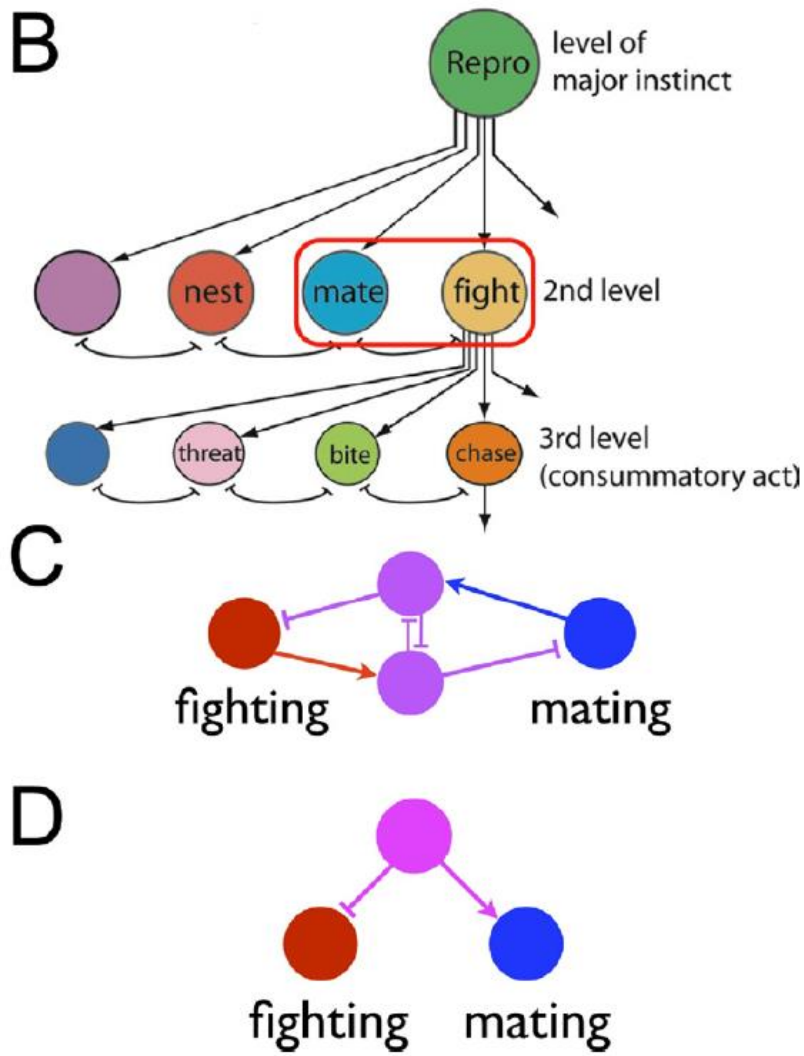




Male sexual behavior

Male aggression

Anderson, 2012; Biol Psychiatry



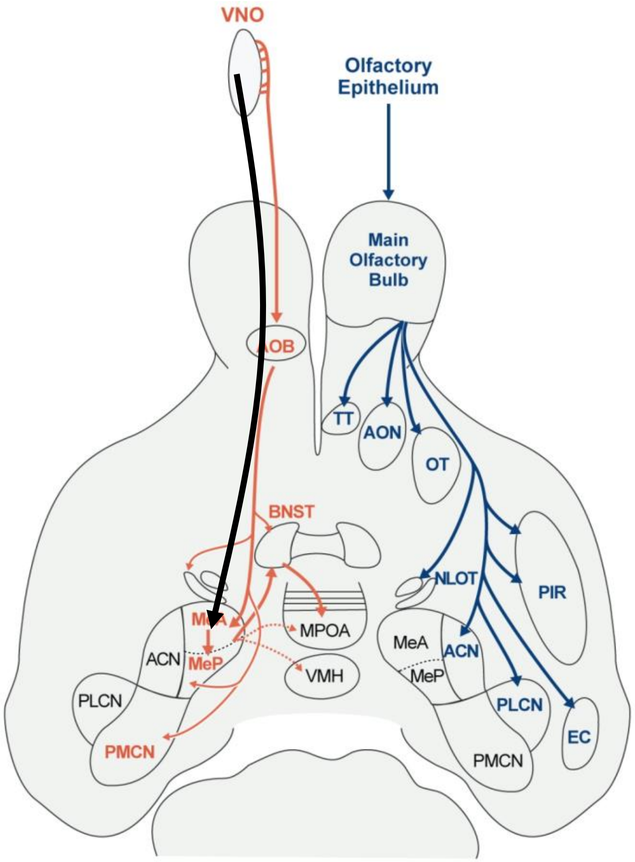
Anderson, 2012; Biol Psychiatry

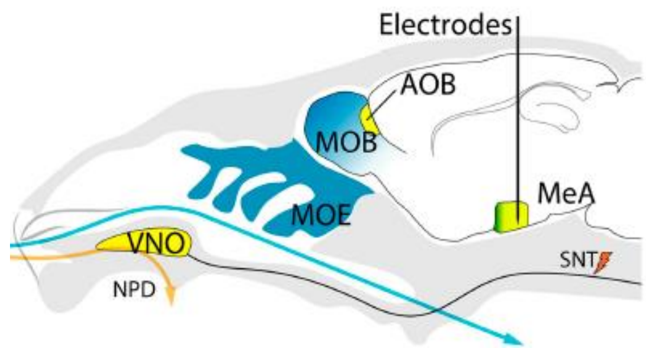
Neuronal coding of sex-specific social behaviors in mice models

Examples:

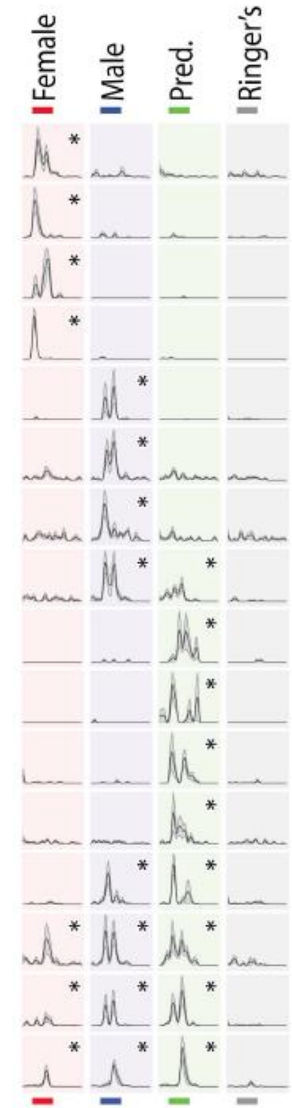
1. Aggressive behavior in the VMH
2. Sex-specific pheromone cues in the AOB and MeA
3. Sexual preference in the MeA

MeA sensory responses to VNO stimuli



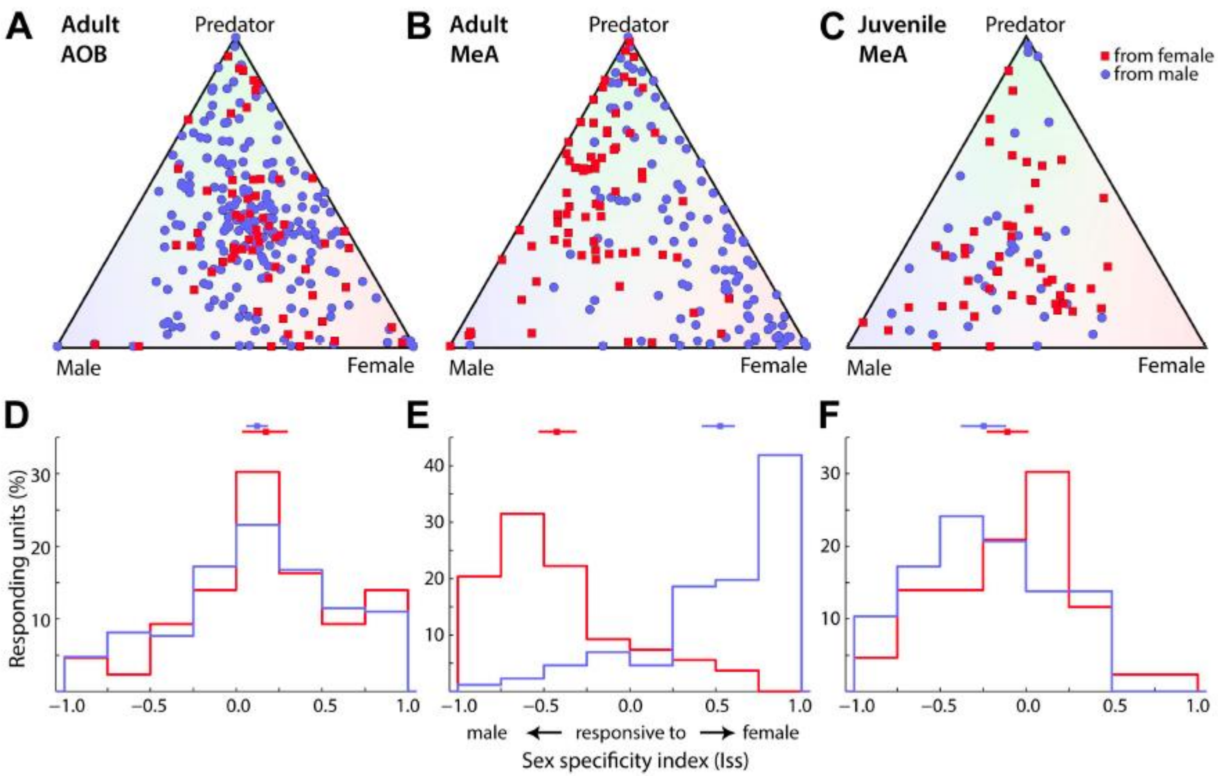


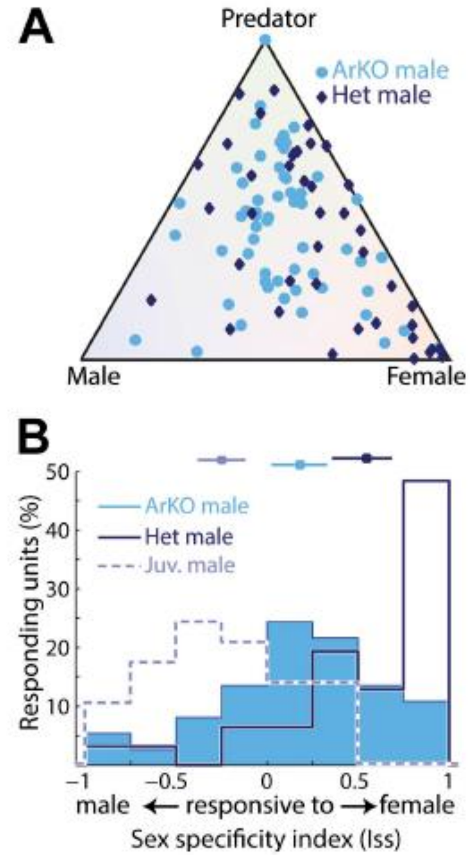
Electrical stimulus to artificially
activate the VNO pump



Bergan et al 2014, eLife

Sexual dimorphism of adult MeA responses to chemosignals





Bergan et al 2014, eLife

Neuronal coding of sex-specific social behaviors in mice models

Examples:

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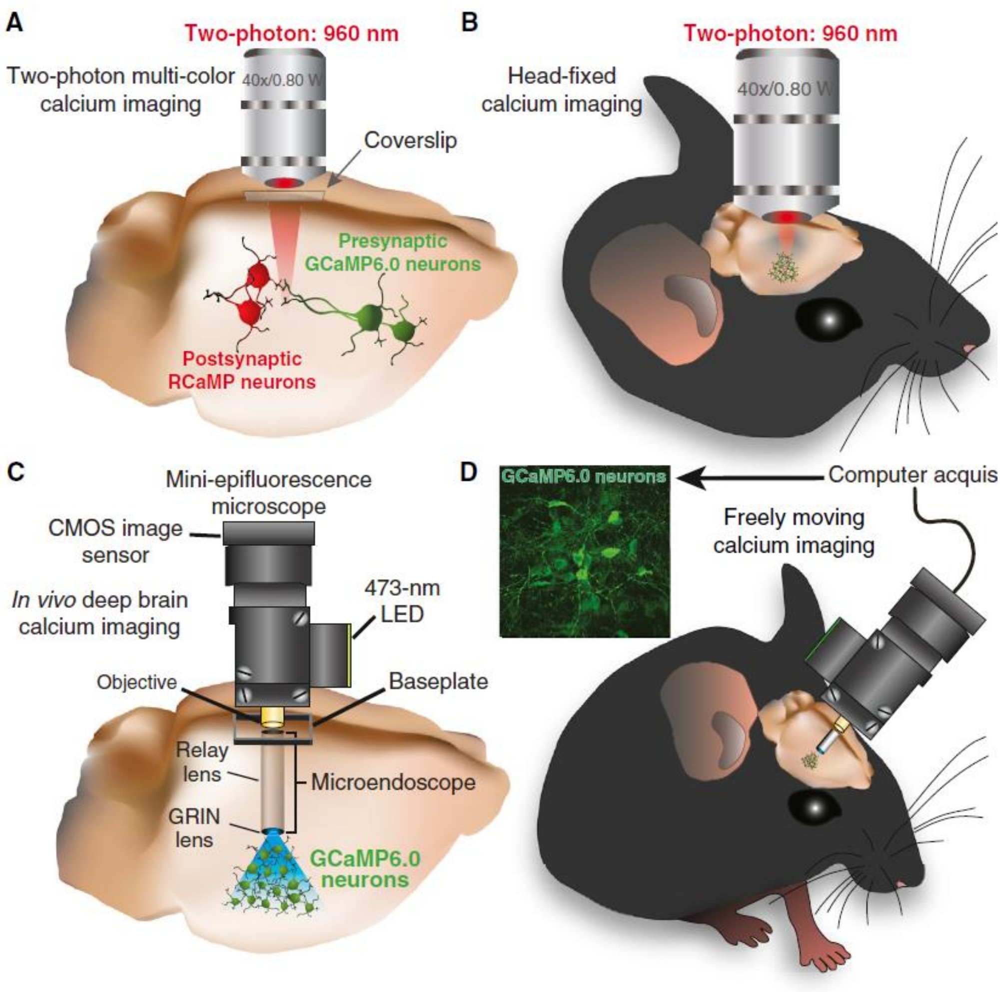
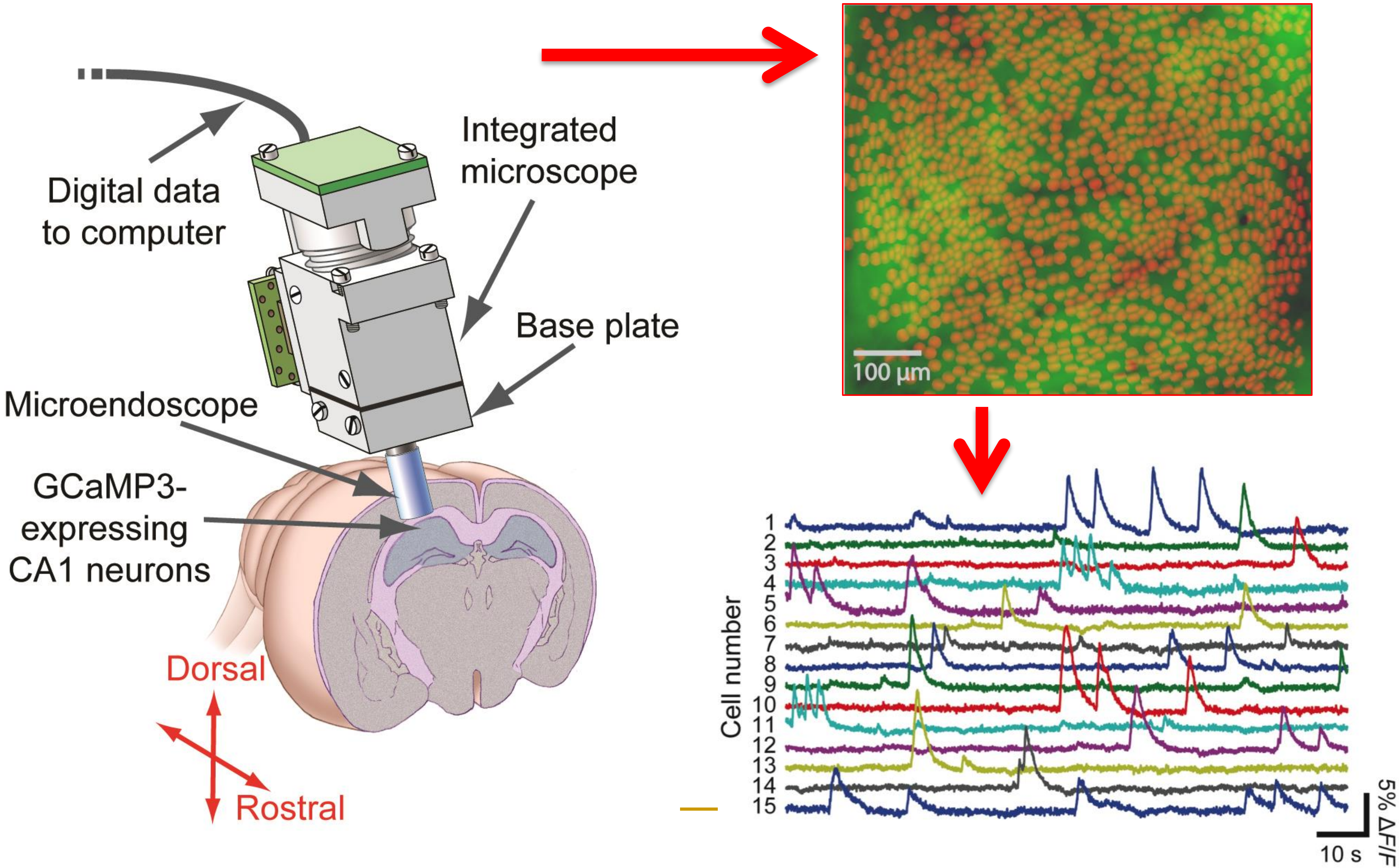
Imaging of neuronal ensemble activity in deep brain circuits



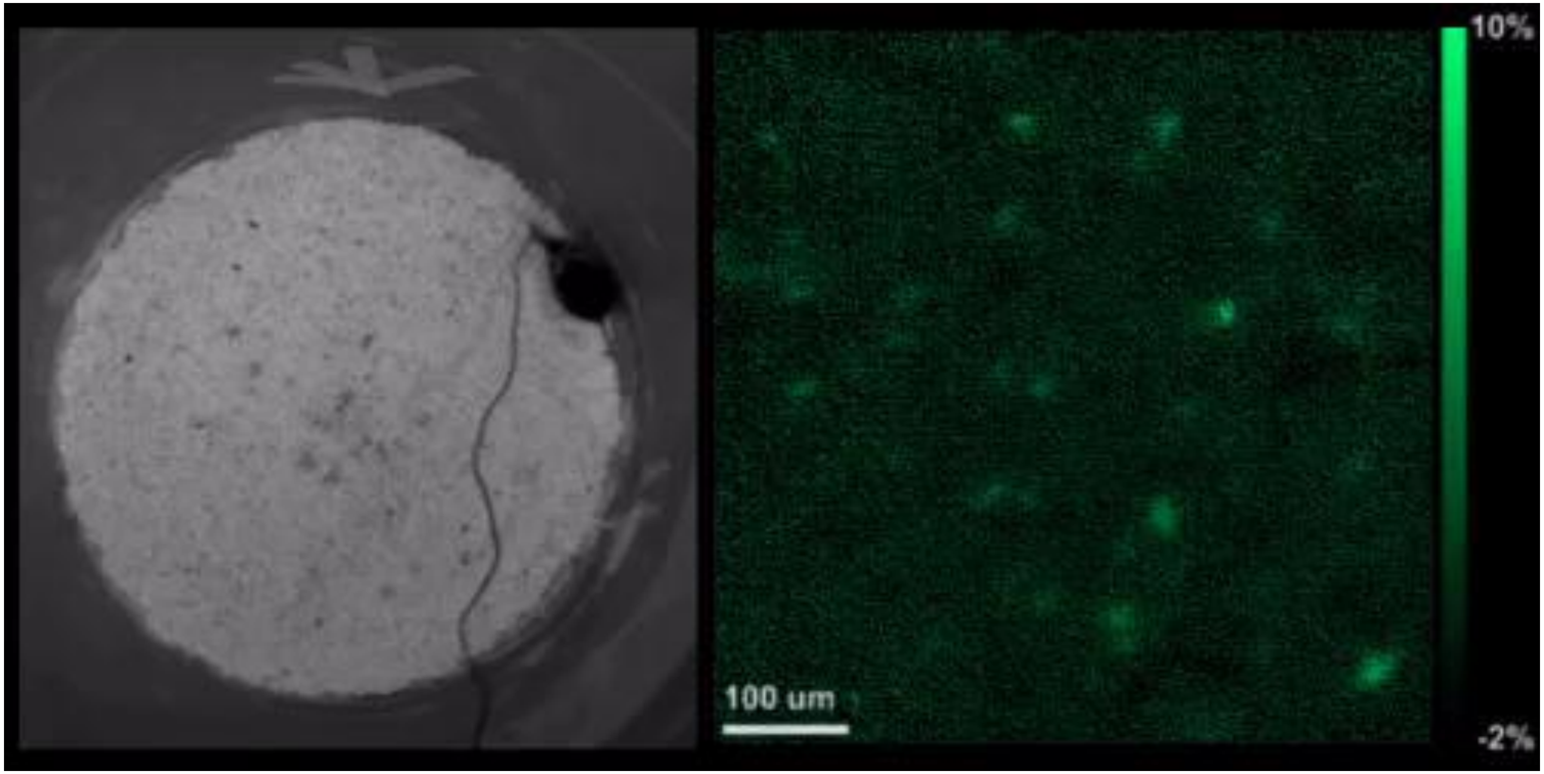
Jennings and Stuber, 2014, Cur Biol

A system for chronic imaging of neuronal ensemble activity in deep brain circuits of freely behaving mice



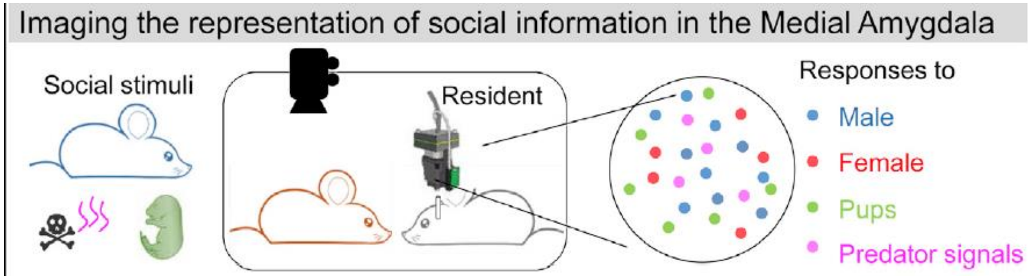
Ziv *et al*, *Nature Neuroscience* 2013

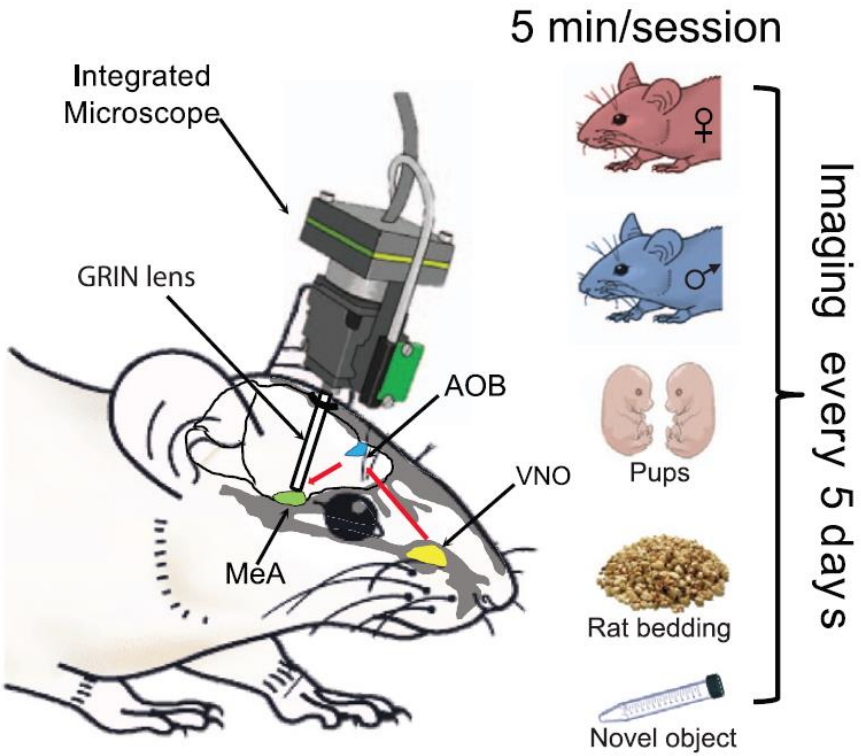
Imaging of Ca^{2+} dynamics in CA1 neurons of freely behaving mice



Ziv et al 2013, *Nature Neurosci*

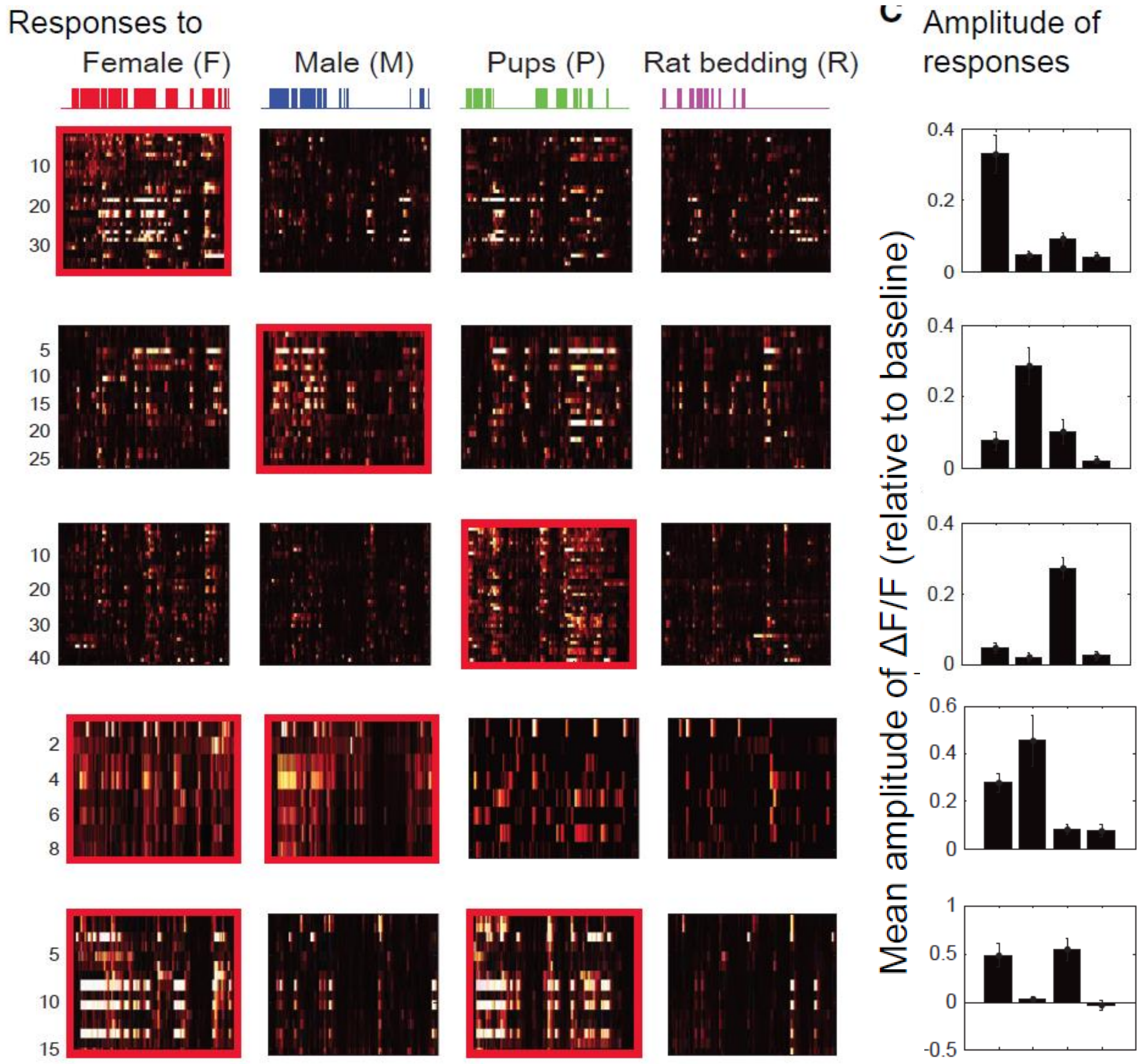
Neuronal representation of social information of awake behaving mice



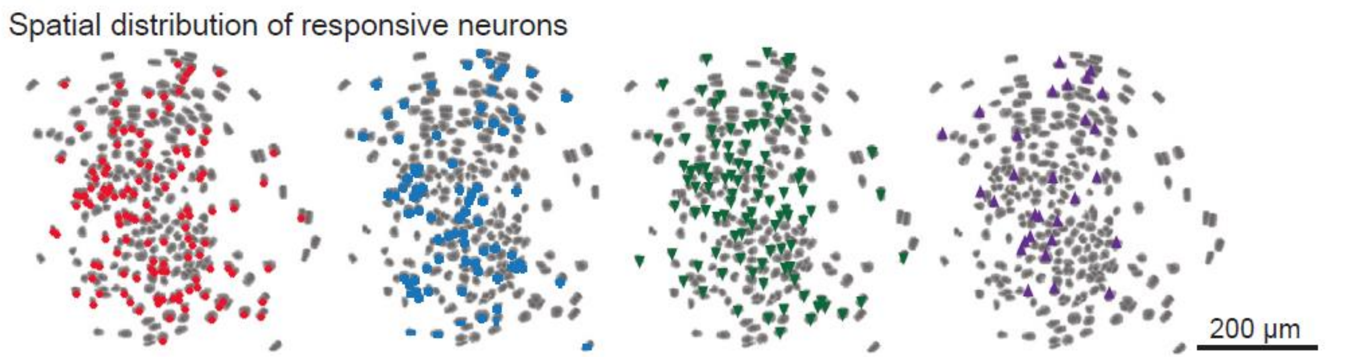


Li et al 2017, Cell

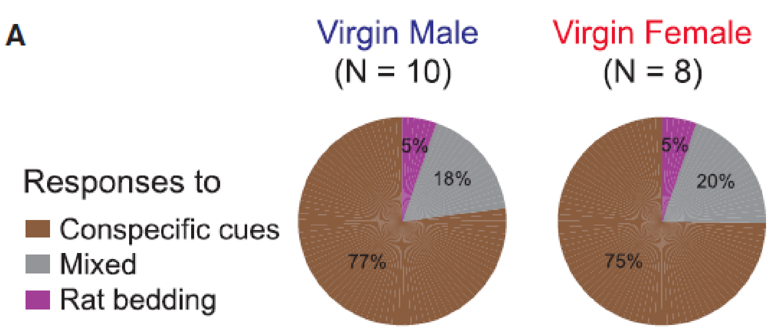
Examples of neurons responsive to one or multiple stimuli

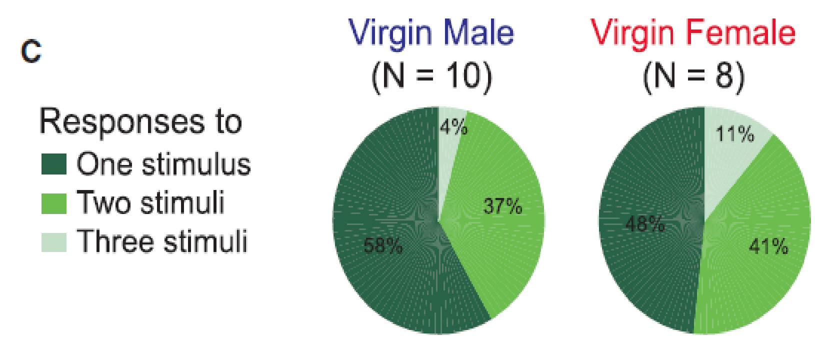


Li et al 2017, Cell



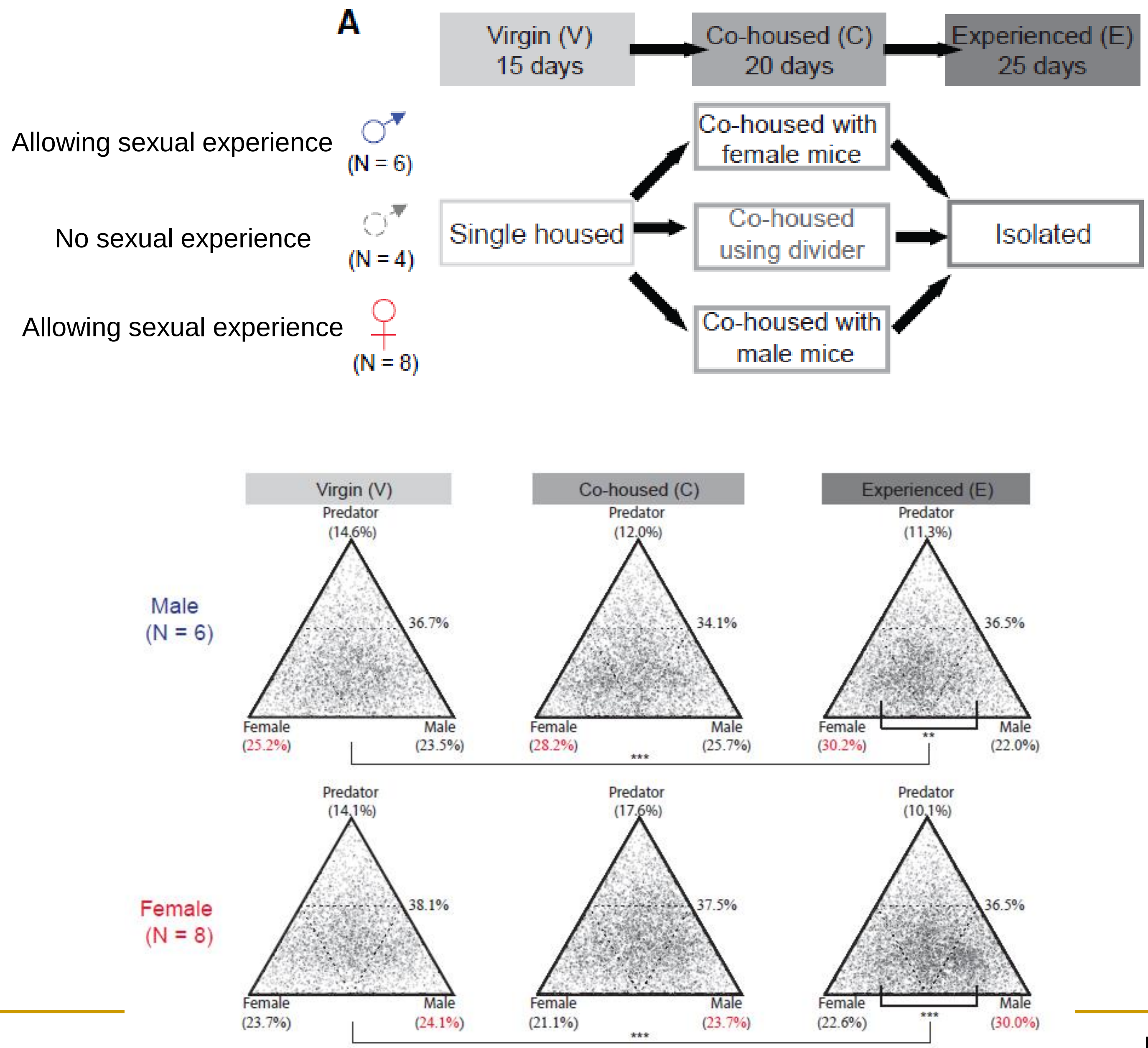
Female Male Pup Rat (predator)





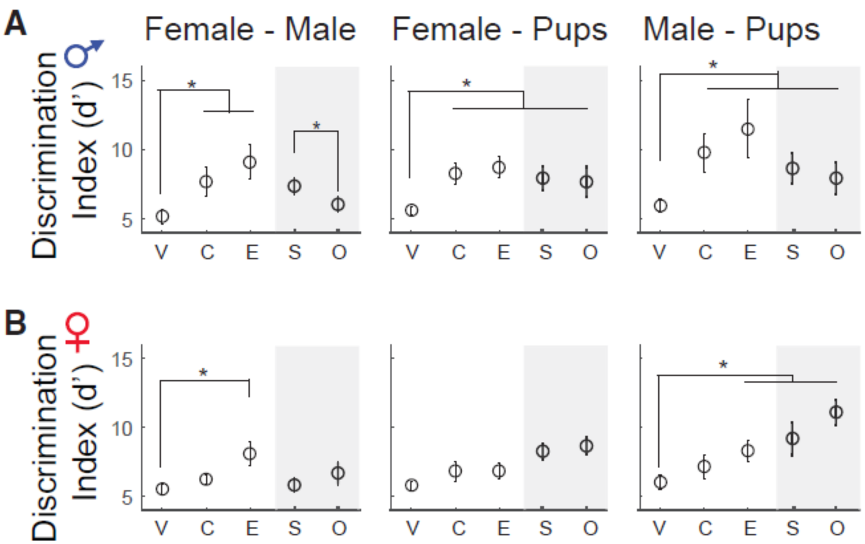
Most neurons in the MeA responded to conspecific cues and only to one stimulus

Li et al 2017, Cell



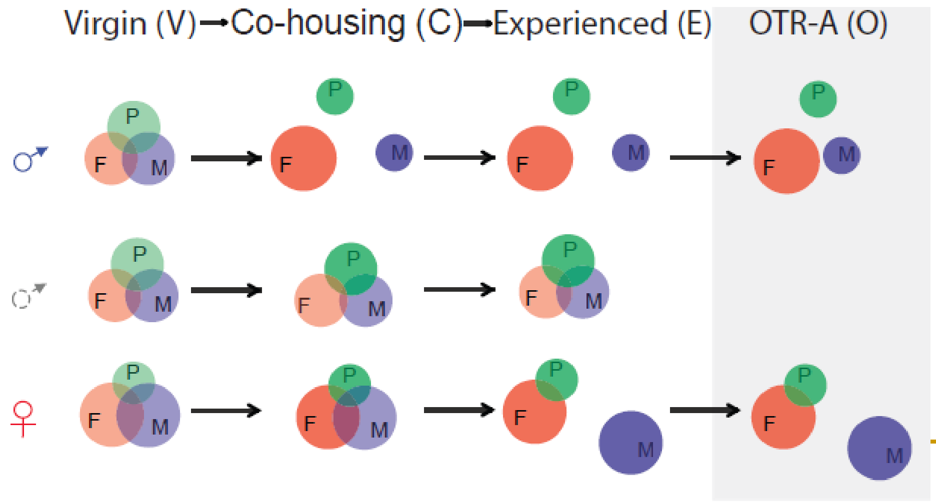
Li et al 2017, Cell

Social discrimination between male and female cues in males is suppressed by oxytocin receptor antagonist



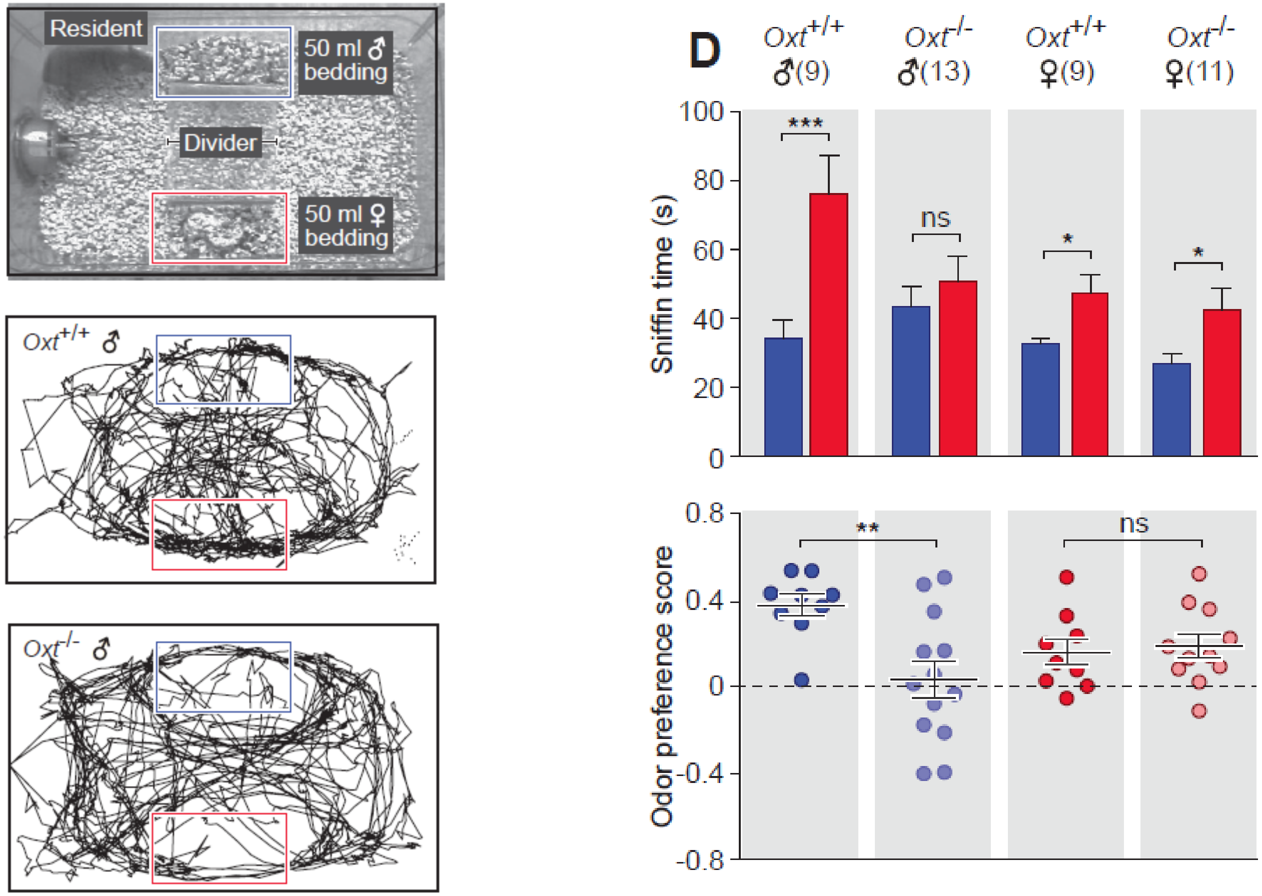
V= Virgin
C= Co-housed
E= Experienced
S= Saline
O= OT-antagonist

* Discrimination index=
The distance (reparability) between each response class



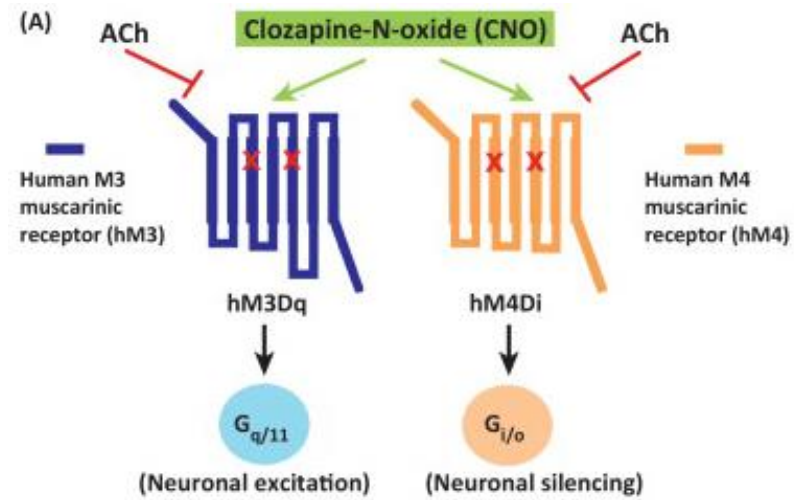
Li et al 2017, Cell

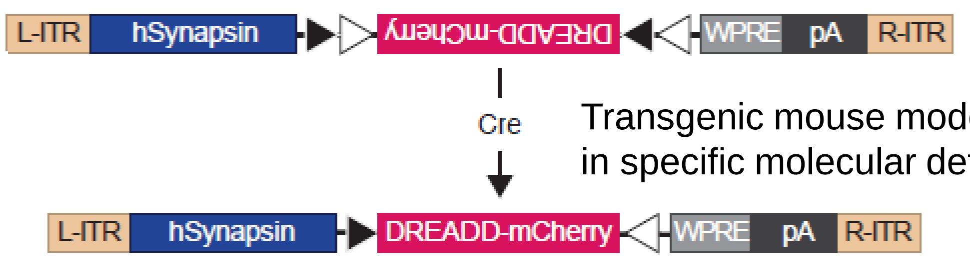
Oxytocin signaling is required for sex discrimination in males



Yao et al 2017, eLife

DREADs (Pharmacogenetics) system to manipulate neuronal activity

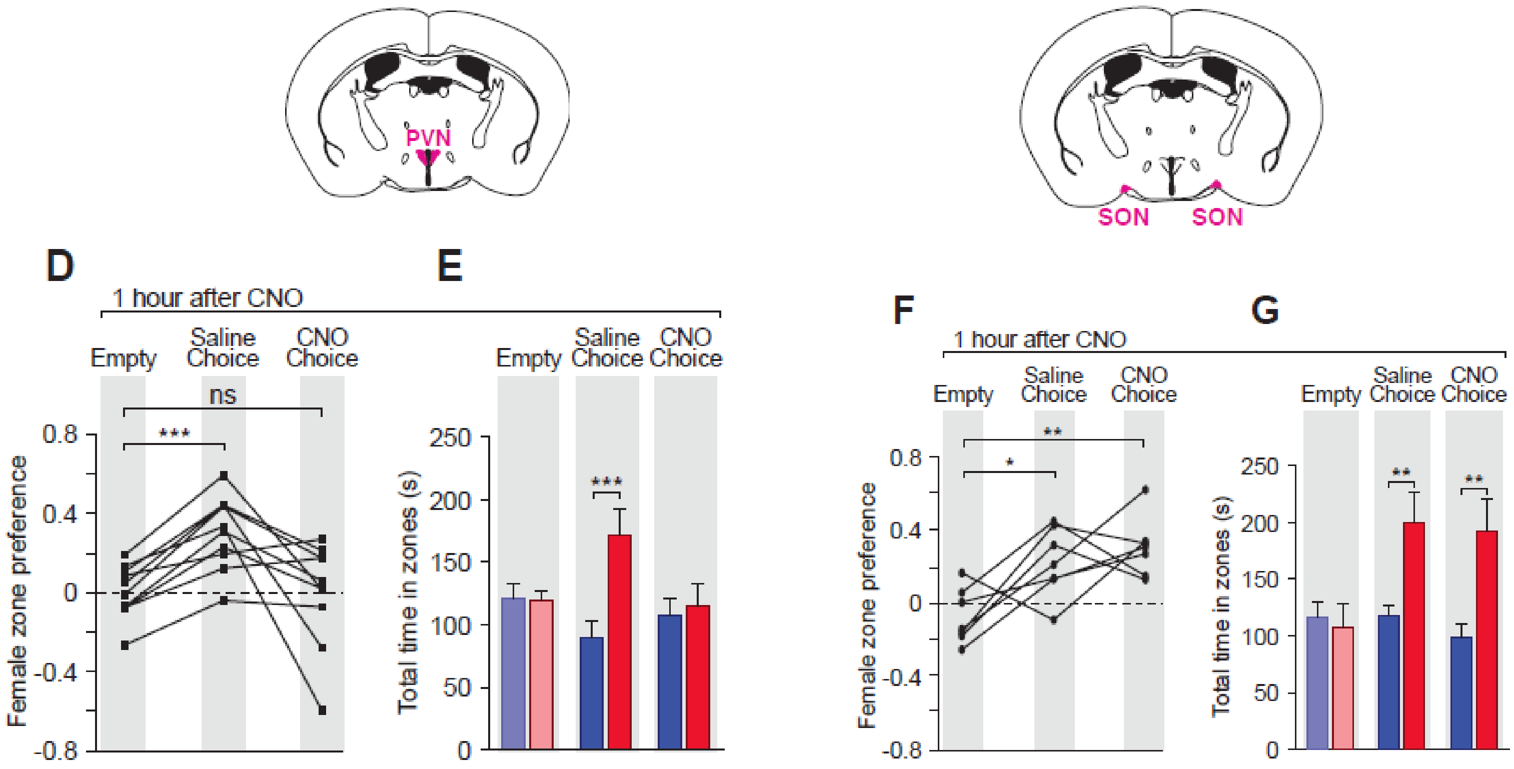




Transgenic mouse model expressing Cre in specific molecular defined neurons

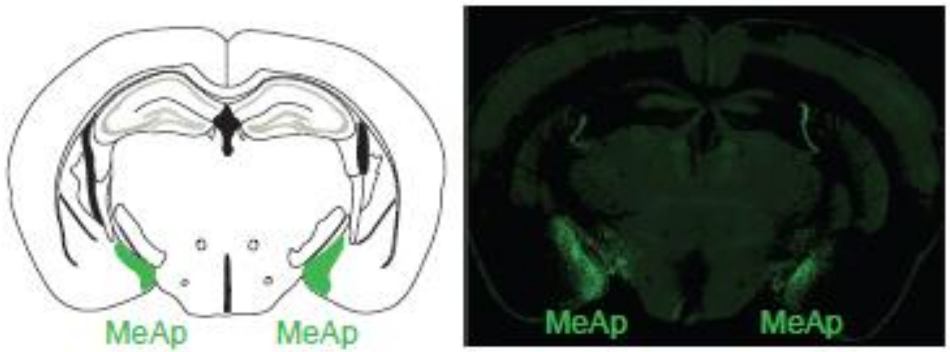
Oxytocin signaling in the PVN (and not SON) is required for sex discrimination in males

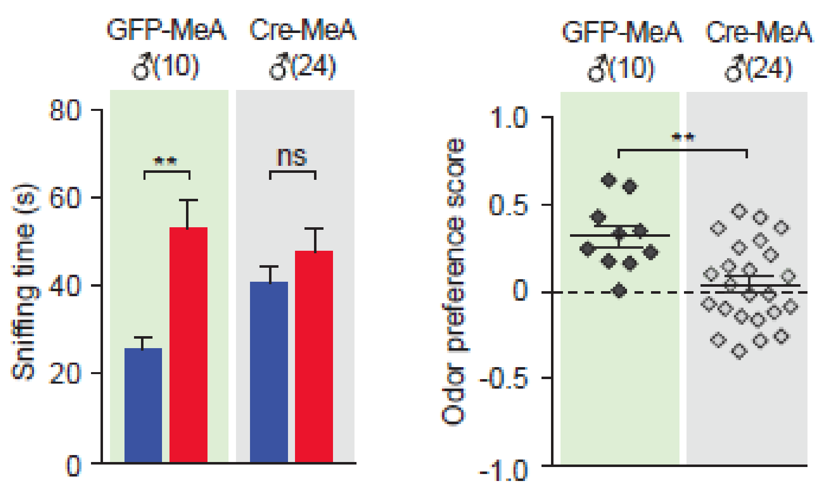
Selective inhibition of neuronal activity of oxytocin expressing neurons (using DREADDs) in adult mice



Yao et al 2017, eLife

Oxytocin signaling in the MeA (and not BNST) is required for sex discrimination in males

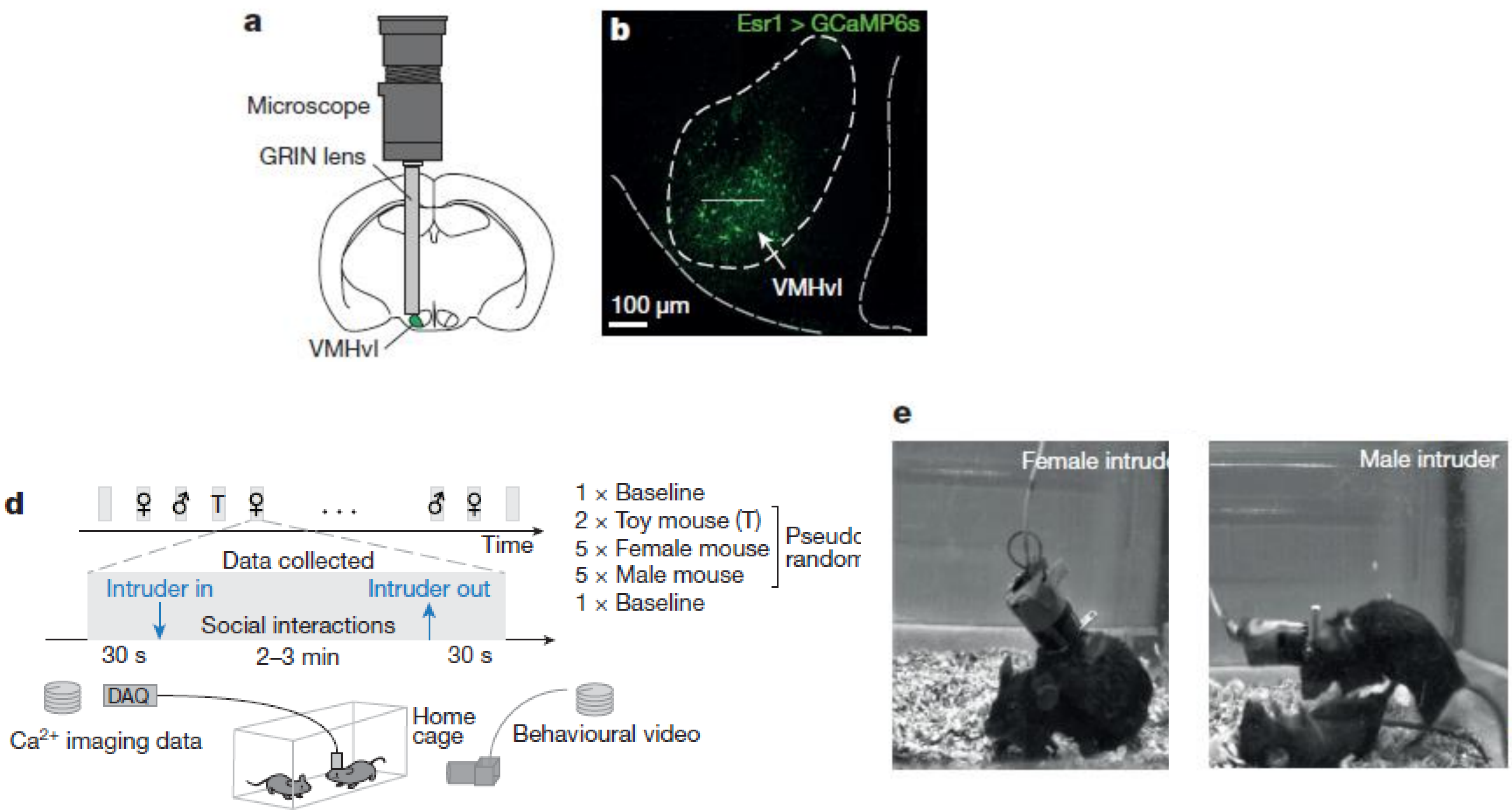




Method: Injection of an AAV expressing Cre to OT-R Floxed mice

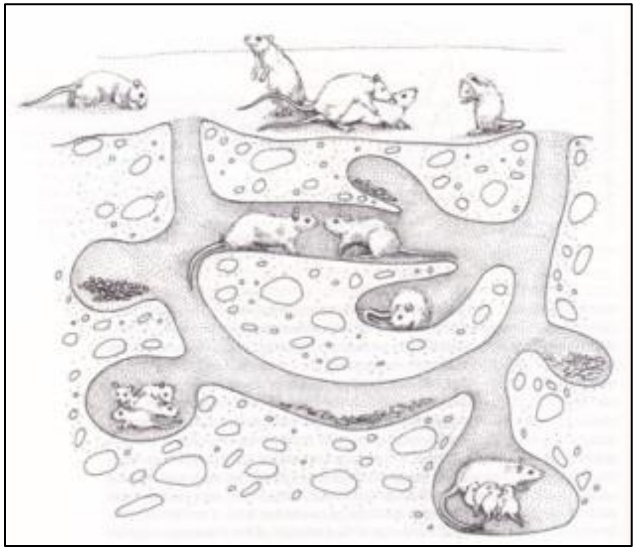
Yao et al 2017, eLife

Social behaviour shapes hypothalamic neural ensemble representations of conspecific sex



Remedios et al 2017, Nature

"Evolution" of social behavior studies in mouse models



Testing conditions:

Natural/field

↓

Lab conditions (restricted/artificial)

Animal model:

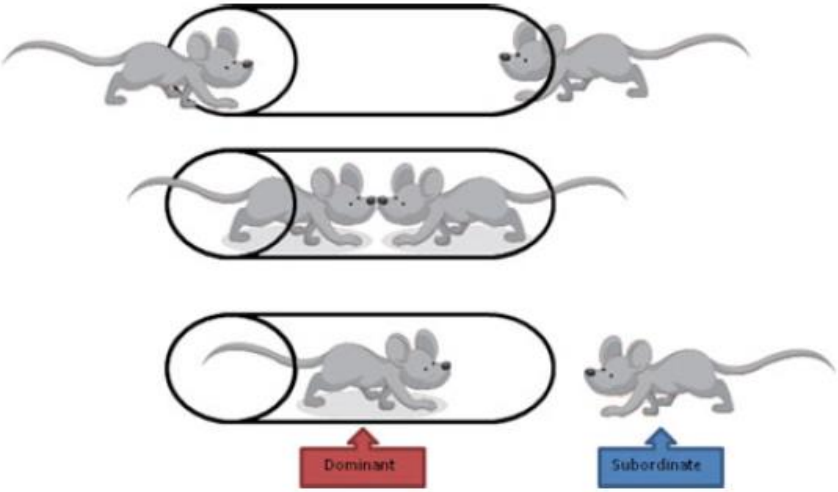
Wild outbred mice

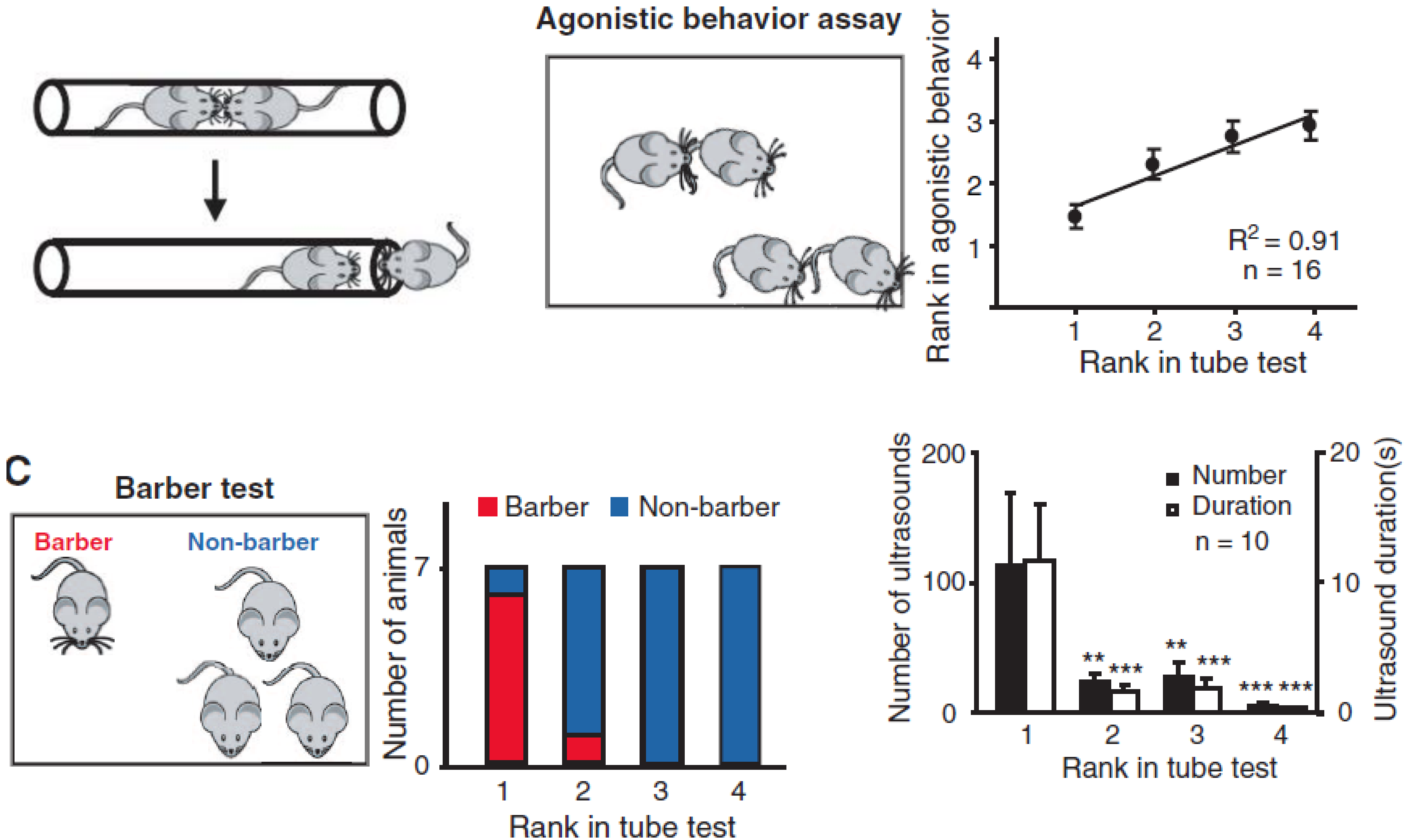
↓

Lab inbred mice
(human selected for specific characteristics)

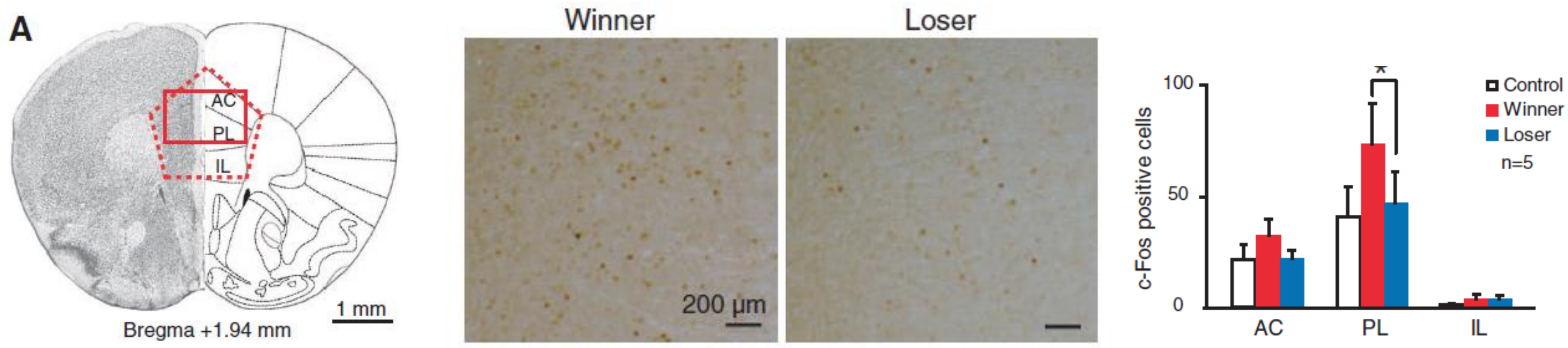
Examples of classical methods for social behavior

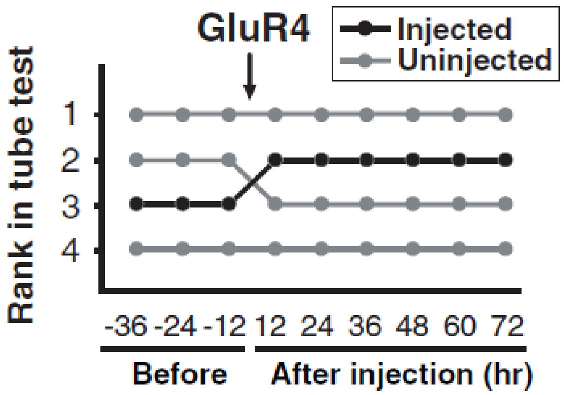
The tube assay: Testing dominance rank in pairwise interactions



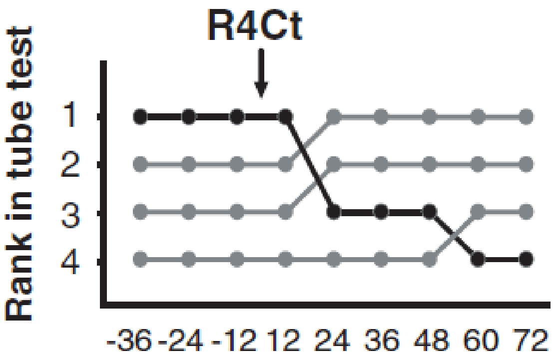


Wang et al 2011; *Science*



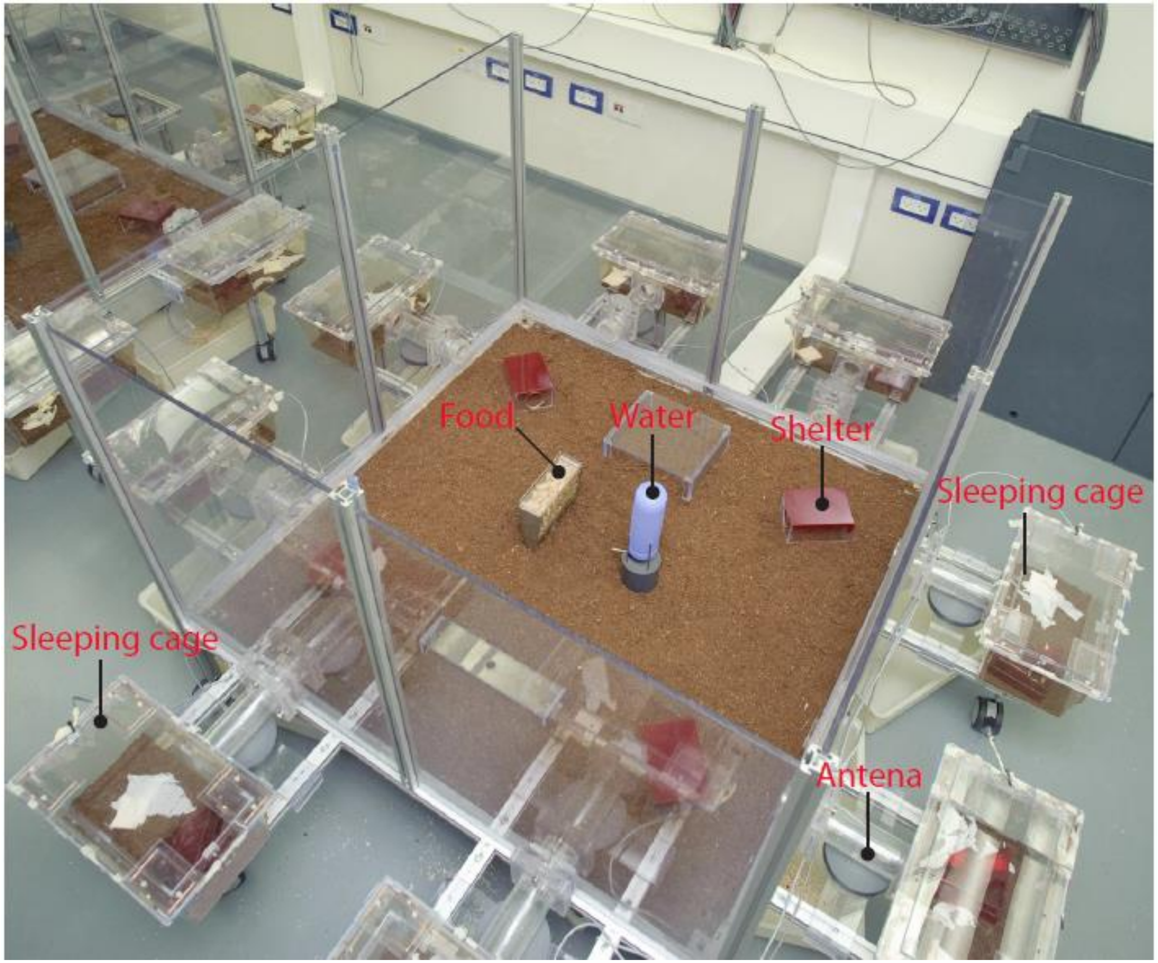


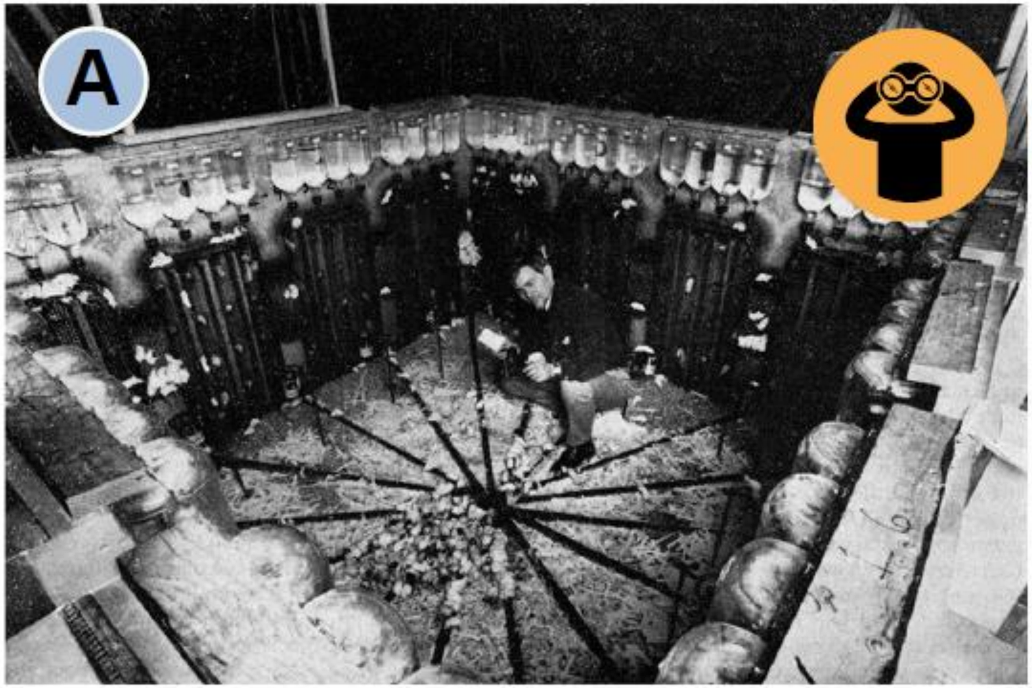
GluR4 = increases the transmission of neural signals



R4Ct= suppresses the transmission of neural signals

Wang et al 2011; *Science*


Food
Water
Shelter
Sleeping cage
Sleeping cage
Antena



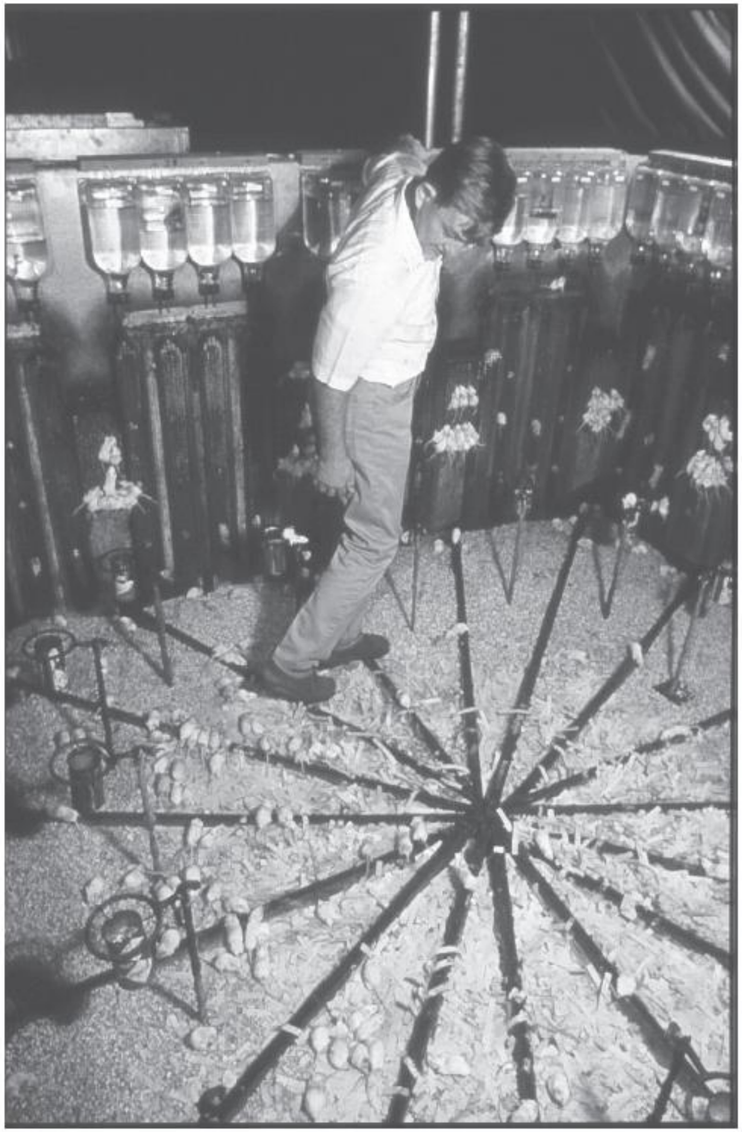
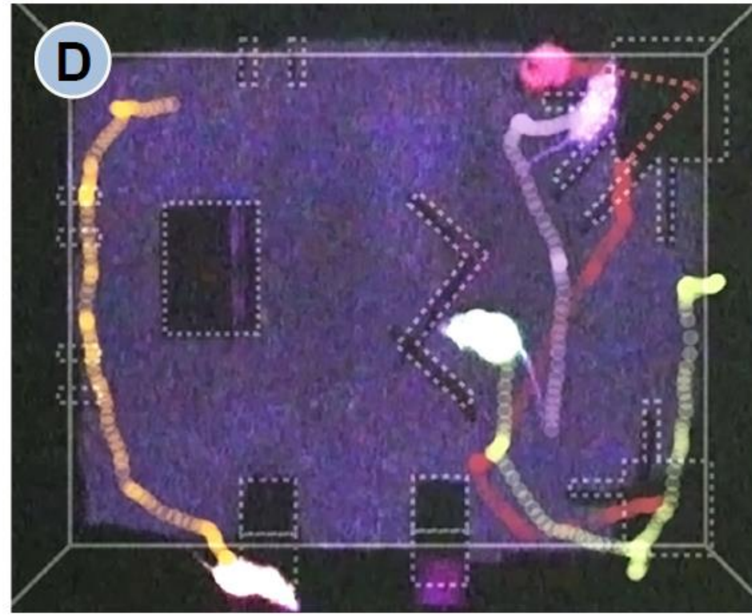
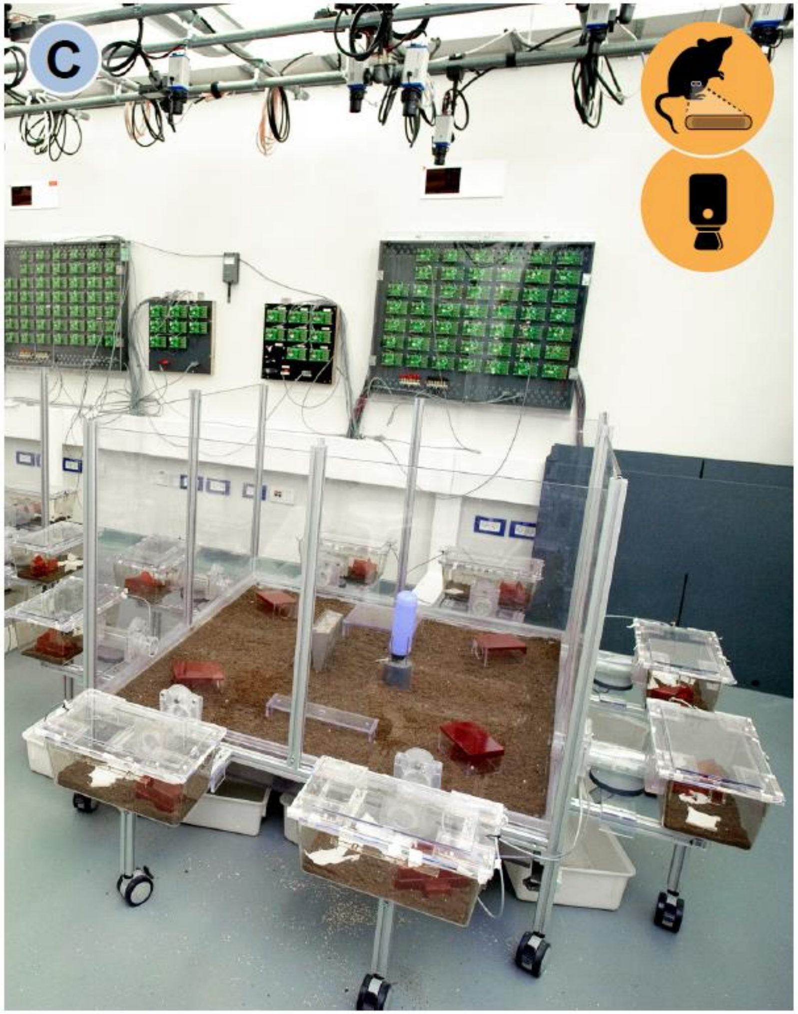


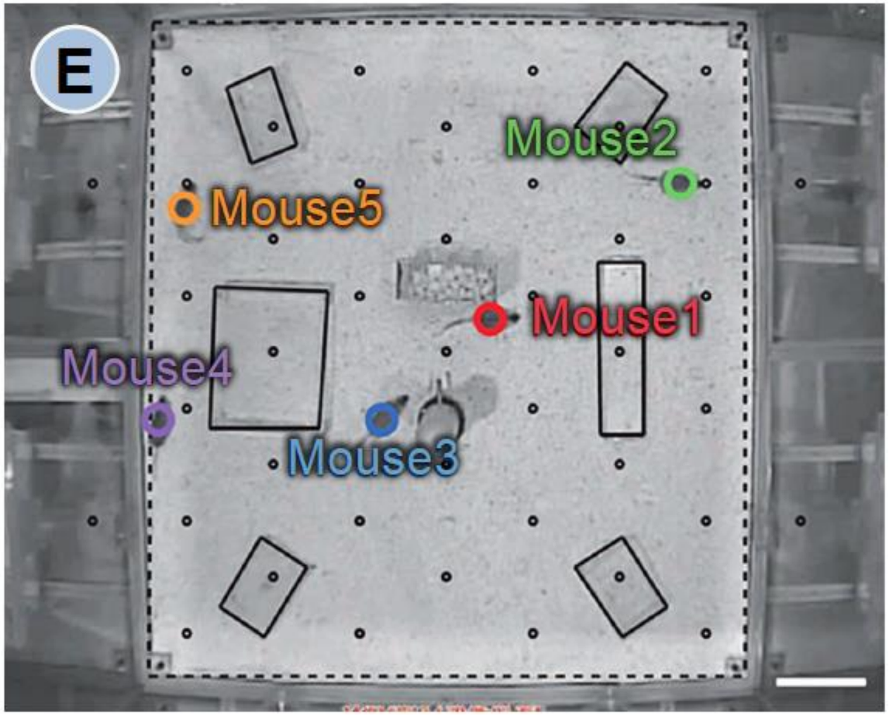
Fig 1. *John B. Calhoun in rodent Universe 133*

Kritzler et al 2006; Proc PTA



Shemesh et al 2013; eLife

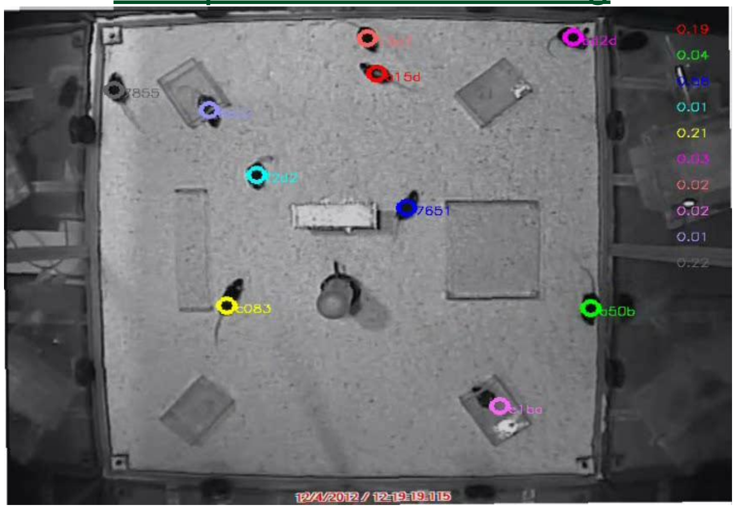




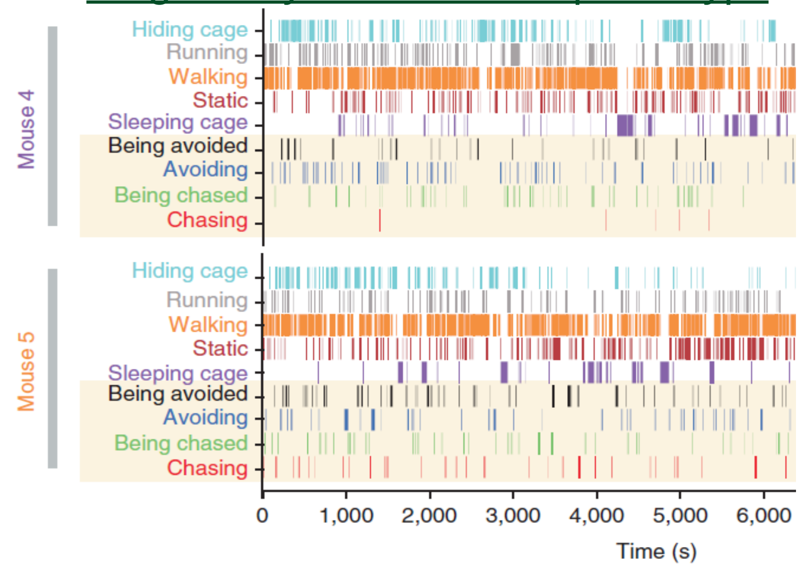
Weissbrod et al. 2013, *Nature Communications*

Automated characterization of social behaviors in a semi-natural environment

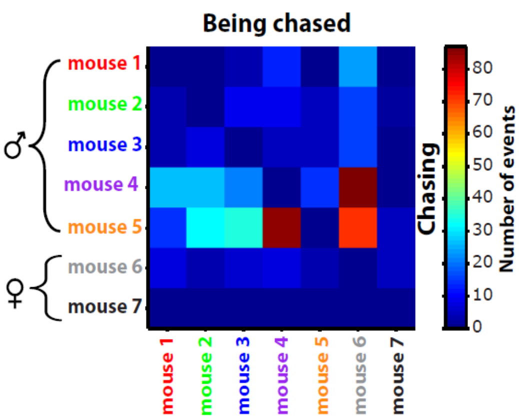
Multiple individual tracking



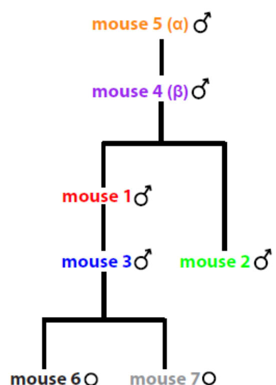
Large array of behavioral phenotyps



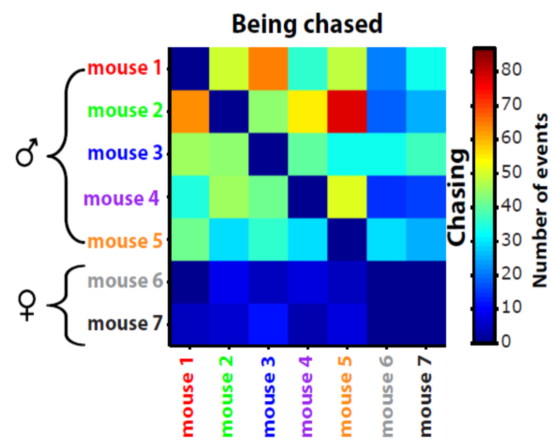
Dyadic social interactions (chasing/being chased)

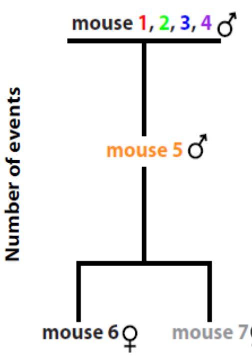


Dominance hierarchy (group organization)



Mouse model for autism





Weissbrod et al. 2013, *Nature Communications*

Scientific problem	Animal model	
Action potential generation →		**Squid** Squids were used to study the mechanisms underlying action potential generation because of their giant axons, which allow the insertion of voltage-clamp electrodes (*5*).
Synaptic transmission →		**Frog** Frogs were used to study the mechanisms of synaptic transmission because of the simple behavior and large size of the synapses involved (*43*).
Retinal physiology and lateral inhibition →		**Horseshoe crab** Horseshoe crabs were used to study mechanisms of retinal physiology, including lateral inhibition, because of the accessibility of individual nerve cells and convenient structure of the compound eye (*44*).
Learning and memory →		**Aplysia** Aplysia was used to study the neurobiology of learning and memory because of its capacity for simple forms of learning and the easily identifiable and accessible neurons that mediate these behaviors (*45*).

Adapted from Yartsev, 2017, *Science*

Laboratory Mouse

Education

Caltech, Oxford, Stanford, Harvard, MIT, Princeton, Cambridge, Imperial, Berkely, Chicago, Yale, ETH Zurich, Columbia, UPenn, John Hopkins, UCL, Cornell, Northwestern, UMichigan, Toronto, Carniege Mellon, Duke, UWashington, UTexas at Austin, GA Tech, Tokyo, Melbourne, Singapore, UBC, Wisconsin-Madison, Edinburgh, McGill, Hong Kong, Santa Barbara, Karolinska Institute, UMinnesota, Manchester ... and just about every other major university, medical school & research institution in the world.

Nobel Prizes

1905 - Transmission and treatment of TB
1906 - Structure of Nervous System
1907 - Role of protozoa in disease
1908 - Immunity to infectious diseases
1928 - Investigations on typhus
1929 - Importance of dietary vitamins
1939 - Discovery of antibacterial agent, Prontosil
1945 - Discovery of penicillin
1951 - Yellow fever vaccine
1952 - Discovery of streptomycin
1954 - Culture of the polio virus
1960 - Understanding of immunity
1970 - Understanding of neurotransmitters
1974 - Structural & functional organisation of cells
1975 - Tumour-viruses and genetics of cells
1977 - Hypothalamic hormones
1984 - Techniques of monoclonal antibody formation
1986 - Nerve growth factor and epidermal growth factor
1990 - Organ transplantation techniques
1992 - Regulatory mechanisms in cells
1996 - Immune-system detection of virus-infected cells
1997 - Discovery and characterisations of prions
1999 - Discovery of signal peptides
2000 - Signal transduction in the nervous system
2004 – Odour receptors and organisation of olfactory systems
2008 - Role of HPV and HIV in causing disease
2010 - Development of in vitro fertilization
2011 - Discoveries around innate and adaptive immunity
2012 - Reprogramming mature cells to pluripotent ones

CV of a Lifesaver

Overview

- Involved in around 75% of research
- Short life-span and fast reproductive rate means mice are suitable for studying disease across whole life cycle
- 98% of genes have comparable genes in humans
- Similar reproductive and nervous systems and suffer many of the same diseases as humans including cancer diabetes and anxiety
- Can be genetically modified to include human genes in enhance biological relevance
- Can act as an avatar for a human cancer to allow drug therapies to be trialled safely

Research Areas

Alzheimer's disease, anaesthetics, AIDS & HIV, anticoagulants, antidepressants, asthma, blindness, bone and joint disease, brain injury, breast cancer, cardiac arrest, cystic fibrosis, deafness/hearing loss, Down's sndrome, drugs for high blood pressure, transplant rejection, Hepatitis B, C & E, Huntington's disease, influenza, leukaemia, malaria, motor neurone disease, multiple sclerosis, muscular dystrophy, Parkinson's disease, prostate cancer, schistomiasis, spinal cord injury, stroke, testicular cancer, tuberculosis,

Contact

www.understandinganimalresearch.org.uk
www.animalresearch.info
www.amprogress.org
www.speakingofresearch.com

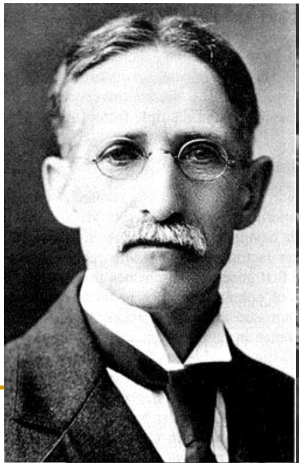
Recent mouse history

Wild mice were first described by the Swedish biologist Carl Linnaeus in 1758

Fancy mouse breeding - Asia, Europe (last few centuries)

Retired schoolteacher Abbie Lathrop collects and breeds these mice Granby, MA – 1900

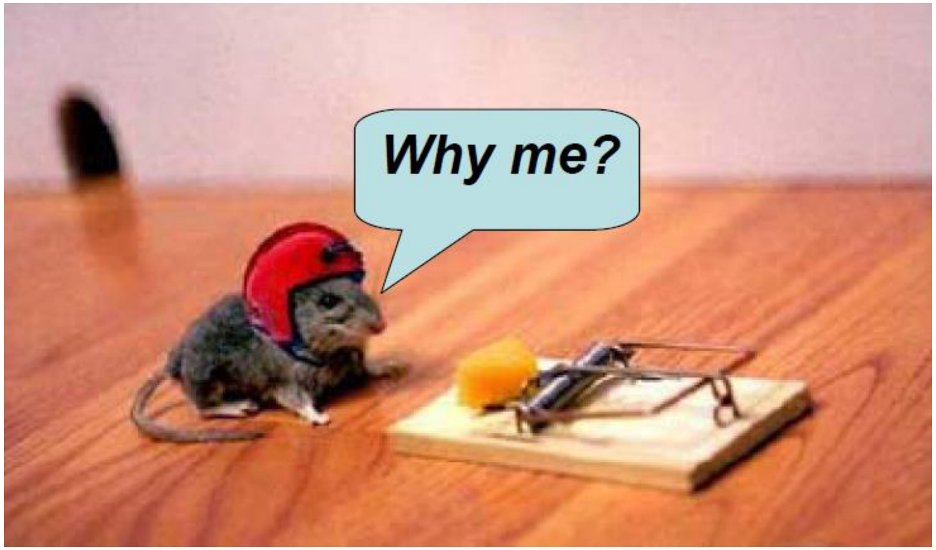
Castle, Little and others form most commonly used inbred strains from Lathrop stock (1908 on)



W.E. Castle

C.C. Little

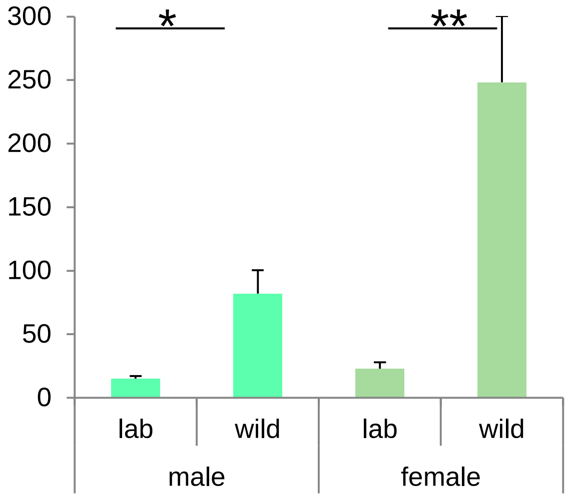
The first inbred mouse strain, DBA, was created in 1909



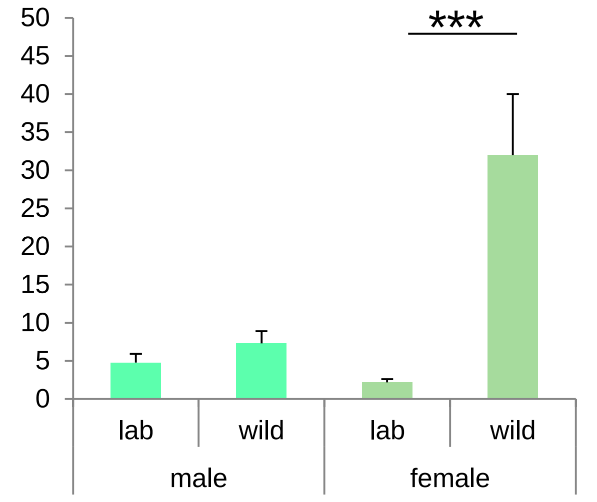
- Mammalian system - close to human
(genetically, physiologically and morphologically)
- Small and easy to handle
- Easy to house and breed
- Inexpensive
- Can be genetically manipulated
- There is a lot of biological knowledge (e.g. Jax lab database, Allen brain atlas)

Behavioral differences between lab and wild mice

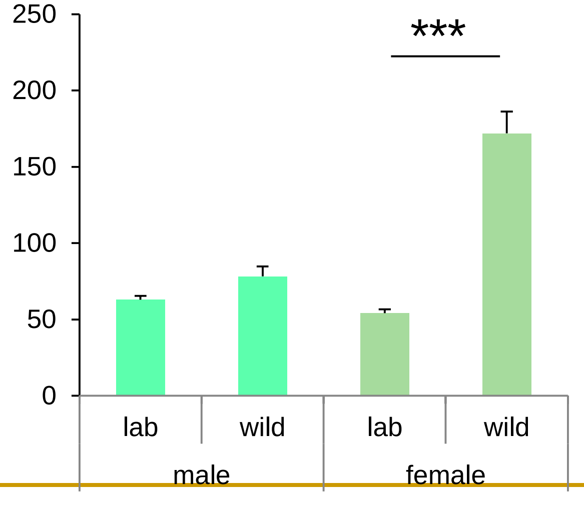
Corticosterone levels (ng/mL)



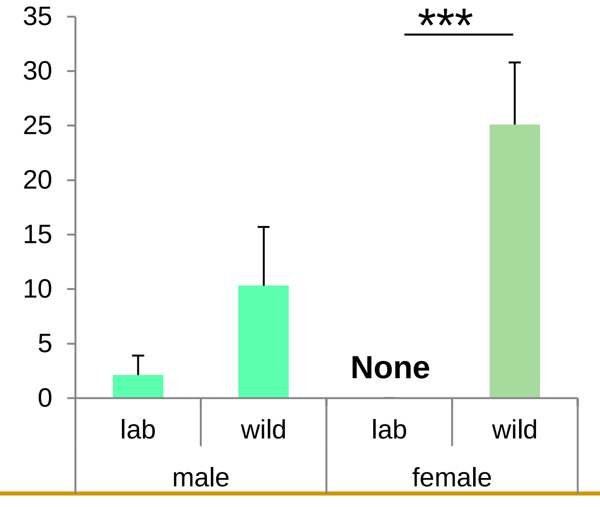
Anxiety behavior (latency to light chamber)



Max velocity (cm/sec)

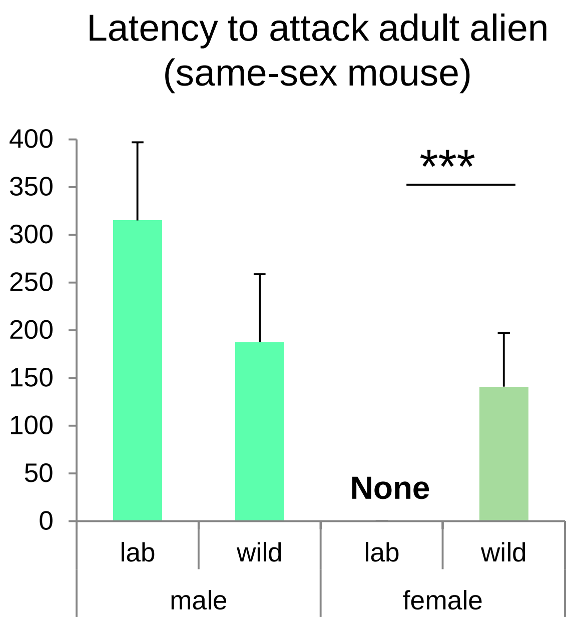


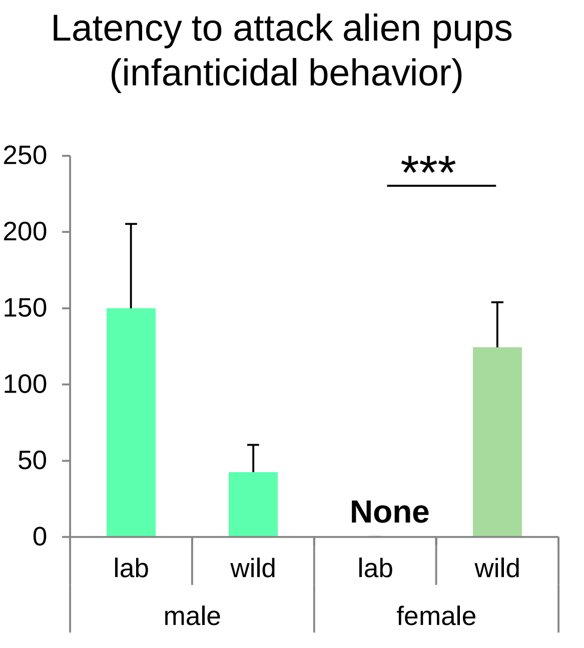
Wildness (Escape jumps)



Chalfin et al 2013; *Nat Commun*

Roust aggressive behavior towards conspecifics in naïve wild females

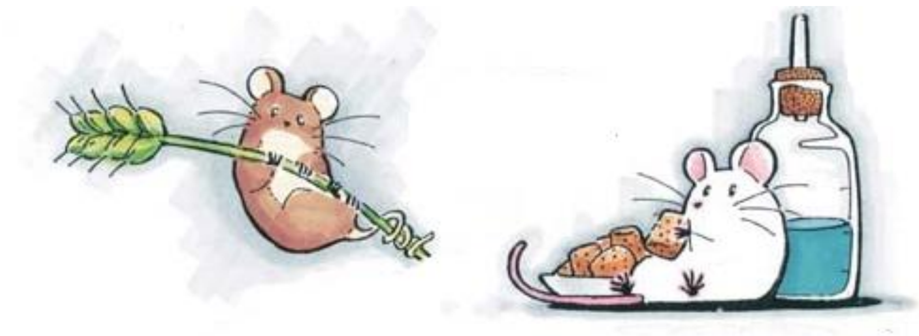


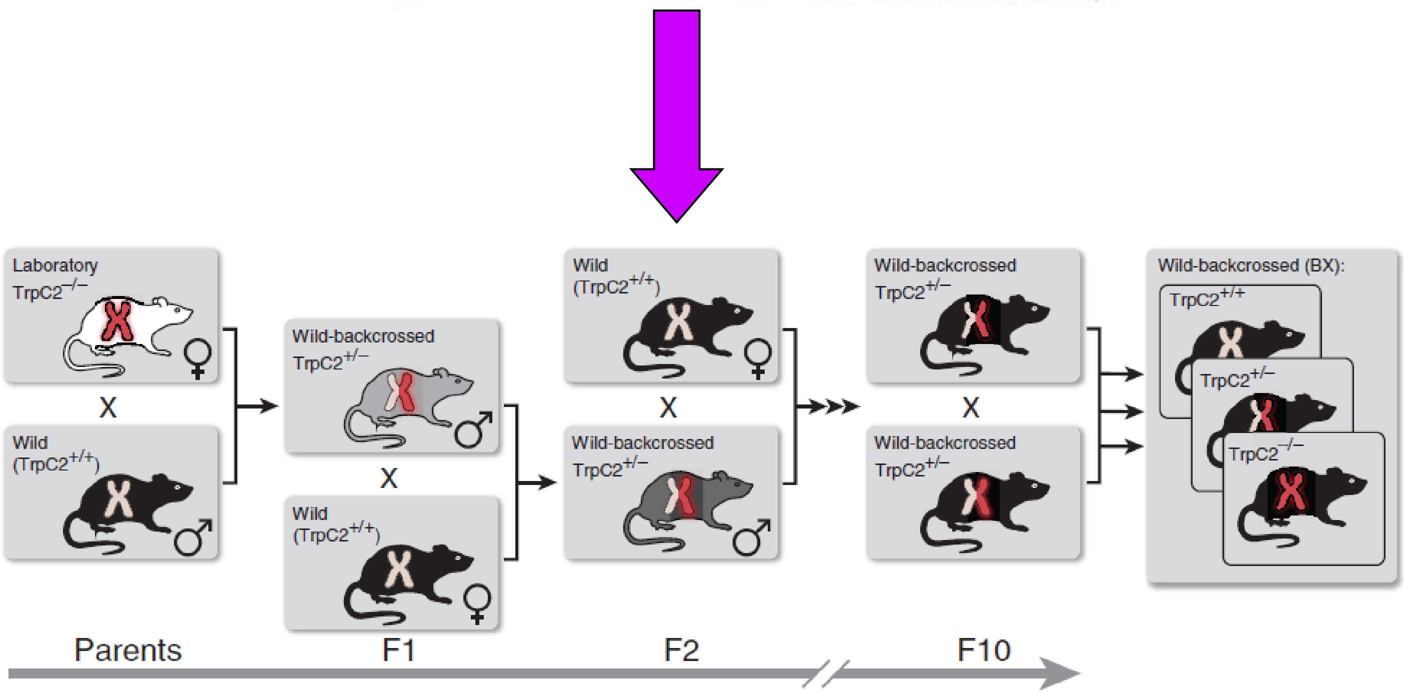


Novel model for studying the mechanisms underlying social behavior (aggression) in female mice

Generating Wild-Background TRPC2 Mice

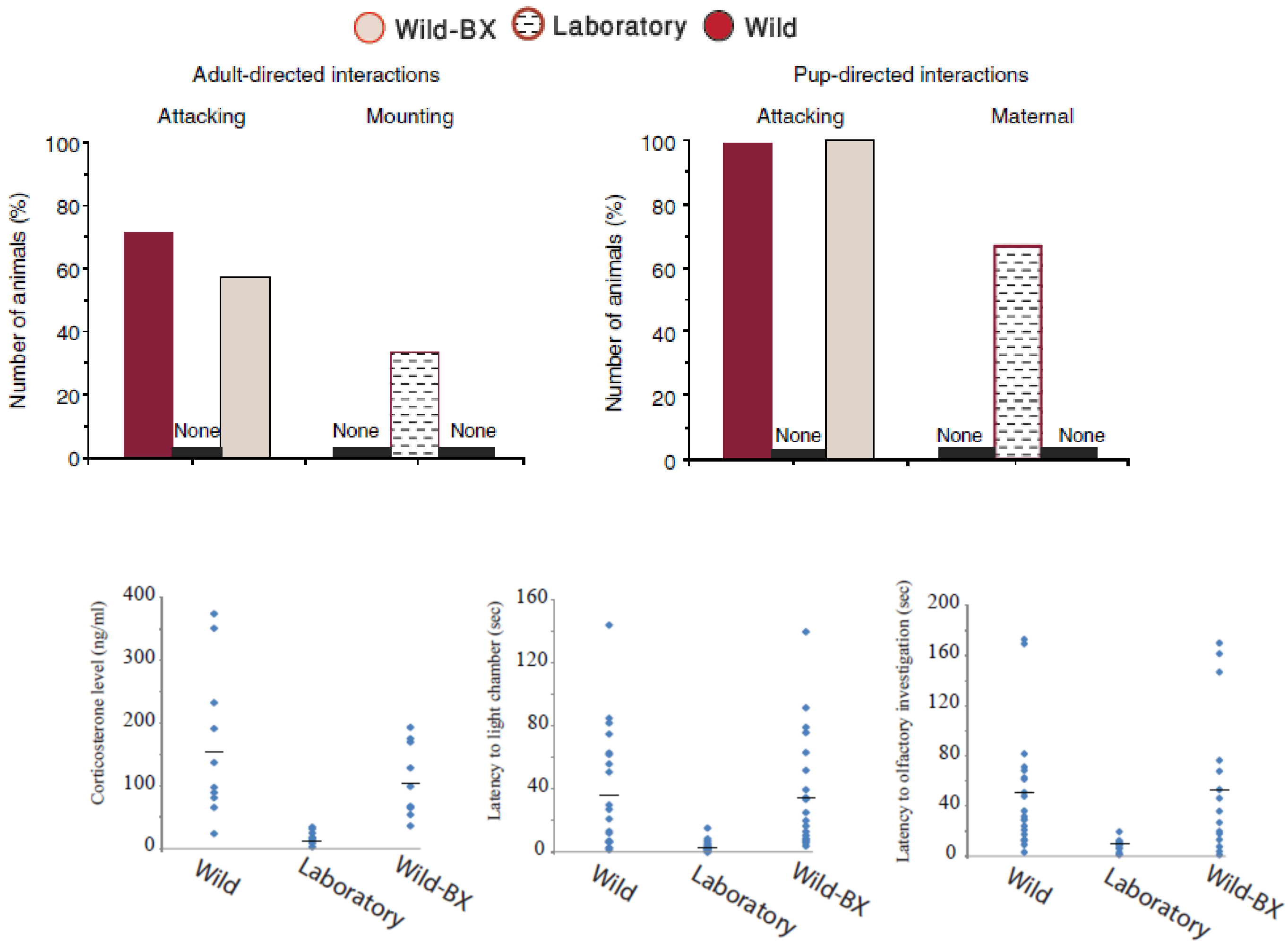
(TrpC2= gene essential for detection of pheromone signals)



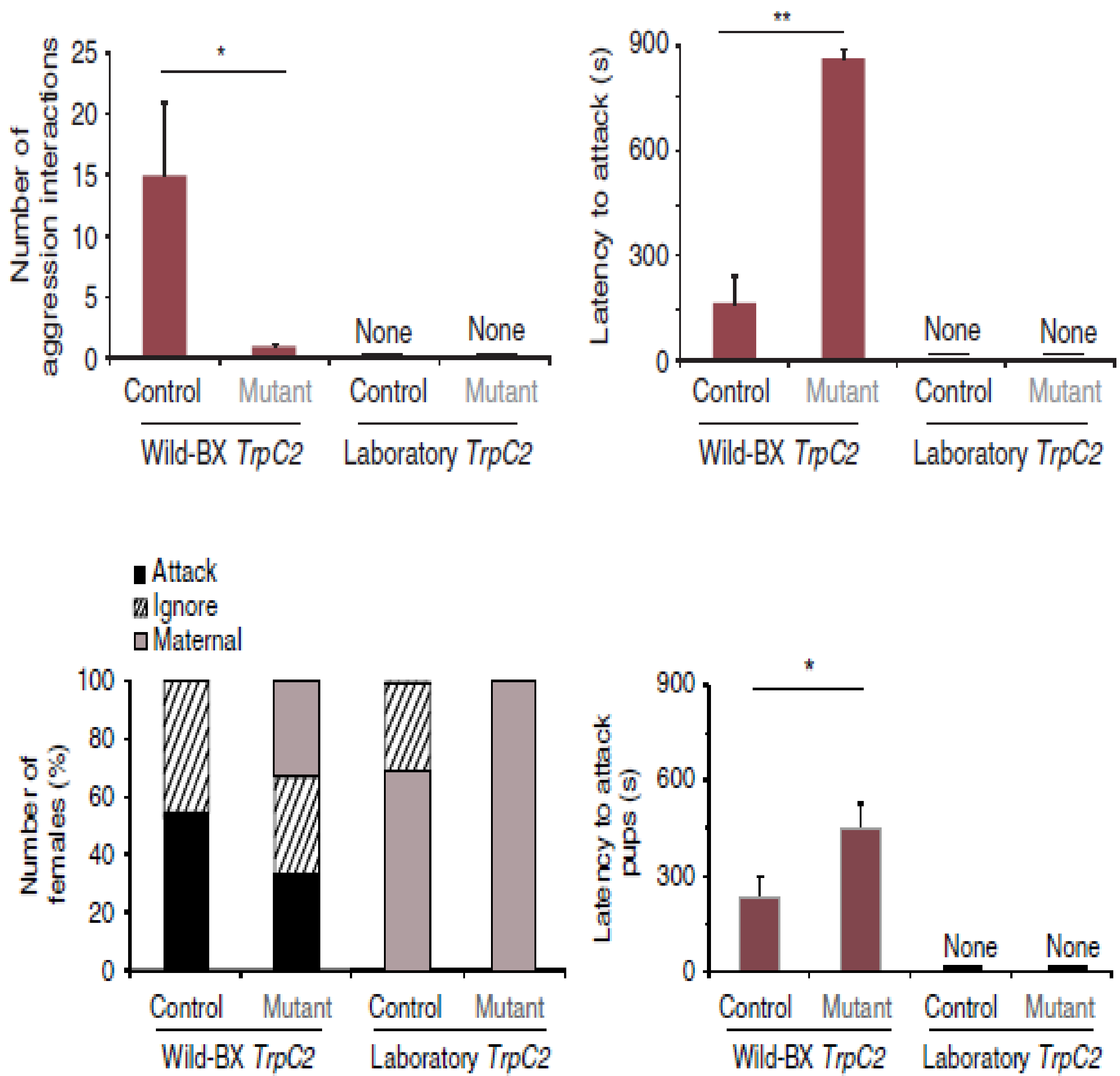


Chalfin et al 2014; *Nat Commun*

Wild-backcrossed females present behavioral traits similar to that of wild-caught females



Chalfin et al 2014; *Nat Commun*



Chalfin et al 2014; *Nat Commun*