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Animal models in neurodevelopmental disorders and neurodegenerative diseases

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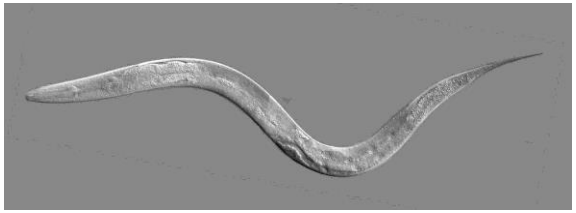
Common model organisms in neuro-research

Drosophila melanogaster

Caenorhabditis elegans



Danio rerio

Mus musculus

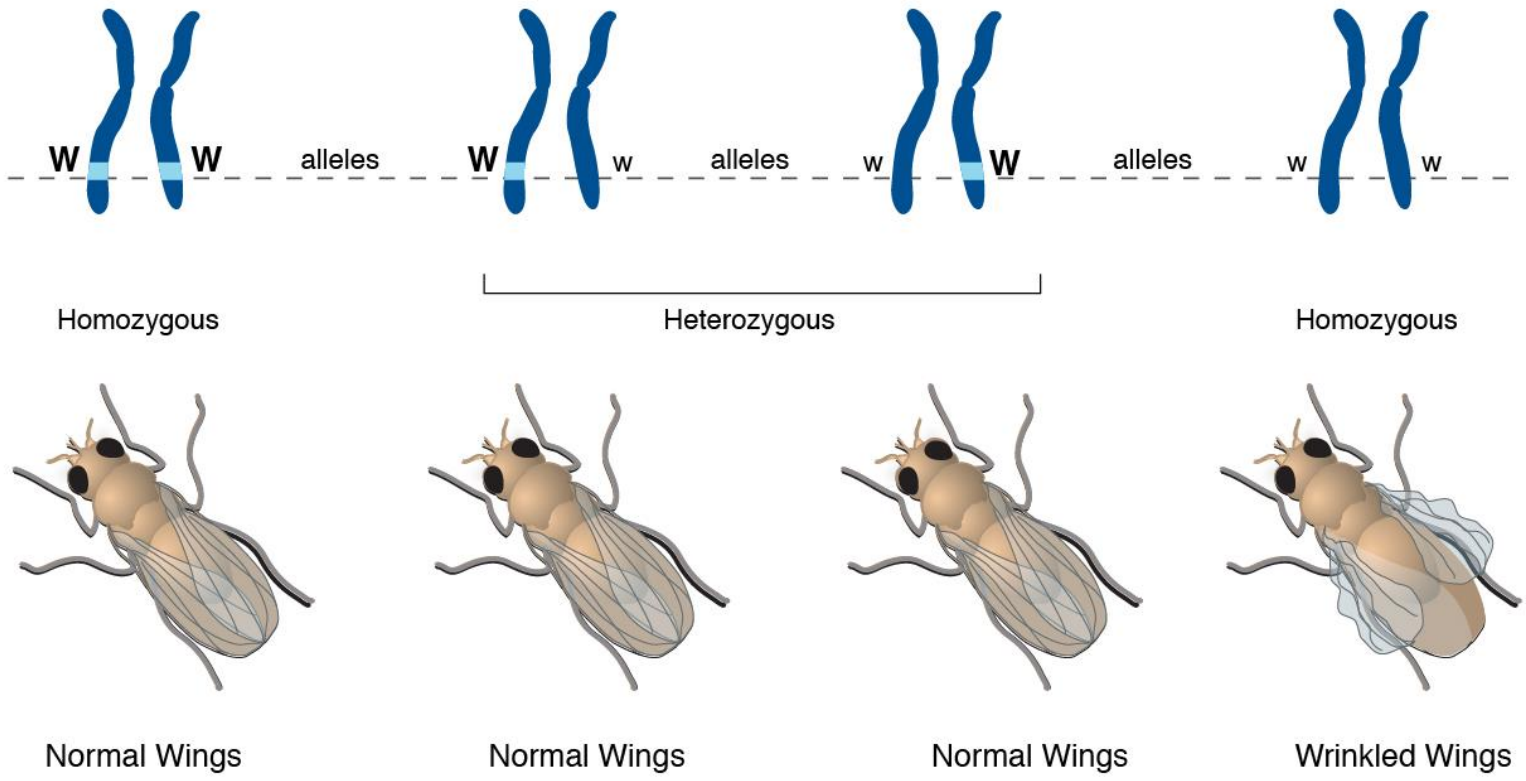
Rattus norvegicus

Macaca mulatta

Genotype and phenotype

- A phenotype is an individual's observable traits
  - height,
  - eye color, and
  - blood type and etc.,
- The genetic contribution to the phenotype is called the genotype.
- Some traits are largely determined by the genotype, while other traits are largely determined by environmental factors.

Genotype and phenotype



Genome.gov

Animal models for neurobiology research

- Useful tools to test specific hypothesis about
  - Impact of genes conferring predisposition on brain development, circuits and/or behavior
  - Impact on behavior of suspected pathophysiological mechanisms
  - Possibility of environmental factors affect on brain function

Types of animal models

- Gene-based animal model or etiological model
- Pharmacological animal model

Neurodegenerative disease

- Neurodegenerative diseases are a heterogeneous group of disorders
- characterized by the progressive degeneration of the structure and function of the central nervous system or peripheral nervous system.

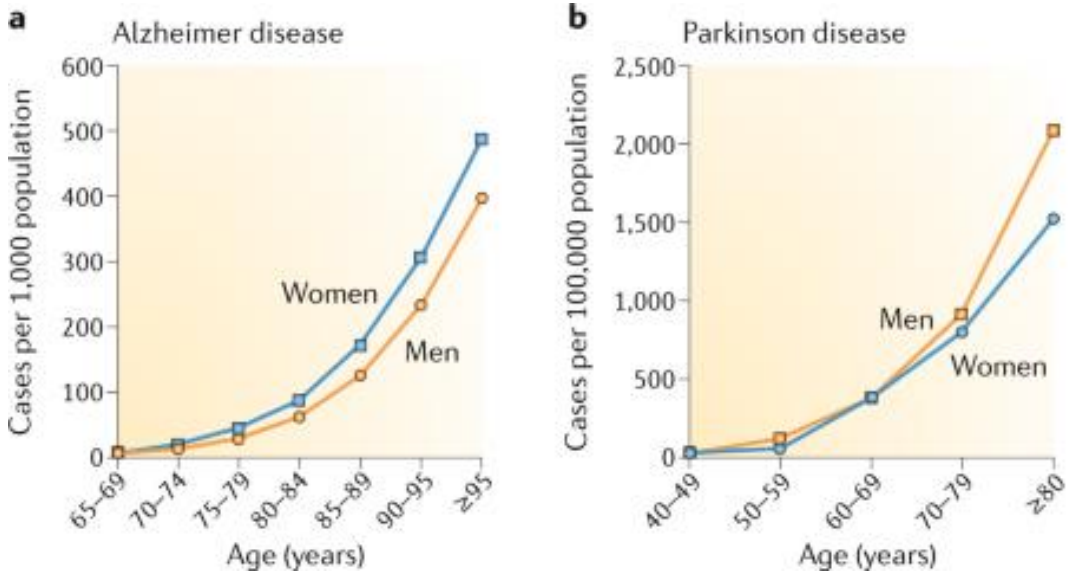
Common neurodegenerative diseases

Table 1 | **Age-related neurodegenerative diseases**

Disease	Prevalence	Major symptoms	Risk factors	Neuropathological hallmarks
Alzheimer disease	5.7 million in the USA in 2018 (REF.[3])	Impairment of learning and memory, speech difficulties	Age, family history, genetics, history of head trauma, female gender, vascular risk factors, environmental factors[195–198]	Aβ plaques, neurofibrillary tangles, neuronal loss, neuroinflammation
Parkinson disease	2–3% of the global population aged >65 years in 2017 (REF.[10])	Muscle rigidity, tremors, alterations in speech and gait	Environmental factors, genetics, male gender, ethnicity, age, psychiatric symptoms[199,200]	α-Synuclein-containing Lewy bodies and loss of dopaminergic neurons, grey matter atrophy[201]
Amyotrophic lateral sclerosis	0.6 cases per million globally in 2016 (REF.[202])	Progressive motor defects, with muscle weakness, atrophy and spasms	Physical activity, familial aggregation, environmental and occupational exposure (for example, to pesticides, solvents or heavy metals), smoking, head injury, genetics[202]	TAR DNA-binding protein 43 aggregation
Huntington disease	5–7 per 100,000 white people in 2007 (REF.[203])	Chorea, dystonia, loss of coordination, cognitive decline, behavioural difficulties	Genetic mutation in *HTT*, inheritance	Striatal atrophy, neuronal loss, psychiatric symptoms[204,205]
Dementia with Lewy bodies	1.3 million in the USA in 2014 (REF.[206])	Visual hallucinations, movement disorders, cognitive problems, sleep difficulties, depression	Age >50 years, male gender, family history[206]	Lewy bodies and Lewy neurites
Ataxia telangiectasia	From 1 in 40,000 to 1 in 100,000 live births worldwide in 2016 (REF.[207])	Cerebellar degeneration, immunodeficiency, radiation sensitivity, diabetes, cancer predisposition	Genetics (mutations in *ATM* gene)	Ataxia and telangiectasias
Cockayne syndrome	2–3 per million globally in 2008 (REF.[208])	Growth failure, neurological disorders, photosensitivity, eye disorders, premature ageing	Genetics (mutations in *CSA* or *CSB* gene)	Growth retardation and neurodegeneration

Hou Y et al., *Nature Rev. Neurology*. 2019

Requirement of neurodegeneration animal model



- Adult onset of relatively specific and progressive tissue or neuron degeneration.
- A behavioral correlate of the particular brain circuitry
- The formation of cellular features as observed in post-mortem clinical brain tissues from patients

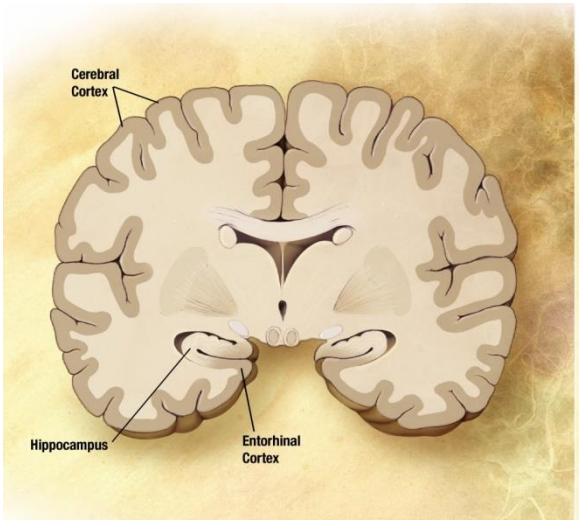
Hou Y et al., *Nature Rev. Neurology*. 2019

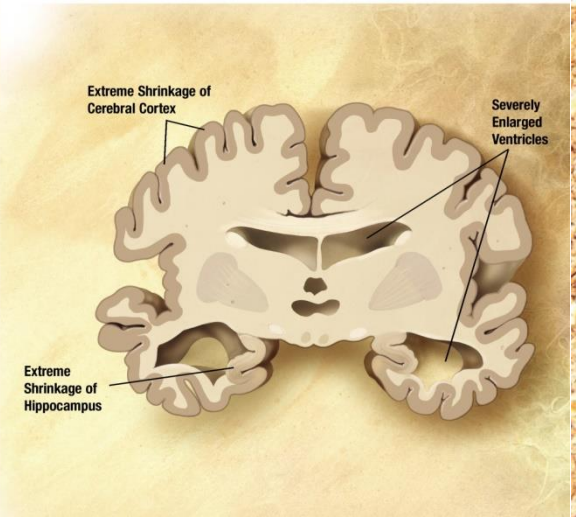
Alzheimer's disease

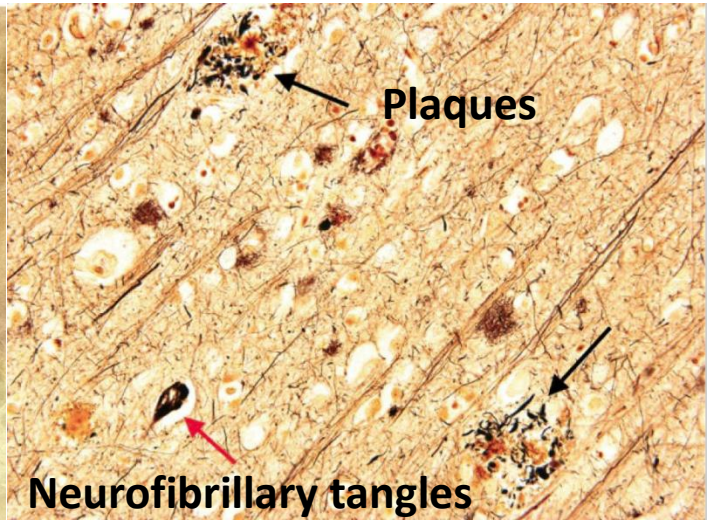
- Alzheimer's disease is a major neurodegenerative disease
  - Affecting approximately 6% of people 65years and older
- characterized by
  - progressive and irreversible decline in memory
  - deterioration of various other cognitive abilities.

Clinical features of brain post-mortem data from Alzheimer's patients

- Brain atrophy (decrease in size and wasting away),
- neuronal loss
- extensive distribution of neuronal tangles and amyloid plaques in brain
- astrogliosis
- vascular alterations

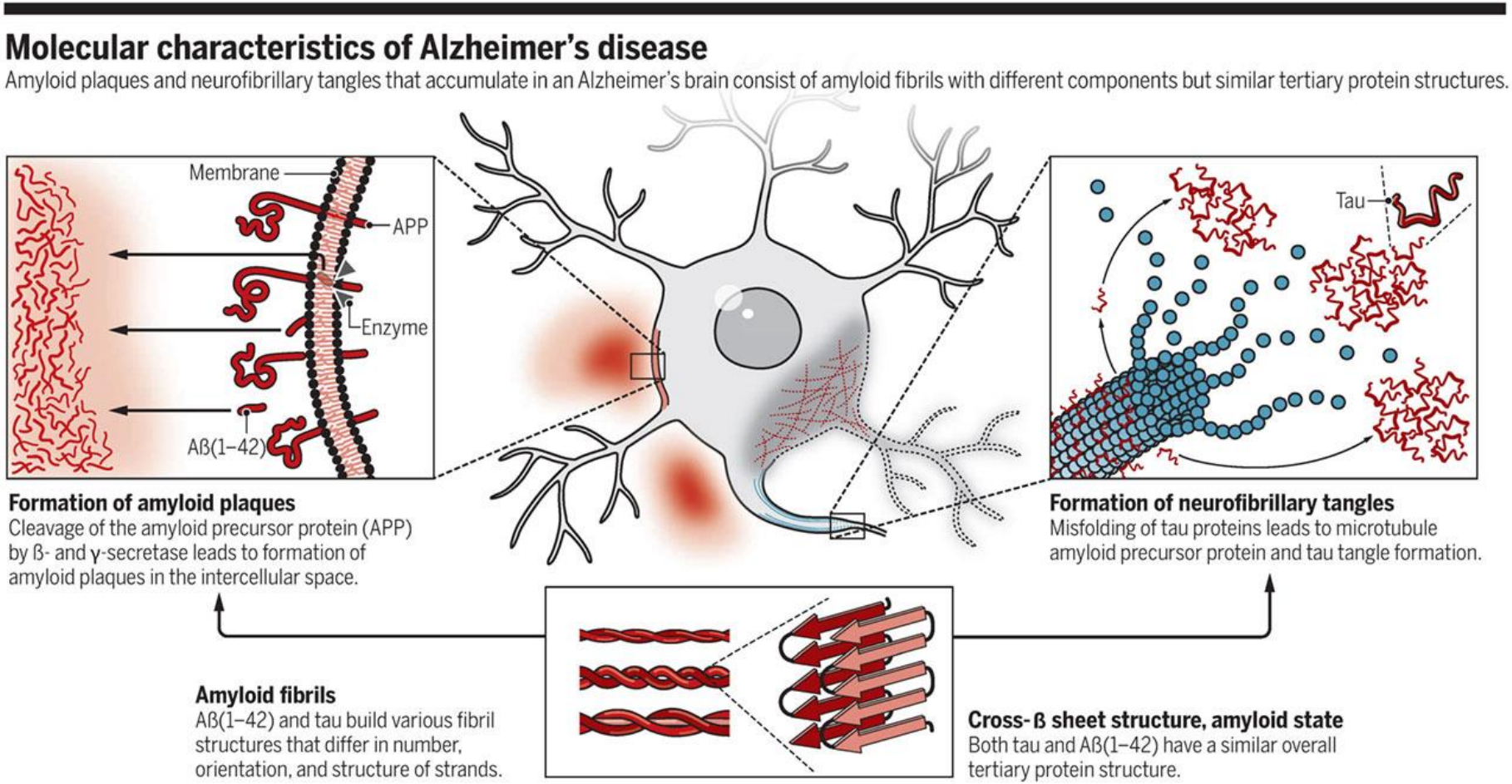






Daniel P. Perl *Mt Sinai J Med*. 2010

Alzheimer's disease



Haas C & Kahle PJ *Nature*. 2000

Animal models of Alzheimer's disease

- Gene-based models
  - APP (amyloid precursor protein)
  - Tau and etc
- Pharmacological models
  - Scopolamine
  - Streptozotocin
  - Ketamine

Drosophila model of Alzheimer's disease

Table 2 | **Models for studying Alzheimer disease**

Organism	Model	Genetic alterations
Drosophila melanogaster	Expression of human proteins: Aβ toxicity[98]	Flies express hAPP, hBACE and *D. melanogaster* γ-secretase presenilin (dPsn) containing AD mutations N141I, L235P and E280A
		Flies express $A\beta_{40/42}$ peptides fused with *D. melanogaster* necrotic gene sequence for secretion
	Expression of human proteins: tau toxicity[98]	Flies express wild-type hTau or hTau containing V337M and R406W mutations
		Flies express phosphorylation-resistant tau variants containing S2A/S11A or S262A mutation
	Overexpression of *D. melanogaster* genes: Aβ toxicity[98]	Flies with overexpression of β-secretase-like protein
		Flies with knockdown of ferritin
	Silencing of *D. melanogaster* genes[98,99]	Flies with loss of dtau or partitioning defective-1 (PAR-1)
		Flies with genetic inhibition of copper-importers (Ctr1C and Ctr1B) or inhibition of zinc importer dZip1 to rescue Aβ42 pathogenesis

Hou Y et al., *Nature Rev. Neurology*. 2019

Mutations in Tau are associated with dementia

- Human Tau (hTau) is a highly soluble and natively unfolded protein that binds to microtubules within neurons.
- Its dysfunction and aggregation into insoluble paired helical filaments is involved in the pathogenesis of Alzheimer's disease

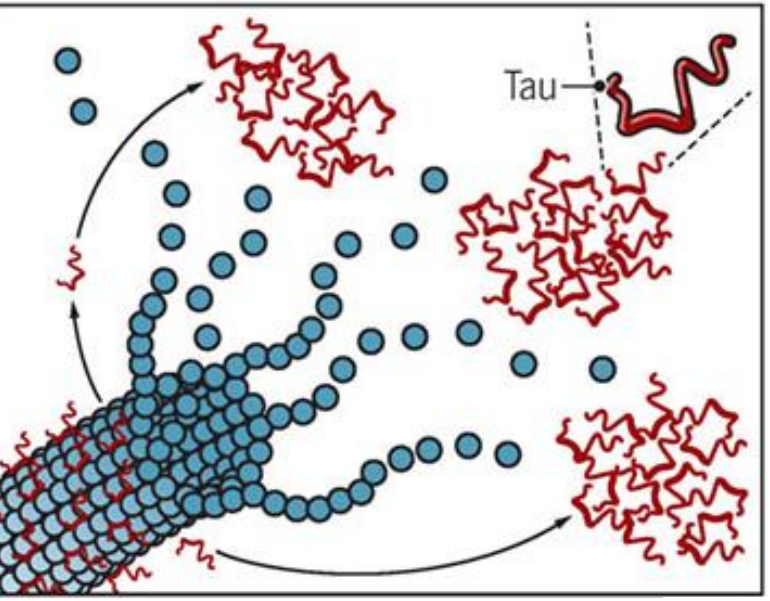
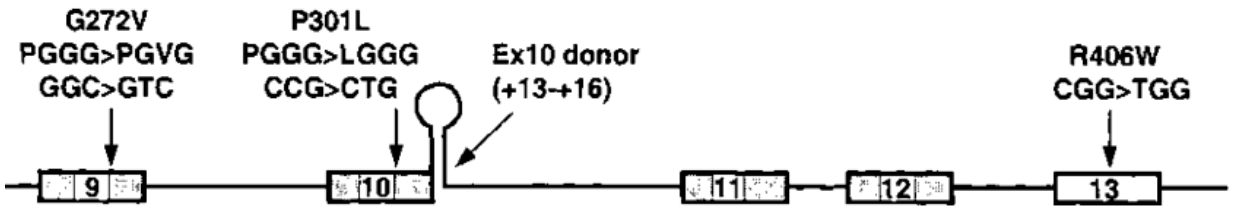


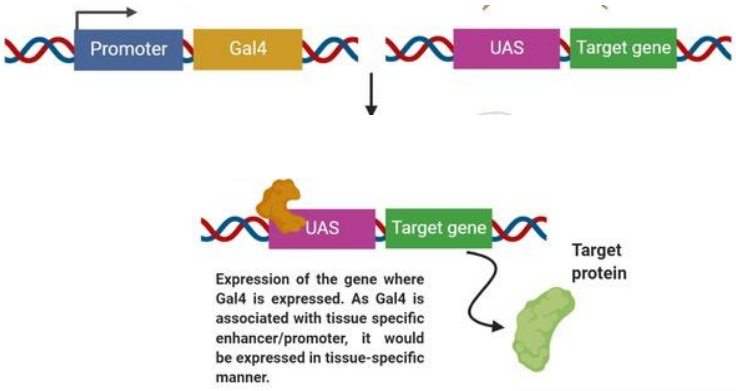
Table 1 Families with segregating mutations in the *tau* gene

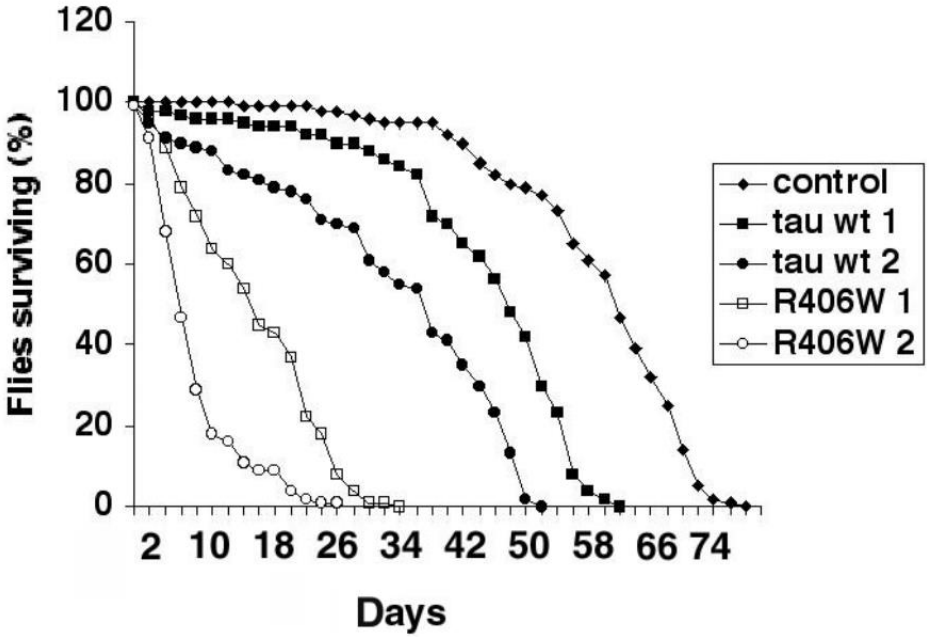
Family	Origin (founder)	Affecteds*	Generations	Mean onset age	Mutation
HFTD2†	Netherlands	34(15)	7	47	G272V
HFTD1†	Netherlands	49(14)	5	50	P301L
FTD003	USA	3(2)	2	45–50	P301L
Man19	UK	3(1)	2	65	Ex10 splice + 13
DDPAC†	Ireland	13(7)	3	44	Ex10 splice + 14
AusI†	Australia (UK)	28(5)	5	53	Ex10 splice + 16
FTD002†	USA	3(1)	2	40	Ex10 splice + 16
Man6	UK	2(1)	1	48	Ex10 splice + 16
Man23†	UK	10(2)	3	51	Ex10 splice + 16
FTD004	USA	10(2)	4	55	R406W



Hutton M et al., *Nature* 1998

Tau-based drosophila model of Alzheimer's disease





elav: pan neuronal promoter

elav–GAL4/+.
elav/+; *UAS–wild-type tau*/+;
elav/+; *UAS–wild-type tau-R406W*/+;

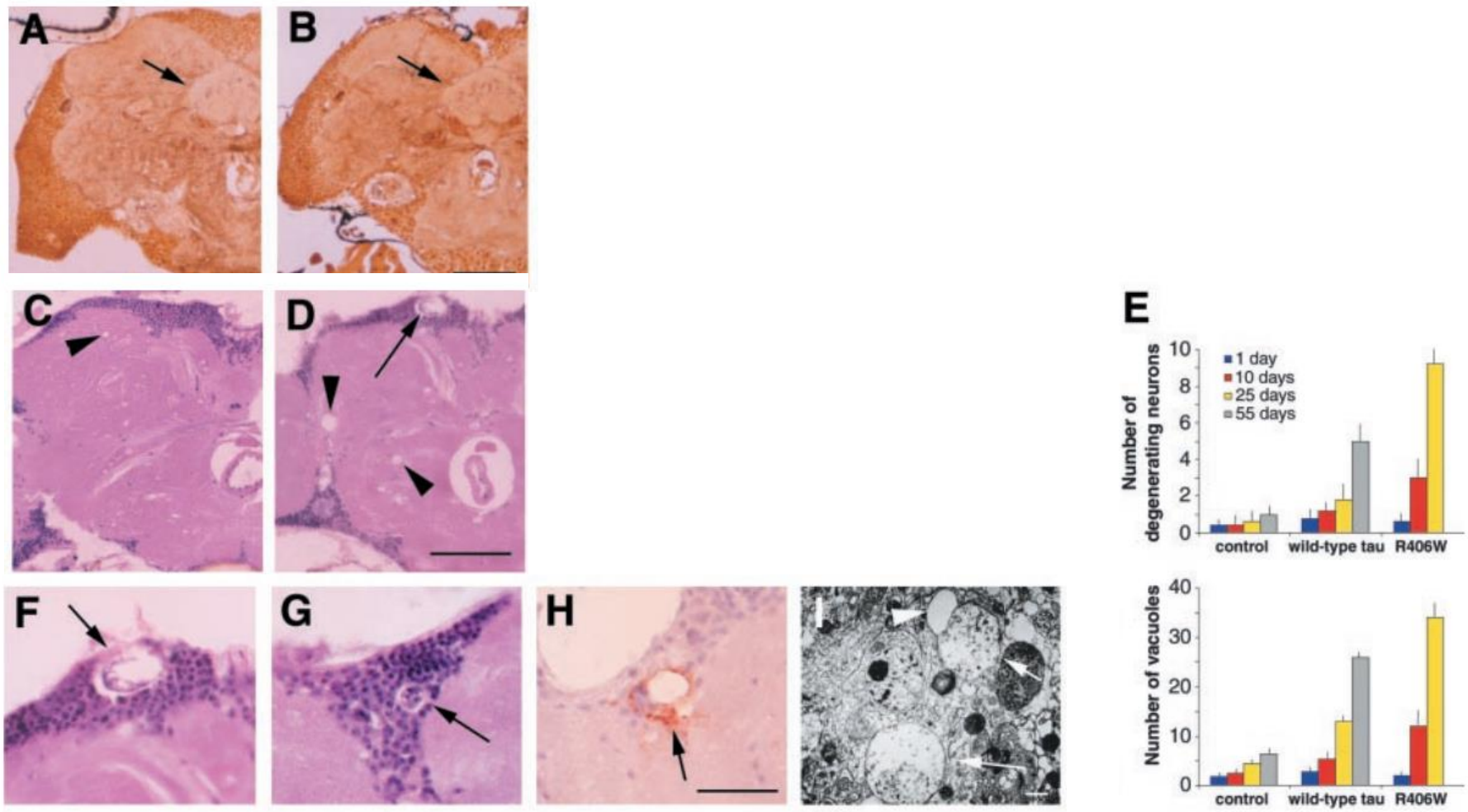
Flies expressing mutated tau in neurons die at an early age

Wittmann CW *et al., Science*. 2001

Tau-based drosophila model of Alzheimer's disease

One-day old flies

Aged flies



Flies expressing mutated tau in neurons exhibit neurodegeneration

Wittmann CW *et al., Science*. 2001

Pros and cons of Tau-based drosophila model

- faithfully replicates a number of features of the human disorders:
  - adult onset,
  - progressive neurodegeneration,
  - accumulation of abnormal tau, and
  - early death.
- However,
  - No large filamentous tau aggregates that are observed in human disease and other experimental models of tauopathy

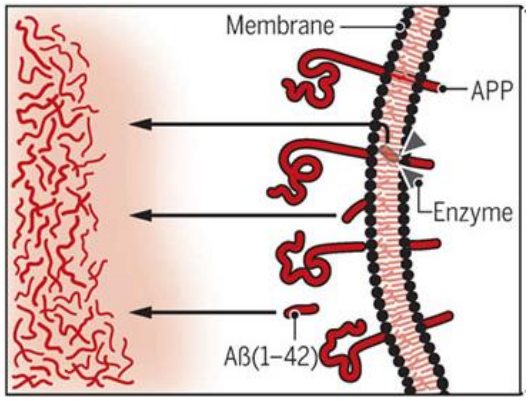
Wittmann CW *et al., Science*. 2001

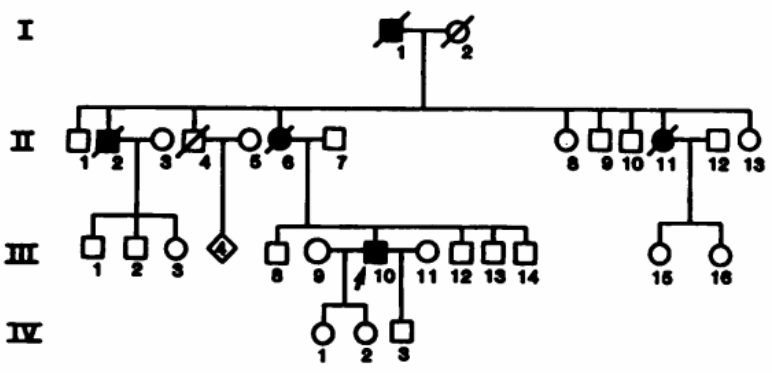
Mouse models of Alzheimer's disease

Model	Genetic alterations
hAPP models	PDAPP mouse line: mice express hAPP695/751/770 with Florida mutation (V717F) at the γ-cleavage site under the regulatory control of the PDGFβ promoter
	J20 mouse line: mice express hAPP695/751/770
	TgCRND8 mouse line: mice express hAPP695, containing the Swedish double mutations (K670N/M671L) at the β-secretase cleavage site and V717F mutation at the γ-secretase cleavage site
	Tg2576, APP23 and RI.40 mouse lines: mice express hAPP695, hAPP751 and entire human APP gene, respectively, containing the Swedish double mutations (K670N/M671L) at the β-secretase cleavage site
	TASD-41 mouse line: mice express hAPP containing the Swedish double mutations (K670N/M671L) at the β-secretase cleavage site and London mutation (V717I) at the γ-secretase cleavage site
Aβ models	BRI-Aβ40 or BRI-Aβ42 mouse lines: mice express fused Aβ and BRI protein, causing amyloid formation
hAPP/PS1 models	APPswe/PS1ΔE9 mouse line: mice express hAPP695 with Swedish mutation (K670N/M671L) and PSEN1 with ΔE9 mutation
	5XFAD mouse line: mice express hAPP with Swedish (K670N/M671L), Florida (I716V), and London (V717I) mutation, and PSEN1 with M146L and L286V mutations
	2xKI mouse line: mice express mAPP with Swedish mutation (K670N/M671L) and humanized Aβ and express mPS1 with P264L mutation
Models with hTau	TAPP mouse line: mice express hAPP695 containing Swedish mutation and human four-repeat tau with P301L mutation and without the amino-terminal sequences
	3xTg mouse line: mice express three mutated AD genes: hAPP695 containing Swedish mutation, human four-repeat tau with P301L mutation and without the N-terminal sequences, and PS1 with M146V mutation
	Htau mouse line: mice express the entire human tau gene

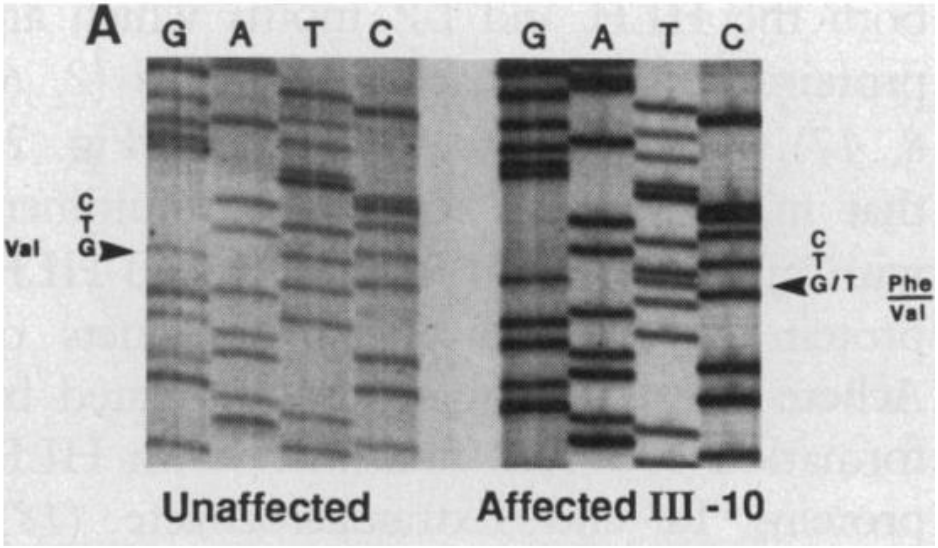
Hou Y et al., *Nature Rev. Neurology*. 2019

Amyloid precursor protein (APP)





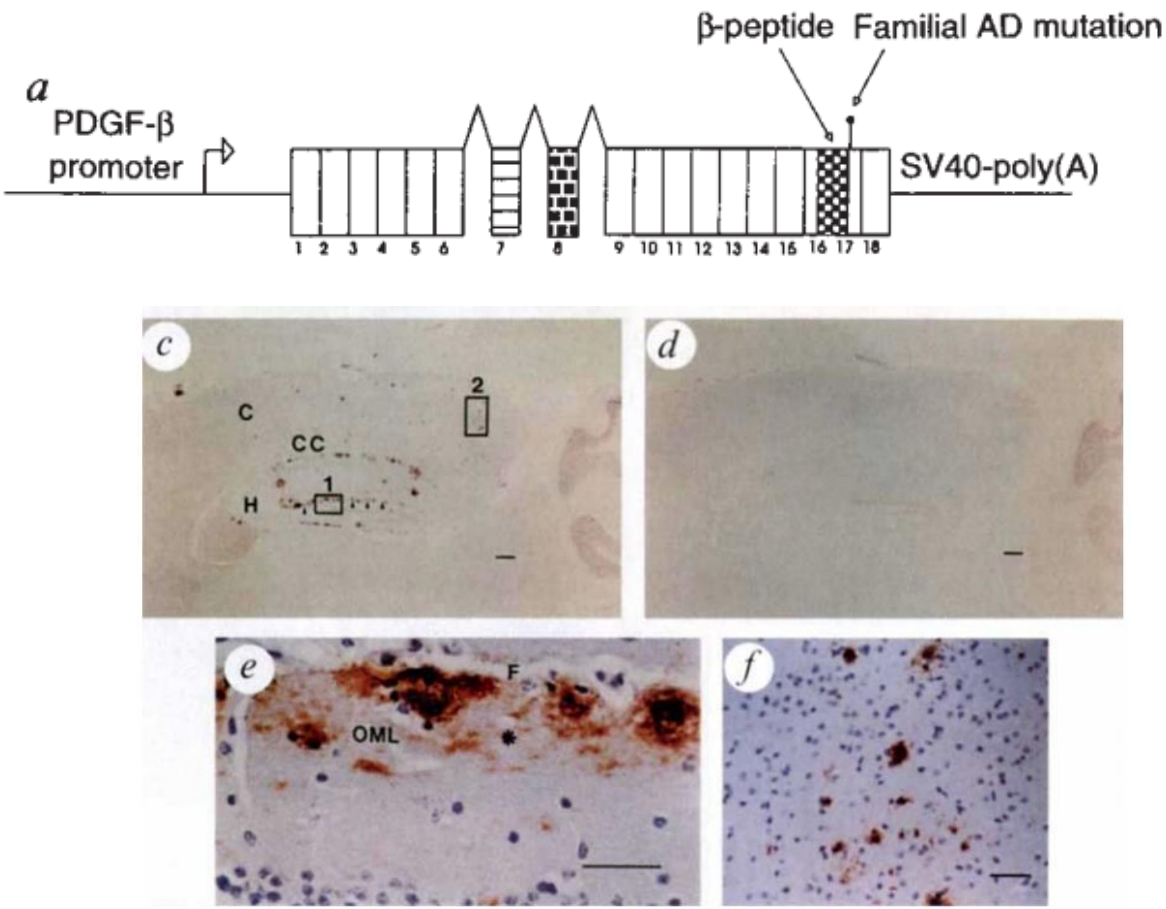
717V->F



Mutations in Amyloid precursor protein (APP) are associated with hereditary Alzheimer's disease

Murrell J et al., *Science* 1991

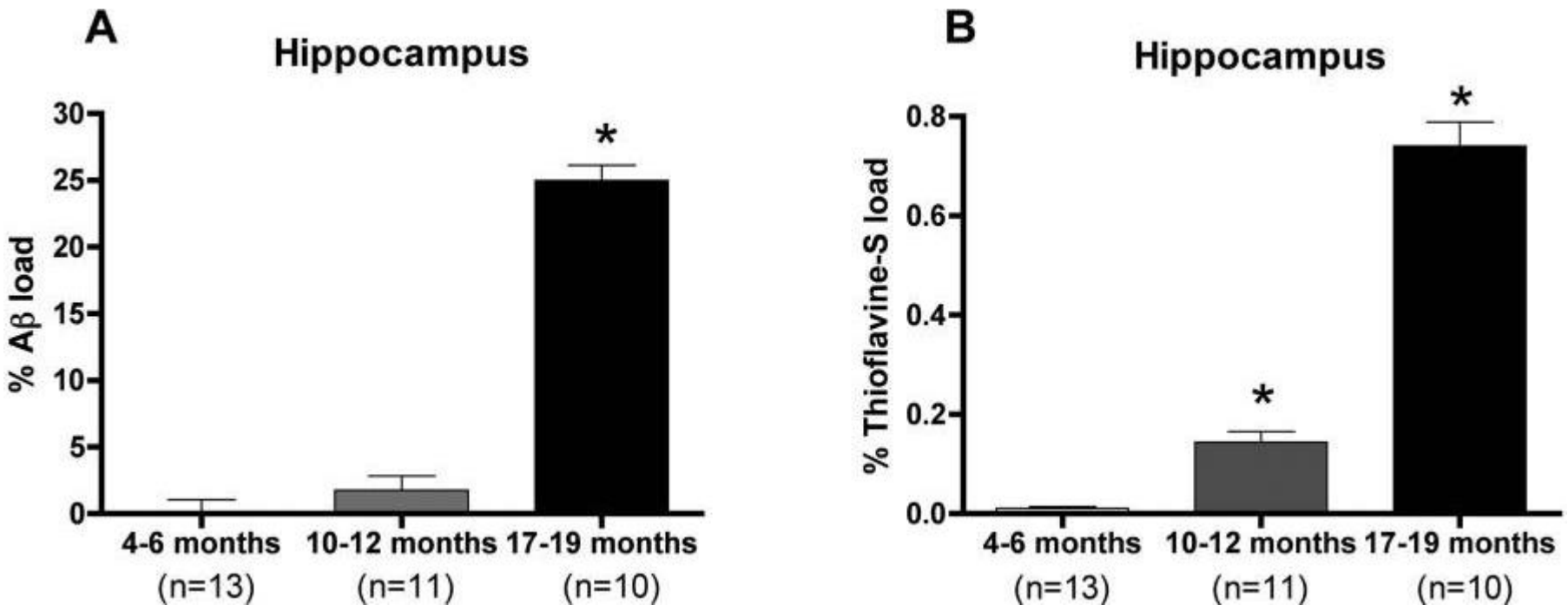
hAPP-based mouse model of Alzheimer's disease



Transgenic mice expressing hAPP mutant, develop amyloid plaques in hippocampus and cortex

Games D et al., *Nature* 1995

hAPP-based mouse model of Alzheimer's disease



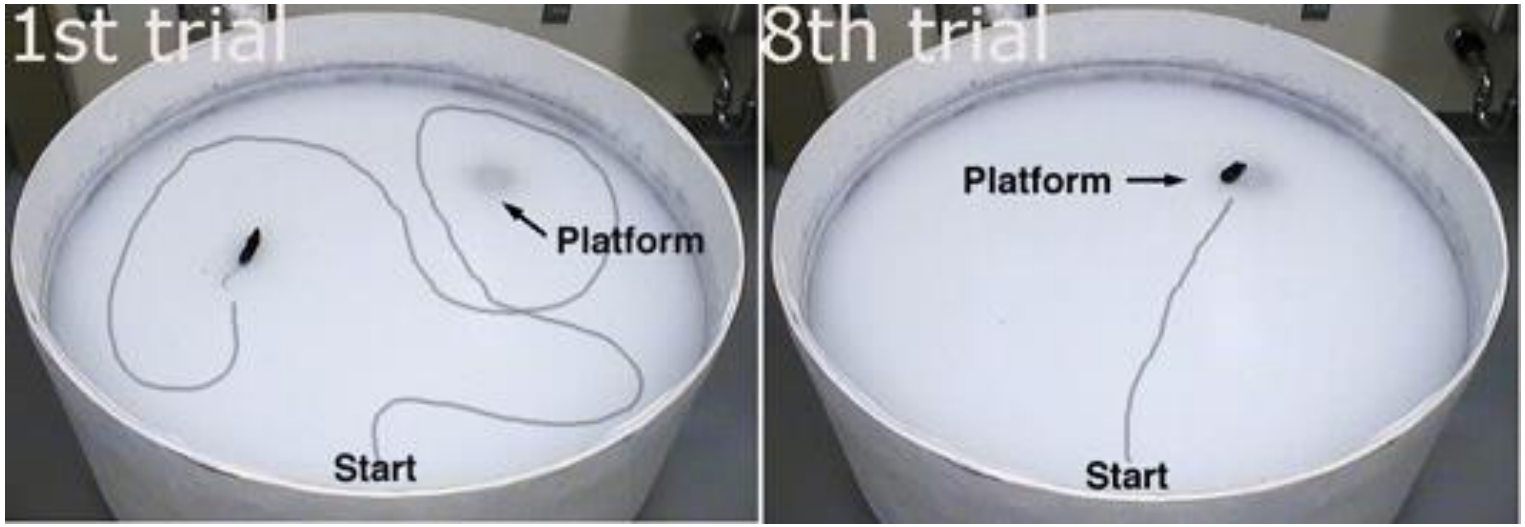
Plaques increase with age

Hartman RE et al., *JNeurosci* 2005

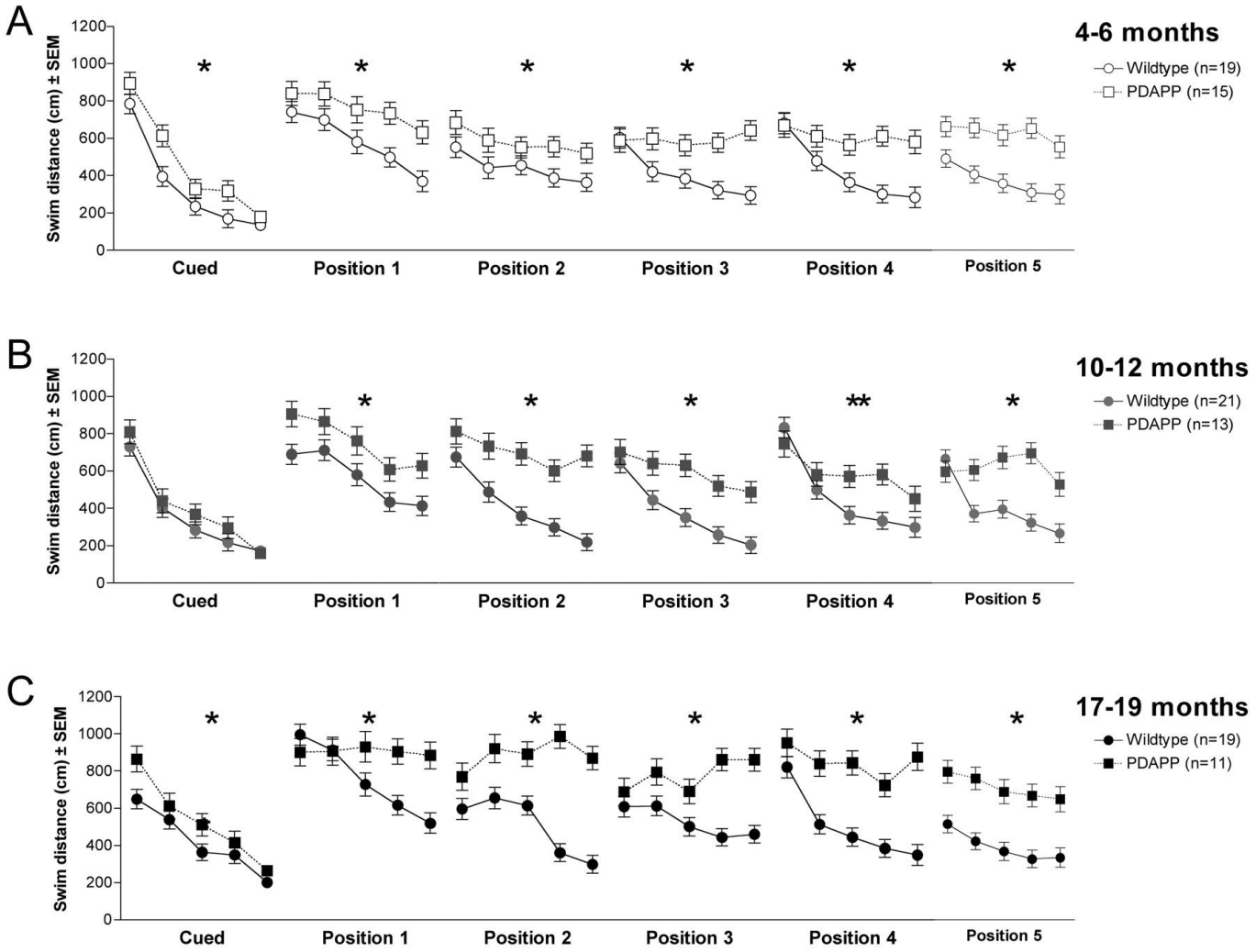
Morris water maze test

To investigate

- working memory,
- reference memory and
- task strategy



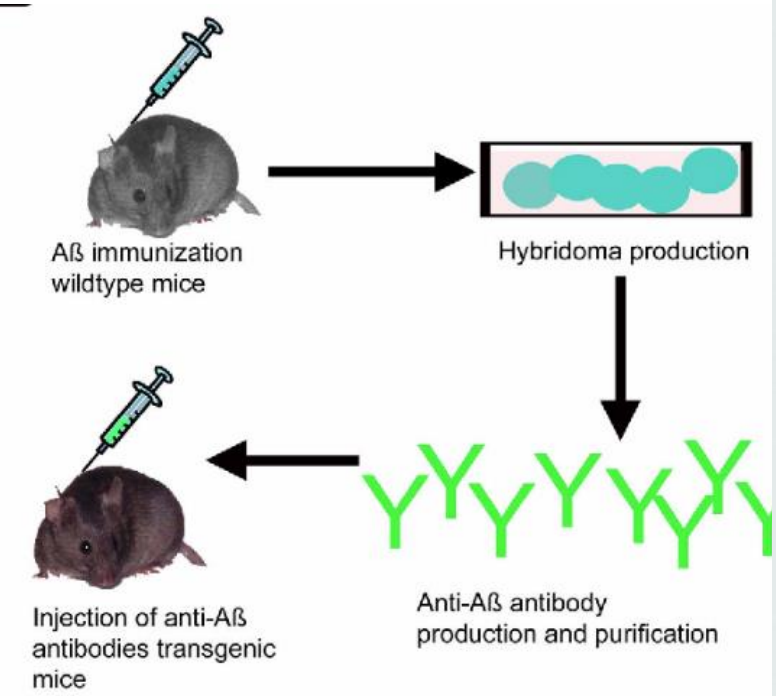
hAPP-based mouse model of Alzheimer's disease

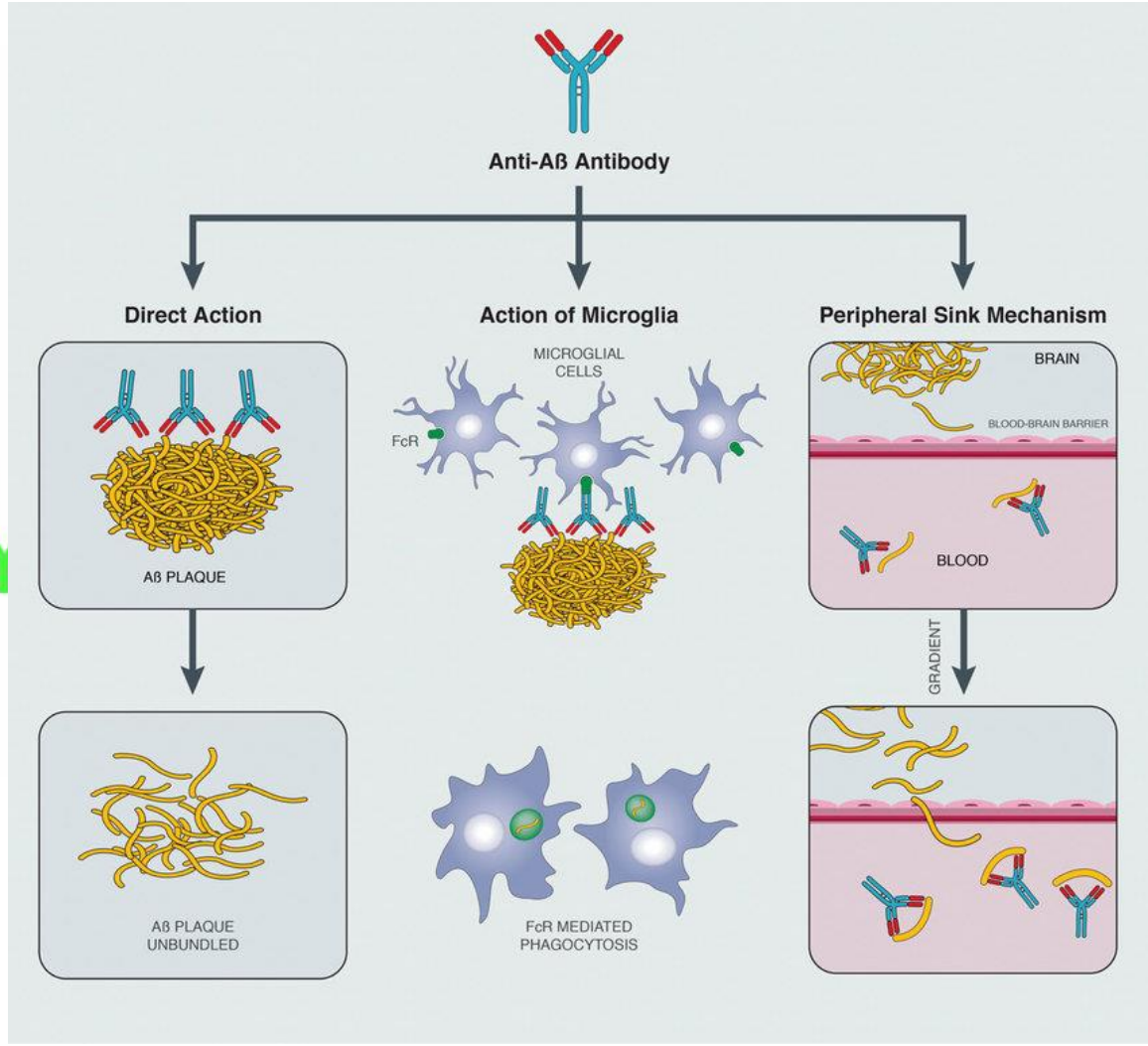


PDAPP mice exhibit impaired spatial learning

Hartman RE et al., *JNeurosci* 2005

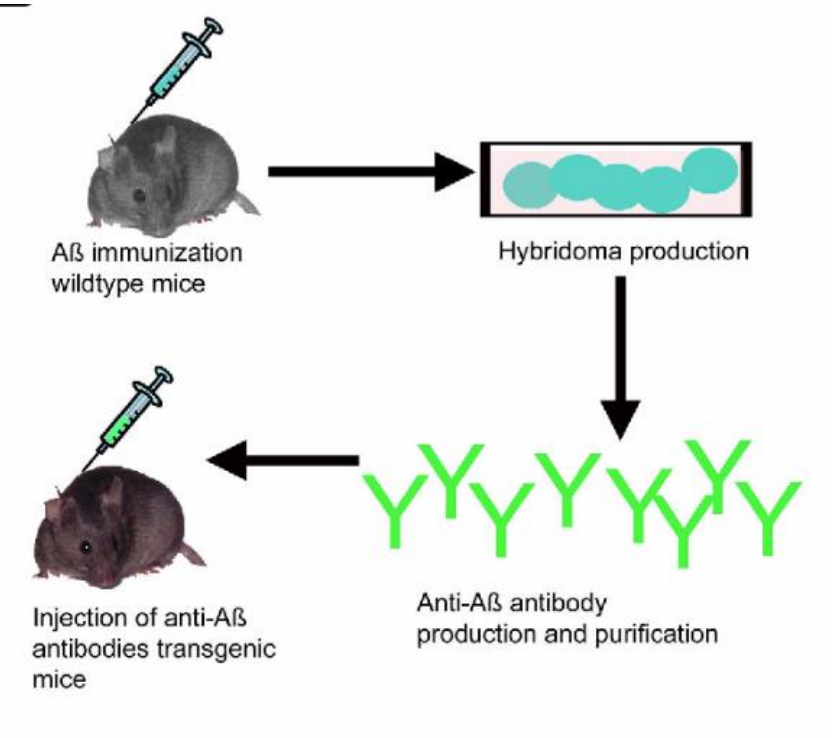
hAPP-based mouse model of Alzheimer's disease

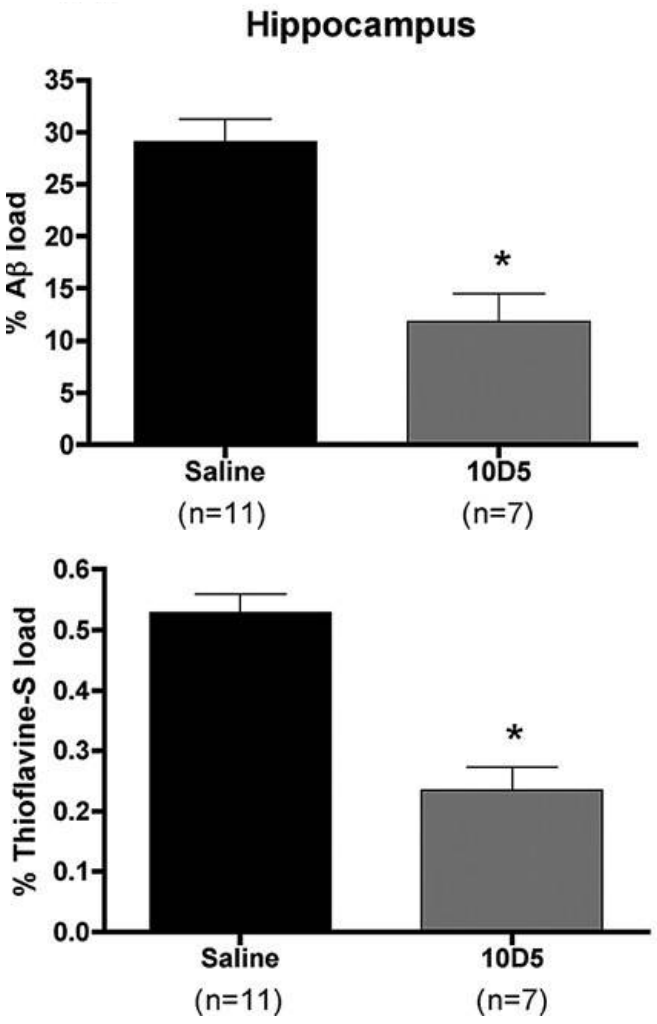




Panza F et al., *Ann Neurol.* 2019
Wilcock D & Colton C *JAD* 2008

hAPP-based mouse model of Alzheimer's disease

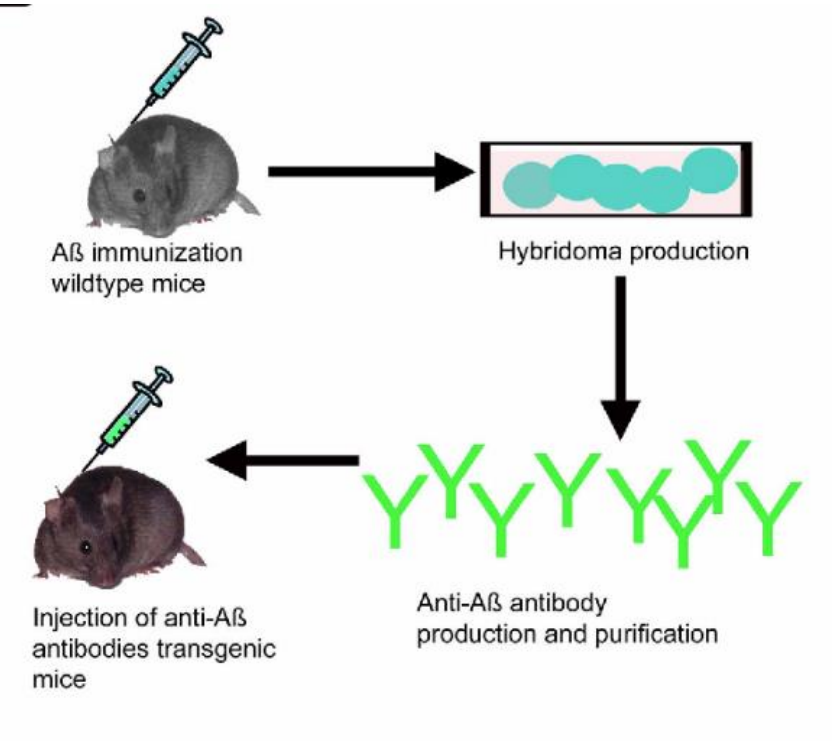


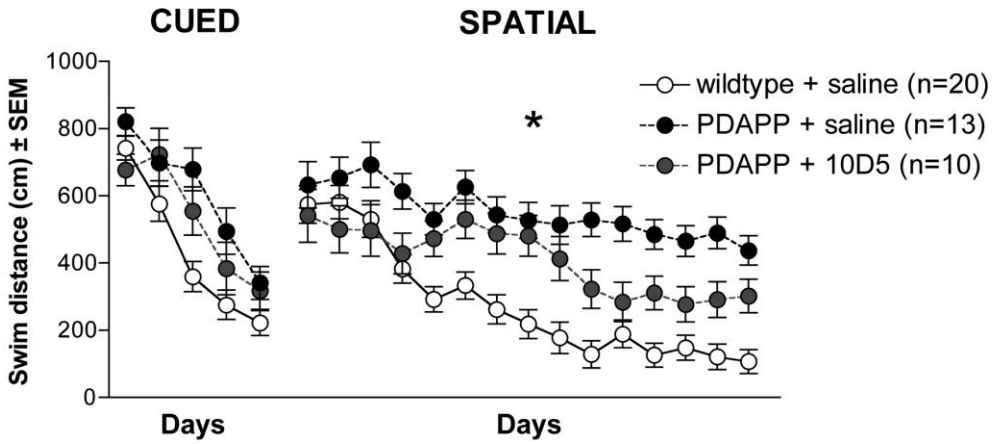


Anti-Aβ antibody injection decreases Aβ load

Wilcock D & Colton C *JAD* 2008
Hartman RE et al., *JNeurosci* 2005

hAPP-based mouse model of Alzheimer's disease





Anti-Aβ antibody injection improves learning

Hartman RE et al., *JNeurosci* 2005

Summary

Animal models can be used

- to study Alzheimer's disease-like phenotypes
- to test the role of a specific gene
- to test the effectiveness of therapeutic options (immunotherapy or drug)

Mice to humans?

TABLE 1. Principal Failed Clinical Studies on Anti-Aβ Therapies in AD and Related Disorders

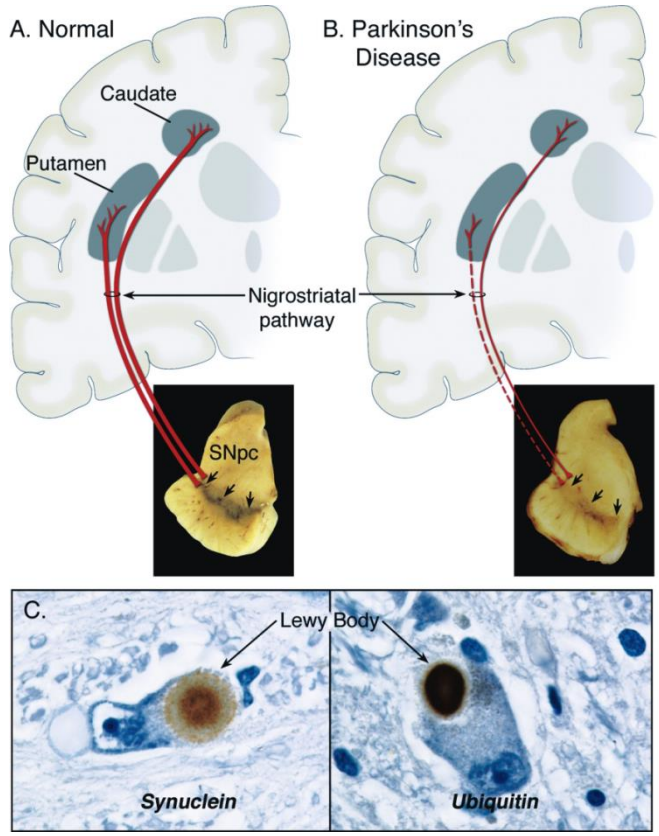
Year (main reference #)	Drug	Mechanism of Action	Subjects	Clinical Phase	Subjects, N	Study Duration, wk	Main Reasons for Failure	Remarks
2002[52]	AN-1792	Aβ antigen	Mild-to-moderate AD	Phase II	372	52	TOX and LOE	
2007[112]	Tramiprosate	Aβ aggregation inhibitor	Mild-to-moderate AD	Phase III	1,052	78	LOE	
2009[39]	Tarenflurbil	γ-Secretase modulator	Mild AD	Phase III	1,684	78	LOE	Worsens global status
2009[42]	Scyllo-inositol	Aβ aggregation inhibitor	Mild-to-moderate AD	Phase II	353	78	TOX and LOE	Increases mortality
2010[113]	Begacestat	γ-Secretase inhibitor	Mild-to-moderate AD	Phase II	17	2	TOX and LOE	
2011[114]	Ponezumab	Anti-Aβ MAb	Mild-to-moderate AD	Phase II	15	24	LOE	
2011[36]	Semagacestat	γ-Secretase inhibitor	Mild-to-moderate AD	Phase III	1,537	76	TOX and LOE	Worsens cognition
2012[115]	Bapineuzumab	Anti-Aβ MAb	Mild-to-moderate AD	Phase III	2,452	78	LOE	
2012[38]	Avagacestat	γ-Secretase inhibitor	Mild-to-moderate AD	Phase II	209	24	TOX and LOE	Worsens cognition
2012[37]	Avagacestat	γ-Secretase inhibitor	Prodromal AD	Phase II	263	104	TOX and LOE	Worsens cognition
2013[57]	Solanezumab	Anti-Aβ IgG1 MAb	Mild-to-moderate AD	Phase II	2,052	78	LOE	
2013[116]	Vanutide	Aβ antigen	Mild-to-moderate AD	Phase II	245	52	LOE	
2013[117]	Immunoglobulin	Anti-Aβ PAb	Mild-to-moderate AD	Phase III	390	78	LOE	
2013[118]	LY2886721	β-Secretase inhibitor	Mild-to-moderate AD	Phase II	70	26	TOX	
2013[119]	AZD3839	β-Secretase inhibitor	Healthy volunteers	Phase I	54	1	TOX	
2014[41]	Affitope AD02	Aβ antigen	Early AD	Phase II	332	78	LOE	Worsens cognition
2014[120]	CAD-106	Aβ antigen	Mild AD	Phase II	121	90	LOE	Worsens cognition
2014[121]	PBT2	Aβ aggregation inhibitor	Prodromal AD	Phase II	42	52	LOE	
2014[61]	Crenezumab	Anti-Aβ MAb	Mild-to-moderate AD	Phase II	433	73	LOE	Binds oligomeric Aβ
2014[58]	Gantenerumab	Anti-Aβ IgG1 MAb	Prodromal AD	Phase II	797	104	LOE	Binds oligomeric Aβ
2014[59]	Gantenerumab	Anti-Aβ IgG1 MAb	Mild AD	Phase II	387	104	LOE	Binds oligomeric Aβ
2016[5]	Solanezumab	Anti-Aβ IgG1 MAb	Mild AD	Phase III	2,129	80	LOE	
2017[122]	Solanezumab	Anti-Aβ IgG1 MAb	Prodromal AD	Phase III	2,450	104	LOE	
2017[123]	Verubecestat	β-Secretase inhibitor	Mild-to-moderate AD	Phase III	1,958	78	LOE	Worsens cognition
2018[34]	Verubecestat	β-Secretase inhibitor	Prodromal AD	Phase III	1,454	104	LOE	Worsens cognition and behavior
2018[124]	Atabecestat	β-Secretase inhibitor	Cognitively healthy subjects at risk of developing AD	Phase III	600	231	TOX and LOE	Worsens cognition
2018[125]	Lanabecestat	β-Secretase inhibitor	MCI and mild AD	Phase III	2,202	104	LOE	Worsens cognition
2018[125]	Lanabecestat	β-Secretase inhibitor	Mild AD	Phase III	1,899	104	LOE	Worsens cognition

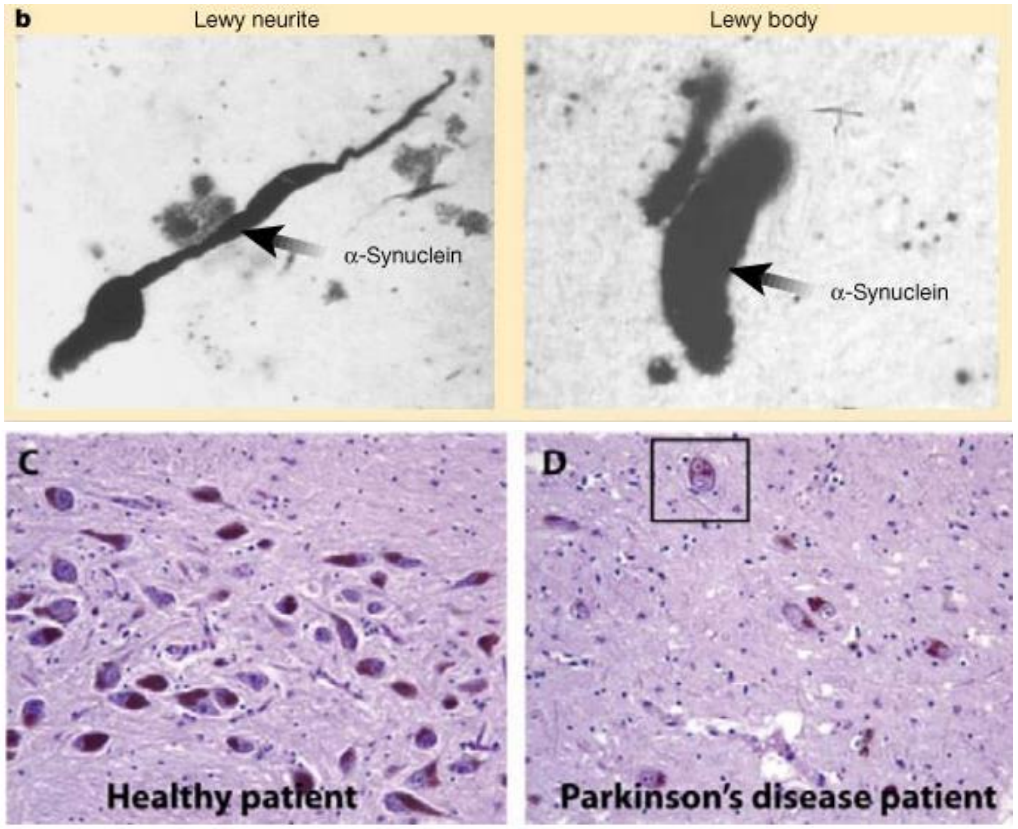
The list is ordered by the year of publication of the main results of the studies.
Aβ = amyloid-β; AD = Alzheimer disease; LOE = lack of efficacy; MAb = monoclonal antibody; MCI = mild cognitive impairment; PAb = polyclonal antibody; TOX = toxicity.

Till date, there are no Aβ-targeted therapeutic options for AD patients

Panza F et al., *Annals. Neurology* 2019

Parkinson disease





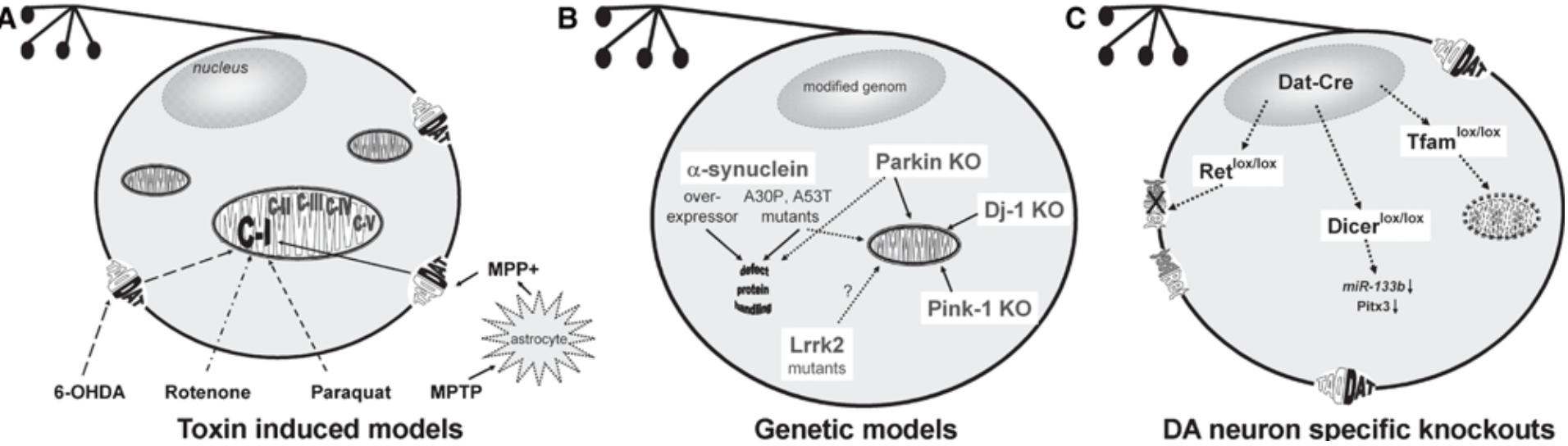
Dauer W & Przedborski S *Neuron* 2003
Haas C & Kahle PJ *Nature*. 2000

Parkinson disease

- Second most common neurodegenerative disorder
- Pathological features in the brain:
  - loss of dopaminergic neurons in the substantia nigra and locus coeruleus
  - formation of filamentous intraneuronal inclusions (Lewy bodies)
- Behavioral features:
  - classical motor signs of
    - bradykinesia (slowness of movement),
    - rigidity, and
    - resting tremor (shaking hands or legs).

Haas C & Kahle PJ *Nature*. 2000

Parkinson's disease animal models



Terzioglu M & Galter D *FEBS J*. 2008

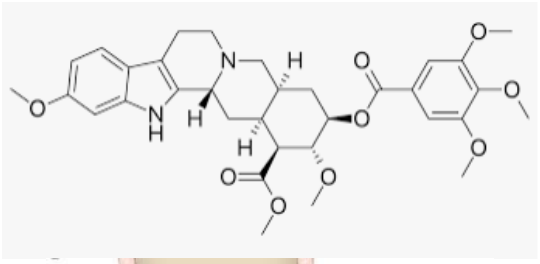
Toxin induced animal models of Parkinson's disease

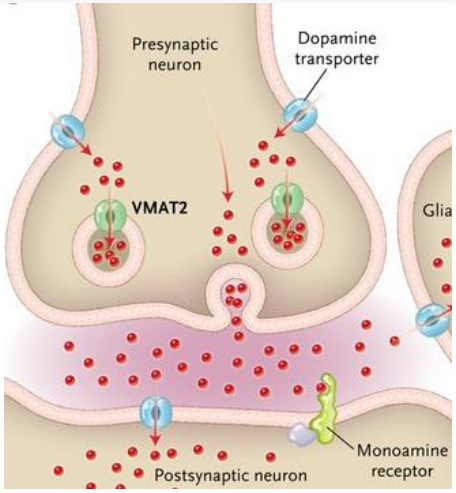
- Reserpine
- MPTP
- 6-OHDA (6-hydroxydopamine)
- Paraquat (herbicide)
- Retenone (insecticide)
- Epoxomicin (proteasomal inhibitor)

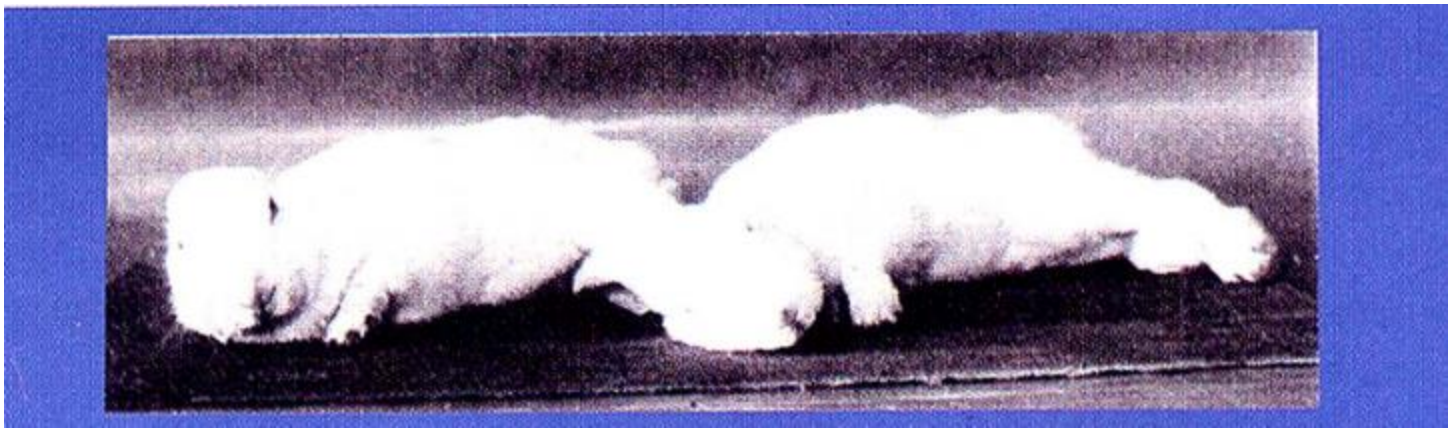
Terzioglu M & Galter D *FEBS J*. 2008

Reserpine model of Parkinson's disease

- Reserpine is an indole alkaloid
- Inhibitor of monoamine transporter VMAT2
- Rabbits injected with reserpine exhibit motor deficits

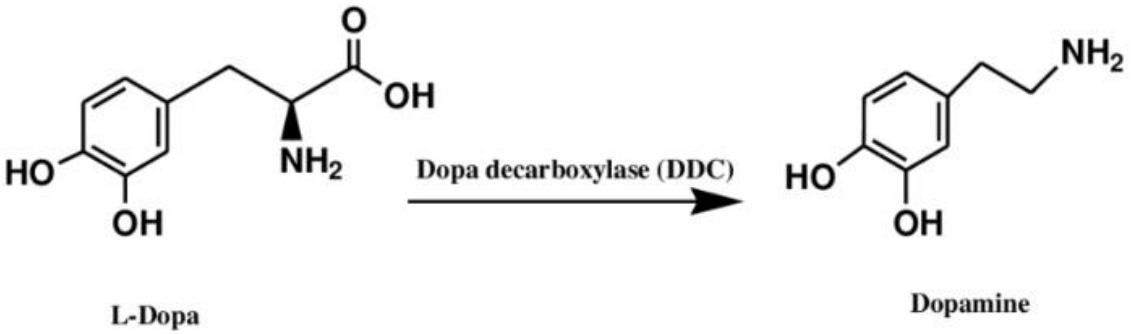






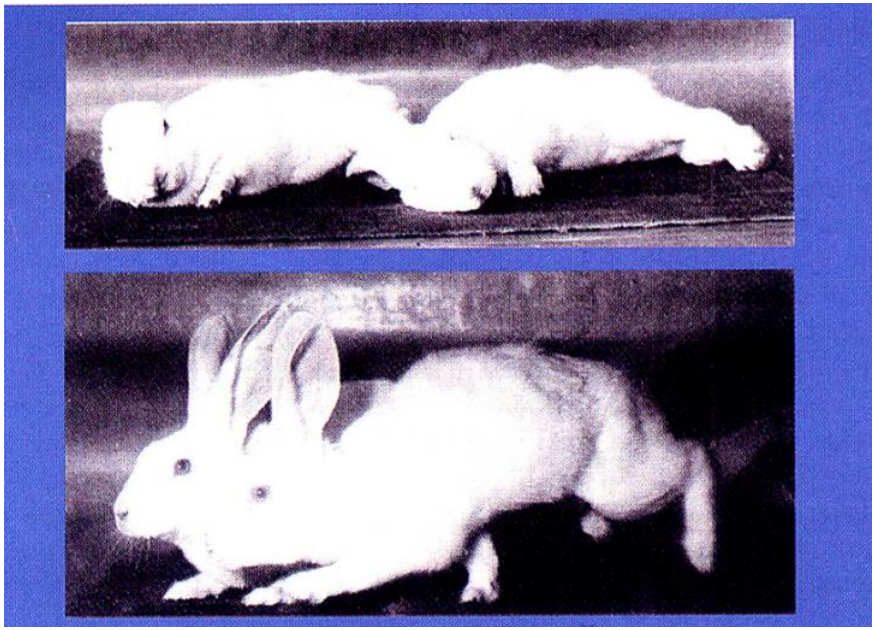
Carlsson A, Nobel Lecture

Reserpine model of Parkinson's disease



L-Dopa

Dopamine



L-dopa injection restores motor activities

Carlsson , Nobel Lecture

The Nobel Prize in Physiology or Medicine 2000

Photo from the Nobel Foundation archive.

Arvid Carlsson

Prize share: 1/3

Photo from the Nobel Foundation archive.

Paul Greengard

Prize share: 1/3

Photo from the Nobel Foundation archive.

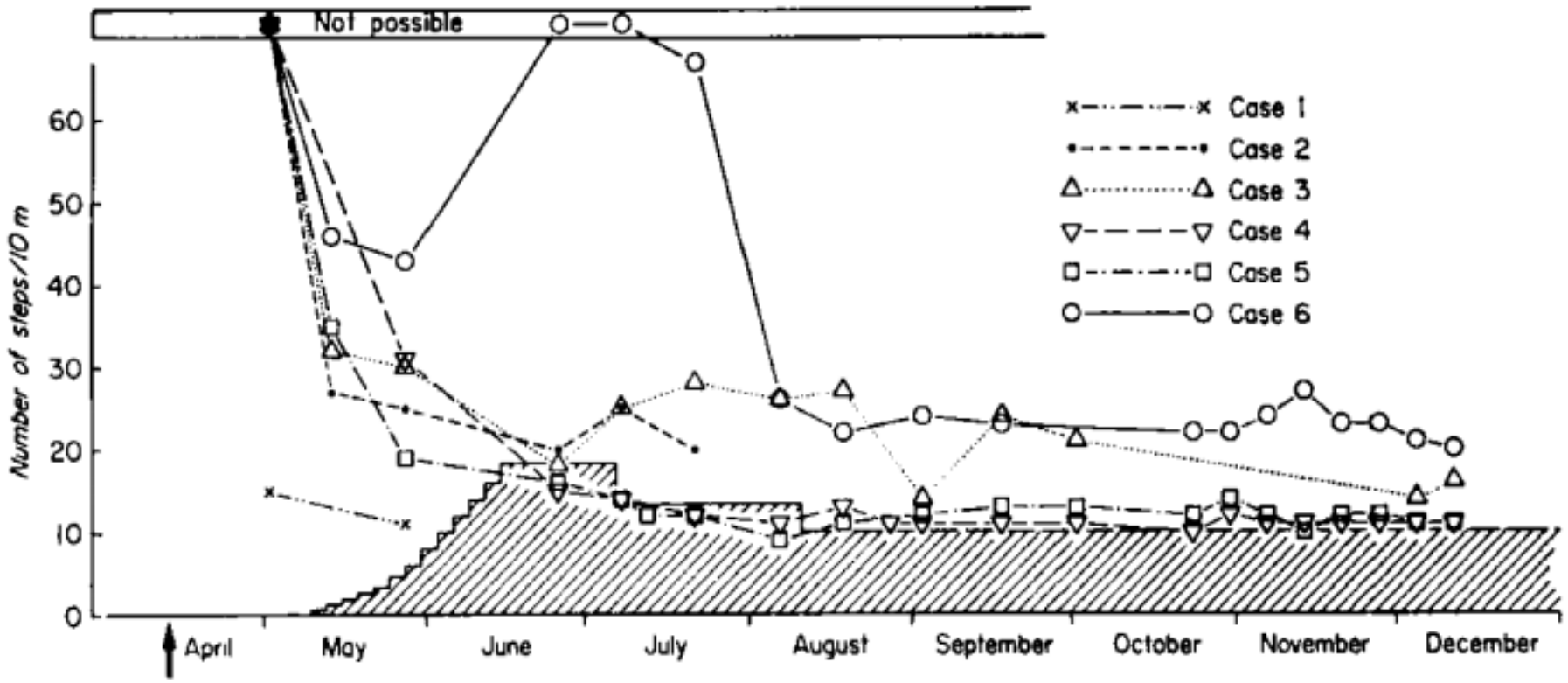
Eric R. Kandel

Prize share: 1/3

"for their discoveries concerning signal transduction in the nervous system"

From animal research to clinical trials in humans

Patients administered with L-dopa



Till date, L-dopa is prescribed to Parkinson patients to treat motor symptoms.

Anden NE et al., *Acta med Scand* 1970

Mystery of ‘New Heroin’ and Parkinson’s disease

Madison.com
Kolata G *Science* 1983

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MPTP - Monkey model of Parkinson's disease

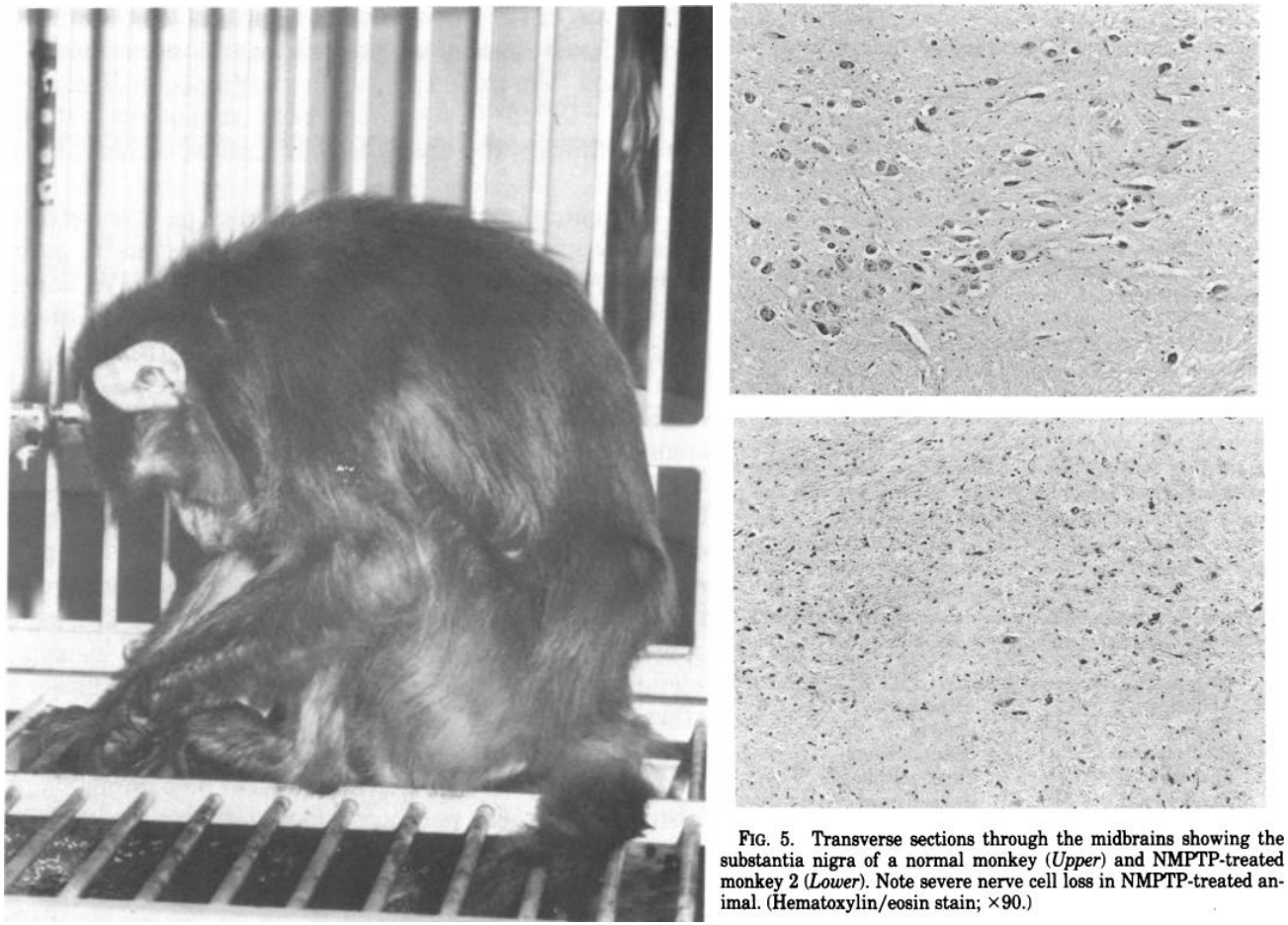


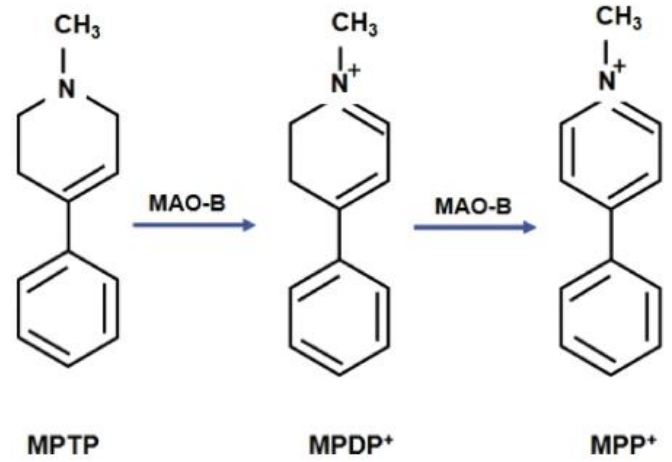
FIG. 5. Transverse sections through the midbrains showing the substantia nigra of a normal monkey (*Upper*) and NMPTP-treated monkey 2 (*Lower*). Note severe nerve cell loss in NMPTP-treated animal. (Hematoxylin/eosin stain; ×90.)

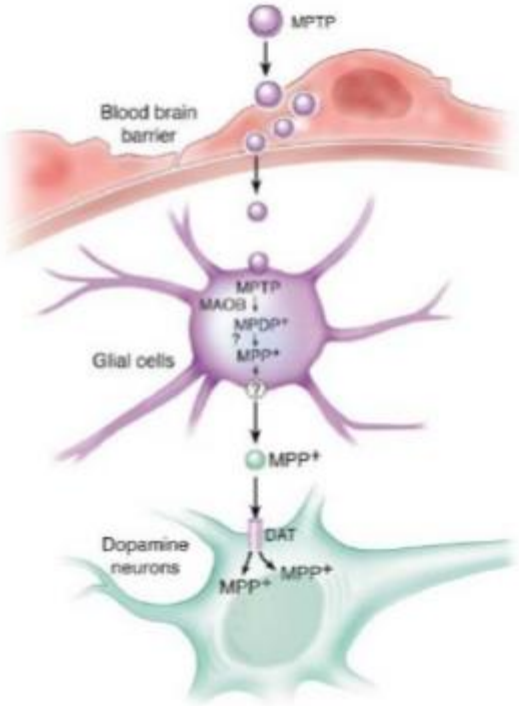
Rhesus monkey injected with NMPTP

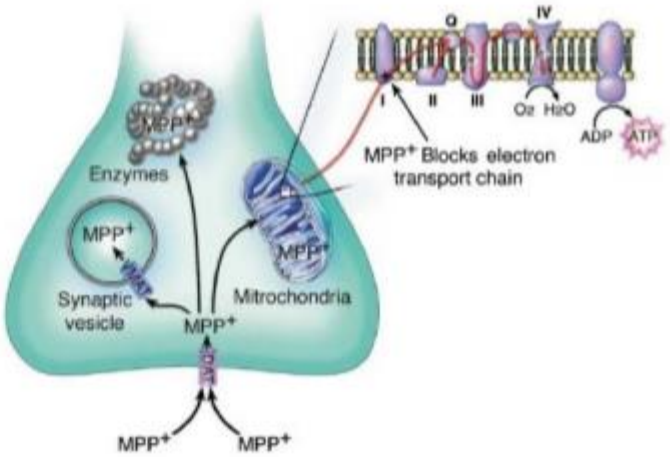
- exhibit parkinsonism disorder features
- Lose neurons in the pars compacta of the substantia nigra

Burns RS et al., *PNAS* 1983

MPTP

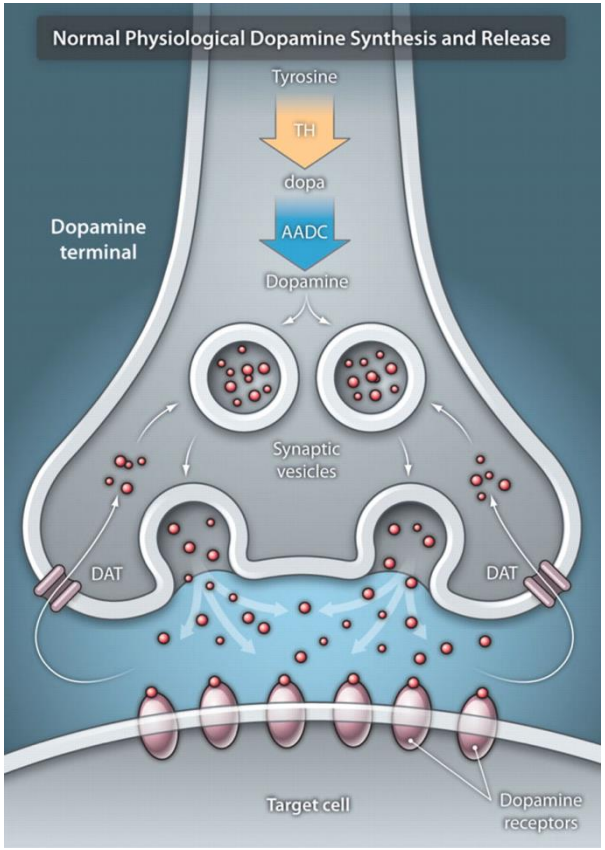


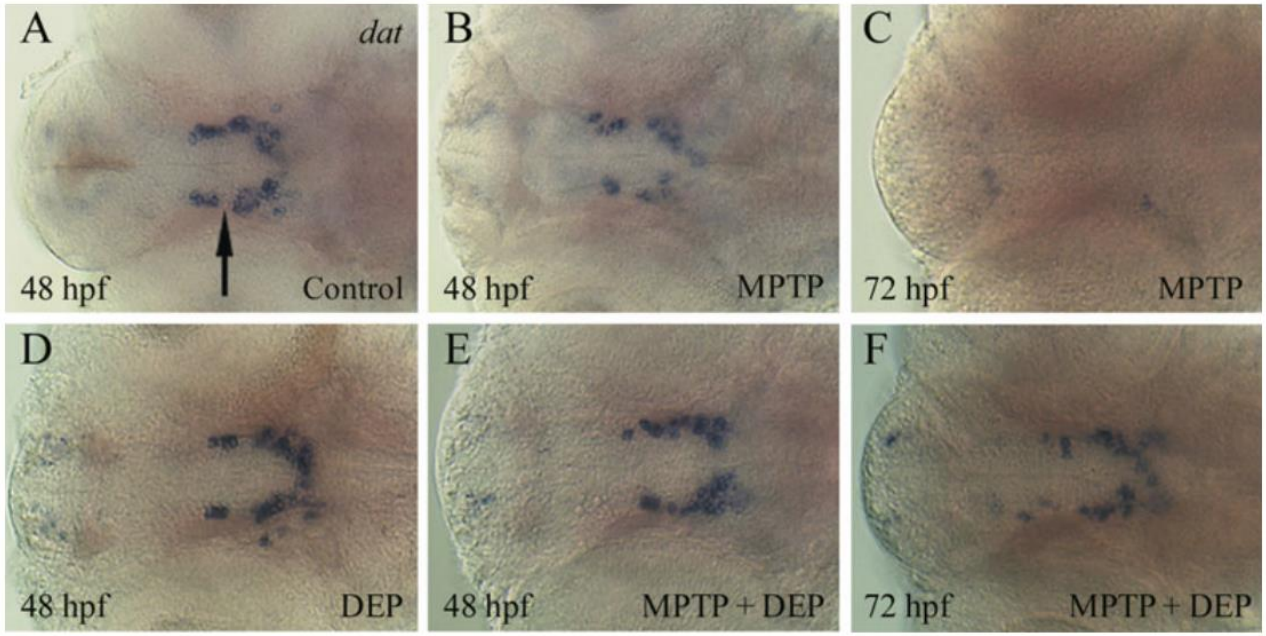




Heikkila R et al., *Science*. 1984

MPTP Zebrafish model of Parkinson's disease

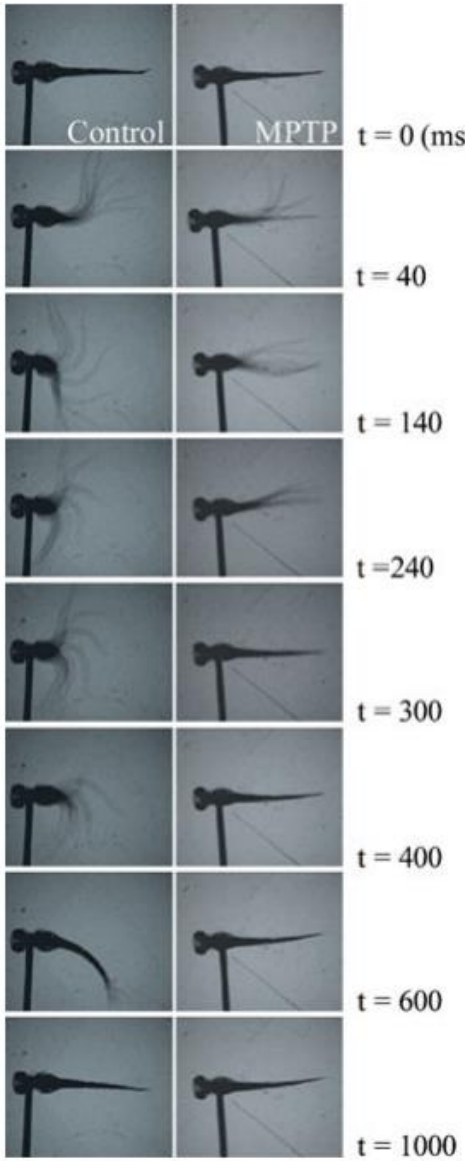




- Zebrafish larvae treated with MPTP lose dopamine transporter *dat* mRNA expression
- The application of deprenyl (DEP) neutralizes the toxicity of MPTP and rescued DAT-positive cells

Björklund A et al., *Sci. Transl. Med.* 2009
Lam CS et al., *E.J,Neurosci*. 2005

MPTP Zebrafish model of Parkinson's disease



MPTP treated larvae exhibit impaired swimming behavior in response to touch stimuli

Lam CS et al., *E.J,Neurosci*. 2005

Genetic model of Parkinson's disease

α-Synuclein Models

Mutations in the α-synuclein gene have been implicated in several cases of familial Parkinson's disease. While most PD patients do not possess these mutations, α-synuclein overexpression models can nonetheless be useful for investigating PD therapies. These models may use the mutations identified in familial PD or a transgene that causes overexpression of full-length α-synuclein or a truncated version.

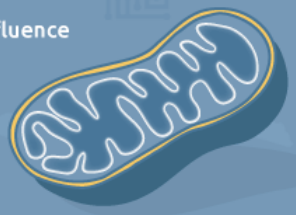
While most cases of human Parkinson's Disease have no known genetic origin, around 10% of PD cases are the result of a genetic mutation. These mutations, which have been localized to 14 different genes, provide insights for creating genetic models of PD.

LRRK2

Dominant mutations in leucine rich repeat kinase 2 (LRRK2) have been observed in familial PD, and the gene also plays a role in sporadic Parkinson's disease. LRRK2 knockout or overexpressing mice do not show gross neurodegeneration, but they do display α-synuclein accumulation due to deficits in Golgi complex function and microtubule-based transport.

PINK1 and DJ1

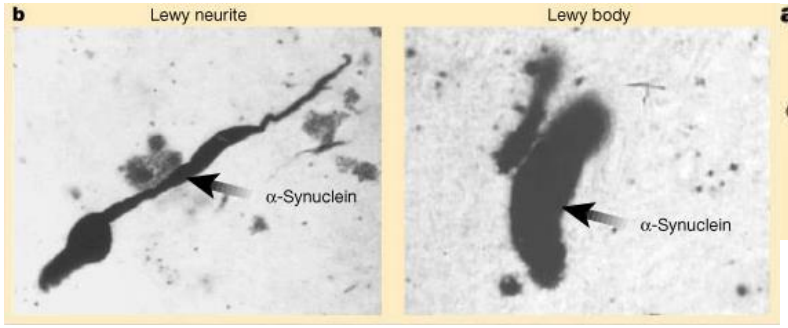
PINK1 and DJ1 knockout mice do not recapitulate PD pathology without additional insults. However, these animals show increased vulnerability to mitochondrial toxins. This could make them useful for studying environmental factors that influence familial PD.

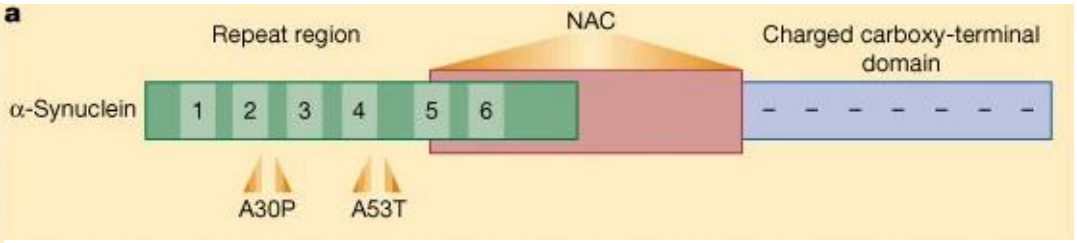


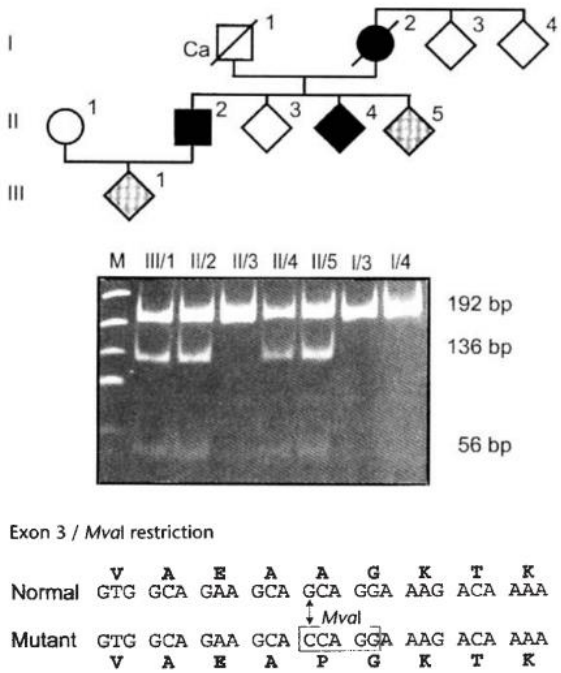
PARKIN

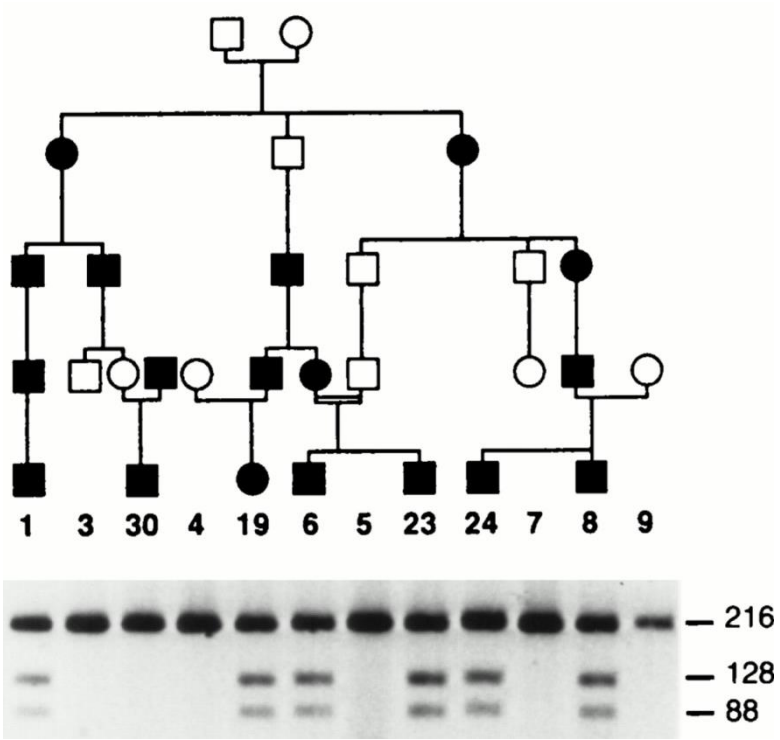
PARKIN knockout mice have mild motor deficits that progress with age, as well as reduced dopamine release, α-synuclein pathology, and increased oxidative stress.

Mutations in α-synuclein gene in hereditary Parkinson's disease





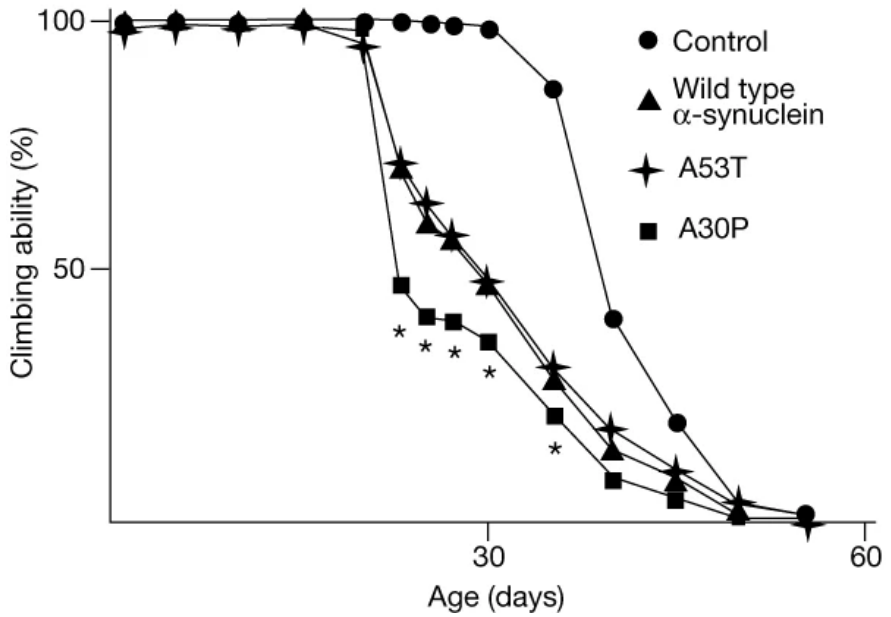




Polymeropoulos, M. H et al., *Science* 1997
Krüger R et al., *Nat Genet*. 1998

α-synuclein based drosophila model of Parkinson's disease

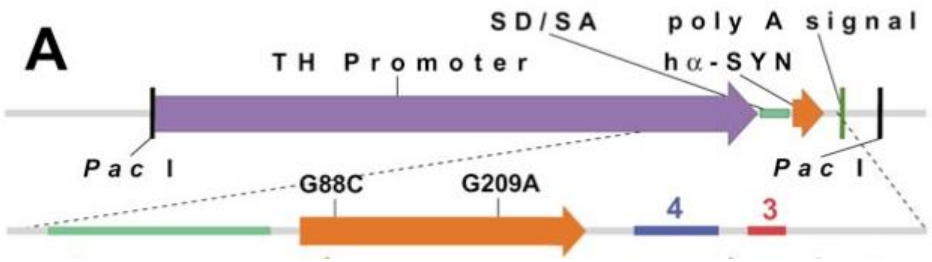
elav–GAL4/+.
elav/+; *UAS–wild-type α -synuclein*/+;
UAS–A30P α-synuclein/*elav*–GAL4;
UAS–A53T α-synuclein/*elav*–GAL4.

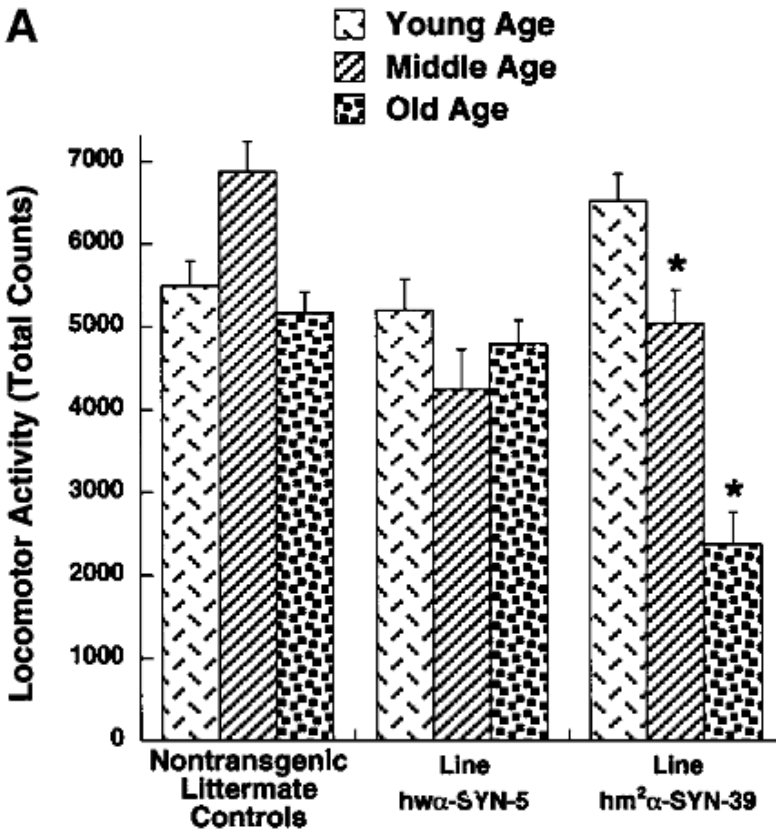


Flies expressing mutated α-synuclein exhibit defective age-associated motor features (climbing ability)

Feany MB & Bender WW *Nature*. 2000

α-synuclein based mouse model of Parkinson's disease

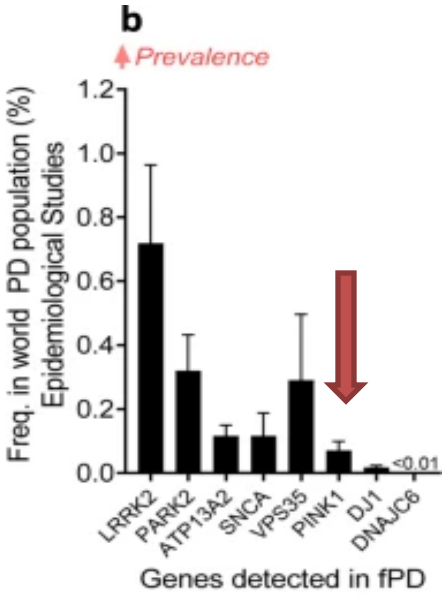


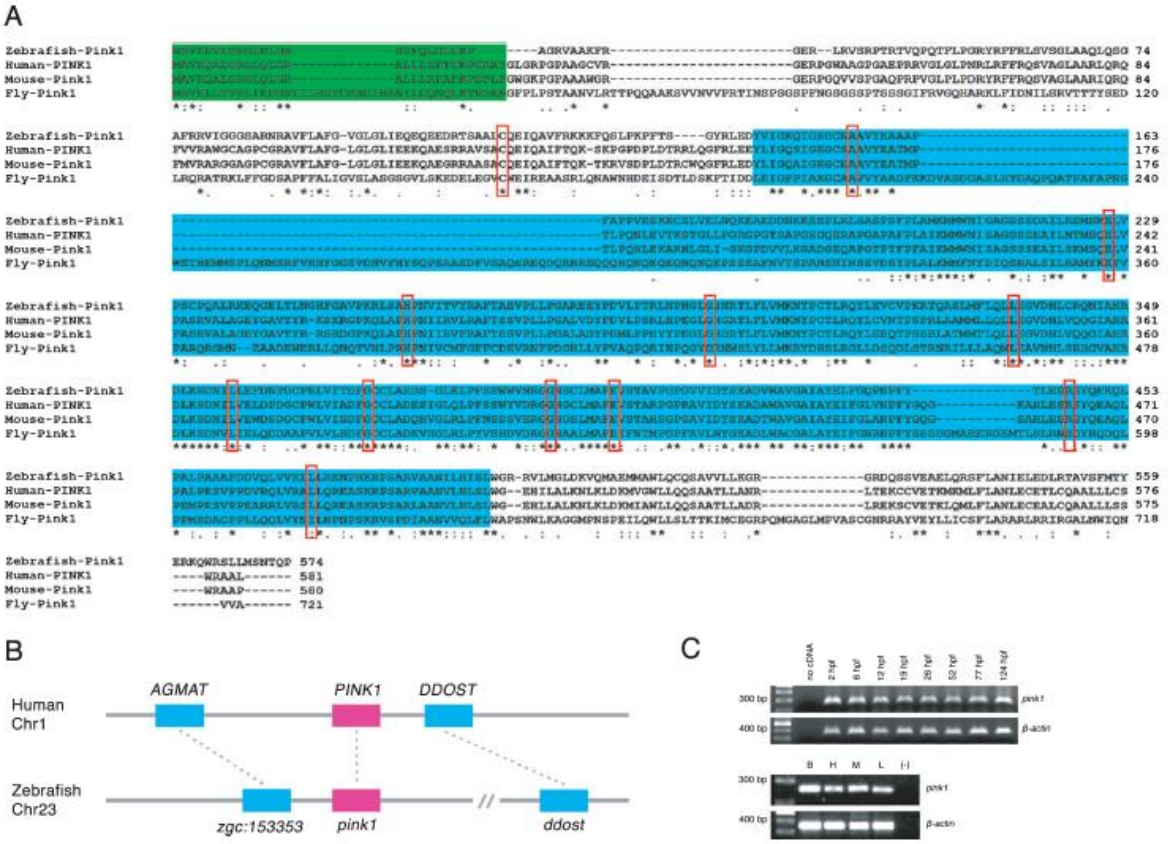


Mice expressing mutated α-synuclein exhibit age-associated defective motor features (locomotor activity)

Richfield E et al., *Expt*. *Neurol.* 2002

PINK1/Hereditary Early-onset Parkinson's disease



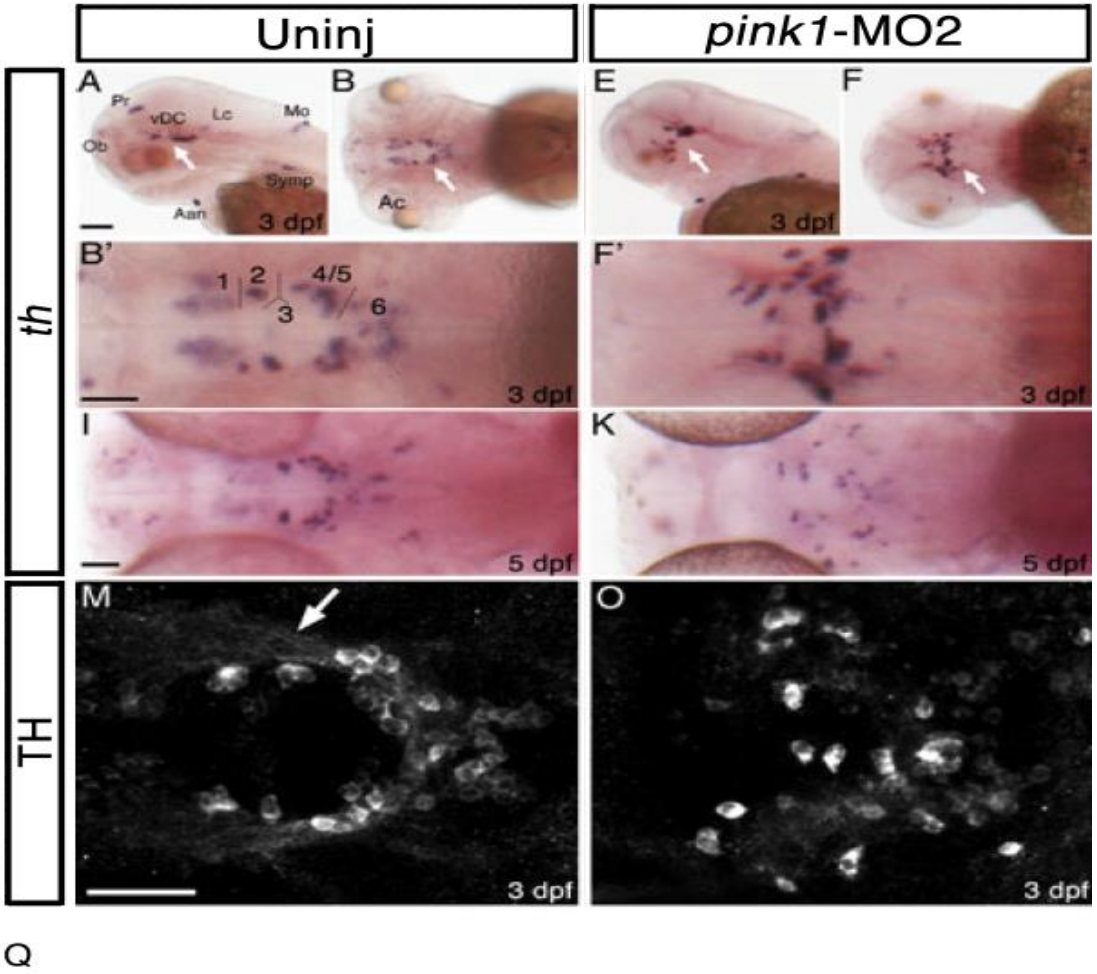


PINK1 gene is evolutionarily conserved

Tran J *et al.,* NPJ 2020

Xi Y *et al.,* Eur. J. Neurosci. 2010

Zebrafish Pink1 model of Parkinson's disease



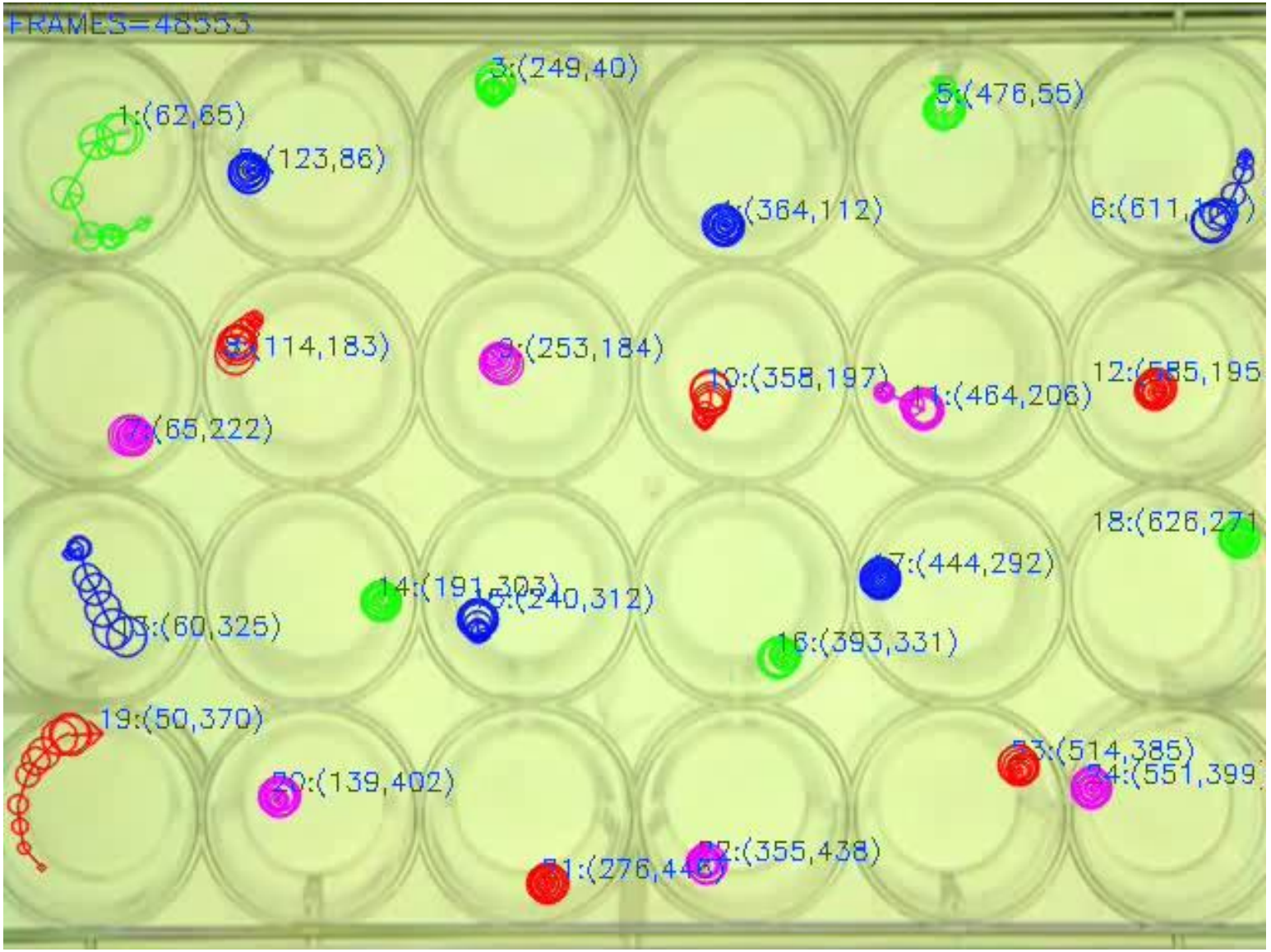
pink1 knockdown **changes tyrosine hydroxylase (TH) neuron organization**

Xi Y *et al., Eur. J. Neurosci.* 2010

Tracking zebrafish swimming behaviour

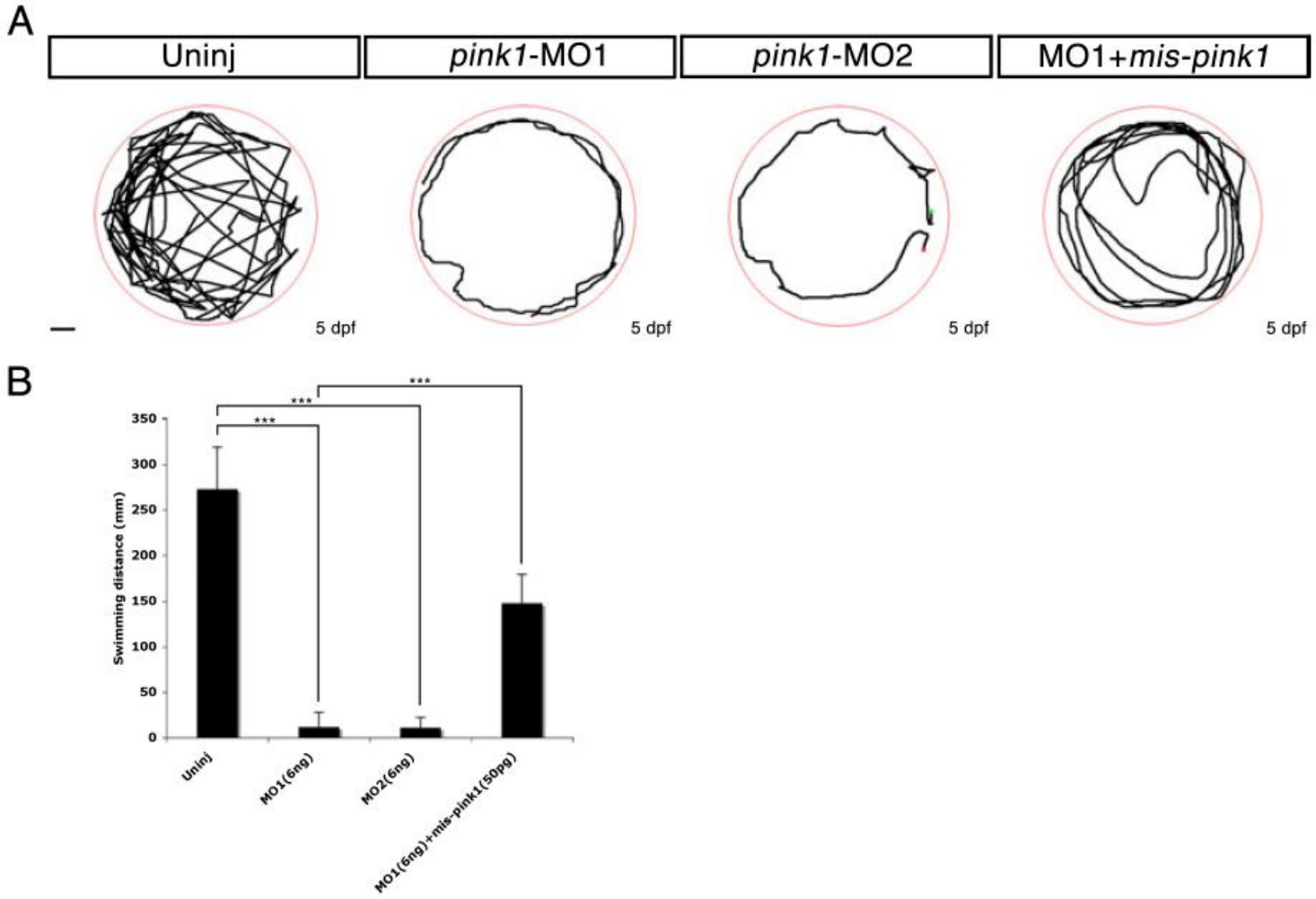
https://www.youtube.com/watch?v=MnGWPK7-Odg&

Tracking zebrafish swimming behaviour



https://www.youtube.com/watch?v=Gi7FiAXWTdY&

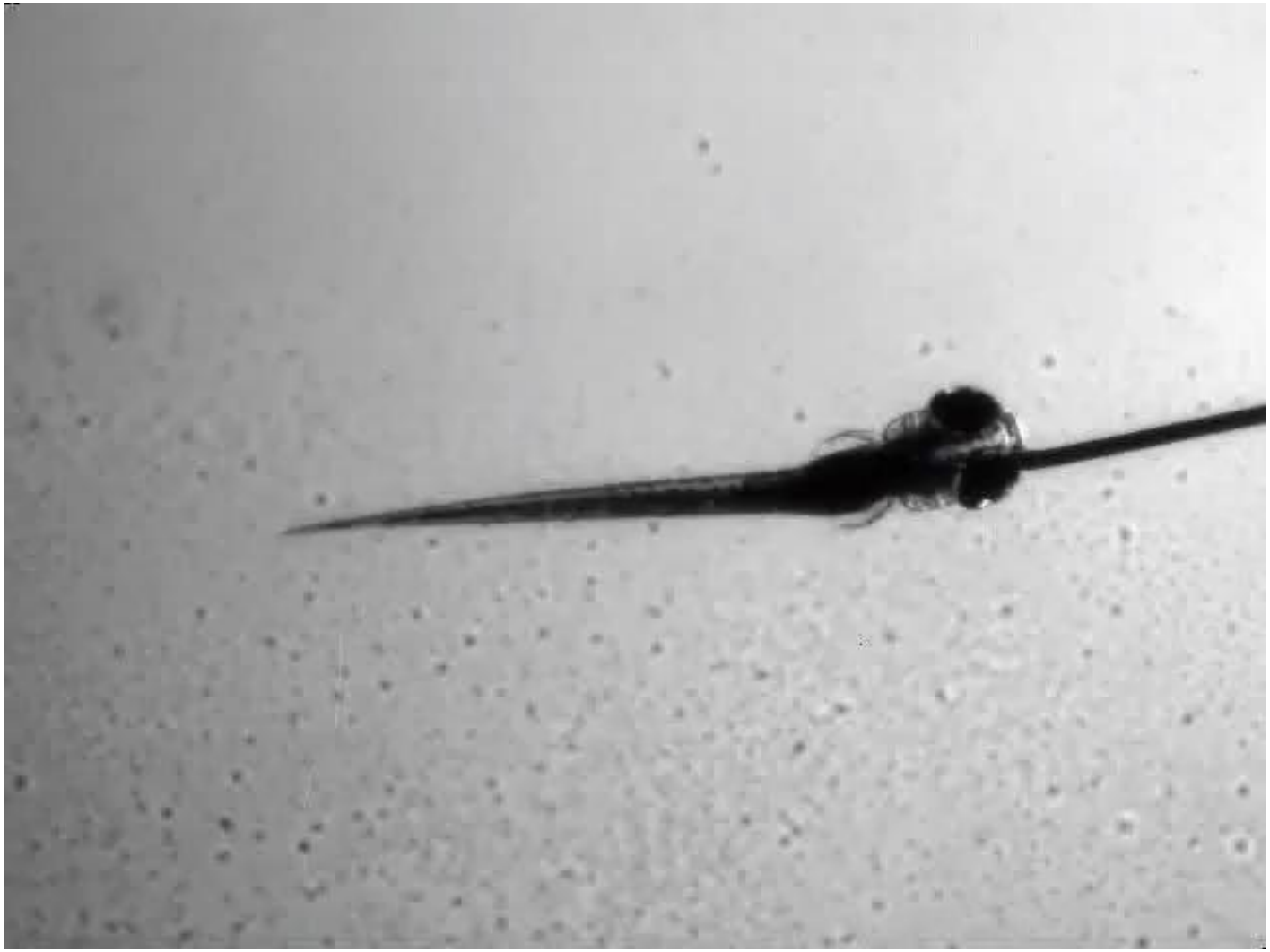
Zebrafish Pink1 model of Parkinson's disease



***pink1* knockdown impairs motor feature (swimming ability)**

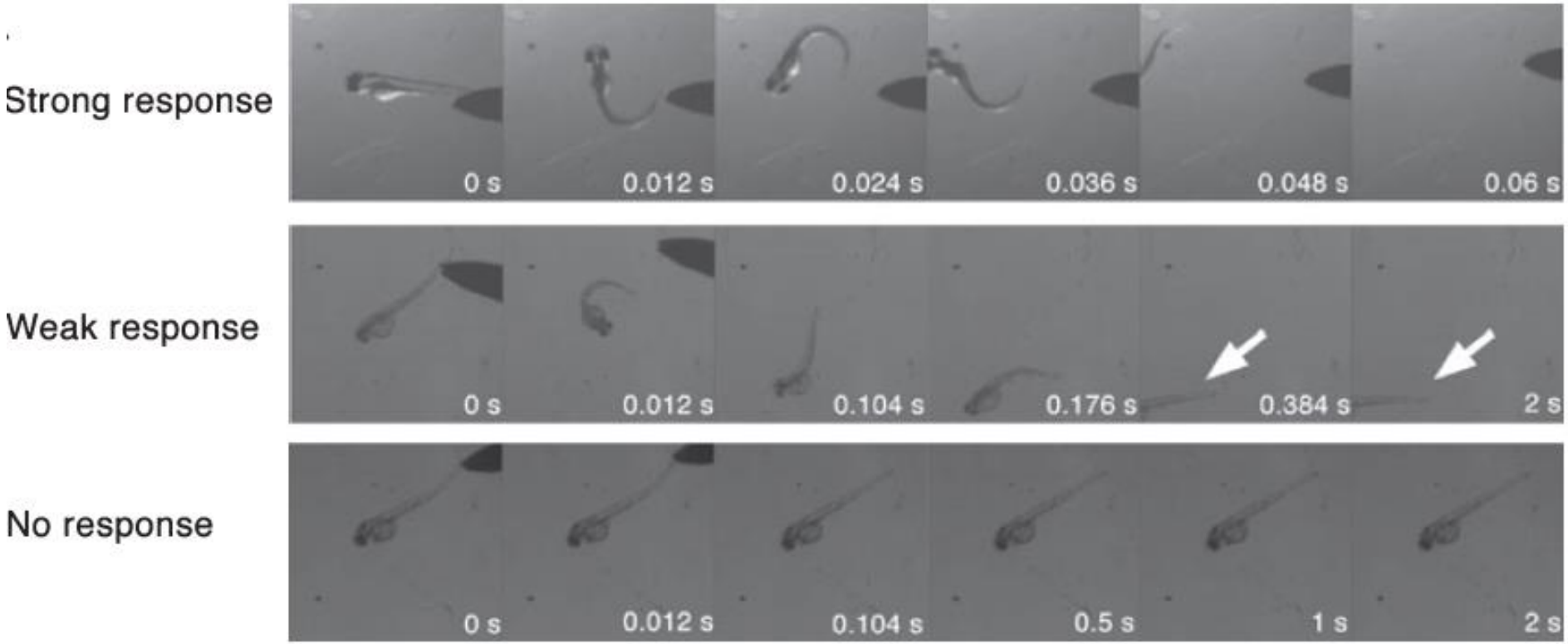
Xi Y *et al., Eur. J. Neurosci.* 2010

Tracking zebrafish touch response



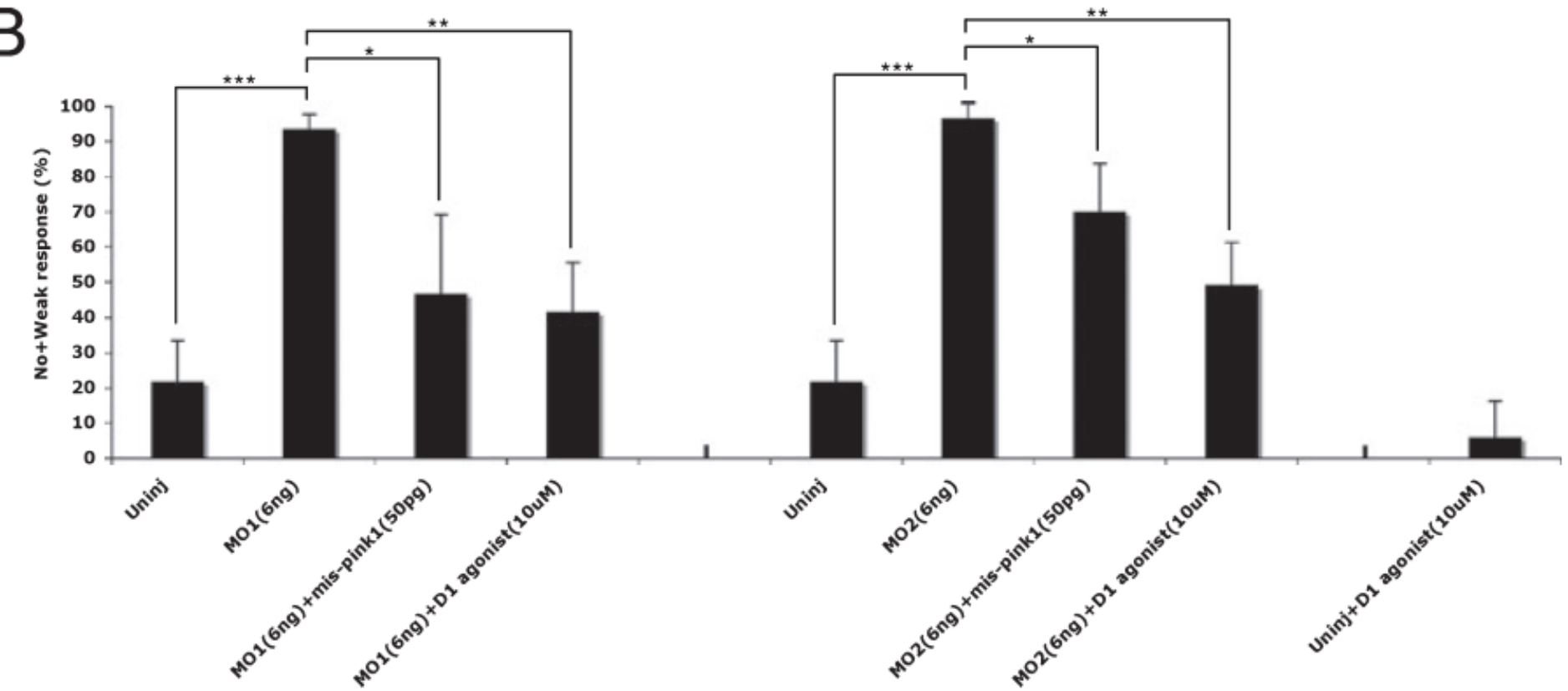
https://www.youtube.com/watch?v=MnGWPK7-Odg&

Tracking zebrafish touch response



https://www.youtube.com/watch?v=MnGWPK7-Odg&

Zebrafish Pink1 model of Parkinson's disease



***pink1* knockdown impairs touch response**

Xi Y *et al., Eur. J. Neurosci*. 2010

Summary

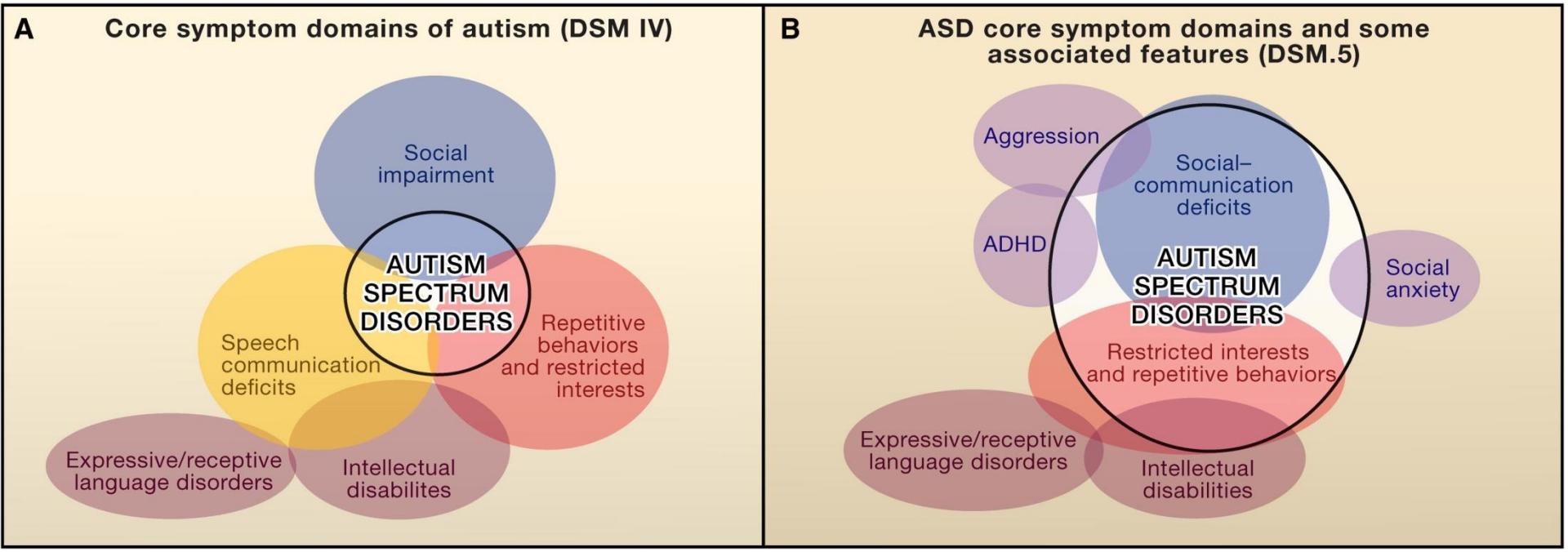
Animal models can be used

- to study Parkinson's disease-like phenotypes
- to test the effect of toxins
- to test the role of a specific gene implicated in the disease
- to test the effectiveness of therapeutic options

Neurodevelopmental disorder

- Group of disorders in which the development of the central nervous system is disturbed.
- And manifest as
  - neuropsychiatric problems or
  - impaired motor function or
  - Impaired learning, language or non-verbal communication.
- Examples:
  - Down syndrome, Fragile-X syndrome
  - Autism Spectrum Disorders

Autism Spectrum Disorders



Lord C *Cell*. 2011

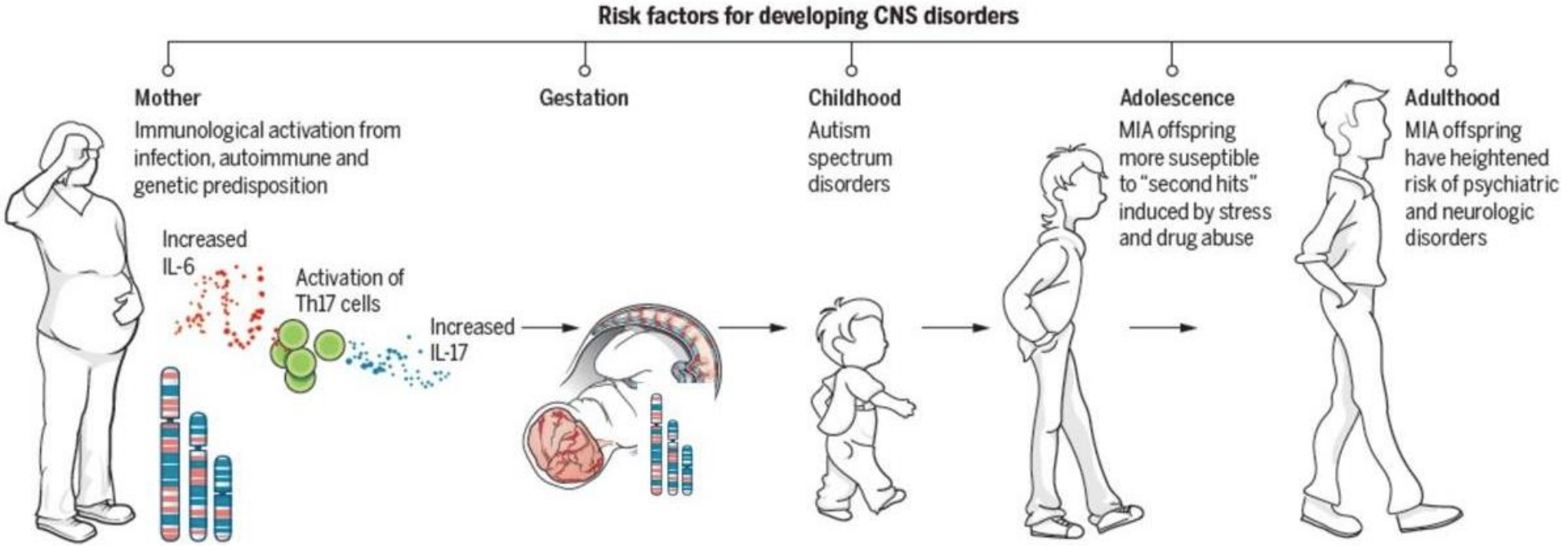
Autism Spectrum Disorders

- Clinically heterogeneous class of neurodevelopmental disorders
- Genetic basis and environmental basis
- Chracterized by
  - dysfunctional reciprocal social interaction
  - delayed or absent speech or difficulties initiating or sustaining a conversation (language delay)
  - abnormal preoccupations, inflexible adherence to routines or rituals, or repetitive motor behaviours

Types of ASD animal models

- Environmental model:
  - Maternal immune activation (MIA) model
- Genetic model:
  - gene-based animal model or etiological model
- *“there are no generally accepted animal models of autism”*

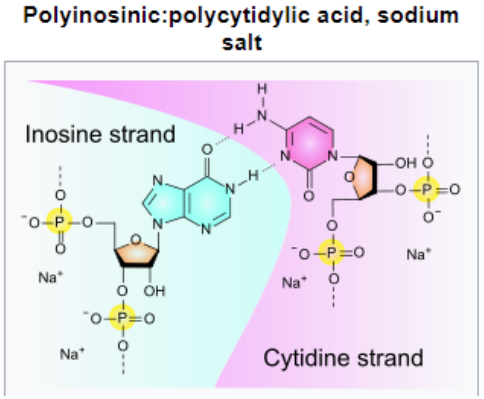
Maternal inflammation in neurodevelopmental disorders

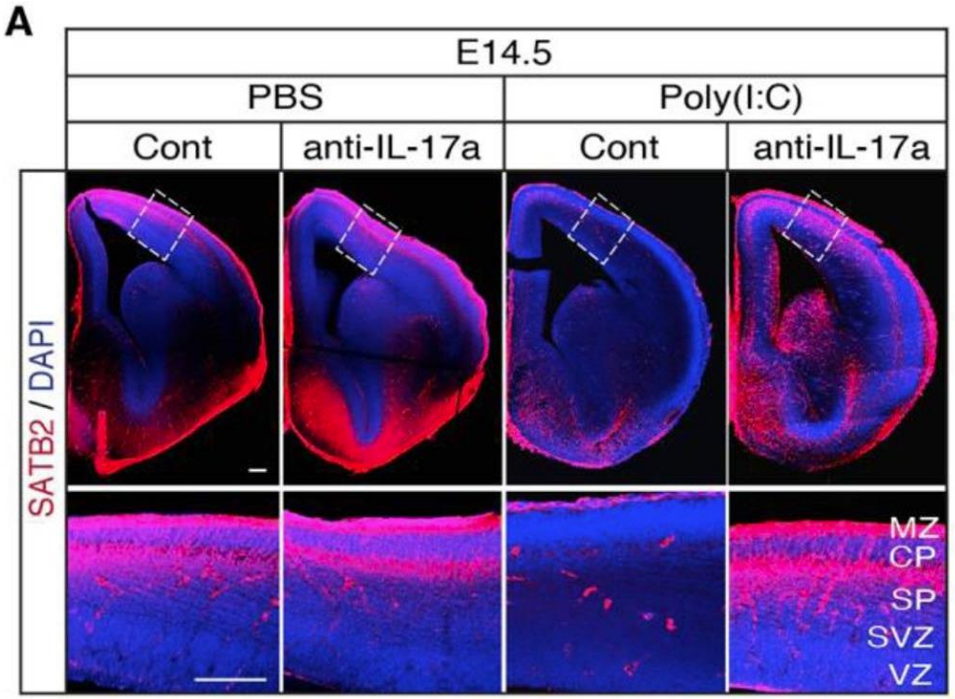


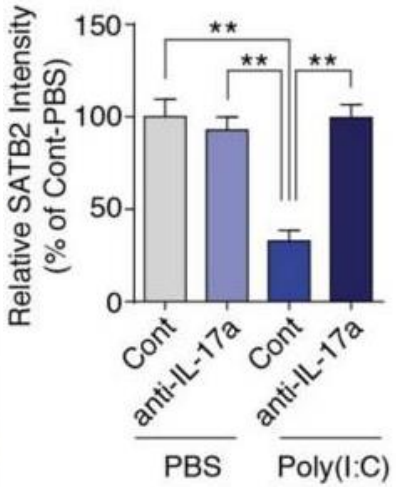
Although controversial, maternal inflammation is linked with development of some neurodevelopmental disorders (ASD, schizophrenia)

Estes ML & McAllister AK *Science*. 2017

Maternal Immune Activation (MIA) model of ASD



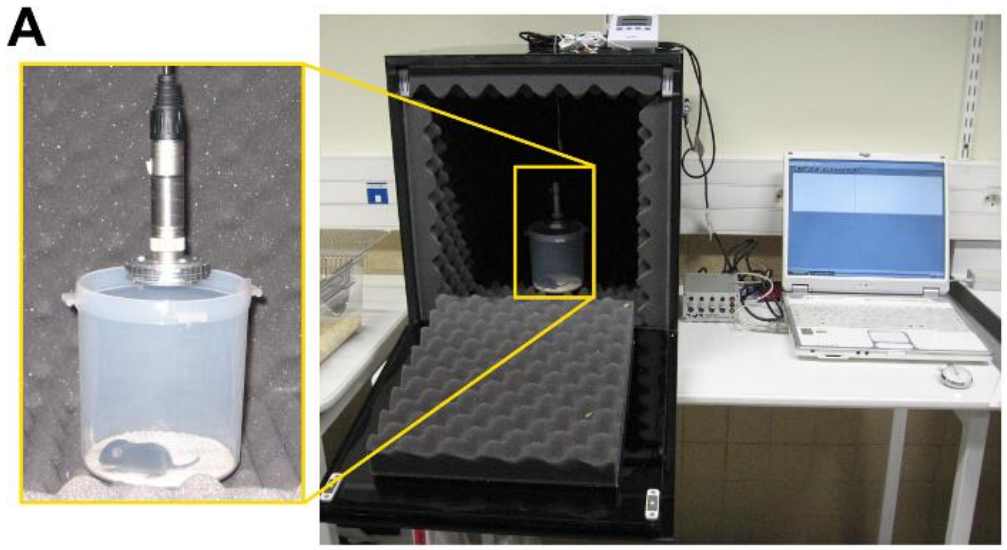


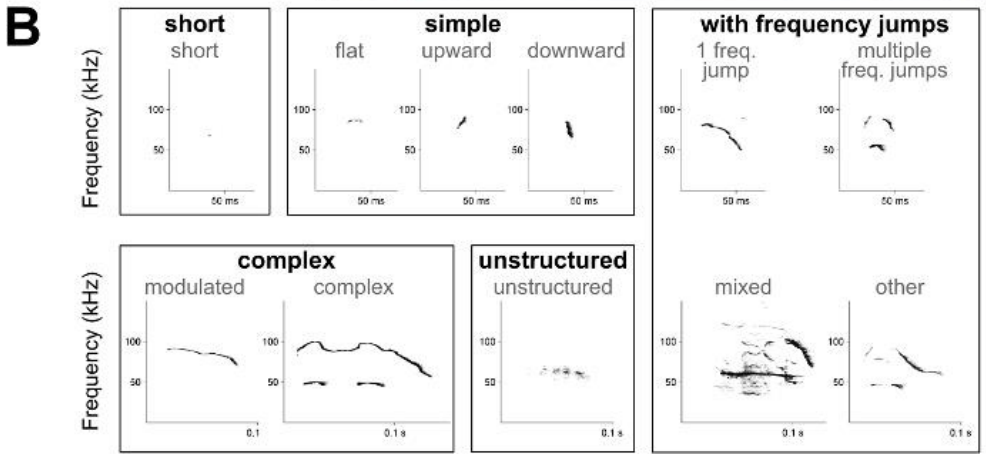


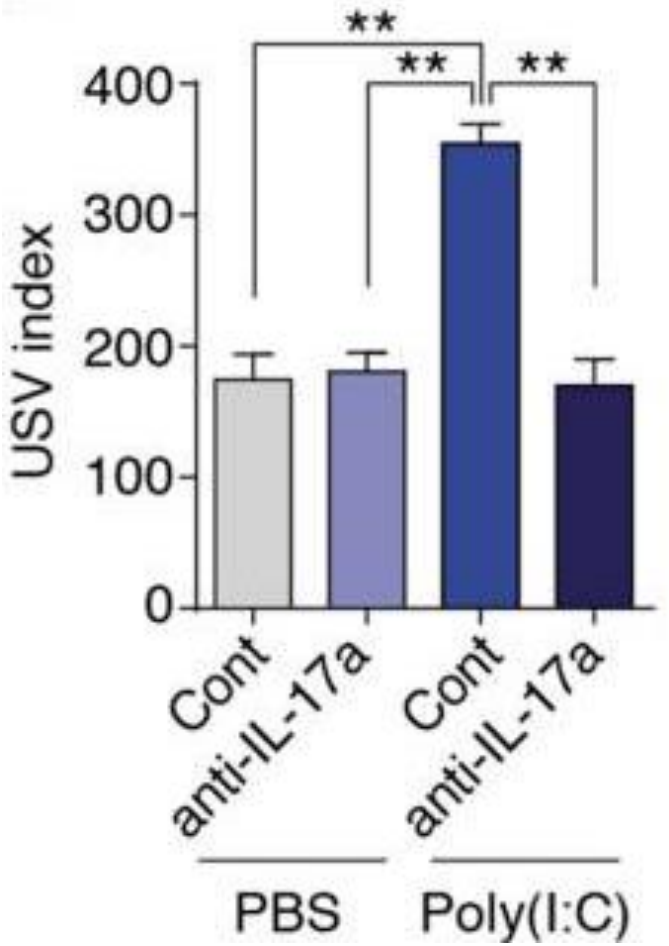
- Maternal poly(I:C) administration affect fetal cortical neurons
- Anti-IL-17a inject can rescue this defect

Choi GB et al., *Science*. 2016

Maternal inflammation and autism risk







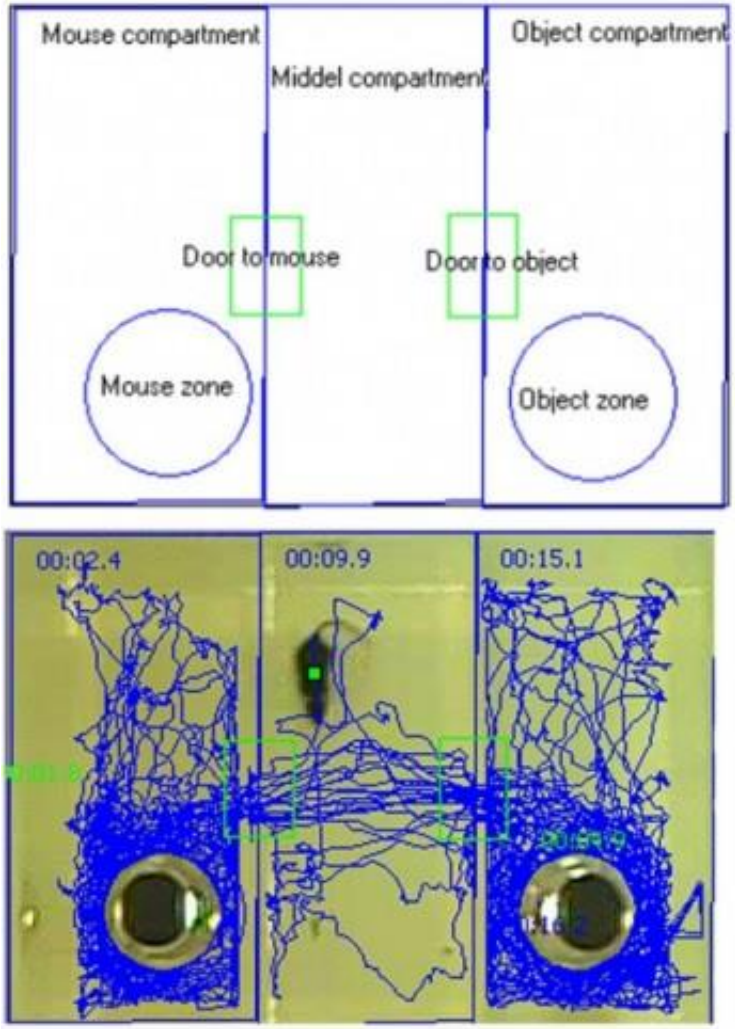
Maternal poly(I:C) administration induces abnormal ultrasound vocalizations in pups

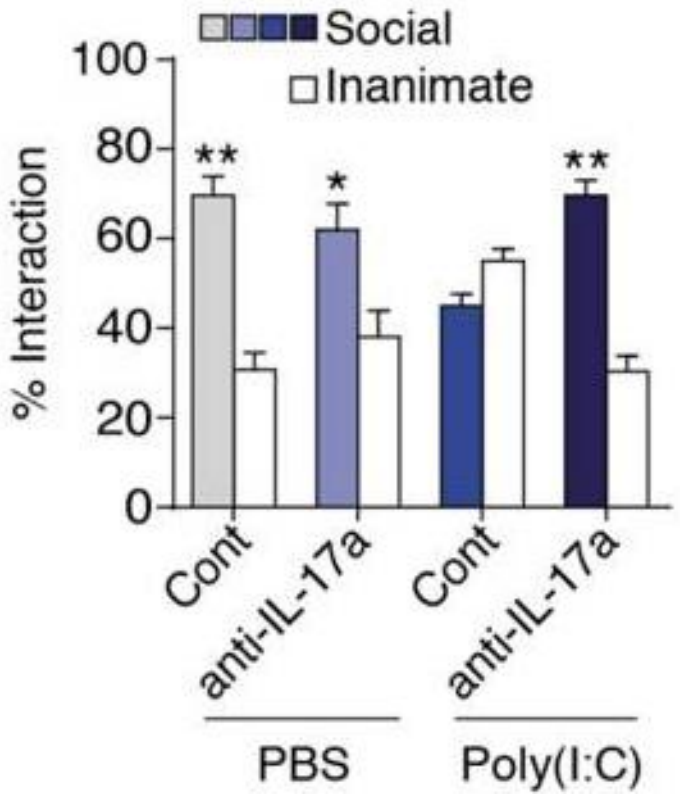
Ferhat AT etl a., *JOVE* 2016
Choi GB et al., *Science*. 2016

Social behaviour tests in mouse models

https://www.youtube.com/watch?v=Ys-HThuruyw&

Maternal inflammation and autism risk



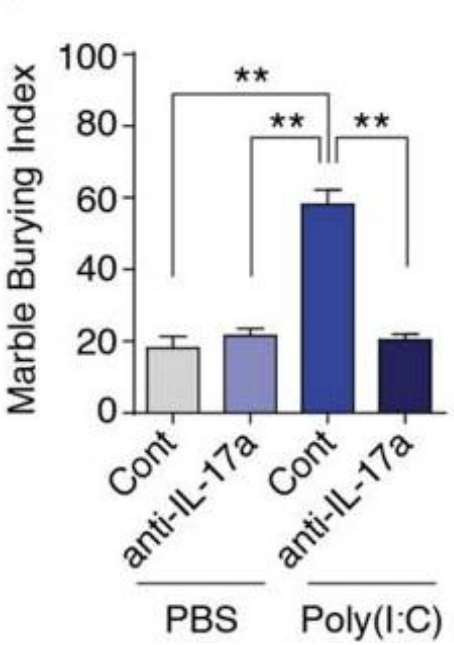


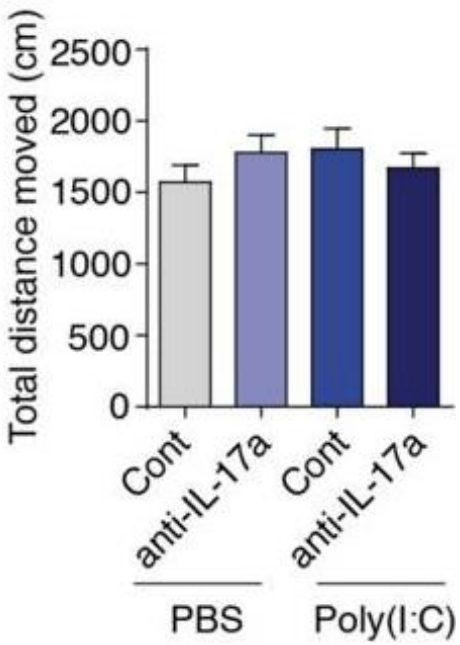
Maternal poly(I:C) administration induces social deficits in adult offsprings

Sylics.com
Choi GB et al., *Science*. 2016

Maternal inflammation and autism risk

The marble burying test is used to measure repetitive and anxiety-related behaviour in rodents.





Maternal poly(I:C) administration enhances marble burying in in adult offsprings

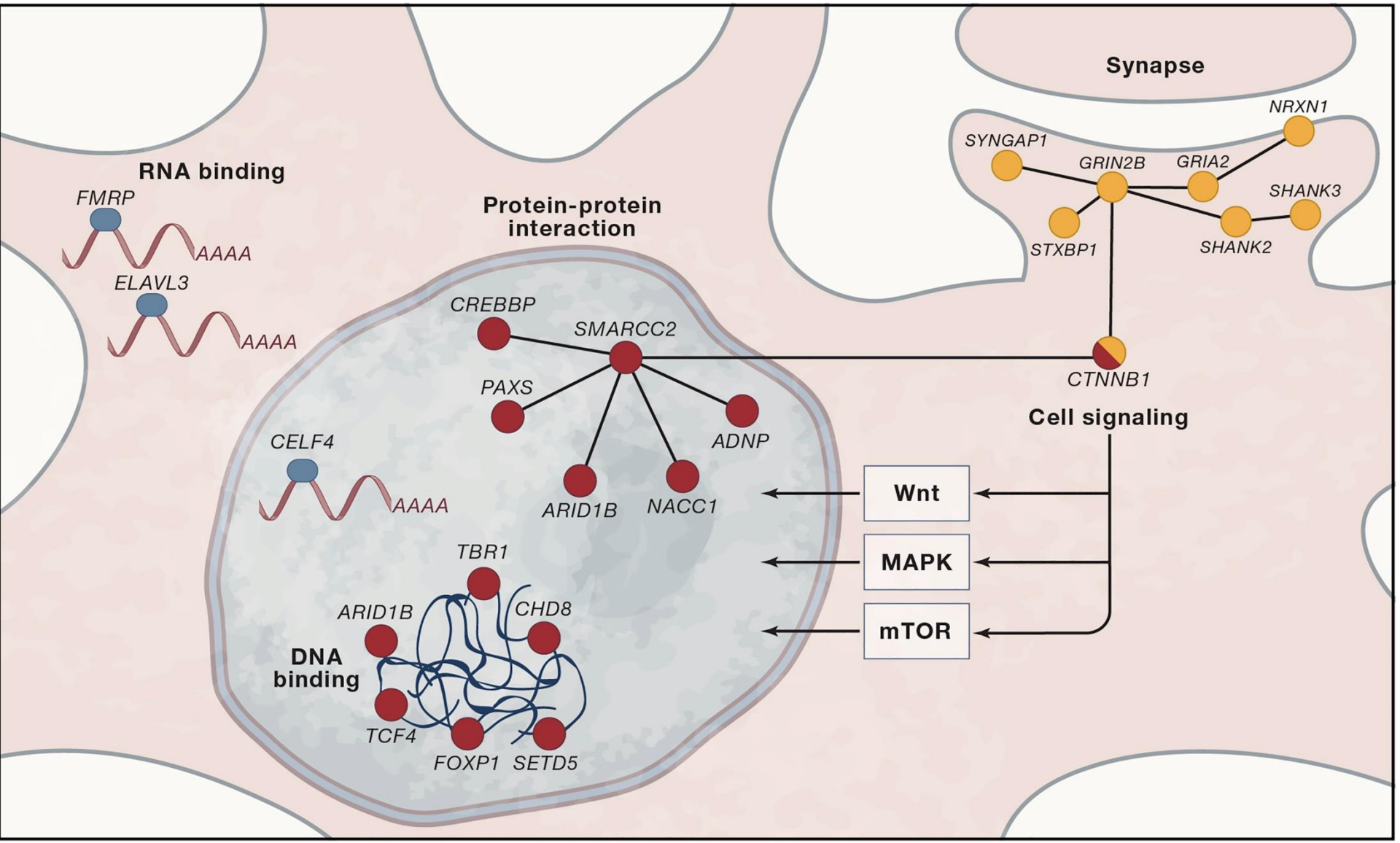
Choi GB et al., *Science*. 2016

Summary

Animal models can be used

- to study effect of maternal inflammation in off-springs
- to test the effect of specific inflammatory agents
- to test the role of a specific inflammatory protein
- to test the effectiveness of therapeutic options

Gene-based ASD animal models



Iakoucheva LM et al., *Cell*. 2019

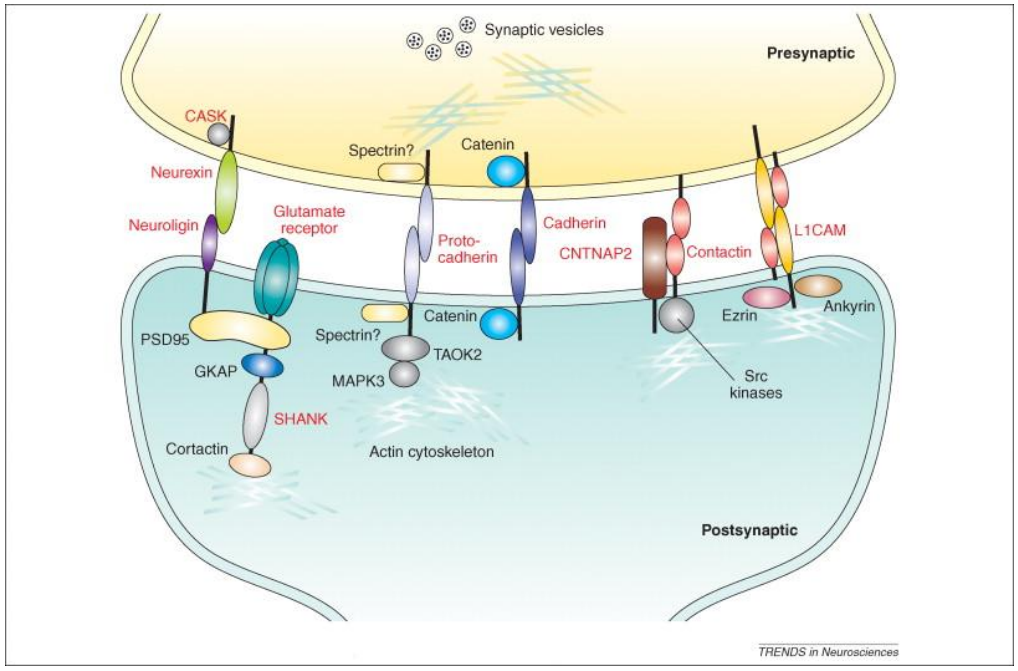
Gene-based mice models of ASD

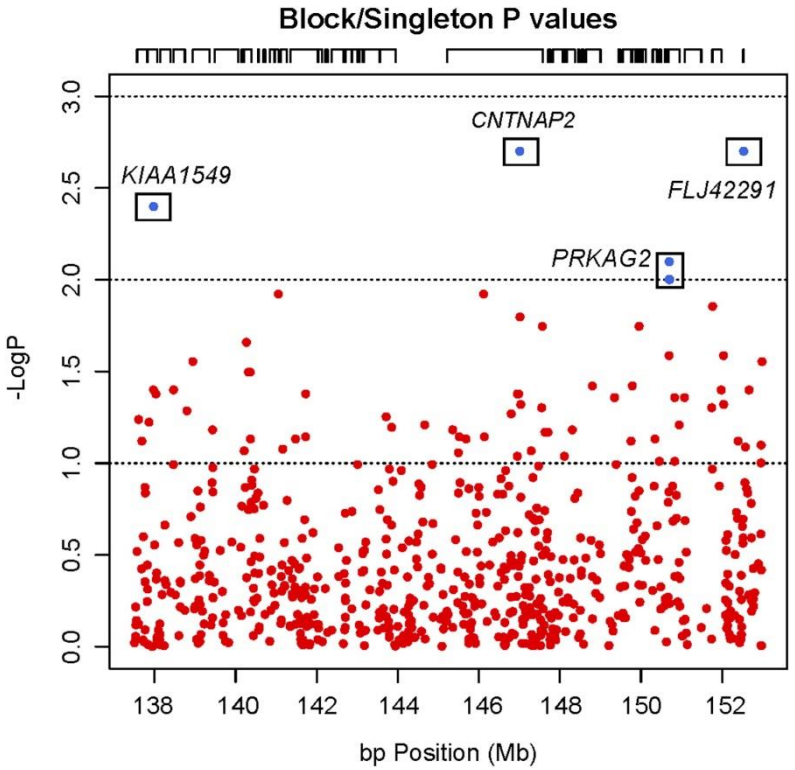
Mouse model	Genetic characteristics	Behavioural phenotypes relevant to the symptoms of autism*
Nlgn4	Null mutation in the murine orthologue of the human *NLGN4* gene[43]	• Reduced reciprocal social interactions[43] • Low sociability[43] • Lack of preference for social novelty[43] • Reduced ultrasonic vocalizations[43]
Nlgn3	Homozygous mutation of humanized R451C mutation of the *Nlgn3* gene[44,45]	• No genotype differences in reciprocal social interactions[44,45] • No genotype differences in sociability[44,45] • No genotype differences in preference for social novelty[44] • Reduced ultrasonic vocalizations[44]
	Null mutation in the murine orthologue of the human *NLGN3* gene[41]	• No genotype differences in reciprocal social interactions[41] • Reduced preference for social novelty[41]
Neurexin 1α	Null mutation in the murine *neurexin 1α* generated by deleting the first exon of the gene[46]	• No genotype differences in reciprocal social interactions[46] • No genotype differences in sociability[46] • Impaired nest-building behaviour[46] • Increased repetitive self-grooming[46]
Nlgn1	Null mutation in the murine orthologue of the human *NLGN1* gene[47]	• No genotype differences in reciprocal social interactions[47] • No genotype differences in sociability[47] • No genotype differences in preference for social novelty[47] • Impaired nest-building behaviour[47]
Pten	Conditional null mutation, inactivated in neurons of the cortex and hippocampus, mouse orthologue of the human *PTEN* gene[68]	• Reduced reciprocal social interactions[68] • Low sociability[68] • Impaired nest-building behaviour[68] • Impaired social recognition[68]
	Pten haploinsufficent mutant line in which exon 5, and thus the core catalytic phosphatase domain, is deleted[48]	• Low sociability in females[48]
En2	Null mutation in the murine orthologue of the human *EN2* gene[49,50]	• Reduced reciprocal social interactions[49] • Increased repetitive self-grooming[49] • No genotype differences in sociability, confounded by low activity levels[50]
15q11–13	Duplication in the genomic region on the mouse chromosome 7 homologous to the human genomic region 15q11–13 (REF. 29)	• Low sociability[29] • Ultrasonic vocalizations elevated in pups and reduced in adults[29] • Impaired reversal learning[29]

Silverman JL et al., *Nat Rev Neurosci.* 2010

CNTNAP2 SNPs are associated with language delay

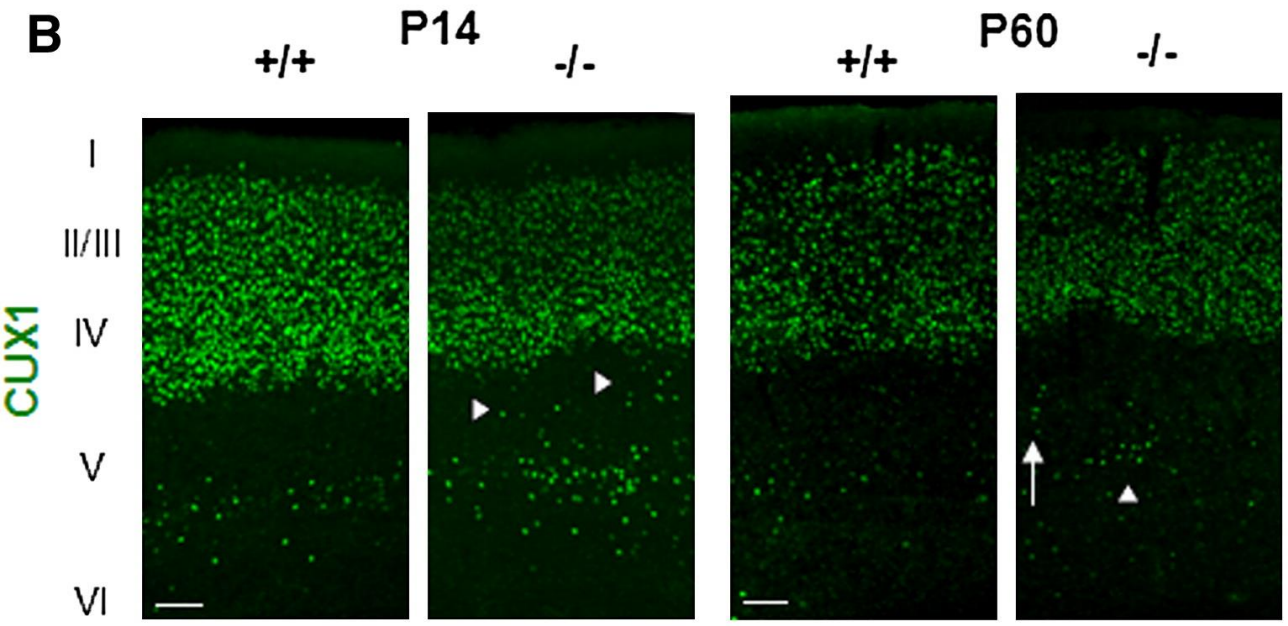
- Epilepsy
- Language delay: lag in the age of the first spoken word or the first spoken phrase
- likely accounts for fewer than 1% of cases of ASD

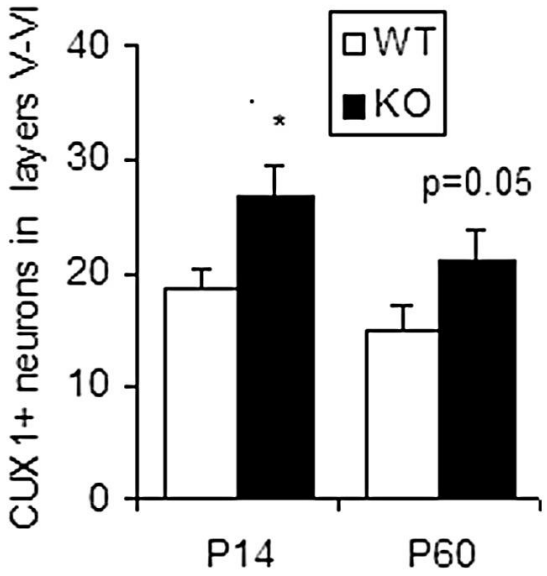




Alarcón M *et al., AJHG*. 2008
Arking DE et al., AJHG 2008
Betancur C *et al., Trends Neurosci.* 2009

CNTNAP2 mice model of autism

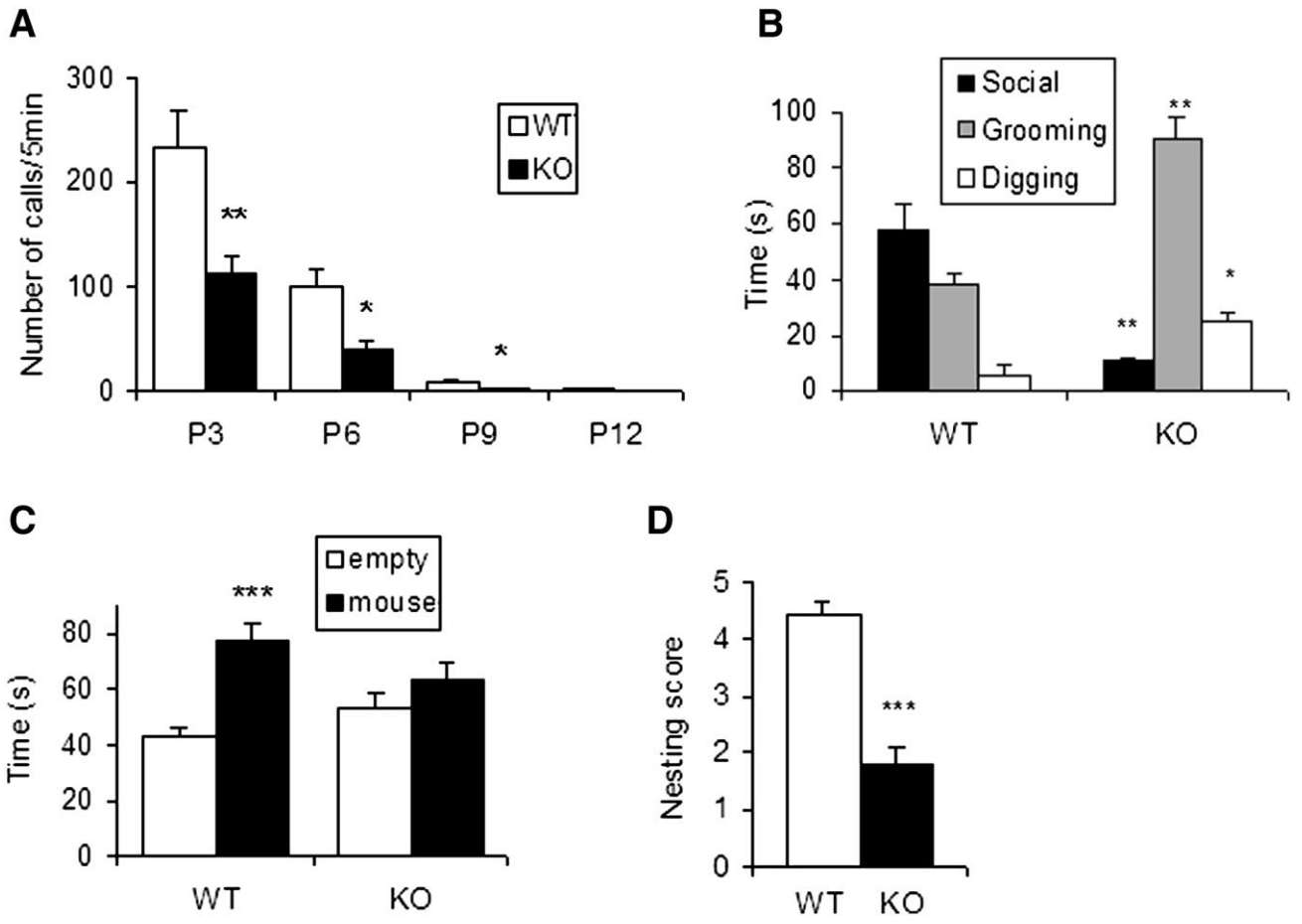




CNTNAP2 mutants exhibit defects in cortical neuronal migration

Peñagarikano O *et al.,* Cell. 2011

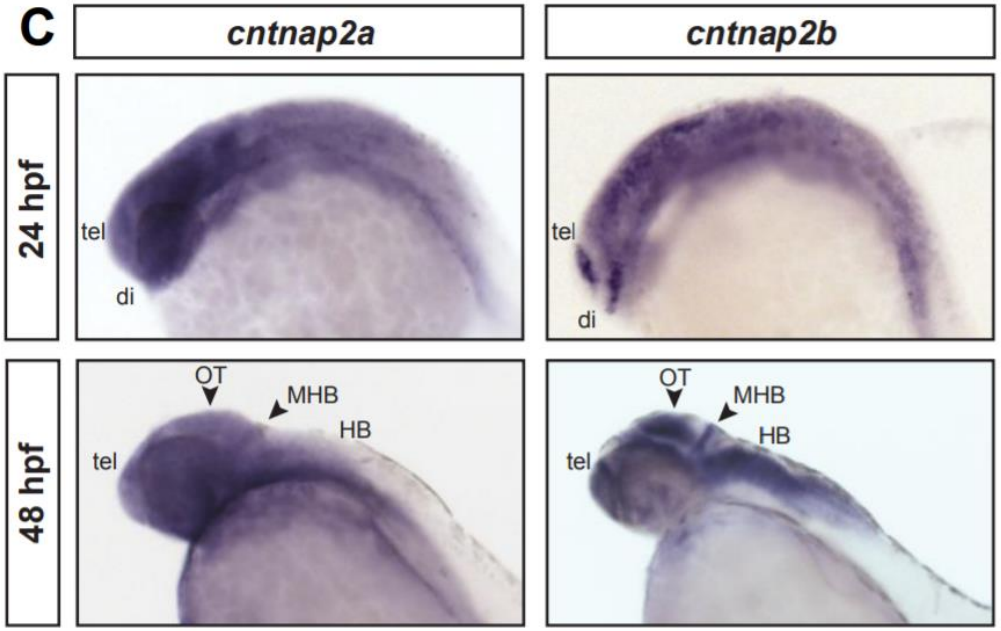
CNTNAP2 mice model of autism

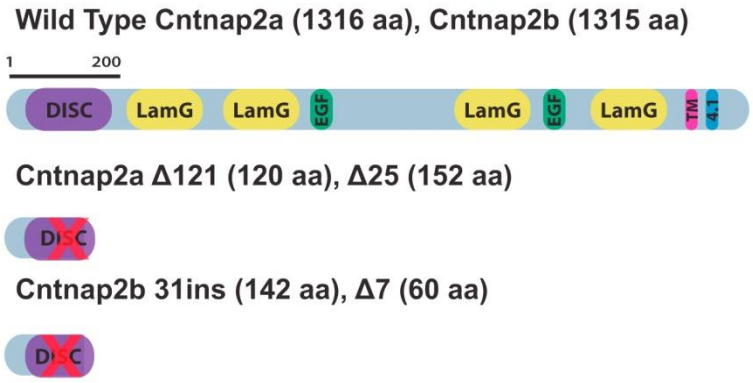


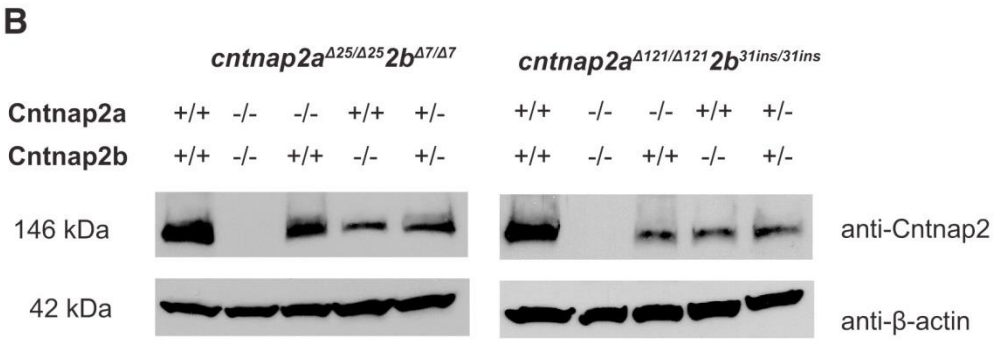
Mice CNTNAP2 mutants exhibit deficits in various social behaviors

Peñagarikano O *et al.,* Cell. 2011

CNTNAP2 zebrafish model of autism

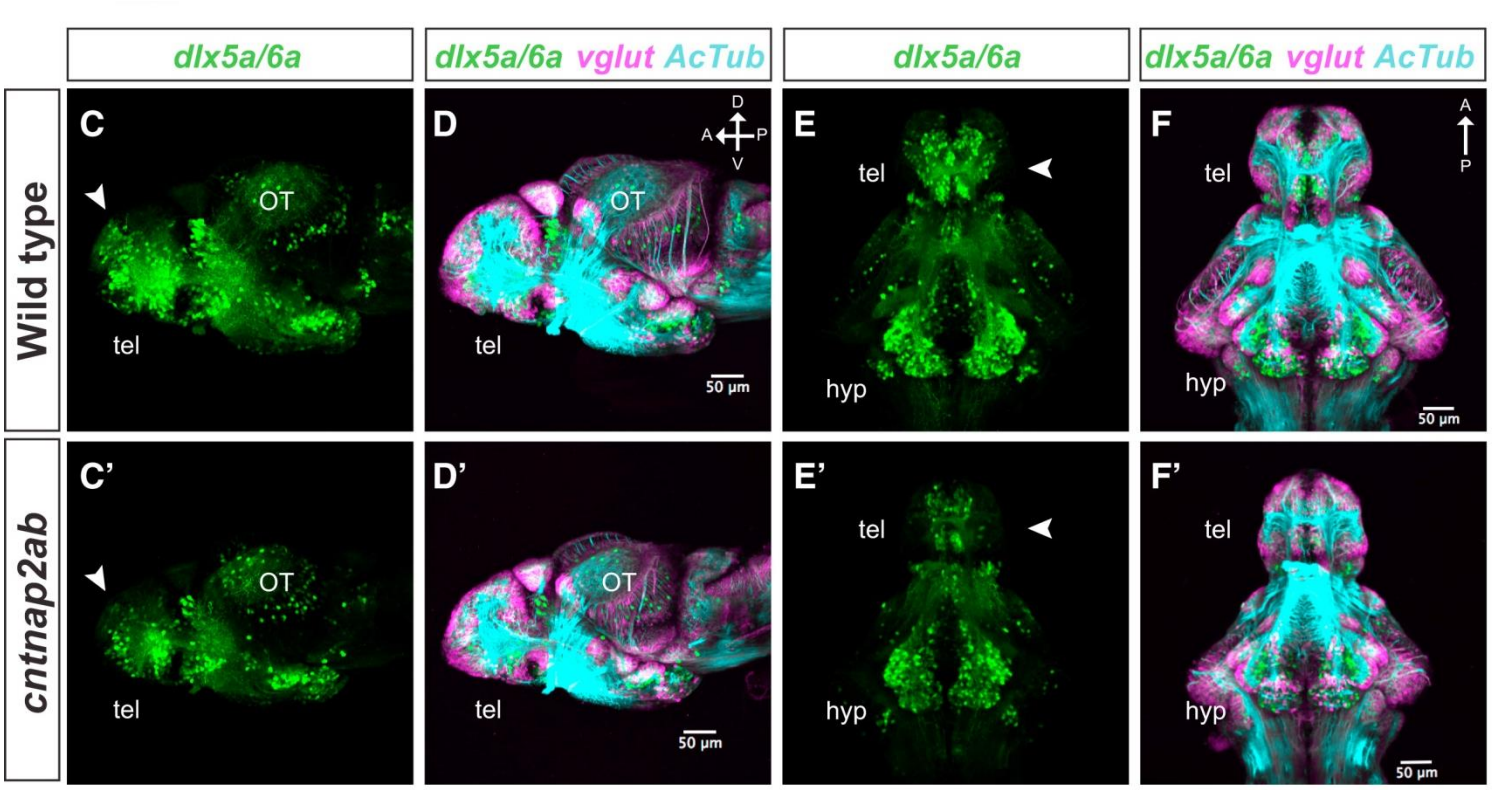


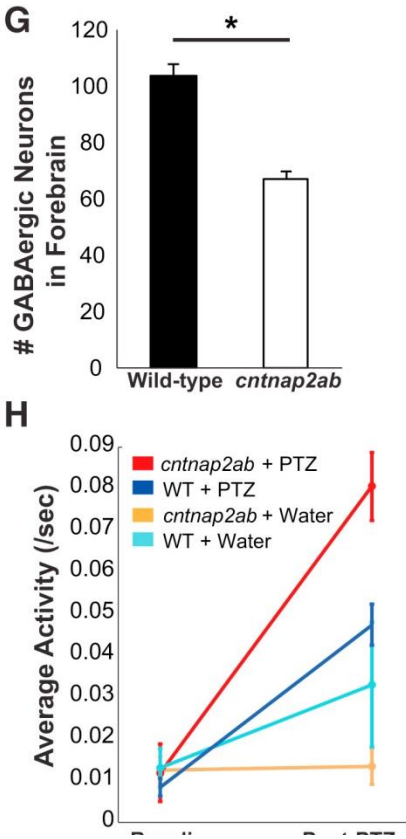




Hoffman E *et al.,* Neuron. 2016

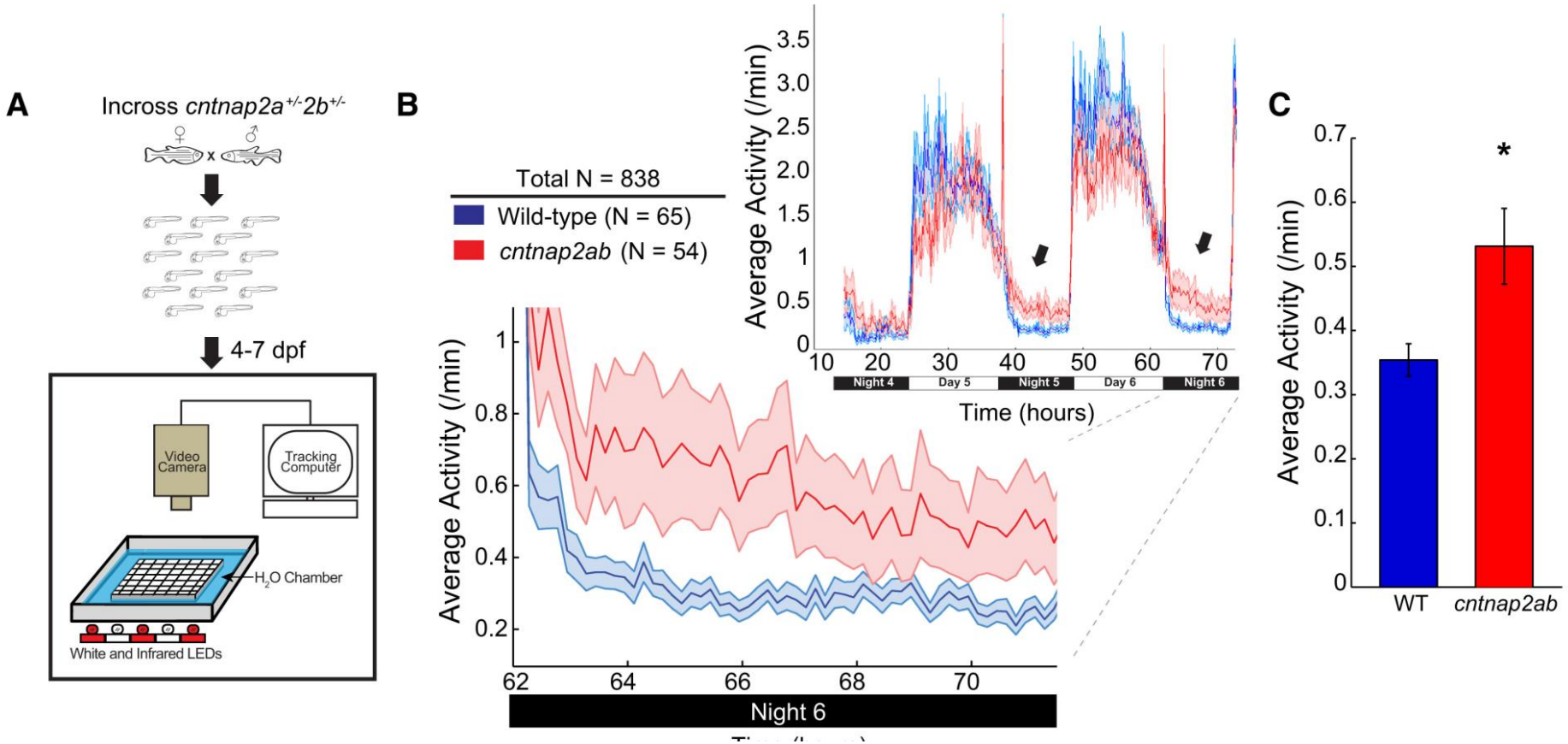
CNTNAP2 zebrafish model of autism





Hoffman E *et al.,* Neuron. 2016

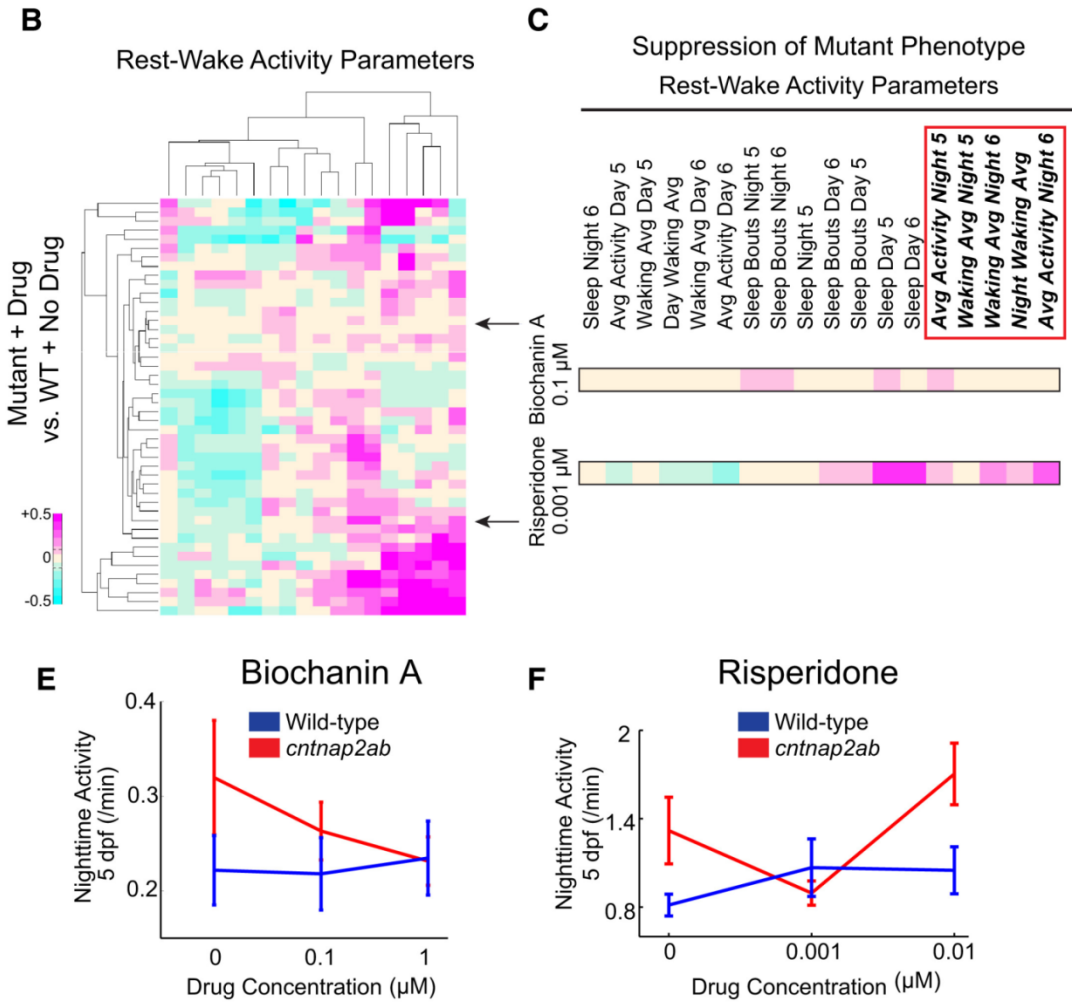
CNTNAP2 zebrafish model of autism



cntnap2ab mutants are hyperactive in dark

Hoffman E *et al.,* Neuron. 2016

CNTNAP2 zebrafish model of autism



cntnap2ab mutants be used to screen for drugs that can affect behavioral activity

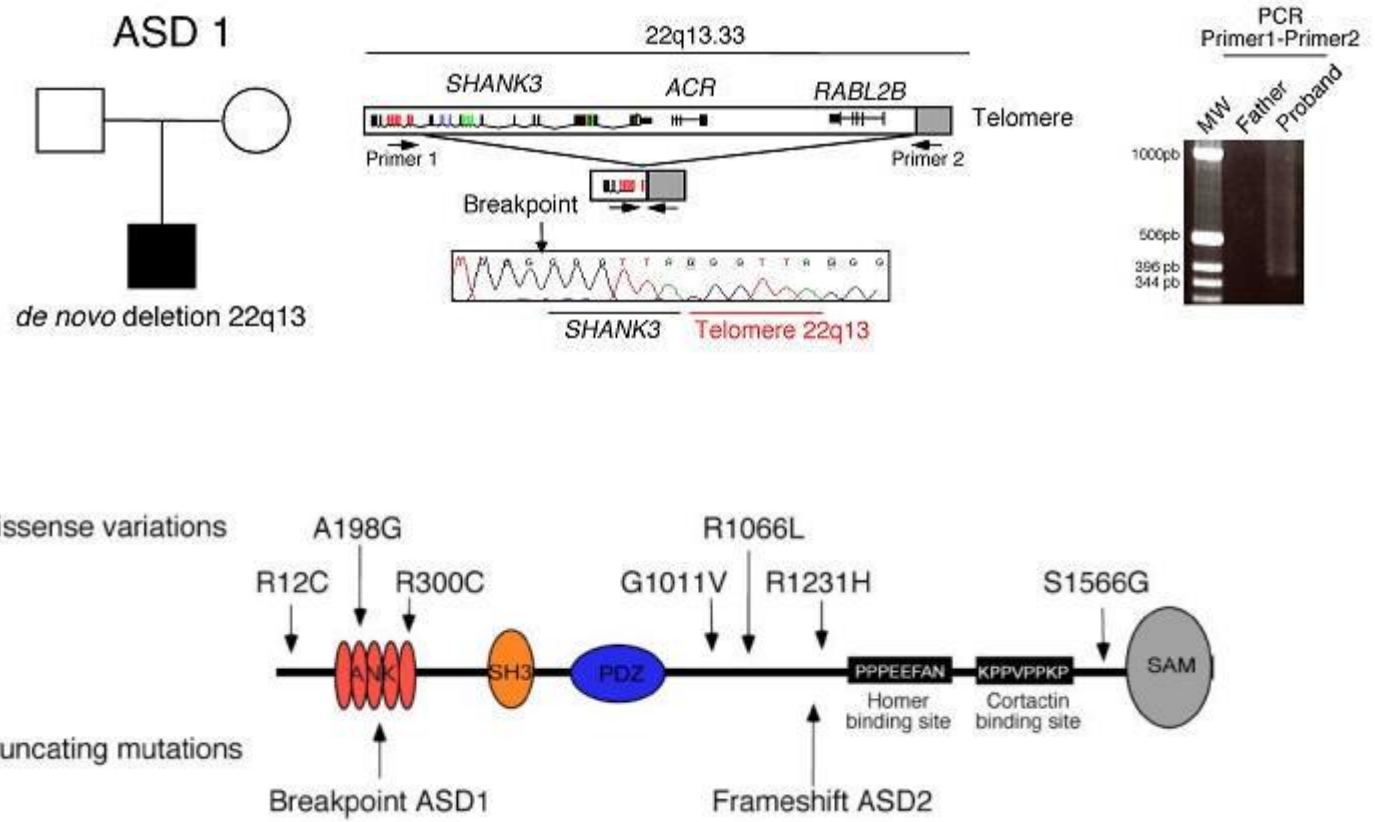
Hoffman E *et al.,* Neuron. 2016

Summary

Animal models can be used

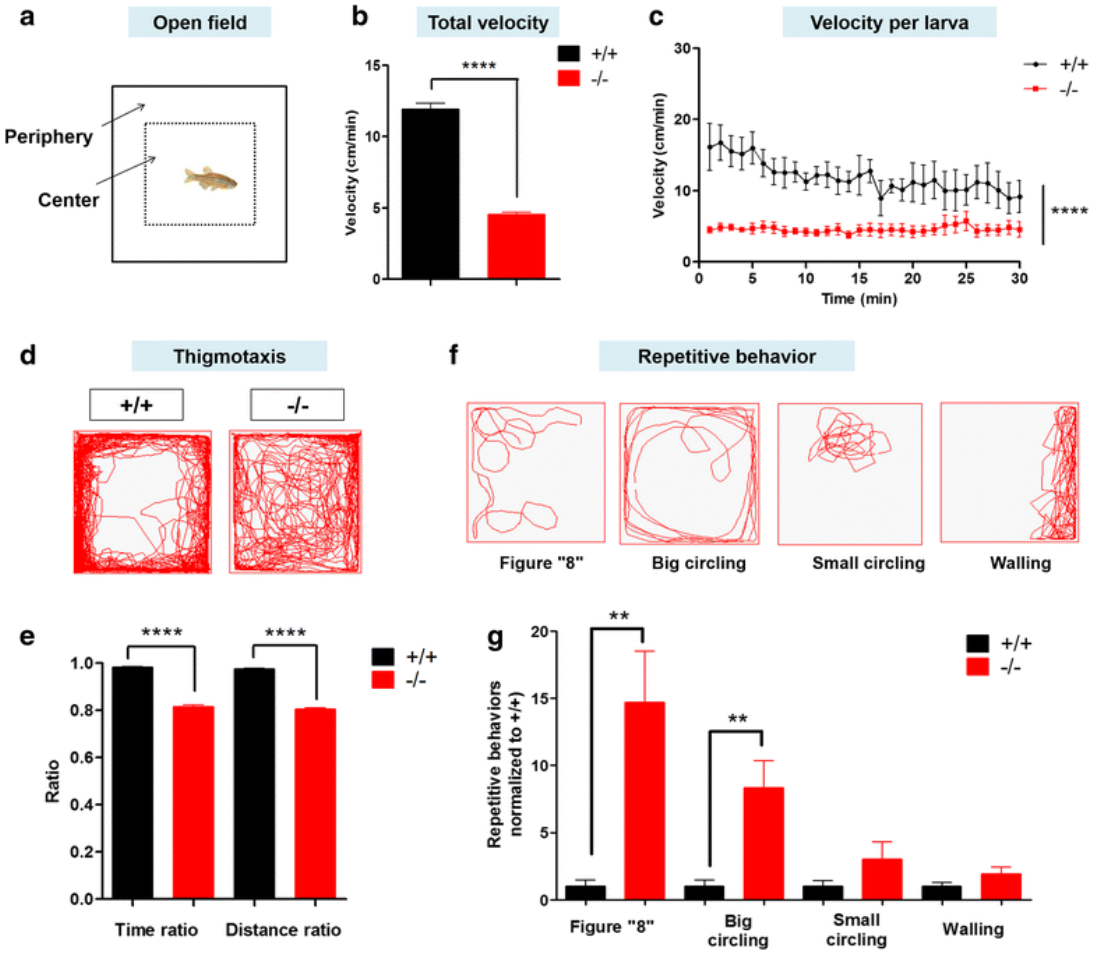
- to test the effect of specific genes implicated in neurodevelopmental disorders
- to characterize robust behavioral phenotypes
- to identify specific chemicals that can reverse behavioral phenotypes

SHANK3 mutations in humans are associated with ASD



Durand *et al.,* Nat Genetics 2007

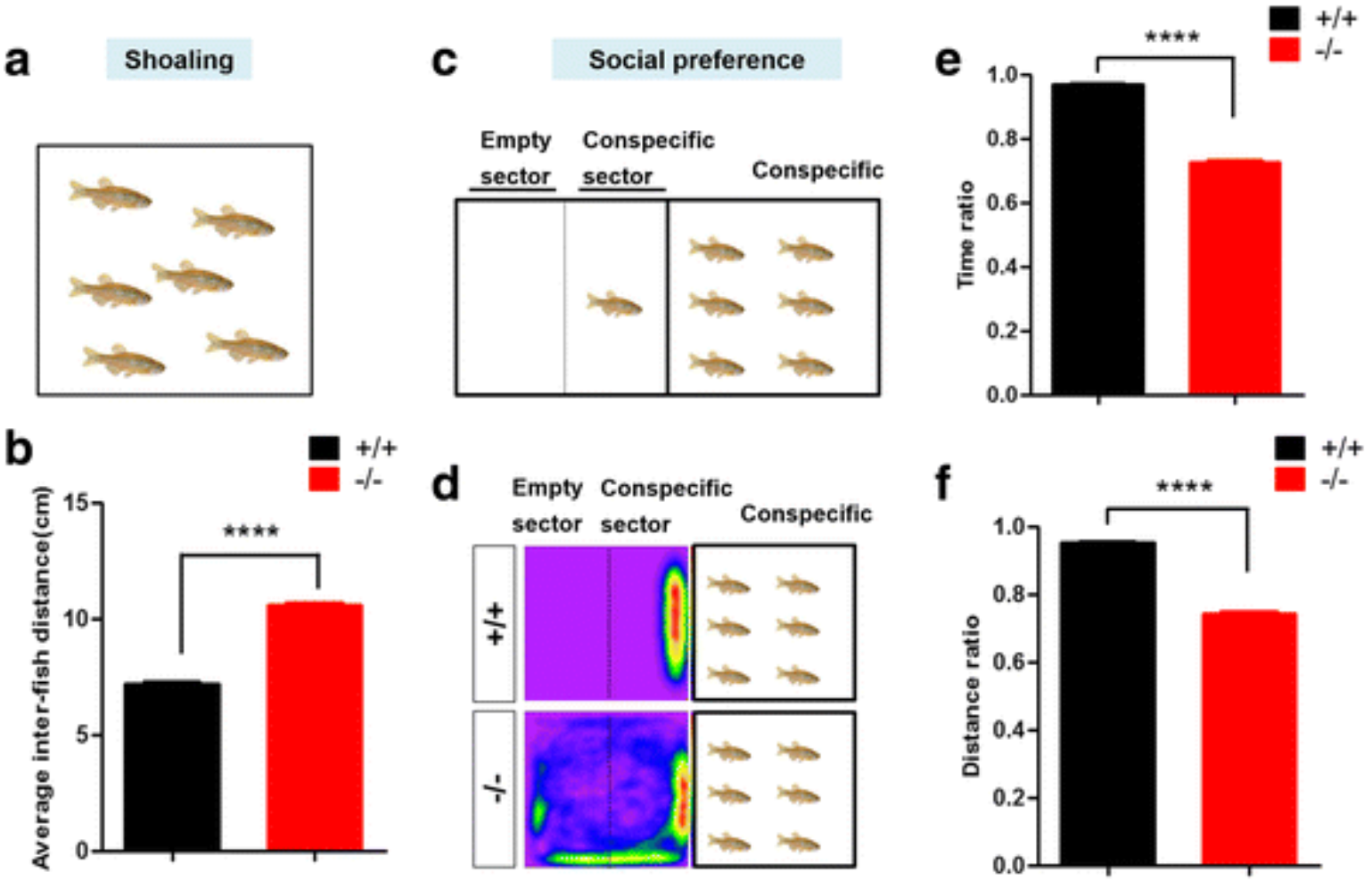
Zebrafish Shank3b model of ASD



Shank3b mutants exhibit impaired swimming behaviour

Liu C *et al.,* Mol. Autism. 2018

Zebrafish Shank3b model of ASD

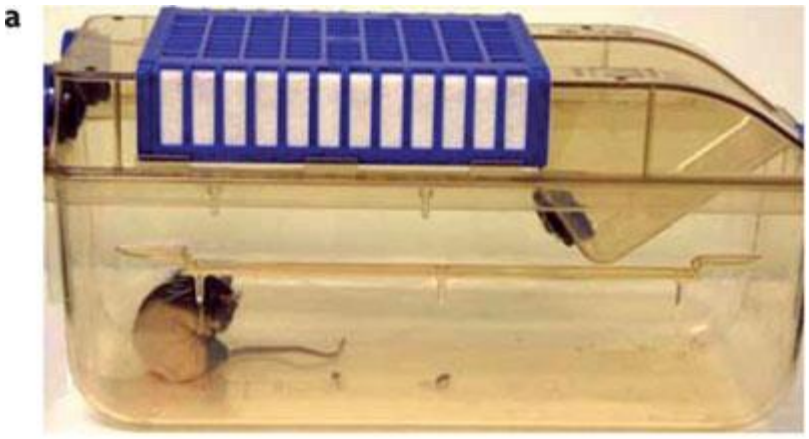


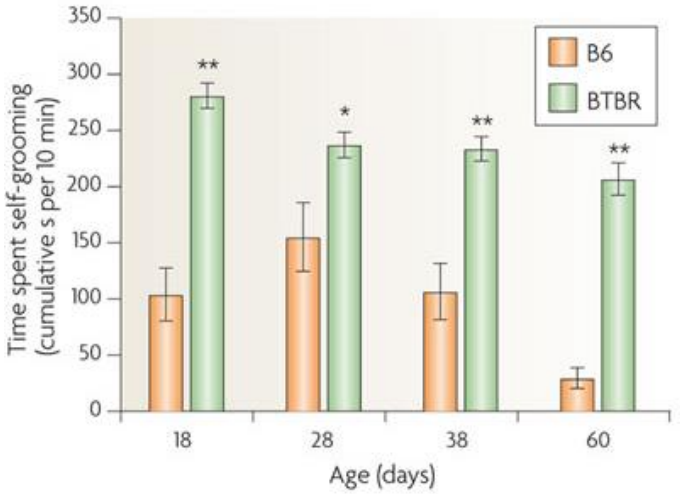
Shank3b mutants exhibit impaired social behaviour

Liu C *et al.,* Mol. Autism. 2018

Autism and repetitive behaviours

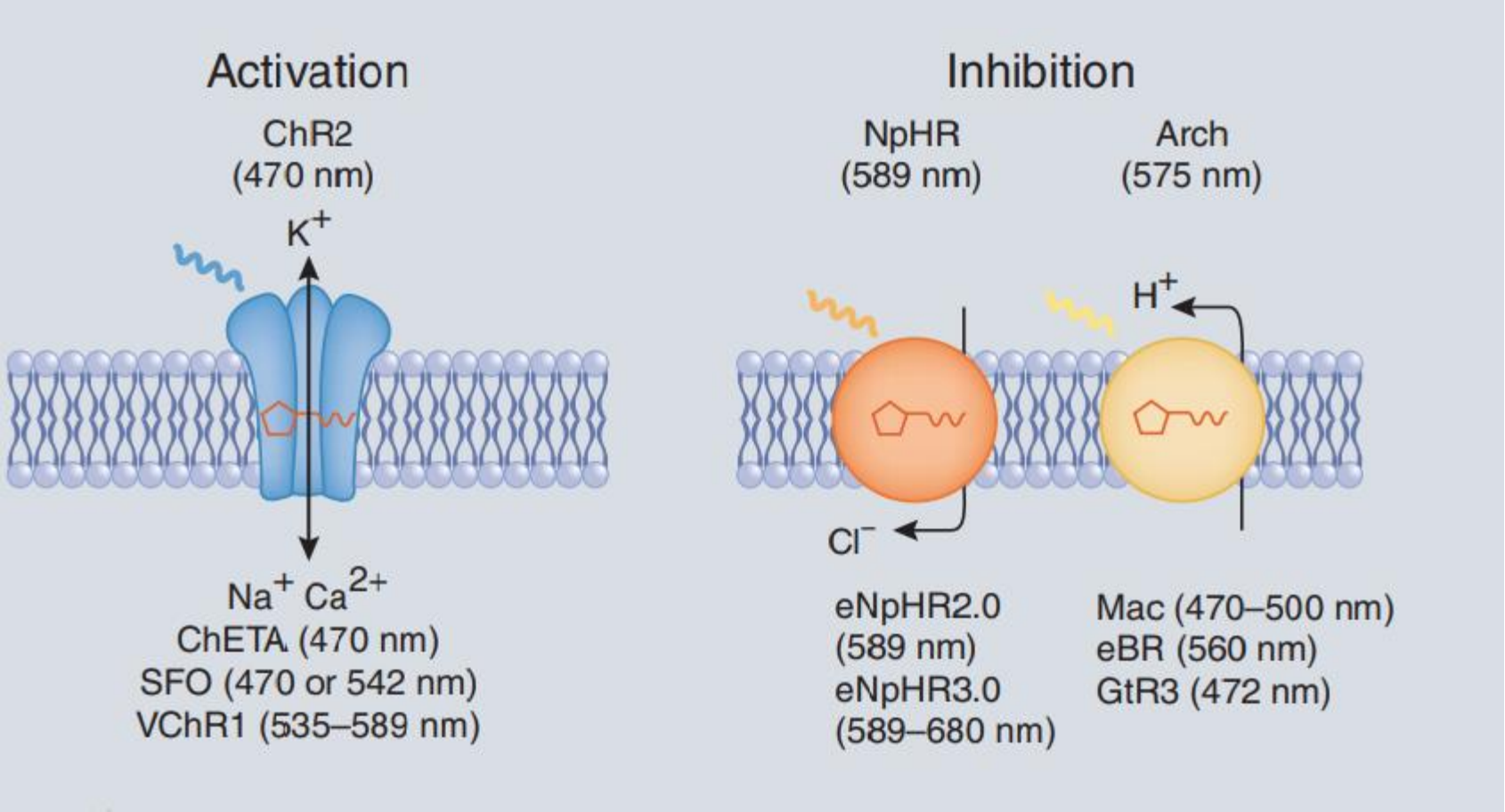
- Restricted and repetitive behaviors and interests are among the three core symptoms of autism.
- lack of understanding about why these restricted and repetitive behaviors develop, the mechanisms underlying them





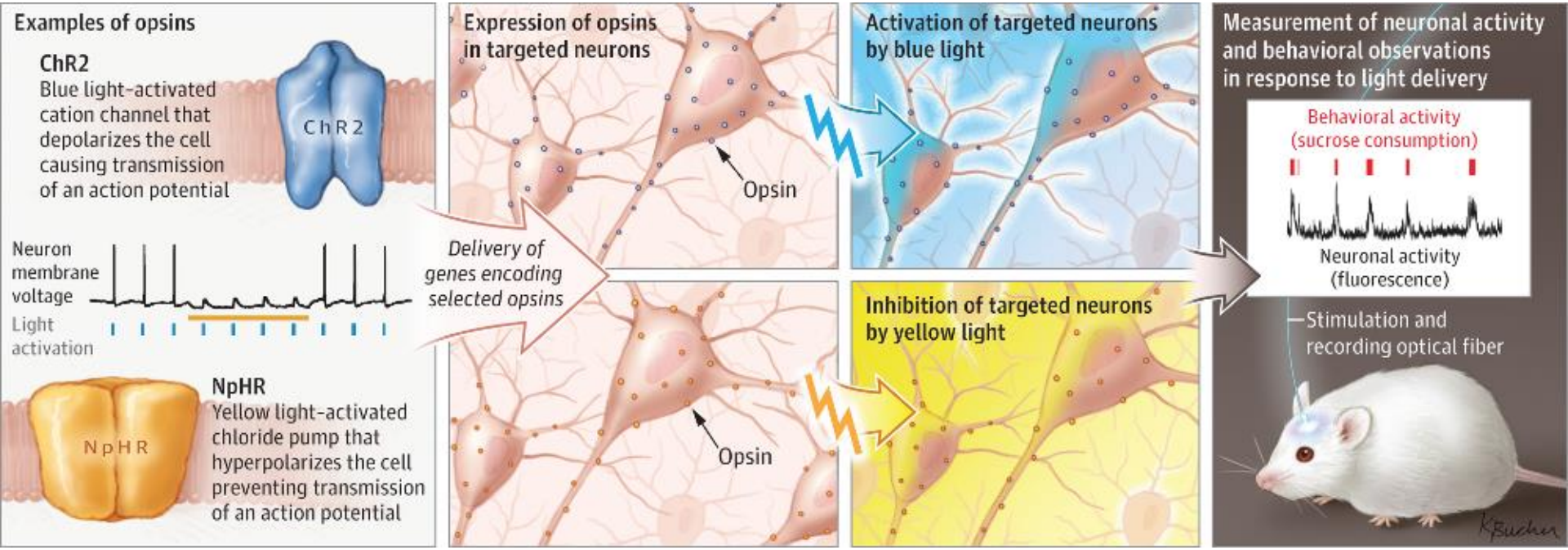
Triyb E et al., *J. Autism Dev Disord.* 2016
Silverman JL et al., *Nat Rev Neurosci.* 2010

Optogenetics



Erika Pastrana., *Nat. Methods.* 2010

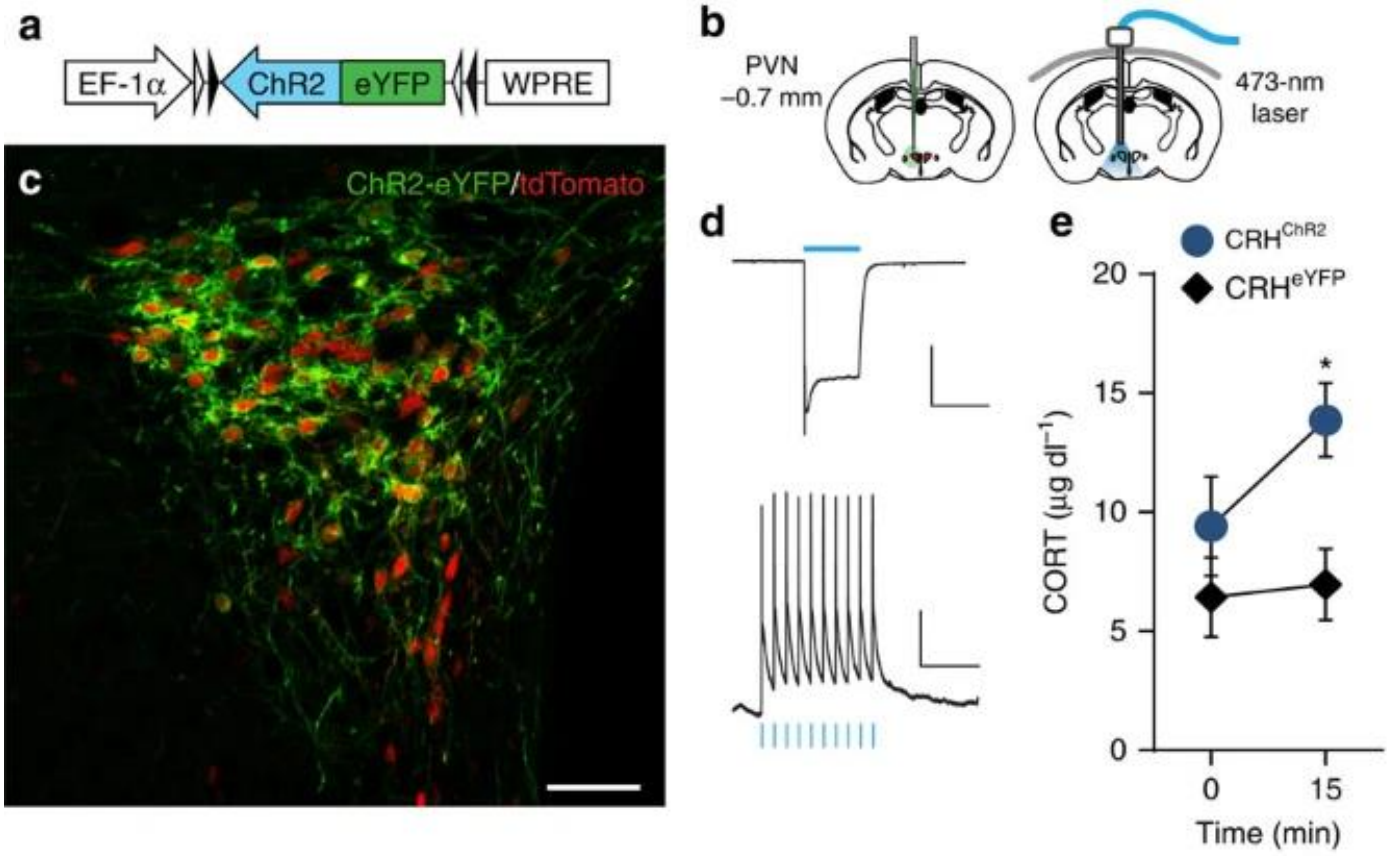
Optogenetics



www.geg-tech.com/

Optogenetics in neuroscience

CRH Cre mice x



Optogenetic stimulation of CRH neurons can trigger neuronal activity and peripheral cortisol release

Füzesi T *et al., Nat. Comm.* 2016

Optogenetics in neuroscience

Optogenetic stimulation of CRH neurons can induce repetitive grooming behaviour

Füzesi T *et al., Nat. Comm.* 2016

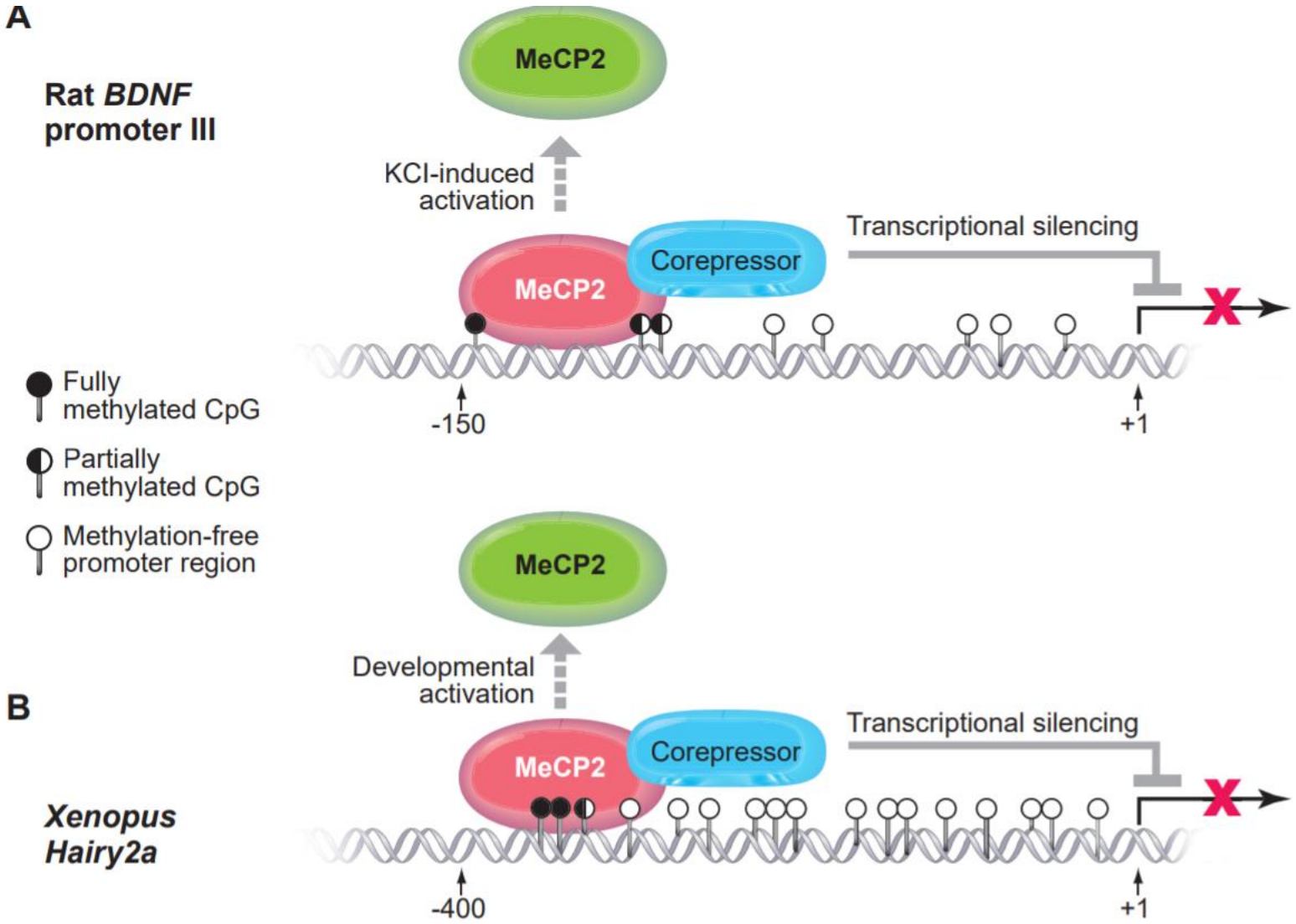
Rett Syndrome (RTT)

- Progressive, X-linked neurodevelopmental disorder that mostly manifests in girls with a morbidity rate of 1:10,000–1:15,000
- four distinct phases of Rett syndrome:
  - stagnation of development after 7 to 18 months,
  - rapid deterioration,
  - a pseudostationary phase, and
  - late motor deterioration
- Almost 95% of RTT is believed to be caused by mutations of an X-linked gene methyl-CpG-binding protein 2 (MECP2)
  - MECP2 mutations are most often embryonic lethal for boys
  - RTT girls seem to have normal development for up to 6–18 months
  - Later exhibit a series of symptoms associated with intellectual disability, loss of acquired language, and compromised cognitive, social, and motor skills, etc.

Rett Syndrome (RTT)

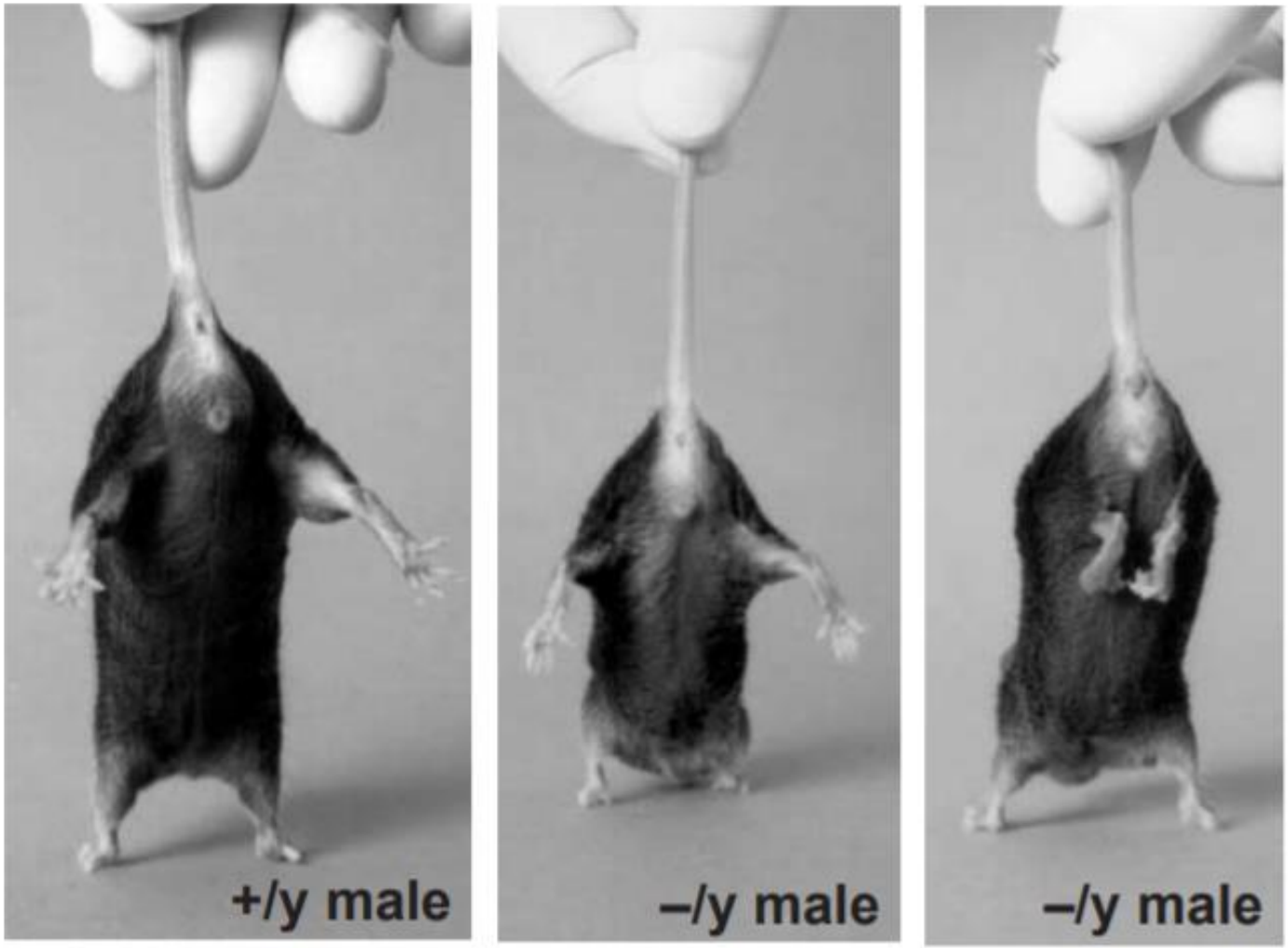
- RTT patients show abnormal neuronal morphology, but not neuronal death
- However, neurons do not die
- An important question for future therapeutic approaches to this and related disorders concerns phenotypic reversibility.
- Can viable but defective neurons be repaired, or is the damage done during development without normal MeCP2 irrevocable?

MECP2 in Rett Syndrome (RTT)



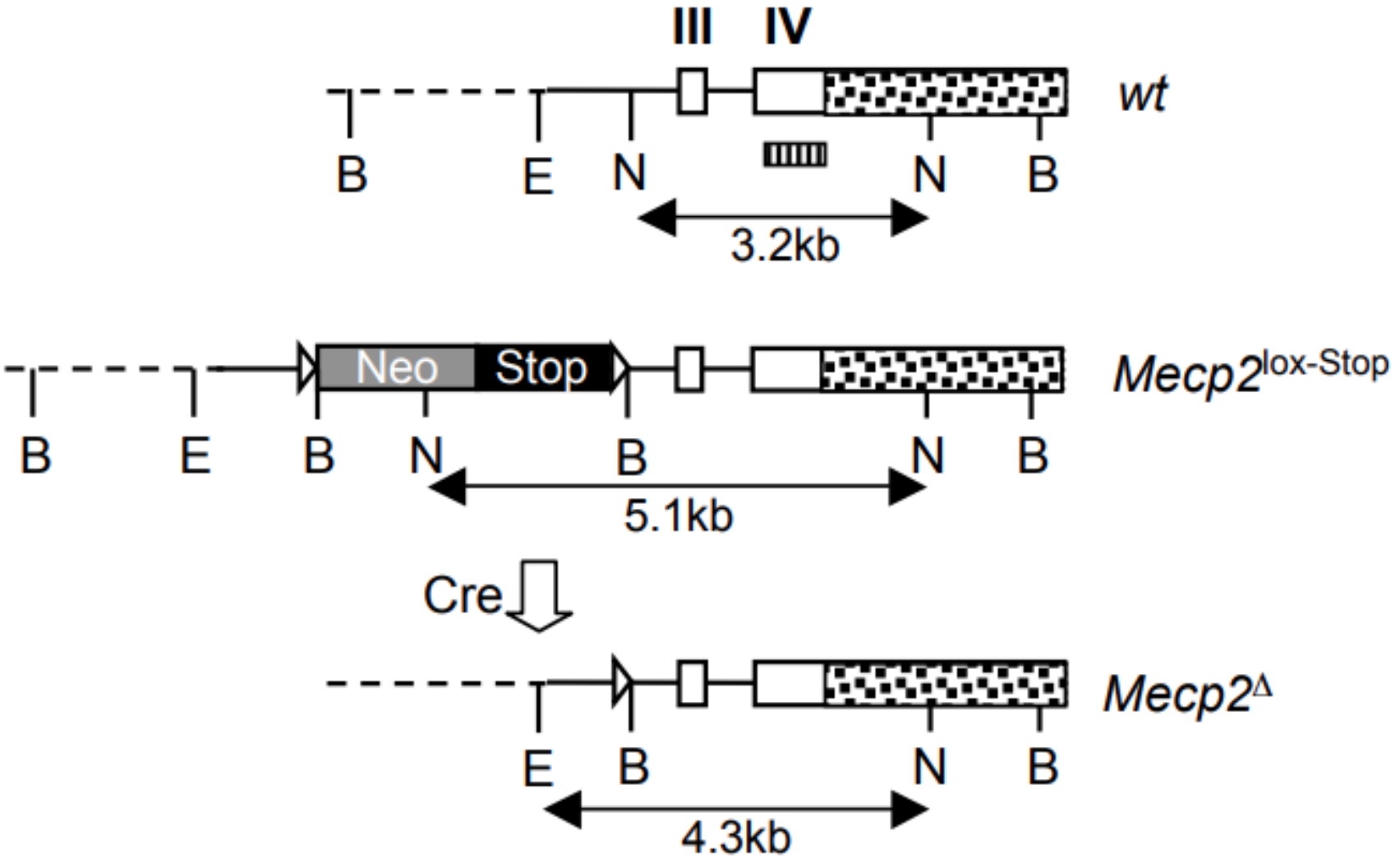
Klose R & Bird A Science. 2003

Mouse model of Rett Syndrome (RTT)



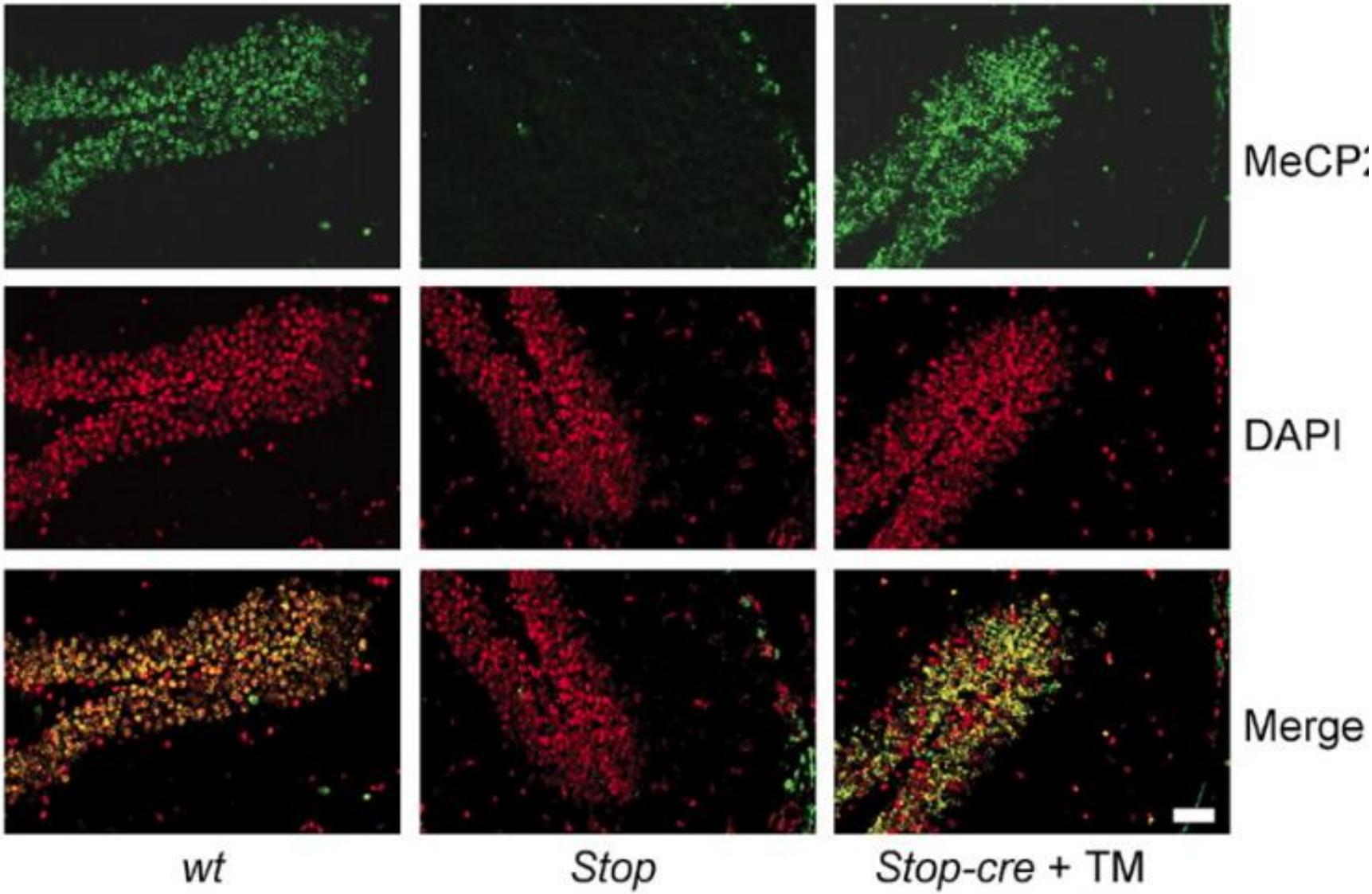
Guy J *et al.,* Nat Genet. 2001

Mouse model of Rett Syndrome (RTT)



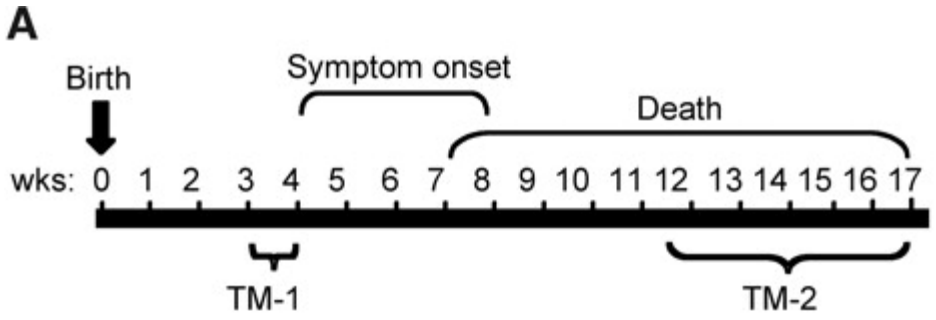
Guy J *et al.,* Science. 2007

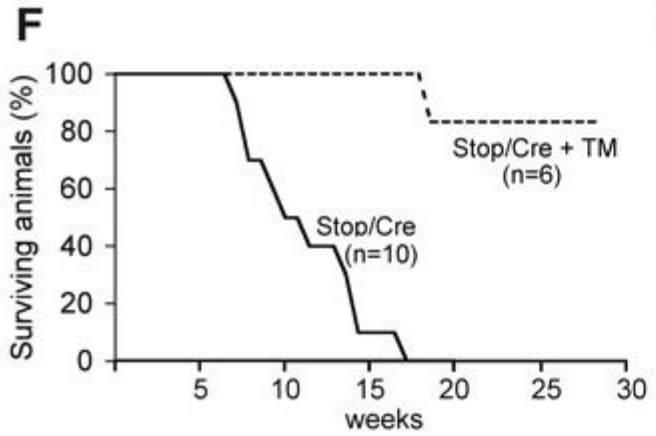
Mouse model of Rett Syndrome (RTT)

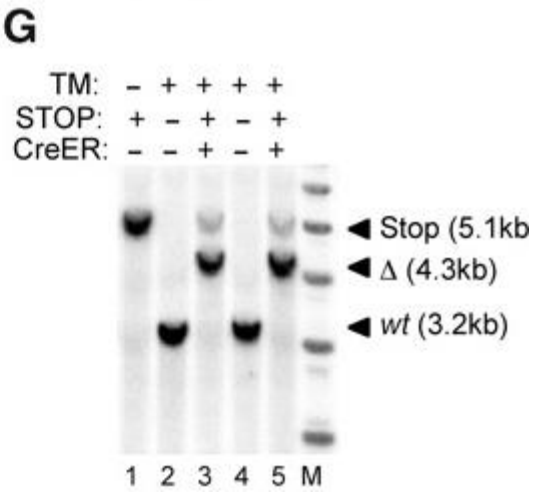


Guy J *et al.,* Science. 2007

Mouse model of Rett Syndrome (RTT)







Guy J *et al.,* Science. 2007

Mouse model of Rett Syndrome (RTT)

Guy J *et al.,* Science. 2007

Summary and outlook

- Animal models have greatly improved our understanding of the
  - role of specific mutations in neurodegenerative diseases, neurodevelopmental disorders
  - role of specific neurons in specific behavior
- Have provided a valuable platform for testing potential therapeutic strategies
- No single animal model can mimic all clinical features.
- Majority of therapeutics show promise in animal models but then fail to elicit predicted effects when tested in humans..