

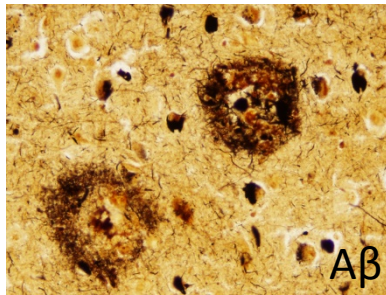
# Rodent models for Alzheimer's disease and respective behavioral paradigms

Prof. Hilal Lashuel & Johannes Burtscher  
LMNN, EPFL  
**12.10.2018**

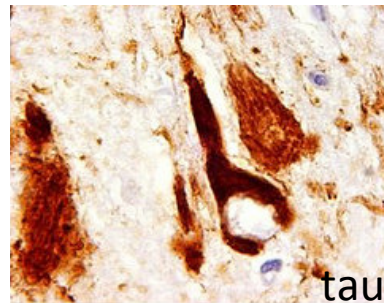
# Pathological hallmark's of Alzheimer's disease: foundation of modelling the disease

## Pathology

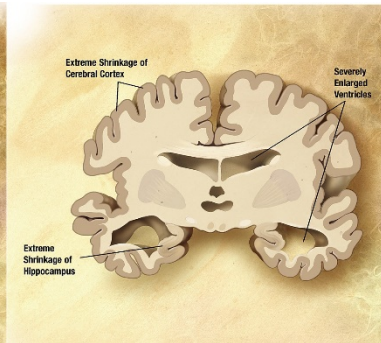
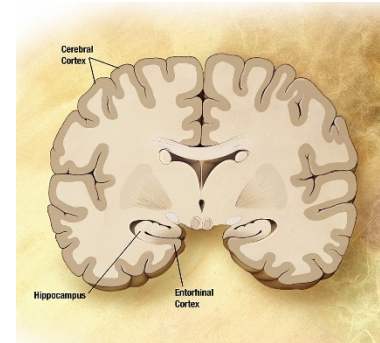
Senile plaques



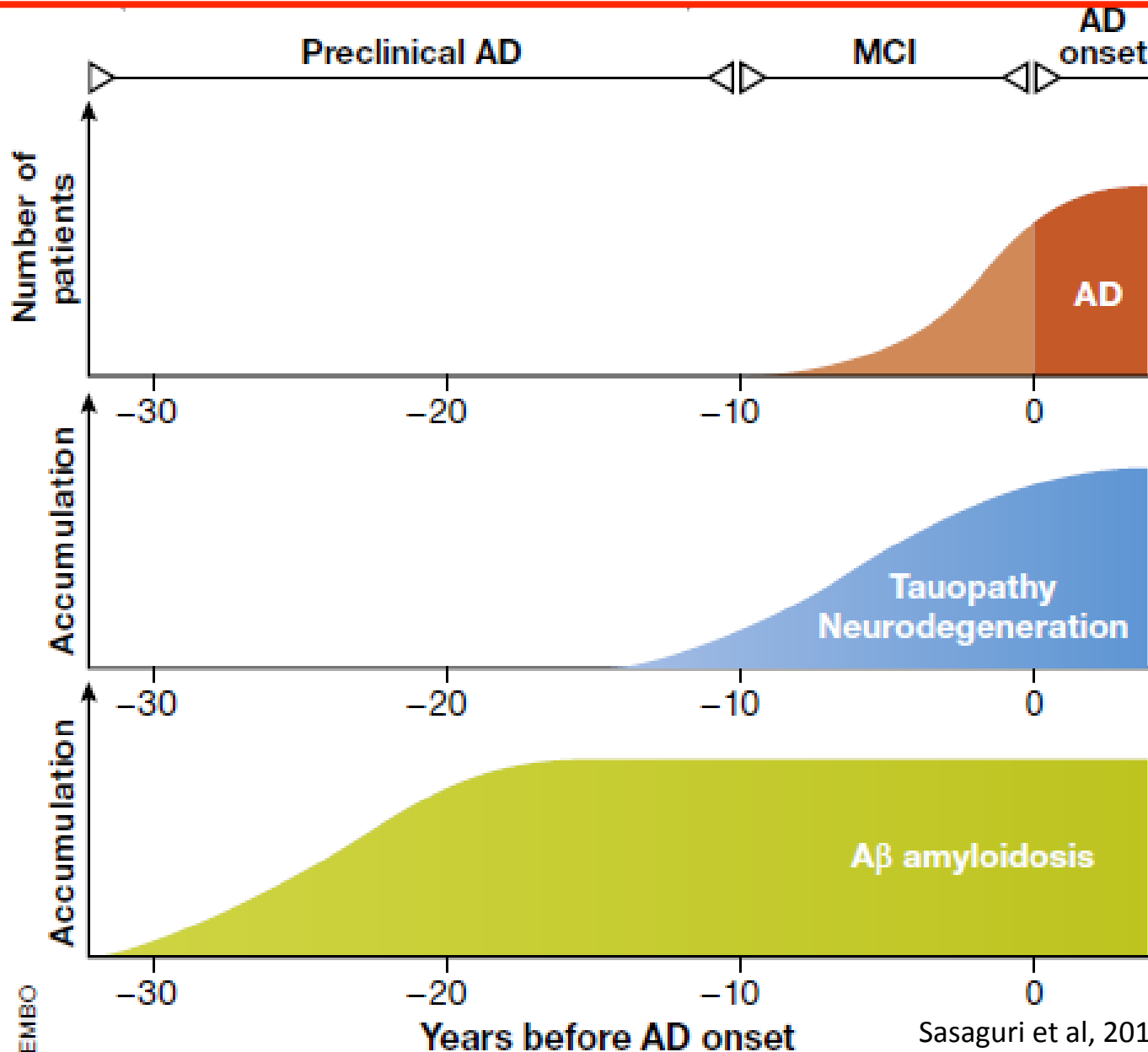
Neurofibrillary tangles



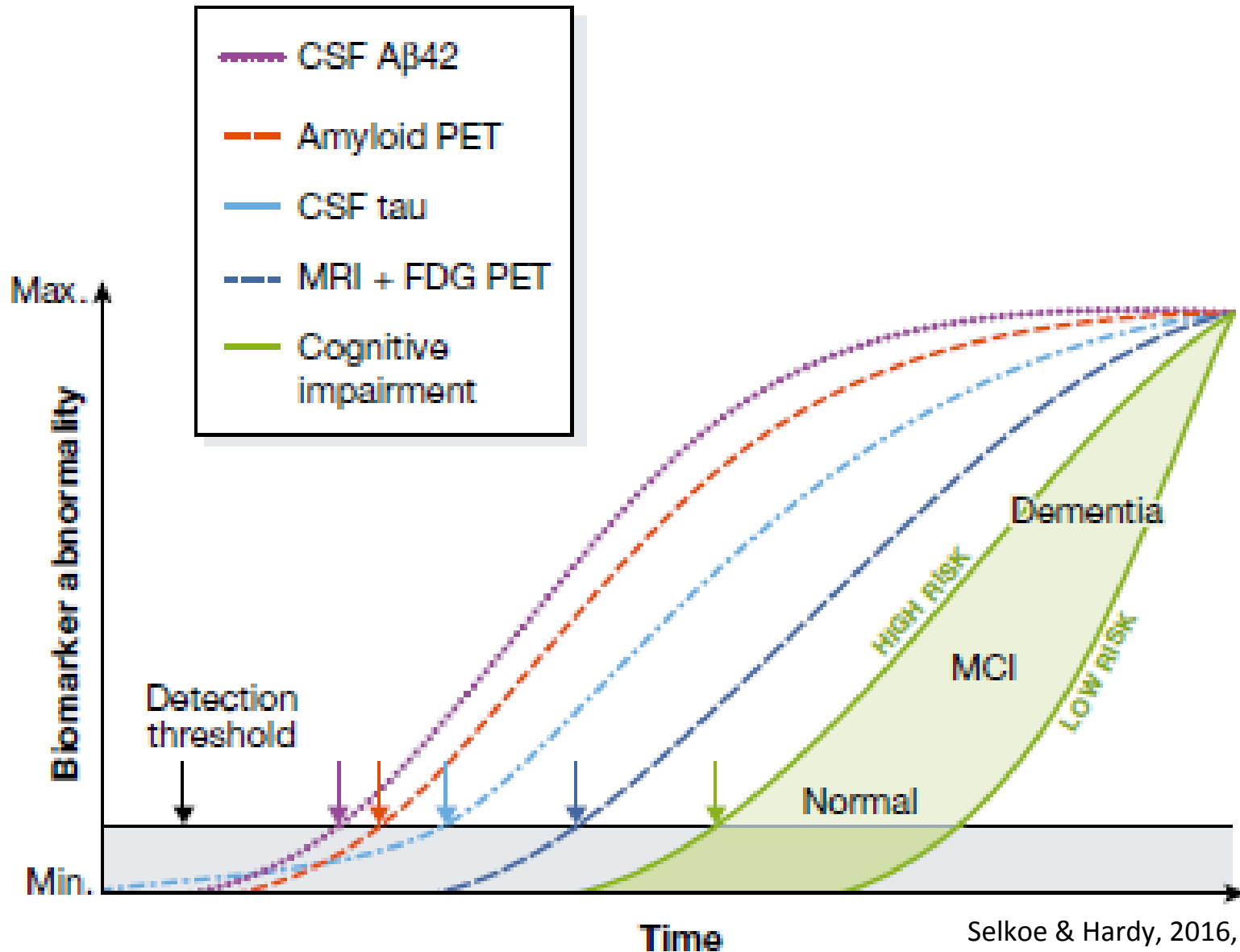
Hippocampal and cortical neurodegeneration



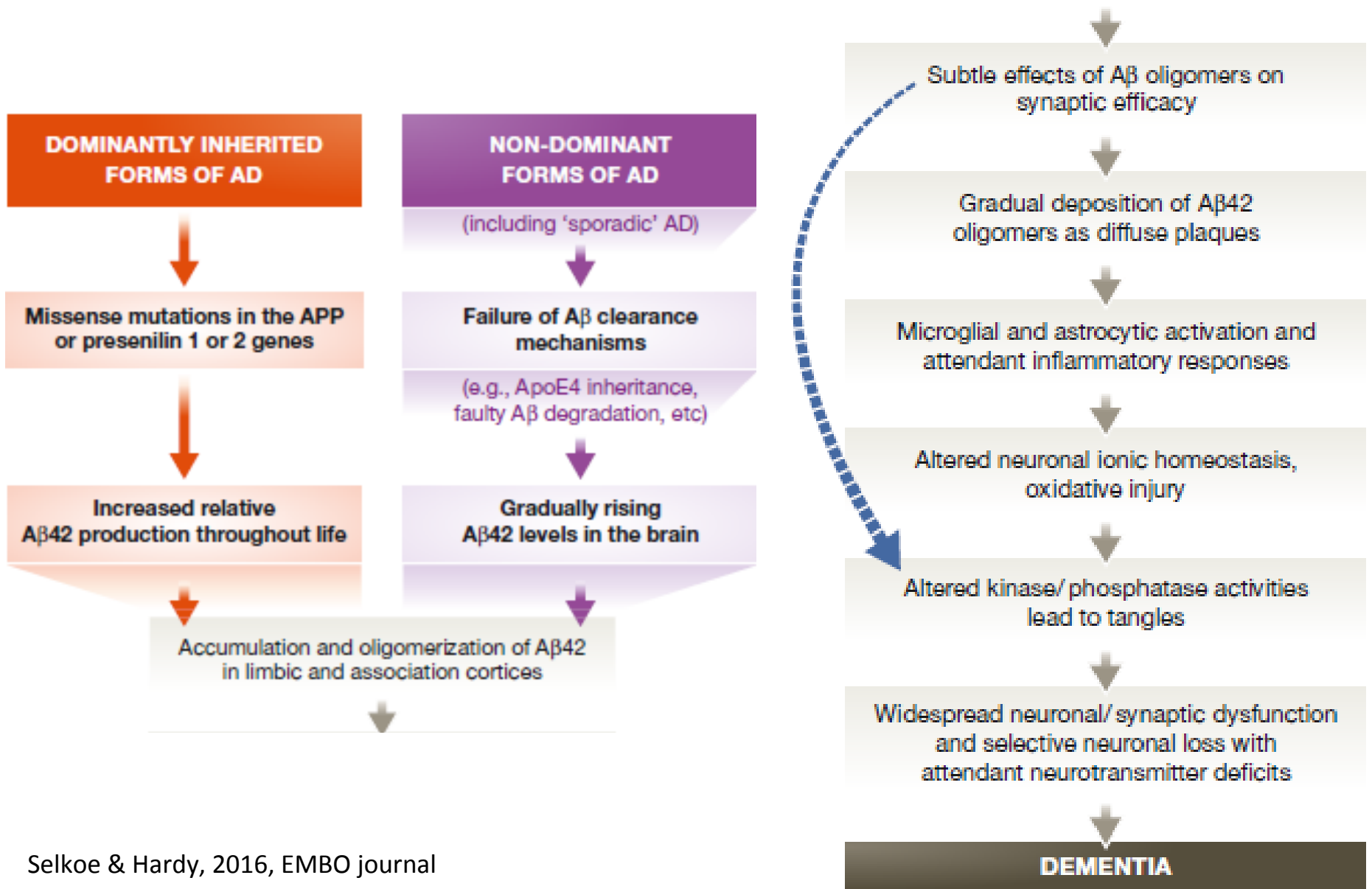
# AD progression & biomarkers; which temporal conditions would ideal models reflect?



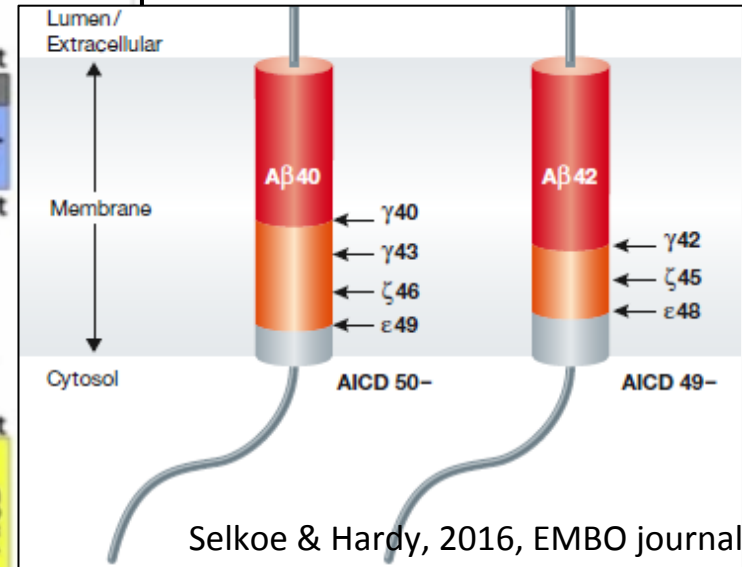
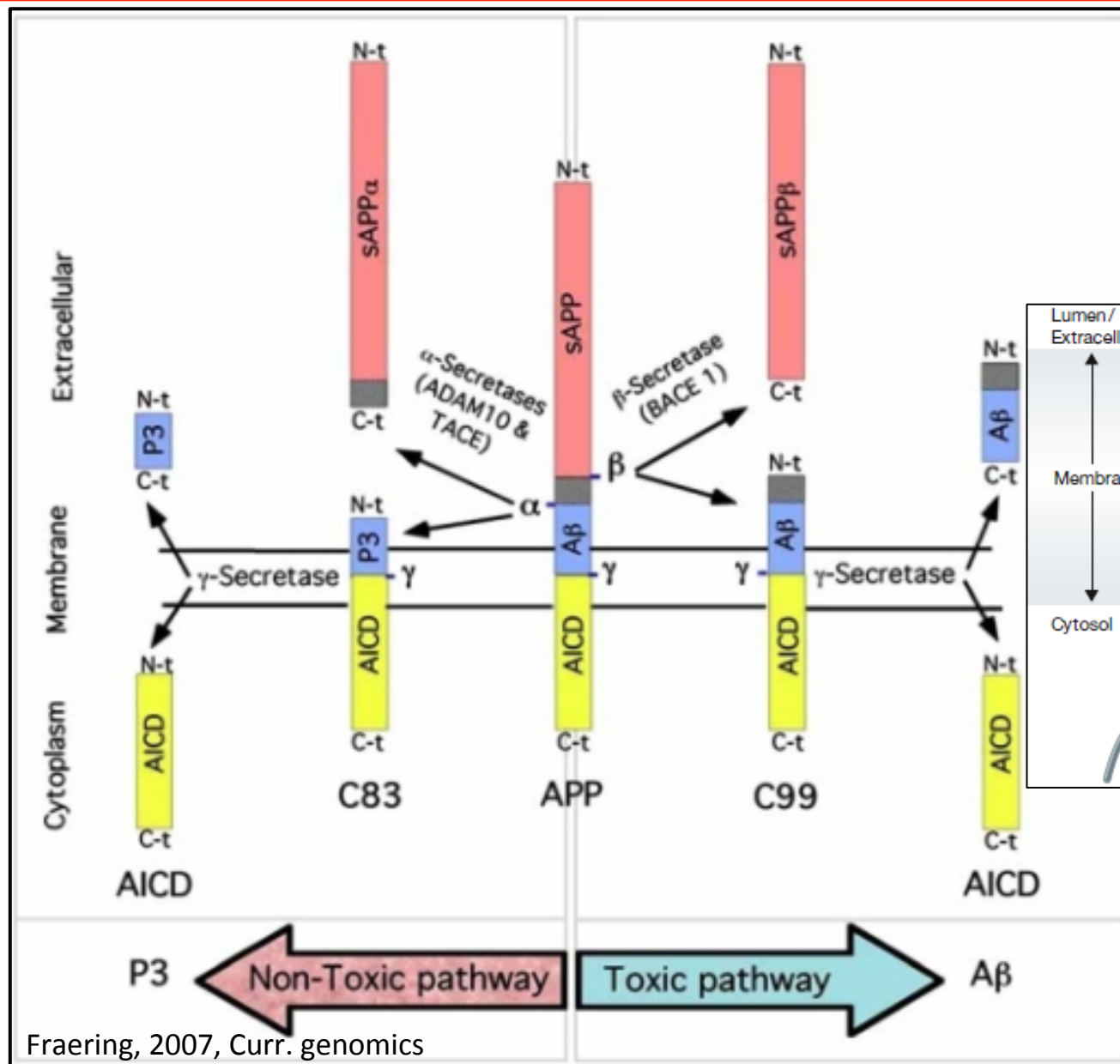
# AD progression & biomarkers; which temporal conditions would ideal models reflect?



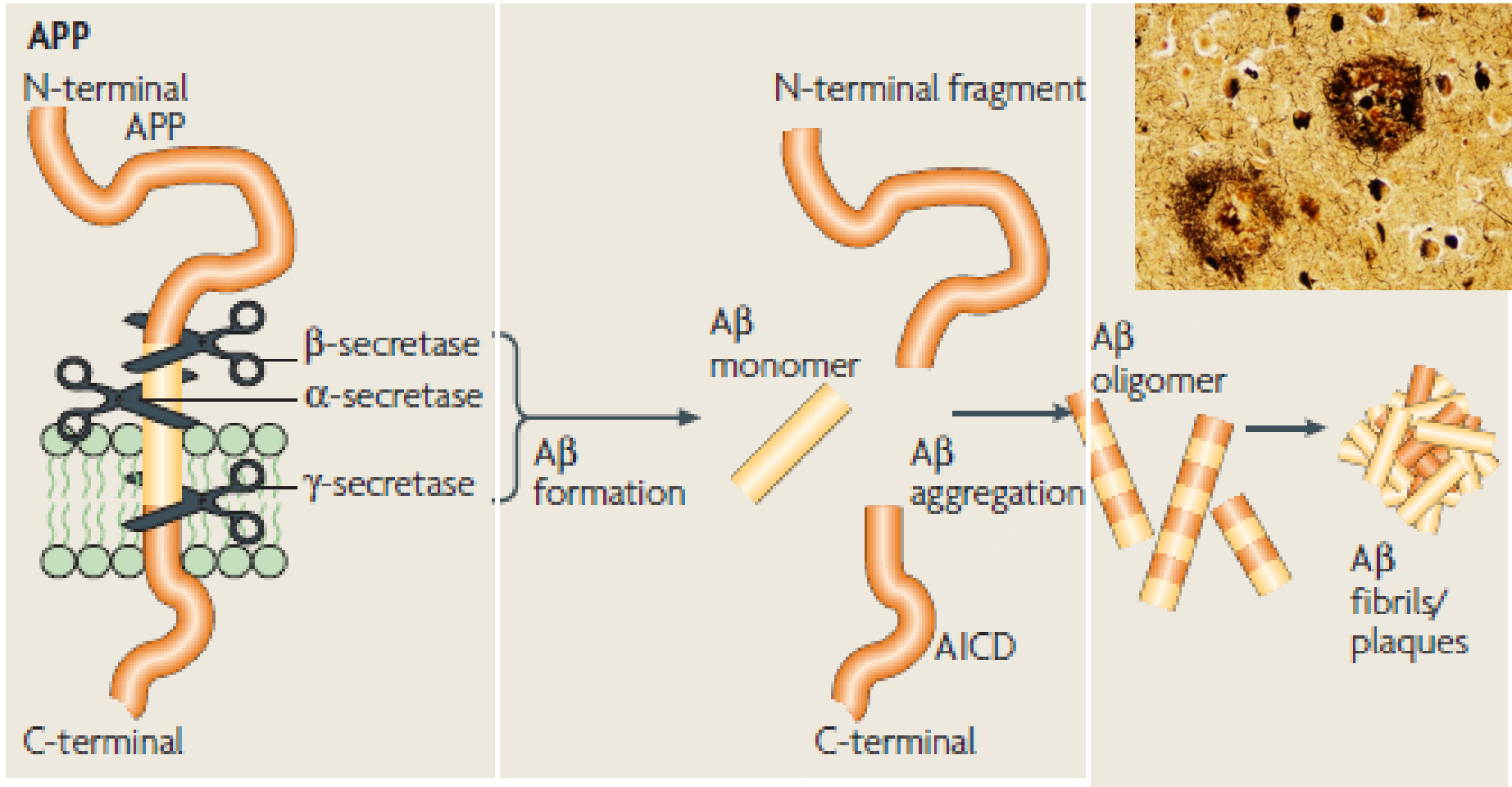
# The amyloid cascade hypothesis



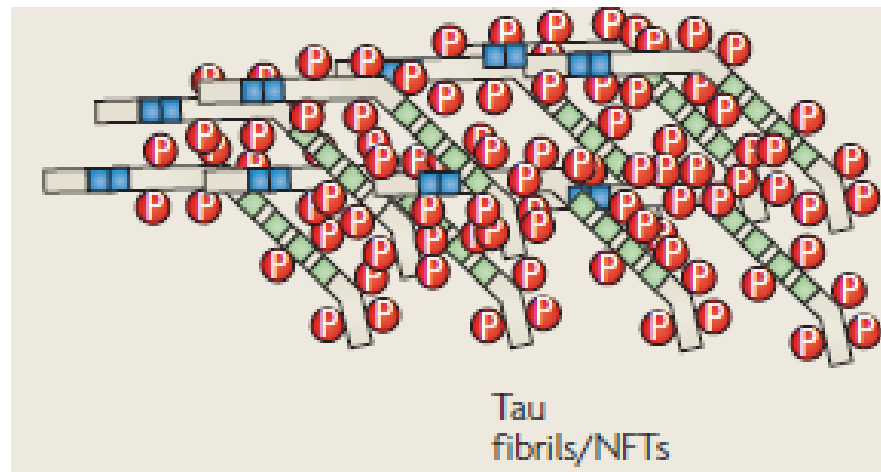
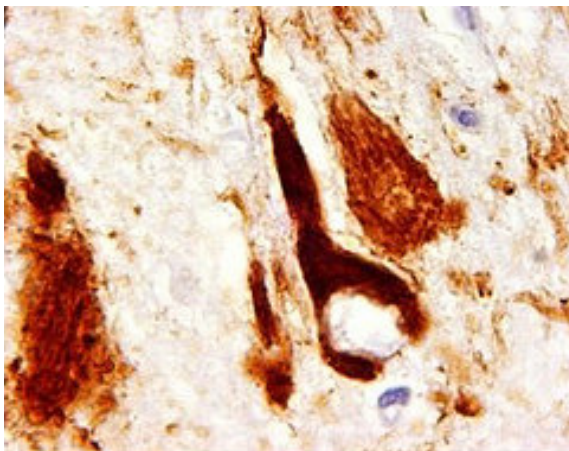
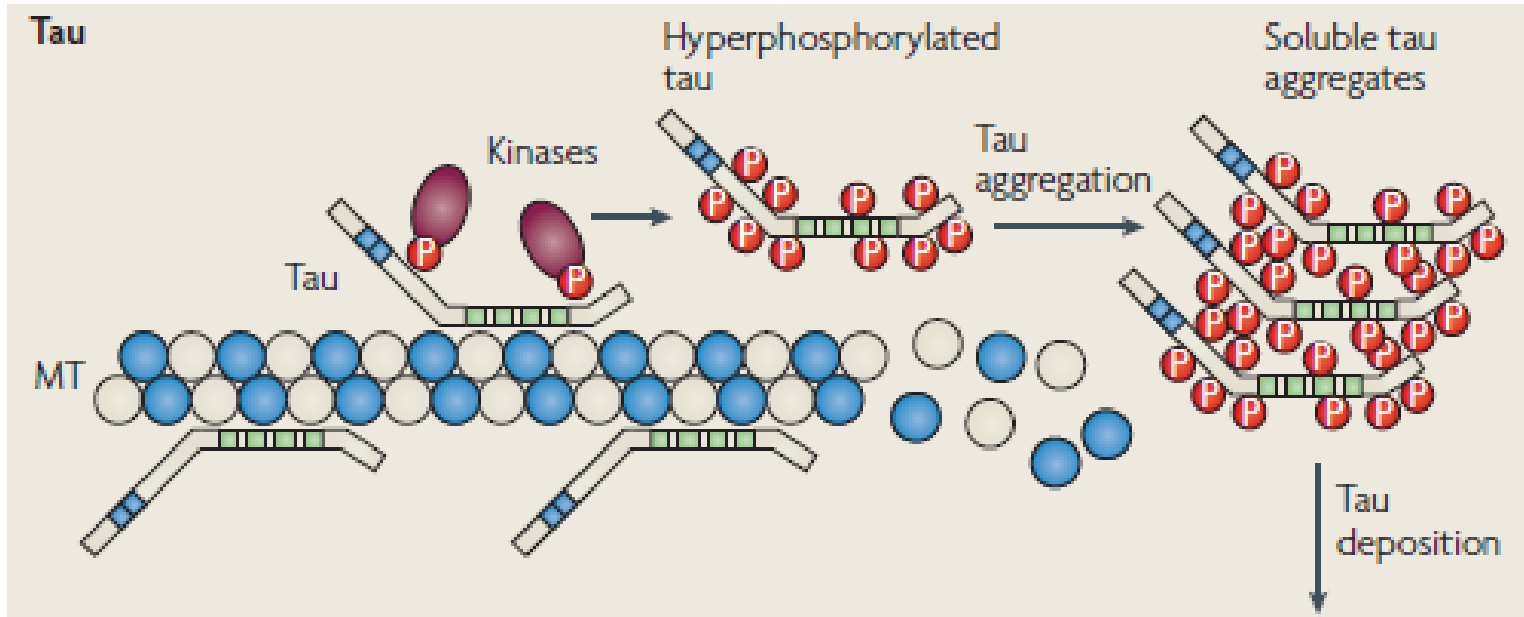
# Processing of A $\beta$



# Aggregation of A $\beta$



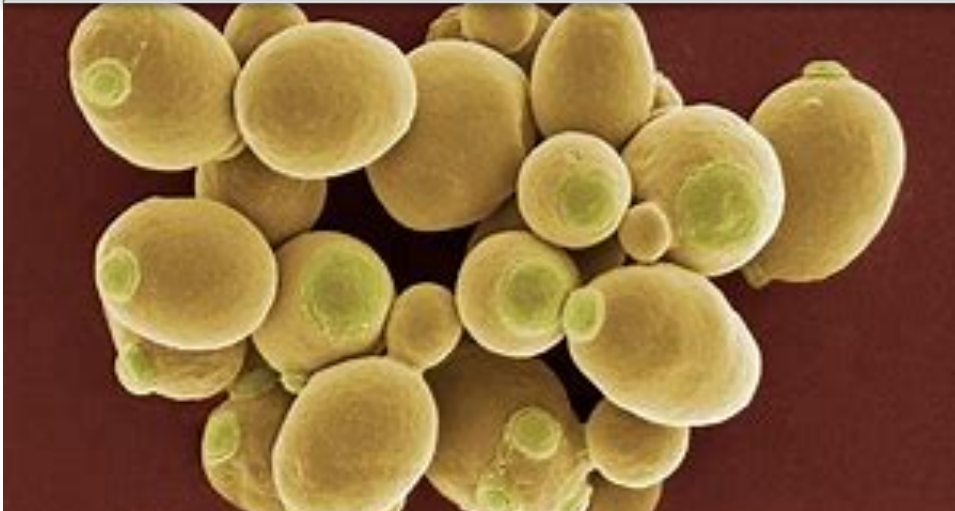
# Aggregation of tau



# Model organism yeast: studying molecular mechanisms of protein folding

## General facts of yeast

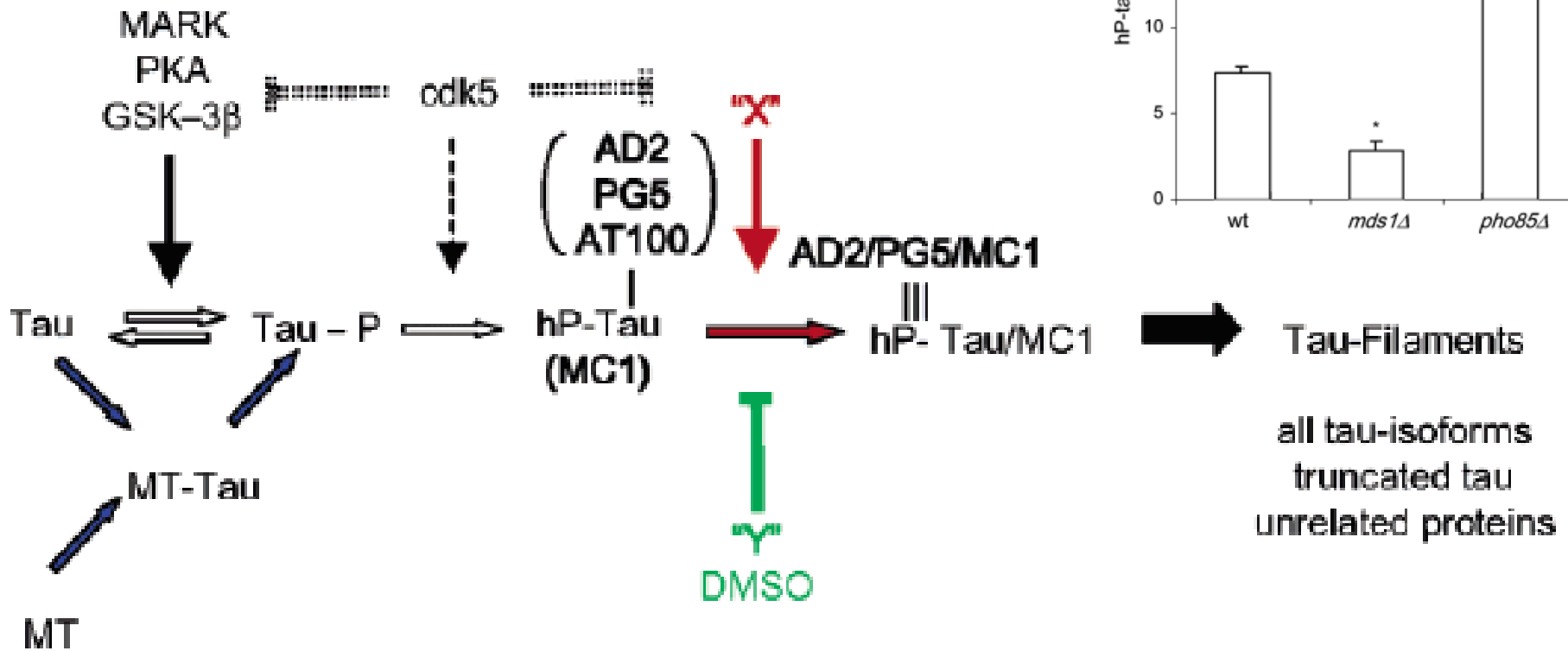
- *Saccharomyces cerevisiae* is a type of yeast, a single-celled organism.
- It is one of the simplest eukaryotic organisms.
- Complete genome sequence of *S. cerevisiae* Published already in 1996; 12,157,105 base pairs, 6,692 genes.
- Used to study the connections between genes and proteins, and their functions



## Benefits of yeast

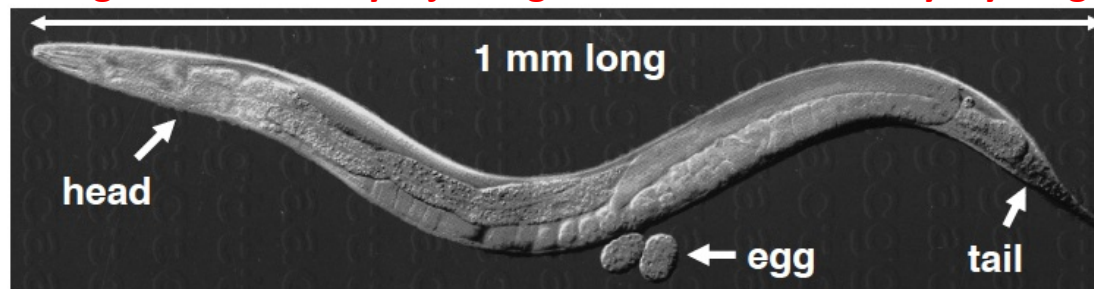
- Yeast cells share many basic biological properties with human cells.
- Genetic manipulation in yeast is easy and cheap
- At least 20 per cent of human genes known to have a role in disease have counterparts in yeast. This suggests that such diseases result from the disruption of very basic cellular processes.
- Thousands of drugs can be tested on yeast cells containing mutated human genes to see if the drugs can restore normal function.

# Studying tau in yeast: molecular mechanisms of protein folding

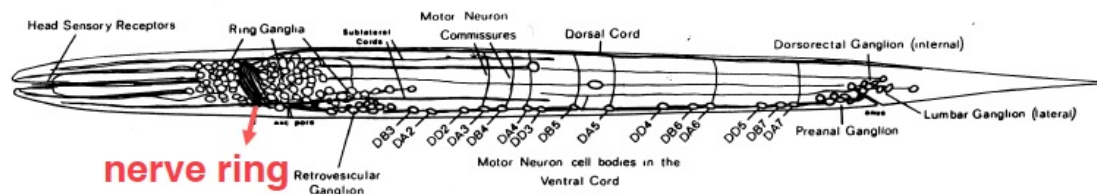


# Model organism *C. elegans*

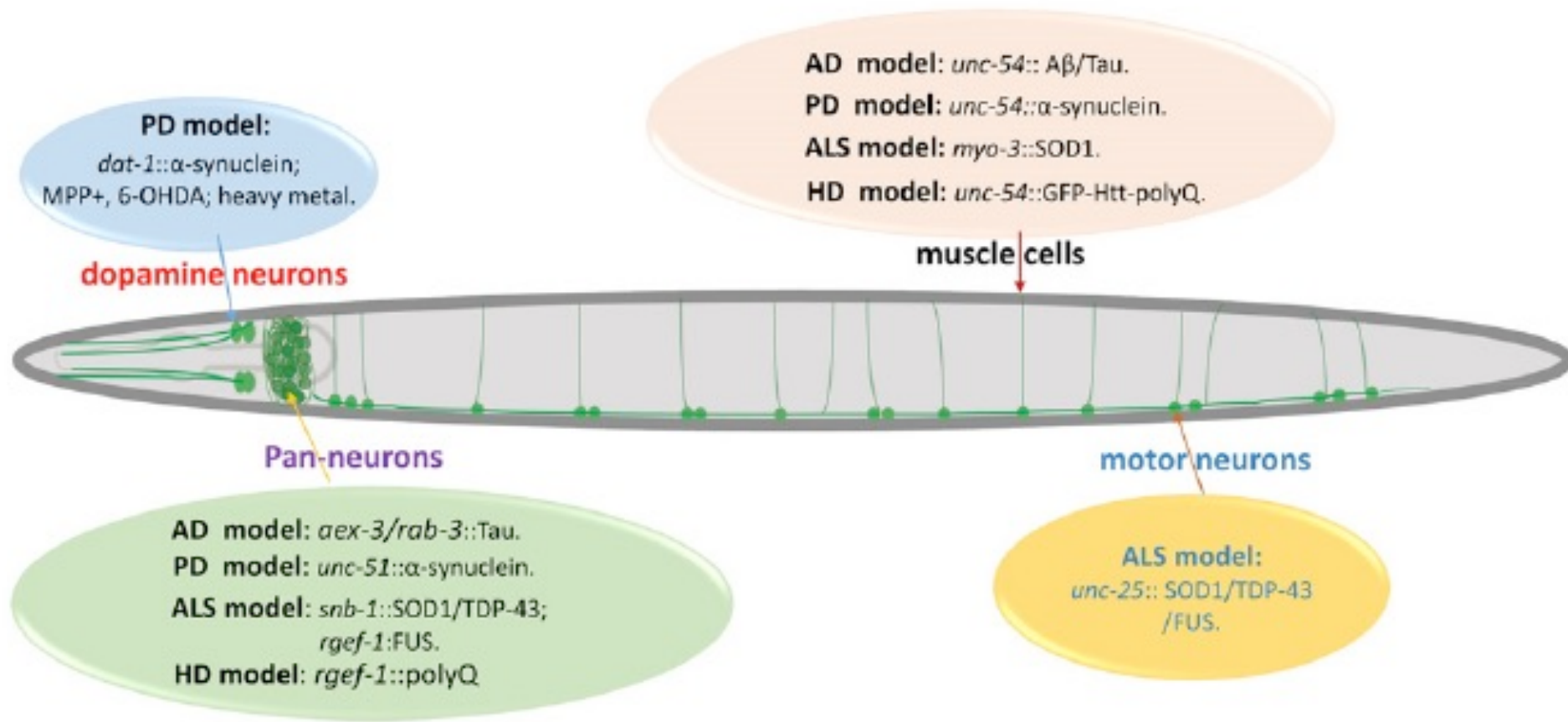
- \*Small, huge brood sizes.
- \*can be safely used in the laboratory.
- \*easily and cheaply cultivated.
- \*Rapid life cycle, 3 days at 20°C, Life span: 2-3 weeks.
- \*Sequenced genome, ~19,000 genes.
- \***Complete cell lineages with constant cell position and cell number, so it is possible to know which cell is derived from which.**
- \*Starved worms can be kept for months, and recovered quickly
- \*Can be frozen at -80°C for long-term storage.
- \*Powerful forward genetic (EMS mutagenesis), reverse genetic (RNAi) and molecular tools.
- \***Transparent cuticle makes it very easy to do live imaging.**
- \***Easy to make transgenic animals by injecting DNA or dsRNA into syncytial gonads.**



*C. elegans* hermaphrodite adult



# Taking advantage *C. elegans* to study neurodegeneration



# Studying Alzheimer's disease in flies: *D. melanogaster*



Spot Magn Det WD | 500 µm  
4.0 34x SE 12.1 EME UKA 10-0500-5

Prüßing et al, 2013, Molecular  
Neurodegeneration

## Advantage

No ethical  
problems/no restrictions according to animal protection laws

Easy and cheap to maintain  
in large quantities, time and cost effective handling

Genetic manipulation is fast  
and inexpensive (3 month, < \$ 500 per transgene)

Plethora of available  
resources/stocks (e.g. genome-wide RNAi-library)

Short generation time (~10 days),  
short life span (2-3 month)- > easy to use for screens

Fully sequenced and annotated genome

Good conservation of basic  
signaling pathways and cellular processes in general

Low redundancy/reduced  
number of paralogous genes compared to vertebrates

Probably best  
analyzed/understood multi- cellular organism

More complex  
organism compare to *C. elegans* and yeast

Balancer chromosomes allow  
the maintenance of mutations/transgenes without genotyping

## Disadvantage

Brain anatomy, cardiovascular system and  
respiration systems differs substantially from humans

No easy measure of complex behavior

Only basic measures of cognitive decline

Sometimes poor  
conservation of proteins/protein function

Maintenance as living cultures only,  
no permanent conservation (e.g. frozen stocks) possible

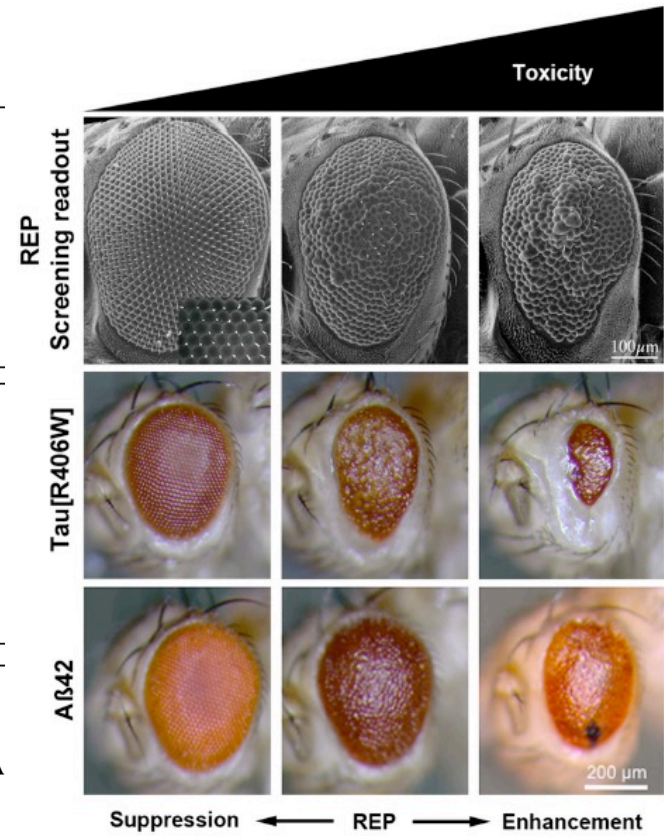
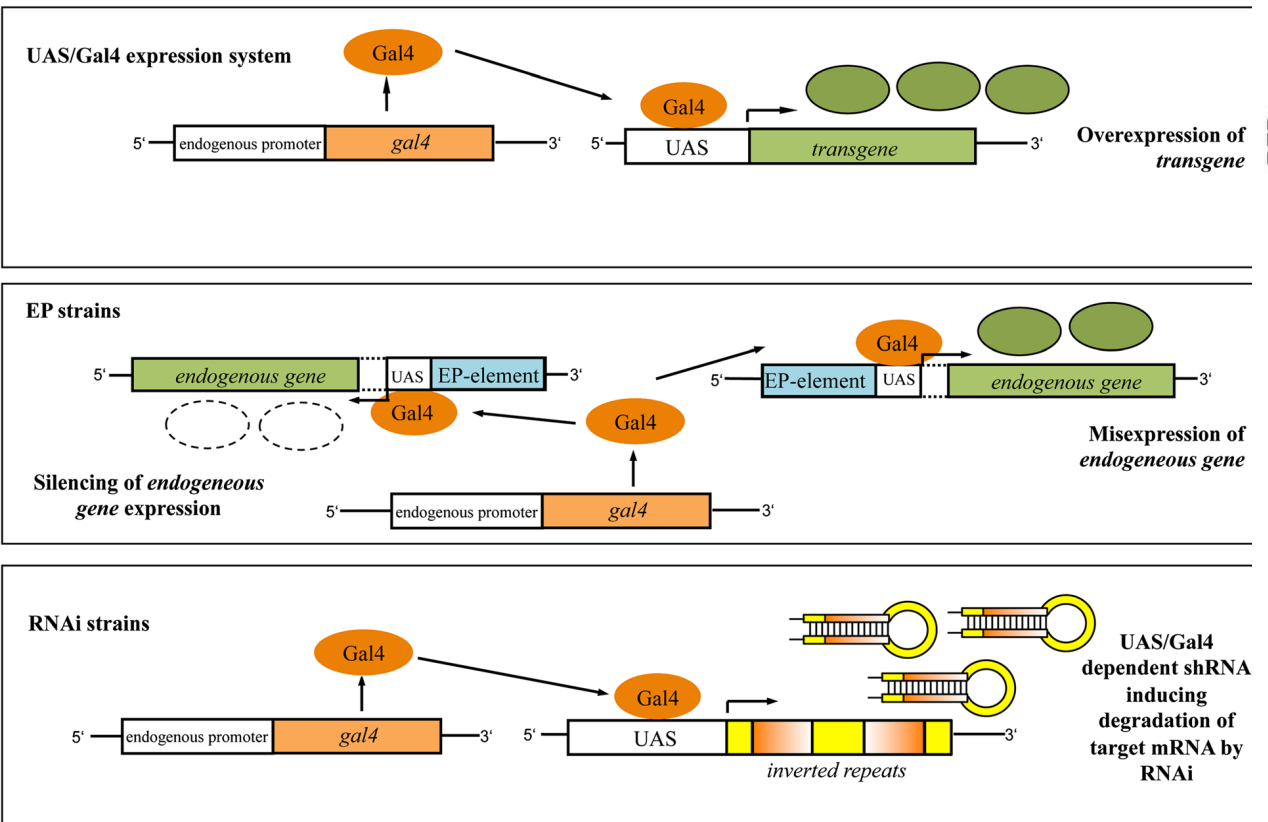
Less complex and adaptive  
immune system as in vertebrates

Effects of drugs on the organism might differ  
strongly (e.g. conversion of pro-toxins to toxins in liver)

# Screens for modifiers of toxicity induced by expression of AD-linked genes in *D. melanogaster*

Transgene causing a REP	Screened library	Results	Reference
hTau[V337M]	2,276 EP strains	<ul style="list-style-type: none"> <li>• Kinases, phosphatases (CDK5, GSK3<math>\beta</math>, PAR1)</li> <li>• Apoptosis</li> <li>• Novel: Ataxin 2, Fmr1</li> </ul>	Shulman & Feany [69]
hTau[V337M]	1,250 P-element strains	<ul style="list-style-type: none"> <li>• Cytoskeletal components</li> <li>• Molecular chaperones</li> <li>• Chromatin remodelling</li> </ul>	Blard <i>et al.</i> [70]
hTau[WT]	920 P-lethal strains 895 EY strains	<ul style="list-style-type: none"> <li>• Kinases, Phosphatases</li> <li>• Autophagy/lysosomal</li> <li>• RNA processing</li> <li>• Chromatin regulation</li> <li>• Cytoskeletal</li> </ul>	Ambegaokar <i>et al.</i> [71]
GMR > A $\beta$ 42	1,963 EP strains	<ul style="list-style-type: none"> <li>• Secretory pathway</li> <li>• Cholesterol homeostasis</li> <li>• Chromatin regulation</li> </ul>	Cao <i>et al.</i> [72]
Pan neural Arctic A $\beta$ 42 life span reduction	3,000 de novo insertions of transposable elements	<ul style="list-style-type: none"> <li>• Fenton chemistry and oxidative stress are involved in AD pathology</li> <li>• Ferritin expression protects from <math>\beta</math>-amyloid toxicity</li> </ul>	Rival <i>et al.</i> [61]

# Gene expression techniques in *D. melanogaster* to study AD-linked genes



rough eye phenotypes (REP) used as a readout for modifier screens.

# Model zebrafish



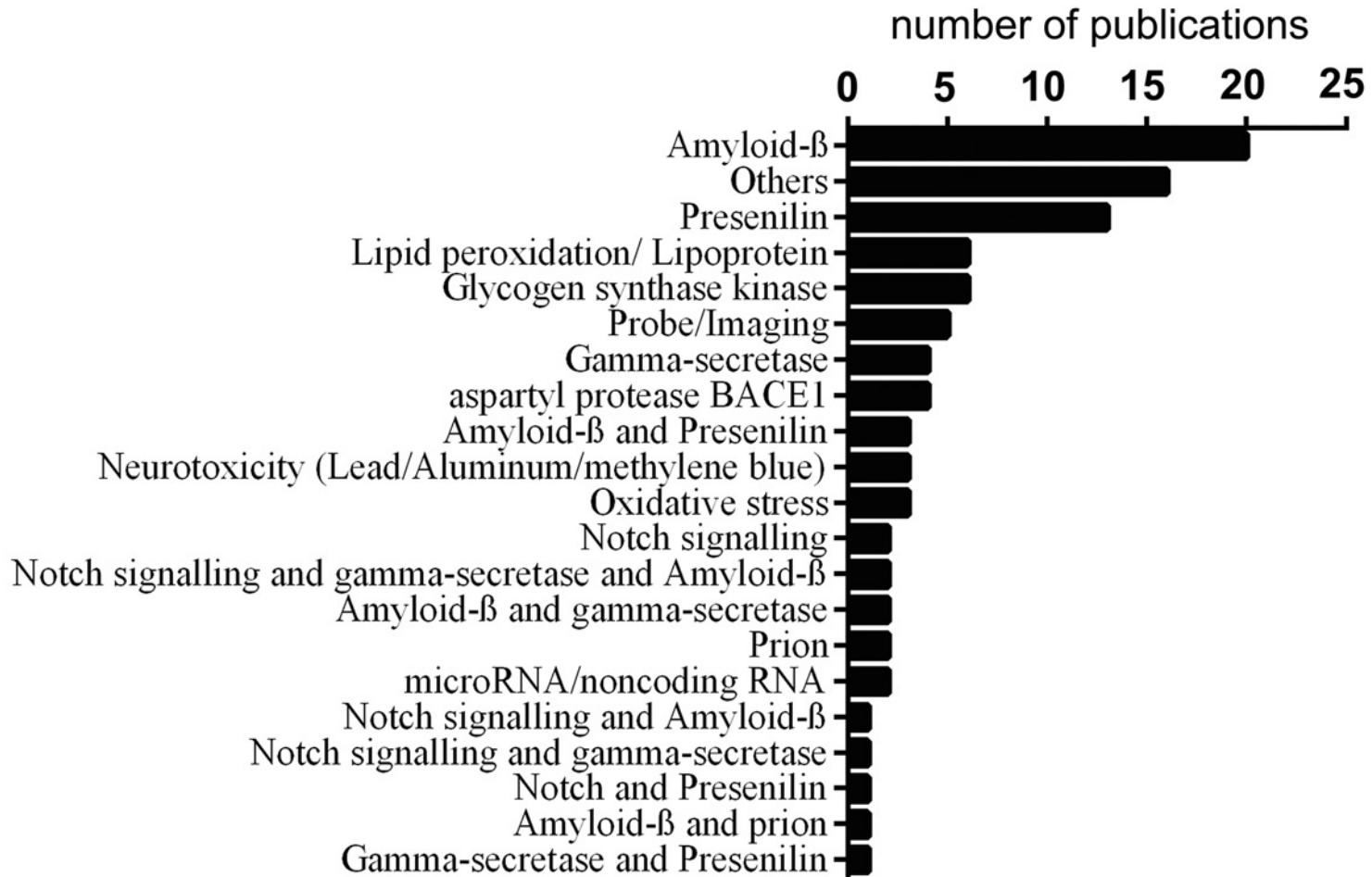
## Key facts

- The use of zebrafish (*Danio rerio*) as a model organism began in the 1960s.
- The zebrafish is a tropical fish native to southeast Asia.
- The zebrafish is about 2.5 cm to 4 cm long.
- In its larval stages it is transparent
- Important model eg for muscular dystrophy, cancers.
- The complete genome sequence of the zebrafish was published in 2013, 1,505,581,940 base pairs in length, 26,247 protein-coding gene.

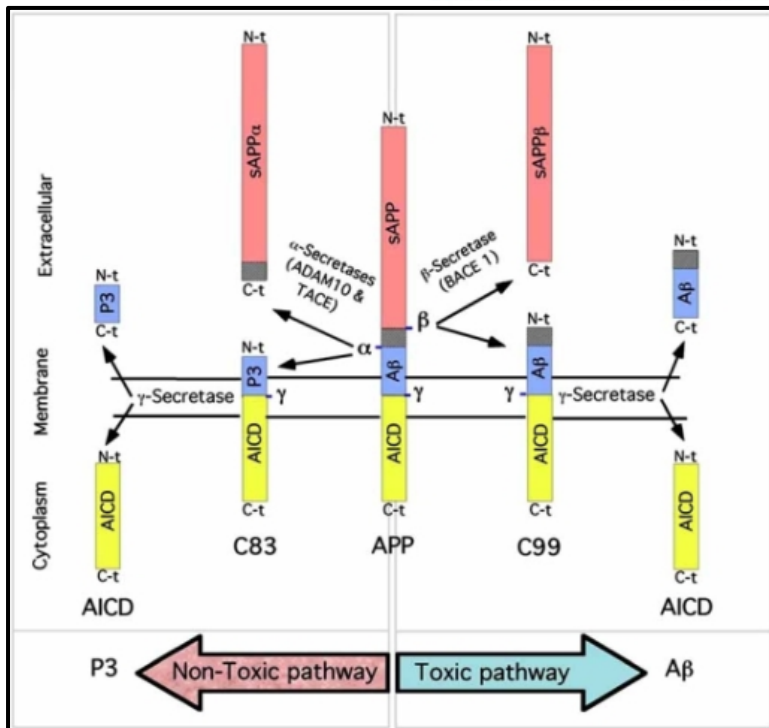
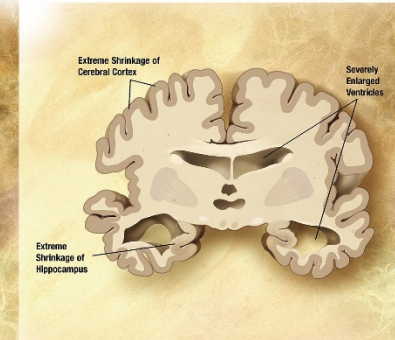
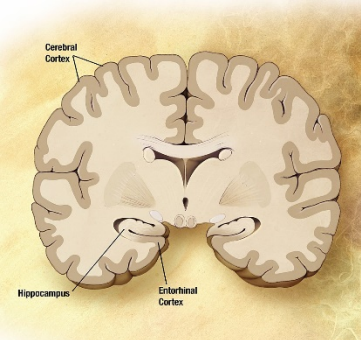
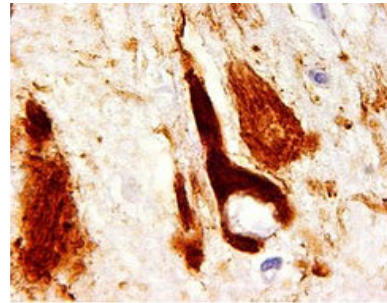
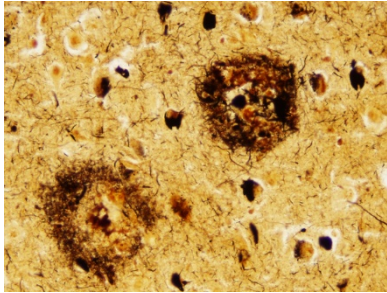
## Benefits of the zebrafish

- small and robust, cheaper to maintain than rodents, weekly hundreds of offspring
- Break of daylight triggers mating in zebrafish (many other fish only lay eggs in the dark).
- grow fast, developing as much in a day as a human embryo develops in one month.
- Zebrafish embryos are nearly transparent.
- Embryos develop outside the mother's body: good early development model.
- share 70 per cent of genes with humans.
- 84 % of human disease genes have zebrafish counterpart.
- As a vertebrate, the zebrafish has the same major organs and tissues as humans. Muscle, blood, kidney and eyes share many features with human systems.
- unique ability to repair heart muscle.
- 
- mutations in more than 14,000 genes created

# Modelling AD in zebrafish: AD-related gene mutations and pathways



# Modelling AD in these model organisms: useful to study gene mutations and pathways, but what about other factors?



# Common neuropsychological assessment tasks seen clinically

Task	Description	Cognitive domains	References
<b>MENTAL STATUS EXAMS</b>			
Mini-Mental State Examination (MMSE)	Nineteen item (30 points) test of general cognitive status	Working memory, attention, memory (semantic), praxis, etc.	Folstein et al., 1975
Montreal Cognitive Assessment (MoCA)	A rapid screening method to assess mild cognitive dysfunction	Working memory, memory (semantic and episodic), attention, visuospatial memory, etc.	Nasreddine et al., 2005
Short Blessed Test (SBT)	A short six item test measuring general cognitive status	Memory (semantic and episodic), working memory, and attention, etc.	Blessed et al., 1968
Alzheimer's Disease Assessment Scale (ADAS)	An 11 part test that measures cognitive dysfunction	Memory (semantic and episodic), and attention, etc.	Rosen et al., 1984
<b>MEMORY TESTS</b>			
Logical memory test I and II	A short story is presented to the patient and used to test immediate memory (test I) and delayed memory (test II)	Memory (episodic), verbal recall, etc.	Wechsler, 1997
Benton Visual Retention Test (BRVT)	Visual based test of general memory	Memory (episodic), and working memory etc.	Benton, 1992

Neuropsychological testing is the only measure that provides information about a patient's current cognitive status and remains the most reliable means to clinically diagnose probable AD

# Laboratory Mouse

## Education

Caltech, Oxford, Stanford, Harvard, MIT, Princeton, Cambridge, Imperial, Berkeley, Chicago, Yale, ETH Zurich, Columbia, UPenn, John Hopkins, UCL, Cornell, Northwestern, UMichigan, Toronto, Carnegie 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



# 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

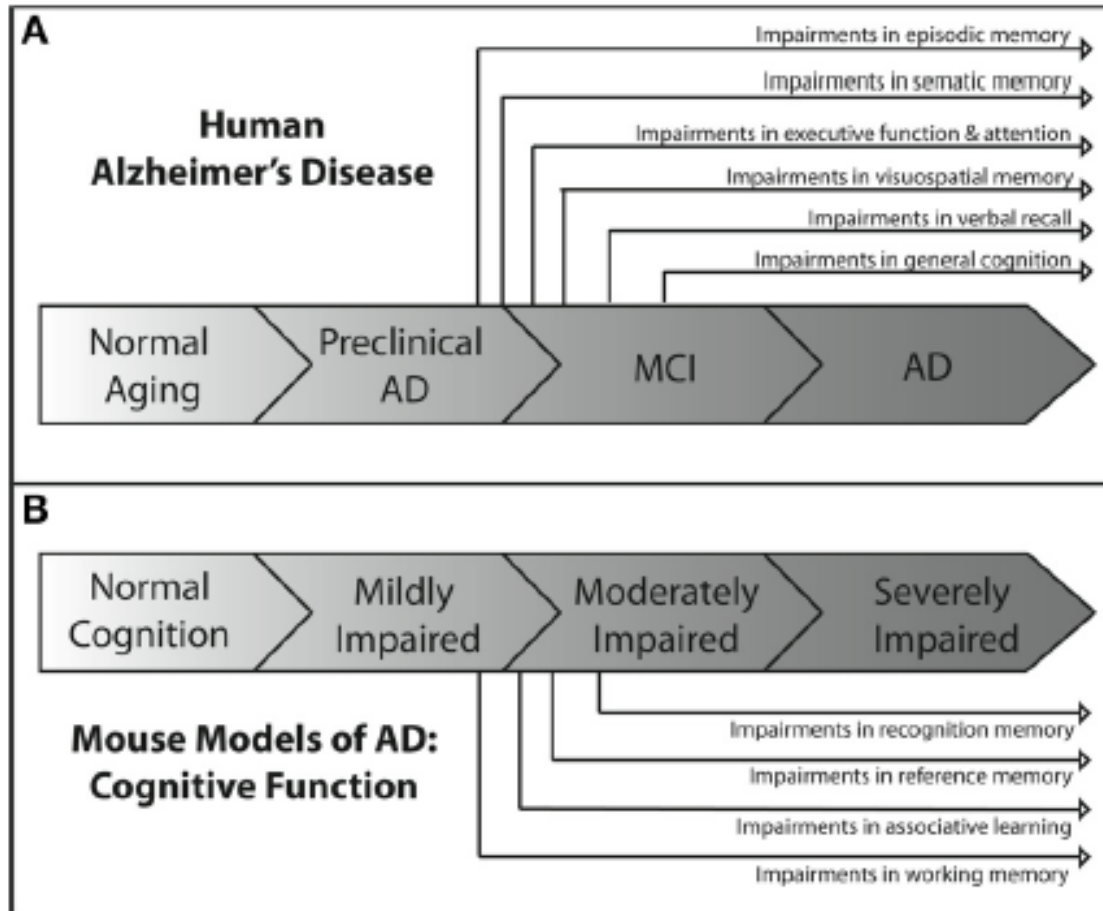
## Research Areas

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 syndrome, 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, schistosomiasis, spinal cord injury, stroke, testicular cancer, tuberculosis,

## Contact

[www.understandinganimalresearch.org.uk](http://www.understandinganimalresearch.org.uk)  
[www.animalresearch.info](http://www.animalresearch.info)  
[www.amprogress.org](http://www.amprogress.org)  
[www.speakingofresearch.com](http://www.speakingofresearch.com)

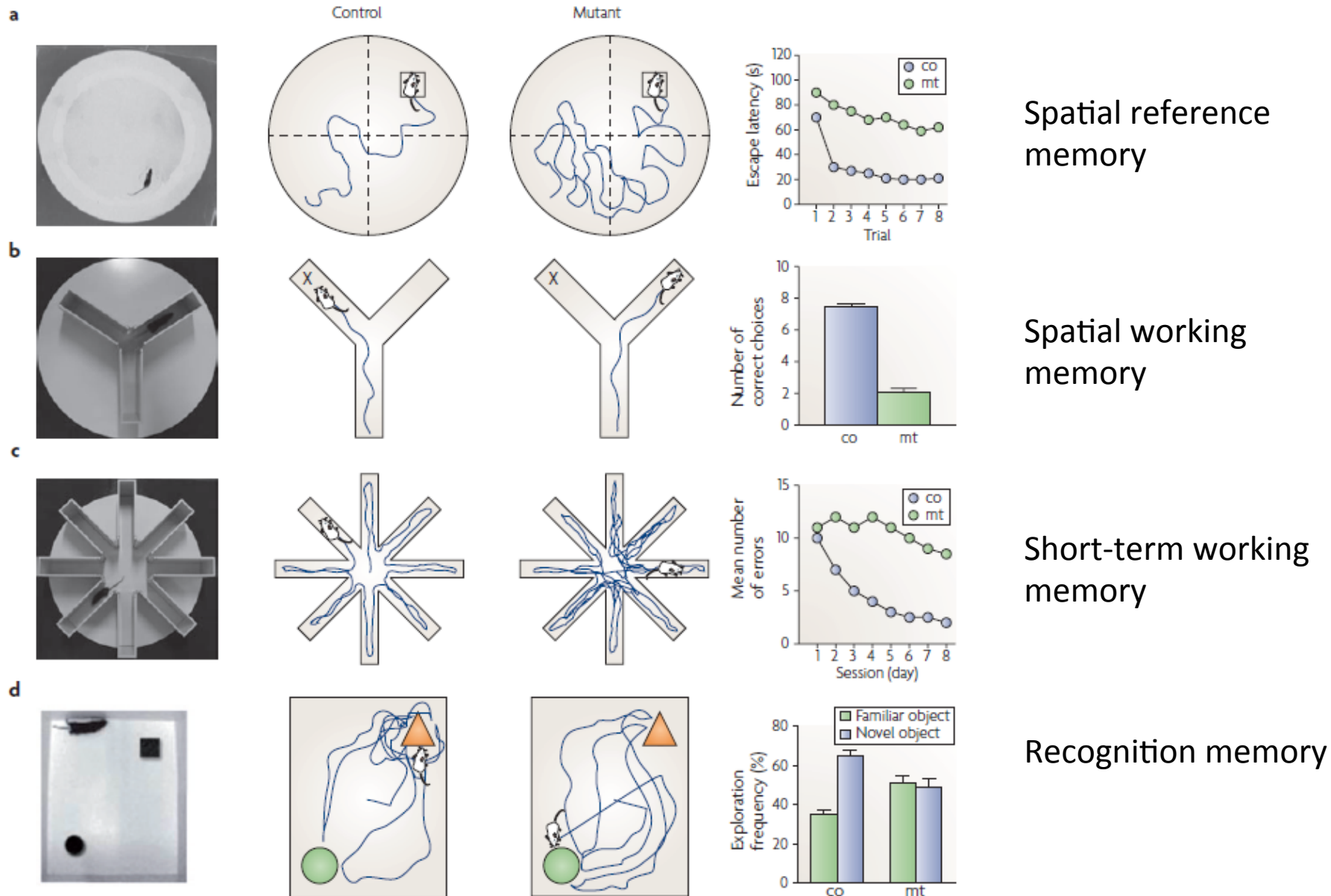
# Progression of cognitive deficits in human patients and mouse models



## Measuring cognitive deficits in rodents

Measurable construct	Exemplary behavioral paradigms
Spatial working memory	Morris water maze, Barnes maze
Associative learning tasks	Fear conditioning, passive avoidance
Reference memory / alternation tasks	Y-maze
Recognition memory	Novel object recognition
Short-term and long-term memory	Passive avoidance

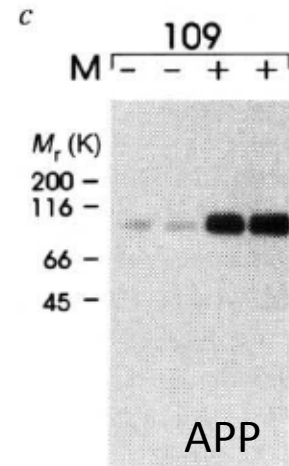
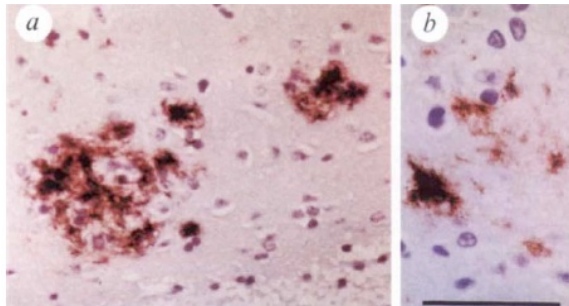
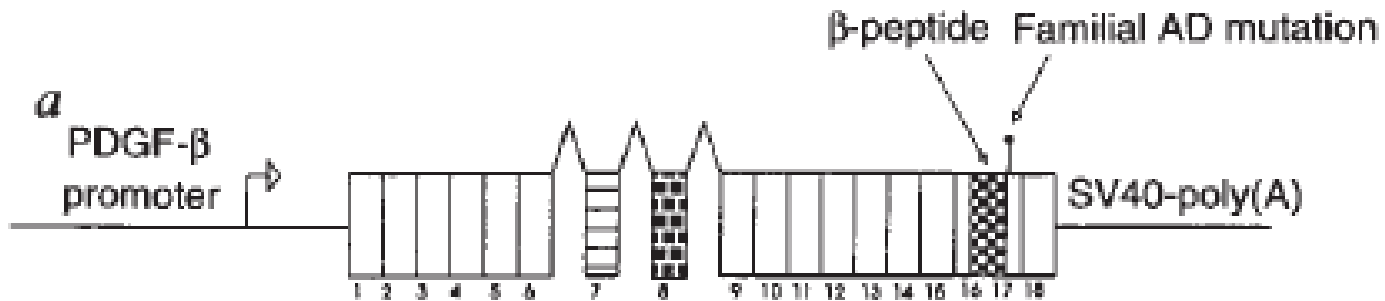
# Some behavioral tests to test cognition in mice



# Current, commonly used APP mouse models

	Strain(s)	Genetic background	Promoter	Mutation(s)	General features
Single transgenic APP-Tg	PDAPP	C57B6 × DBA2	PDGF-β	App <sup>V717F</sup>	Moderate behavioral phenotype Neuronal loss in some models
	Tg2576	B6; SJL mixed background	hamster prion protein (PrP)	App <sup>K.M67 Q/671NL</sup>	
	APP23	C57BL/6	mouse Thy1	App <sup>K.M67 Q/671NL</sup>	
	J20	C57BL/6	PDGF-β	App <sup>K.M67 Q/671NL,V717F</sup>	
	TgCRND8	Hybrid C3H/He-C57BL/6	hamster prion protein (PrP)	App <sup>K.M67 Q/671NL,V717F</sup>	
Double transgenic APP-Tg × PSEN1-Tg or KI	APPPS1	C57BL/6J	mouse Thy1 (APP, PS1)	App <sup>K.M67 Q/671NL</sup> PS1 <sup>I266P</sup>	Moderate behavioral phenotype Aβ accumulation from early stage Neuronal loss in some models
	5XFAD	(C57BL/6 × SJL)F1 and C57BL/6J	mouse Thy1.2 (APP, PS1)	App <sup>K.M67 Q/671NL,I716V,V717I</sup> PS1 <sup>M146L,I286V</sup>	
Triple transgenic	3xTg-AD	C57BL/6J	mouse Thy1.2 (APP, Tau) endogenous (PS1)	App <sup>K670N,M671L</sup> PS1 <sup>M146V</sup> MAPT <sup>P301L</sup>	Moderate to severe behavioral phenotype NFT formation Neuronal loss
Single App knock-in	NL-F	C57BL/6J	endogenous APP	App <sup>K.M67 Q/671NL,I716F</sup>	Minor behavioral phenotype No overexpression of APP and byproducts except for CTFβ
	NL-G-F	C57BL/6J	endogenous APP	App <sup>K.M67 Q/671NL,E693G,I716F</sup>	

# Single transgenic APP mouse models



Games et al, 1995, Nature

# Single transgenic APP mouse models – behavioral phenotypes

Strain	Passive avoidance	Fear Conditioning	Y/T-Maze Alternation	NOR	RAWM	MWM	Barnes Maze	Age in Months
PDAPP: [1]					[2]	[2]		1-2m
				[3]		[4]		3-5m
				[3] [5]	[6]			6-8m*
				[3]		[4]		9-11m
				[6]		[6]		12-14m
					[2]	[4]		15-18m
				[6]		[6]		≥ 19m

Data not found

Same as control

Increased from control

Decreased from control

Webster et al, 2014, Frontiers in genetics

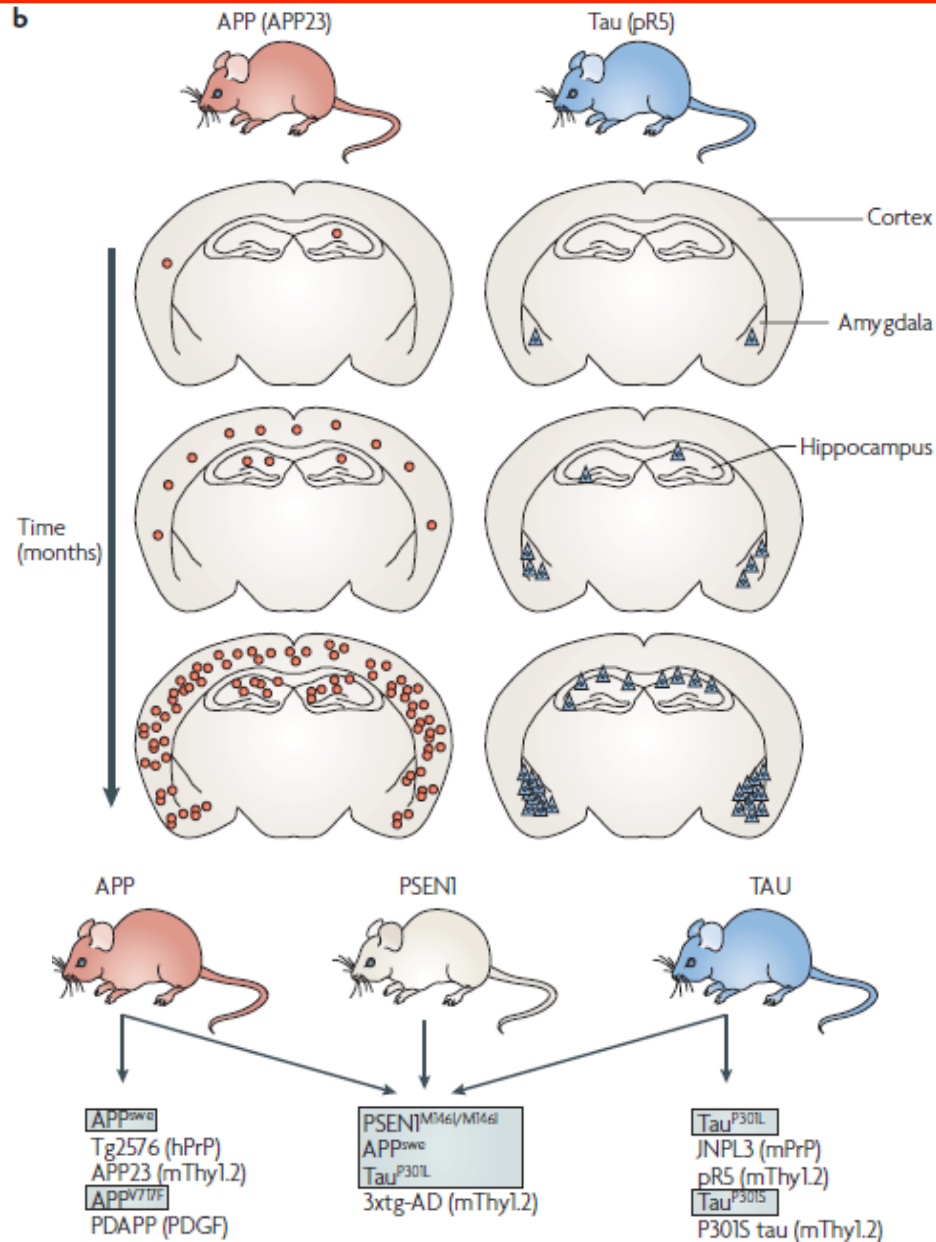
# Conclusions: Single transgenic APP mouse models

Mutations in APP or one of the presenilins are sufficient to cause the complete AD phenotype of progressive cognitive impairment, plaques, tangles and neuron loss in the **human** brain → when expressed in mouse brain, human transgenes with these same mutations replicate some, but not all, aspects of AD.

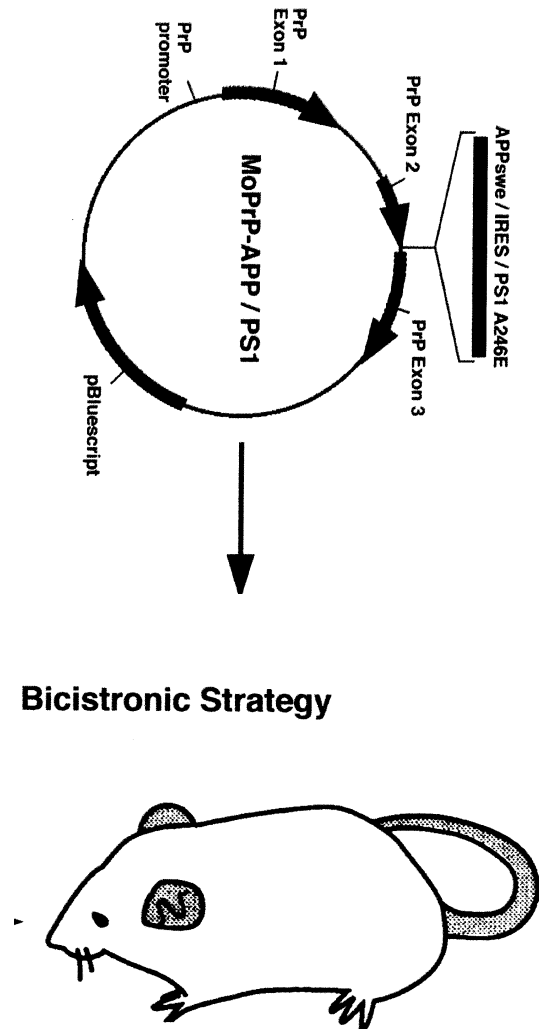
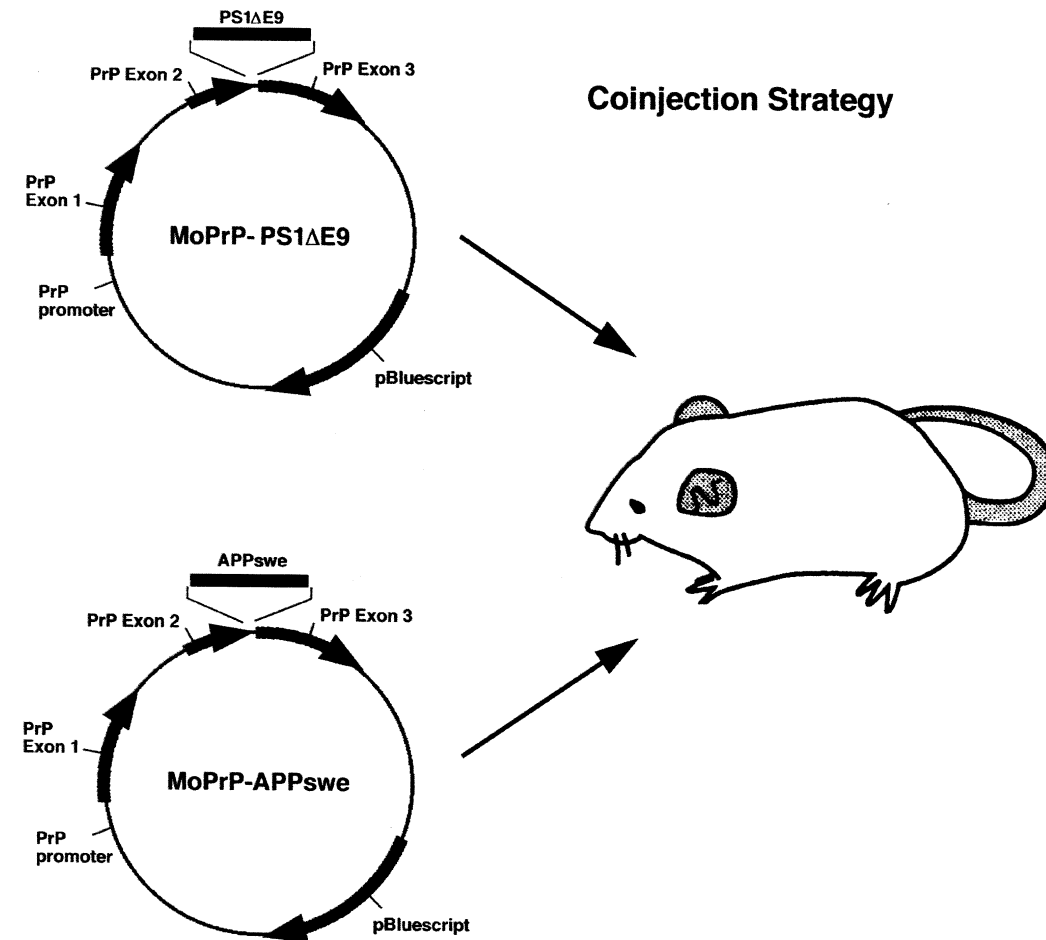
APP transgenic **mice** develop memory loss and plaques, with no NFTs and little or no neuron loss. Although APP transgenic mice fail to replicate the full human disease, they appear to faithfully simulate the pre-dementia phase of AD. Presenilin variants produce no neuropathology, but potentiate plaque deposition in APP transgenic mice.

Short-term working  
Memory

# Limitations of single transgenic APP mouse models



# Double transgenic AD mouse models –



# Double transgenic AD mouse models – behavioral phenotypes

Strain	Passive avoidance	Fear Conditioning	Y/T -Maze Alternation	Novel Object Recognition	RAWM	MWM	Barn es Maze	Age in Months
APP/PS1: [1]								1-2m
					[2]			3-5m*
		[3] [4]	[5, 6]		[7]	[6]	[4]	6-8m
		[3]			[8]	[3]		9-11m
	[9]	[10]			[2]	[11]		12-14m
						[11]	[12]	15-18m
								≥19m

Data not found

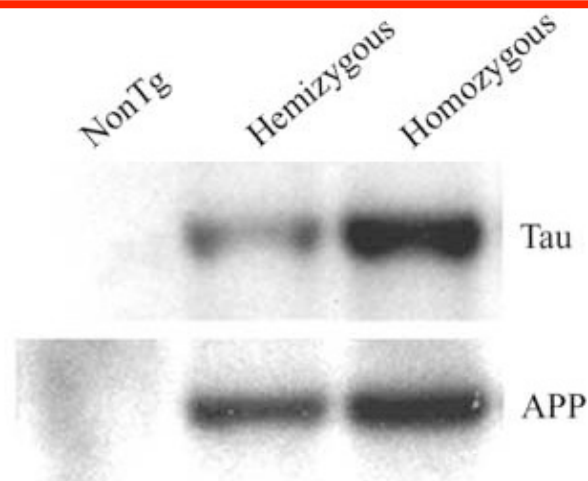
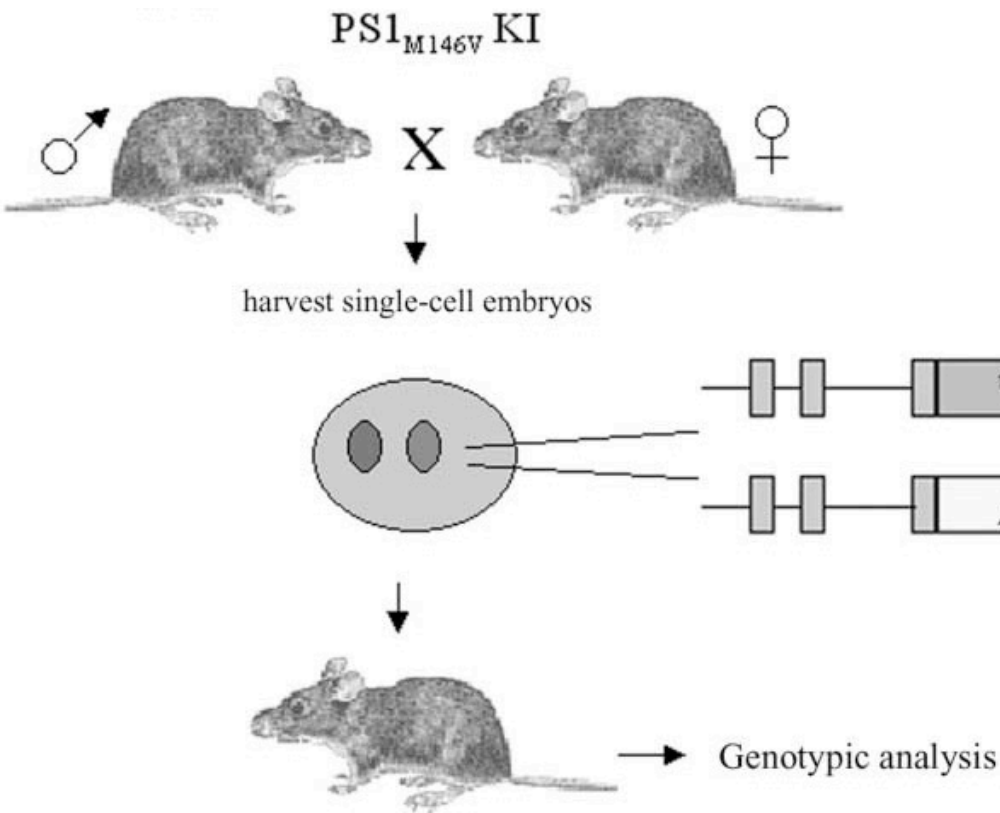
Same as control

Increased from control

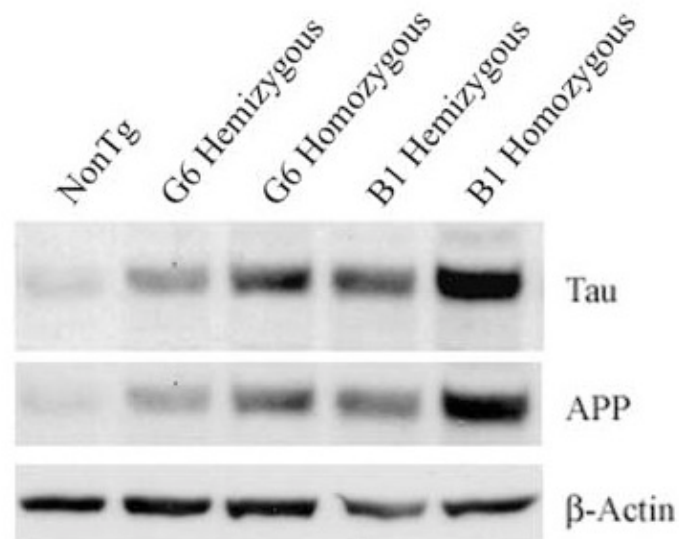
Decreased from control

Webster et al, 2014, Frontiers in genetics

# 3xTg-AD model; generation of the mice

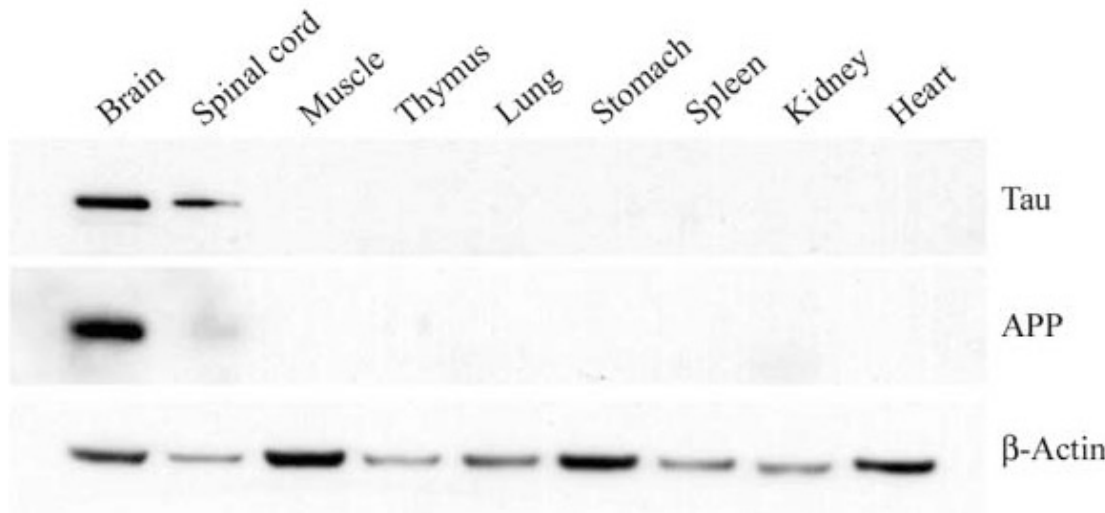


Southern blot

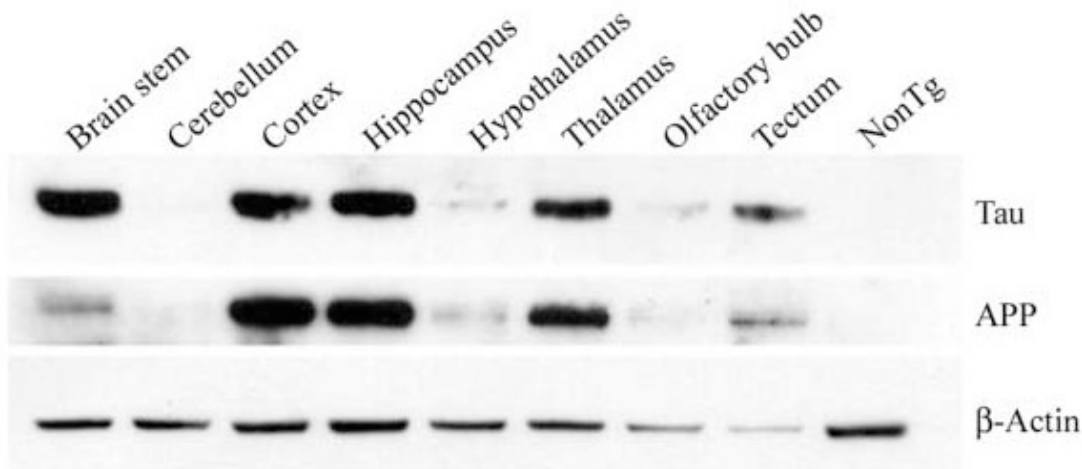
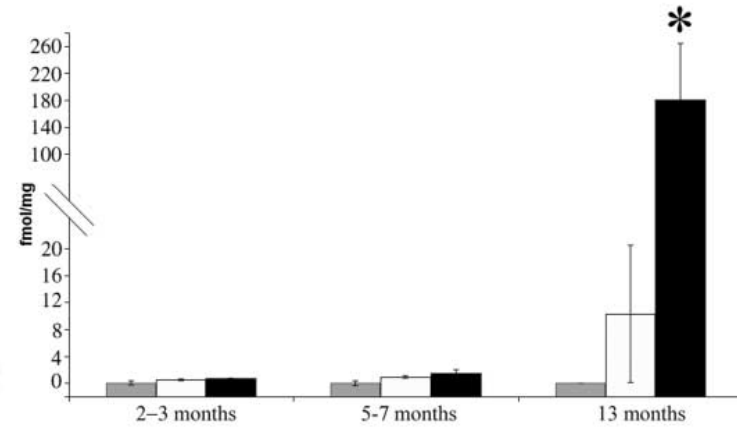


Western blot

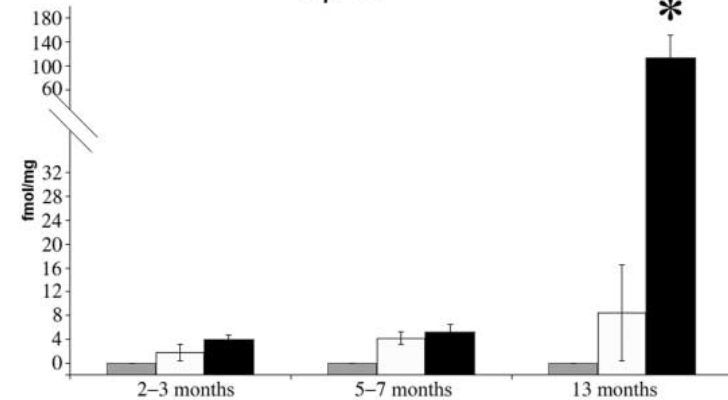
# 3xTg-AD model; characterization



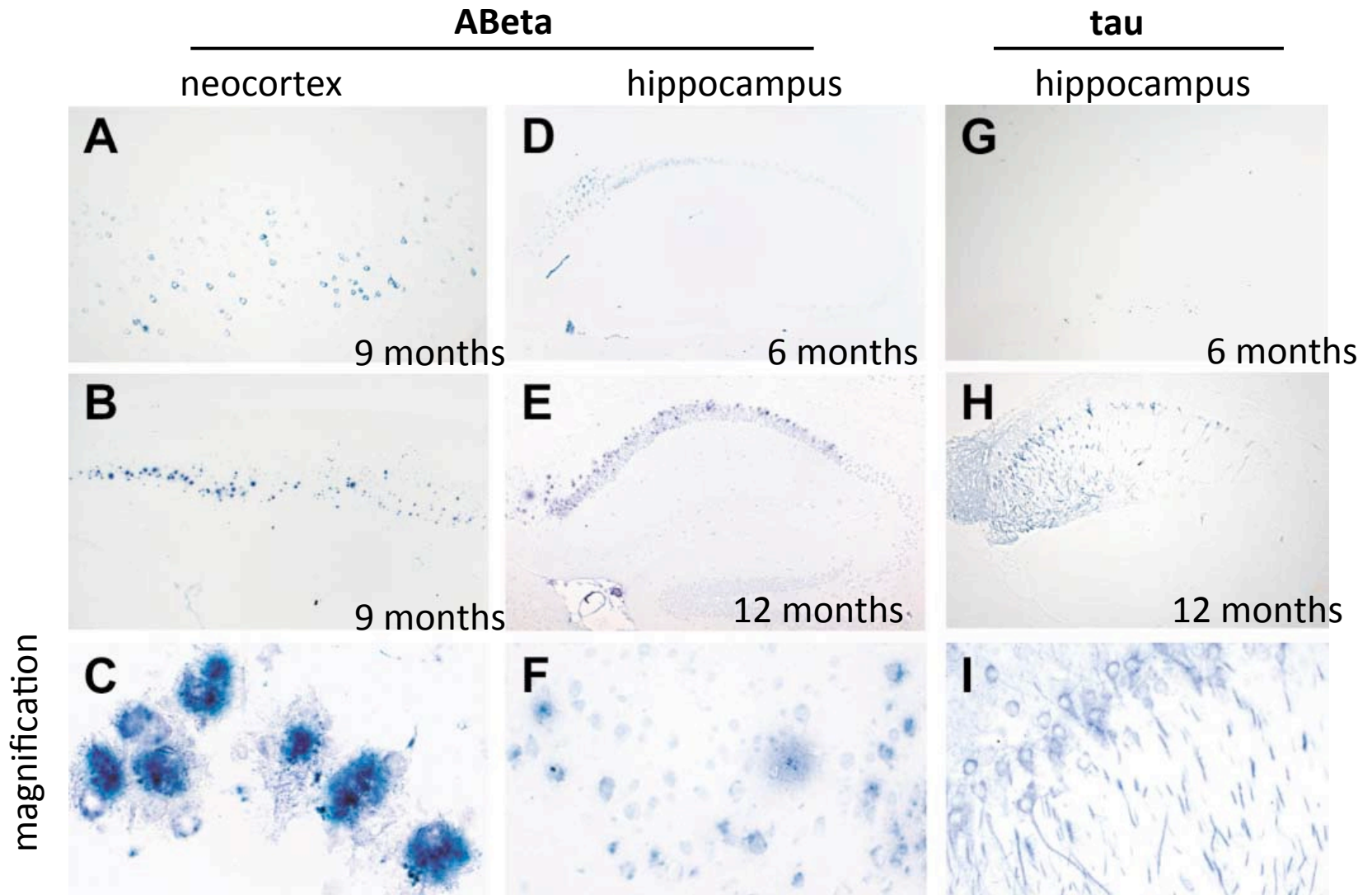
A $\beta$  42



A $\beta$  40

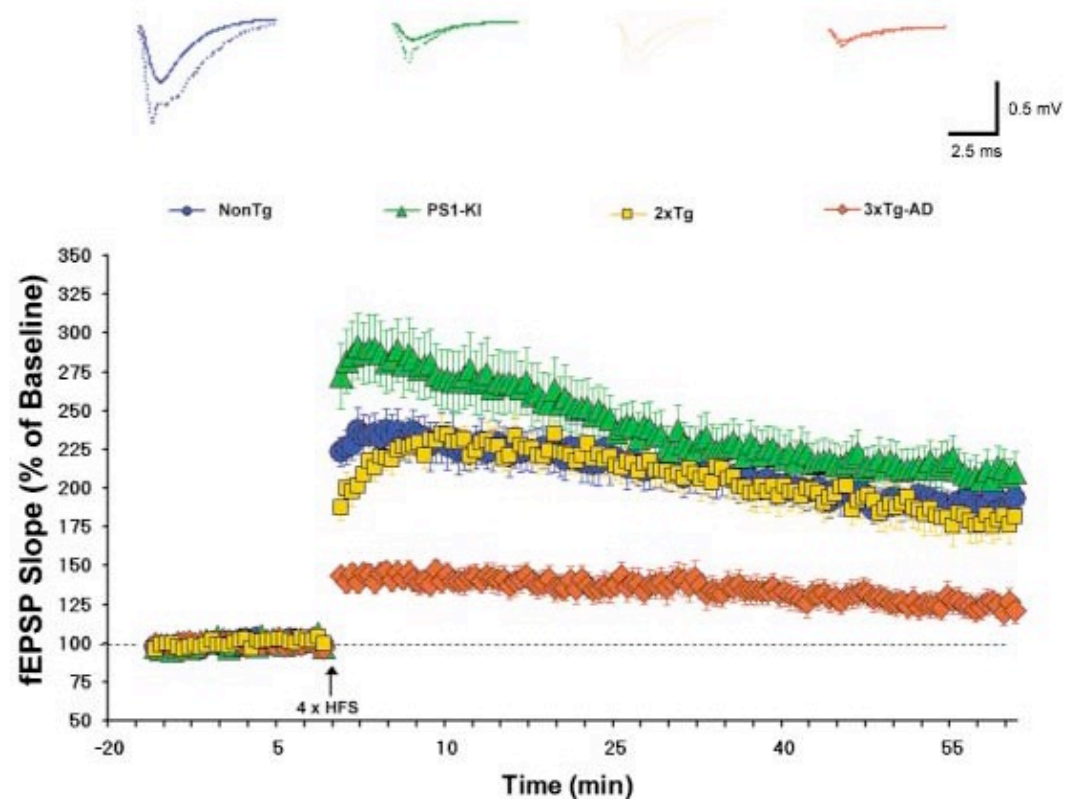
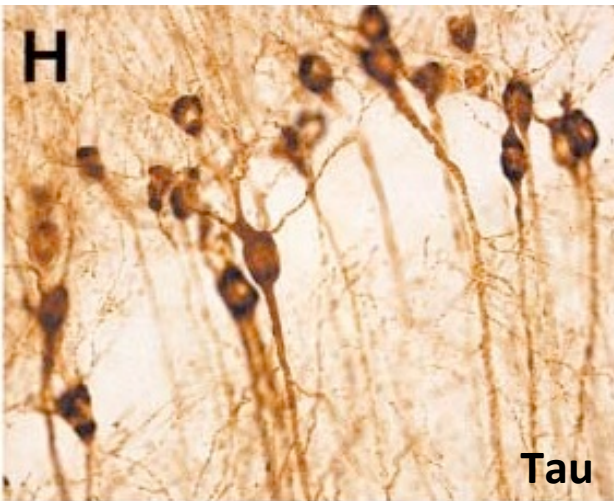
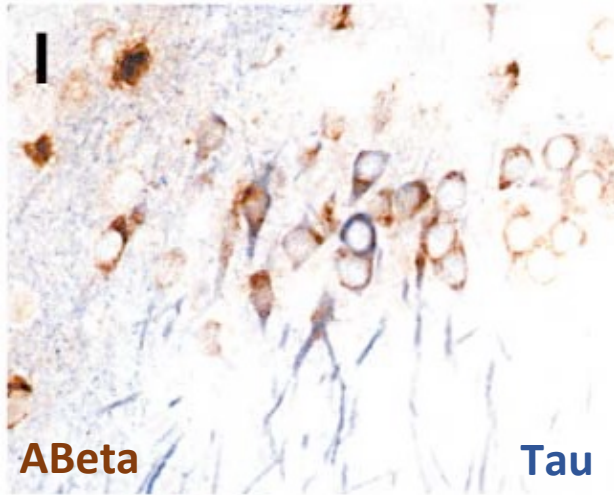


# 3xTg-AD model; ABeta pathology precedes tau pathology



# 3xTg-AD model; characterization

Oddo et al, 2003, Neuron

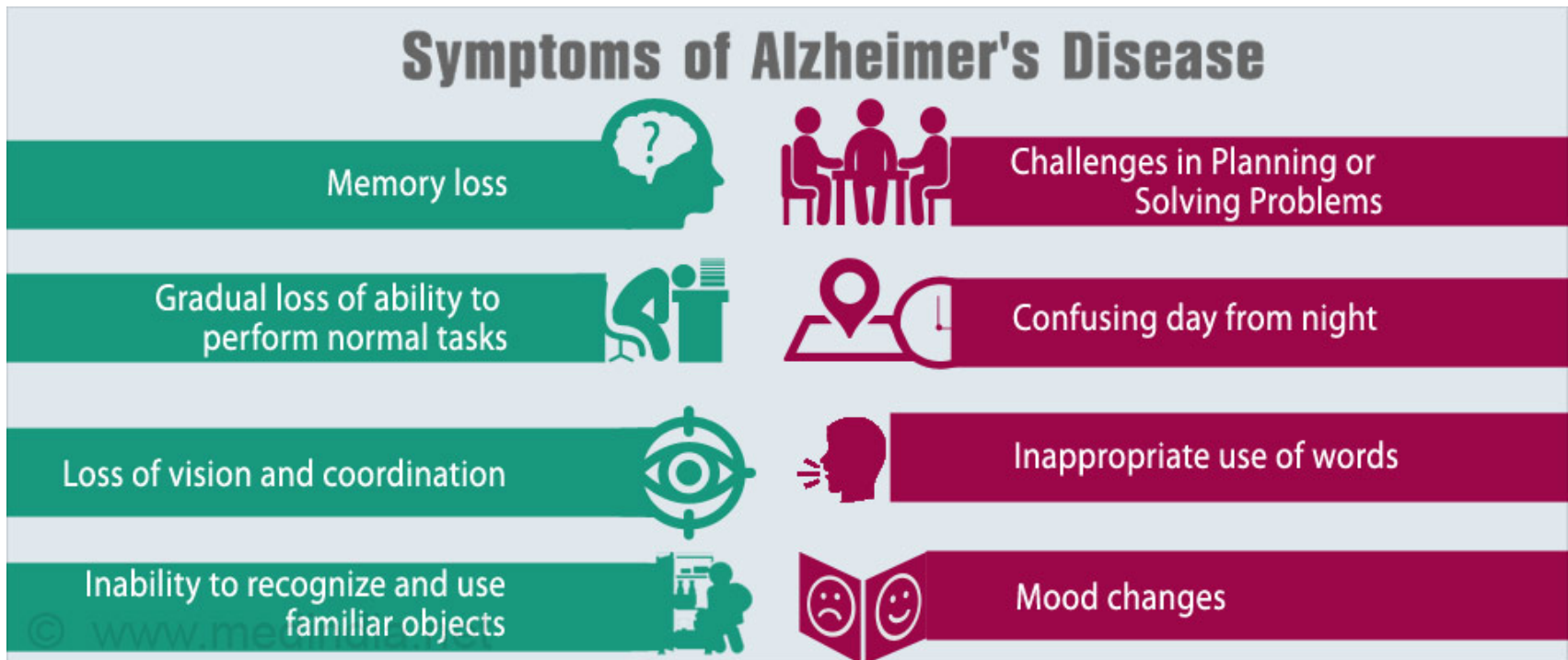


# Behavioral paradigms to study AD – learning & memory

Strain	Passive avoidance	Fear Conditioning	Y/T-Maze Alternation	NOR	RAWM	MWM	Barnes Maze	Age in Months
PDAPP: [1]					[2]	[2]		1-2m
				[3]		[4]		3-5m
				[3] [5]	[6]			6-8m*
				[3]		[4]		9-11m
				[6]		[6]		12-14m
					[2]	[4]		15-18m
				[6]		[6]		≥19m

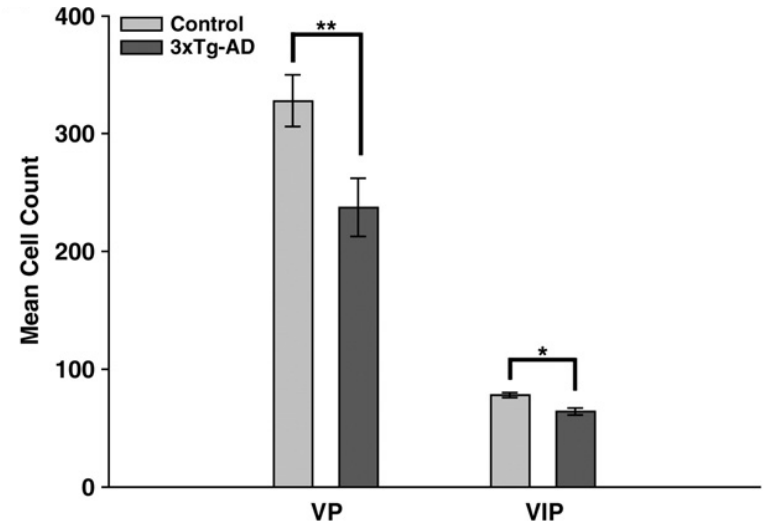
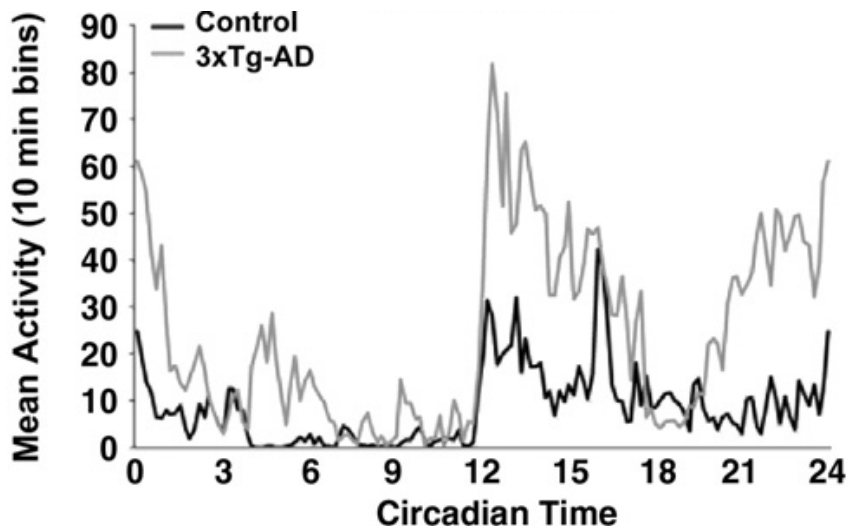
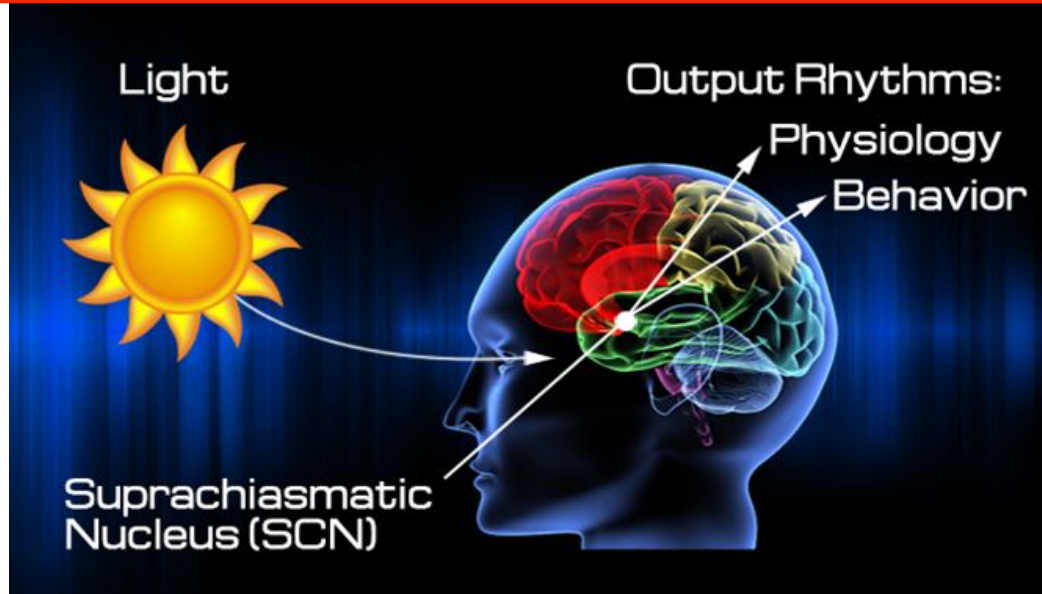
3xTgAD: [30]				[31]		[31]		1-2m
	[31]		[32]			[31] [33]		3-5m*
		[34]	[35]	[31]		[34]		6-8m
	[35]		[32]	[31]		[36]		9-11m
	[37]		[32]	[37, 38]		[33]	[39]	12-14m
				[40]		[36]		15-18m
								≥19m
Data not found		Same as control		Increased from control		Decreased from control		

# AD-like, cognitive and non cognitive symptoms assessed in behavioral paradigms



Measureable construct	Exemplary behavioral paradigms
Anxiety disturbances	Elevated plus maze, open field test, light-dark test, marble burying
Depressive symptoms	Forced swim test, sweet-preference test
Aggressive behaviors	Resident intruder test
Locomotion deficits, circadian activity	Home cage activity, rotarod-test, catwalk test

# Triple transgenic model; behavioral characterization, circadian rhythm (7.5-11 months)

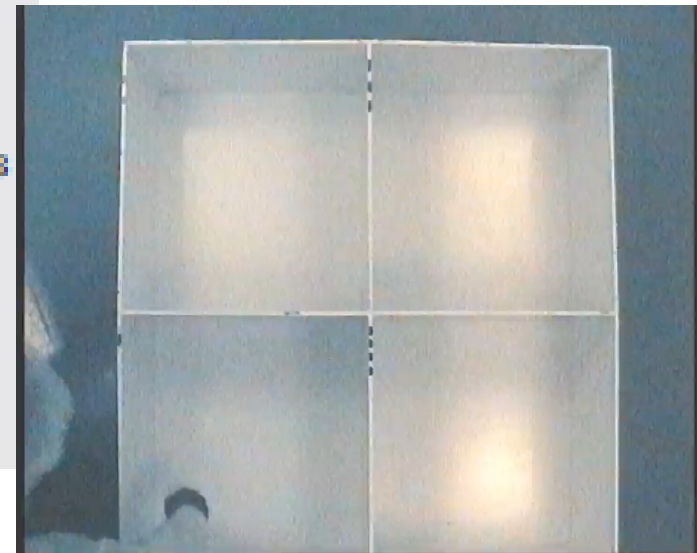
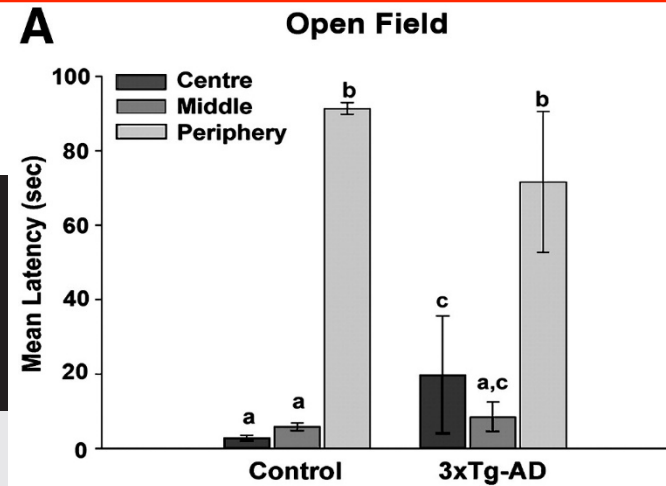


Neurons in suprachiasmatic nucleus

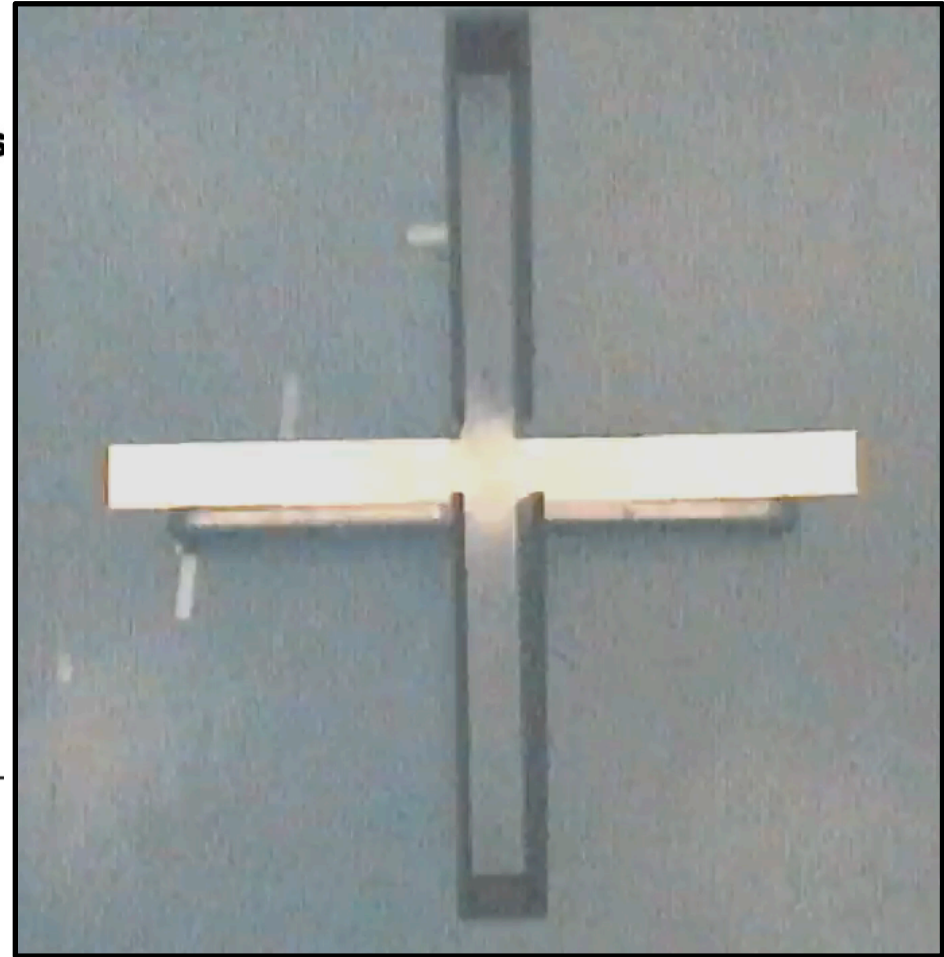
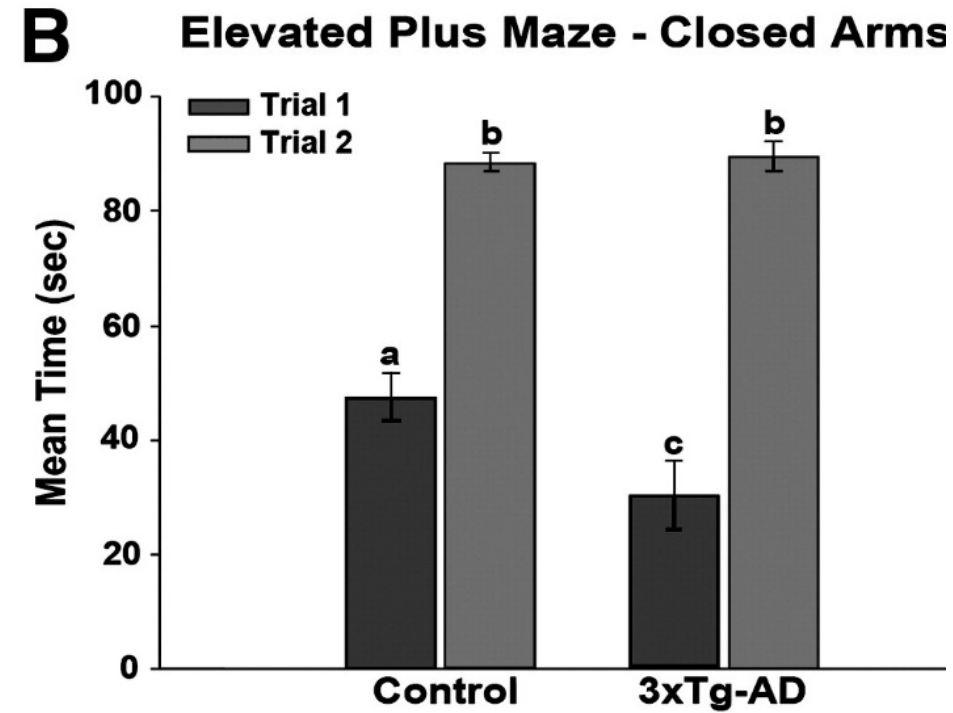
# 3xTg-AD model; behavioral characterization – anxiety, motor, learning (7.5-11 months)

**Table 1 – Summary of the behavioral test battery and circadian analyses for female 3xTg-AD mice.  $P < 0.05$  indicates a significant difference between genotypes, and n.s. a non-significant finding.**

Behavior	Significance
Test battery	
Open field	$p < 0.05$
Elevated plus maze	n.s.
Neuroassessment	$p < 0.05$ for 6/46 tests
Wire hang	n.s.
Rotarod	n.s.
Horizontal ladder	$p < 0.05$
Passive avoidance	$p < 0.05$
Active avoidance	n.s.



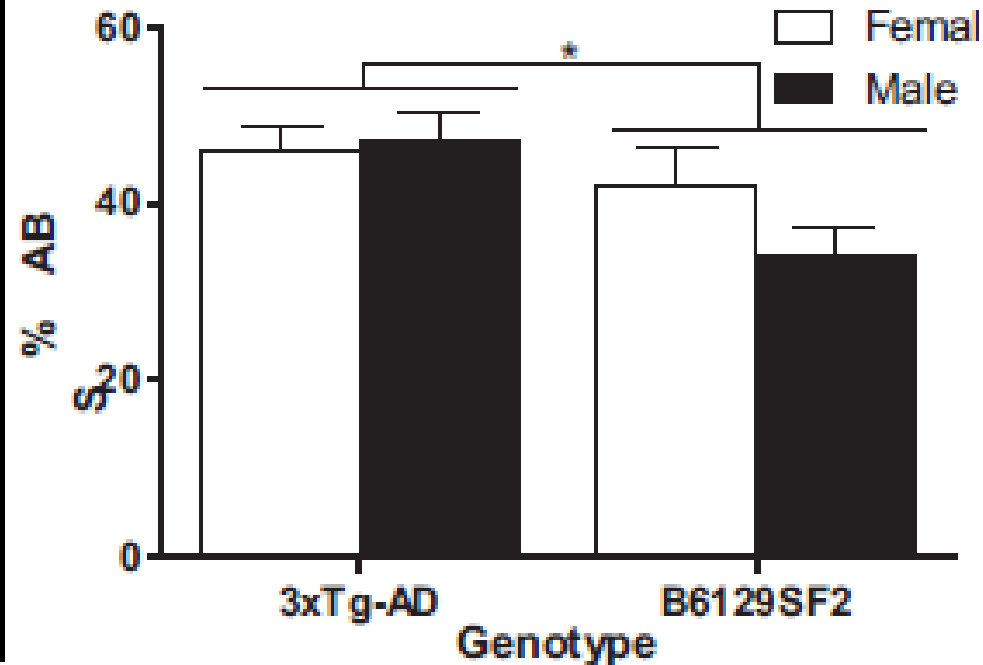
# 3xTg-AD model; behavioral characterization – anxiety (7.5-11 months)



# Impaired working memory in the triple KO (6.5 months): Y-maze

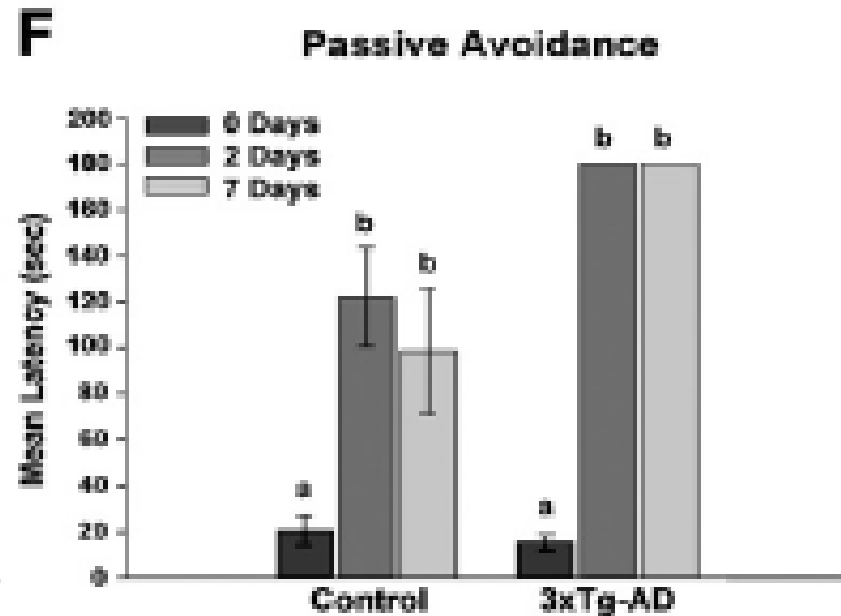
Working memory is a system for temporarily storing and managing the information required to carry out complex cognitive tasks such as learning, reasoning, and comprehension.

Spontaneous alternation behavior



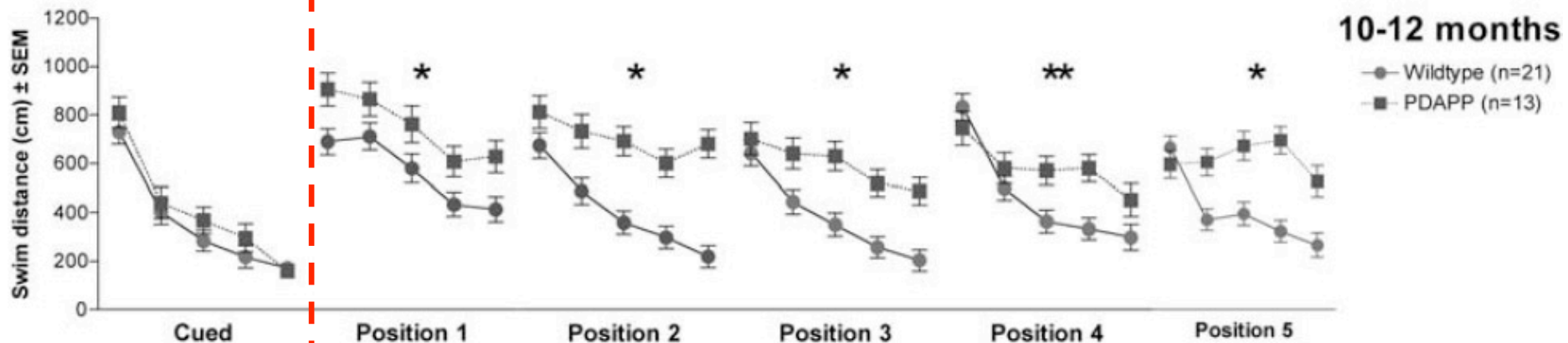
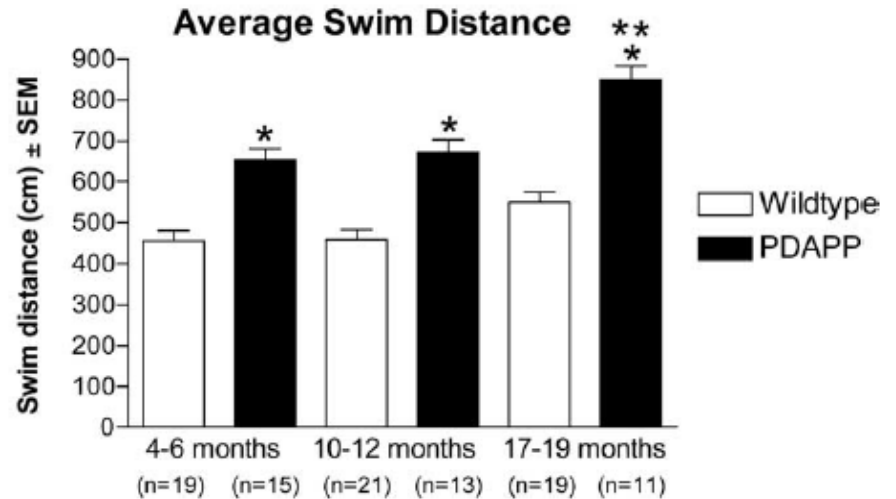
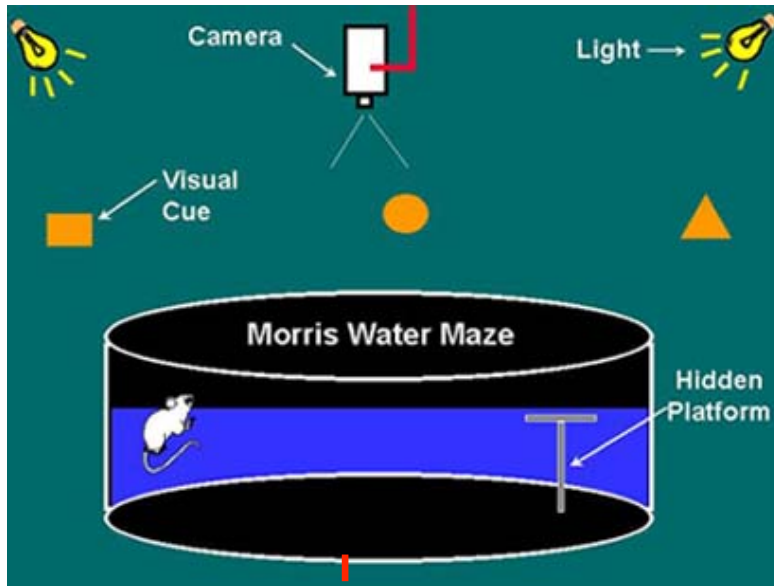
Stover et al, 2015, Behavioral Brain Research

# Triple transgenic model; behavioral characterization (7.5-11 months)



Mean latency to escape

# Example: Morris water maze

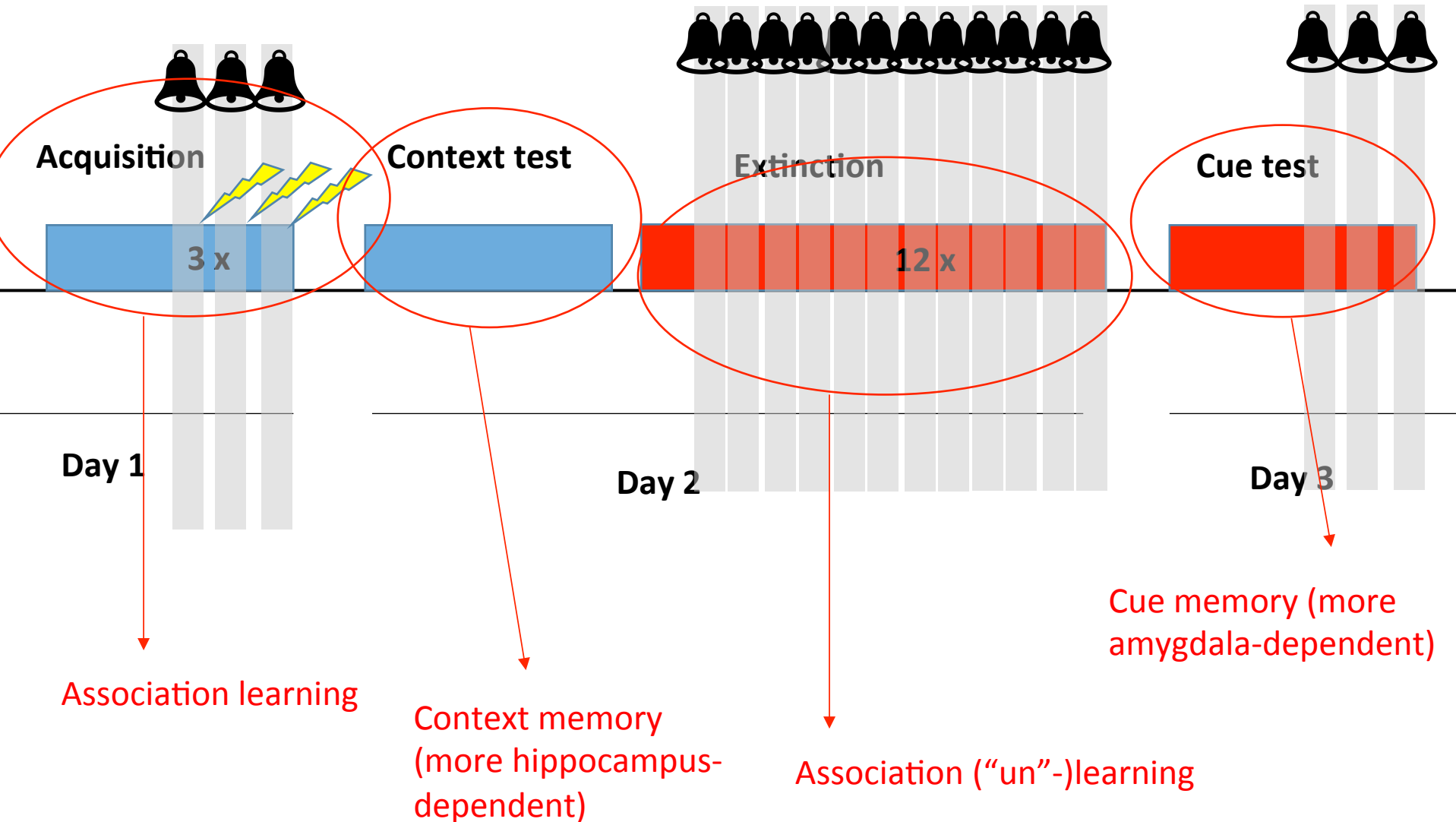


Platform visible

Spatial testing: Platform submerged

[JOVE video](#)

# Example: Fear conditioning



# Example: Fear conditioning – acquisition (classical, or Pavlovian conditioning)



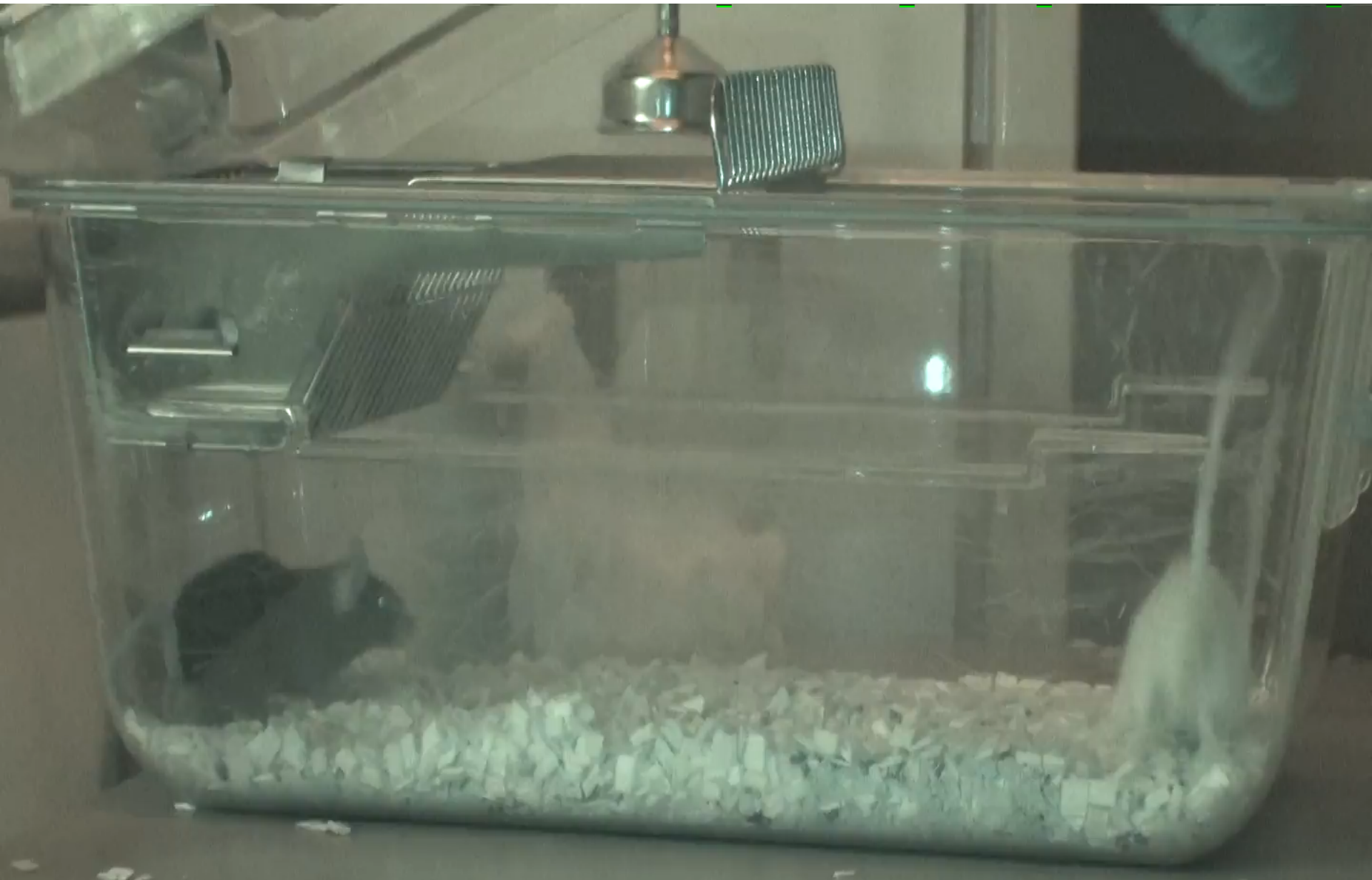
# Example for other learning tests: Self administration paradigms



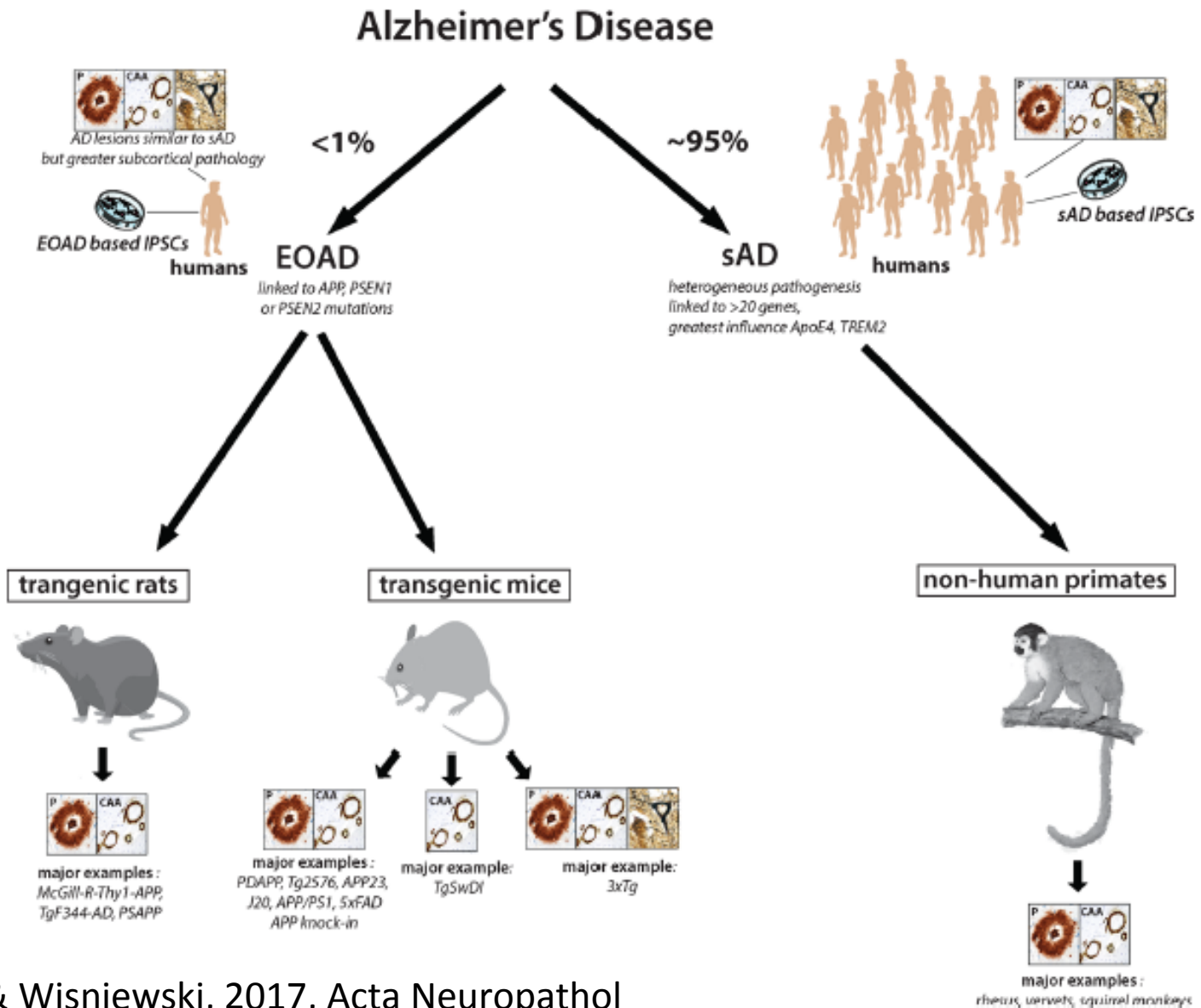
# Example: forced swim test



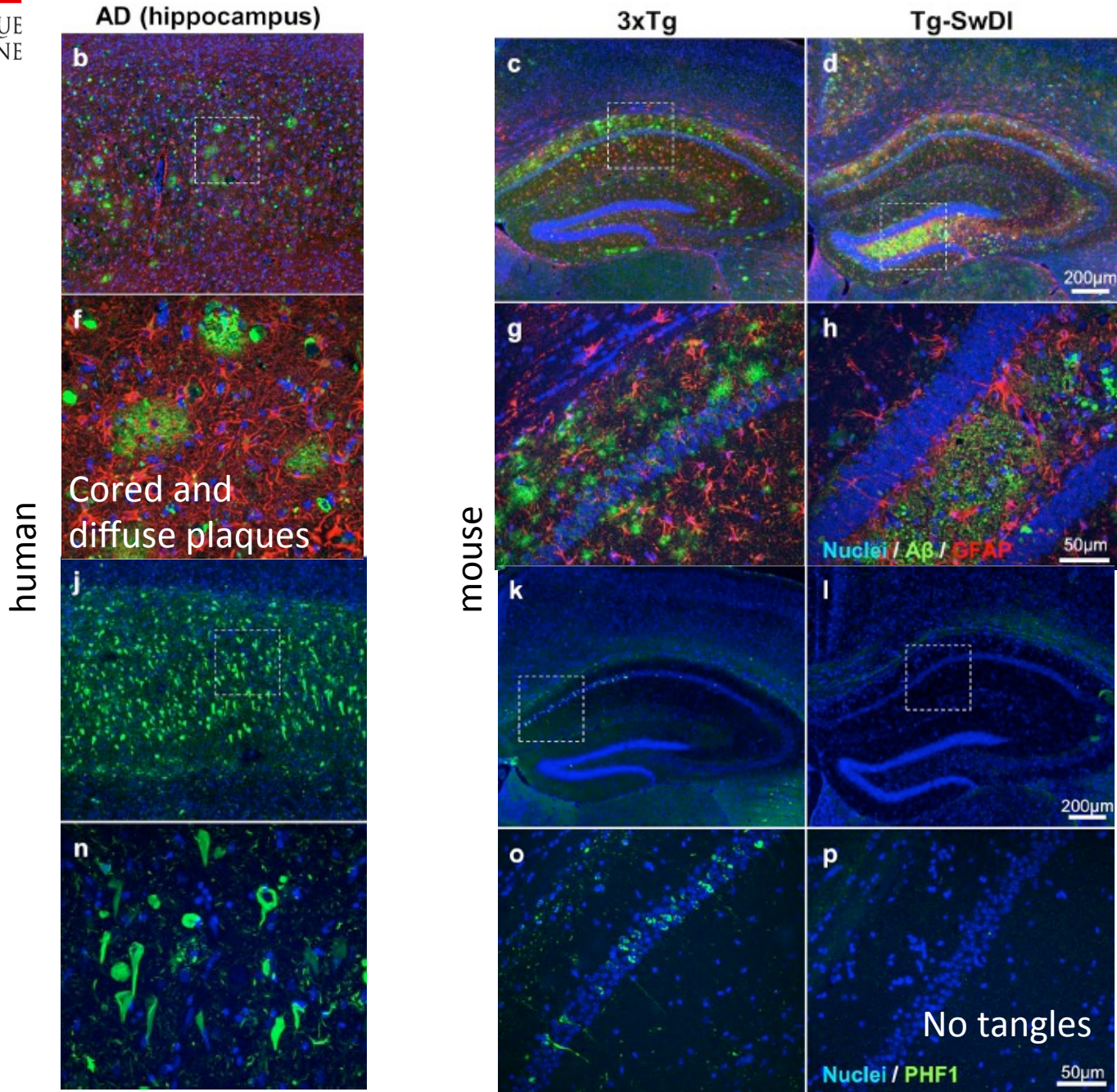
# Example: resident intruder test –measuring aggressive behavior



# Limitations of rodent models



# Limitations of rodent models

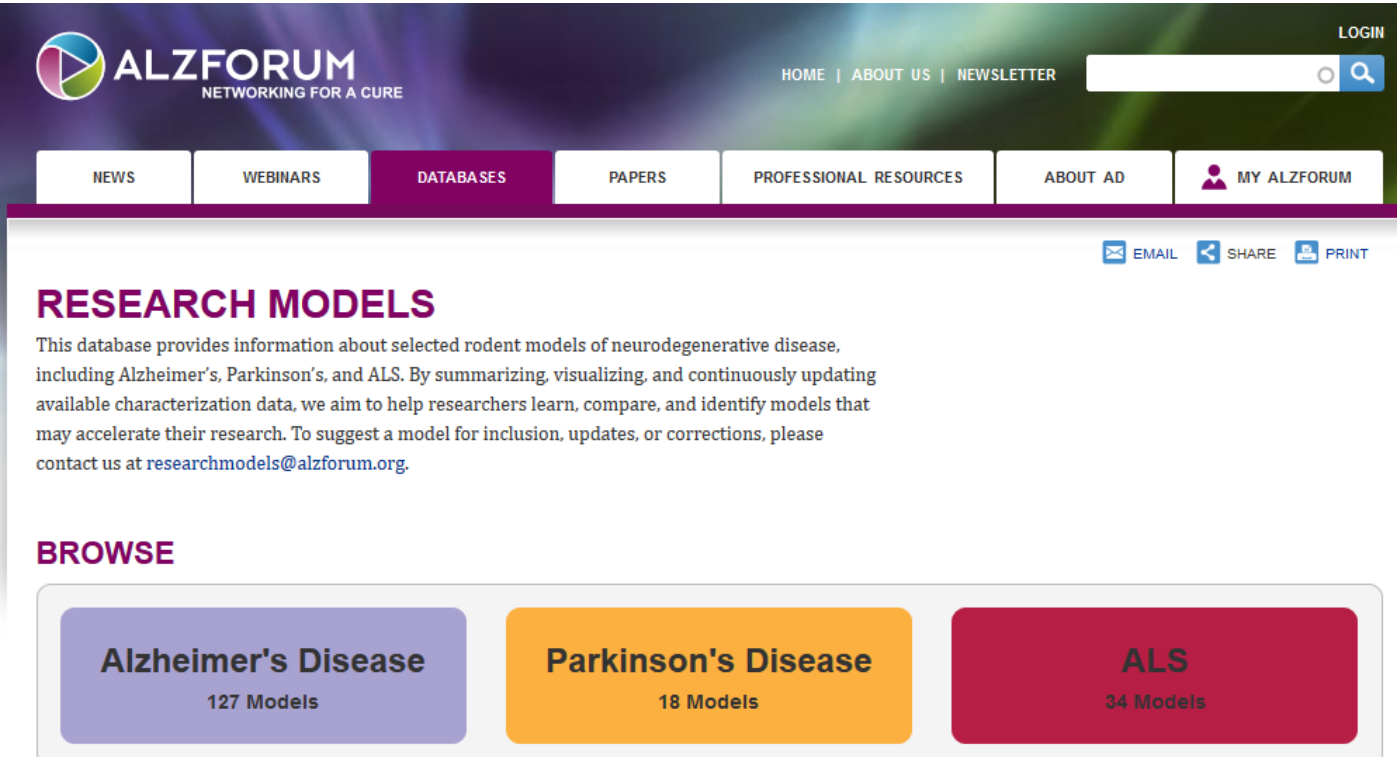


mouse

# Conclusions

- Many different rodent models available → incomplete models by themselves
- Distinct symptoms can be studied in great detail in appropriate rodent models
- Selection of the appropriate model is key
- Clinically relevant behavioural deficits can be studied in a wide variety of validated behavioural tests
- Validation with human disease is necessary

# Resources on rodent models for neurodegenerative disorders



The screenshot shows the ALZFORUM website interface. At the top left is the ALZFORUM logo with the tagline 'NETWORKING FOR A CURE'. To the right are navigation links for 'HOME | ABOUT US | NEWSLETTER' and a search bar. Below the navigation is a menu with 'NEWS', 'WEBINARS', 'DATABASES' (highlighted), 'PAPERS', 'PROFESSIONAL RESOURCES', 'ABOUT AD', and 'MY ALZFORUM'. On the right side of the page, there are icons for 'EMAIL', 'SHARE', and 'PRINT'. The main content area features the heading 'RESEARCH MODELS' followed by a paragraph: 'This database provides information about selected rodent models of neurodegenerative disease, including Alzheimer's, Parkinson's, and ALS. By summarizing, visualizing, and continuously updating available characterization data, we aim to help researchers learn, compare, and identify models that may accelerate their research. To suggest a model for inclusion, updates, or corrections, please contact us at [researchmodels@alzforum.org](mailto:researchmodels@alzforum.org).' Below this is a 'BROWSE' section with three buttons: 'Alzheimer's Disease' (127 Models), 'Parkinson's Disease' (18 Models), and 'ALS' (34 Models).

<http://www.alzforum.org/res/com/>

<https://www.jax.org/research-and-faculty/tools/alzheimers-disease-center/alzheimers-mouse-model-repository>