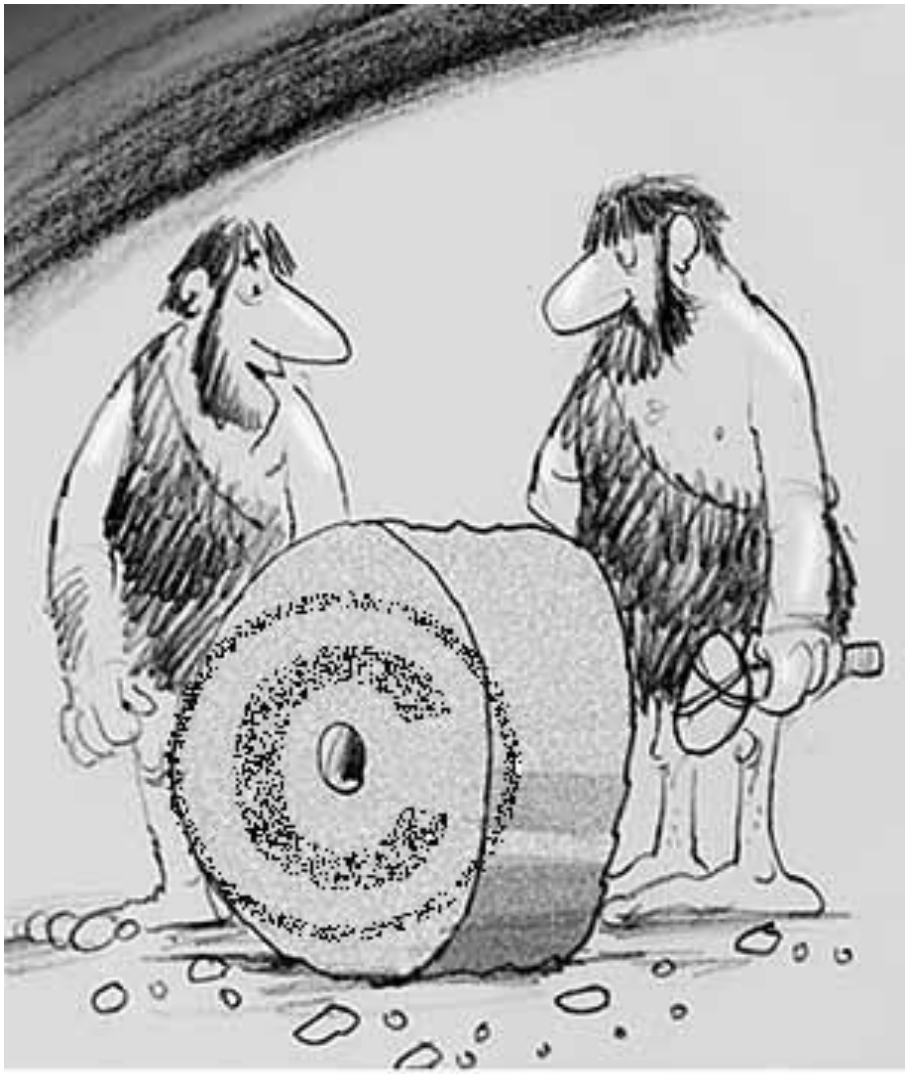
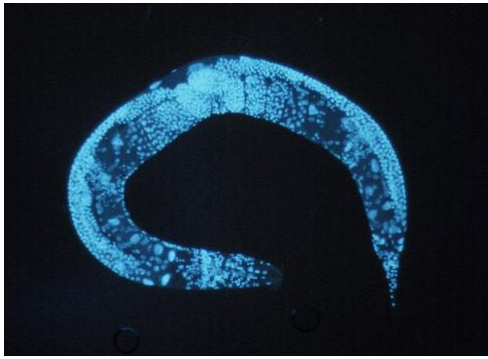
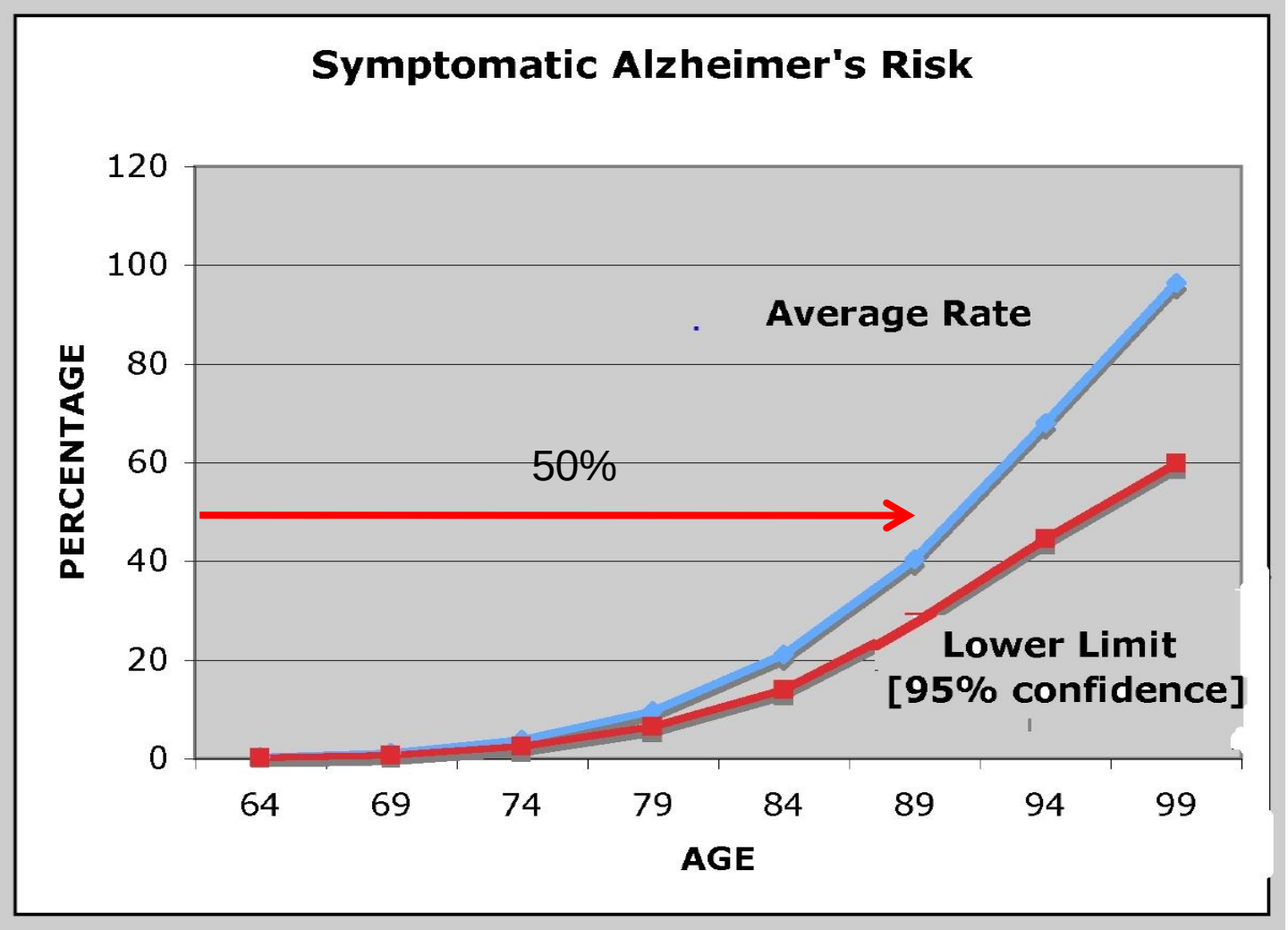
Animal models of Disease



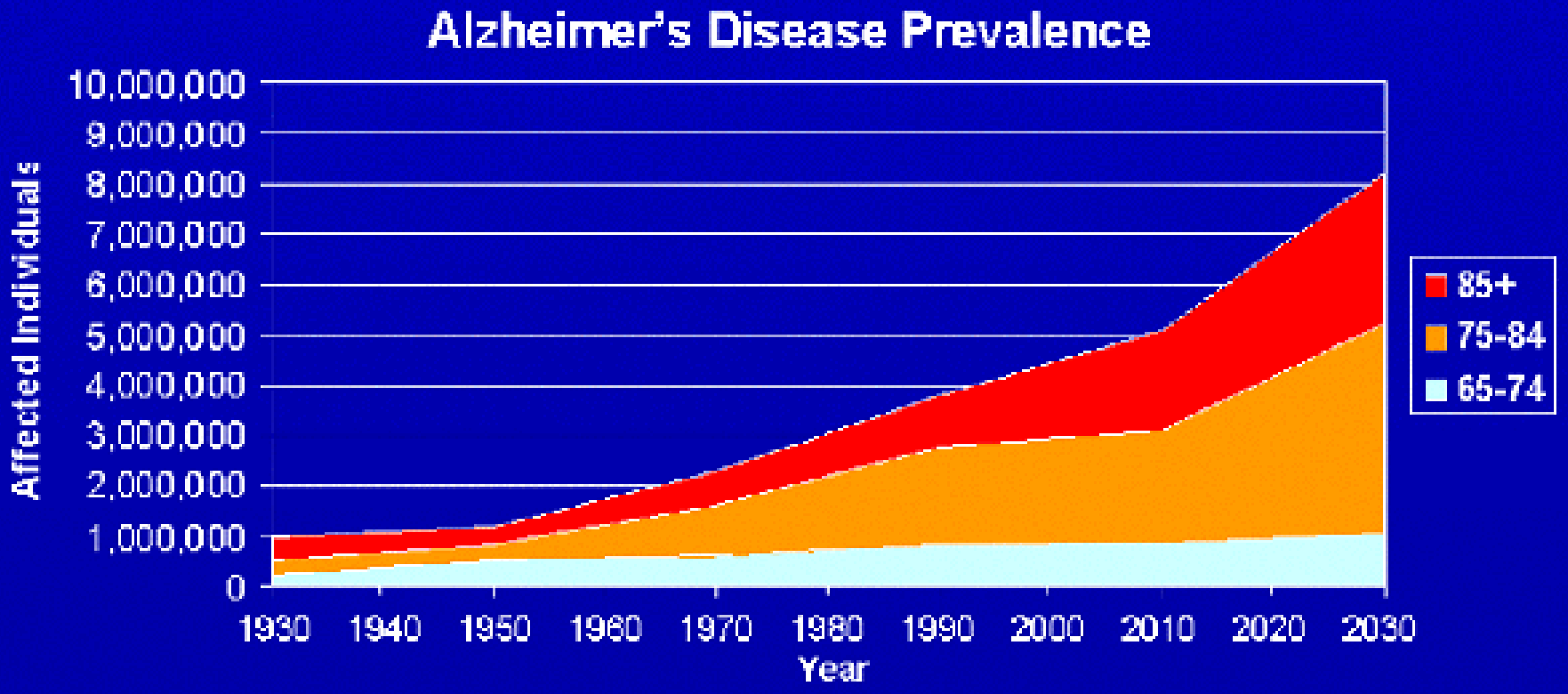
"Nope, not quite...what I had in mind was more a sort of double helix."



Risk of AD


Symptomatic Alzheimer's Risk
PERCENTAGE
120
100
80
60
40
20
0
64
69
74
79
84
89
94
99
AGE
Average Rate
50%
Lower Limit
[95% confidence]

Prevalence of AD Through 2030



Brumback, RA, Leech RW, J. Ohio State Med Assoc. 1994: 87, 103-111

Alzheimer's Disease

Early

Insidious Memory Dysfunction
Cognitive problems (finances, driving, following instructions)
Lost during walking

Middle

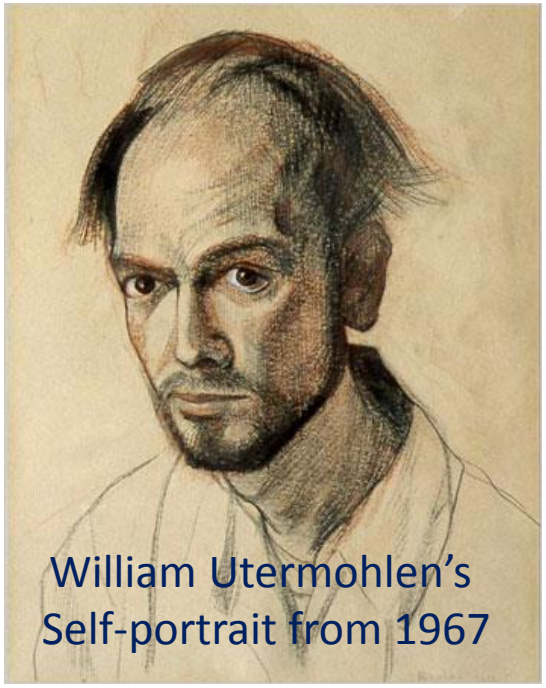
Can't work
Lost and confused
Deficiencies in motor tasks (dressing, eating etc.)
Needs services of a caregiver

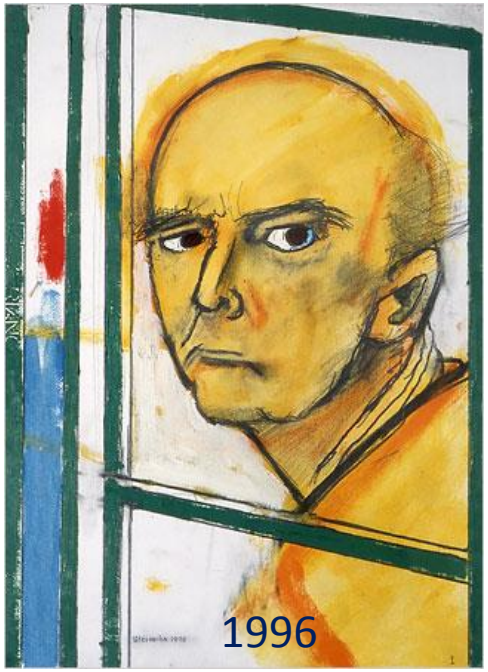
Late

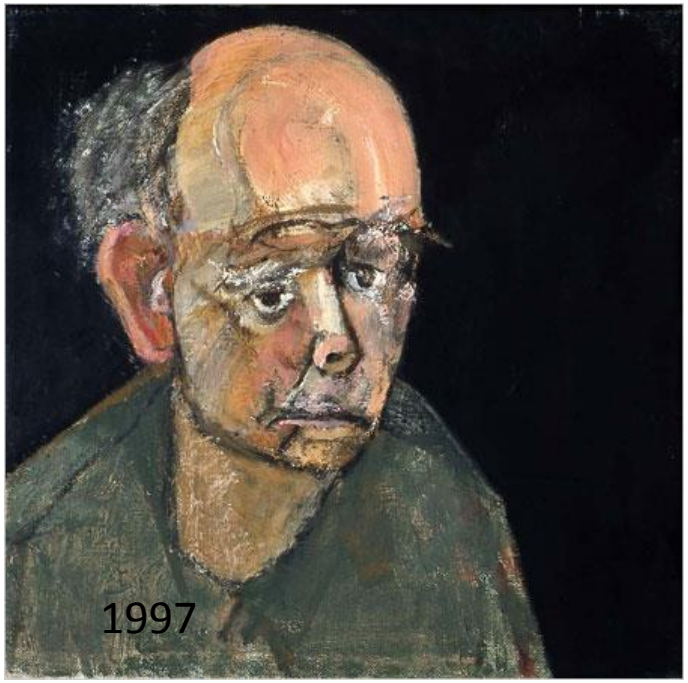
Complete loss of cognitive abilities
Wander aimlessly
Hallucinations
Delusions

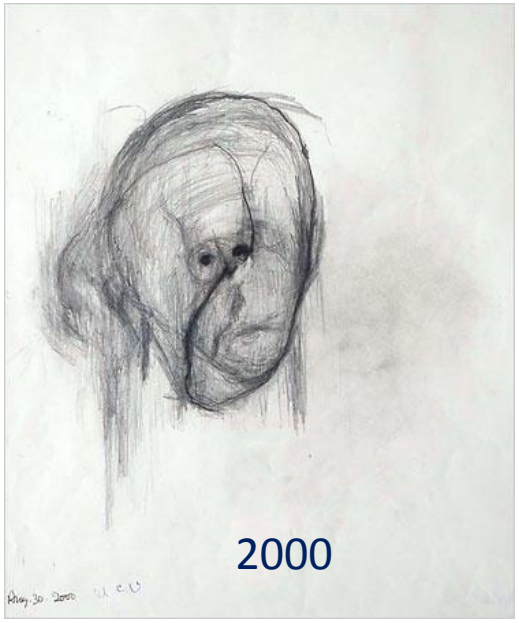
Death

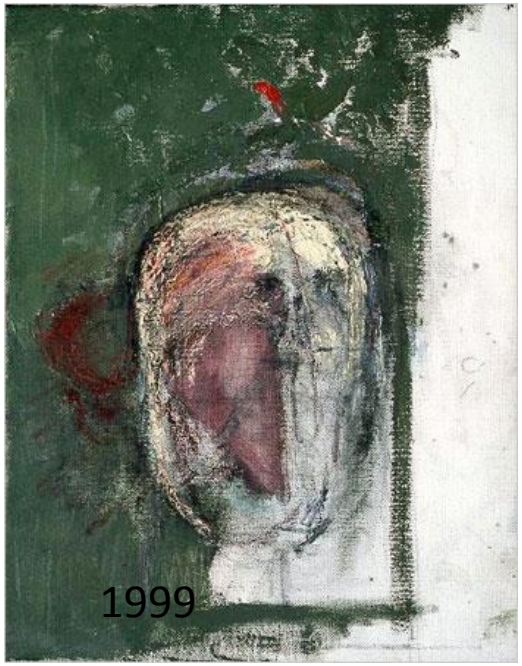
Due to malnutrition, infections, heart disease

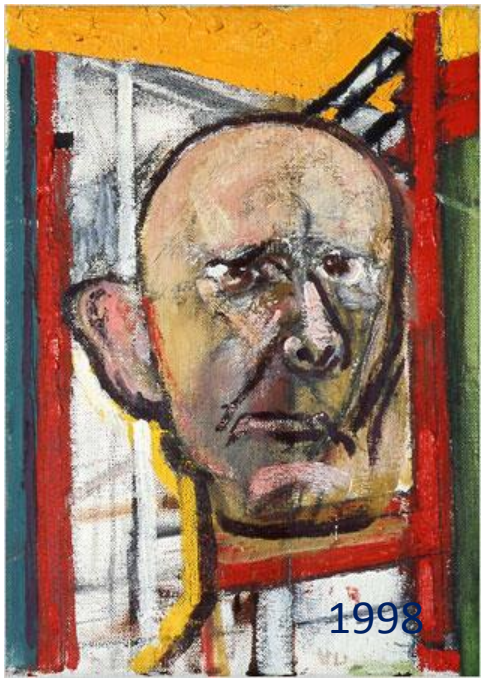

William Utermohlen's
Self-portrait from 1967


1996


1997


2000


1999

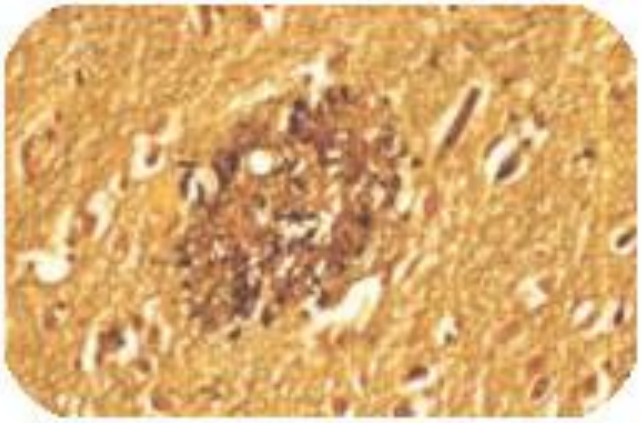

1998

Alzheimer's Disease and the Brain

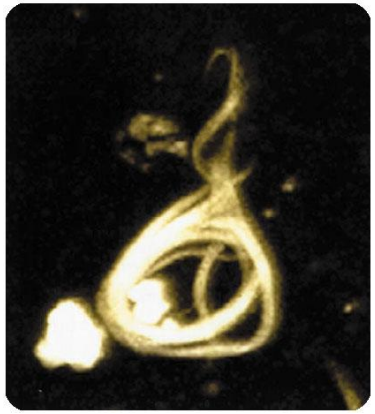
Plaques and Tangles: The Hallmarks of AD

The brains of people with AD have an abundance of two abnormal structures:

- beta-amyloid plaques, which are dense deposits of protein and cellular material that accumulate outside and around nerve cells
- neurofibrillary tangles, which are twisted fibers that build up inside the nerve cell

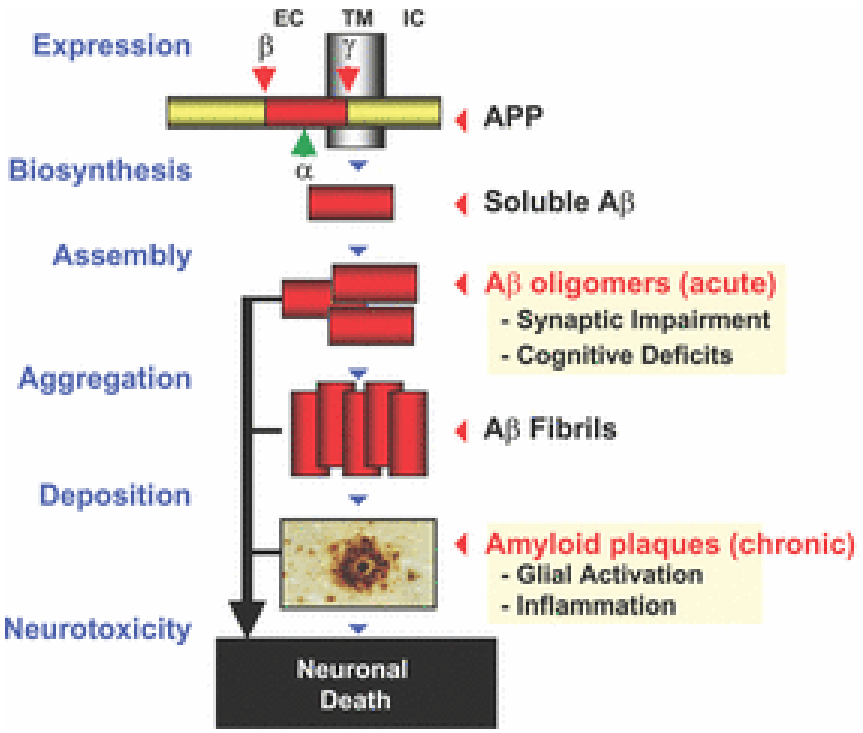


An actual AD plaque

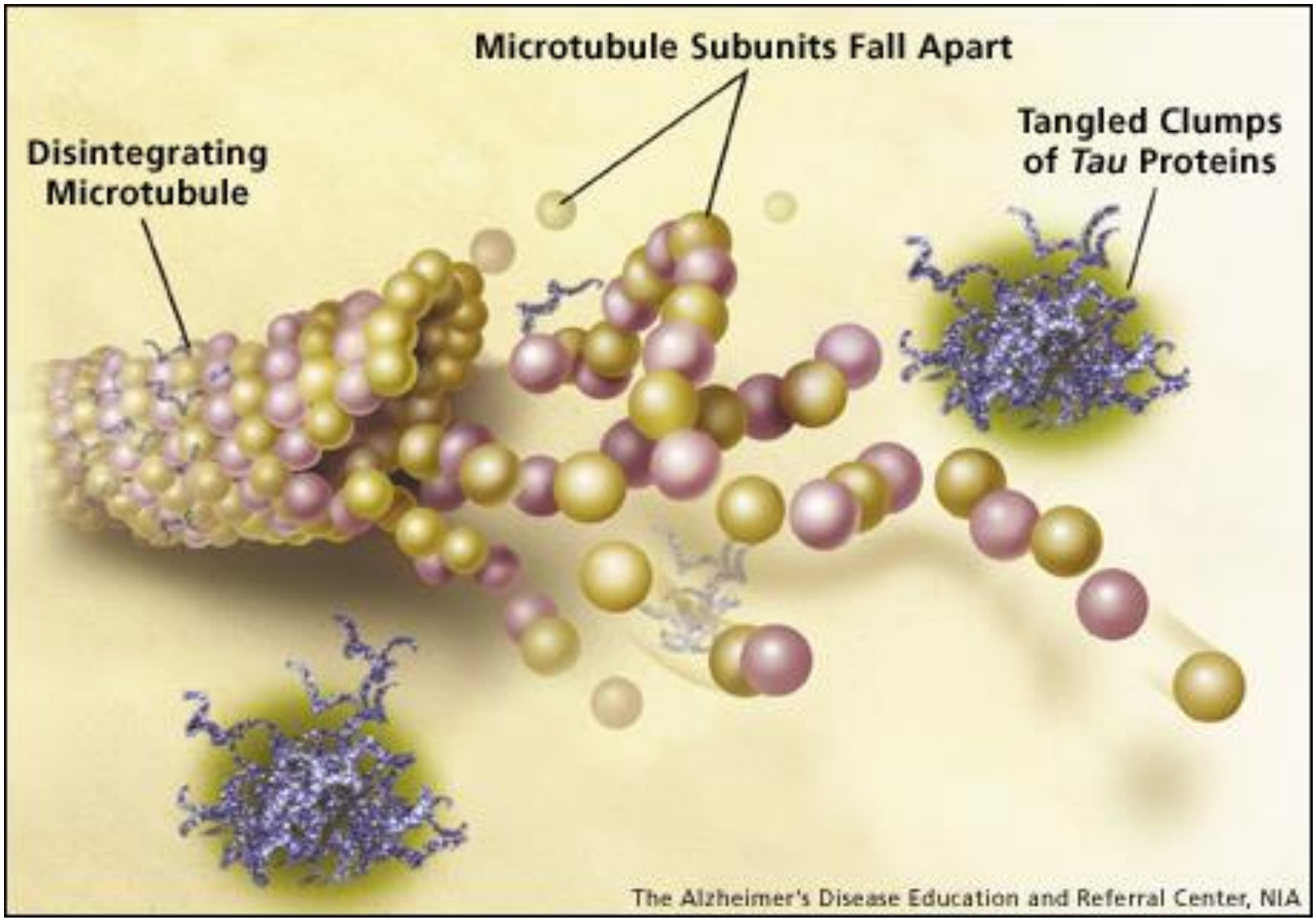


An actual AD tangle

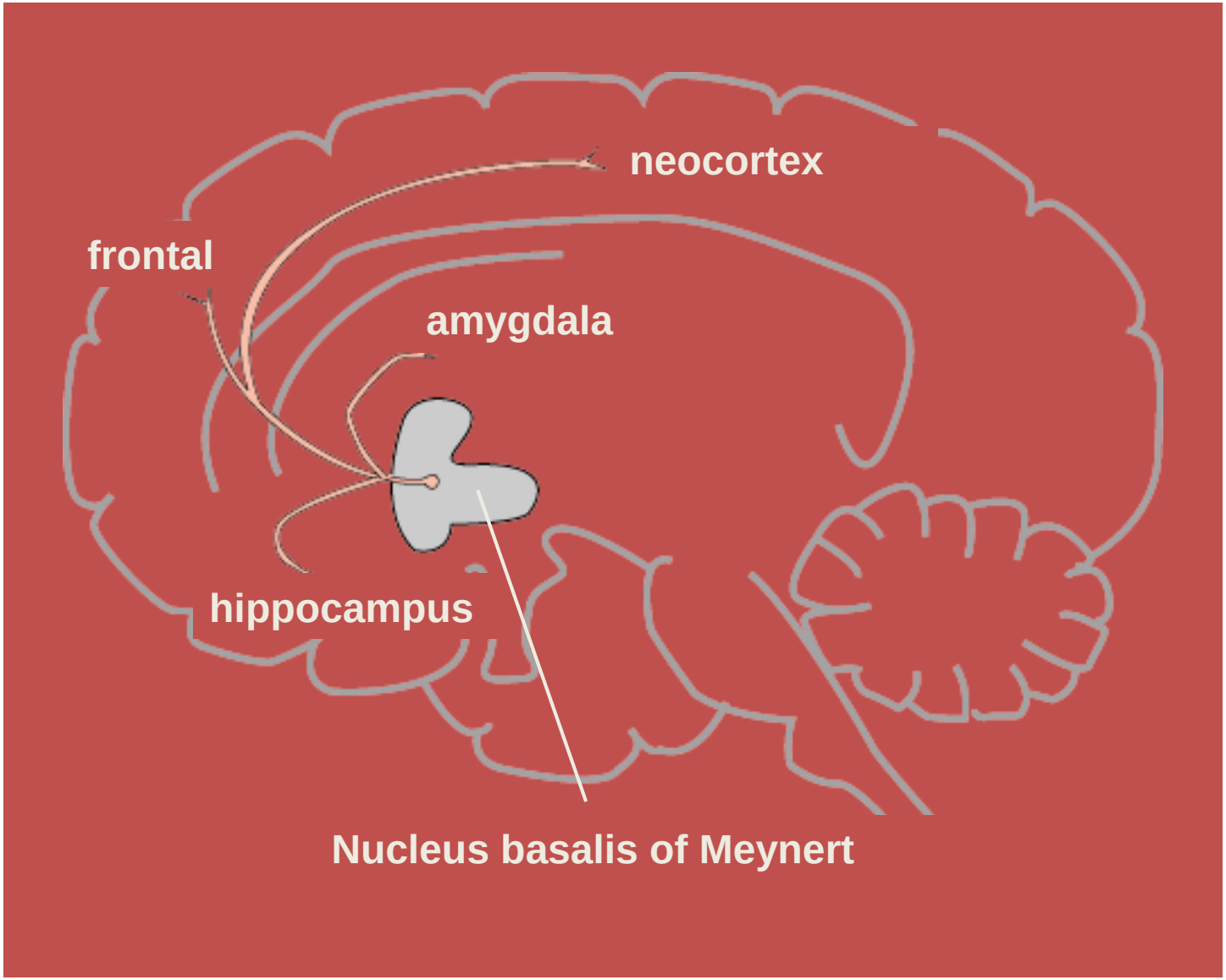
AMYLOID HYPOTHESIS



- **Aβ biosynthesis**
  - γ-secretase
  - β-secretase
- **Aβ degradation**
  - α-secretase
  - IDE
  - Neprilysin
  - Plasmin


Microtubule Subunits Fall Apart
Disintegrating Microtubule
Tangled Clumps of *Tau* Proteins
The Alzheimer's Disease Education and Referral Center, NIA

Acetylcholine Pathways



Stahl, S. (2008) Essentials of Psychopharmacology

Stahl S M, Essential Psychopharmacology (2000)

Proposed Chronology of Changes in AD

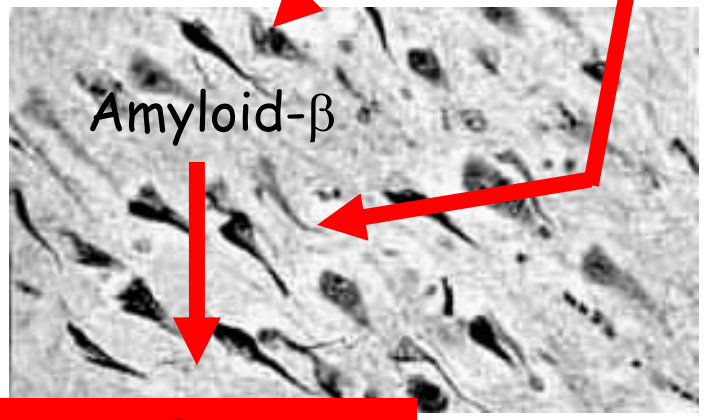
OXIDATIVE STRESS

BACE

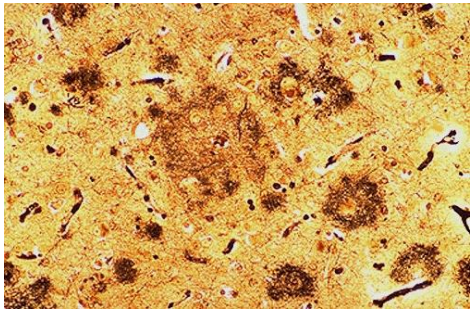
SAPKs

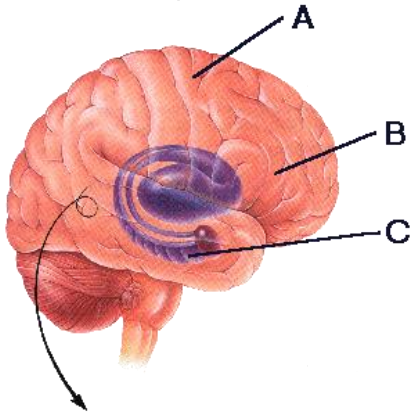
Phospho-Tau

HNE Modification



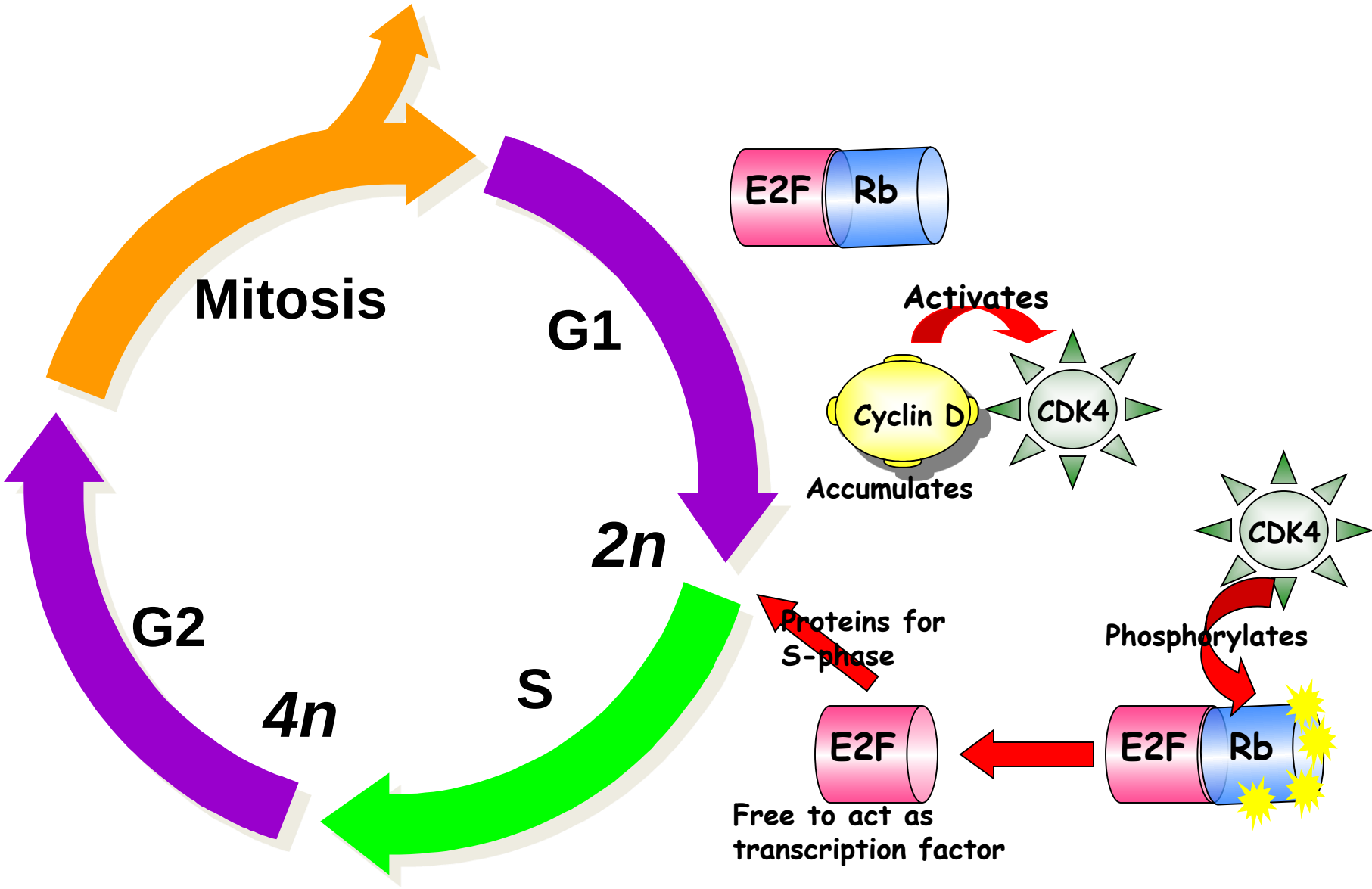
Amyloid-β Deposition
[*Senile Plaque*]





Tau Phosphorylation
[*Neurofibrillary Tangles*]

Neuronal Exit from Quiescence in AD

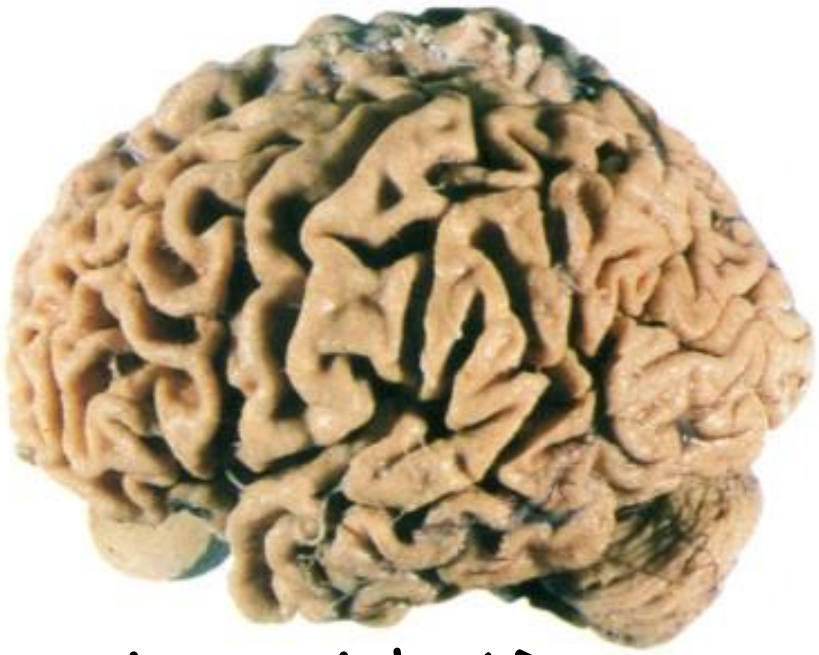


Alzheimer's Disease & the Brain

- Loss of nerve cells within brain "shrunken, shriveled"

Hippocampus affected first

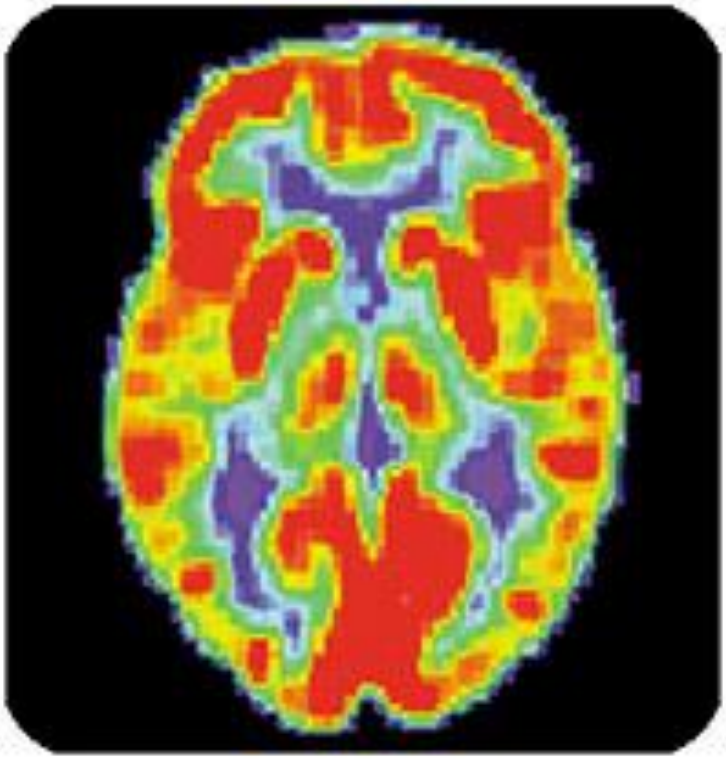
- Reduced activity of neurotransmitter acetylcholine



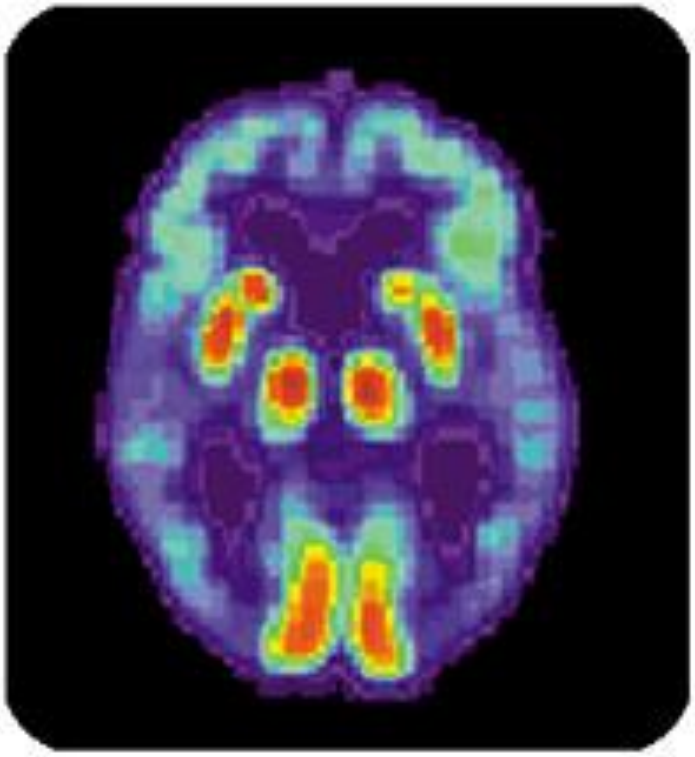
patient with AD

healthy patient

The Changing Brain in Alzheimer's Disease



Normal Brain



Alzheimer's Disease Brain

Animal/Not animals that we can use to Model disease

- **Vertebrates**
  - **Primates**
  - **Mice**
  - **Rats**
  - **Zebrafish**
  - **Dogs/Cats**
- **Invertebrates**
  - **Flies**
  - **Worms**
- **Single Cell Organisms**
  - **Phage**
  - **Bacteria**
  - **Yeast** (Prion disease)

We chose models based on the phenotype that we are aiming to study

Genetic/structural/behavioral
Size
Complexity of what we are trying to observe

Types of animal Models

Natural

- Dogs/Primates to study AD

Semi-natural (based on spontaneous mutations/Irradiation)

- Accelerated aging mouse to study AD

Lesion (Ablation of regions of interest)

- Hippocampus to study AD

Chemical (Neurotoxins)

- Cholinergic toxins or Abeta to study AD

Transgenic Models

- The newest and most used – Models for everything… Are they good though?

What is a good Model?

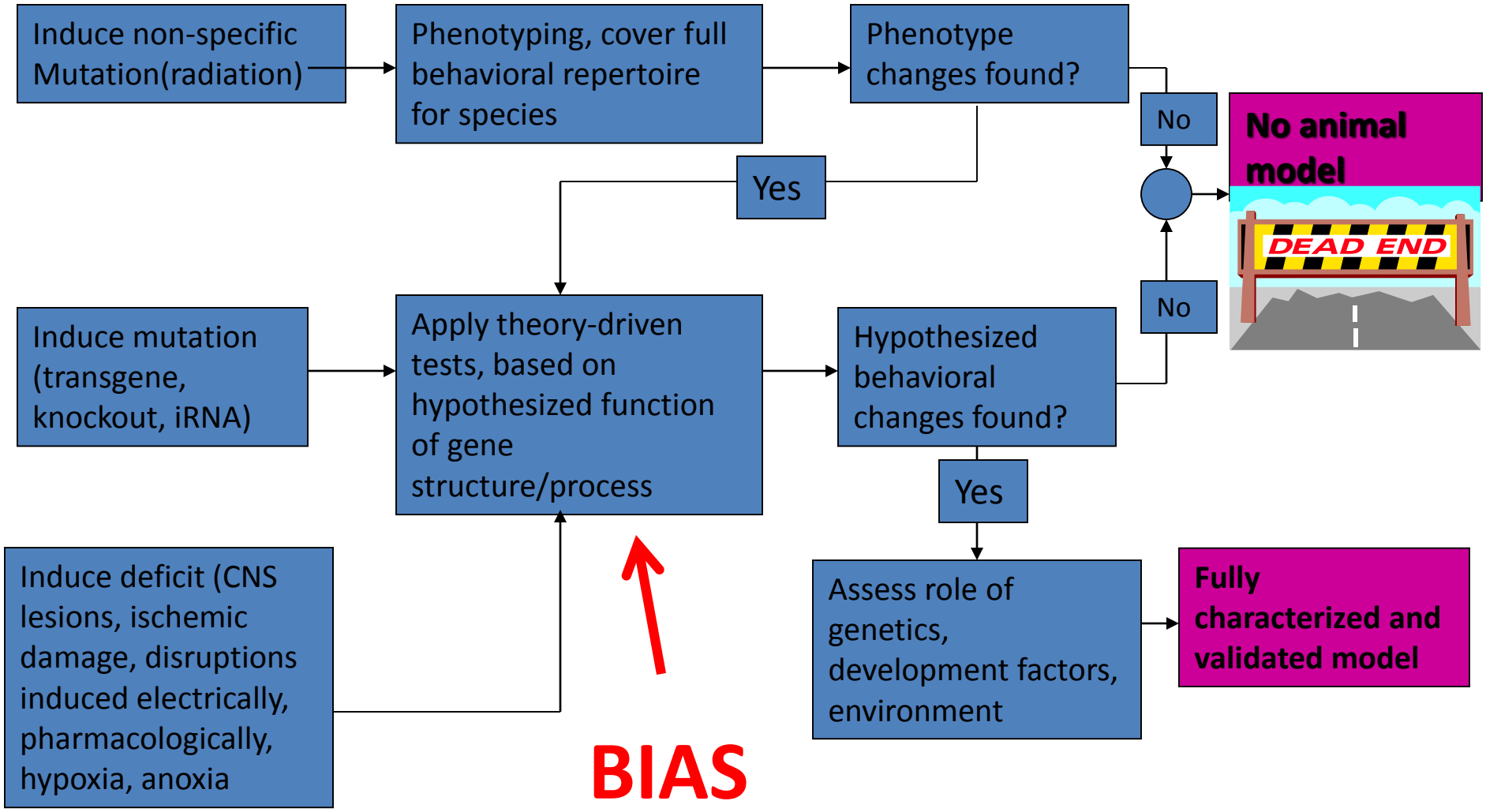
- **Face validity** – Does the animal "look the same" as the species to be compared to? – If not – **NO/INCOMPLETE MODEL**

- **Reliability and Replicability** – Can the phenotype be reliably observed and replicated? – If not, Idiopathic– **NO/INCOMPLETE MODEL**

- **Construct Validity - Generalizablility** – Can you achieve the same results?
  - Across species
  - Environment (Different labs)
  - Behavioral tests that measure the same thing

- **Predictive Validity– Can you predict outcomes after:**
  - **Pharmacological manipulations that are relevant to the model**
  - **Time**

Development of an Animal Model of "Disease"



“Natural Models”

- Good - Most related to the natural disease, most likely to portray all aspects of the disease

- Bad - Restrictive in terms of time and types of animals used, also in terms of animals developing illnesses (in aging studies).

- 1)Expensive 2) Long studies 3) Restrictive at an experimental level

“Semi-Natural Models”

- Good – Usually good models to study disease because they are based on phenotypical presentations of the disease.

- Bad – Often lack appropriate controls because they are bred for a phenotype and the genetics are unknown.

Lesion Models

- Good to study the function of a structure and how the loss of that structure affects other neuronal populations

- Bad because most diseases are not specific to a structure or a neuronal population – High mortality & variability
    - HM – Hippocampus removal does not lead to AD, it leads to retrograde amnesia!

Chemical Models

- Good to study selective depletion of specific neuronal populations and examine the role of neurotransmitters in disease, more specific than lesions and sometimes leads to full phenotype expression (MPTP in drug addicts)

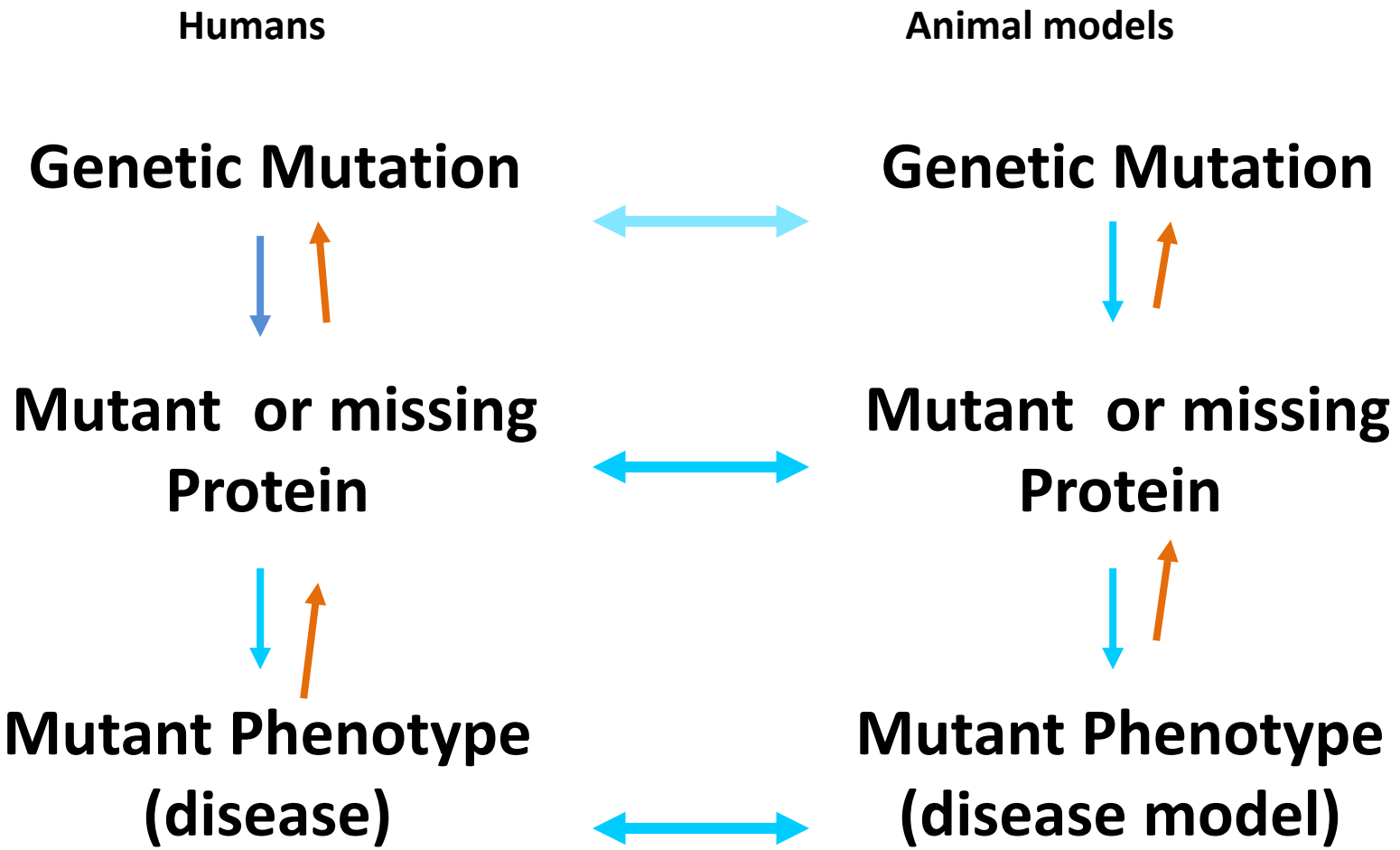
- Bad because it is artificially induced and is incomplete with regards to phenotype (pathology/not pathology), it does not always translate to the real population (rotenone and farmers)

Transgenic Models

- **Good** excellent to study the role of genes and genetic mutations in disease

- **Bad** because diseases like all biological events are a mix of environment and genetics so a lot of times the genetic mutations do not recapitulate the disease.
- Also, while some of the genetics are highly conserved a mouse/fish/fly/worm do not respond the same way than humans do nor do animals of the same species!!! (BACKGROUNDS!!!)

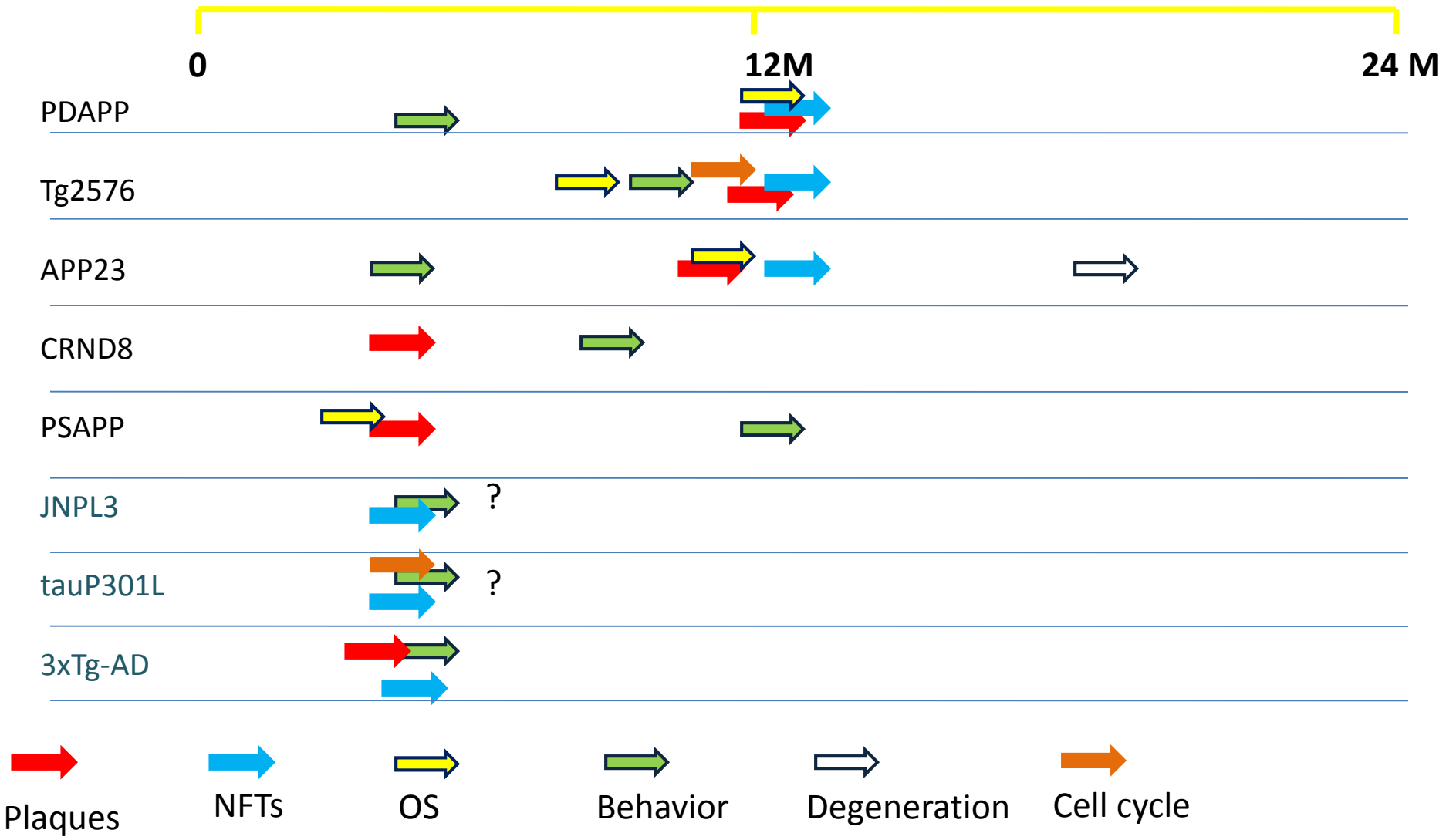
Animal disease models:



Transgenic models of neurodegeneration

- Alzheimer's disease:
- APOE null mice
- APP transgenic
- Tau transgenic
- GSK3beta transgenic

The endless list of AD transgenics



What does a good model of AD need to show?

- Face validity – Have plaques and tangles, cognitive deficits and neurodegeneration.
- Reliability/Reproducibility – Needs to be able to be observed consisitently in the same animals.
- Construct validity – Observed in different environments, in different tests measuring the same thing AND across species.
- Predictive validity – It needs to show temporal relevance to the disease and it needs to show that treatment aimed at the insult will make it better.

Transgenic mice recapitulate part of Alzheimer pathology

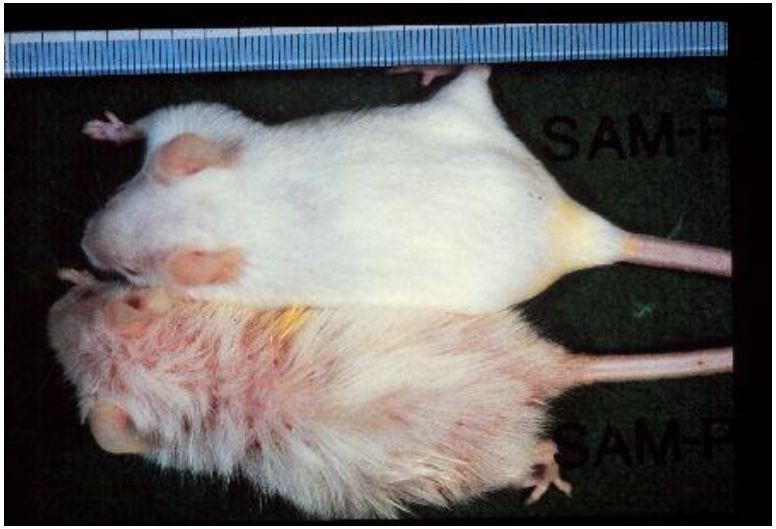
- Presence of amyloid plaques
- Presence of hyper-phosphorylated tau
- **OR BOTH**

Memory deficits in Tg2576 transgenic mice, which overexpress mutant APP, can be reversed by $A\beta_{42}$ vaccination.

ALL BASED ON OVER-EXPRESSION OF MUTATIONS ASSOCIATED WITH EARLY ONSET AD. Therefore excellent to study the biology of the peptide but less relevant to disease dynamics

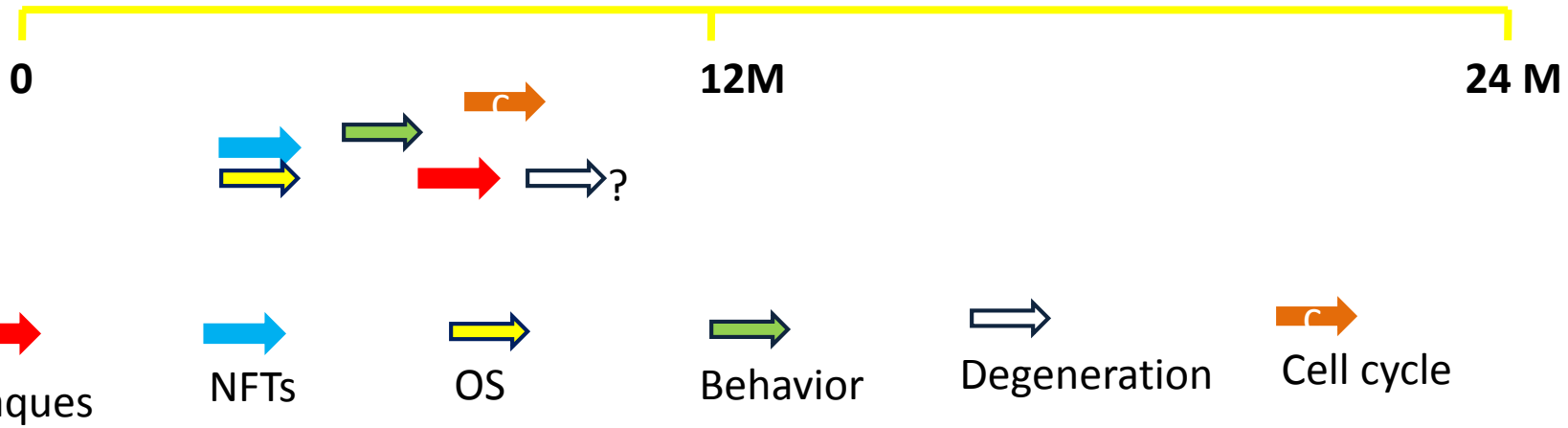
- **How CAN we study late-onset AD?**
  - Aged animals – Expensive, low availability
  - Mice do not develop AD so we have to study higher level animals like dogs and primates
    - More expensive/ethically complicated
    - life span of dogs/primates is much longer than 3 years so even less available!

Age-Accelerated SAMP8 Mouse Model



12 months of age

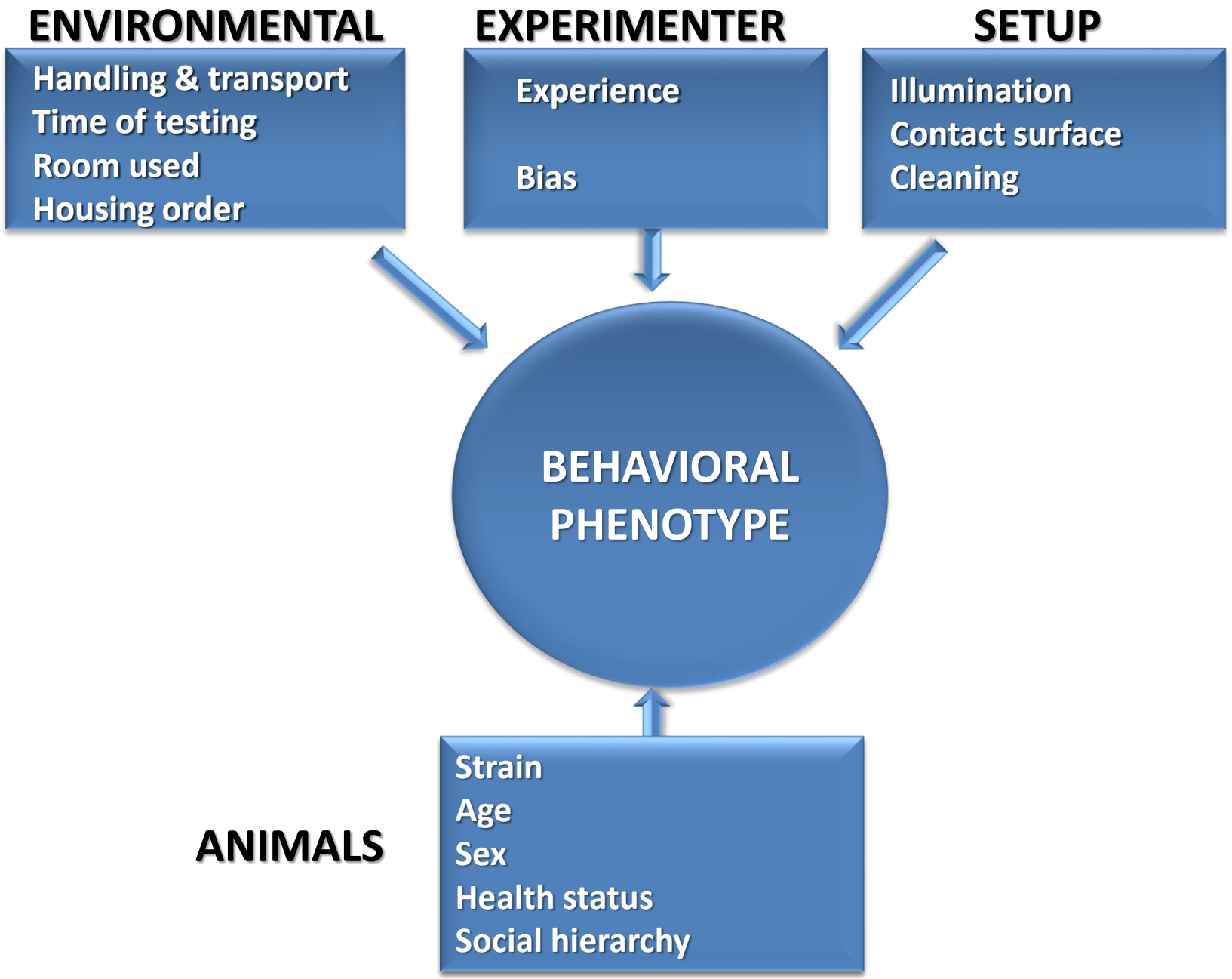
- The SAM strain of mice is derived from AKR/J strain.
- littermates which became senile at an early age in life and had a shorter life span were selected as the progenitors of the SAMP.
- Littermates in which the aging process seemed normal were also selected as the progenitors of SAMR.
- Retrospective pedigree selection and inbreeding were applied based on the degree of senescence, the lifespan and the age-associated pathologic phenotypes.



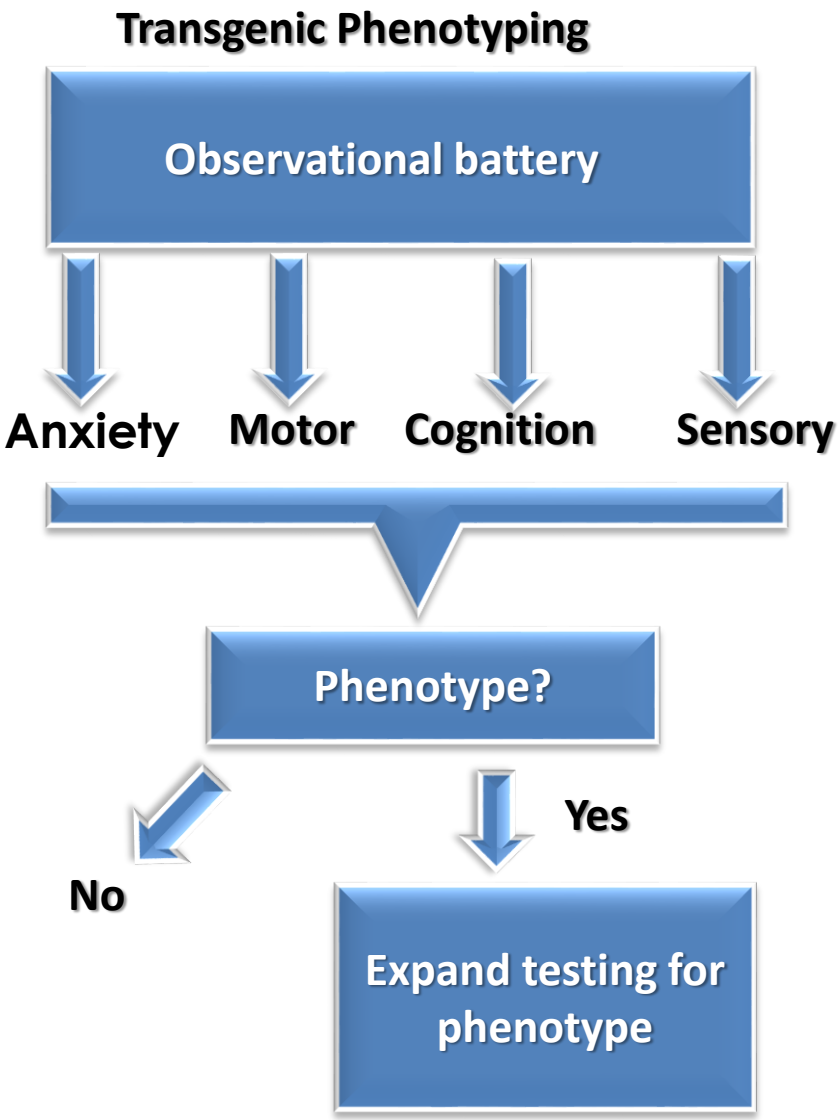
MICE versus MEN

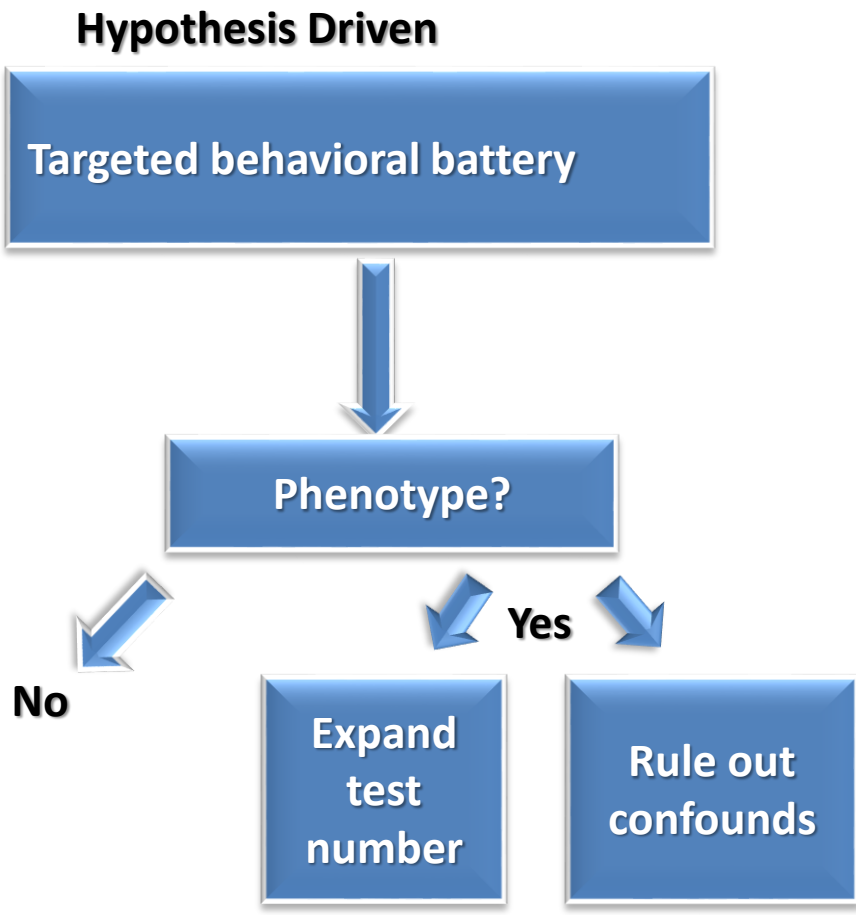
Treatment	Mice	Humans
Acetyl cholinesterase Inhibitors (Aricept)	Cognitive benefits	Marginal - up to 1 year stabilization in 30-50%
NMDA Inhibitors (Mementine)	Cognitive benefits	Marginal - up to 6 months stabilization
Vitamin E trial	Cognitive benefits and reduction of plaques	Failed
Ibuprophen trial - Failed	Cognitive benefits and reduction of plaques	Failed
Estrogen replacement	Cognitive benefits and reduction of plaques	Failed
Amyloid beta Vaccine	Cognitive benefits and reduction of plaques	Reduction of plaques but no cognitive benefits + inflammation
GSK3 beta inhibition	Cognitive benefits and reduction of tangles	Marginal doing larger trial

Phenotyping of Complex Behavioral Traits to Assess Nervous System Function and Dysfunction


ENVIRONMENTAL
Handling & transport
Time of testing
Room used
Housing order
EXPERIMENTER
Experience
Bias
SETUP
Illumination
Contact surface
Cleaning
BEHAVIORAL PHENOTYPE
ANIMALS
Strain
Age
Sex
Health status
Social hierarchy

How do we go about this?





Strain differences on cognitive performance

Morris Water Maze

Great	Adequate	Impaired	Blindness
FVB129F1	C57BL/6J	129/SvJ	A/J
FVBB6F1	C57BL/10J	DBA/2	SJL/J
129B6F1	BALB129F1	BALB/cByJ	C3H/lbg
129/Svev	B6SJLF1		FVB/NJ
	LP/J		BuB/BnJ

Fear Conditioning

Great	Adequate	Impaired	Freezers
FVB129F1	C57BL/6J	FVB/NJ	A/J
FVBB6F1	C57BL/10J	DBA/2	
129B6F1	BALB129F1	BuB/BnJ	
129/Svev	B6SJLF1	C3H/lbg**	
129SvJ	LP/J		

Behavior tests

Motor Function

- Rota-rod
- CatWalk
- BMS scale
- Muscle strength
- Inclined screen
- Beam Walk
- Sticky tape test
- Open-field
- Home-cage
- Treadmill Exercise
- Pole test

Cognition

- Morris Water Maze
- Fear Conditioning
- Passive avoidance
- Object recognition
- T-maze
- Y-maze

Emotion

- Plus maze
- Light-dark box
- Open-field
- Startle response
- Fear - startle
- PPI
- Aggression Beh
- Maternal Behav
- Social Behav.
- Feeding Behav.
- Forced swim test

Sensory

- Hot-plate
- Tail flick
- PPI
- Visual Cliff
- Visual placing
- Visual WM
- Sticky-tape

Phenotyping of coordination impairment in a mouse model of Ataxia

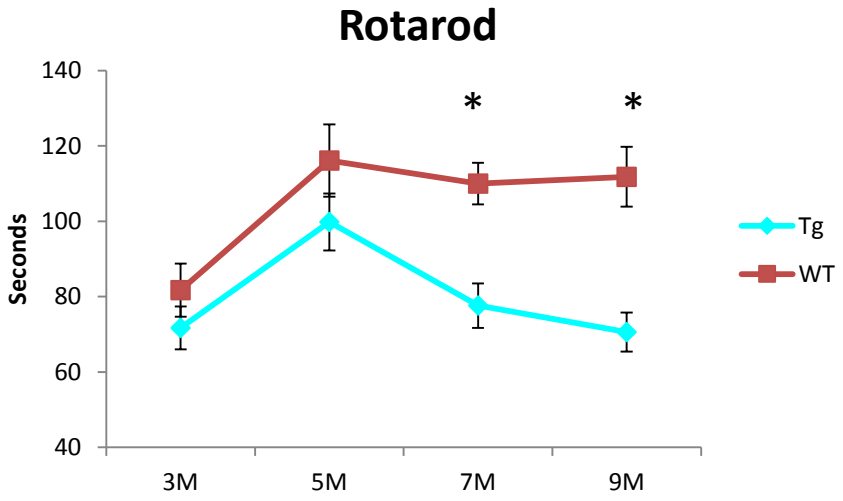
- Ataxia is a disorder that presents with lack coordination associated with dysfunction in CNS regions that control movement and balance, such as the cerebellum.

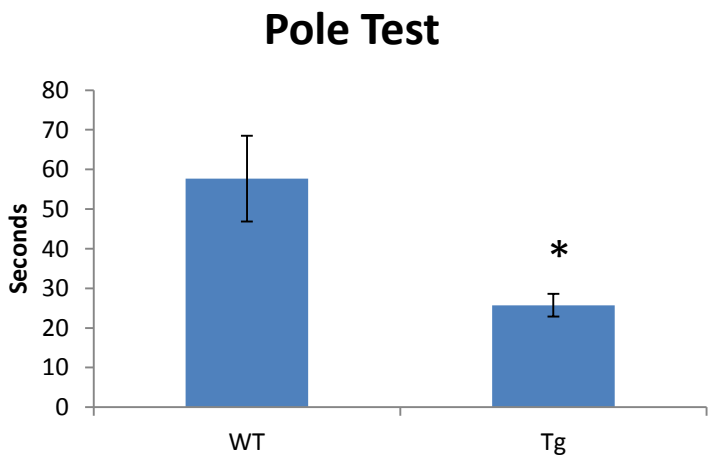
- Determine the face validity of a novel transgenic mouse model of Ataxia

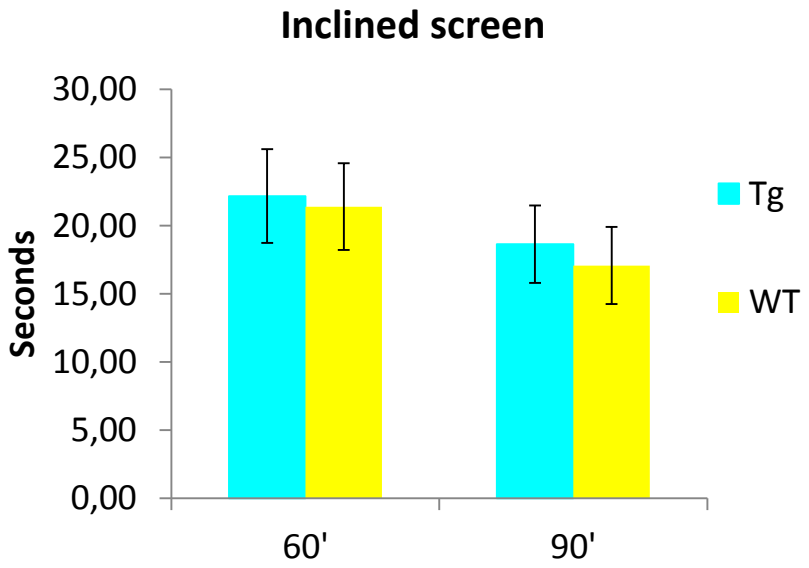
- Transgenic manipulation lead to specific changes in motor coordination

Motor Tests

- Coordination tests progressively declines







Importance of the Findings

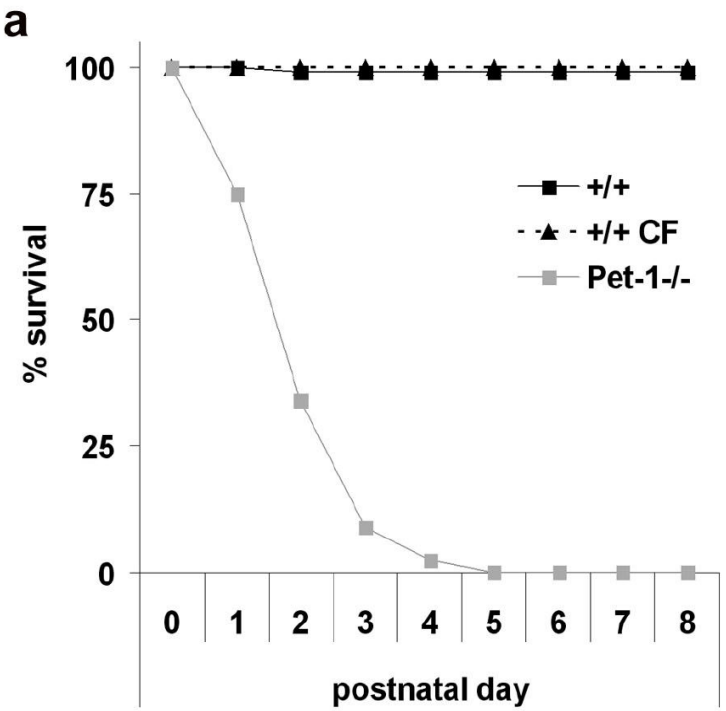
- The genetic manipulation has an effect that is consistent with the disease studied.

- Face validity from an animal model stand-point
  - Allows to test treatments
  - Dissect molecular mechanisms further

Role of PET-1 on Maternal Behavior

- The Pet-1 transcription factor was found to be essential for serotonergic differentiation

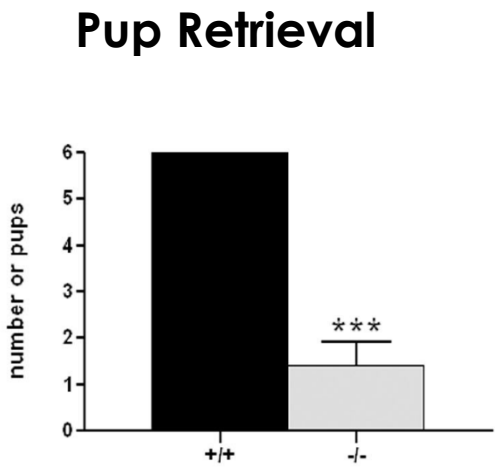
- Pet-1 mutant mice exhibited anxiety and aggression. They also had very low survival of Litters



Maternal behavior Observations

- Is the poor survival of the pups due to physiological problems of the mother or poor maternal behavior?

Retrieval Test



Importance of Findings

- **The serotonergic nervous system is important for:**
- Anxiety and aggression

- **Enables normal maternal behavior that ensures the survival of offspring**

- Published in Nature Neurosciences (2008)