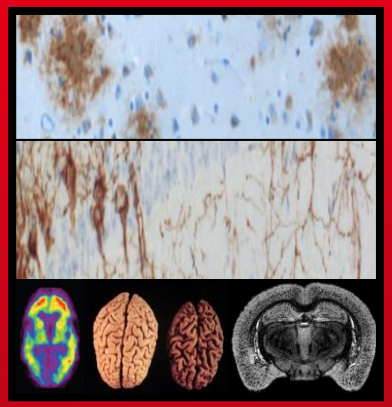
Preclinical Models of neurodegenerative diseases
Exemple of Alzheimer's disease



Marc Dhenain

Multimodal Imaging of
Neurodegenerative Diseases
and Therapies

MIRCen, CEA-CNRS UMR 9199
Fontenay-aux-Roses

Master 2 Biothérapies Tissulaires, Cellulaires et Géniques
UE3 Modèles Animaux
UE3 Animal Models

MIND
Multimodal Imaging of
Neurodegenerative Diseases
and Therapies

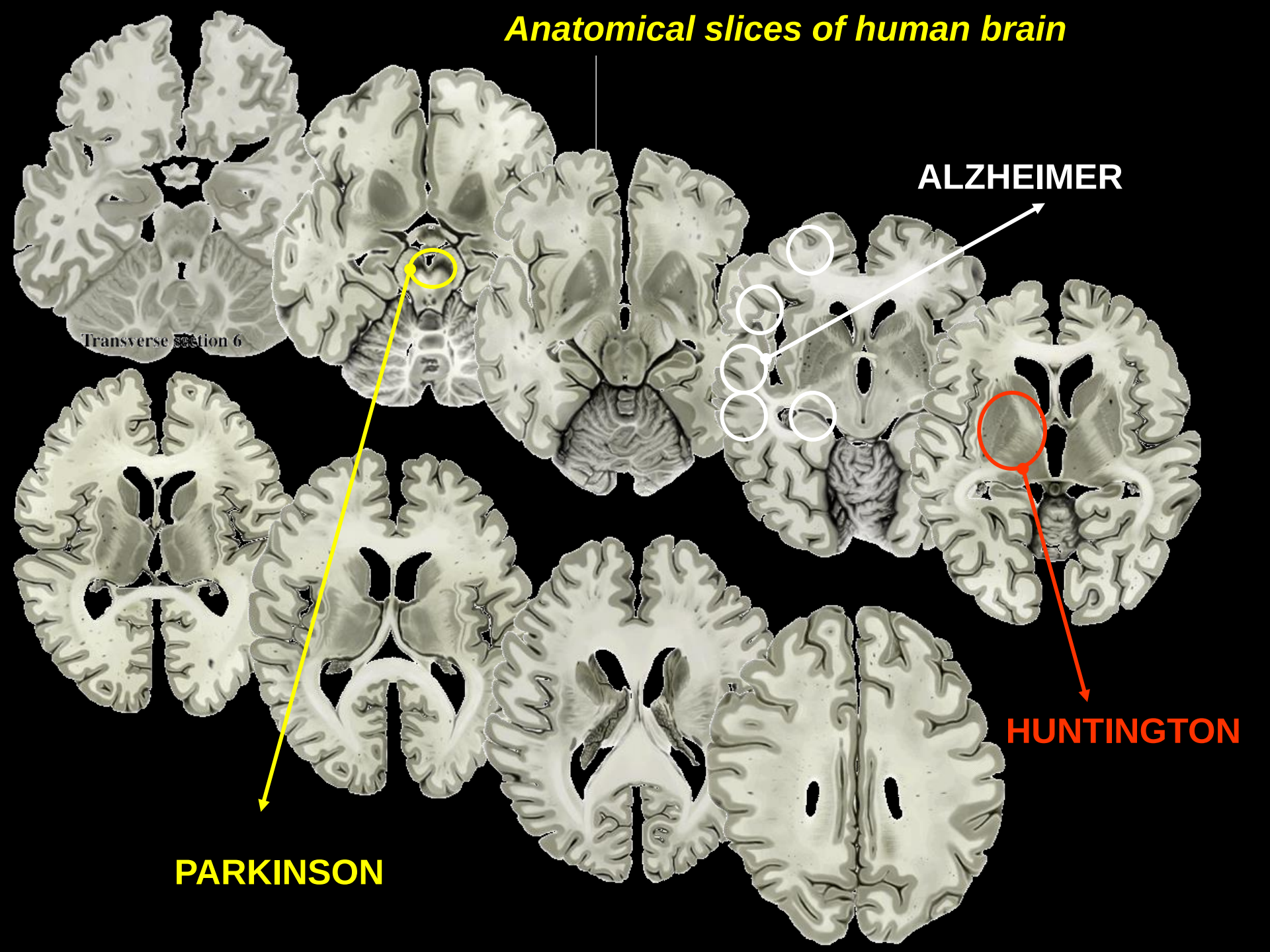
NEURODEGENERATIVE DISEASES

Definition :

- **Diseases of the nervous system caused by a loss or incapacitation of neurons.**

Examples :

- **Multiple sclerosis (Sclérose en plaques) (Myelin loss)**
- **Alzheimer's disease (loss of cholinergic neurons)**
- **Parkinson's disease (loss of dopaminergic neurons)**
- **Huntington's disease (loss of GABAergic neurons)**


Anatomical slices of human brain
ALZHEIMER
Transverse section 6
HUNTINGTON
PARKINSON

NEURODEGENERATIVE DISEASES

Disease	*Anatomy*	*Patients (Fr)*
Alzheimer	cortex	860 000
Parkinson	subst. nigra	80 000
Huntington	striatum	6 000
Spino-cereb. ataxia	cerebellum	<5 000
Amyotrophic Lat. Scler.	cortex, medulla	<5 000
Multiple Sclerosis	cortex, stem, medulla	60 000

ALZHEIMER'S DISEASE

Symptoms

Dementia

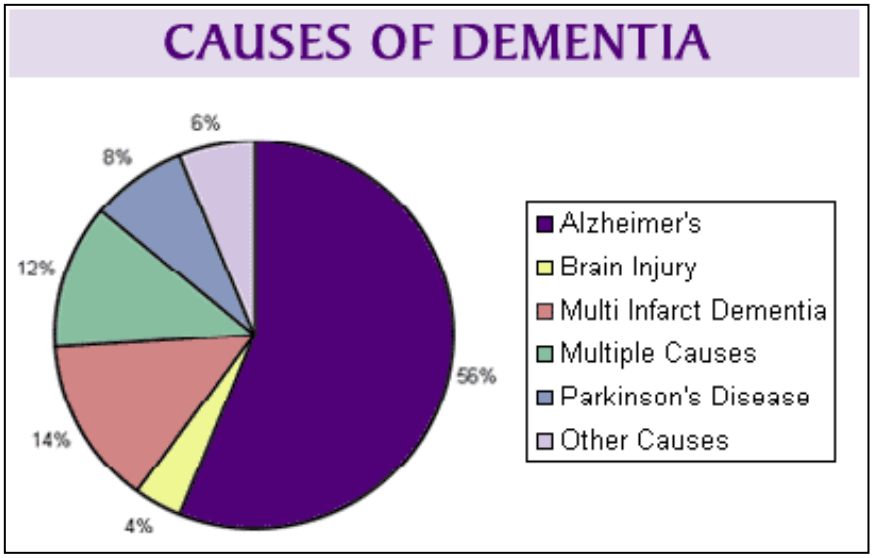
- spatio-temporal disorientation

- Alteration of short term memory (episodic)

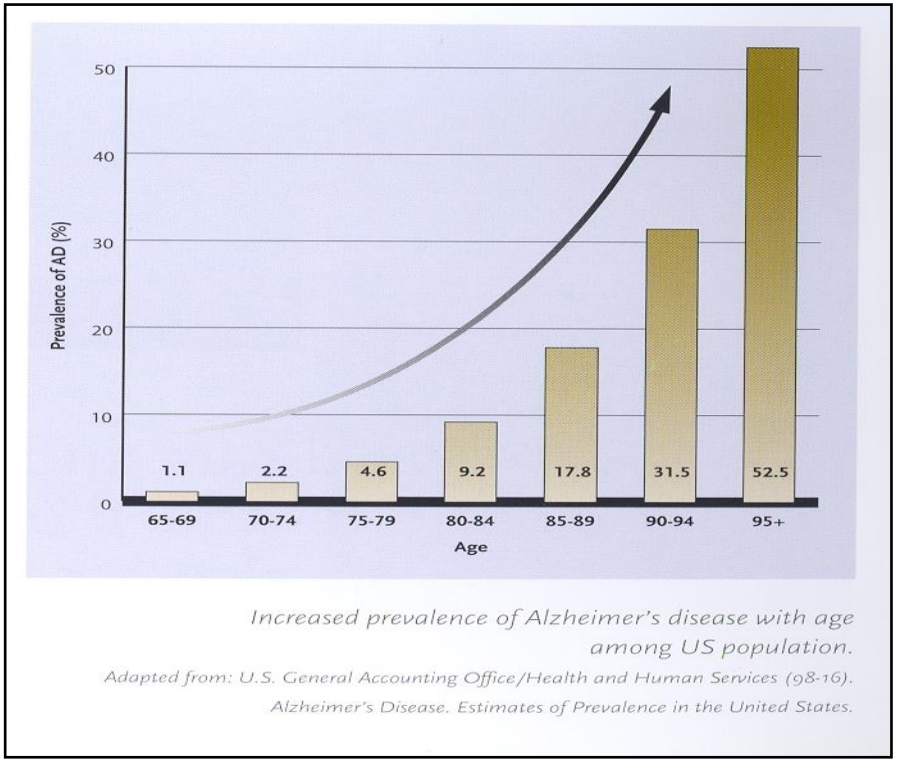
- language, visual recognition

ALZHEIMER'S DISEASE

Main cause of dementia



Aging is the first risk factor



Increased prevalence of Alzheimer's disease with age among US population.

Adapted from: U.S. General Accounting Office/Health and Human Services (98-16). Alzheimer's Disease. Estimates of Prevalence in the United States.

RISK FACTORS (ALZHEIMER)

Age

Education level

Familial History

Positive genotype Apolipoprotein E 4/4

Arterial hypertension

Hyperinsulinemia

ALZHEIMER'S DISEASE: TARGETS AND THERAPIES

1900 1910 1970 1980 1990 2000 2010

1906: Alois Alzheimer

860 000 cases in France

Acetylcholinesterase Inhibitors
1993 95 97 2007 2010
Tacrine patch generics
Galantamine
Donepezil
Rivastigmine

Anti NMDA
2002
Memantine

Anti amyloid (immuno)therapies era
2000

Anti Tau era ?
2010

ALZHEIMER'S DISEASE
CURRENT STATE OF AD DRUG DEVELOPMENT

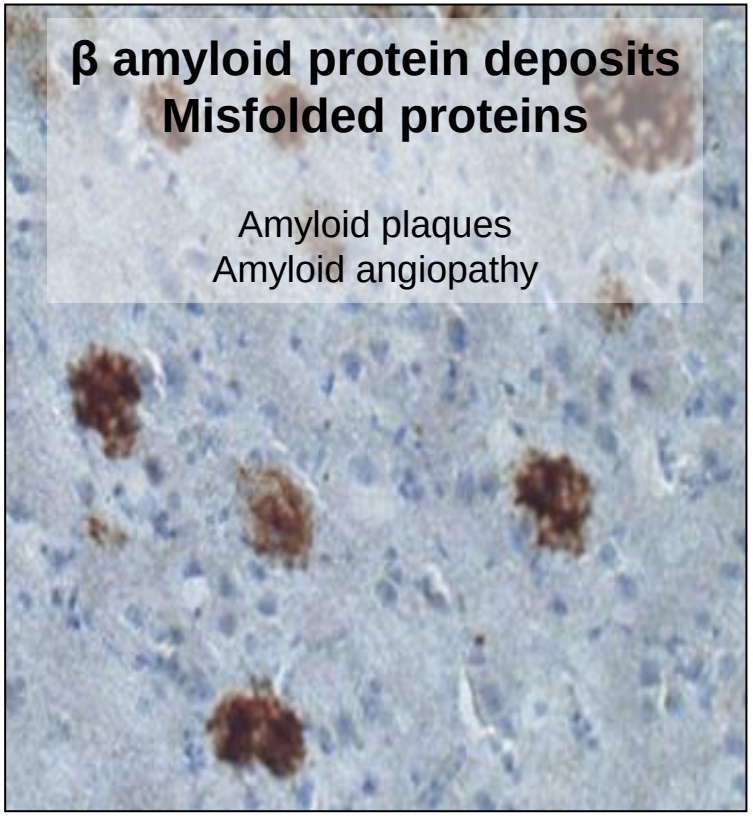
- Only five approved drugs (four cholinesterase inhibitors, one NMDA antagonist)

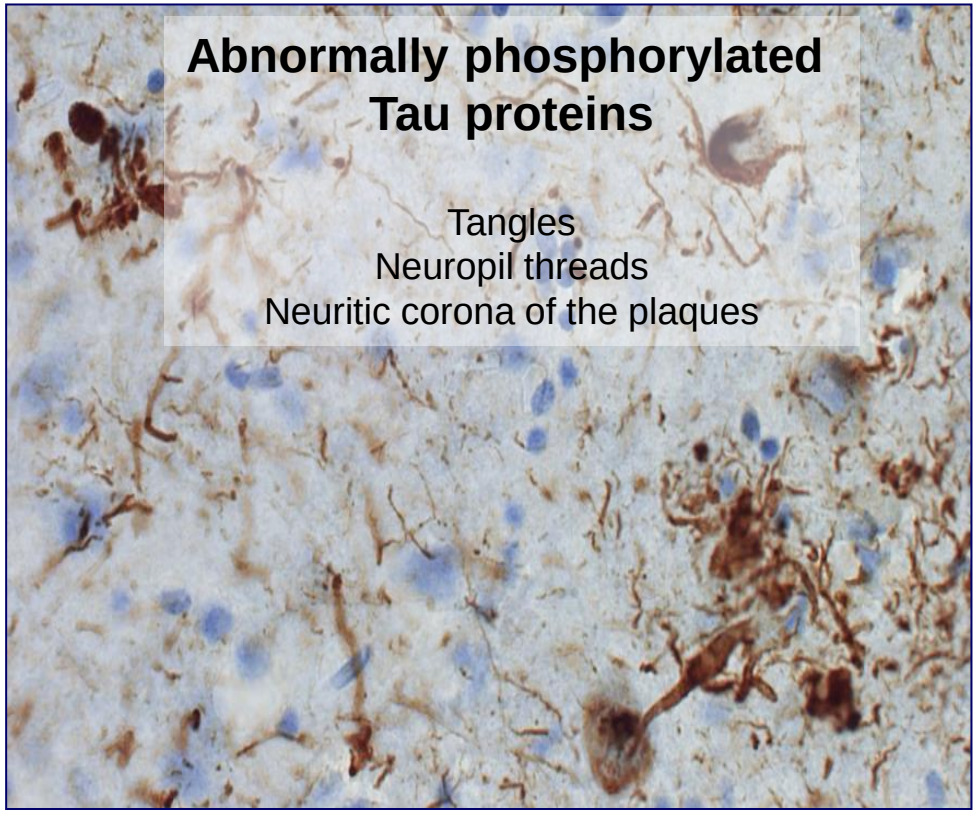
- 413 trials
  124 in Phase 1
  206 in Phase 2
  83 in Phase 3

- **Attrition rate of 99.6%!**

Data from clinicaltrials.gov looking at period 2002-2012
Analysed by Cummings et al. 2014

ALZHEIMER'S DISEASE
POST-MORTEM HALLMARKS

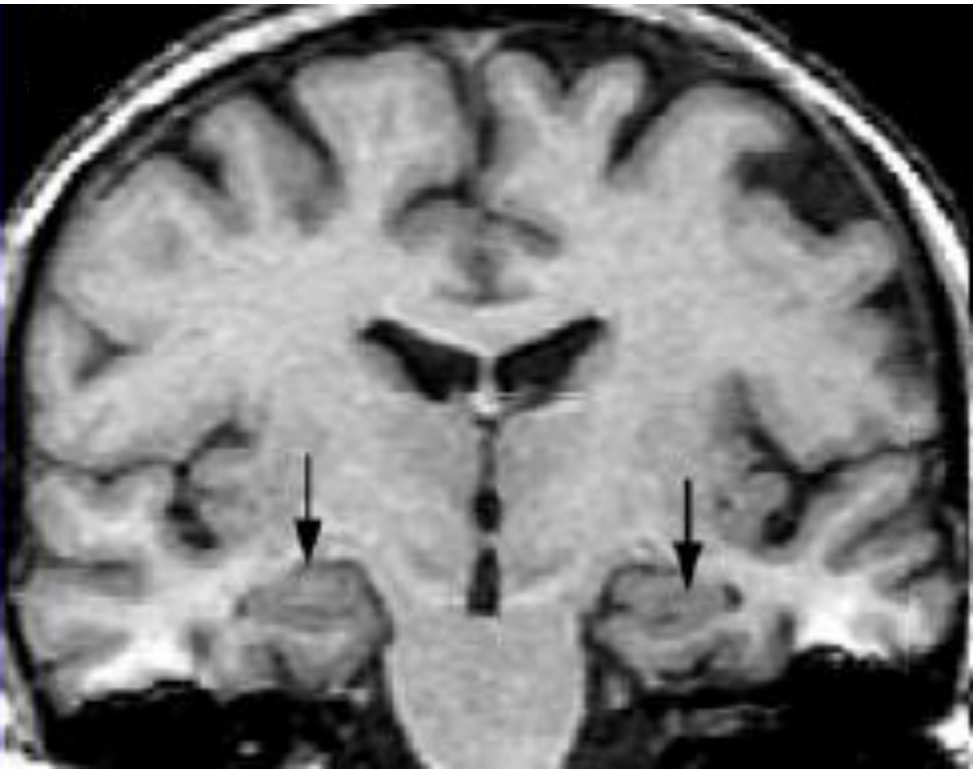


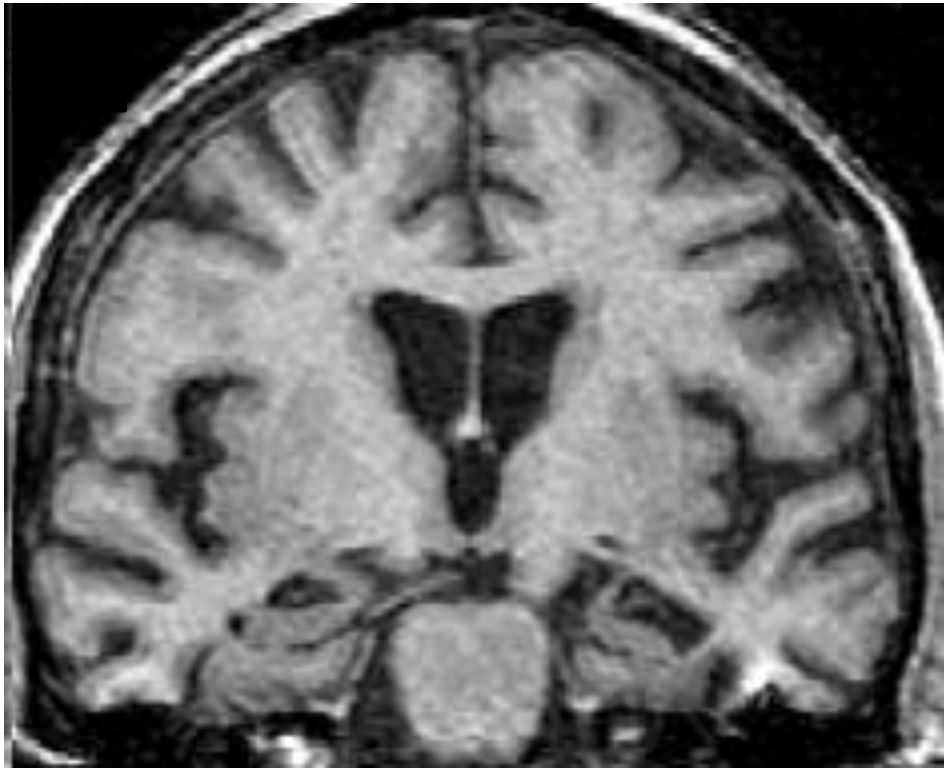


Duyckaerts C et al. Acta Neuropathol. 2009

DIAGNOSTIC OF ALZHEIMER'S DISEASE
CONTRIBUTION OF IN-VIVO IMAGING

Cerebral atrophy



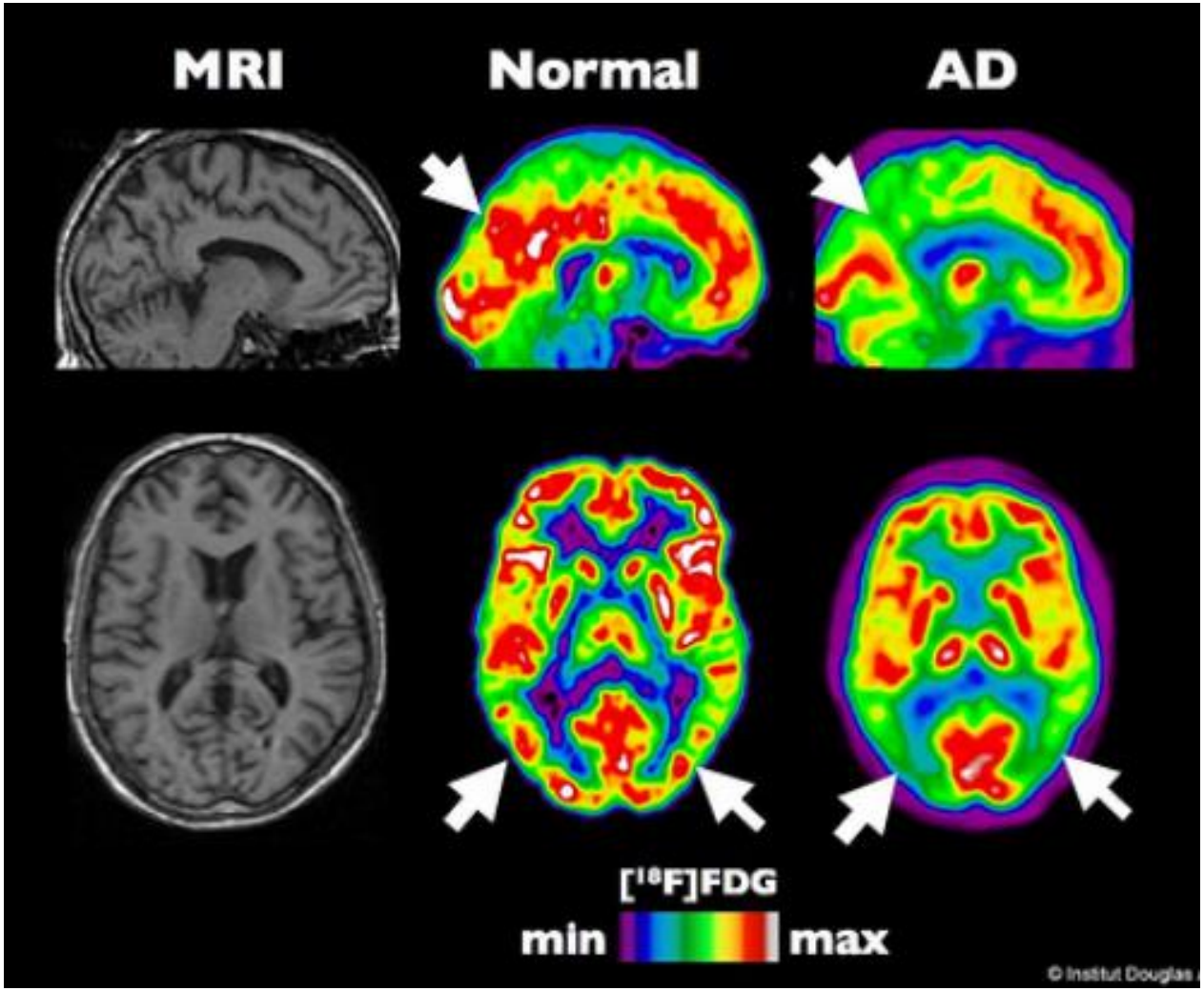


Normal aging

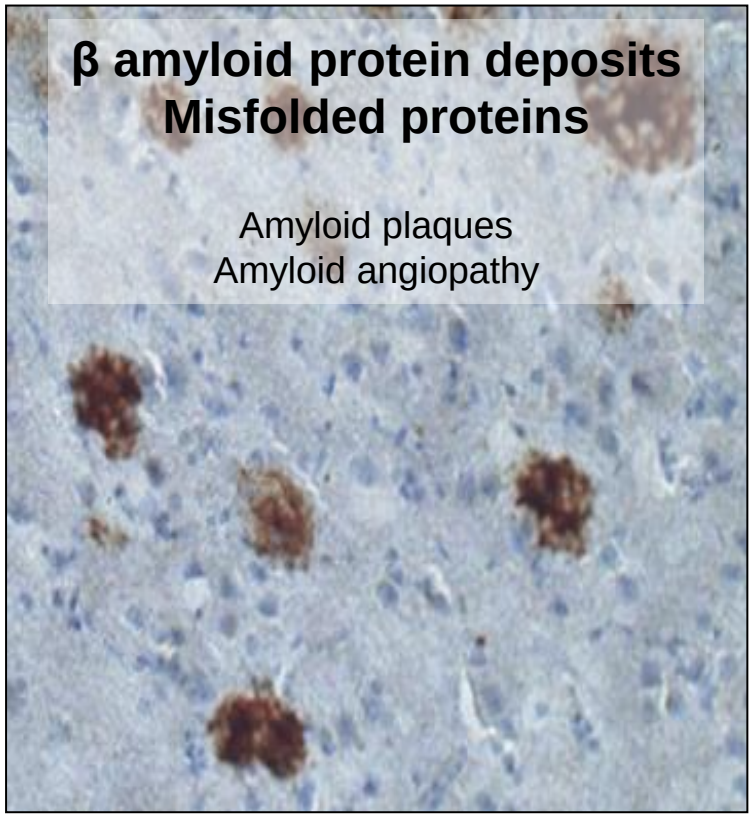
Alzheimer
Moderate form
40%
Temporal atrophy

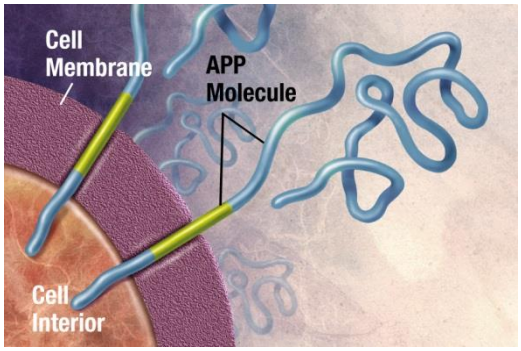
DIAGNOSTIC OF ALZHEIMER'S DISEASE
CONTRIBUTION OF IN-VIVO IMAGING

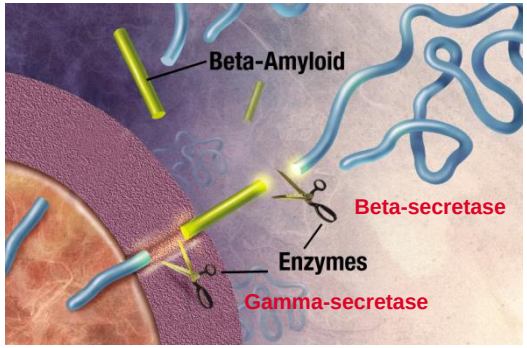
Reduced glucose metabolism

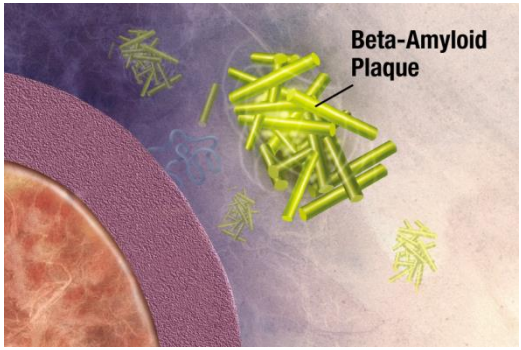


ORIGIN OF AMYLOID PLAQUES



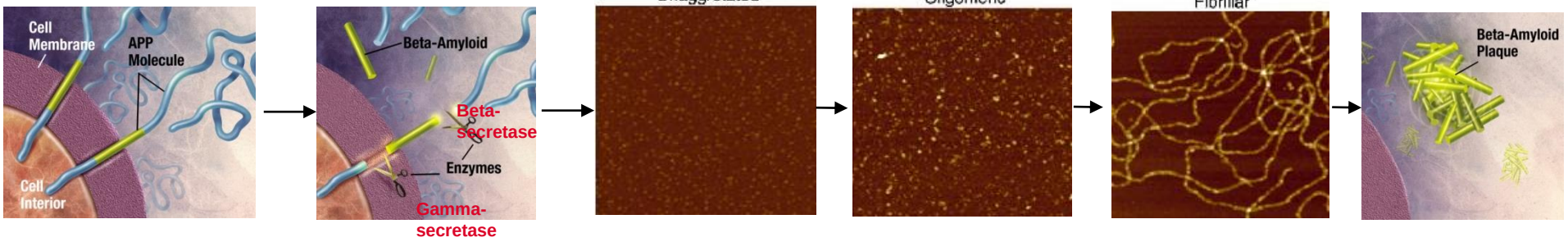






APP : Amyloid Protein Precursor

EX. OF AMYLOID PLAQUES
FROM APP TO AGGREGATED FORMS OF AMYLOID



soluble
Invisible

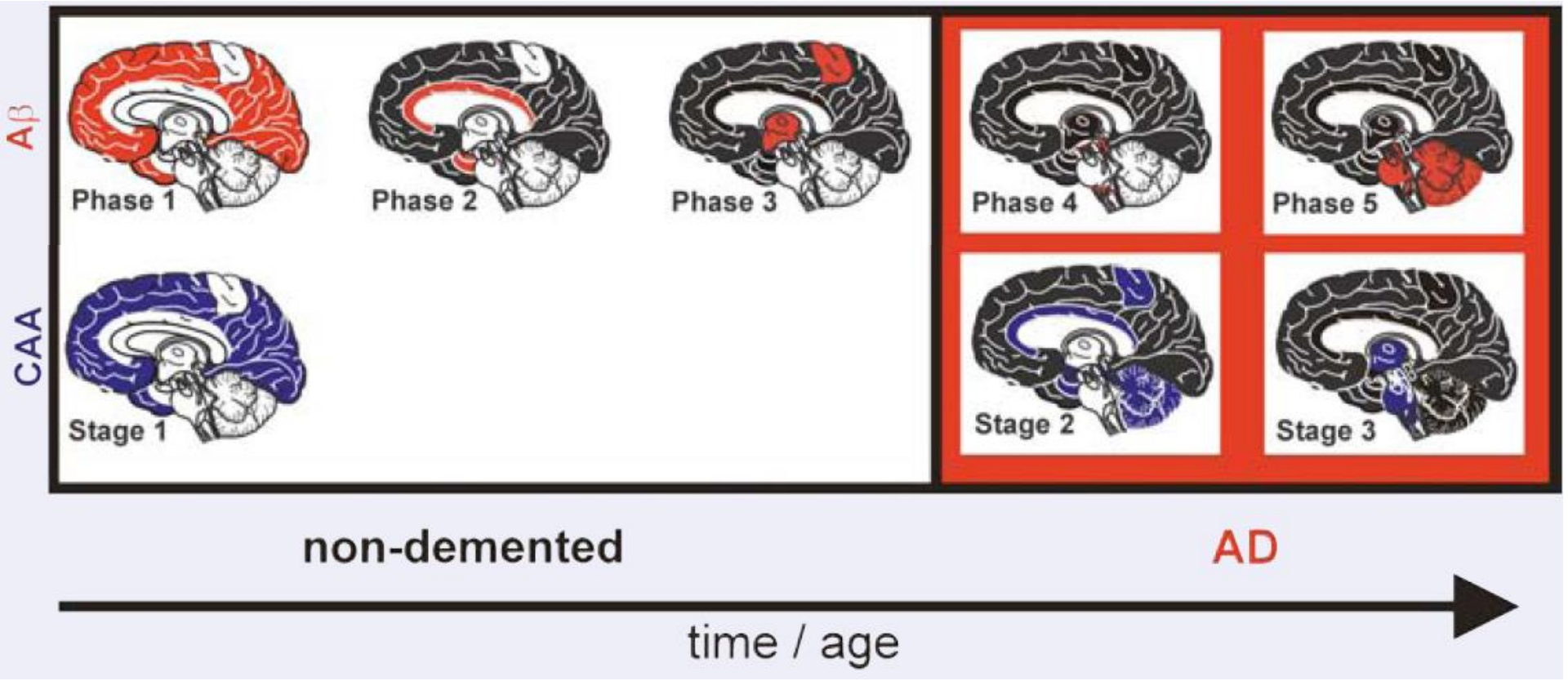
soluble
~ Invisible

~Invisible

Visible

APP : Amyloid Protein Precursor

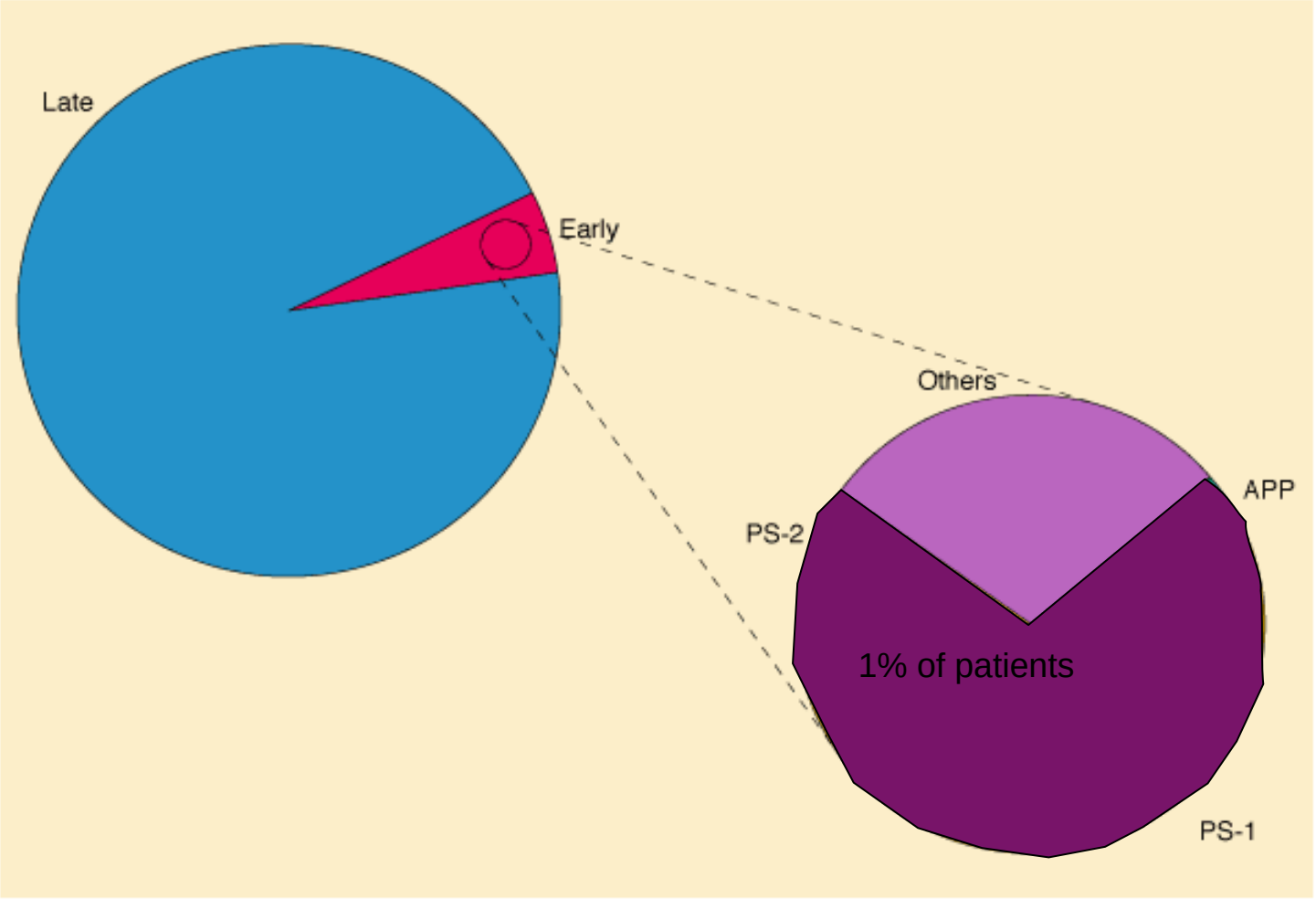
STAGES OF AMYLOID DEPOSITION



Thal, D. R., W. S. Griffin and H. Braak (2008). J Cell Mol Med **12(5B): 1848-1862.**

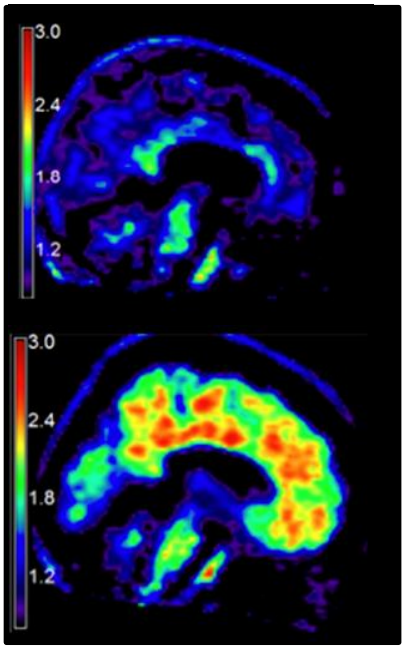
ALZHEIMER'S DISEASE : FEW GENETIC CAUSES
TOWARDS AMYLOID HYPOTHESIS OF ALZHEIMER'S DISEASE

Relative frequency of early and late-onset Alzheimer's and the proportion of early-onset cases attributed to mutations in specific genes such as APP, PS1, PS2 or others



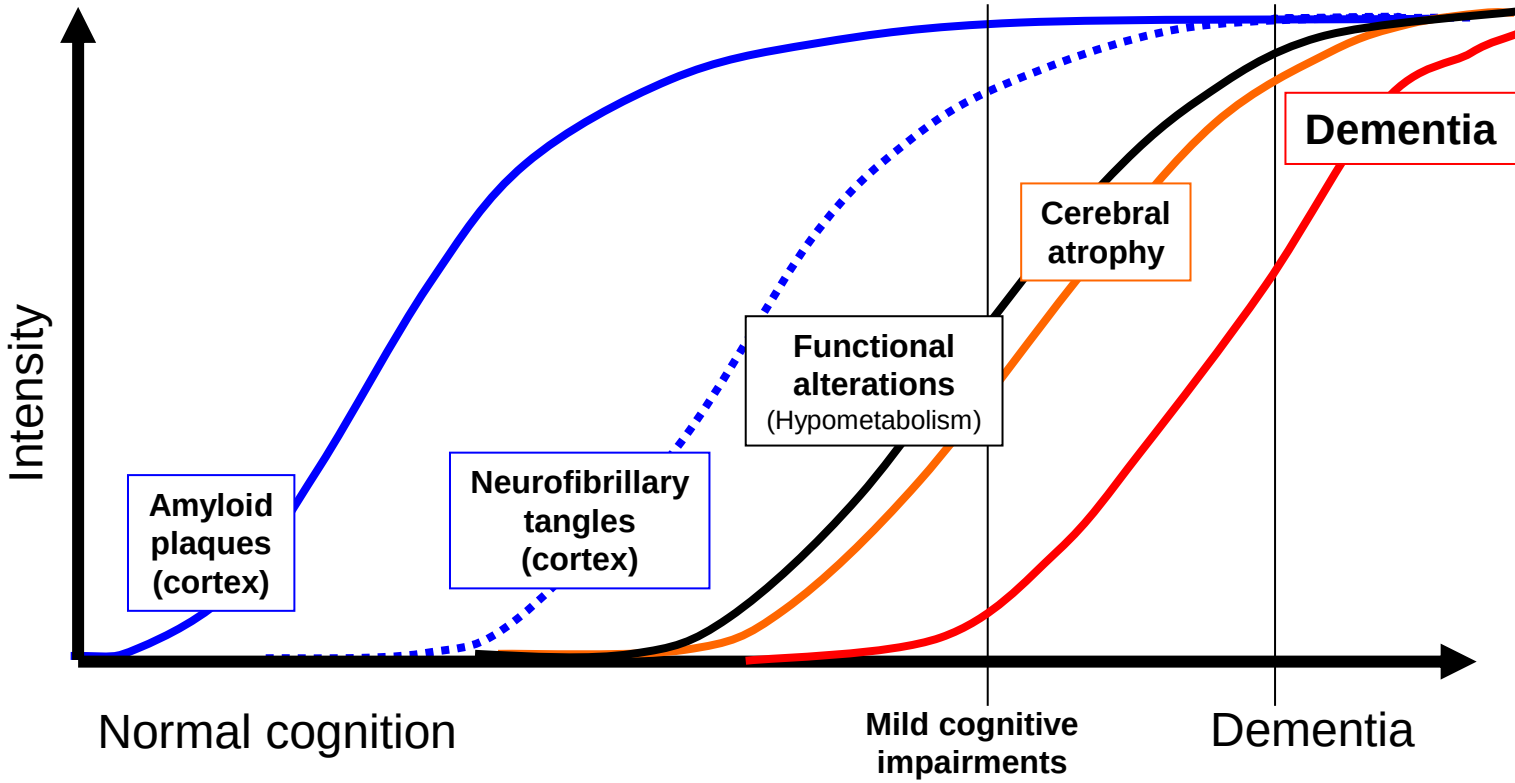
NATURAL HISTORY OF AD BASED ON IMAGING BIOMARKERS ?

Amyloid
(PET)



Klunk, 2004

Jack et al, Lancet Neurol. 2013

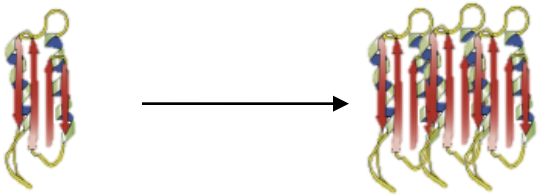


Probably wrong

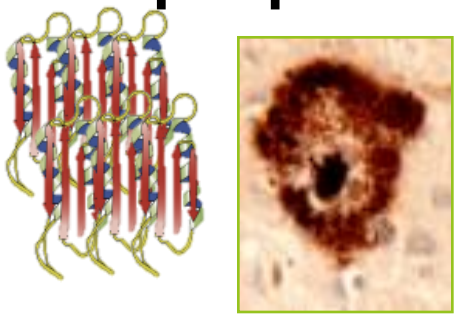
ANIMAL MODELS BASED ON AMYLOID HYPOTHESIS OF AD

Amyloid precursor Protein (APP)

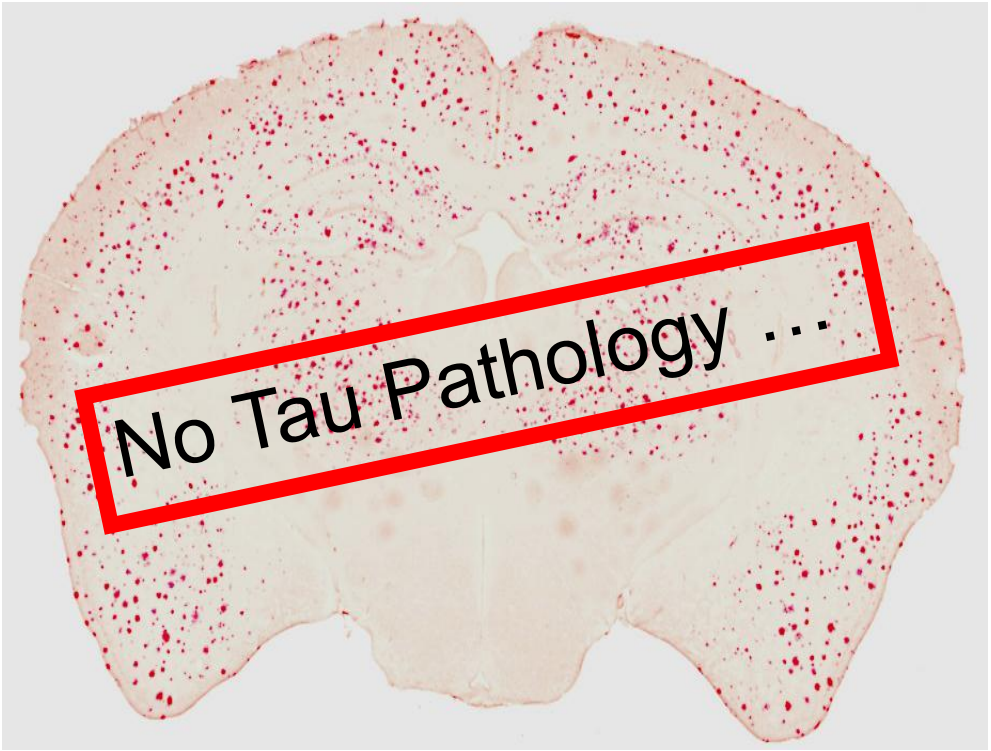
Amyloide oligomeric (soluble)



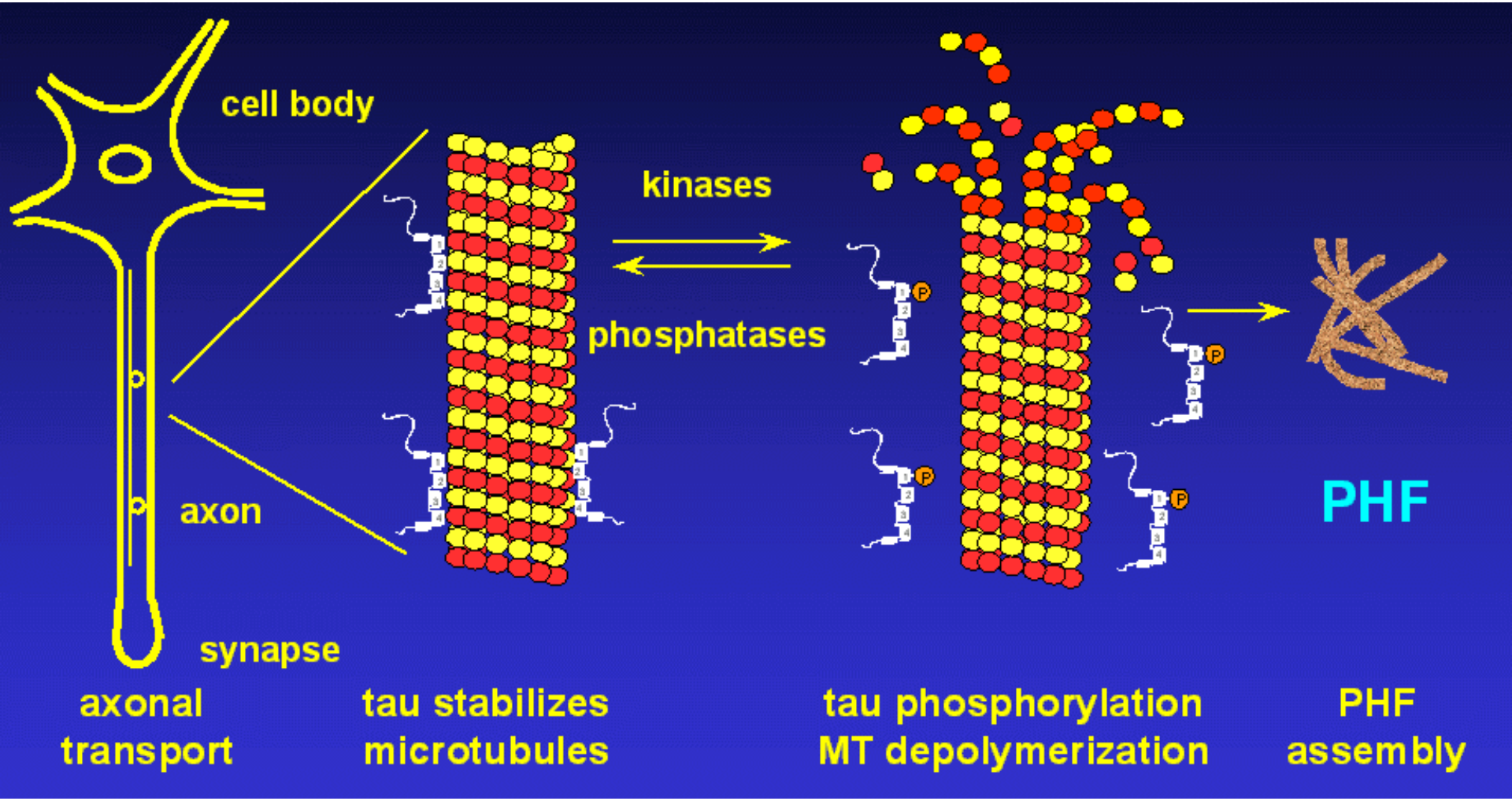
Amyloid plaques



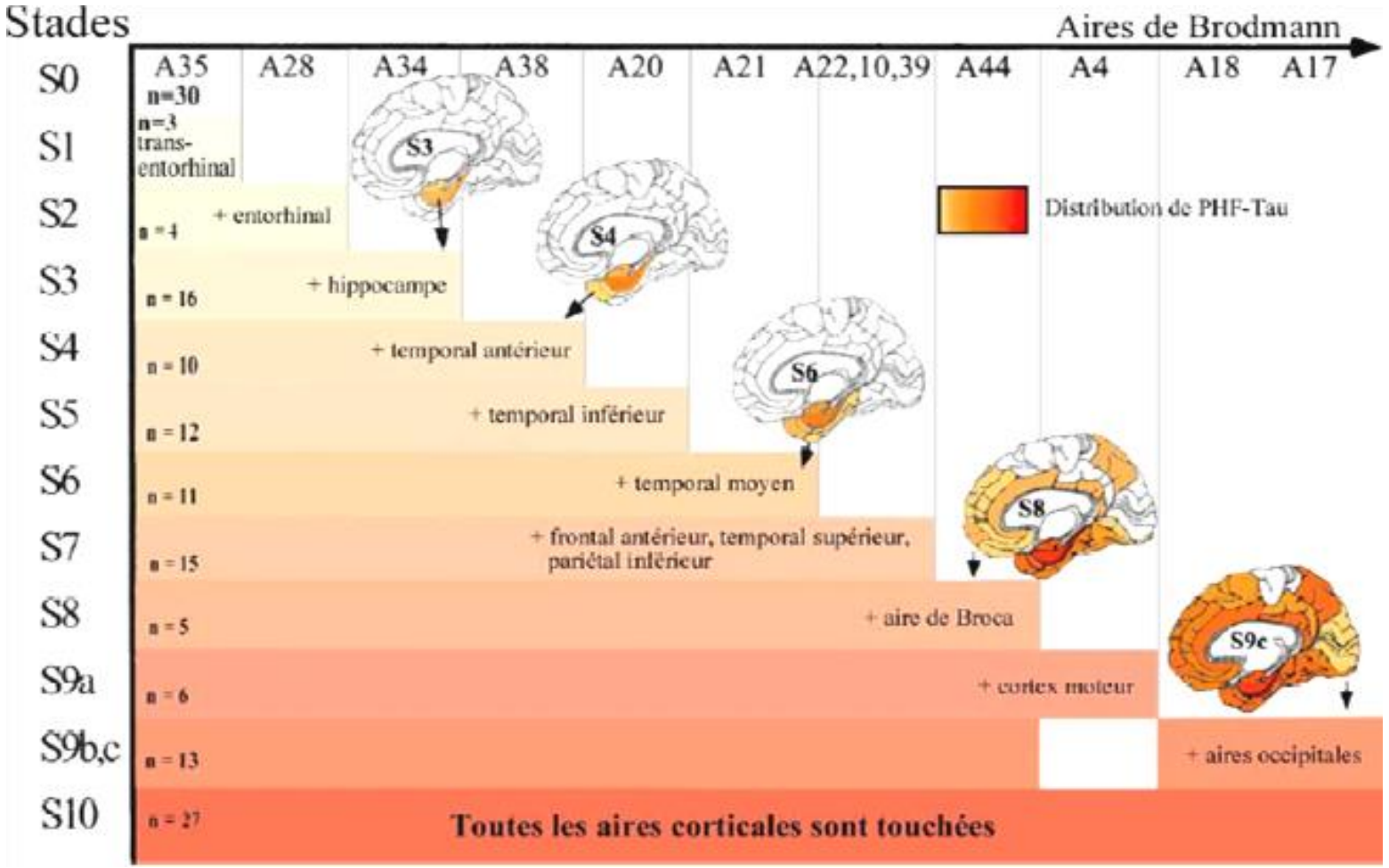
APP Mutations



ORIGIN OF TAU LESIONS

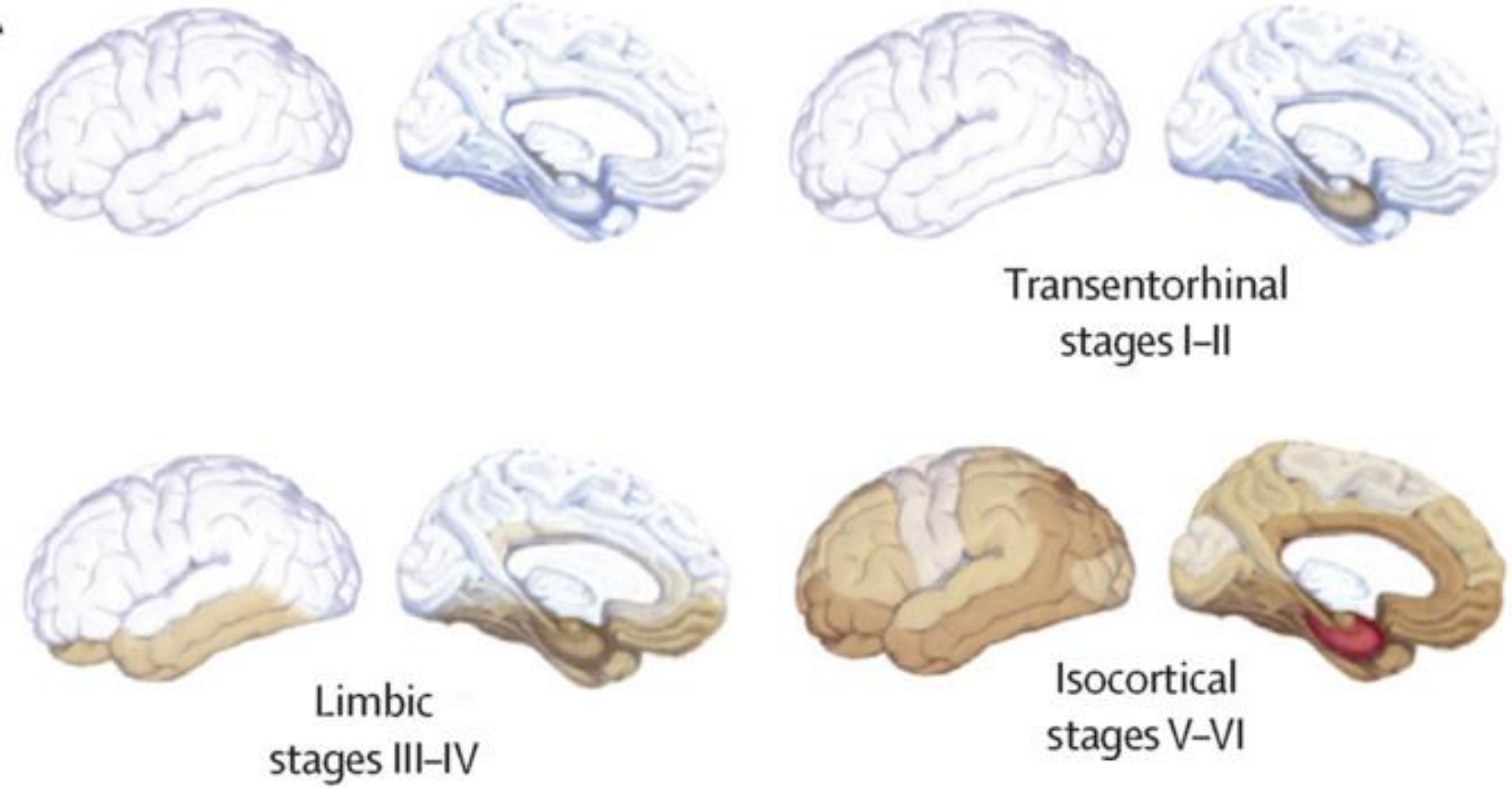


TAU LESIONS
PROGRESSIVE COLONISATION OF THE BRAIN



Delacourte, A., (1999). Neurology **52(6): 1158-1165.**

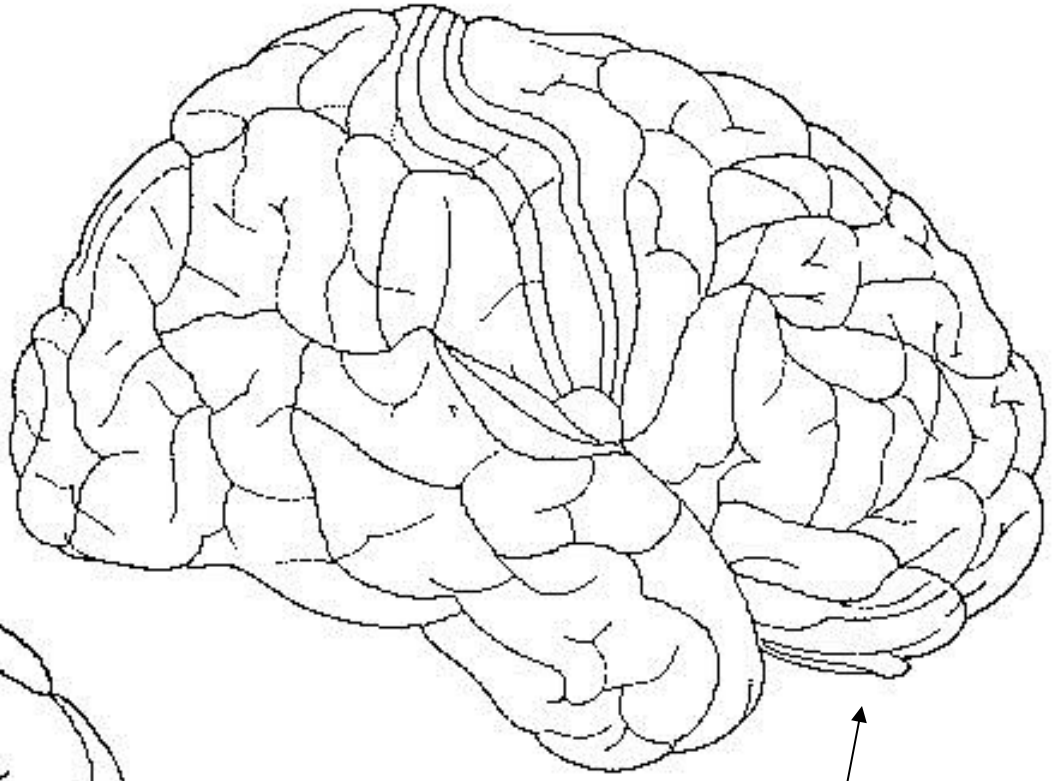
TAU LESIONS
PROGRESSIVE COLONISATION OF THE BRAIN



Braak, H. and E. Braak (1991). Acta Neuropathologica **82: 239-259.**

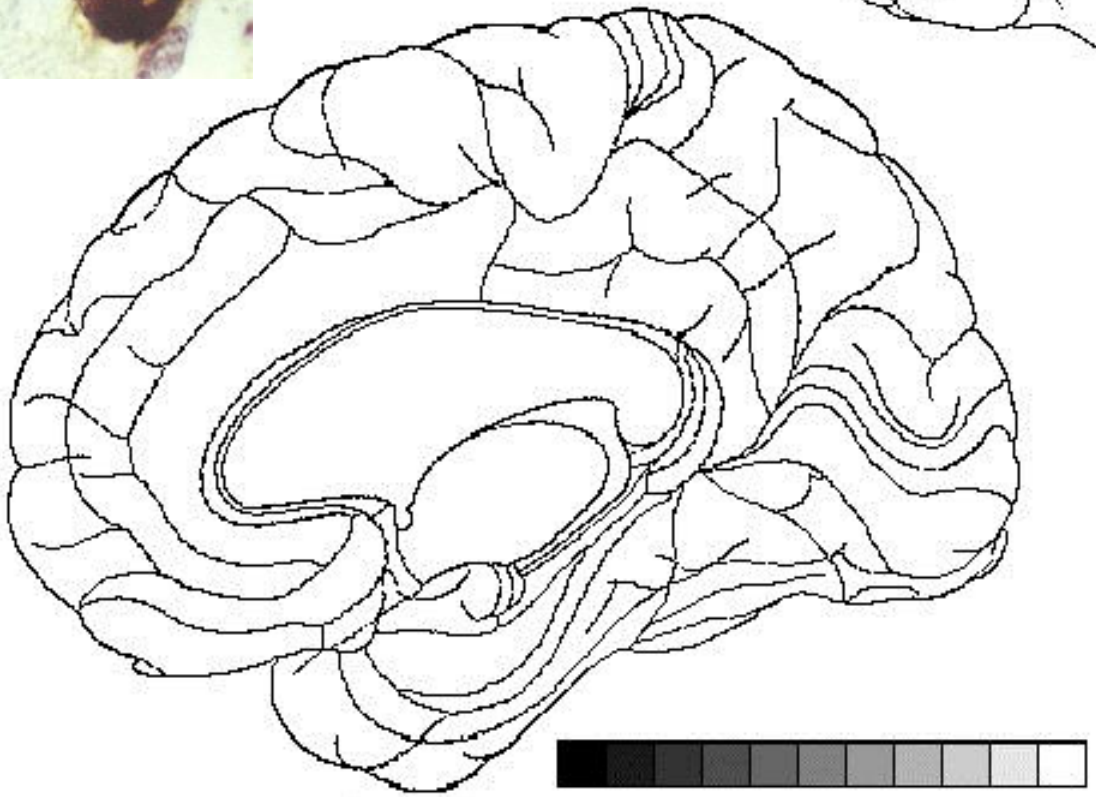
Delacourte stade 0
~ BRAAK STAGE 0

Occipital



External view

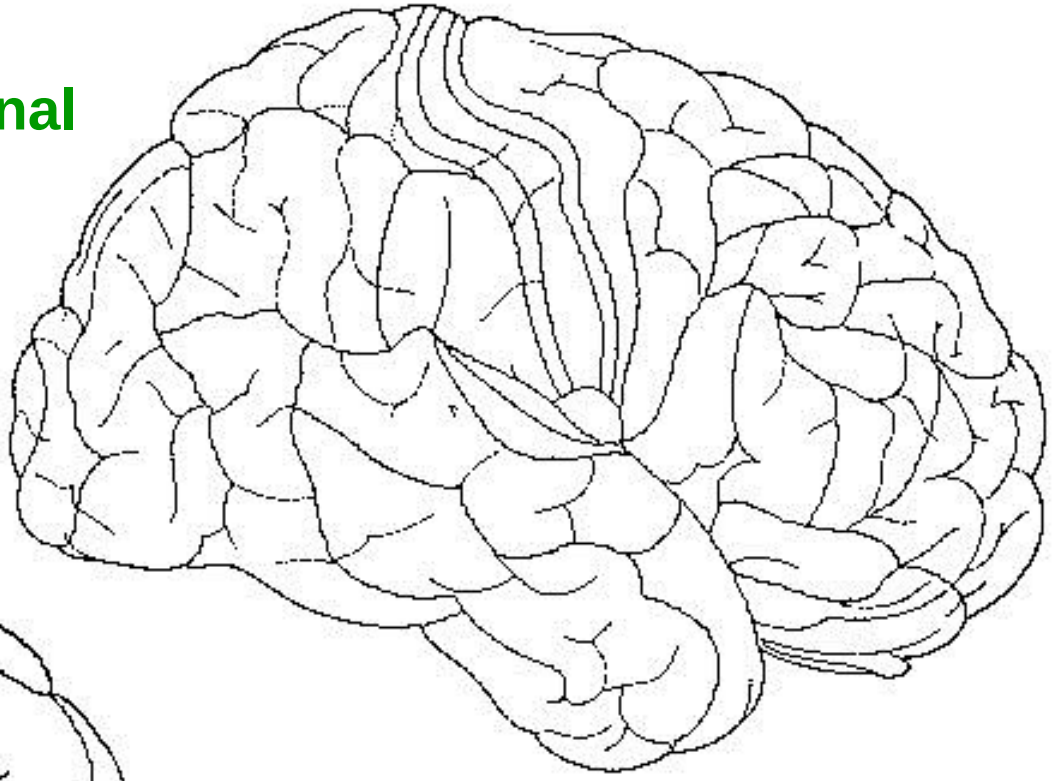
Frontal

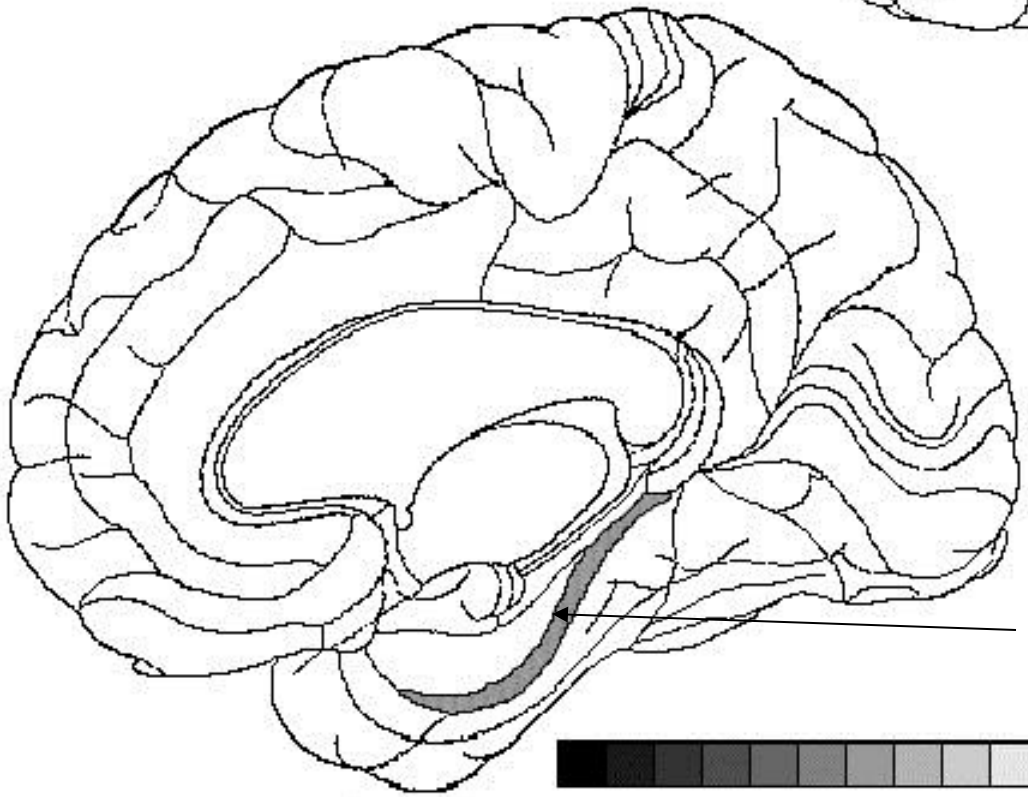


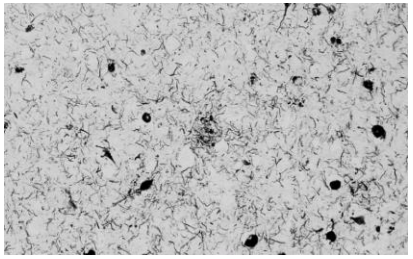
Internal view

Adapté de A. Delacourte

Delacourte stade 1
~ Braak stage I - Transentorhinal



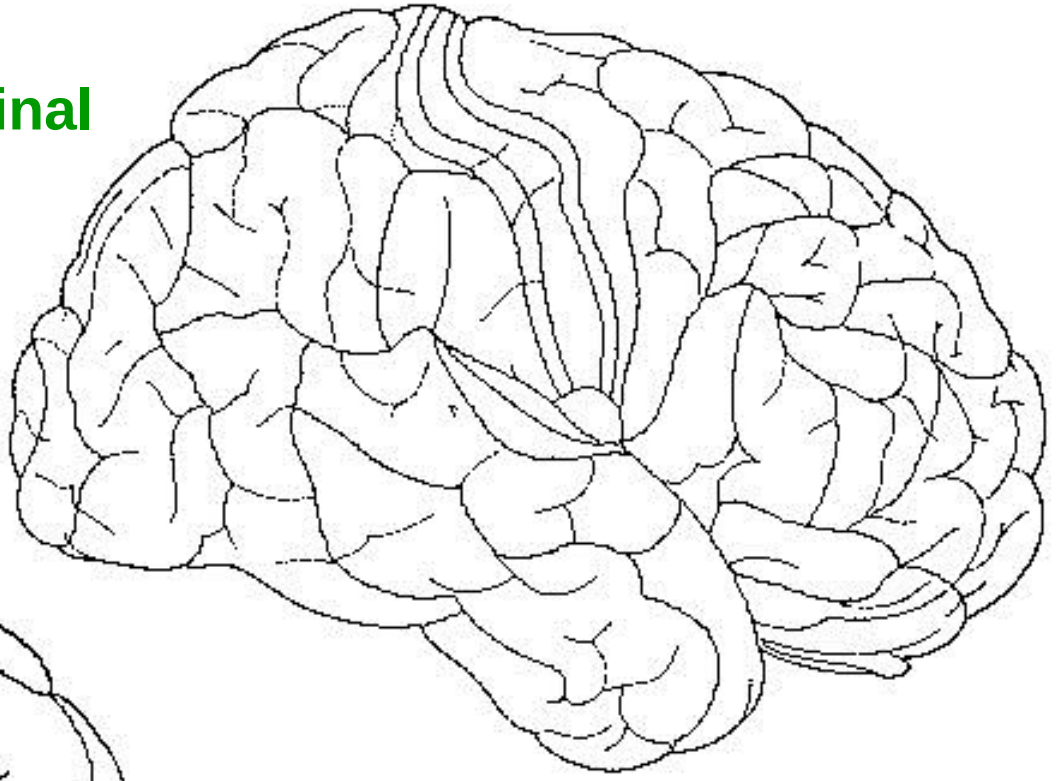


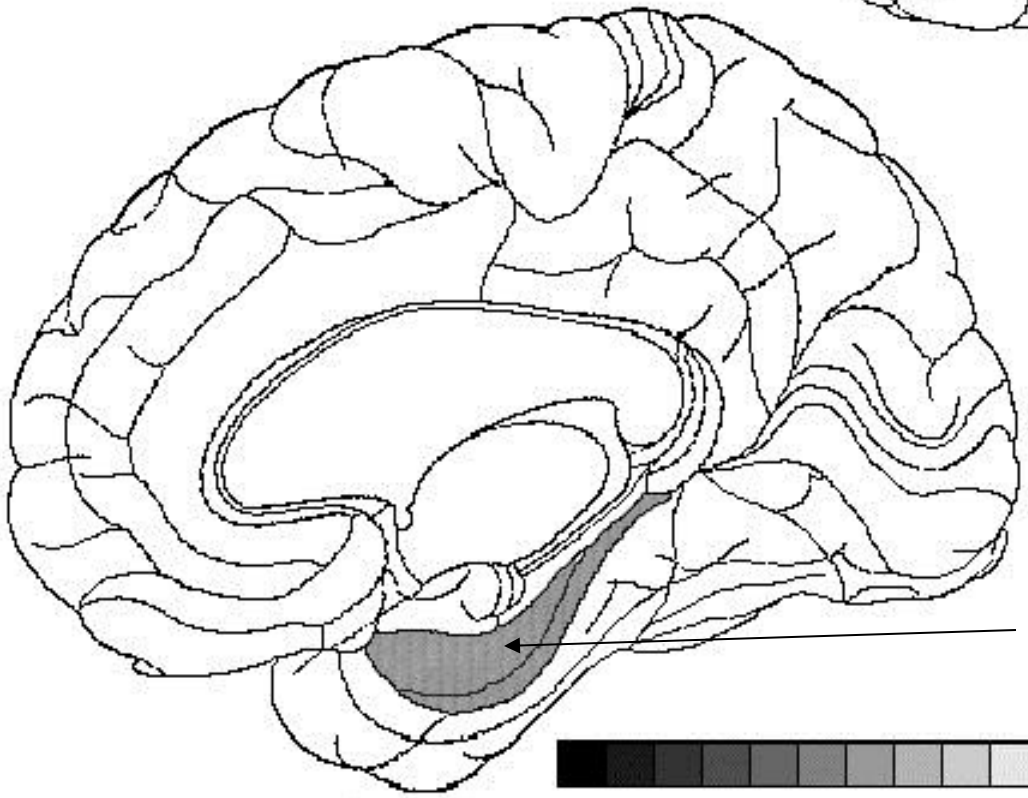


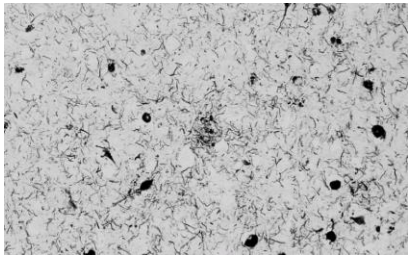
Trans-entorhinal Cortex

Adapté de A. Delacourte

Delacourte stade 2
~ Braak stage II - Transentorhinal





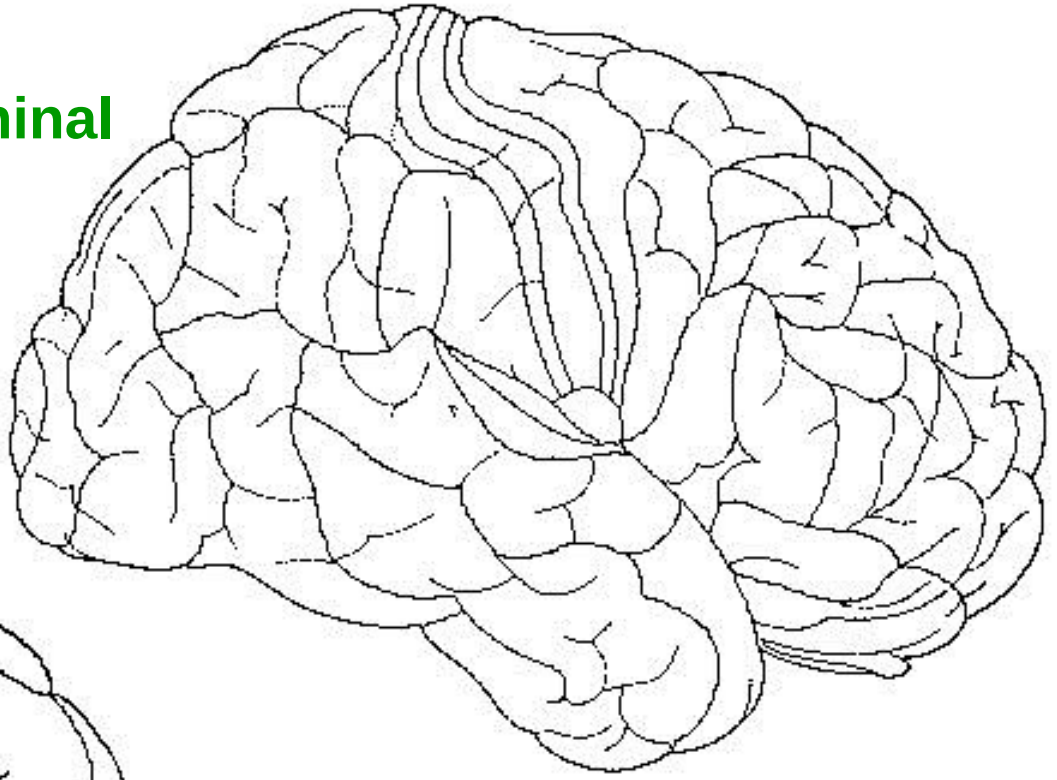


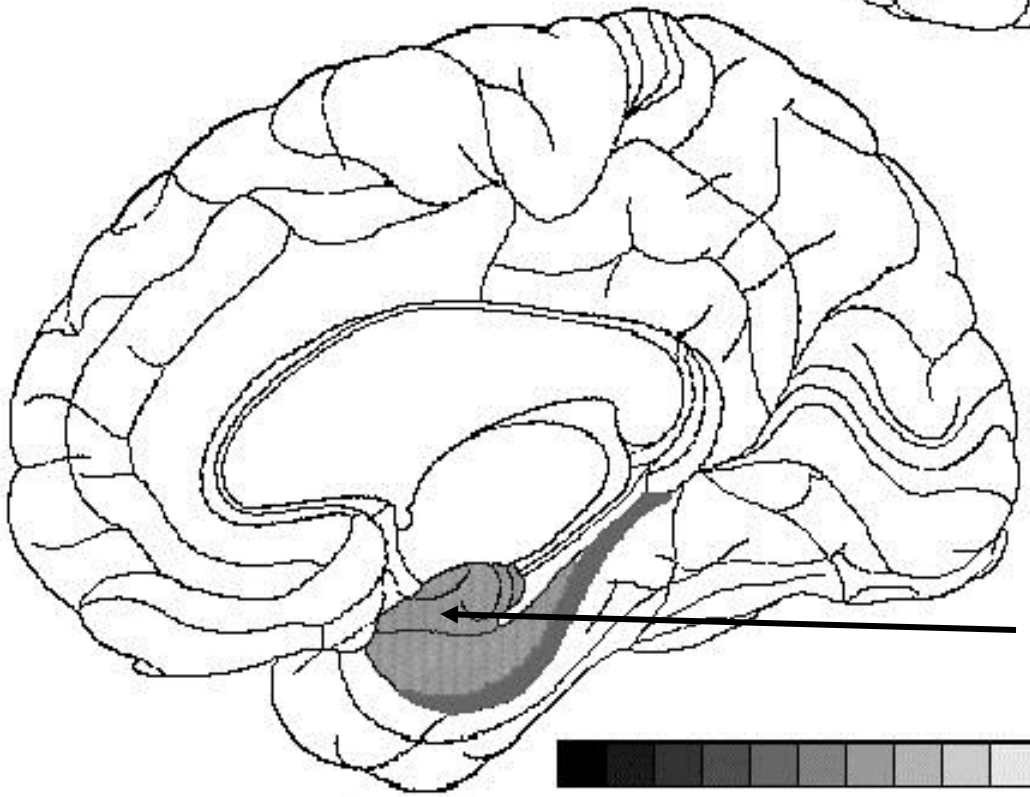
Entorhinal Cortex (aera 28)

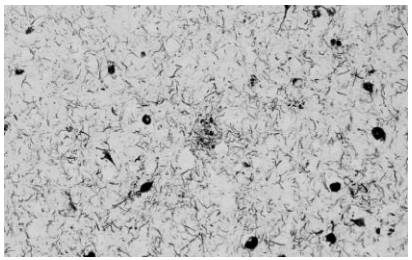
Adapté de A. Delacourte

Delacourte stade 3
~ Braak stage II - Transentorhinal

Mild Cognitive Impairment
start







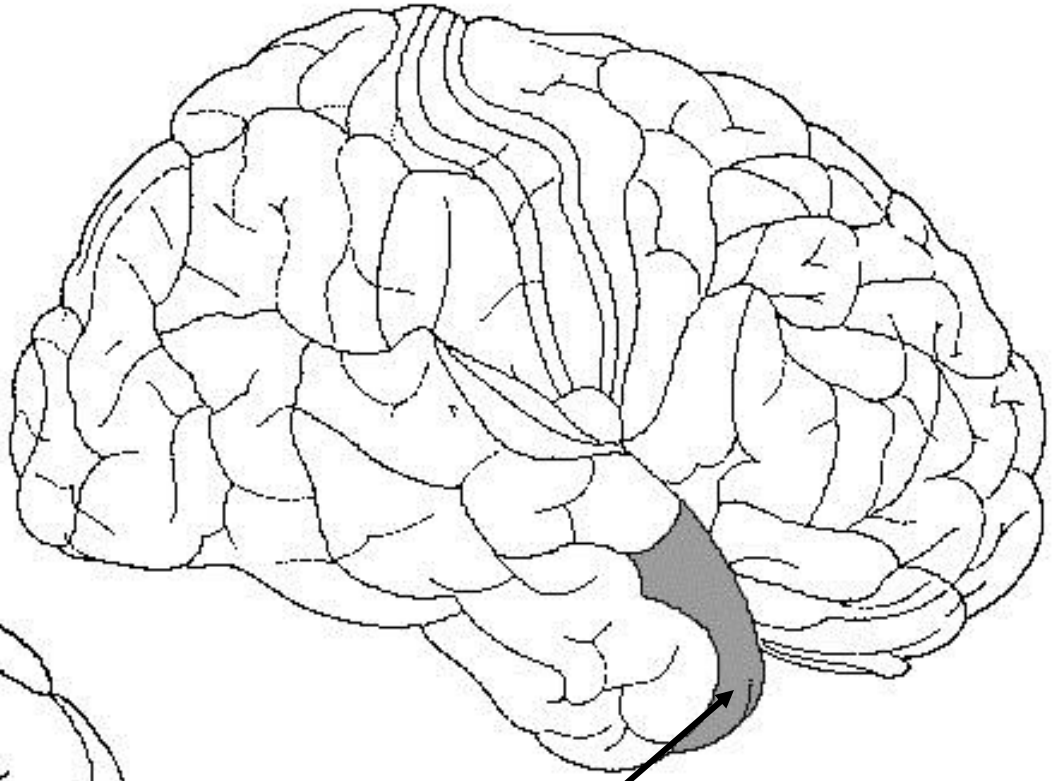
Hippocampus

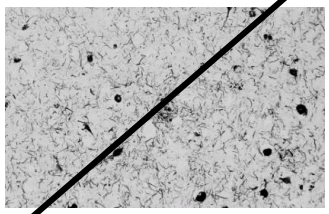
Adapté de A. Delacourte

Delacourte stade 4
~ Braak stage III - Limbic

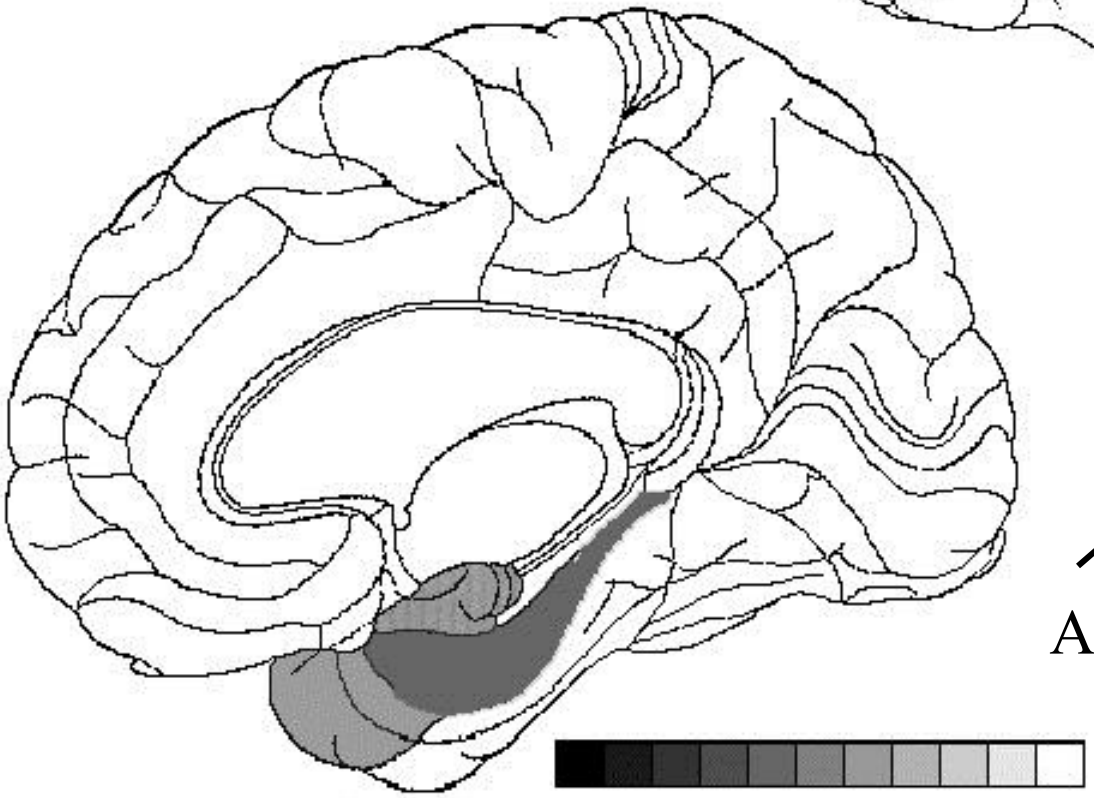
MCI probable

Slight amyloid deposition





Anterior temporal cortex

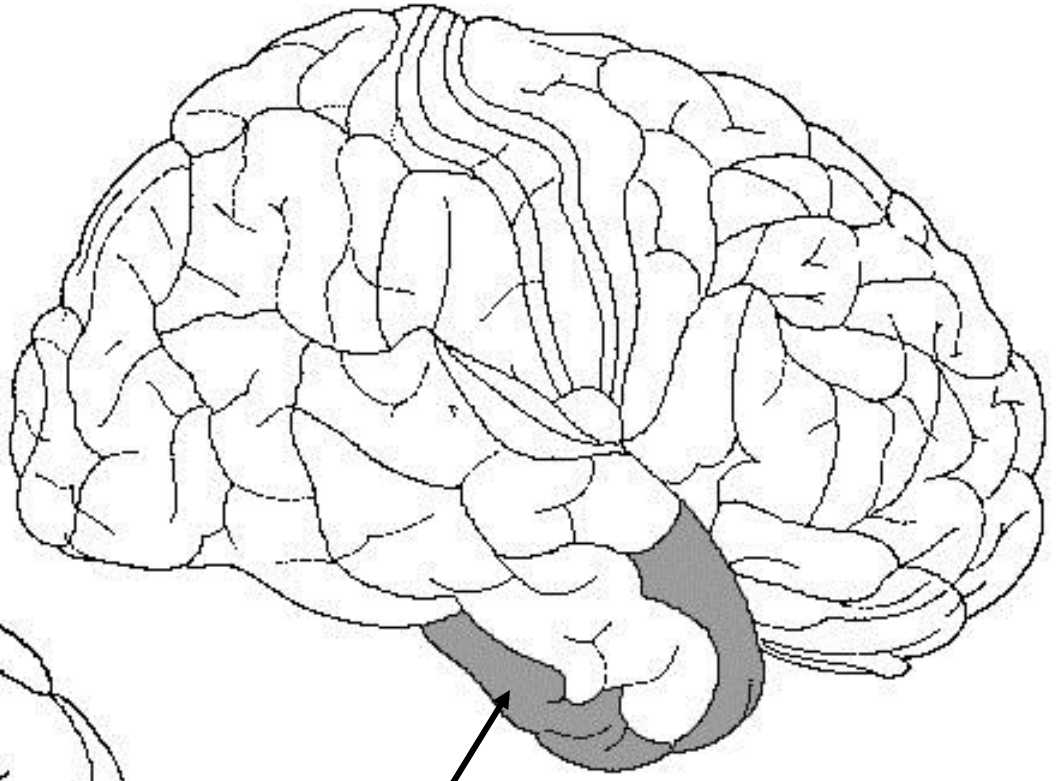


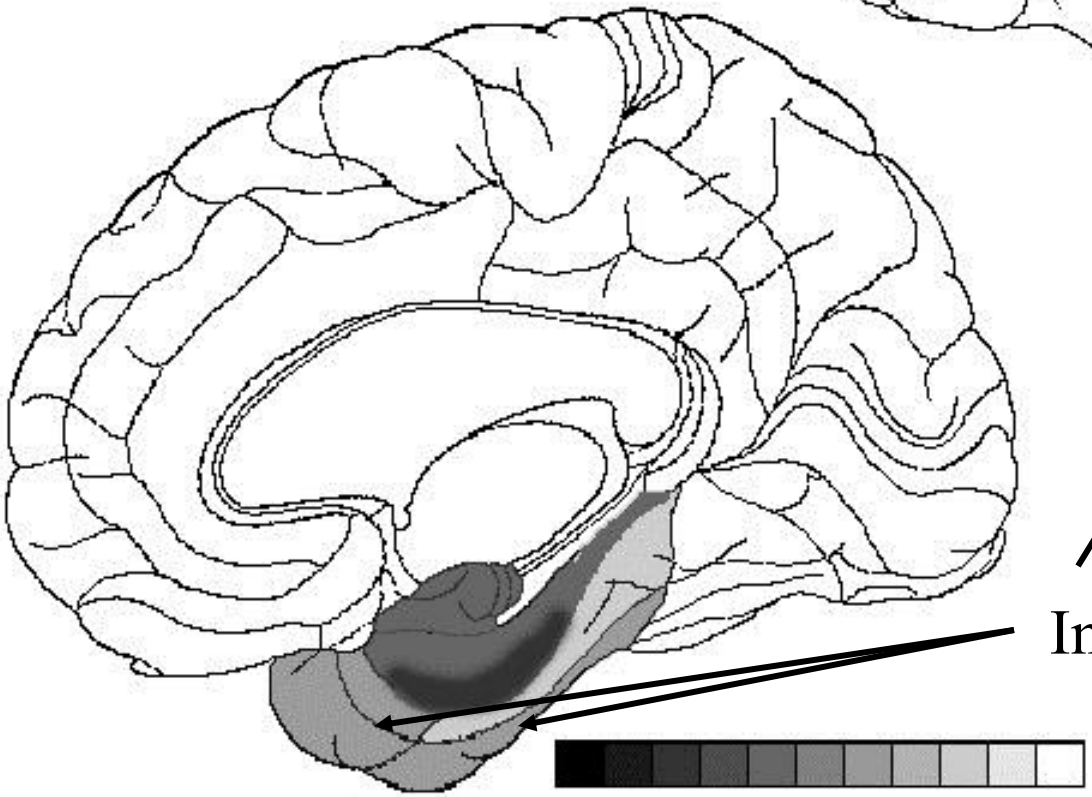
Adapté de A. Delacourte

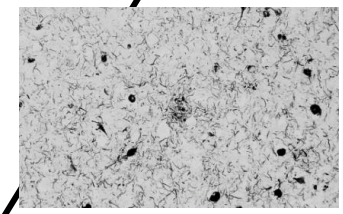
Delacourte stade 5
~ Braak stage III - Limbic

MCI probable ++

Abeta 42 aggregation





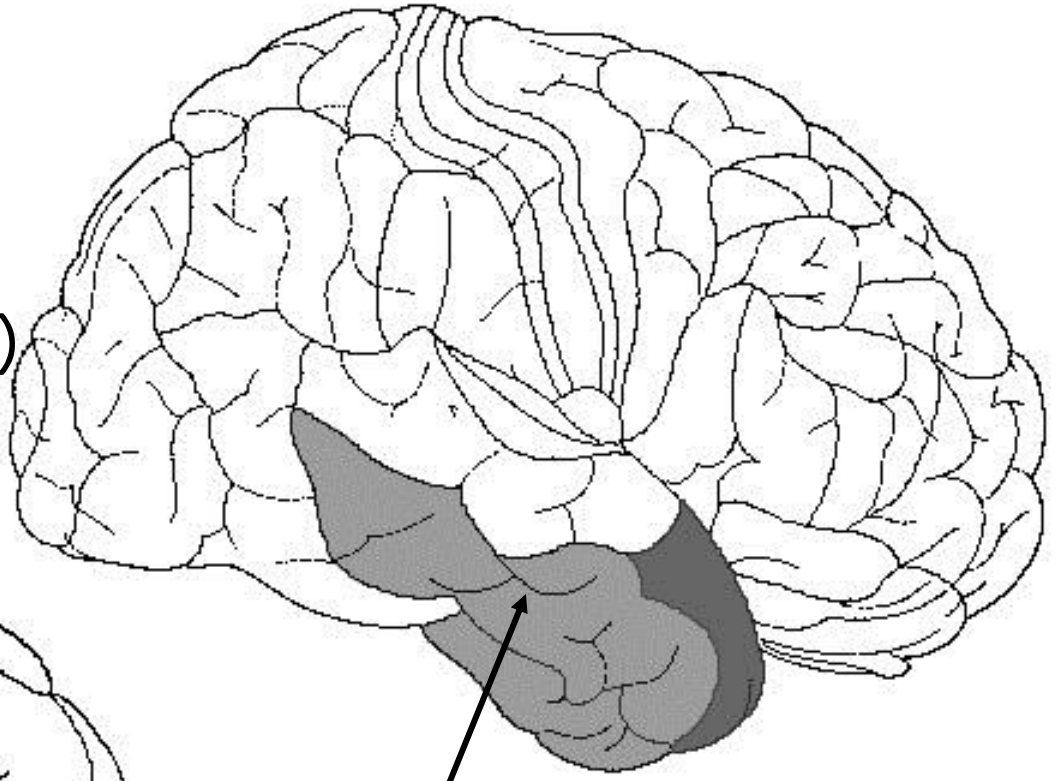


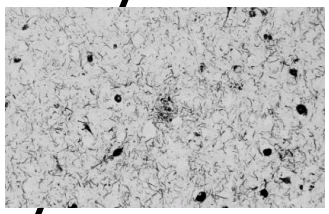
Inferior temporal cortex

Adapté de A. Delacourte

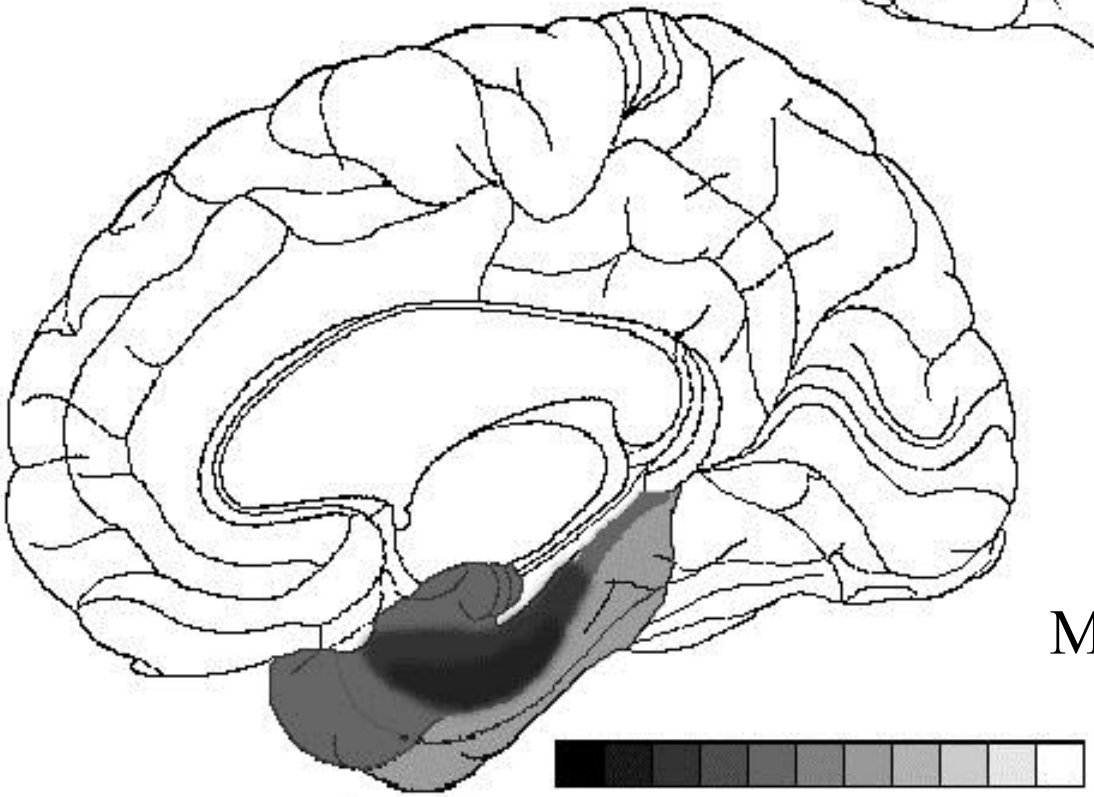
Delacourte stade 6
~ Braak stage IV - Limbic

Cognitive alteration start
(memory, language, praxies)





Median temporal cortex



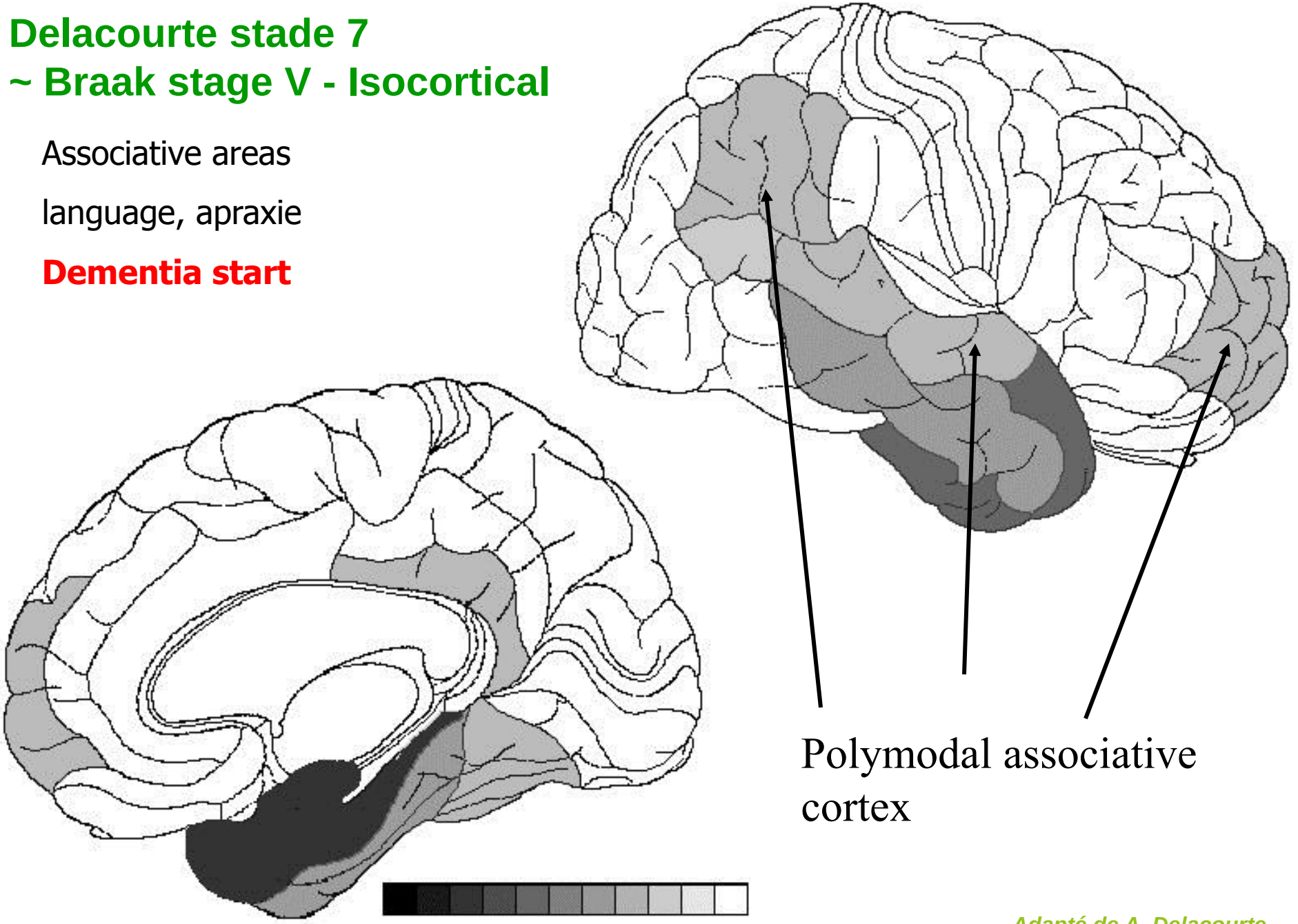
Adapté de A. Delacourte

Delacourte stade 7
~ Braak stage V - Isocortical

Associative areas

language, apraxie

Dementia start



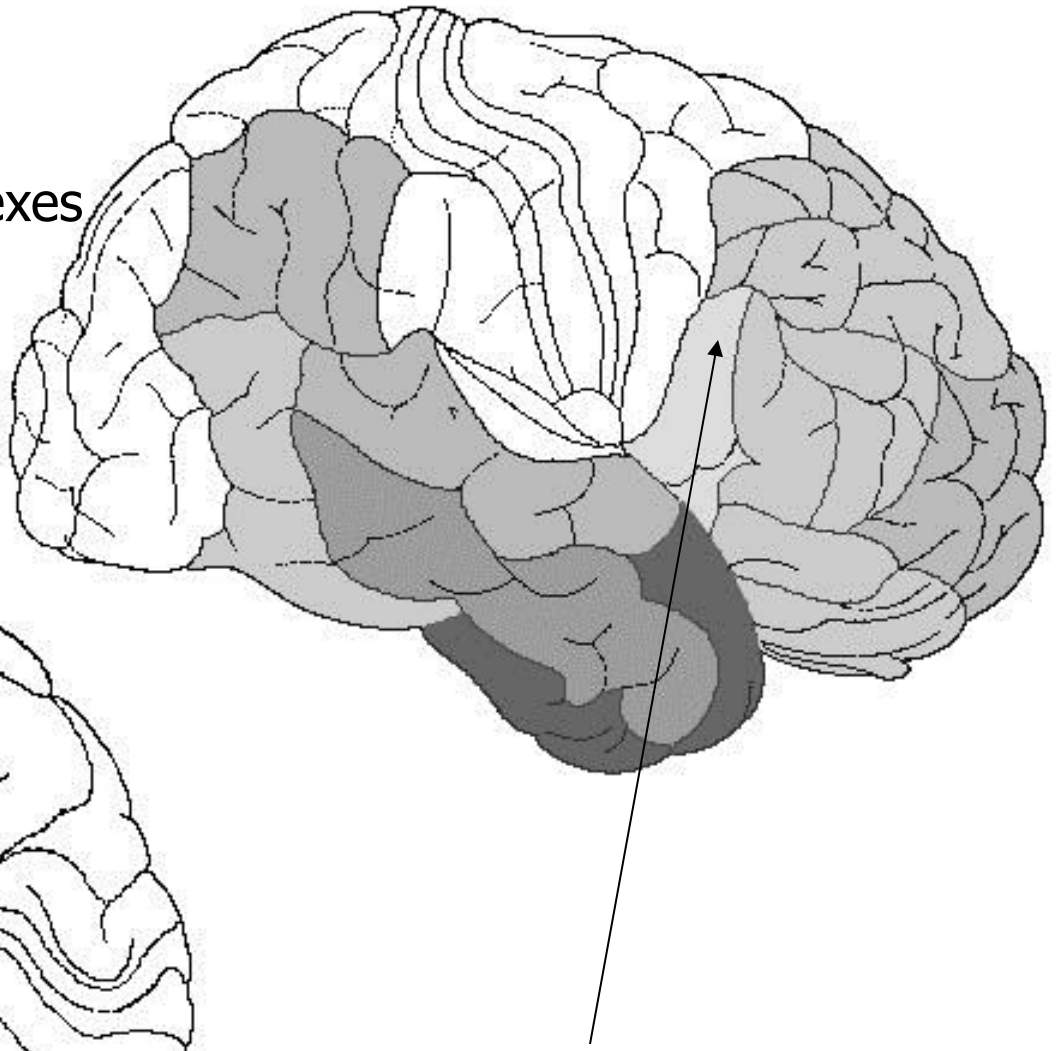
Polymodal associative cortex

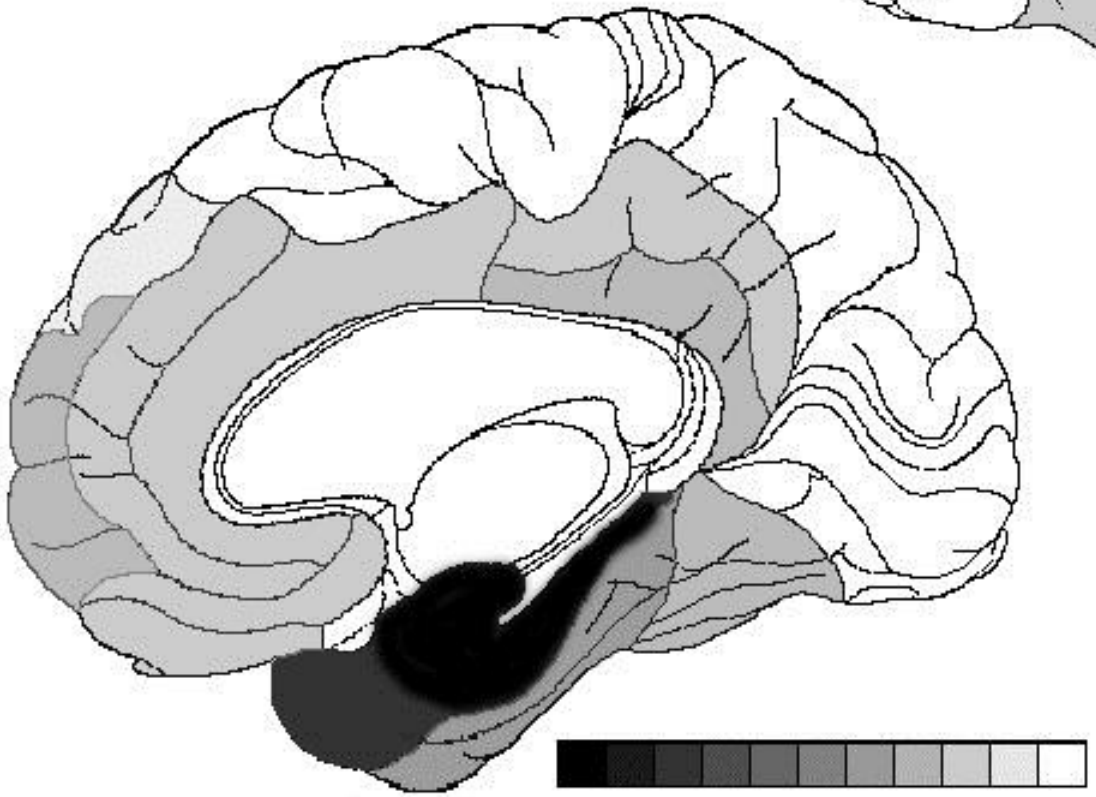
Adapté de A. Delacourte

Delacourte stade 8
~ Braak stage V - Isocortical

Sensitive or motor associative cortexes

Dementia (slight to moderate)





Broca area

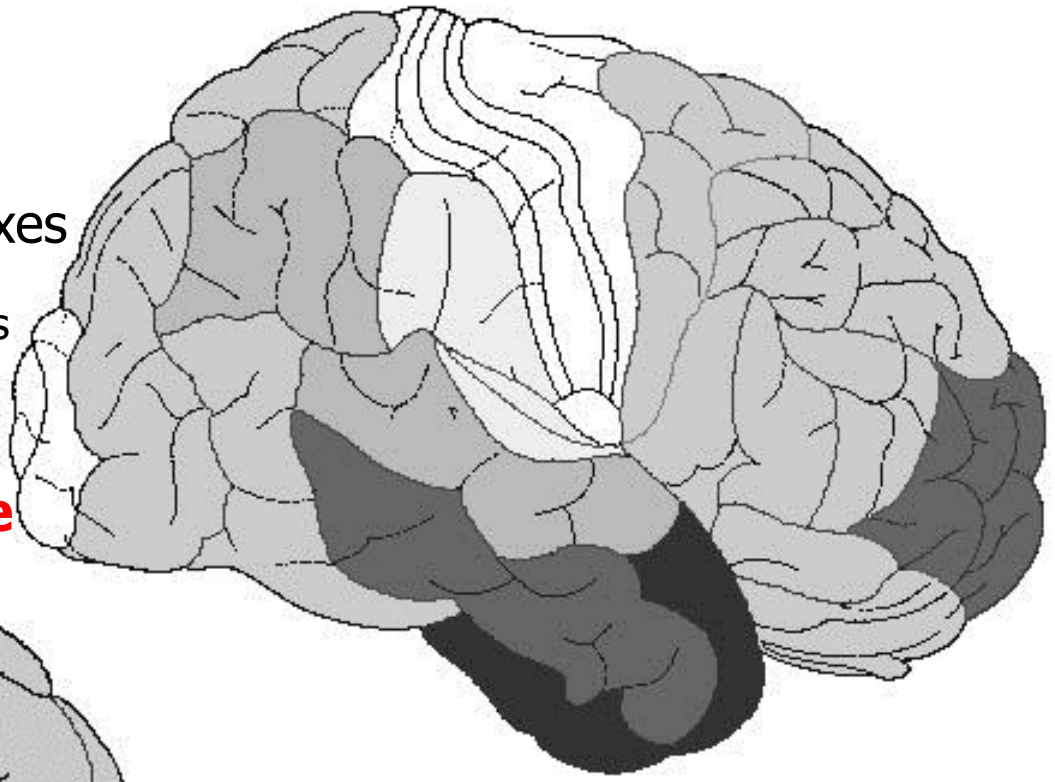
Adapté de A. Delacourte

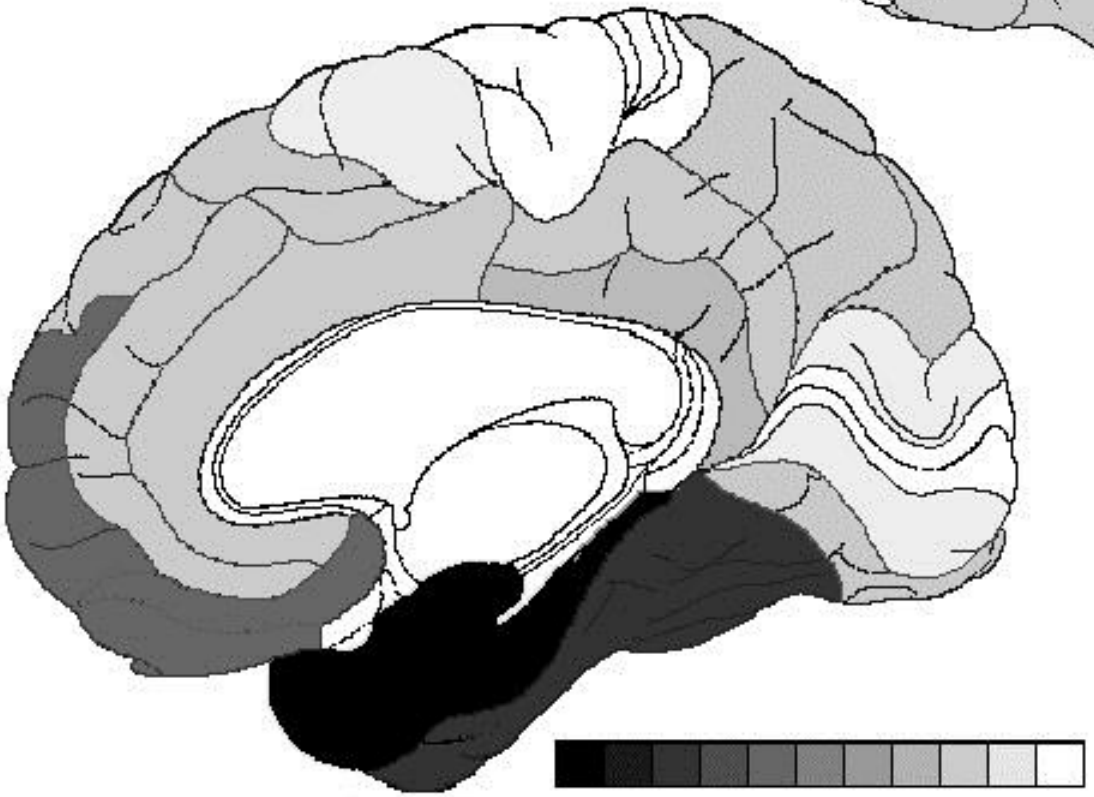
Delacourte stade 9
~ Braak stage V - Isocortical

Sensitive or motor secondary cortexes

Sensitive, visual ou motor primary cortexes

Dementia (moderate to) severe





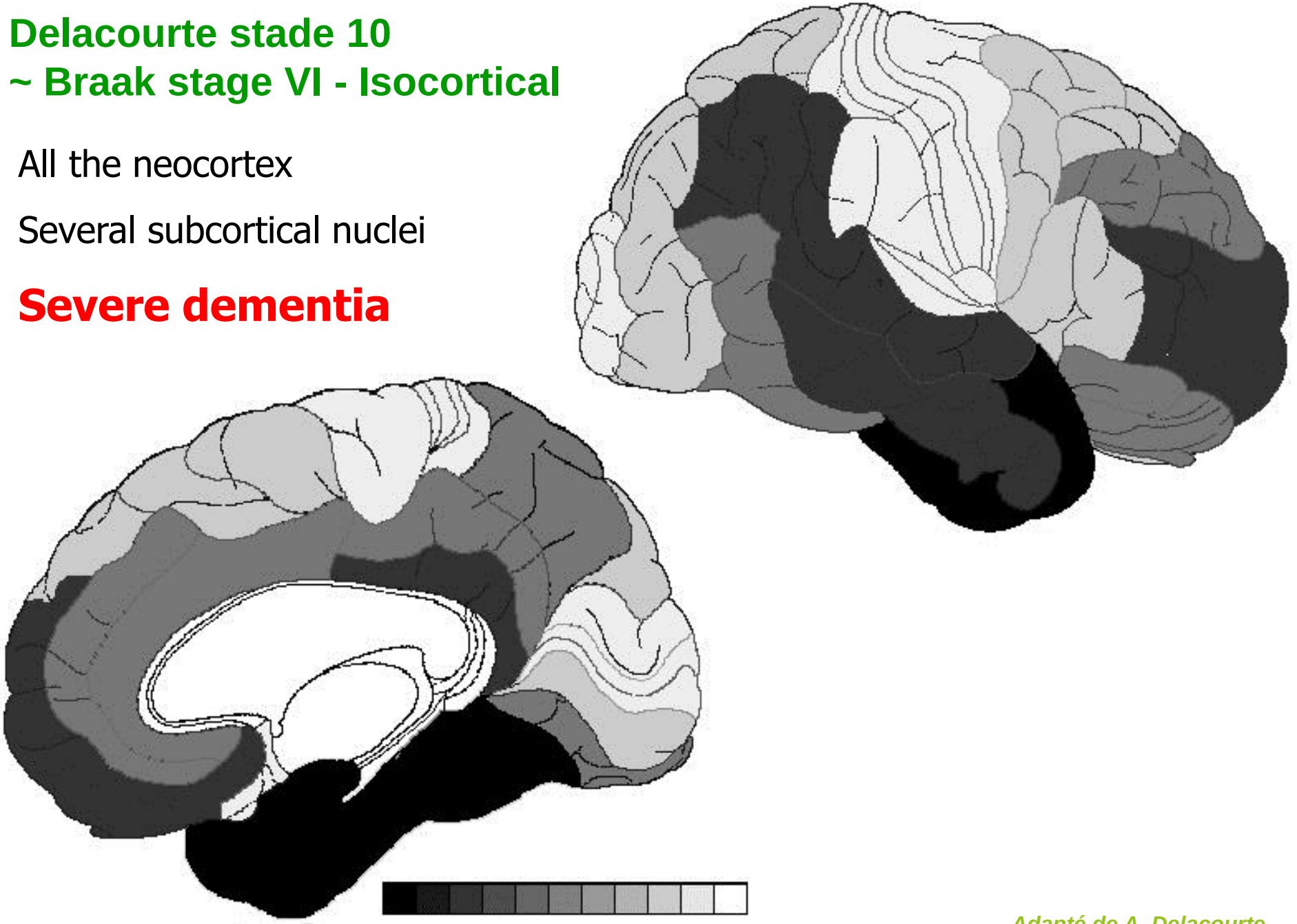
Adapté de A. Delacourte

Delacourte stade 10
~ Braak stage VI - Isocortical

All the neocortex

Several subcortical nuclei

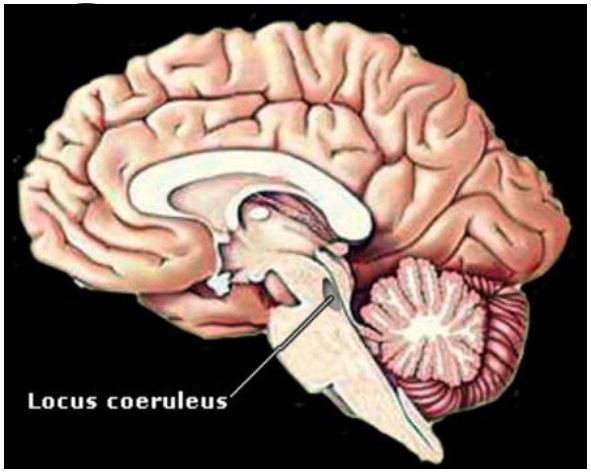
Severe dementia

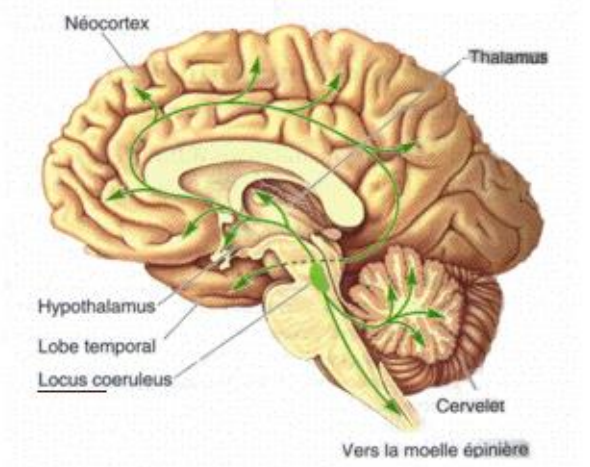


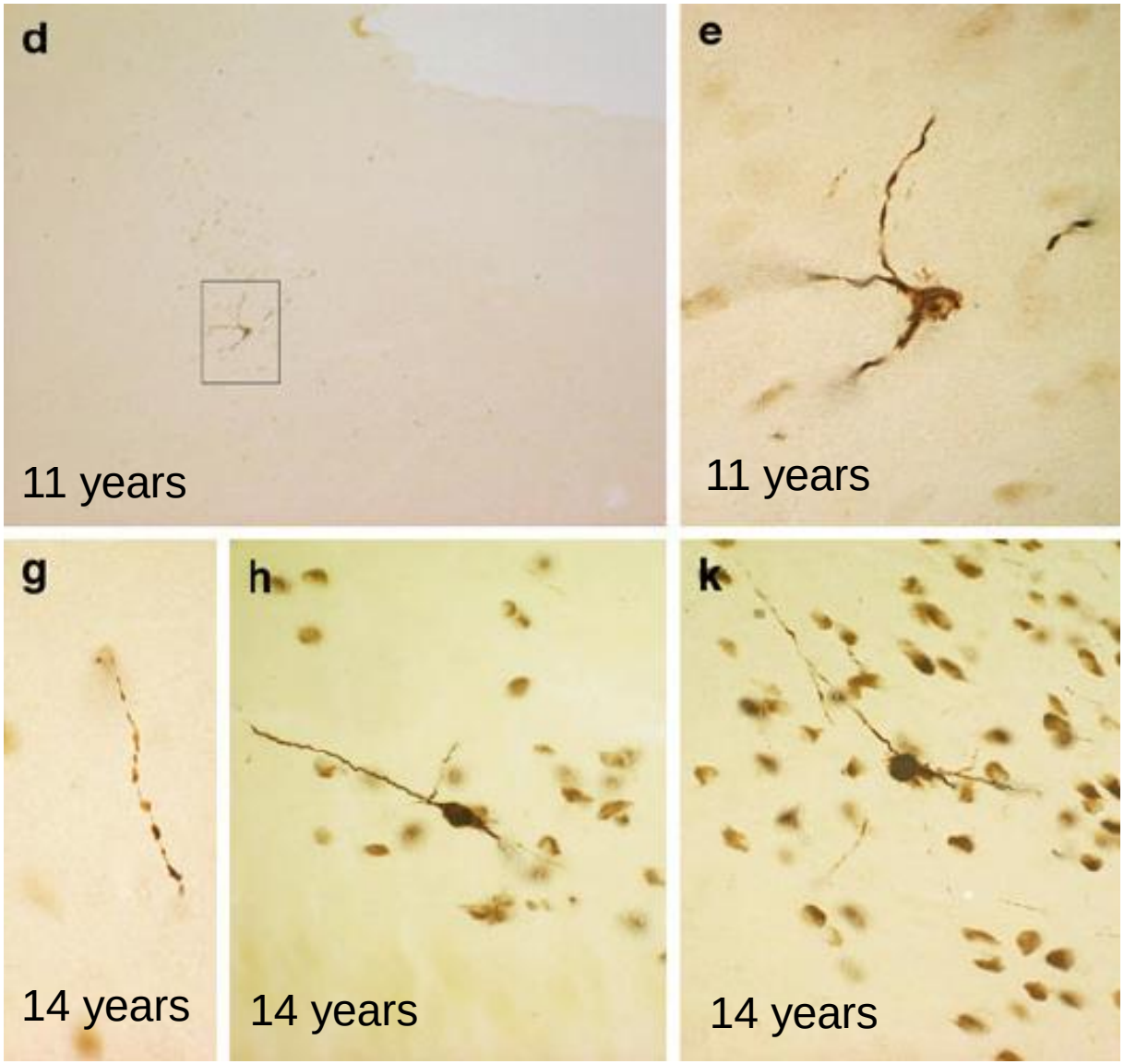
Adapté de A. Delacourte

TAU LESIONS START IN LOCUS COERULEUS IN CHILDHOOD

PROGRESSIVE COLONISATION OF THE BRAIN

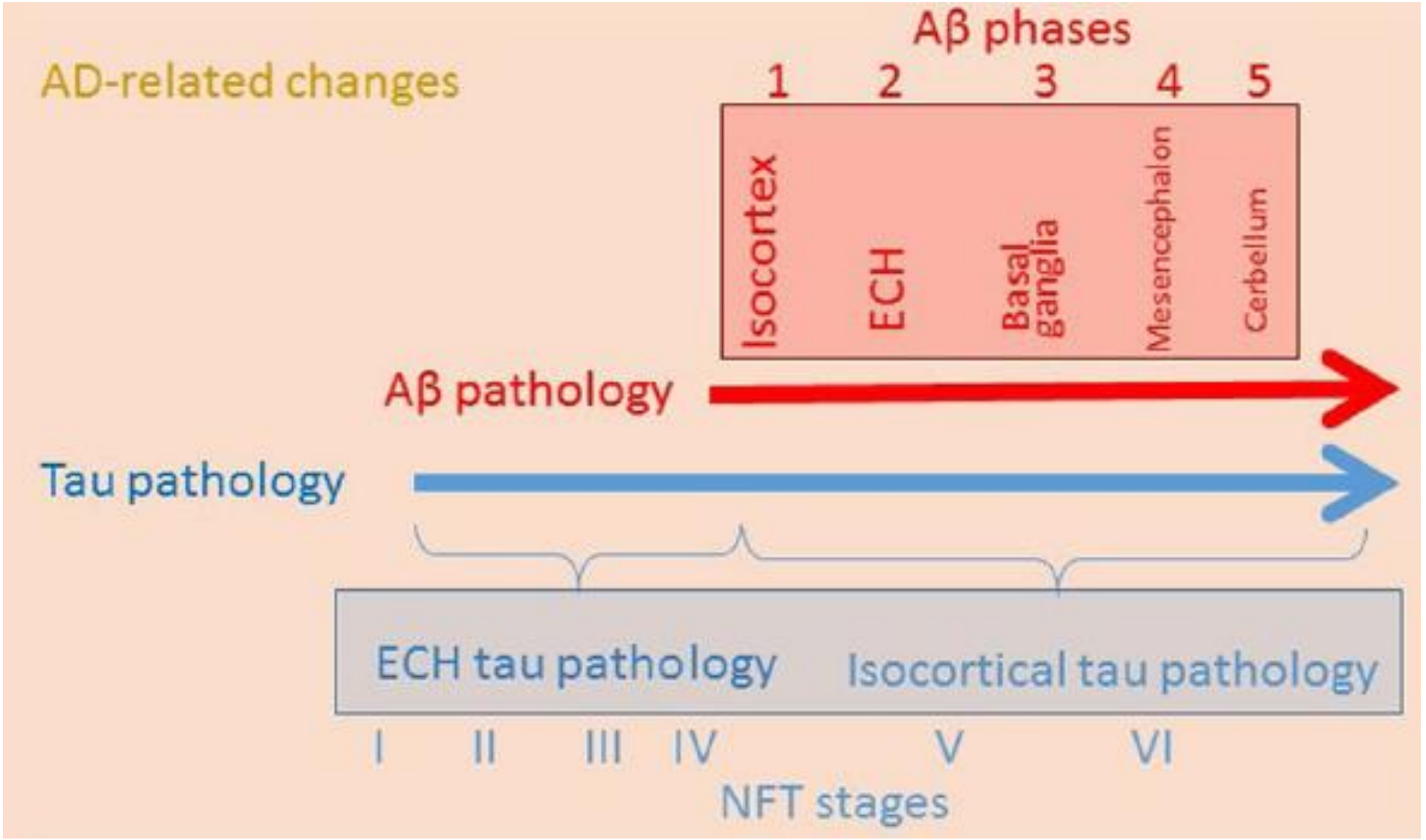






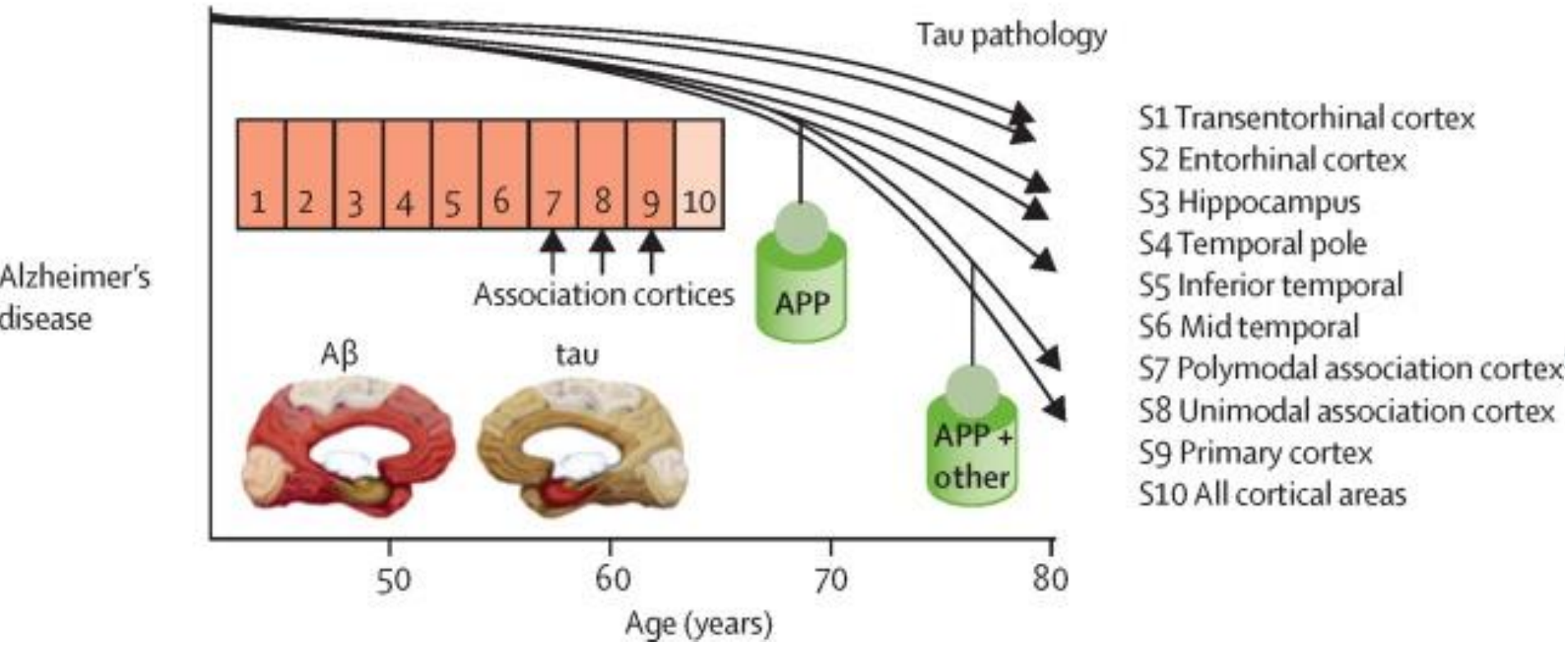
Braak, Acta Neuropathol, 2011

CURRENT VIEW OF AD LESIONS



Duyckaerts, C. (2015). Acta Neuropathol **129(5): 749-756.**

POSSIBLE INTEGRATED VIEW OF AD PATHOLOGY



Villemagne, V. L. (2015). Lancet Neurology **14(1): 114-124.**
Concept initially proposed by A. Delacourte

ANIMAL MODELS FOR ALZHEIMER'S DISEASE

Causes ?

Mechanisms ?

Early diagnosis ?

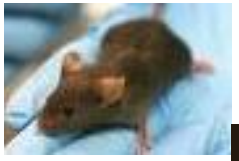
Therapy ?

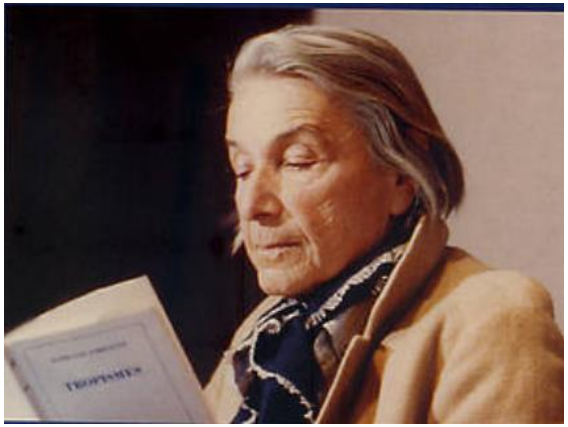
Aging

Or

?

Alteration of cognitive abilities





Preservation of cognitive abilities

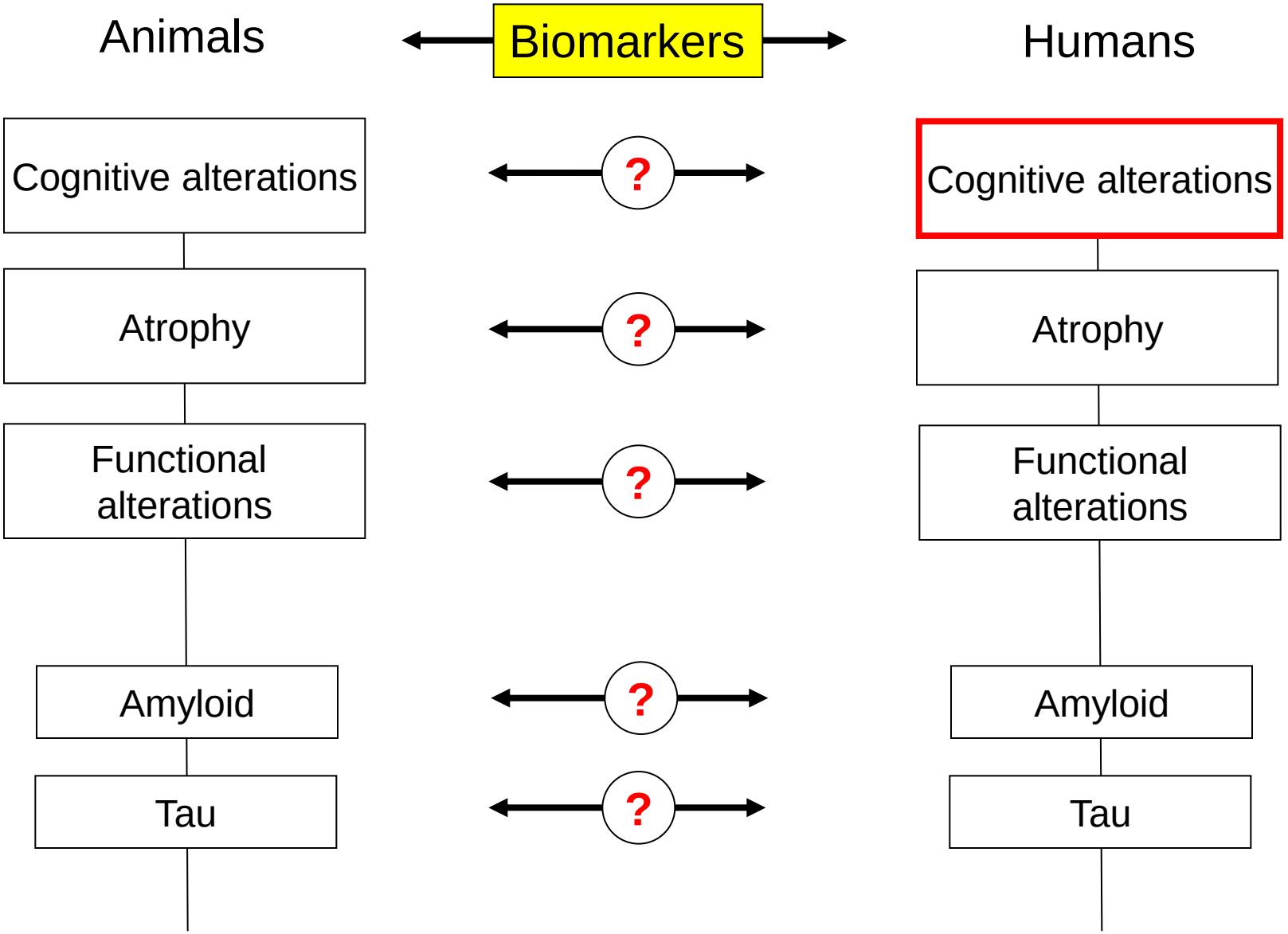
WHAT IS A GOOD ANIMAL ?

- Construct validity
  - Biological construction for example (aging…)
  - Genetic construction
  - …

- Face validity
  - Phenotypic
  - Endophenotypic
    - Lesions: Amyloide, Tau, Neurodegeneration
    - Endophenotypes accessibles with biomarkers

- Prediction validity
  - Cross talk with clinical trials in humans to validate animal models

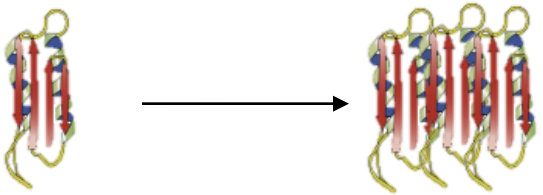
HOW TO FOLLOW-UP ANIMAL MODELS ?



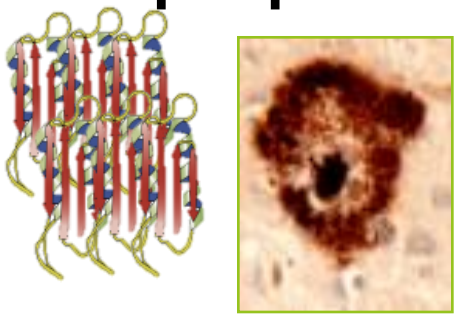
MOUSE MODELS BASED ON AMYLOID HYPOTHESIS OF AD

Amyloid precursor Protein (APP)

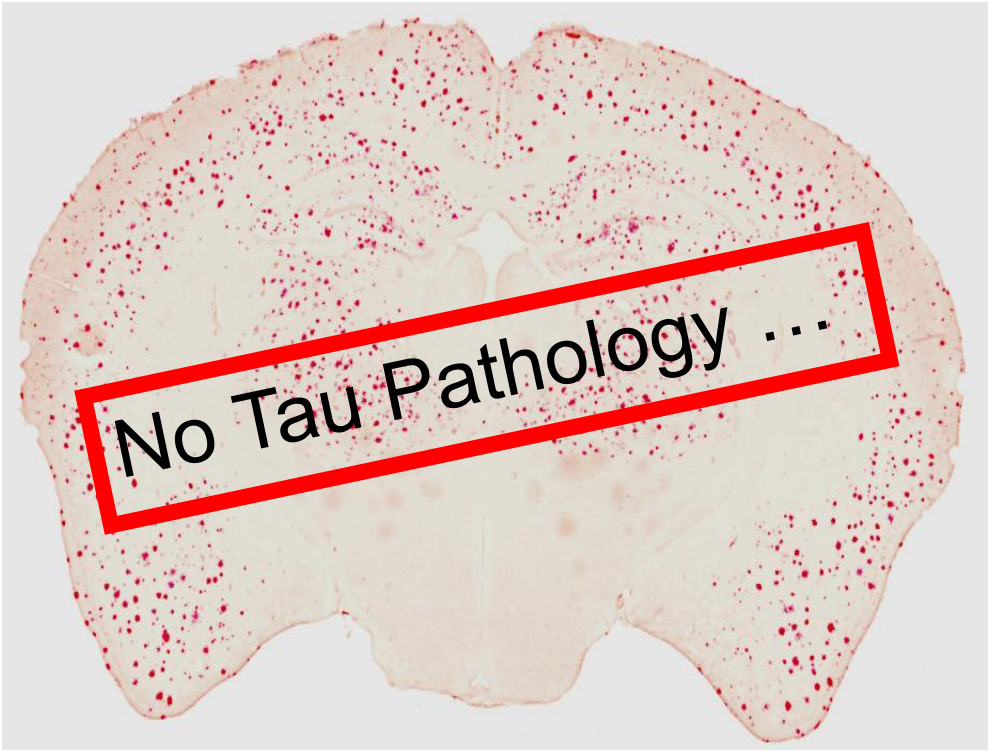
Amyloide oligomeric (soluble)



Amyloid plaques



APP Mutations



BEHAVIORAL STUDIES

Rationale: Alzheimer is a dementia

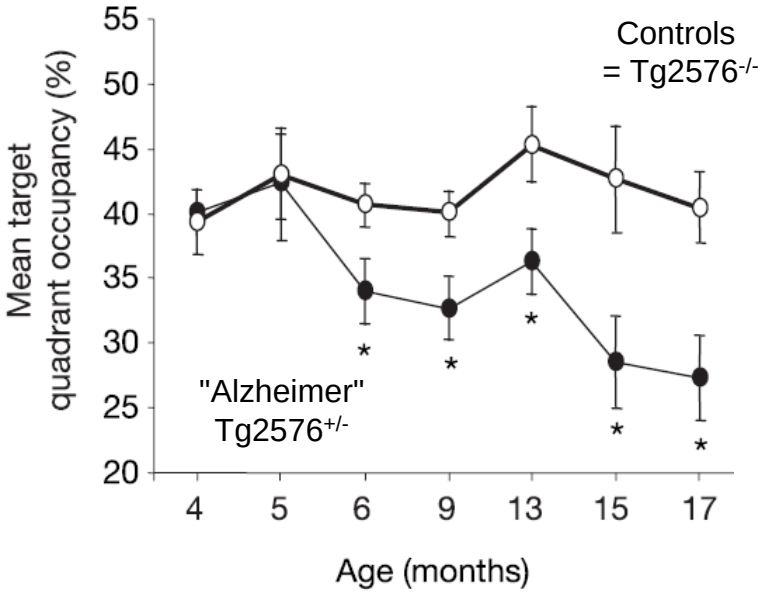
Let's look a behavioral alterations in animals to predict drug efficacy…

BEHAVIORAL ALTERATIONS IN RODENTS

Ex. Morris water maze

- Spatial memory (reference memory)
- Hippocampal integrity
- Widely used

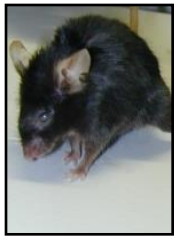
Less time spent in good quadrant in old mice



Cognitive alterations
But no dementia

Lesne, Nature, 2006

DIFFERENT ORIGIN OF BEHAVIORAL ALTERATIONS IN HUMAN AND ANIMAL



Mice

Cognitive alterations
(not homologous
to human alterations)

oligomers

No Tau

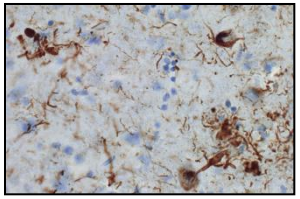
Amyloid

Homol
Prédictive

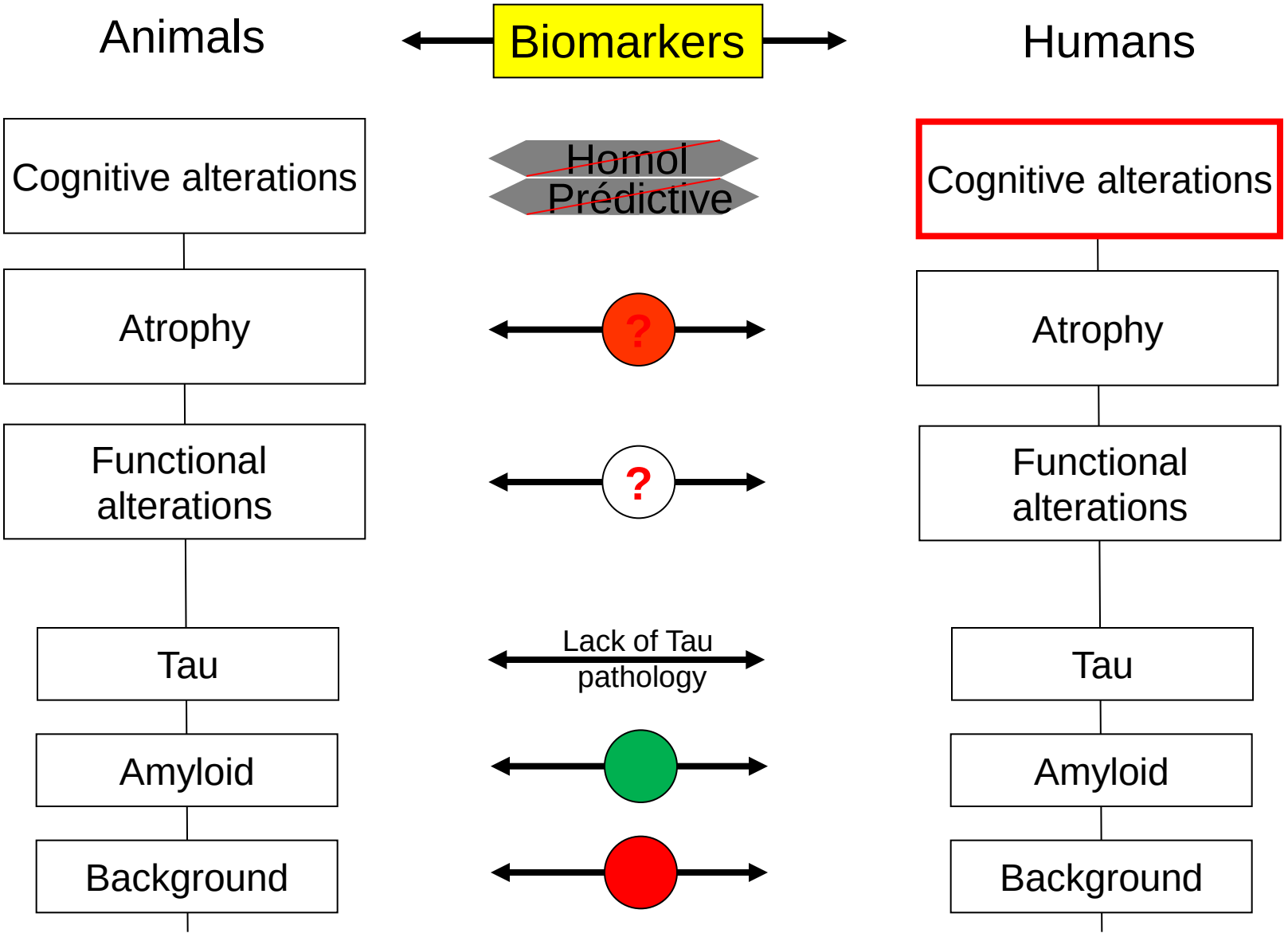
Human

Cognitive alterations

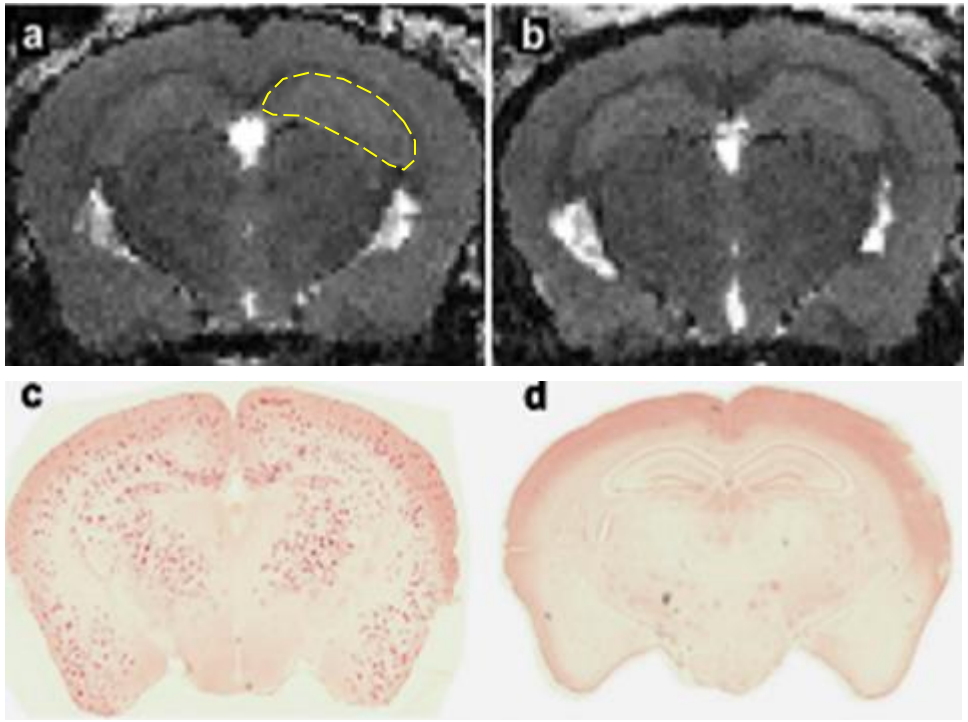
Tau

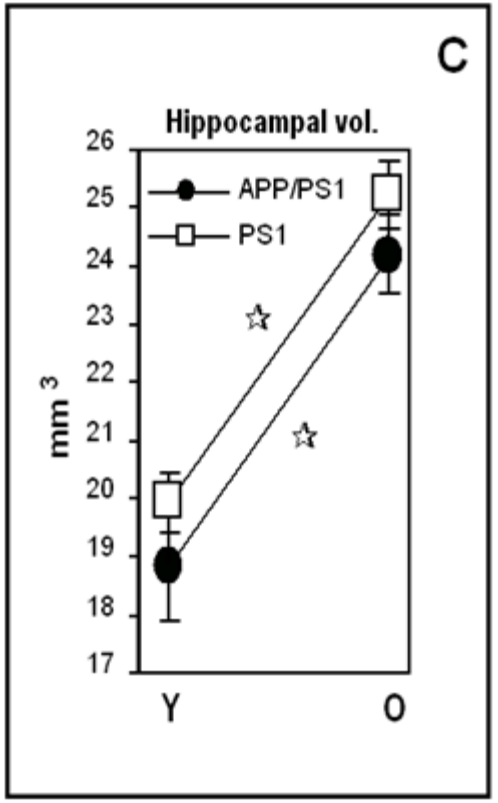


Amyloid


Animals
Biomarkers
Humans
Cognitive alterations
Homol
Prédictive
Cognitive alterations
Atrophy
?
Atrophy
Functional alterations
?
Functional alterations
Tau
Lack of Tau pathology
Tau
Amyloid
Amyloid
Background
Background

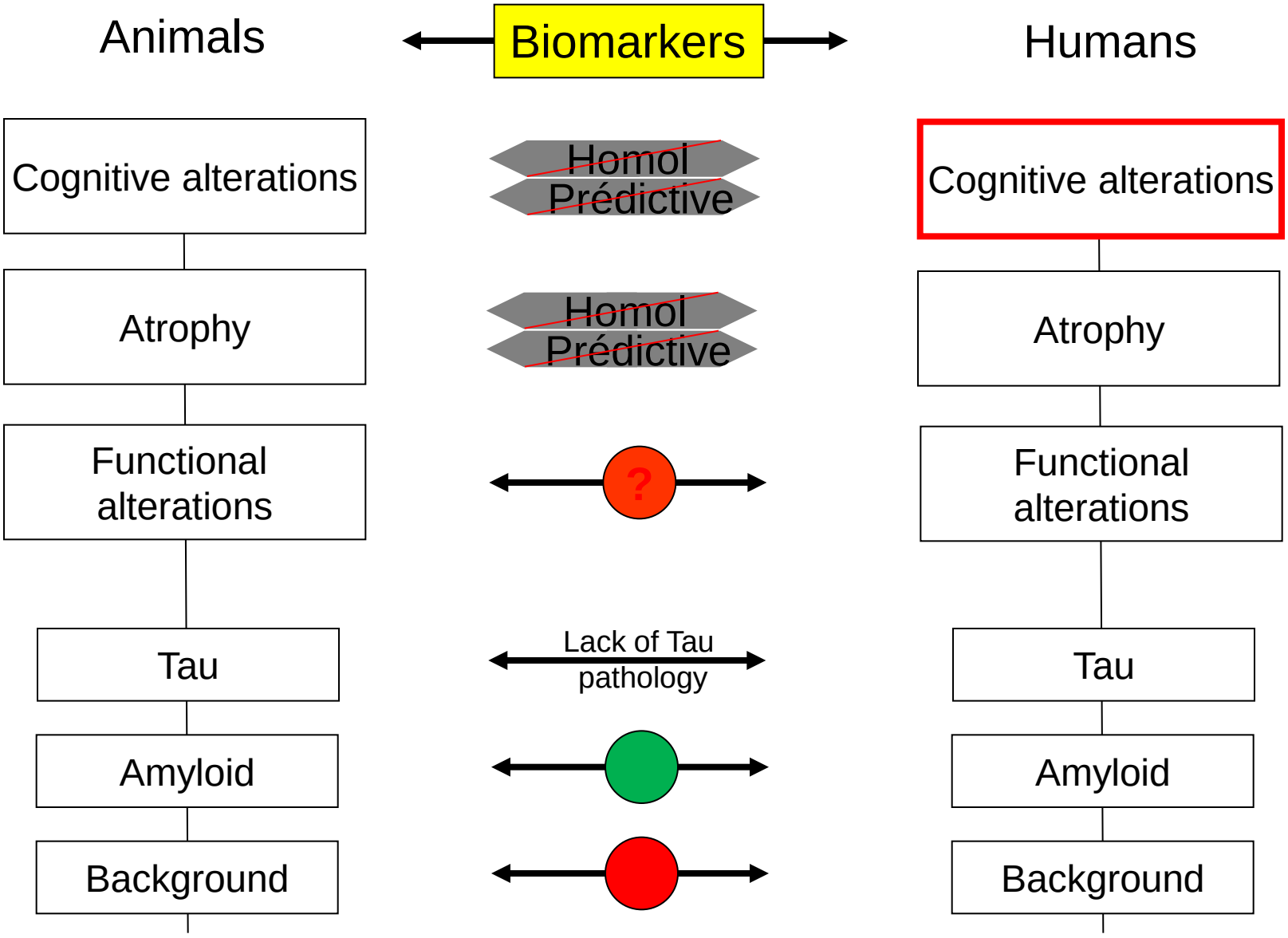
CEREBRAL ATROPHY IN TRANSGENIC MOUSE MODEL OF AMYLOIDOSIS

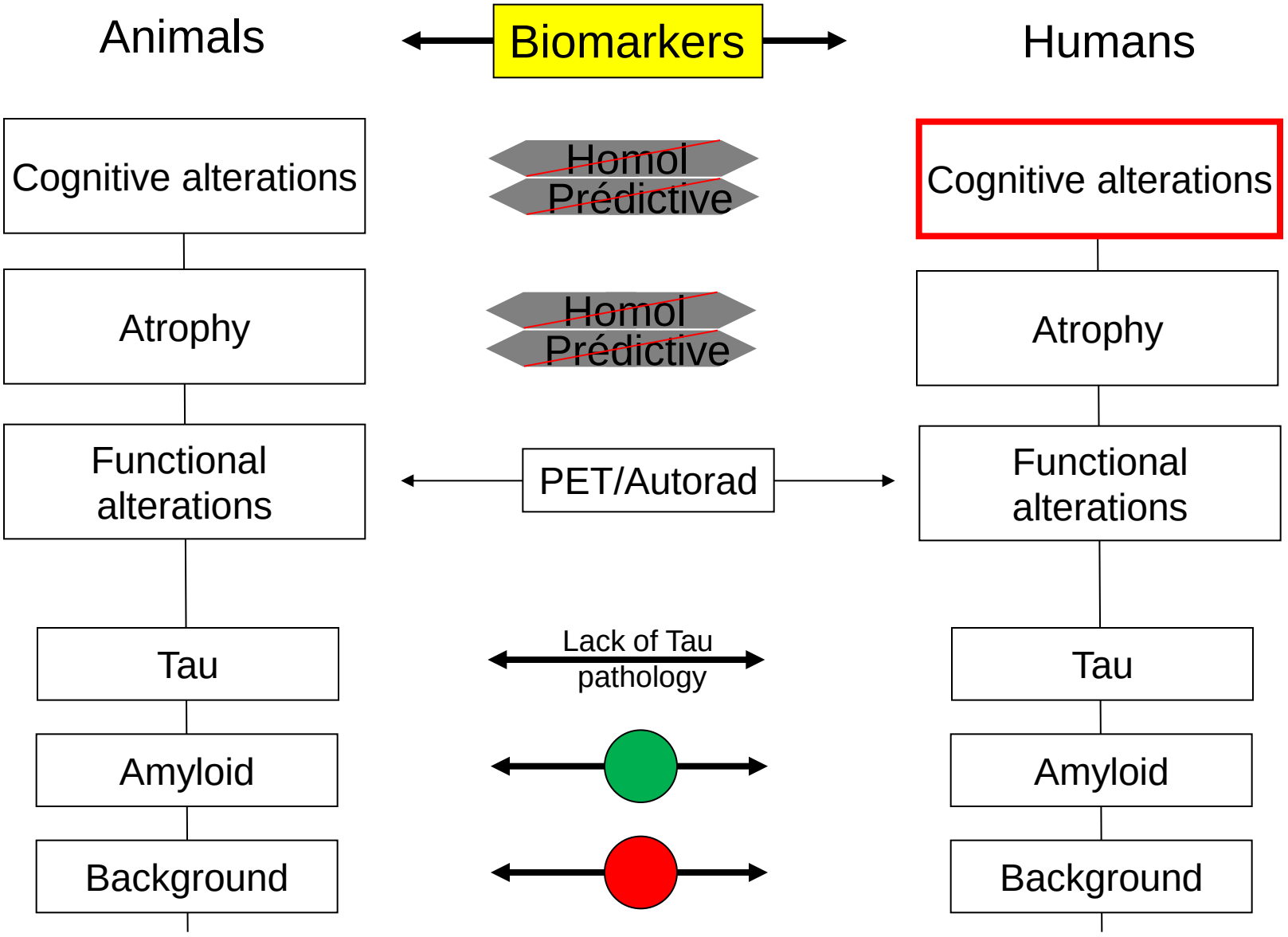




Brain and hippocampal growth
even in the presence of amyloid deposits…

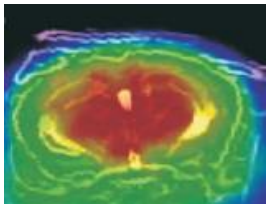
Delatour B. et al.. Neurobiol Aging, 27(6), 835-847; 2006.

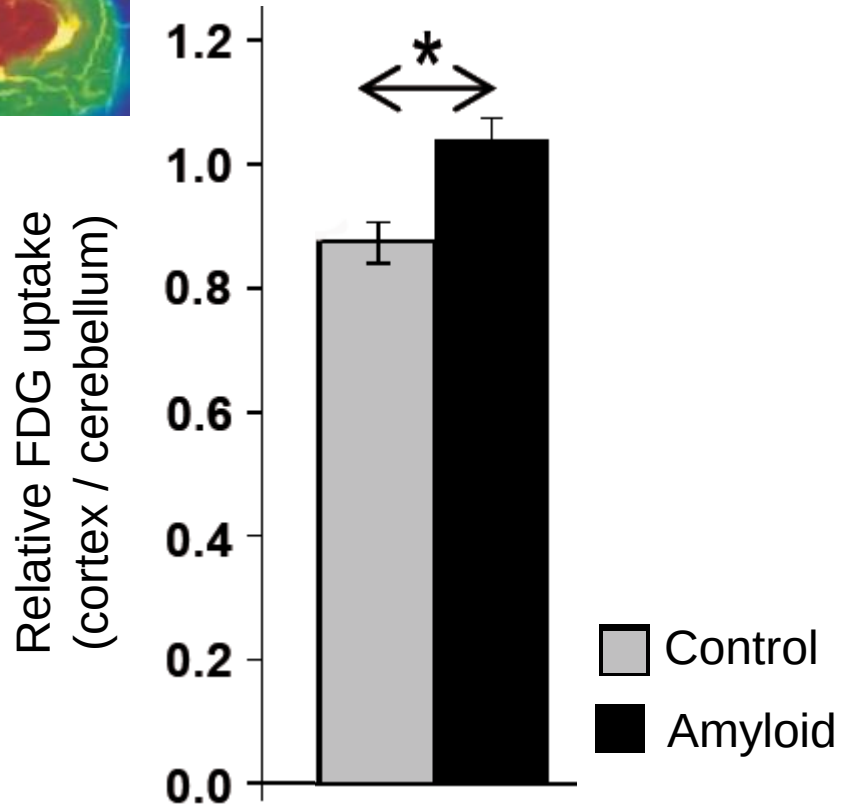

Animals
Biomarkers
Humans
Cognitive alterations
Homol
Prédictive
Cognitive alterations
Atrophy
Homol
Prédictive
Atrophy
Functional alterations
?
Functional alterations
Tau
Lack of Tau pathology
Tau
Amyloid
Amyloid
Background
Background


Animals
Biomarkers
Humans
Cognitive alterations
Homol
Prédictive
Cognitive alterations
Atrophy
Homol
Prédictive
Atrophy
Functional alterations
PET/Autorad
Functional alterations
Tau
Lack of Tau pathology
Tau
Amyloid
Amyloid
Background
Background

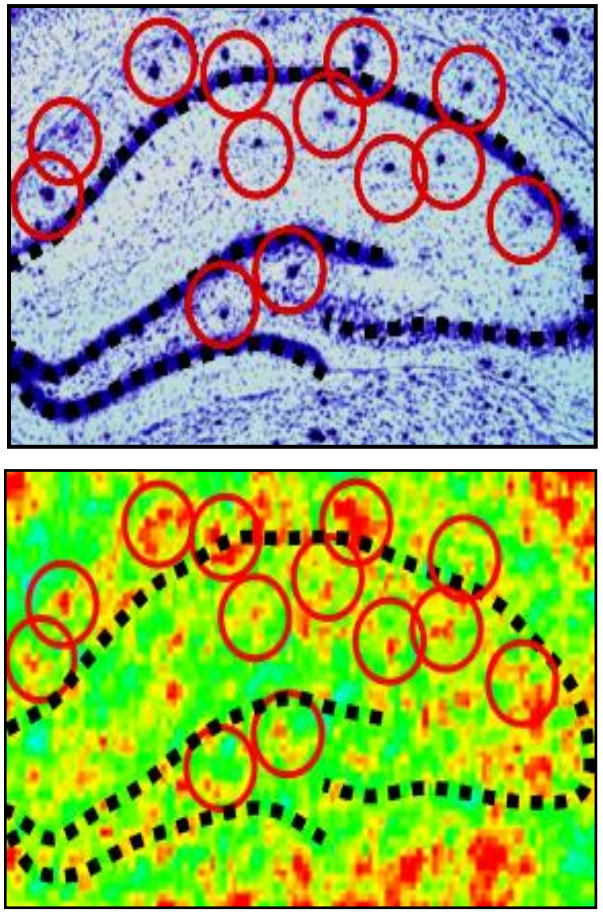
CEREBRAL HYPERMETABOLISM IN MOUSE MODELS OF AMYLOIDOSIS

Index of cerebral metabolism (FDG-PET)

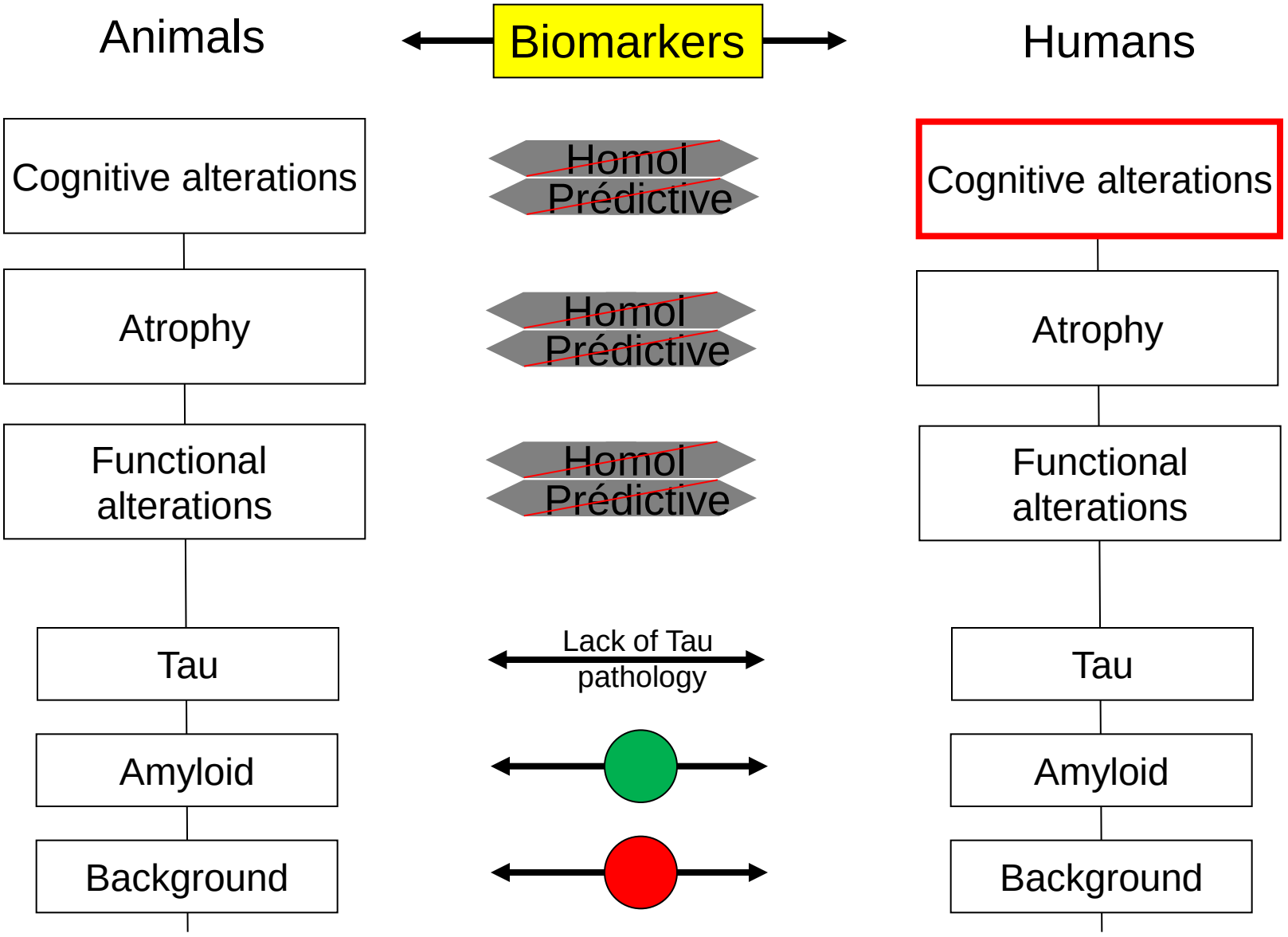




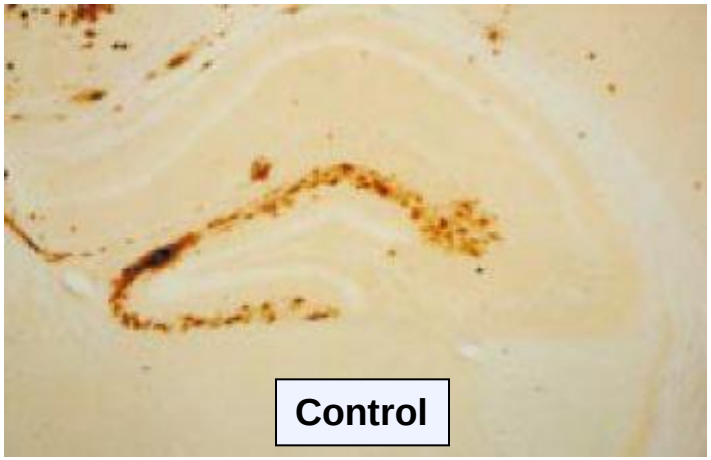
Index of micro-metabolism (2DG Autoradiography)

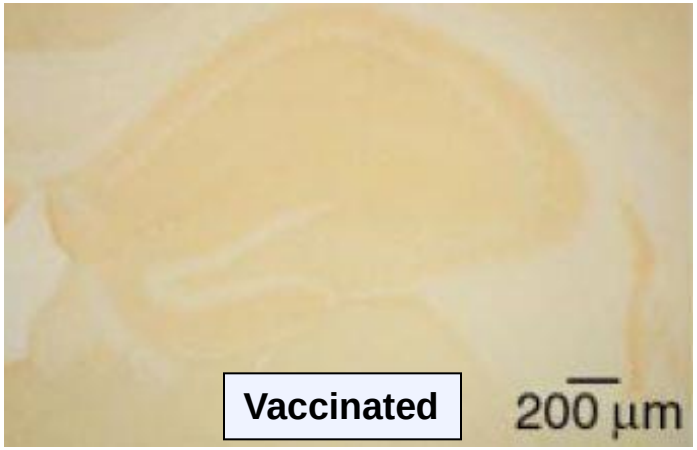


Poisnel et al, Neurobiol Aging. 2012


Animals
Biomarkers
Humans
Cognitive alterations
Homol
Prédictive
Cognitive alterations
Atrophy
Homol
Prédictive
Atrophy
Functional alterations
Homol
Prédictive
Functional alterations
Tau
Lack of Tau pathology
Tau
Amyloid
Amyloid
Background
Background

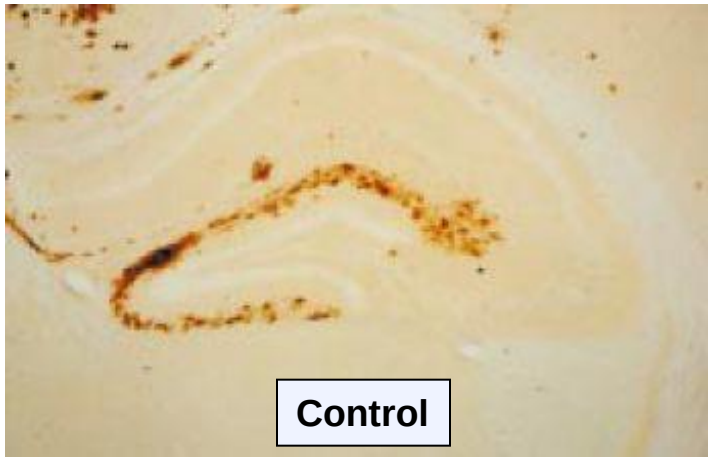
IMMUNOTHERAPIES IN AMYLOID MICE



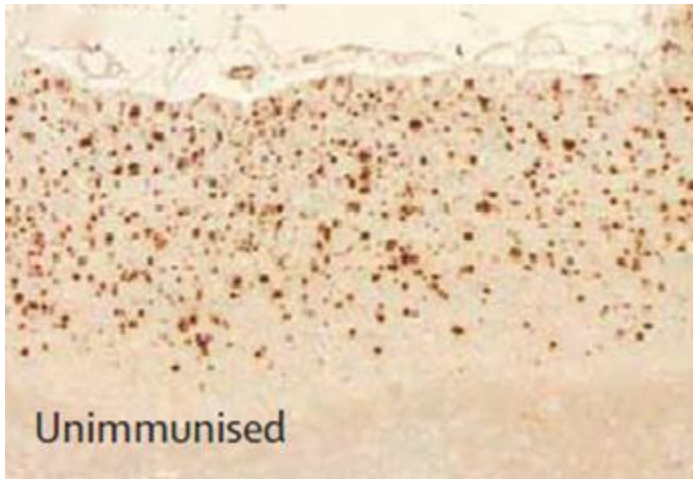


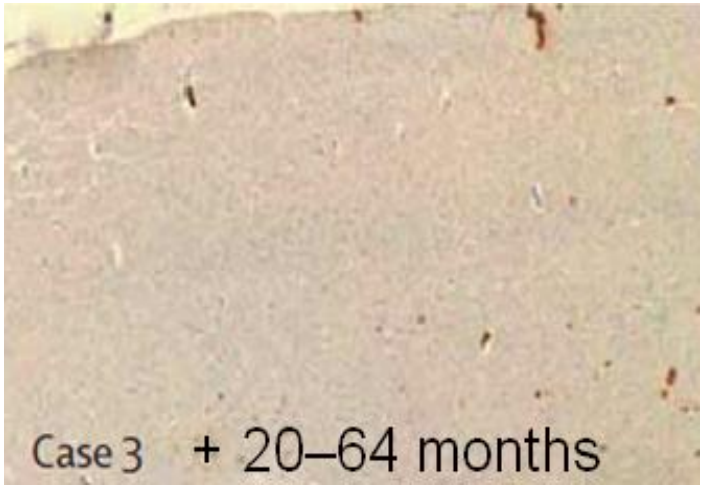
(Schenk et al, 1999)

DISCOVERY OF NEW THERAPY STRATEGIES IN AMYLOID MICE



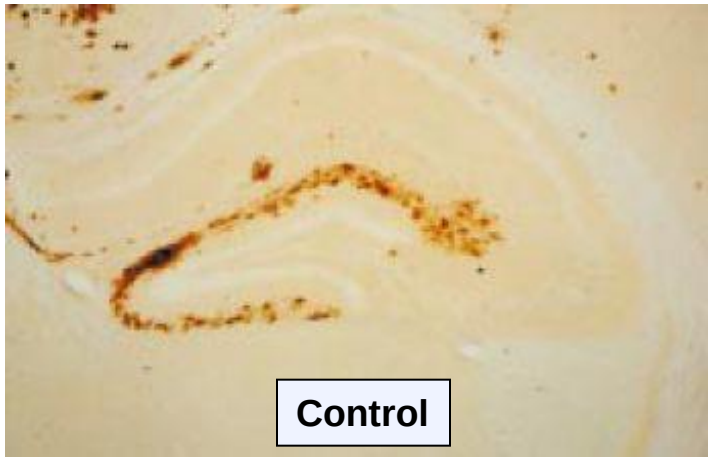
(Schenk et al, 1999)



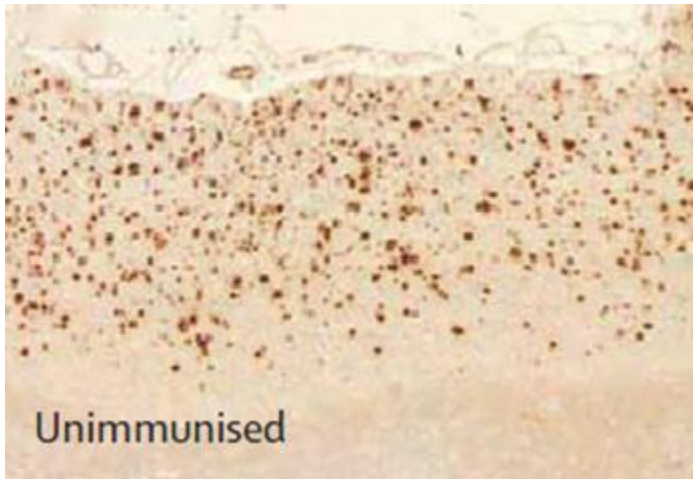


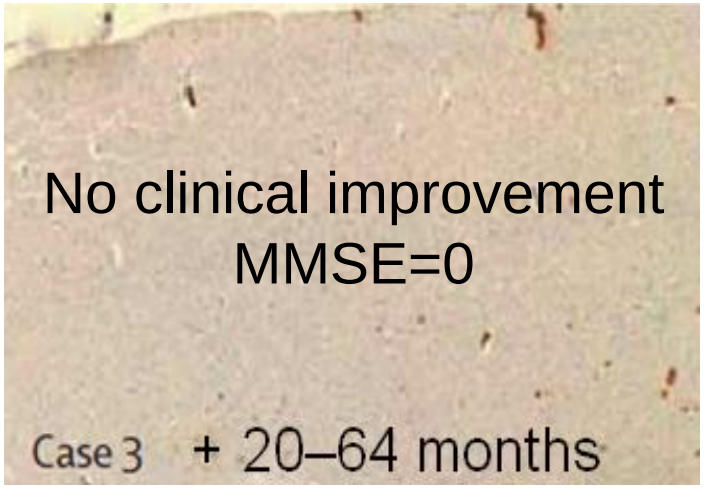
(Holmes et al, 2008)

DISCOVERY OF NEW THERAPY STRATEGIES IN AMYLOID MICE



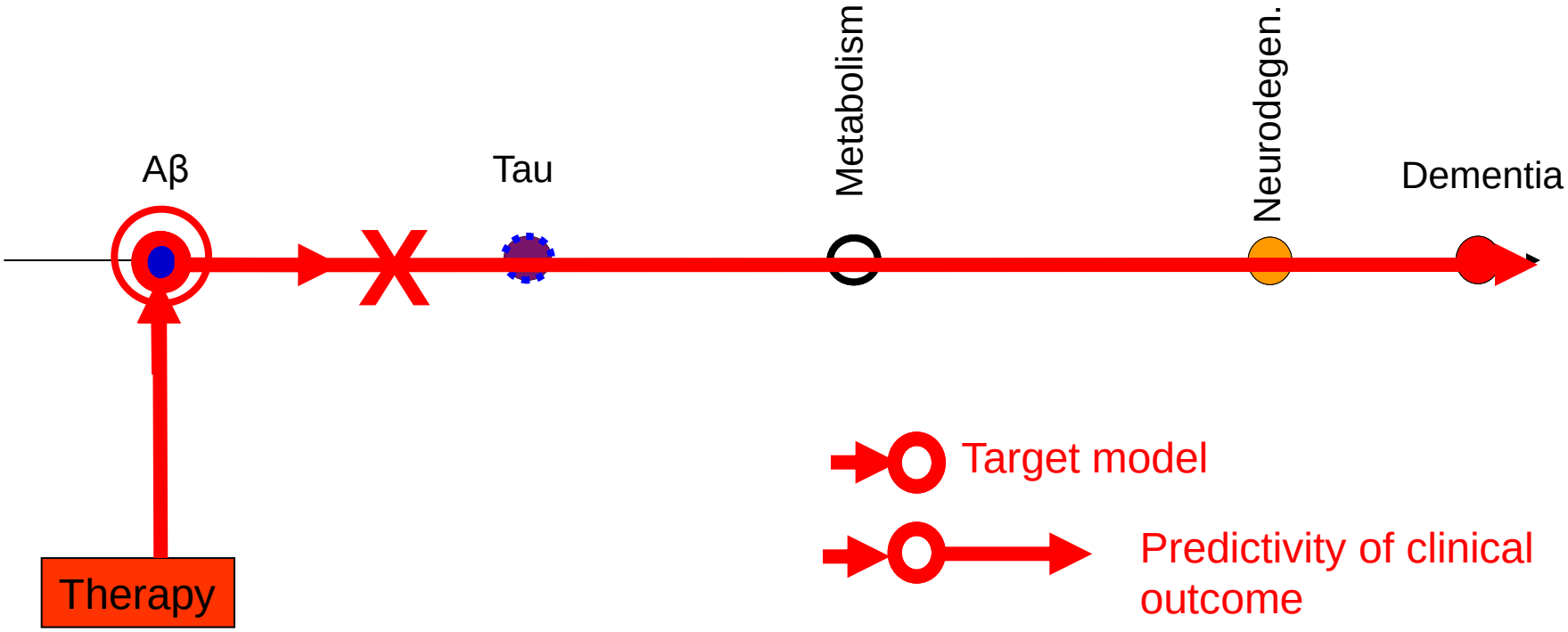
(Schenk et al, 1999)





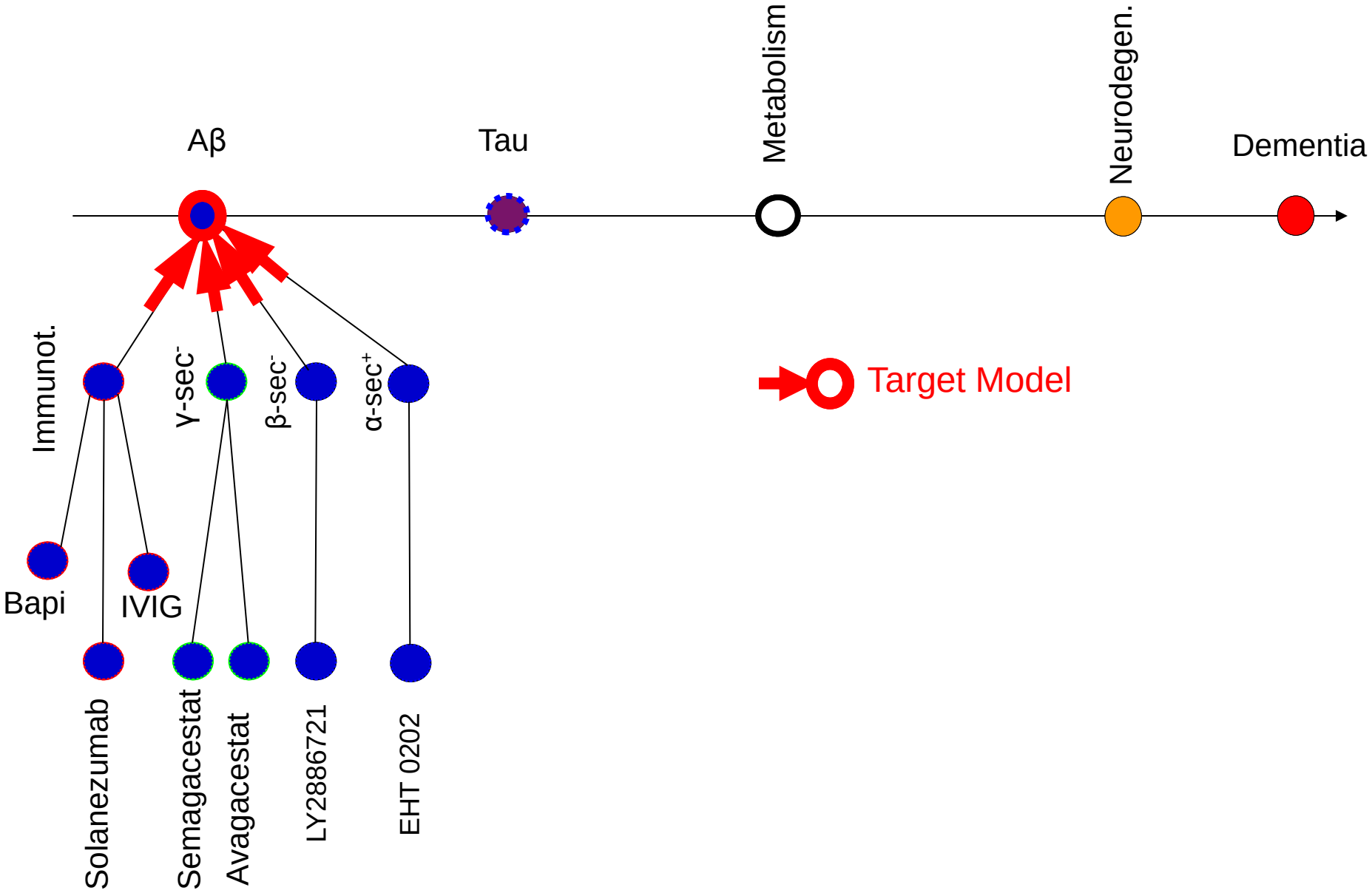
(Holmes et al, 2008)

TARGET MODELS VERSUS CLINICAL MODELS



- Predicting clinical efficacy is impossible with rodent models

TARGET MODELS ARE USEFUL TO EVALUATE MULTIPLE ANTI-AMYLOID THERAPIES

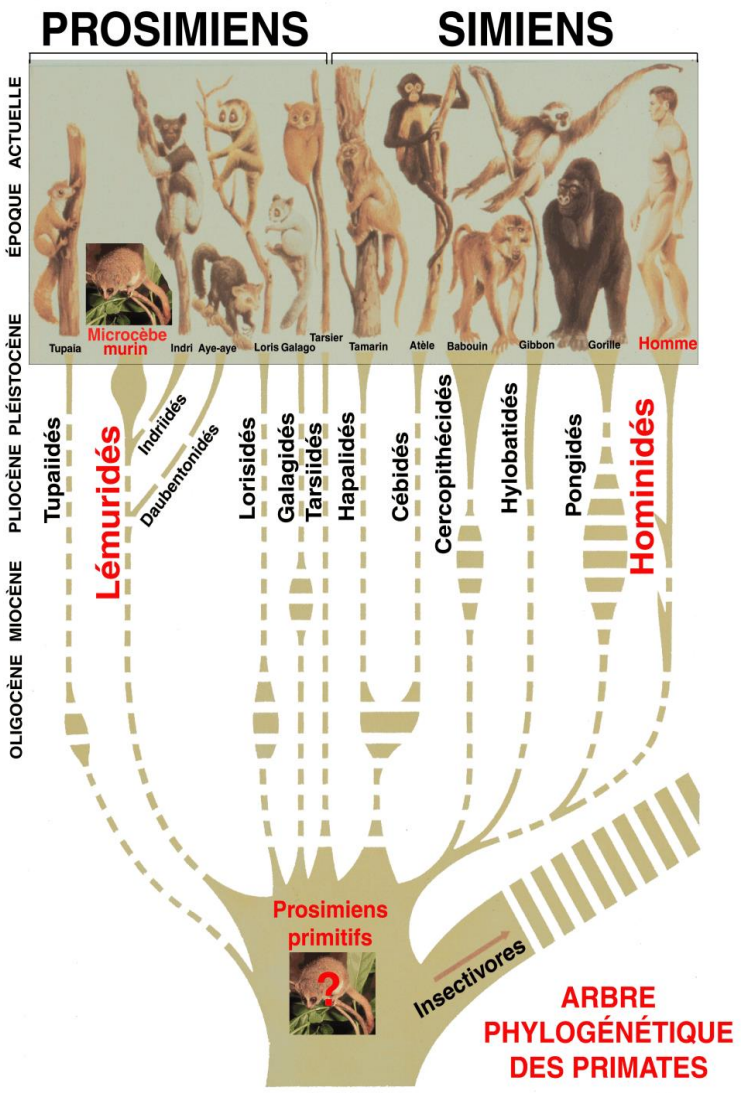


FIRST TAKE HOME MESSAGE

- Do not speak of animal model of Alzheimer's disease

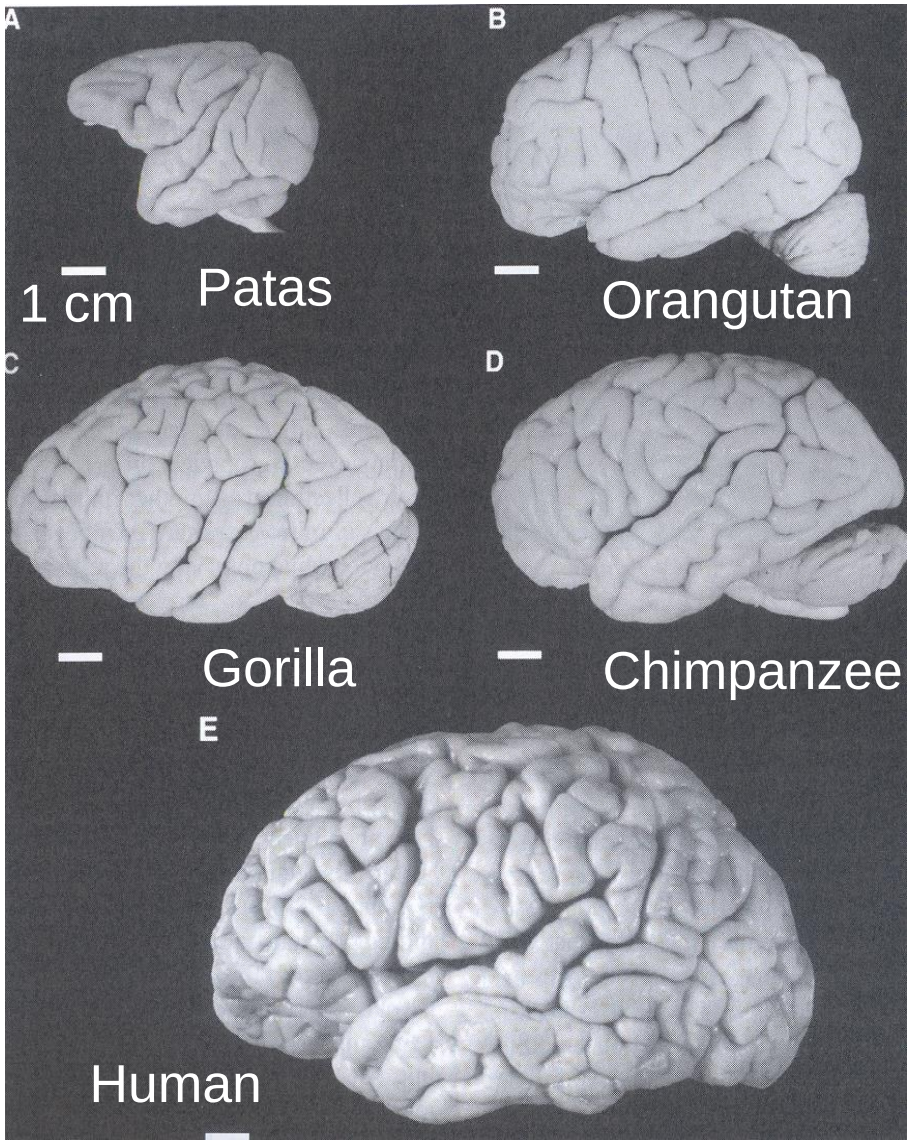
- Use a more specific language
  - Model of amyloidosis
  - Target model for amyloidosis

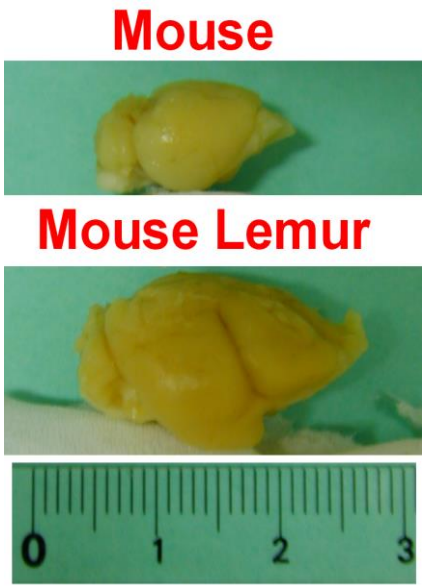
PRIMATE MODELS
PRIMATE HETEROGENEITY



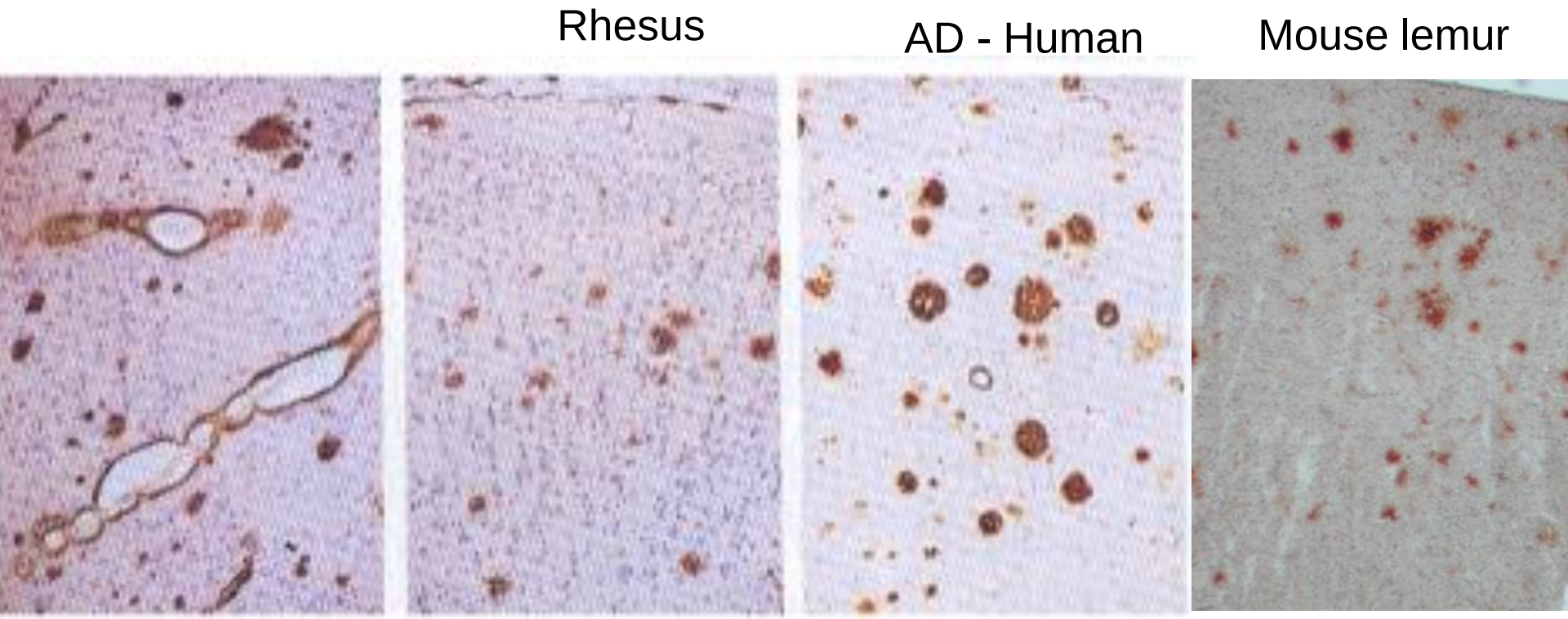
Species	Maximum life span (years)
Primates	
Human	122
Chimpanzee	59
Rhesus monkey	40
Squirrel monkey	27
Mouse lemur	12
Tree shrew	12
Polar bear	34
Sheep, goat	20
Dogs	
Small size (Pekinese)	20
Middle size (Beagle)	16
Large size (Saint Bernard)	14
Cat	~30
Guinea pig	8
Rodents	
Mouse	3.5
Rat	4

BRAIN HETEROGENEITY IN PRIMATES





MICROSCOPIC ALTERATIONS
AMYLOID DEPOSITS



Gearing et al, PNAS, 1994

http://m.lemur.free.fr

MICROSCOPIC ALTERATIONS
AMYLOID DEPOSITS

Animal species	Maximum amyloid deposits density	References
AD brain	256 /mm²	Hyman, 1993
Rhesus monkeys	8 /mm²	Walker, 1987
New world monkeys Squirrel monkeys	4-5 /mm²	Walker, 1987
Lemurian primates Mouse lemurs	16 /mm²	Bons, 1993
Tree Shrews	0 /mm²	Pawlik, 1999
Polar Bears	8-10 /mm²	Cork, 1988
Dogs	Similar or exceeding severe cases of AD	Cummings, 1996

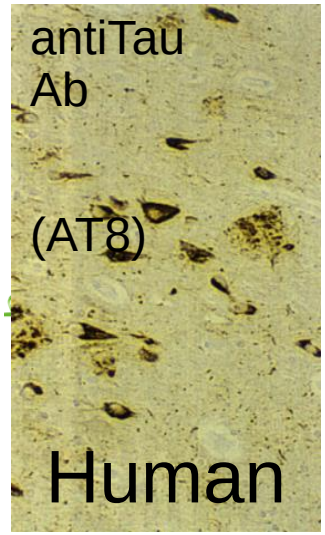
(Dhenain, Handbook of Neuropsychology (2nd ed, 2001))

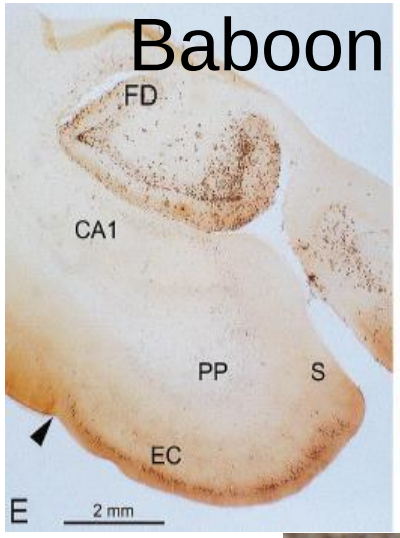
SEQUENCE HOMOLOGIES
APP – BETA AMYLOID

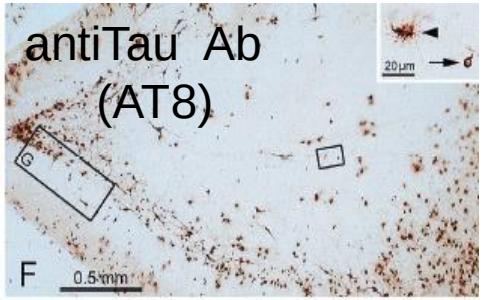
Animal species	β-APP	A β Sequence	Mutations
Cynomolgus monkeys	Homology 100%	Homology 100%	Not reported
New world monkeys Squirrel monkeys	Difference 3 amino acids	Homology 100%	Not reported
Lemurian primates Mouse lemurs	??	Homology 100%	Not reported
Tree Shrews	Difference 3 amino acids	Homology 100%	Not reported

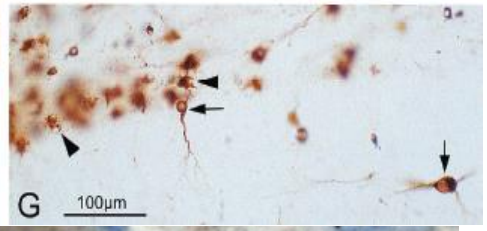
(Dhenain, Handbook of Neuropsychology (2nd ed, 2001)

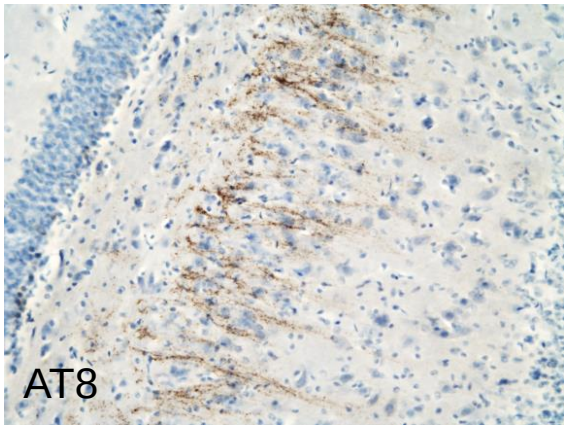
NEUROFIBRILLARY ALTERATIONS

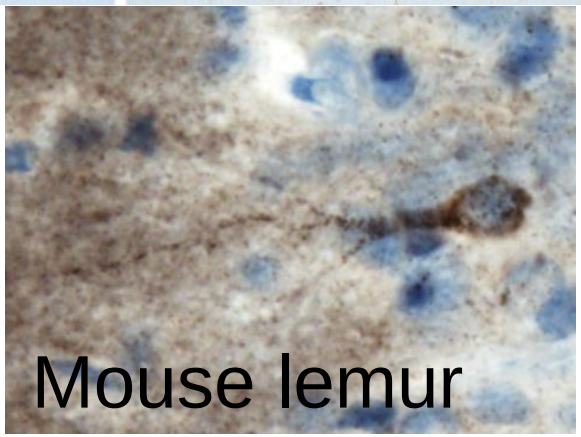


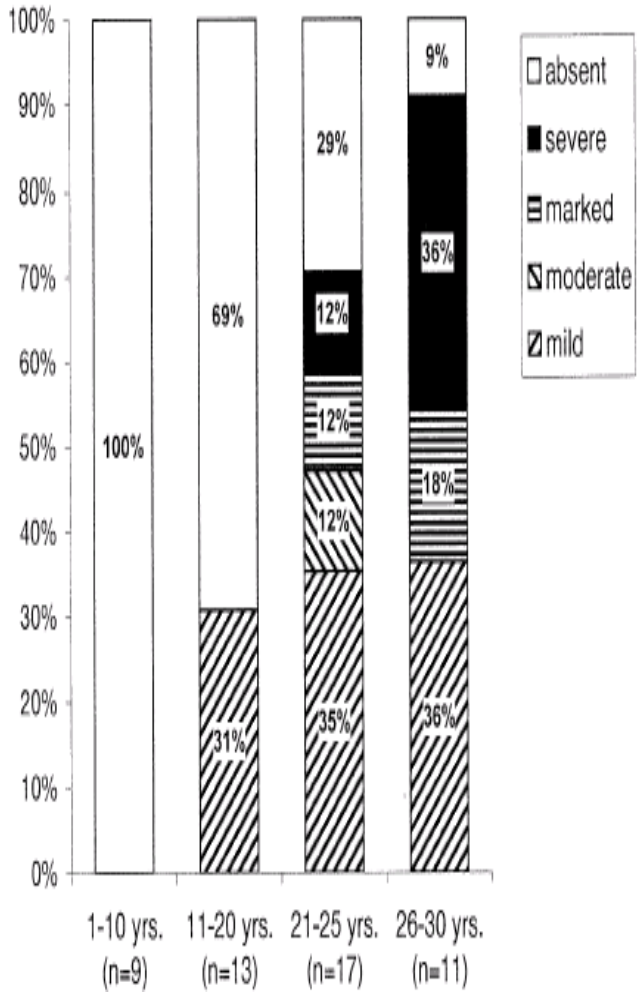






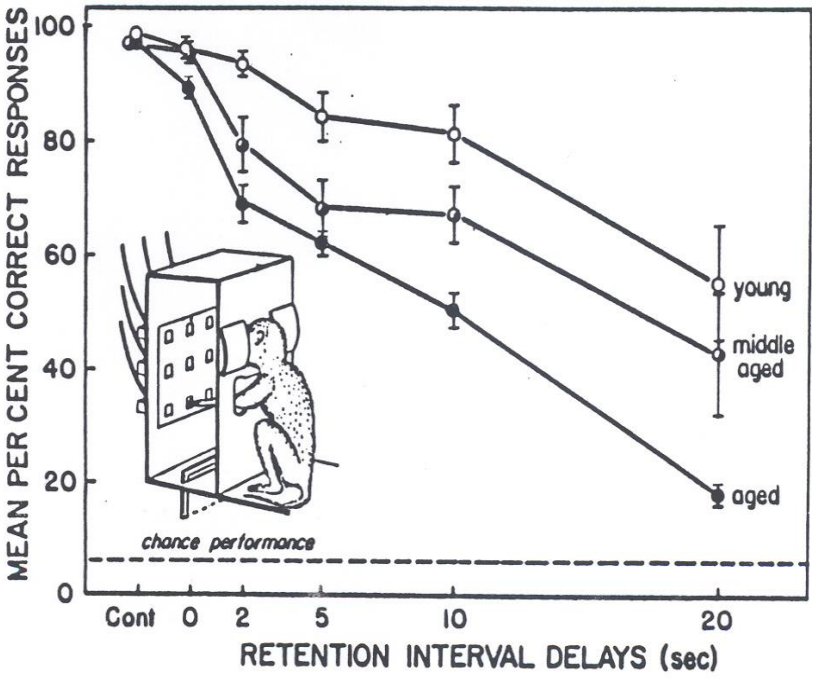


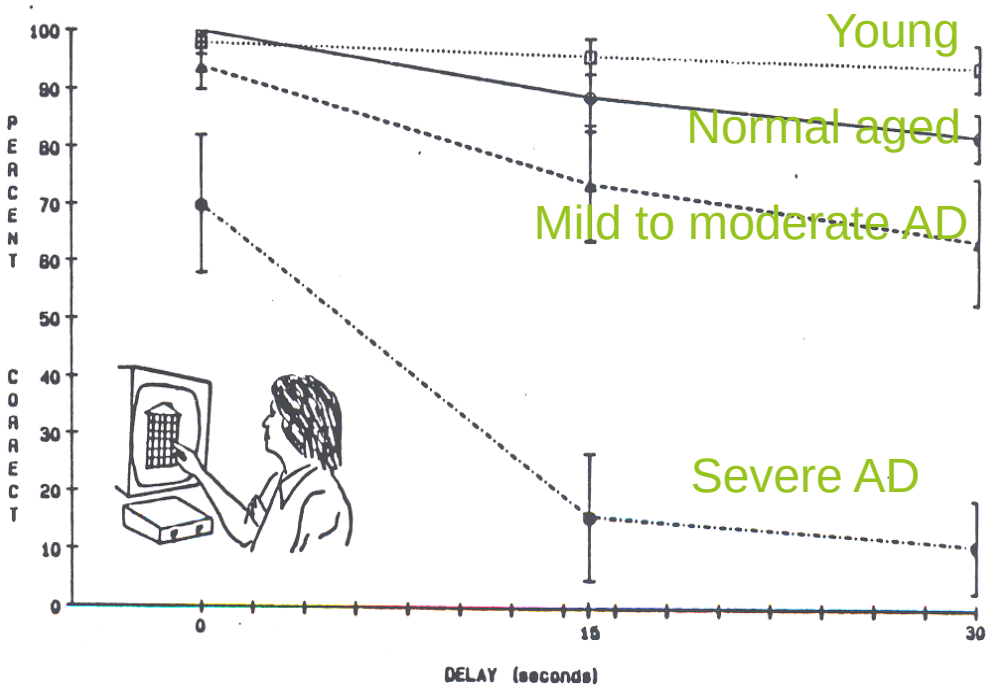




(Schultz, Neurob Aging, 2000)

AGE RELATED COGNITIVE ALTERATIONS





Delayed Response

(Bartus and Dean. Normal Aging, Alzheimer's disease and senile dementia, Aspects on Etiology, Pathogenesis, Diagnosis and Treatment, 1985)

AGE RELATED COGNITIVE ALTERATIONS

Prefrontal impairments, perseveration

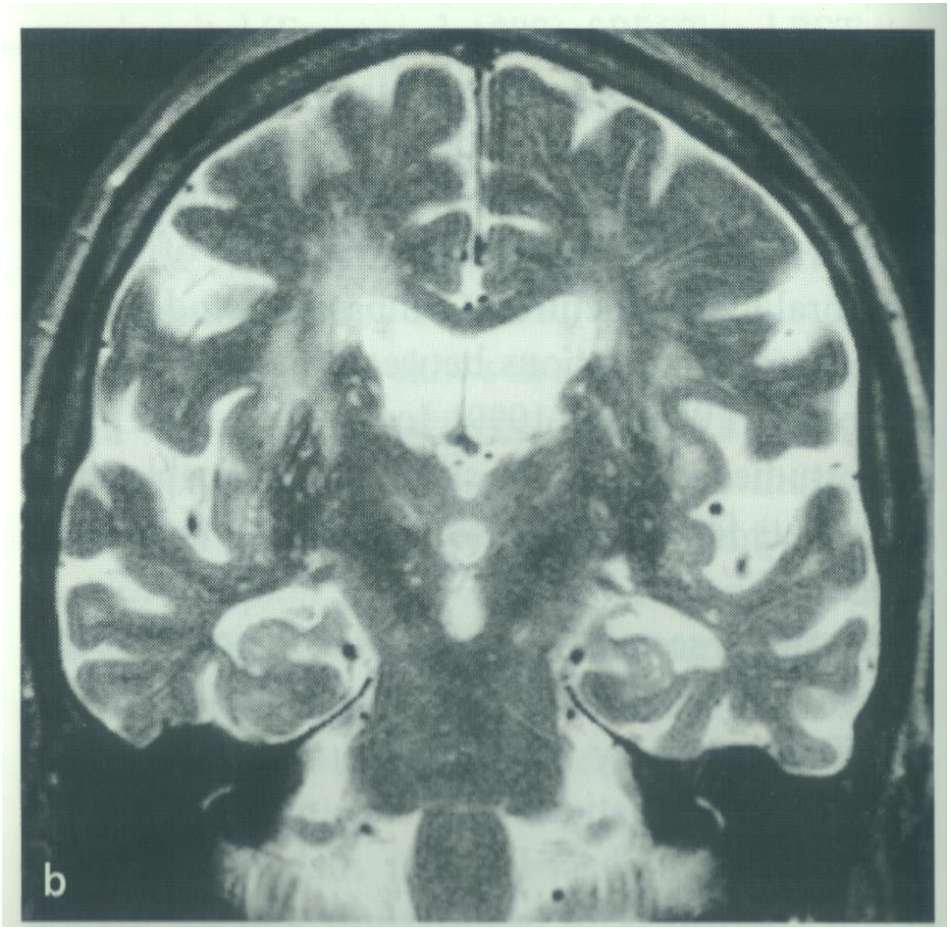
~ 15-20 years in Rhesus monkeys
Very constant in different animals

Tasks depending on medial temporal areas

~25-30 years in Rhesus monkeys
(But) Interindividual variations

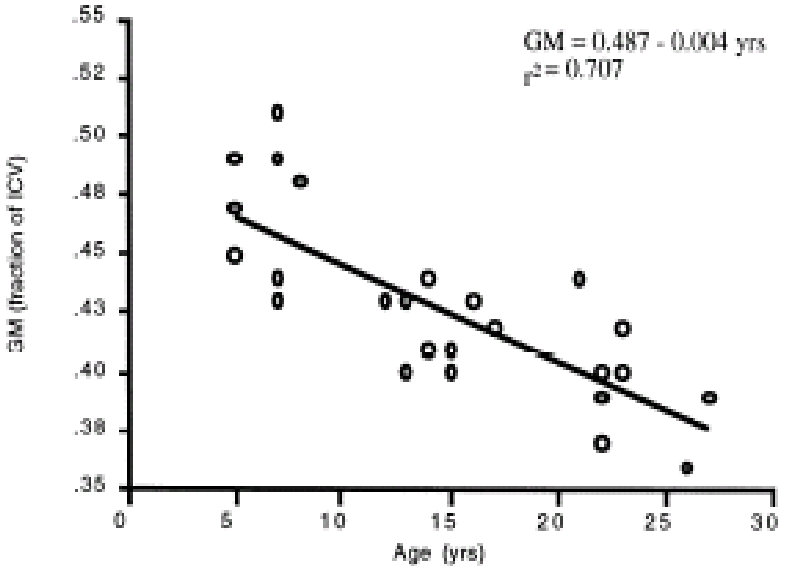
What is responsible for these alterations ?

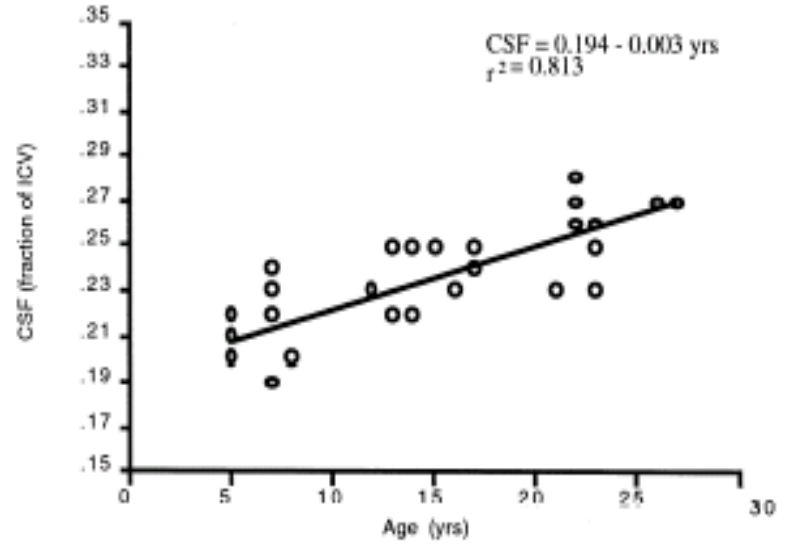
CEREBRAL ATROPHY



CEREBRAL ATROPHY IN HUMAN

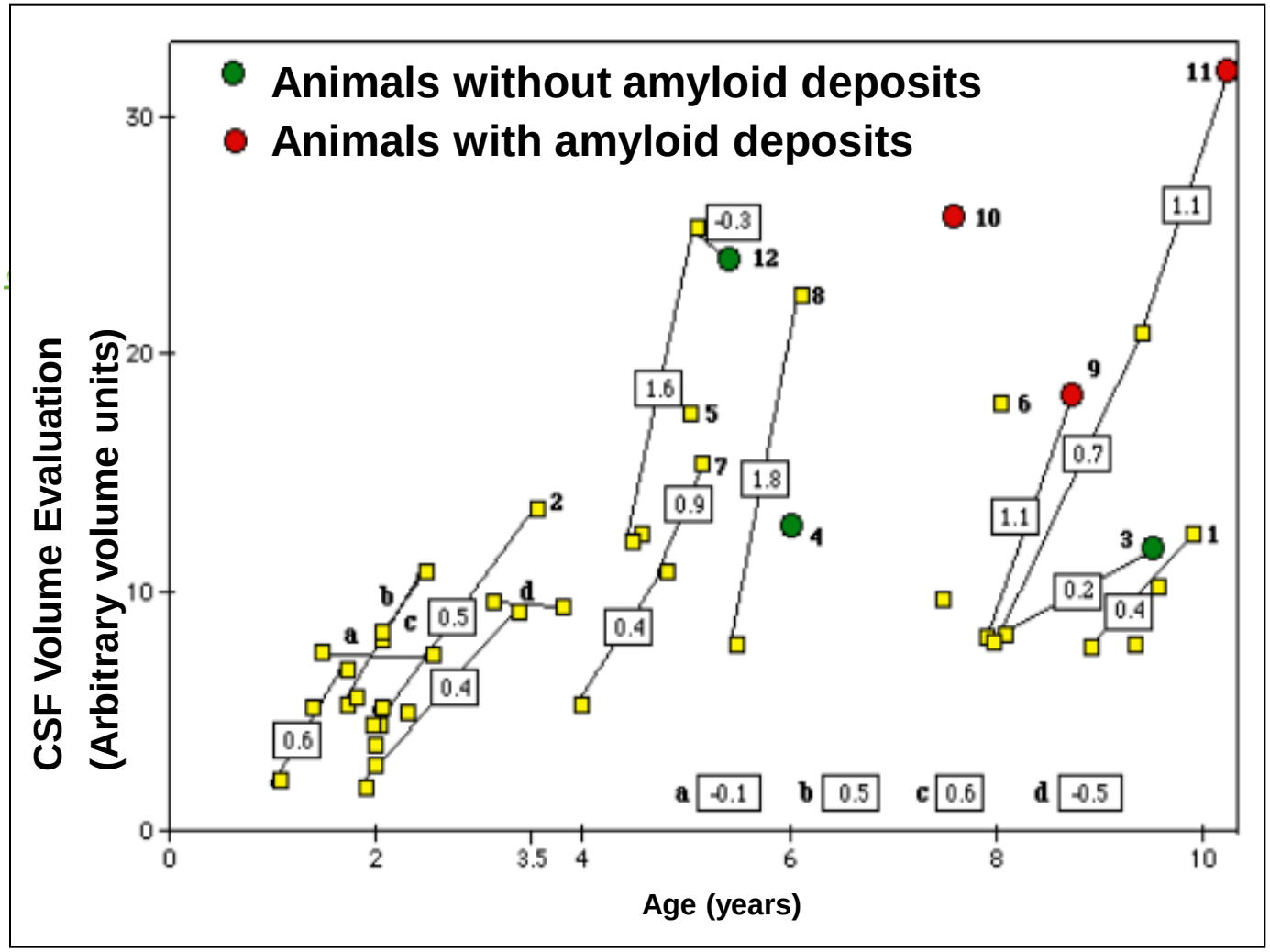
CEREBRAL ATROPHY IN RHESUS MONKEY

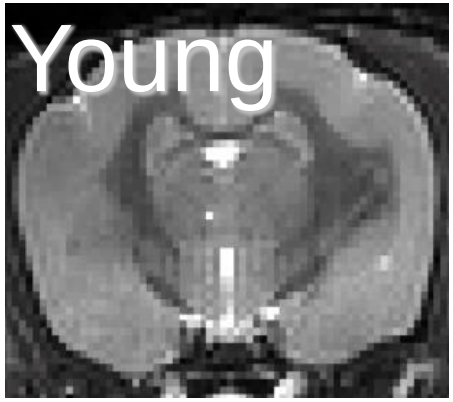


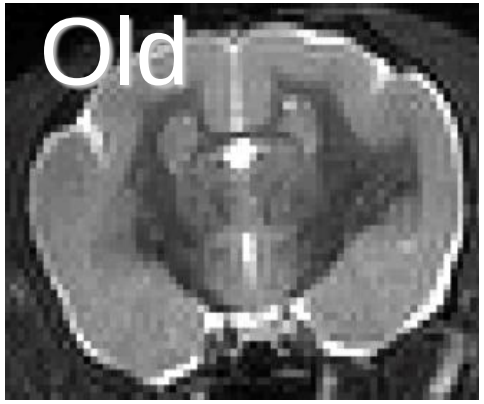


(Andersen et al., Brain Research, 1999)

TEMPORO-PARIETAL ATROPHY IN MOUSE LEMURS



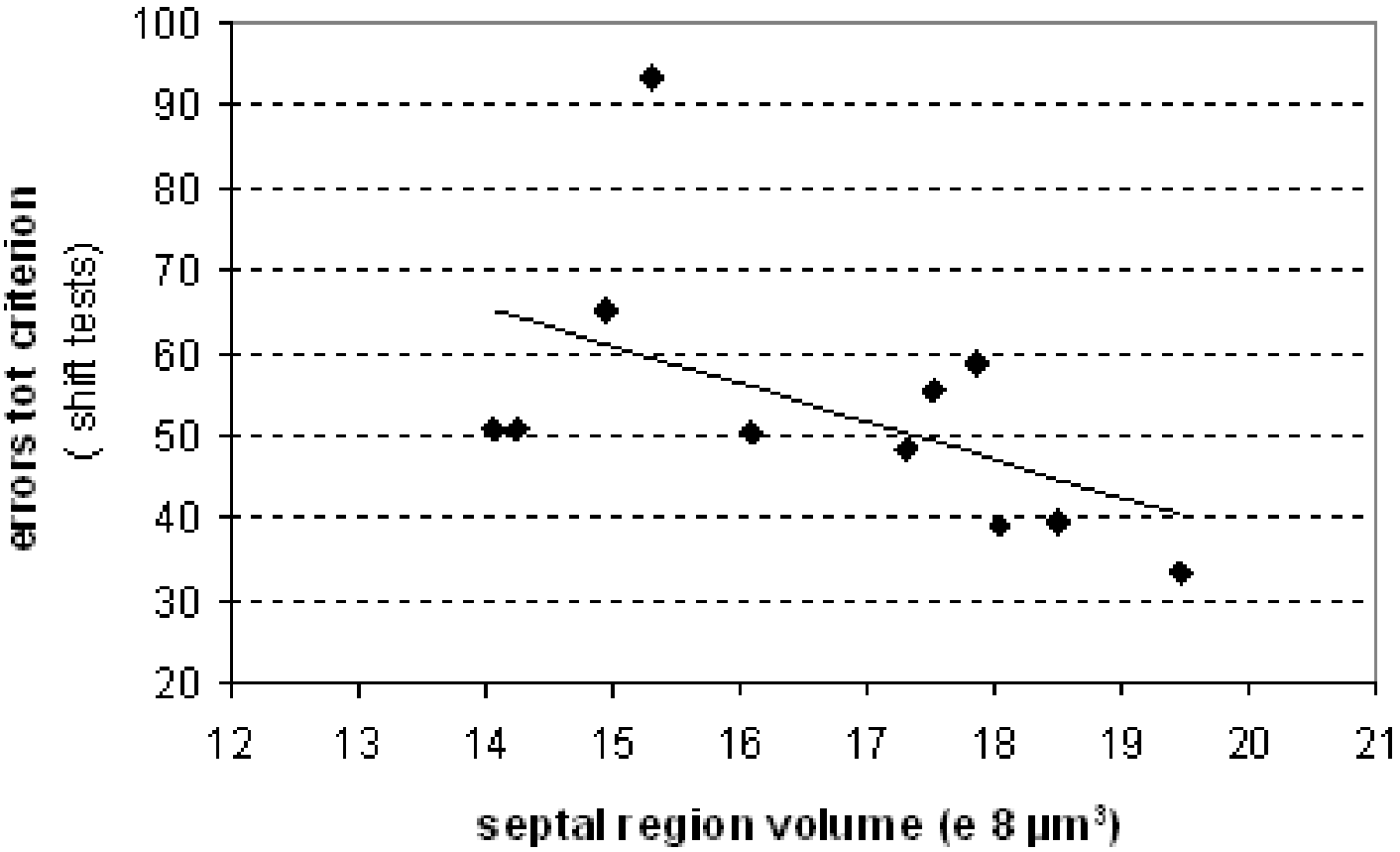




(Dhenain et al., Neurob. Aging, 2000)

- Fast evolution when the process is started

LINK BETWEEN BEHAVIORAL ALTERATIONS AND ATROPHY IN AGED MOUSE LEMURS OR MACAQUES



Correlation between macroscopic brain atrophy and age-related cognitive alterations

Picq et al. Neurobiology of Aging, 2012, Shamy et al. Cereb Cortex, 2011.

FUNCTIONAL CONSEQUENCES OF NEUROPATHOLOGICAL ALTERATIONS

No correlation between amyloid deposits and behavioral alterations

No study concerning neurofibrillary / behavioral alterations (especially in baboons)

ALTERATION OF THE NEUROTRANSMISSION

Acetylcholine

Monoaminergic

Serotonin
Noradrenaline

Somatostatin

…

Correlation between occurrence of neurotransmission alterations and behavioral alterations

PRIMATE MODELS

- Models to evaluate neurotransmission-based therapies

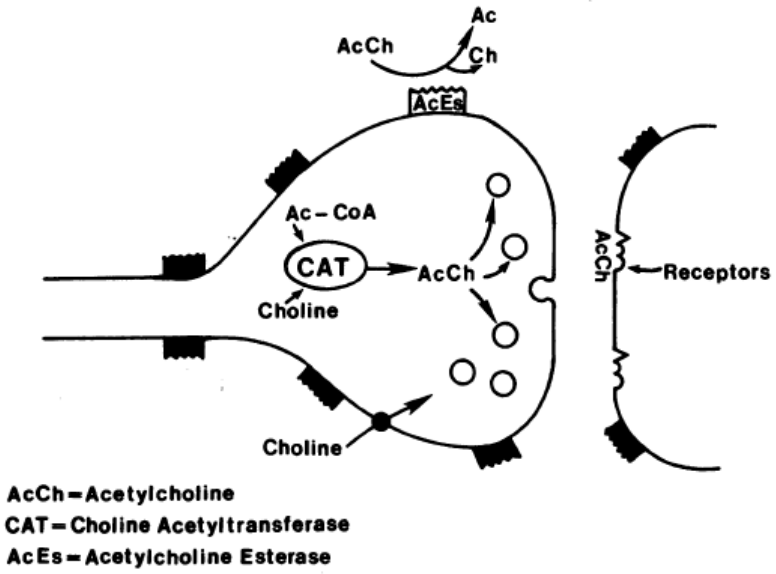
- Models to study amyloid-based therapy

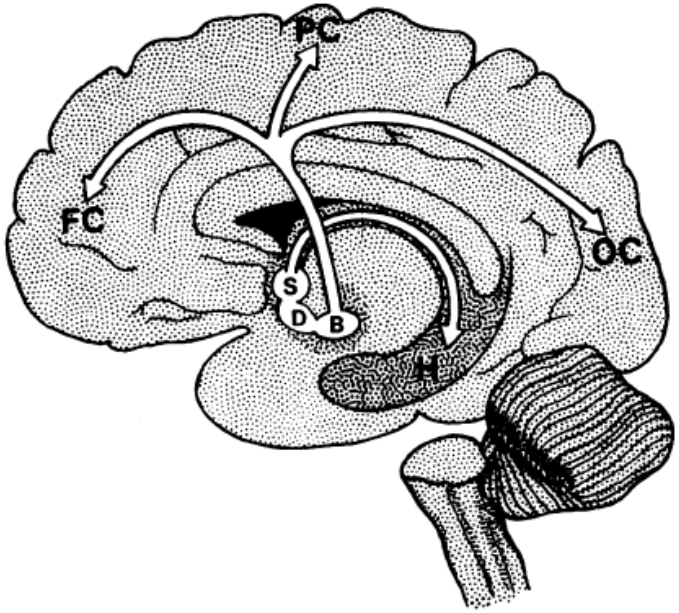
MODULATION OF THE NEUROTRANSMISSION

1900 1910 1970 1980 1990 2000 2010

1906: Alois Alzheimer

1978 (Perry)
Ach Alteration in Alzheimer





Reduced activity of CAT in Alzheimer patients
Reduction of AcEs in Alzheimer patients (less specific)

Coyle et al. Science, 1983

MODULATION OF THE NEUROTRANSMISSION

1900 1910 1970 1980 1990 2000 2010

1906: Alois Alzheimer

1978 (Perry)
Ach alteration in Alzheimer

Animal models of cholinergic alterations

Acetylcholinesterase inhibitors
1993 95 97 2007 2010
Tacrine patch generics
Galantamine
Donepezil
Rivastigmine

Anti NMDA
2002
Memantine

ANIMAL MODELS OF CHOLINERGI ALTERATIONS

Pharmacologic blockage of cholinergic system

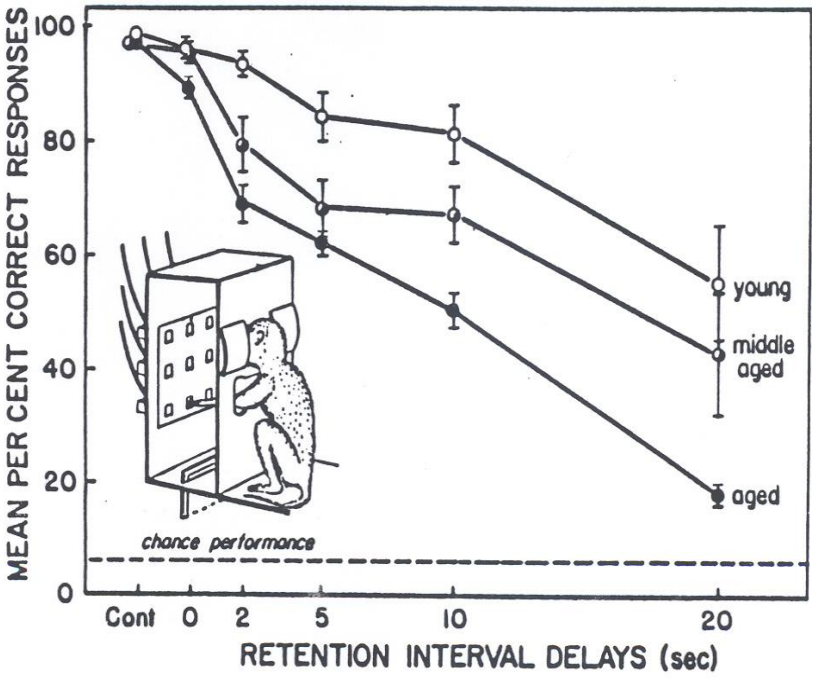
Scopolamine

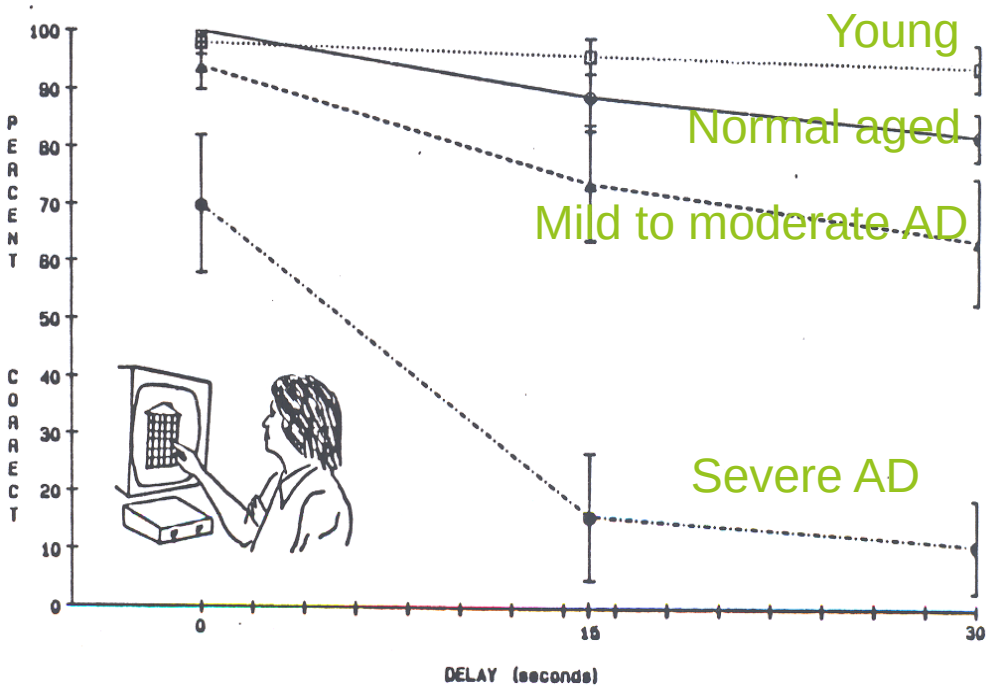
Lesions of cholinergic neurons

Ibotenic acid for ex.

Aged animals

AGE RELATED COGNITIVE ALTERATIONS





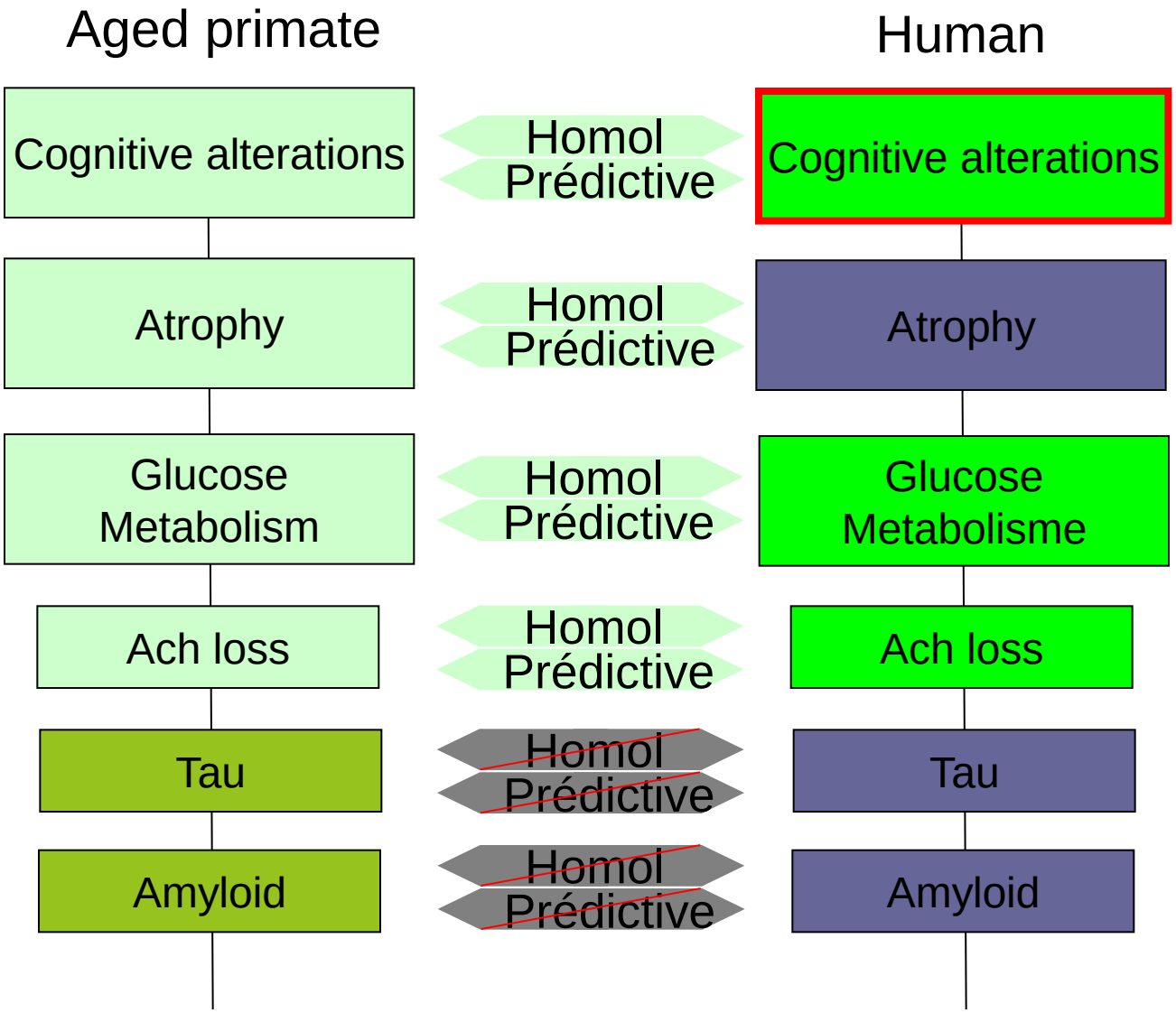
Delayed Response

(Bartus and Dean. Normal Aging, Alzheimer's disease and senile dementia, Aspects on Etiology, Pathogenesis, Diagnosis and Treatment, 1985)

EVALUATION OF THERAPIES MODULATING NEUROTRANSMISSION

Traitement	Classe	Amélioration Primates âgés	Date étude
Physostigmine	Anticholinestérase	Oui	Bartus, 1979
Tetrahydroaminoacridine	Anticholinestérase	Oui	Bartus, 1983
Arecoline	Agoniste muscarinique	Oui	Bartus, 1980
Oxotremorine	Agoniste muscarinique	Oui	Bartus, 1983
Choline	Cholinergique Precurseur de phospholipides	Non	Bartus, 1980
Apomorphine	Agoniste dopaminergique	Non	Bartus, 1983
Muscimol	Agoniste GABA	Non	Bartus, 1983
Clonidine	Agoniste α agoniste	Non	Bartus, 1983

AGED PRIMATE MODELS



Incomplete model but predictive to develop therapies

CONCLUSION PRIMATES

No case of AD in primates = Models for normal aging

No mutation reported for AD-like lesions
Few animals evaluated

Evaluation of the factors that are responsible for inter-individual differences

Clinical approach in animals with well known historical records

Factors modulating cognitive aging

Neuroendocrinologic factors, Biological rhythms,…

Thank you for your attention