MODEL-AD: Characterization of Familial AD Models (5xFAD, APP/PS1, hTau, 3xTg-AD)

MODEL-AD
MODEL ORGANISM DEVELOPMENT AND EVALUATION
FOR LATE-ONSET ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia, without an effective treatment. Animal models of AD have been valuable tools to understand familial or early onset AD, but to date have not been predictive for translational research. The objective of the Model Organism Development and Evaluation for Late-onset AD (MODEL-AD) Center is to develop, validate, and distribute novel mouse models of late-onset AD (LOAD) that can be used to develop novel therapeutics. We have established pipelines to test current fAD models across three sites and characterize mice using biochemistry, histology, functional assays and *in vivo* MRI and PET imaging, to determine a standard testing paradigm across all sites. MODEL-AD is a consortium involving Indiana University, The Jackson Laboratory, University of California-Irvine and Sage Bionetworks.

METHODS

Mice will be assessed for biochemistry, histology, transcriptional profiling and *in vivo* imaging. For biochemistry/histology, mice will be sacrificed at designated time points, perfused, and tissue harvested. Half of the brain will be frozen and the other half fixed. For biochemistry, we will assess AD relevant changes in tissue samples using antibodies against the following proteins: 22C11 (APP); 6E10, 4G8 (Aβ antibodies); Tau-46 (pan-tau); CP13, PHF1 (phospho-tau); NeuN, NFL, MBP, SYN and PSD-95 (neurons); GFAP, COL4 (astrocytes); IBA1, CD68, TREM2 (microglia, monocytes); PDGFRB (pericytes); and Fibrin, Albumin (vascular integrity). We will use RNA-seq to stage AD in existing models. Multiple RNA profiling datasets exist for human AD and therefore RNA-seq of mouse models will provide a valuable tool to directly assess human relevance. Models will undergo in vivo MRI and PET scanning as well as secondary validation with autoradiography studies.

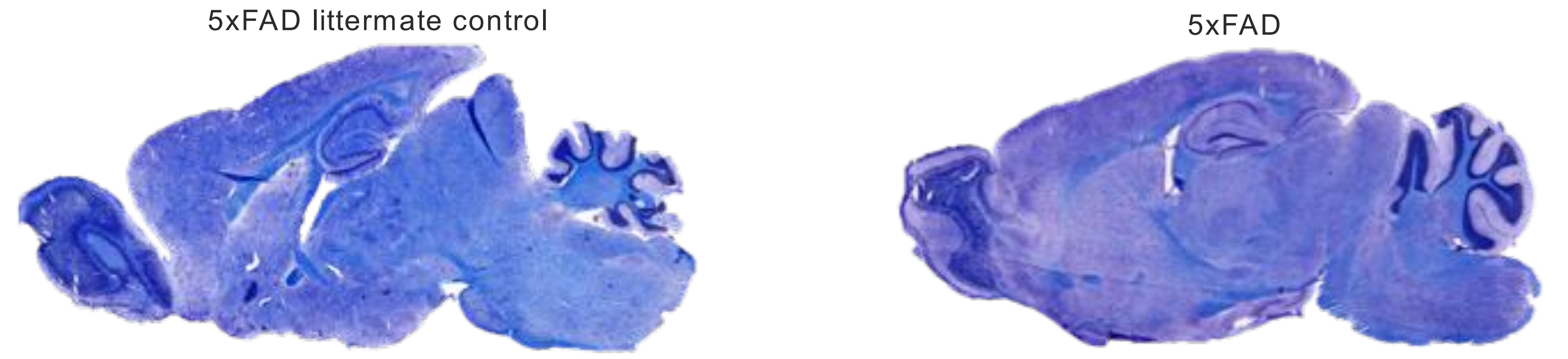
STRAINS ASSESSED

Common Name	Strain Nomenclature	JAX ID
5xFAD	B6.Cg-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax	8730
APP/PS1	B6.Cg-Tg(APPswe,PSEN1dE9)85Dbo/Mmjax	5864
3xTG-AD	B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1^{tm1Mpm}/Mmjax	4807
hTau	B6.Cg-Mapt$^{tm1(EGFP)Klt}$Tg(MAPT)8cPdav/J	5491

5xFAD model

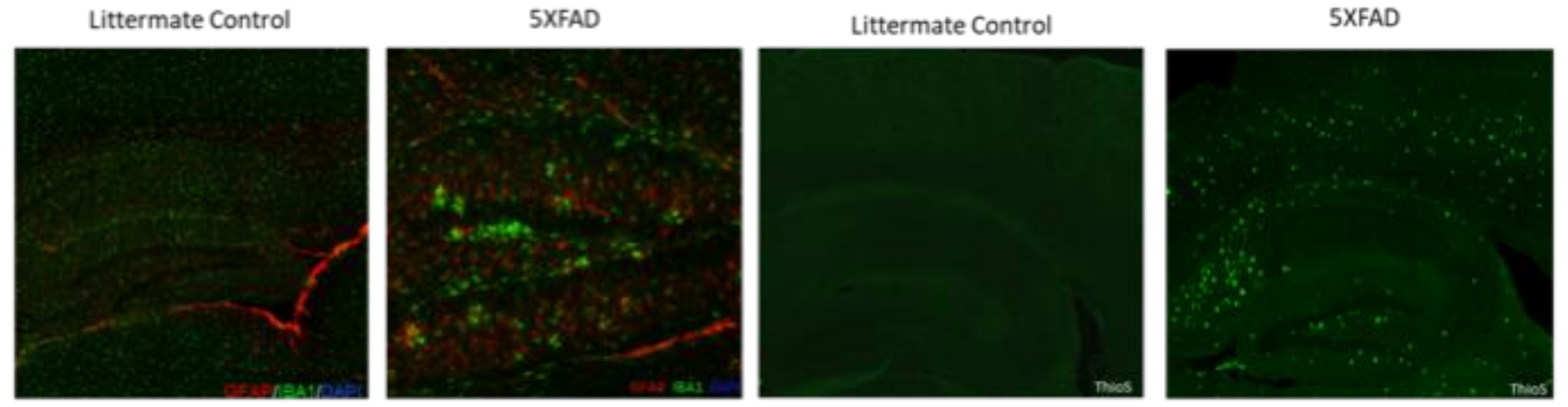
5XFAD transgenic mice overexpress both mutant human APP(695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) Familial Alzheimer's Disease (FAD) mutations and human PS1 harboring two FAD mutations, M146L and L286V. Expression of both transgenes is regulated by neural-specific elements of the mouse Thy1 promoter to drive overexpression in the brain.

Cresyl Violet and Luxol Fast Blue



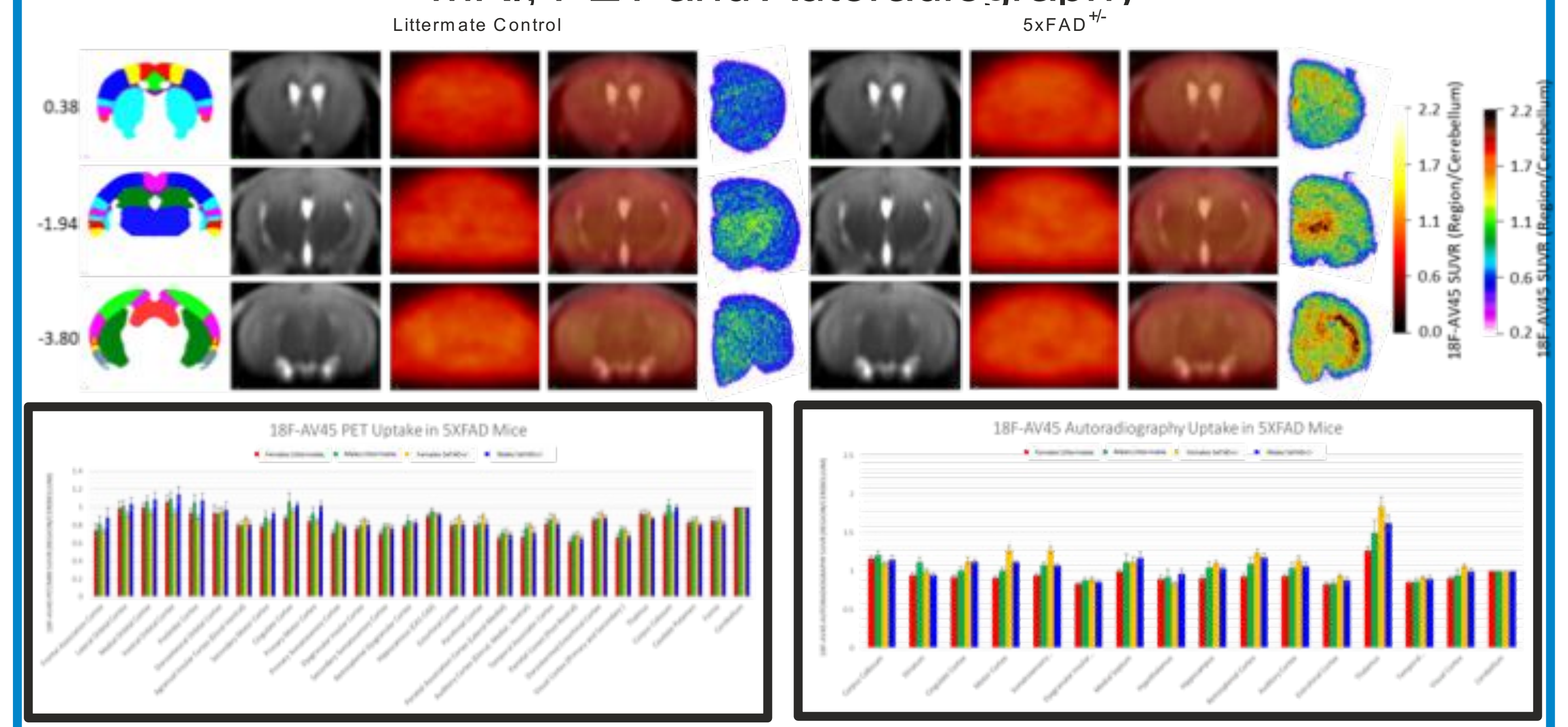
Tissue sections are stained with cresyl violet and counterstained with luxol fast blue to observe the white matter. Sections were then scanned using a Leica Aperio Versa system and uploaded to an eSlide manager for analysis.

Immunohistochemistry



Immunohistochemical staining from 8 month old mice. GFAP (red) stains reactive astrocytes; IBA1 (green) stains microglia and DAPI (blue) stains nuclei, comparing neuroinflammation between 5xFAD and C57BL/6J controls. Thio S (green) stains amyloid beta plaques in 5xFAD and littermate controls.

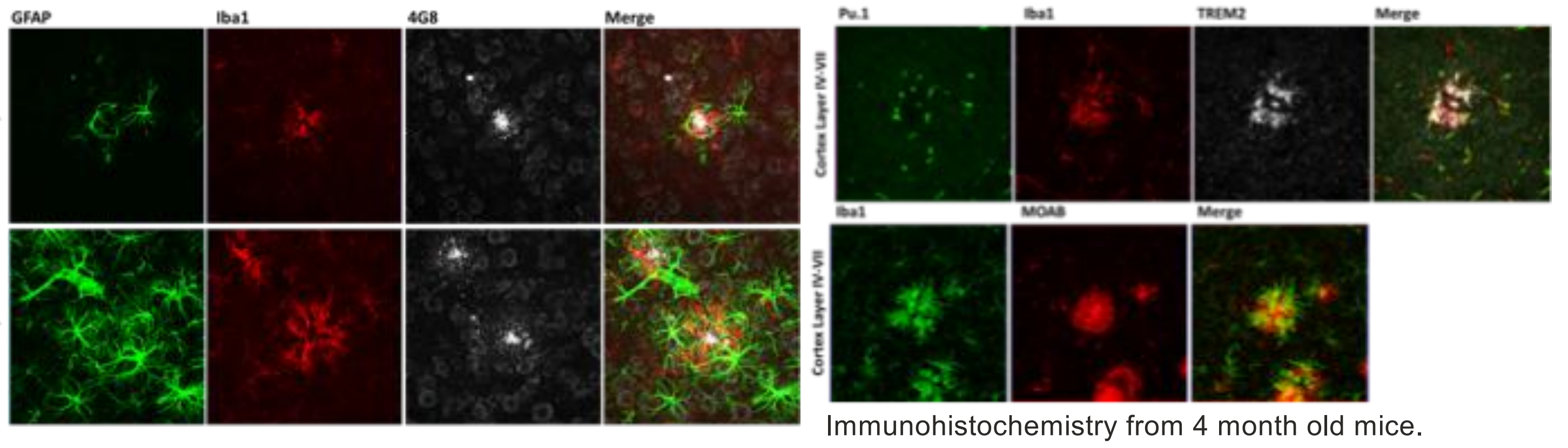
MRI, PET and Autoradiography

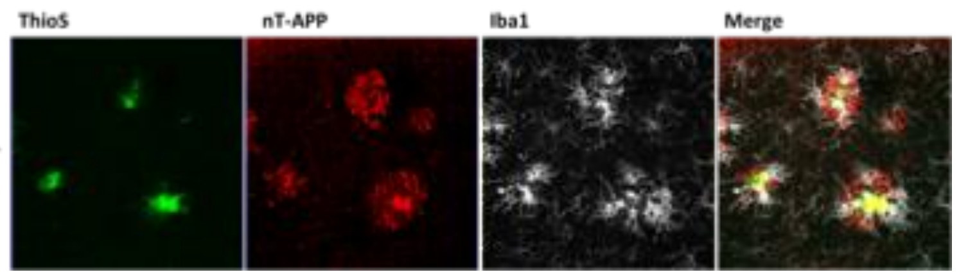


All PET scanning (15 min/ea.) was performed on the IndyPET3 scanner and post mortem brains were extracted and frozen for autoradiography (Autorad). MRIs were acquired (10 min/ea.) on a Siemens 3T Prisma scanner outfitted with a 4 channel phased array head coil. PET/MRI images were co-registered to Paxinos-Franklin atlas and 27 brain regions were extracted. For Autoradiography, frozen brains were sectioned at 20 um in sextuplet at 3 bregma targets, exposed on phosphor plates, scanned and manually segmented for 16 brain regions. Increased uptake is observed in the 5xFAD+/- mice compared to littermate controls.

APP/PS1 model

Double transgenic mice express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) both directed to CNS neurons. The "humanized" Mo/HuAPP695swe transgene allows the mice to secrete a human A-beta peptide.

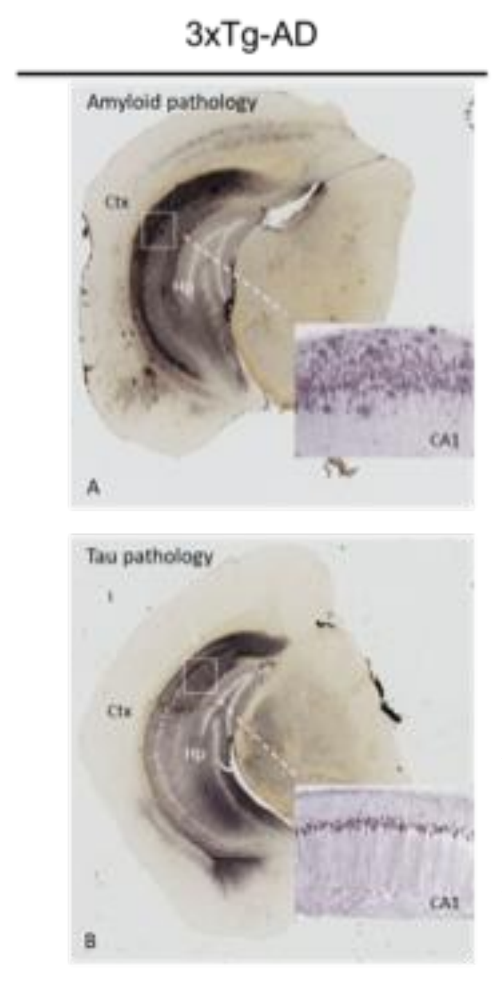


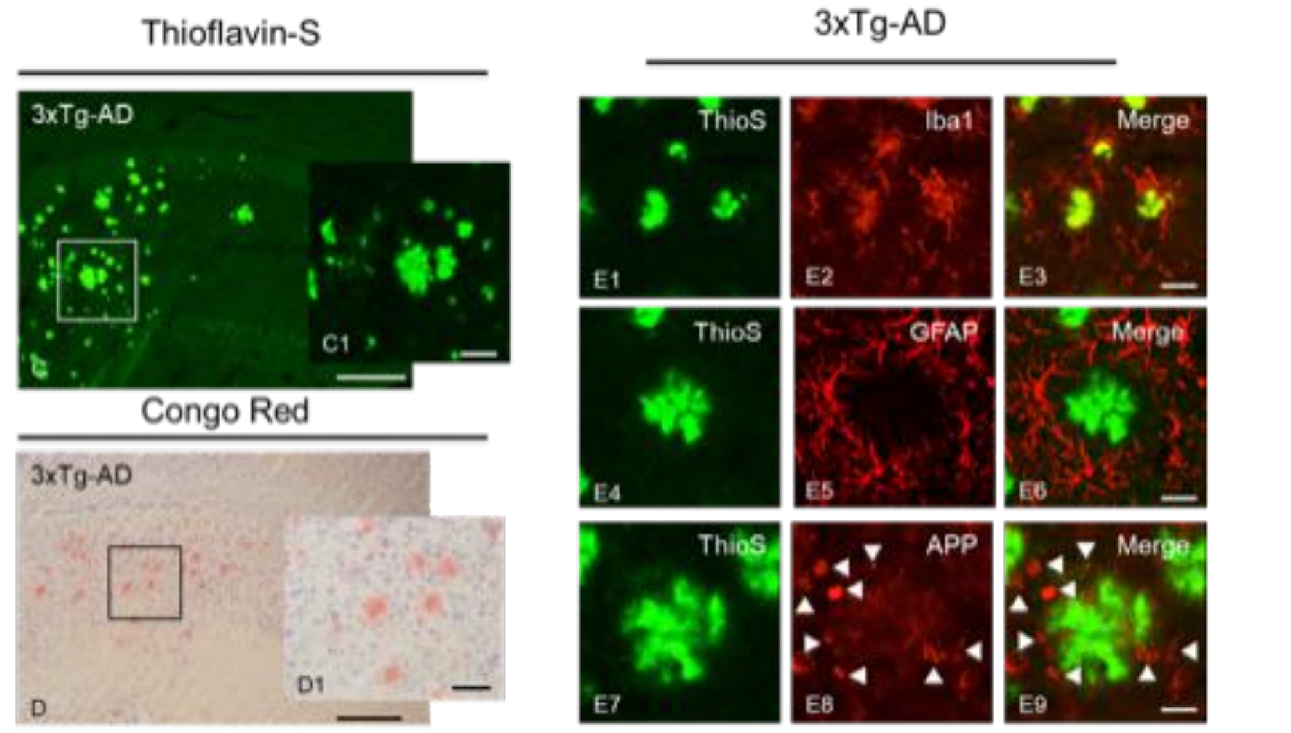


Immunohistochemistry from 4 month old mice.
Top Left: Immunofluorescence staining for astroglia (GFAP), microglia, and A-beta plaques. Note the differential expression of these cells types in the superficial vs. deep layers.
Bottom Left: Immunofluorescence staining for A-beta plaques and microglial cells.
Top Right: Immunofluorescence staining for IgG, microglia and Trem2.
Bottom Right: Immunofluorescence staining for microglia and A-beta.

3xTg-AD model

Mice are homozygous for all three mutant alleles (3xTg-AD; homozygous for the Psen1 mutation and homozygous for the co-injected APPSwe and TauP301L transgenes (Tg(APPSwe,tauP301L)1Lfa)) display no initial gross physical or behavioral abnormalities. Characterization of this mouse indicates a progressive increase in amyloid beta peptide deposition, with intracellular immunoreactivity as early as 3-4 months. Synaptic transmission and long-term potentiation are impaired at 6 months of age. Between 12-15 months aggregates of conformationally altered and hyperphosphorylated tau are detected in the hippocampus.

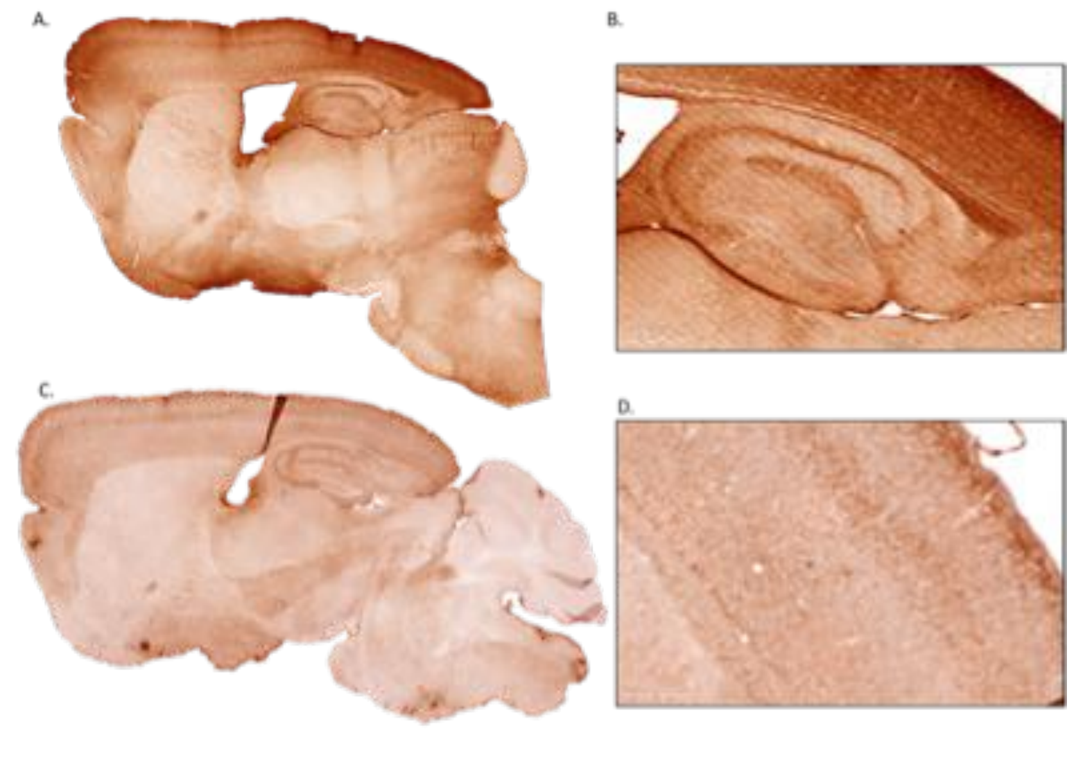




A and B) Light microscopic images 3xTg-AD animal model of AD. Elevated number of plaques (A) and intraneuronal tau (B) accumulation is observed in the hippocampal area of 3xTg-AD mice at 18 months of age. C and D) Accumulation of ThioS (C) and Congophilic (D) plaques is observed in 3xTg-AD mice at 20 months of age. E) Important gliosis and formation of neuritic dystrophies is observed in the hippocampus of 3xTg-AD at 20 month of age. Hp: Hippocampus; Ctx: Cortex. Scale bar: 200 μm (C and D), 50 μm (C1 and D1), 22.62 μm (E1 and E9).

hTau model

Mice express no endogenous mouse MAPT and all six isoforms (including both 3R and 4R forms) of human MAPT are expressed. Hyperphosphorylated MAPT is detected in cell bodies and dendrites by three months of age. Paired helical filaments of aggregated insoluble MAPT can be isolated from brain tissue as early as two months of age.



Immunohistochemistry from 6 month old mice.
A: Immunohistochemistry staining for AT8 in an hTau mouse at 12 mo of age. Panel shows the distribution of hyperphosphorylated tau at Ser202/Thr205.
B: Hyperphosphorylated Tau throughout the hippocampus and dentate gyrus.
C: Immunohistochemistry staining for AT180 in ah hTau mouse at 12 mo of age. Panel shows the distribution of hyperphosphorylated Tau at Thr231.
D: Hyperphosphorylated tau is observed throughout the cortex, but is more pronounced in cortical layers II/III and VI.

CONCLUSIONS

- All models, protocols, and data sets will be made widely available to researchers. We seek input and collaborations from research and pharma/biotech communities. For more information see www.model-ad.org.

FURTHER INFORMATION

- MODEL AD: www.modelad.org
- AMP-AD Knowledge Portal: http://www.synapse.org/ampad
- JAX AD models: https://www.jax.org/alzheimers
- AlzForum research models: http://www.alzforum.org/research-models

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