

Alzheimer's Models

Alzheimer's disease is characterized by both behavioral and histopathological symptoms that develop with age, and NDI offers animal models that capture these different aspects. Especially comprehensive are two naturally occurring aged-animal models that exhibit AD-like cognitive, non-cognitive, as well as histopathological symptoms.

AGED BEAGLES

Background

- Beagles develop beta amyloid accumulations, starting around 8 years of age.
- The predominant form of beta amyloid is the 42-peptide molecule, which is also the predominant form in humans.
- Beta amyloid also accumulates in the vascular system.
- Development of beta amyloid is structure specific, starting first in prefrontal cortex.
- A β deposition increases progressively. By 11 years of age, over 80 percent of these animals have A β deposition in prefrontal and entorhinal cortex.

Behavior testing (*Cognition*)

- Acquisition of a visuospatial memory task shows progressive deterioration with age (Delayed-Non-Matching to Position Task).
- Allocentric spatial ability decreases with age (Landmark Discrimination Task).
- Control tests are procedural learning and egocentric spatial learning tasks, which show little age sensitivity.
- Complex discrimination learning and reversal learning tasks are also age sensitive (Oddity Task and Discrimination Reversal Tasks).

Behavior testing (*Non- Cognitive Behaviors*)

- General activity is lessened (Open Field Test).
- Specific exploratory behavior is diminished (Curiosity Task).
- Social behavior is reduced (Human Interaction Task).
- Sleep-Wakefulness rhythms are disrupted.

Neuropathological Assessment

- Immunohistochemical staining for beta amyloid deposition
- Counts of apoptotic neurons
- Measurement of brain volume (MRI)

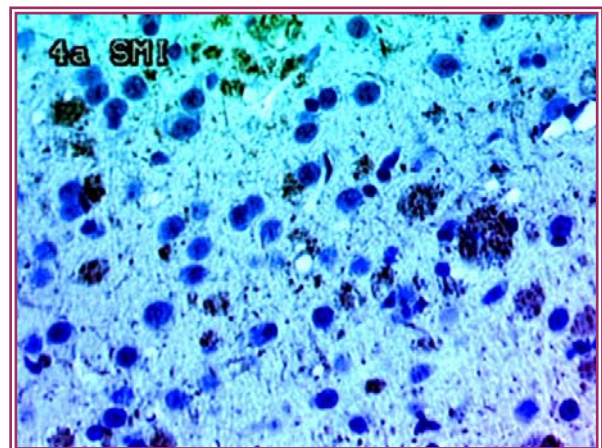
Compounds we have tested in the aged beagle model:

- Cholinesterase inhibitors
- Nicotine receptor agonists
- Muscarinic receptor modulators.
- Glutamate antagonists
- Estrogen receptor agonists/antagonists
- Calcium channel blockers
- Vitamins
- Anti-oxidants



FBN/F1 AGED RAT

Both males and females of the FBN/F1 hybrid rat strain progressively develop specific cognitive impairments during aging, in a manner characteristic of the development of human cognitive deficits in dementia. The order in which these deficits develop is: (1) an attention deficit (seen in a T-maze test and in a modification of the Barnes maze), (2) a memory deficit (seen in the moving-platform version of the watermaze test), (3) a learning deficit (seen in the classic Morris version of the watermaze), and (4) a global cognitive impairment (seen in the foraging portion of the modified Barnes maze task). Importantly, these impairments in cognition are detectable before motor impairments develop, which in other aged rat strains occurs in parallel with age-related behavioral impairments and compromises the “cognitive” interpretation of those behavioral impairments. In addition the FBN/F1 strain displays age-related increases in ubiquitin expression in specific brain regions implicated in its cognitive impairments, making it a model for an early, pro-AD state characterized by elevated inflammatory responses and gradual cognitive loss.



Increased ubiquitin staining in entorhinal cortex of 32-month-old FBN rat. Density of staining correlates with extent of short-term memory deficit.

A β INJECTION-INDUCED DEGENERATION (RAT)

An animal model of neurodegeneration has been developed that induces neuron loss by injections of β -Amyloid (A β) fragments into the rat hippocampus (Kowall et al., 1992; Miguel-Hidalgo et al., 1998; Alvarez et al., 1998). This A β model of Alzheimer's disease is appropriate for *in vivo* investigation of compounds with neuroprotective potential. Implants of the A β 1-40 or 1-42 fragment induce degeneration of neurons of the hippocampal CA1 subfield, and produce behavioral, neurochemical and immuno-histochemical changes resembling those occurring in Alzheimer's disease.

Quantification of plaque load can be performed using a single antibody (e.g. 6E10). Levels of soluble Abeta can be determined for both Abeta 1-40 and Abeta 1-42 in 4 fractions (TBS, Triton, SDS, FA) as well as in plasma and CSF (all duplicates).

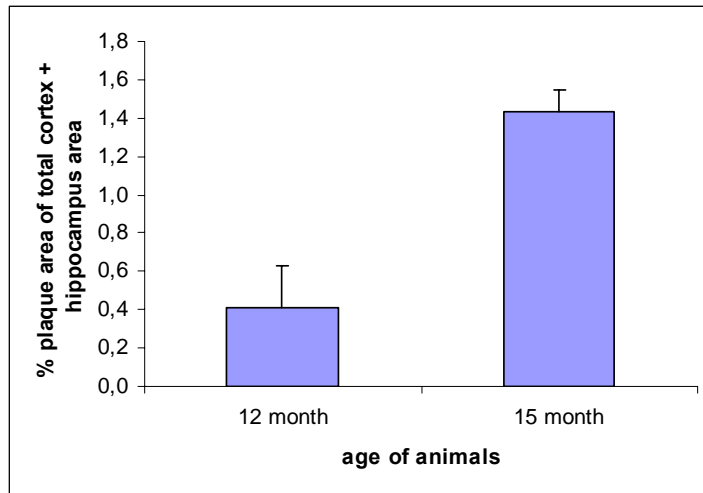
Bilateral injections are used if behavior is measured; unilateral injections may be used if only histological measures are used (allowing comparisons of lesioned vs. unlesioned side). Typically, immunohistochemical measures are used if the intent is to test a treatment effect on reducing A β -induced neuronal damage, broadly speaking (glial and macrophage staining levels). Apoptosis can also be measured specifically if the intent is to test a treatment's ability to reduce cell death, but this measure is indirect; counts of apoptotic cells are made, which do not include neurons that have already died by the time the animals are sacrificed, and neurons that are in the process of dying via a non-apoptotic pathway.



Neuronal degeneration produced by β -amyloid 1-40, and neuroprotection. Left: section through the hippocampus of an unaffected rat. Center: Hippocampus of a rat with a deposit of A β 1-40. Even in absence of major mechanical damage there is a dramatic loss of neurons in the CA1 subfield and in the dentate gyrus. Right: Hippocampus of a rat treated with a neuroprotective compound. Neuronal loss in CA1 and dentate gyrus is greatly reduced.

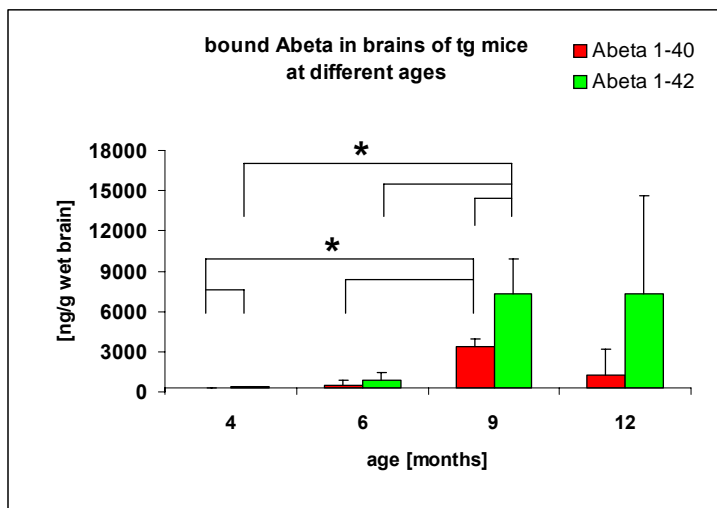
hAPP DOUBLE MUTATION MOUSE

This mouse over-expresses human (h) APP751 (amyloid precursor protein) containing the London (V717I) and Swedish (K670M/N671L) mutations under the regulatory control of the murine (m) Thy-1 promoter (mThy-1-hAPP751). We have data from three-, six-, nine- and twelve-months old transgenic and non-transgenic control mice. The animals have been investigated extensively using motor and cognitive tests, and the tg mice have exhibited no deficits on any motor test but profound deficits on the two most common tests of cognition, the Morris watermaze and object recognition. Biochemical results using ELISA show that A β 1-40 and especially A β 1-42 are clearly increased in brains of transgenic mice. Furthermore these animals show a pronounced A β -histopathology, with amyloid plaques in the cortex at ~4 months of age and in the hippocampus at ~7 months of age.



Each column represents mean +/- of bound hA β 1-42 concentrations [ng/g] in brain samples of tg animals of four different ages determined via ELISA. Sensitivity limits were calculated as 33pg/ml.

Significant differences are marked with asterisk. The level of significance was set at $p \leq 0.05$.

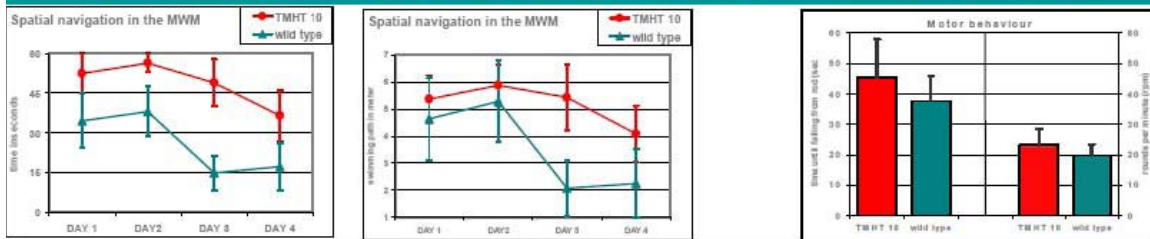


TAU TRANSGENIC MOUSE

A second tg mouse model available is the TAU transgenic mouse over-expressing the human TAU441 gene with two mutations, V337M and R406W, under control of a tissue specific murine Thy1-promoter and with a C57BL6 background. These mice display:

- Well known AD pathology
- No motor deficits
- Behavioral deficits in the place learning version of the watermaze (at 9 months of age)

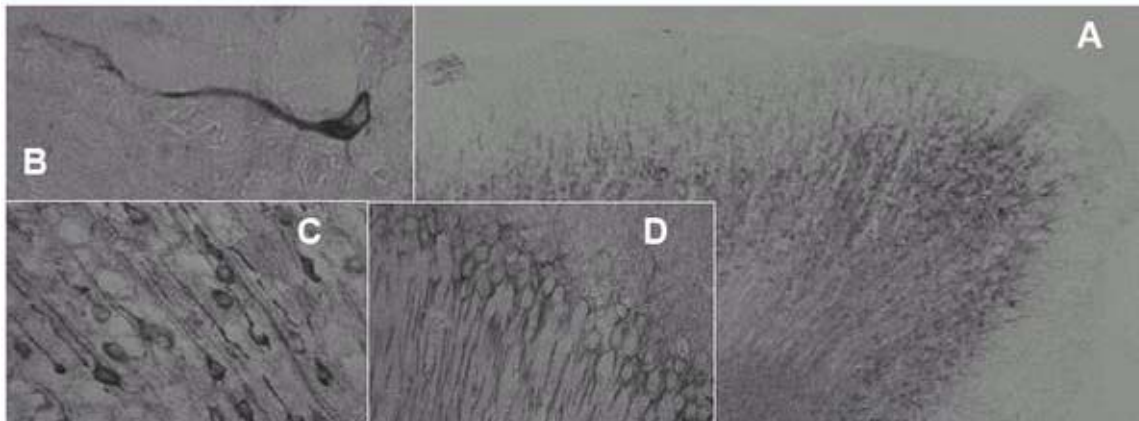
TAU441 expression on behavioural outcome measures



Spatial navigation behaviour in the MWM is severely disturbed in 9 months old TMHT 10 mice compared to littermates.

Motor behaviour of TMHT 10 mice is not disturbed at all.

Evaluation of TAU over-expression in cortex and hippocampus

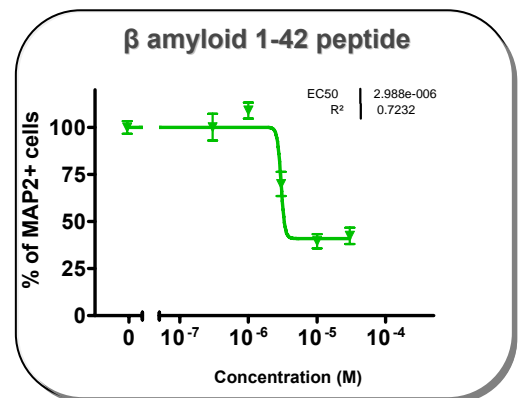
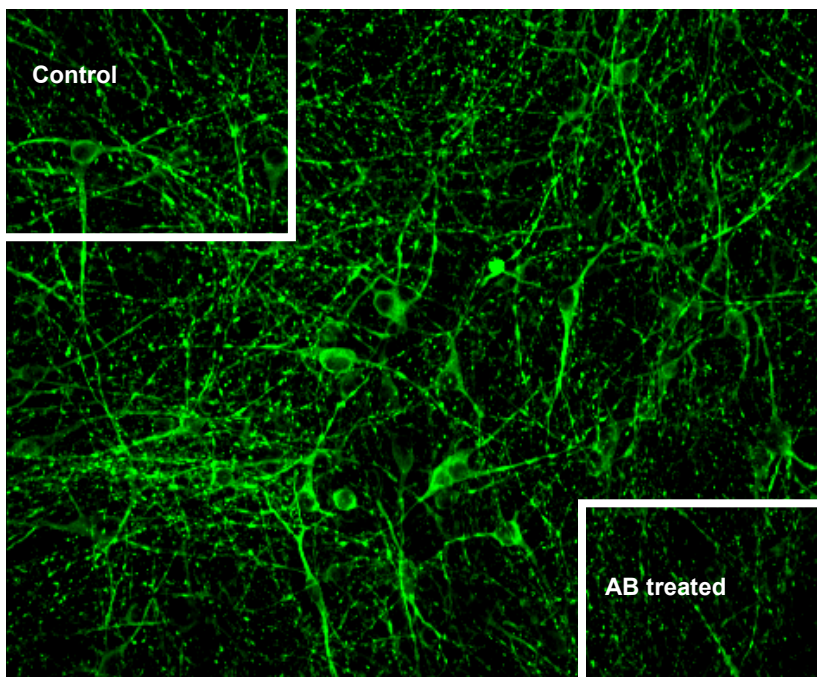


A: Cortex – AT180 overview staining: numerous densely packed tau-positive lesioned i.e., abnormal TAU-immunoreactive neurophil fibers and tangles. Antibody- AT180 detects PHF and tangles (Thr231).
 B: Cortical cell with massive tau-deposition in neuronal soma and axon, which is densely packed with TAU.
 C: Cortical cells showing neuronal perikaria and neuritis (axons and dendrites) massively loaded with TAU, typical tangles detectable.
 D: Cortex / Hippocampus: in the hippocampus accentuated TAU depositions, which are apparently seen in the periventricular matter.

in vitro AD ASSAYS

A β -induced neurotoxicity (mesencephalic cells)

We have developed a novel *in vitro* assay for screening potential neuroprotective agents, that uses mesencephalic neurons and MAP2 (neuron-specific) quantification. An additional capability of this assay is detection of neurotrophic as distinct from neuroprotective effects. The cultures contain dopaminergic neurons taken from embryonic rat mesencephalon, which fully differentiate *in vitro*, and survive for at least 30 days.



A β -induced neurotoxicity (NT2N cells)

This assay is useful for screening compounds that interfere with APP processing. The formation of intracellular A β is measured in NT2N cells that have differentiated into neurons. NT2N cells are used because they appear to process APP in ways similar to human neurons.