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*How To Slice Mice – Transgenically*

# Neurome Scientists Try Method For Viewing Alzheimer's Brain Tissues – Eventually Pre-Mortem

**By David N. Leff**
**Science Editor**

Post-mortem examination of Alzheimer's disease (AD) brains can have important value for probing the still-mysterious etiology of the disorder. By definition, post-mortems are of no use whatsoever to the individuals whose autopsy specimens are being probed.

However, scientists at Neurome Inc. in La Jolla, Calif., foresee the day when their new analytic technology will provide pre-mortem data for AD patients.

"At present," observed researcher John Reilly, Neurome staff scientist, "our new approach still involves the examination of post-mortem tissue. This has two important implications. One is the possibility that by inspecting amyloid-beta in three dimensions, we may pick it up earlier and monitor the pattern it forms in AD senile plaques. In future study, it would have a significant impact in predicting the severity of the disease. In terms of human AD, of course, that prospect is a ways off. It's going to depend on other technologies in terms of visualizing amyloid with some sort of noninvasive methods. (See *BioWorld Today*, April 15, 2003.)

Reilly is lead author of a paper in the current *Proceedings of the National Academy of Sciences* (*PNAS*) dated April 15, 2003. Its title: "Amyloid deposition in the hippocampus and entorhinal cortex: Quantitative analysis of a transgenic mouse model."

"Our overall finding in that *PNAS* paper," Reilly told *BioWorld Today*, "was the observation of the increasing deposition of amyloid over time in human AD. Plus the fact that deposition occurs in this transgenic mouse model in the same circuit that's pathologically affected in human AD. What's novel," he continued, "is the 3-D visualization of amyloid lakes and ribbons that we were able to detect. Most typical post-mortem analyses are based on 2-dimensional studies, and you don't really appreciate where the amyloid tracks in three dimensions. So the ability to see where it is in 3-D space gives us a clue as to how it might be deposited in the AD brain areas where it accumulates.

"The entorhinal cortex," Reilly explained, "is an anatomic region of the brain. It's a fissure or groove along the cerebral surface, back behind the occipital cortex lobe. Like a fingerprint, each fold is slightly different. Mice also have that fissure."

### Elan, Neurome Groom Human AD Mice

The Neurome co-authors ran a transgenic mouse model that mimics human AD. It carries a mutant gene for the amyloid precursor protein (APP), which throws off two AD-toxic alpha-beta peptides and inflicts the familial AD version of the disorder. "The general theme," Reilly noted, "is to take the human gene responsible for familial AD. It resulted in AD-like deposition of amyloid plaques in all of the animals."

That model is now used by Elan Corp. plc, of Dublin, Ireland. It has entered into collaborative agreements with other universities and other companies – among them Neurome – to do analyses on this animal.

"Our joint goal is a more complete characterization of the AD animal model," Reilly said.

In this *PNAS* paper, Reilly and his group reported what they actually determined from their new technology. "One result," he recounted, "was really the first detailed quantitative study of the way that amyloid accumulates in this mouse model over time. Although there have been biochemical studies showing a temporal increase in amyloid in certain grossly defined brain regions – the whole hippocampus, for example – this is the first analysis that actually looked in detail at specific anatomical regions, and tracked progression of amyloid deposition. That's what enabled us to come to the conclusion that amyloid is preferentially deposited in this mouse model in the exact same places where it's deposited over time in human AD.

"We started out at six months with almost no amyloid and progressed out to 22 months of age. The largest amyloid increase was between 12 and 15 months. We were thinking, 'Is that somehow a set of A-beta peptides cleaved off of

the APP and secreted into the extracellular space?' They somehow nucleated and plaques began to form on the neurons like a crystal growing. Over time, once you got a certain basal level of beta amyloid, the addition of beta peptides secreted can glom on to the growing plaques and be deposited."

**Next Stage: Study Human AD Changes**

Reilly described how their method enabled 3-D observation.

"It's essentially a 3-D reconstruction of the 2-D mouse brains. We took the whole brains, sliced them up on a microtome into very thin microscopic slices. All of these we placed on glass microscope slides, labeled, in this case with antibodies for immunostaining. After we scanned these slides into our database, all of the sections were compiled into a single 3-D data set. We called it the 'mice slice.'

"Now ongoing," he said, "we are taking this examination of AD pathology down to the next level. Our forthcoming paper further defines the pathological changes occurring in the dentate gyrus of the hippocampus, looking at the individual neurons and cells. When these connect with one another they do so at synapses because the neurons are branched structures. Other neurons that want to communicate will connect at point synapses. If a pathological change is going to occur that will affect that neuron, it will be reflected in the number of synapses or branches. So what we're currently engaged in doing in conjunction with Elan in their APP transgenic mouse model is looking at pathological changes in the branching patterns of neurons and cells within the dentate gyrus.

"I think the advantage that we have in the possible therapeutic benefits to this approach are first, the identification of this pathological alteration that occurs very early on in the disease. The current ongoing study is high time to start thinking of AD long before your patient has a brain full of plaques. The whole idea is you really want to do it completely prophylactically, and catch these changes before they lead to development of plaques in the brain.

"The idea is to take a drug when you were 30 years old, and never develop AD even if you were someone who was predicted to develop it. That, I think," Reilly concluded, "is the ultimate therapeutic application of what we are working on." ■