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Staining of Amyloid Beta (Abeta) Using (Immuno) Histochemical Techniques and Abeta42 Specific Peptides

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1. Introduction

In the elderly, Alzheimer's disease (AD) is the most common form of dementia (Hebert et al., 2003). The two pathologies that characterize the disease are the presence of large numbers of intracellular neurofibrillary tangles (NFTs) and extracellular neuritic plaques in the brain (e.g., Braak and Braak, 1991; 1998; Selkoe, 2001). Neurofibrillary tangles consist of hyperphosphorylated, twisted filaments of the cytoskeletal protein tau (e.g., Duff, 2006), whereas plaques are primarily made up of amyloid β ($A\beta$ [Selkoe, 2001; Dickson and Vickers, 2002]), a 39-43 amino acid long peptide derived from the proteolytic processing of the amyloid precursor protein (APP [Selkoe, 2001; Vetrivel and Thinakaran, 2006]). When APP is sequentially cleaved by the β -secretase and γ -secretase, one of the resulting breakdown product is $A\beta$, in contrast, initial cleavage by α -secretase (in the middle of the $A\beta$ sequence) leads to production of $APP_{s\alpha}$ and the C83 peptide (Selkoe, 2001).

Most cases of AD are sporadic, however approximately 5 % of AD cases are familial (Price and Sisodia, 1995; Selkoe, 2001), these cases are related to mutations in the genes for APP, and presenilin 1 and 2 (PS1 and PS2 [Price and Sisodia, 1995; Hardy, 1997; Selkoe, 2001]). Transgenic mice expressing mutated human AD genes offer a powerful model to study the role of $A\beta$ in the development of pathology (e.g., Duff and Suleman, 2004; McGowan et al, 2006). The present study employs three lines of transgenic mice expressing both human APP^{swe} and/or PS1 mutations. These lines of mice develop elevated levels of $A\beta_{42}$ at different ages, and at different locations (Van Groen et al., 2005; Wang et al., 2003).

2. Materials and methods

2.1 Animals

Two lines of APP and PS1 single and double transgenic mice (**AP/PS**) were used in the present study. The first line of mice was generated from matings between APP^{swe} and

HuPS1-A246E transgenic mice, this mouse line was originally produced at the Johns Hopkins University (Borchelt et al., 1996), and was bred locally on a C57BL/6J background. The second line of APP/PS1 mice was the APP^{swe}+PS1 Δ 9 line, originally produced at the Johns Hopkins University (Jankowsky et al., 2001), we acquired these mice from JAX at the age of six weeks. The animals were housed 4/cage in our facility; in a controlled environment (temperature 22°C, humidity 50-60%, light from 07:00-19:00), with food and water were available *ad libitum*. All procedures were conducted in accordance with the local Institutional Animal Care and Use Committee (IACUC) guidelines.

2.2 Peptides

In short, a mirror image phage display approach was used to identify novel and highly specific ligands for Alzheimer's disease amyloid peptide A β 1-42 (Wiesehan et al., 2003). In short, a randomized 12-mer peptide library presented on M13 phages was screened for peptides with binding affinity for the mirror image of A β 1-42 (Wiesehan et al., 2003). After four rounds of selection and amplification the peptides were enriched with a dominating consensus sequence. The mirror image of the most representative peptide (i.e., D1) was shown to bind A β 1-42 with a dissociation constant in the submicromolar range (Wiesehan et al., 2003). The D2 and D3 peptides come from two other phage display selections against A β 42. The D1 peptide has a higher affinity for A β 42 monomers, D2 has a low specificity, whereas D3 has a high affinity for A β 42 oligomers (Funke et al., 2010). To study the binding characteristics of the D-peptides in more detail, an L-peptide version of the D1-peptide was made, and a scrambled (sequence) peptide of similar length was also made. In the binding experiments the peptides that were used had been conjugated with a FITC molecule for visualization purposes, except in a few experiments. In those experiments D1 conjugated with other fluorophores were tested to study the interaction of the fluorescent moiety with binding characteristics of the D1 peptide.

2.3 Histopathological techniques

In short, mice were anesthetized, transcardially perfused with saline followed by 4% paraformaldehyde and the brains were removed from the skull. After postfixation (4h) and cryoprotection (24h in 30% sucrose), six series (1 in 6) of coronal sections were cut through the brain. The first series of sections was mounted unstained, and the second, third and fourth series were stained immunohistochemically according to published protocols (Kadish et al., 2002; Van Groen et al., 2006) the other two series were stored in at -20°C in antifreeze for future analysis. One half of the second series was stained for human A β using the W0-2 antibody (mouse anti-human A β ₄₋₉; Ida et al., 1996), the other half of the second series was stained for mouse A β (rabbit anti-rodent A β ; Covance; Van Groen et al., 2006). The first half of the third series was stained for A β 40 (mouse anti-A β 40, Covance) the other half for A β 42 (mouse anti-A β 42; Covance). In some animals, one half of the fourth series was stained for GFAP (mouse anti-GFAP; Sigma), whereas the other half was stained for CD11b (rat anti-mouseCD11b; Serotec), a marker of microglia, to analyze inflammation in the brain. Some of these sections were double stained with either Congo red, thioflavine S or thiazine red to visualise β sheets, i.e., A β plaque cores in our material, in a few animals methoxy-X04 (Klunk et al., 2002) was infused during the perfusion to label all A β in the brain. The sections destined for immunohistochemical A β staining were pretreated for 30 min with hot (85°C) citrate buffer. The series of sections were transferred to a solution containing the

primary antibody (W0-2, mouse monoclonal), this solution consists of TBS with 0.5 % Triton X-100 added (TBS-T). Following incubation in this solution for 18 h on a shaker table at room temperature (20°C) in the dark, the sections were rinsed three times in TBS-T and transferred to the solution containing the appropriate secondary antibody (goat anti-mouse*biotin; Sigma). After two hours, the sections were rinsed three times with TBS-T and transferred to a solution containing mouse ExtrAvidin® (Sigma), following rinsing the sections were incubated for approximately 3 min with Ni-enhanced DAB (Kadish et al., 2002). In a small number of sections, the A β deposits were double labeled for A β 40 and A β 42 using fluorescent secondary antibodies. All stained sections were mounted on slides and coverslipped.

Histochemical stains. Thioflavine-S staining (Guntern et al, 1982), sections are mounted on gelatinized slides, and air-dried. When dry the slides are immersed in distilled water for 5 min twice to rehydrate, then they are immersed in the Thioflavine-S solution (1g of Thioflavine-S in 100 ml distilled water) for 20 min. Then the slides are rinsed quickly twice in distilled water and air dried, when dry they are rinsed in xylene, and coverslipped with DPX. The staining procedure is performed in the dark, i.e., the solution of Thioflavine-S is kept in a opaque container, similarly the staining procedure is done in opaque containers. Thioflavine-T staining (Morimatsu et al, 1975), sections are mounted on gelatinized slides, and air-dried. When dry the slides are immersed in distilled water for 5 min twice to rehydrate, then they are immersed in the Thioflavine-T solution (1g of Thioflavine-T in 100 ml distilled water) for 20 min. Then the slides are rinsed quickly twice in distilled water and air dried, when dry they are coverslipped with DPX. The staining procedure is performed in the dark, i.e., the solution of Thioflavine-T is kept in a opaque container, similarly the staining procedure is done in opaque containers. Congo red staining (Glennner, 1981), slides with brain sections are put overnight in 4% paraformaldehyde solution, the next day slides are rinsed twice with distilled water for 5 min, then put in the pretreatment solution (a 80% ethanol saturated NaCl solution with 1% sodium hydroxide added [1ml per 100 ml) for 20 min. Then the slides are transferred to the Congo red staining solution (a 80% ethanol saturated NaCl solution with 0.2 g Congo red per 100 ml) for 25 min, the slides are rinsed quickly in distilled water and air dried. Thiazine red (Uchihara et al, 2000) slides with sections are rinsed in distilled water for 5 min, and put in the Thiazine red solution (0.1g Thiazine red in 300 ml 0.001 M Naphosphate buffer, pH 7.4) for 15 min. Then the slides are rinsed quickly twice in distilled water and air dried, when dry they are coverslipped with DPX. Methoxy-XO4 staining, slides with sections are rinsed in distilled water for 5 min, and put in the Methoxy-XO4 solution (3.3 mg Methoxy-XO4 in 100 ml 40% ethanol/60%distilled water at pH 10.00) for 10 min. Then the slides are rinsed quickly twice in distilled water and air dried, when dry they are coverslipped with DPX.

3. Results

3.1 In vitro staining

The staining of sections of paraformaldehyde fixed brains of **AP/PS** mice with histochemical methods revealed that all methods that are used for staining amyloid also stain amyloid β and stained all dense A β deposits, i.e., plaques (Figure 1). However, it should be noted that in the sections of the AP/PS mice that have large amounts of diffuse A β deposits, most these deposits were not stained. Staining intensity of the A β deposits was directly related to the method that was used, the solutions that we used contained the optimal concentrations of

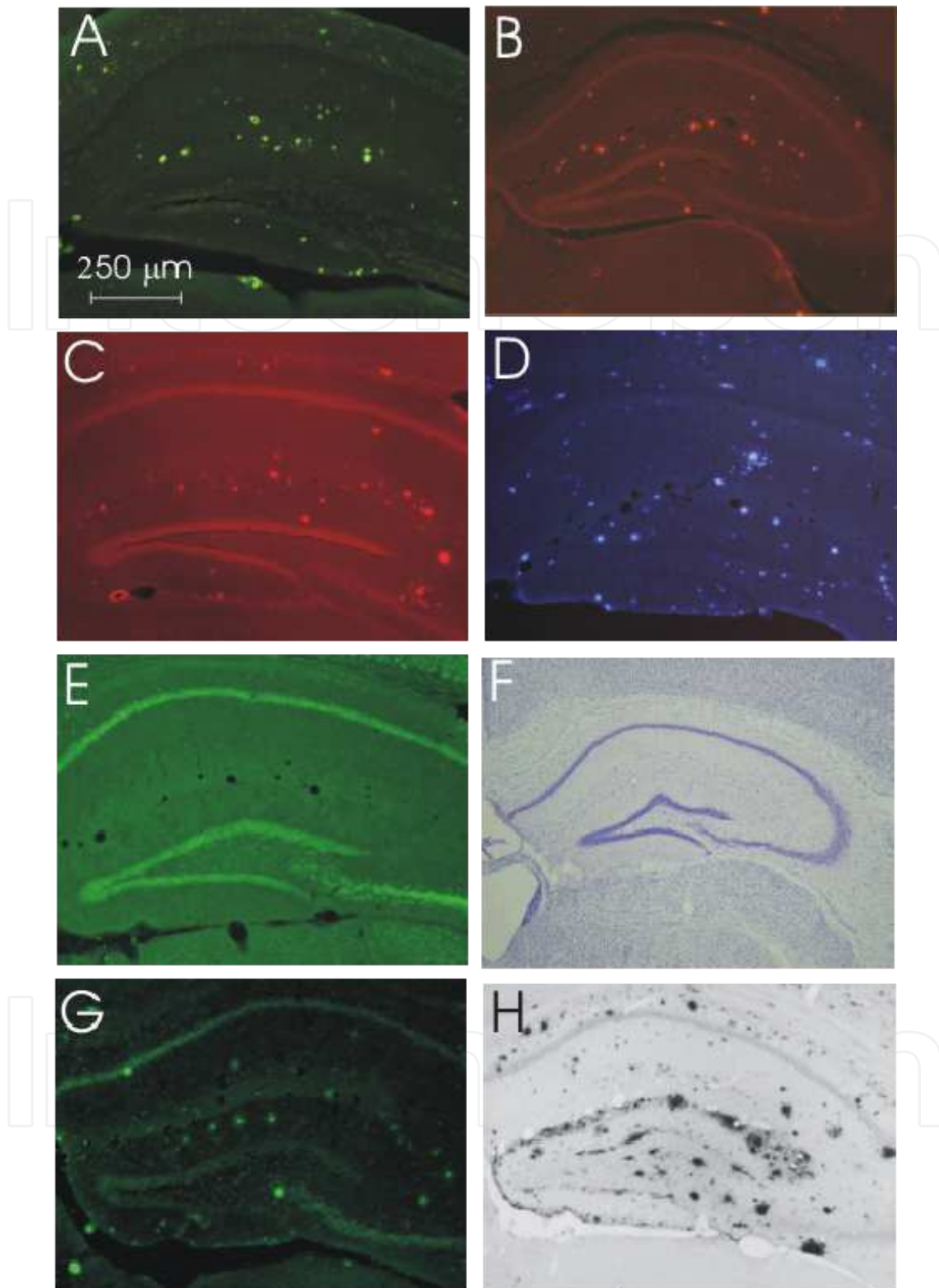


Fig. 1. Eight photomicrographs of coronal sections of the hippocampal formation of a Tg AD model mouse brain. **A**, section stained with thioflavine-S; **B**, section stained with Congo red; **C**, section stained with thiazine red; **D**, section stained with methoxy-X0; **E**, section stained with thioflavine-T; **F**, section stained with cresyl violet (Nissl stain), **G**, section stained with D3, and **H**, section stained for A β .

dye for these sections. They have the highest concentration that stains optimally in the shortest time. Longer time periods increased the background staining and did not improve staining quality (i.e., the signal/noise ratio; not illustrated).

The staining of sections of paraformaldehyde fixed brain sections of **AP/PS** mice with the three D-peptides revealed that all peptides (i.e., D1-D3) bound to all dense A β deposits, i.e., plaques (Figure 2). However, it should be noted that in the sections of the AP/PS mice that have large amounts of diffuse A β deposits, these deposits were not stained (Figure 1). Staining intensity of the A β deposits was directly related to both the D-peptide concentration that was tested (0.01, 0.001 and 0.0001 mg/ml), with the highest concentration staining optimally in the shortest time. At the highest concentration the optimal staining time was less than 5 min (i.e., with the best signal/noise ratio), whereas at the lowest

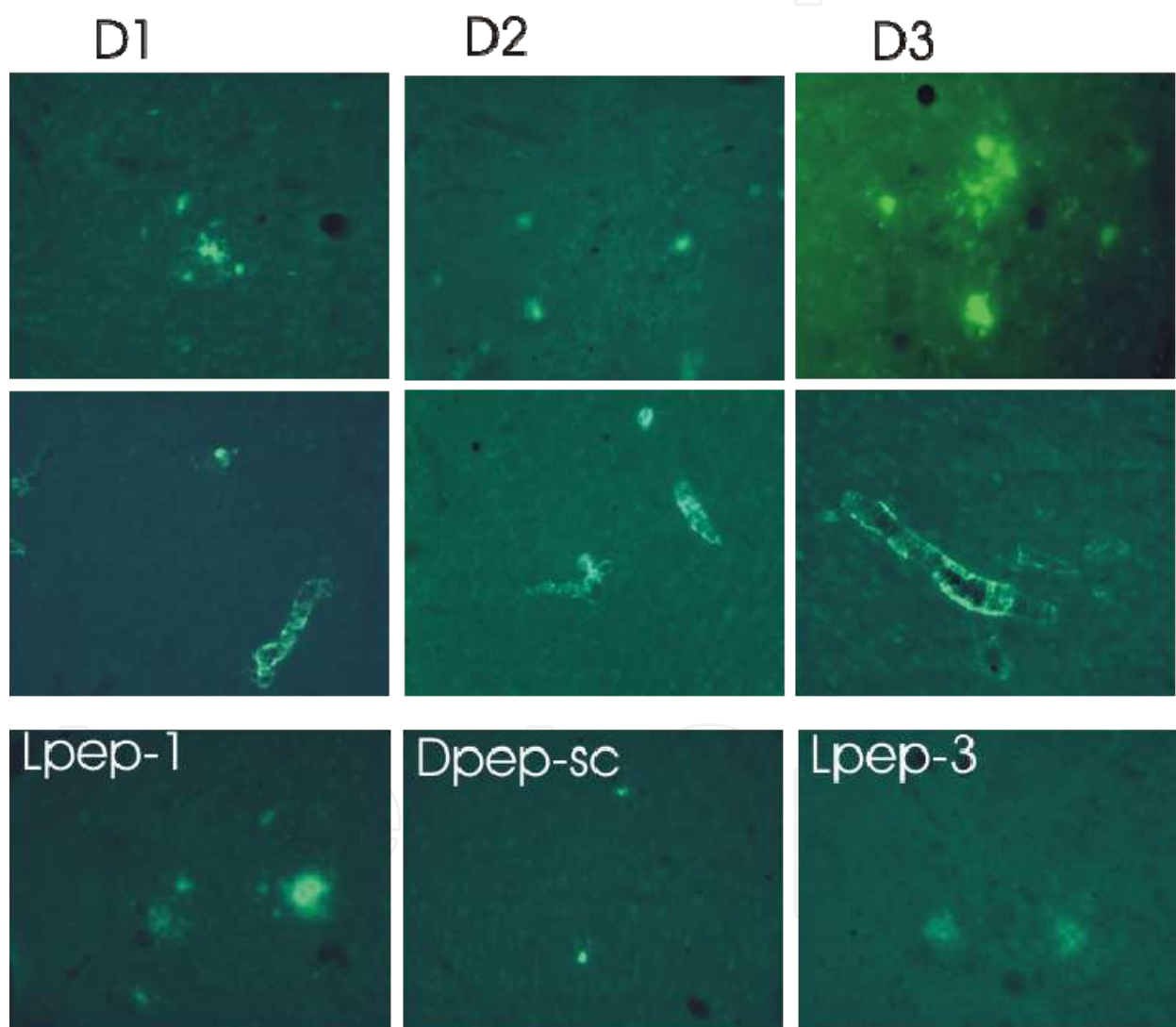


Fig. 2. Nine high power photomicrographs of adjacent coronal sections through the parietal cortex of an 18-month-old APP/PS1 mouse. The top six photomicrographs demonstrate the typical staining of plaques and blood vessels with respectively, D1, D2, and D3. The lower 3 photomicrographs show the typical staining of L-D1, sc-D1, and L3, respectively. Please note the lack of staining by the scrambled D-peptide (sc-D1), arrowhead indicates plaque core

concentration (0.0001 mg/ml) the time was more than 6 hours. Longer time periods increased the background staining and did not improve staining quality (i.e., the signal/noise ratio). It should be noted that with the lower concentrations, and appropriate longer staining time, the amount of non-specific staining (i.e., background) was significantly decreased. Similarly, post-staining rinsing of the stained sections in buffer decreased the amount of background staining, but even 24 h washing in buffer did not change the intensity of the specific binding.

Further, in general, the D3 peptide gave rise to slightly higher levels of specific staining than the D1 peptide. Further, very little A β was stained in the **AP** mouse brain sections, but the APP mutation in these mice is in the A β sequence and thus leads to A β proteins with a different amino acid sequence.

Comparison of the D-peptide stainings with the amyloid staining with A β 40 and A β 42 antibodies of sections of the **AP/PS** and **AP/PS Δ** mouse brains showed that there was nearly complete overlap between the location of A β 42 staining (i.e., plaques) and the D-peptide binding (Figure 3; van Groen et al, 2009). Similarly, comparison of the immunohistochemical staining of the adjacent sections for human amyloid β (with the W0-2 antibody which is specific for human A β ₄₋₁₀ sequence) showed that there was complete overlap between the location of dense A β staining (i.e., plaques) and the binding of the three D-peptides (D1-D3), but that the diffuse amyloid β deposits were not stained, neither in the hippocampus or in the cortex (Figure 1).

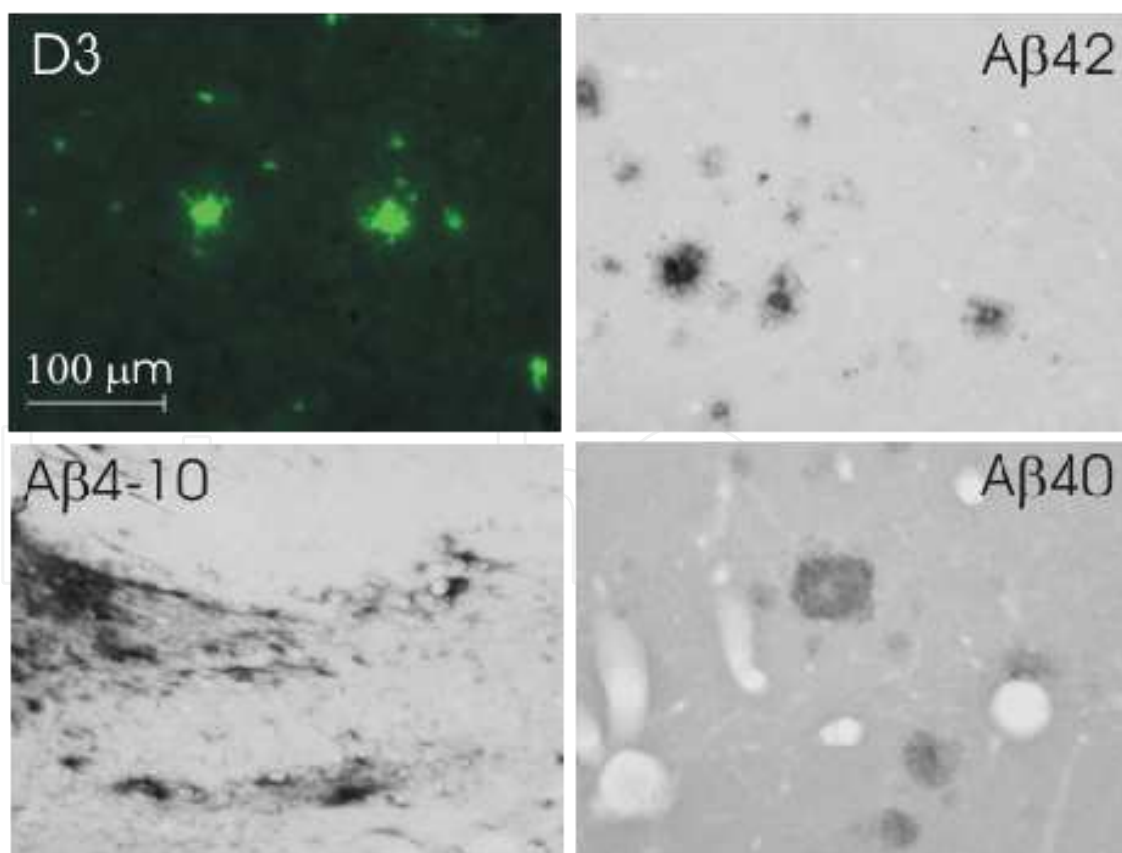


Fig. 3. Four high power photomicrographs of coronal sections through the hippocampus of a Tg AD model mouse. Sections were stained for D3, A β 40, A β 42, and A β , respectively. Please note the correspondence between D3 and A β 42 stained sections.

Immunohistochemical staining for A β 40 or A β 42 of the sections that were adjacent to the sections stained with the D-peptides showed that the distribution of the D-peptides corresponded more closely to the distribution of A β 42 than to the distribution of A β 40 labeling (Figure 3). Both A β 42 and D3 stain predominantly the core of the plaques, whereas A β 40 stains mainly the outside of the plaques, i.e., the rim (Figure 3). Sections that were double-stained for both A β 42 and the D-peptide demonstrated a total overlap between the site of dense A β 42 deposits and the location of D1 and D3 (not illustrated). Furthermore, staining with β -sheet markers such as thioflavine-S, Congo red, or thiazine red revealed that all A β deposits with a β -sheet positive core also stained with the D-peptides (Figure 1). The staining of fixed brain sections of Tg AD model mice (APP-PS) from different ages revealed that in old mice (over 18 months of age), when blood vessel walls contain some A β 42 deposits, they were stained by the D1 and D3 peptides (Figure 2), but not by the D2 peptide. It should be noted that at earlier ages only A β 40 is found in the blood vessel wall, and that at that age no labeling with D-peptides is present. Labeling of sections of non-transgenic littermates or control animals did not show any staining at any place in the brain.

To analyze further the binding characteristics of the D-peptides in more detail, an L-peptide version of the D1-peptide (i.e., L-D1) was also tested, likewise a scrambled (sequence) peptide of similar amino acid length (i.e., sc-D1) was tested (Table 1). We used both 0.001 and 0.0001 mg/ml concentrations of L-D1 and sc-D1 on fixed brain sections of old (18 months of age) **AP/PS** mice. L-D1 bound quite similarly to A β deposits compared to its D-peptide analog, i.e., it labeled dense A β deposits, but not diffuse deposits, and it lightly labeled blood vessel walls with showed A β deposits. It should be noted that similar amounts of the L-peptide showed less labeling compared to the D1 peptide. Finally, in contrast to both the L- and D-peptide, the sc-D1 peptide showed significantly reduced binding to A β at any type of amyloid deposit (Figure 3). Further, we tested a peptide that was generated against the L-form of A β 42, i.e., L3, this peptide showed very similar characteristics to the D3 peptide.

In these binding experiments all peptides that were used had been conjugated with a FITC molecule for visualization purposes, therefore we tested in a final set of experiments whether the D1 conjugated with different fluorophores would show distinct binding characteristics, i.e., study the interaction of the fluorescent moiety with the A β binding. The data show that no differences in specific binding are present at the 0.001 and 0.0001 mg/ml concentrations between these D-peptides (Figure 4). The D1 conjugated to Oregon green, which is similar in size and charge to FITC, bound A β 42 similar to the D1*FITC, but the D1*Bodipy (Bodipy is smaller and more polar than FITC) showed significantly increased background staining (Figure 4).

Peptide	Sequence	Description
D1	qshyrhispaqv	Dominating sequence selection 1, Target: D-A β
L-D1	QSHYRHISPAQV	L-enantiomer of D1
sc-D1	hsspqivhqayr	Scrambled D1
D2	giswqqshhlva	Dominating sequence selection 2, Target: D- A β
D3	rprtrlhthrn	Dominating sequence selection 3, Target: D- A β
L3	LRMMLQIKRIPR	Dominating sequence selection 3, Target: L- A β

Table 1. Showing the nomenclature and amino acid sequence of the peptides used in this study.

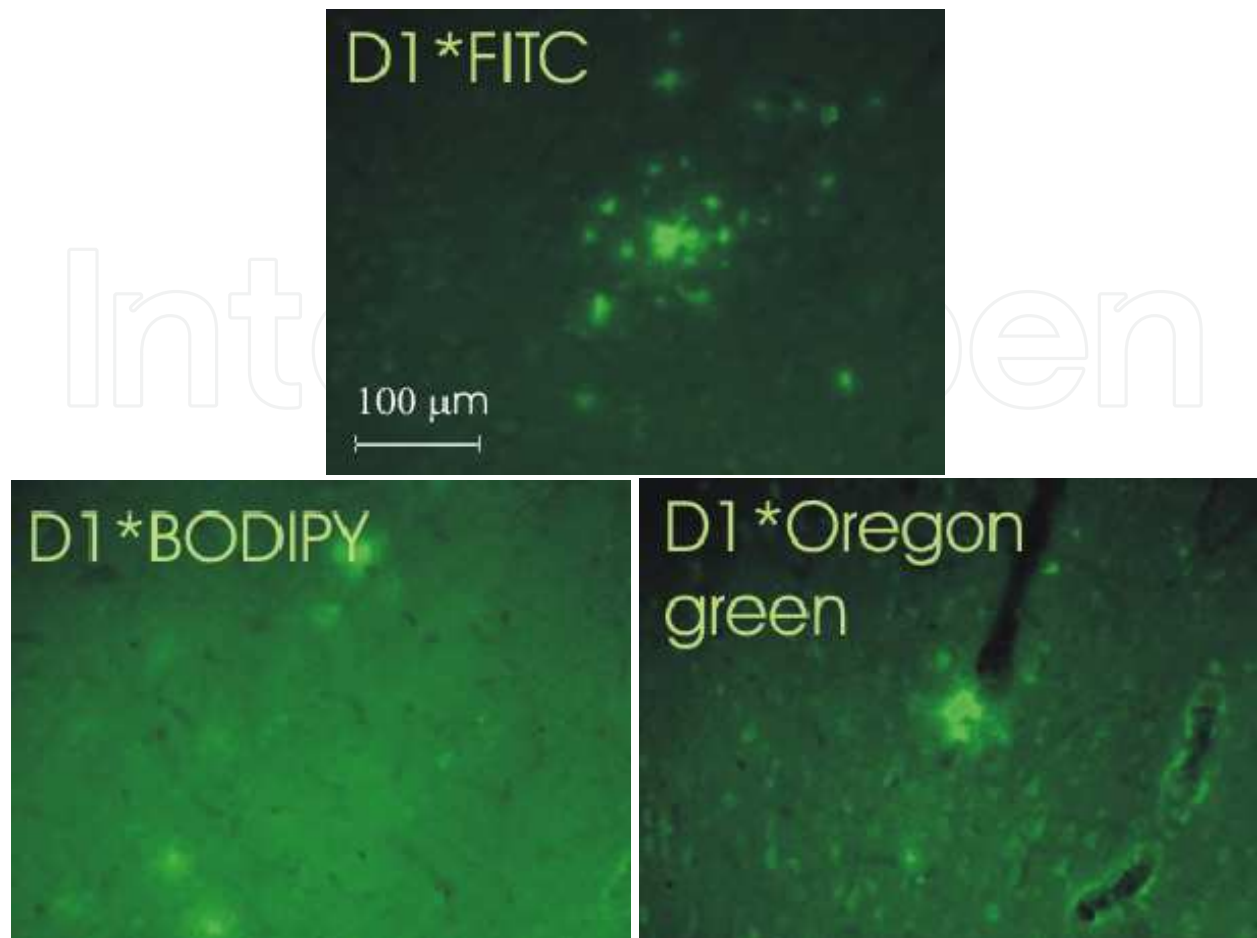


Fig. 4. Three photomicrographs of coronal sections of the parietal cortex of an 18-months-old APP/PS1 mouse stained with the D1 peptide conjugated with different fluorophores, showing staining with D1*FITC, D1*Bodipy, and D1*Oregon green, respectively. Please note the increased background staining with the D1*Bodipy, also note similarity of staining between D1*FITC and D1*Oregon green.

4. Discussion

In this study we compared the staining characteristics of three small D-amino acid peptides (i.e., D1, D2, and D3) that were designed to specifically bind A β 42 (D1, Wiesehan and Willbold, 2003; Wiesehan et al., 2003; D3,) with the two traditional histochemical methods for amyloid (thioflavine-S and Congo red) and two newer techniques. We examined the labeling of A β deposits in Tg AD model mouse brain, in the hippocampus, cortex and in blood vessel walls. The data demonstrate that all dense A β deposits (plaques) are labeled with the D-peptides, but not diffuse deposits. This corresponds to the distribution of the A β staining in the brain when it is labeled with A β 42 specific antibodies. Finally, the binding of the D-peptides corresponds closely to the localization of A β 42 in the brain, more closely than to the localization of A β 40.

Similarly, in brain tissue sections derived from AD patients, amyloid β plaques and leptomeningeal vessels containing A β are stained positively with the fluorescence-labeled derivative of D1 (Wiesehan et al, 2003). In contrast, fibrillar deposits derived from other amyloidosis are not labeled by D1 (Wiesehan et al, 2003). It should be noted that none of the D-

peptides showed any binding to A β deposits in the brains of mice which express the APP^{swe/dutch/iowa} mutation van Groen et al, 2009). This is to be expected since the structure of the A β peptide with these mutations (i.e., the Dutch and Iowa mutations) is predicted to be different from the "normal" A β peptide (Demeester et al., 2001; Kumar-Singh et al., 2002; Tsubuki et al., 2003; Watson et al., 1999). It should be noted that these mutations are in the A β peptide sequence of APP, in contrast to the Swedish mutation (Selkoe, 2001).

The data demonstrate that none of the three D-peptides binds to diffuse A β deposits, whereas they do bind to dense A β deposits, i.e., plaques. Earlier we have shown that the diffuse A β deposits do not stain with thioflavine S, Congo red or thiazine red, whereas the core of plaques does. Furthermore, the diffuse deposits consist primarily of N-terminal fragments of A β , they contain some A β 40 but do not contain stainable amounts of A β 42 (Van Groen et al., 2003), in contrast to plaques that consist of significant amounts of both A β 40 and A β 42. We have suggested earlier that the diffuse deposits consist of A β that has a different length (and structure) from the A β 42 and A β 40 that is present in plaques, even if A β fibrils are present in the diffuse deposits (Van Groen et al., 2003). Together these data indicate that the D-peptides bind very specifically to only A β 42.

Furthermore, it has been shown that A β 42 is actively taken up by astrocytes and microglia (Nagele et al., 2003; Rogers and Lue, 2001). In contrast, surprisingly, no D-peptides are visible in astrocytes and microglia, the phagocytosing cells in the brain (Rogers et al, 2002). Activated microglial cells are present in the brains of AD model mice but these cells never show any presence of intracellular A β (e.g., Stalder et al., 2003; but see Paresce et al., 1996).

We have used these peptides to treat AD model mice and we have shown that a brain infusion with D3 significantly reduces pathology and cognitive deficits in AD model mice (van Groen et al, 2009, Funke et al, 2011). In contrast D1 infusion does not improve cognition (van Groen et al, 2009). Similarly it has been demonstrated that Congo red (Inouye and Kirschner, 2005, Lee, 2002) and thioflavine-S improve pathology (Alavez et al, 2011).

Together, we have demonstrated that 1) D-peptides that specifically bind to A β 42 and, 2) that the D-peptides staining is similar, but more specific, to most traditional histochemical amyloid staining methods. Thus, our data strongly suggest that these novel and highly specific Abeta42 ligands have potential application(s) in the diagnosis and therapy of Alzheimer's disease (Masters and Beyreuther, 2006; Monaco et al., 2006), especially since these D-peptides are much more resistant to proteolysis than natural L-peptides.

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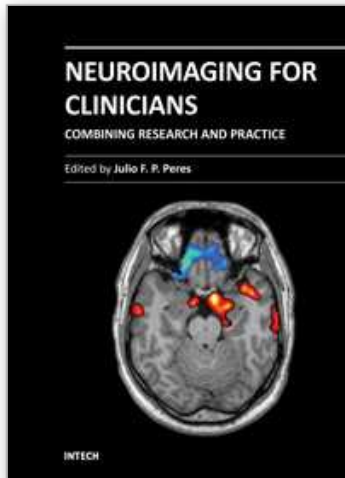
6. References

- Alavez S, Vantipalli MC, Zucker DJ, Klang IM, Lithgow GJ. Amyloid-binding compounds maintain protein homeostasis during ageing and extend lifespan. *Nature*. 2011 472:226-229.
- Benkirane N, Friede M, Guichard G, Briand JP, Van Regenmortel MH, Muller S, Antigenicity and immunogenicity of modified synthetic peptides containing D-amino acid residues. *J Biol Chem.*, 1993 268:26279-26285.

- Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada C-M, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M, Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS, Familial Alzheimer's disease-linked presenilin 1 variants elevate A β 1-42/1-40 ratio in vitro and in vivo. *Neuron*, 1996 17:1005-1013.
- Braak H, Braak E, Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991 82:239-259.
- Braak H, Braak E, Evolution of neuronal changes in the course of Alzheimer's disease. *J. Neural. Transm.*, 1998 53:127-40.
- D'Andrea MR, Nagele RG, Wang HY, Lee DH. Consistent immunohistochemical detection of intracellular beta-amyloid42 in pyramidal neurons of Alzheimer's disease entorhinal cortex. *Neurosci Lett.*, 2002 333:163-166.
- Demeester N, Mertens C, Caster H, Goethals M, Vandekerckhove J, Rosseneu M, Labeur C. Comparison of the aggregation properties, secondary structure and apoptotic effects of wild-type, Flemish and Dutch N-terminally truncated amyloid beta peptides. *Eur J Neurosci.*, 2001 13:2015-2024 .
- Dickson TC, Vickers JC, The morphological phenotype of beta-amyloid plaques and associated neuritic changes in Alzheimer's disease. *Neuroscience* 2001 105:99-107.
- Duff K, Normal and abnormal tau neurobiology. *Alzheimer Dis Assoc Disord.*, 2006 20:202-205.
- Duff K, Suleman F, Transgenic mouse models of Alzheimer's disease: how useful have they been for therapeutic development? *Brief Funct Genomic Proteomic.* 2004 3:47-59.
- Funke SA, van Groen T, Kadish I, Bartnik D, Nagel-Steger L, Brener O, Sehl T, Batra-Safferling R, Moriscot C, Schoehn G, Horn AHC, Muller-Schiffmann A, Korth C, Sticht H, Willbold D. Oral treatment with the D-enantiomeric peptide D3 improves the pathology and behavior of Alzheimer's disease transgenic mice. *ACS Chemical Neuroscience* 2010 1:639-648.
- Glenner GG. The bases of the staining of amyloid fibers: their physico-chemical nature and the mechanism of their dye-substrate interaction. *Prog Histochem Cytochem.* 1981 13:1-37.
- Guntern R, Bouras C, Hof PR, Vallet PG. An improved thioflavine S method for staining neurofibrillary tangles and senile plaques in Alzheimer's disease. *Experientia.* 1992 48:8-10.
- Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol.* 2003 60:1119-1122.
- Ida N, Hartmann T, Pantel J, Schroder J, Zerfass R, Forstl H, Sandbrink R, Masters CL, Beyreuther K, Analysis of heterogeneous A4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. *J. Biol. Chem.*, 1996;271:22908-14.
- Inouye H, Kirschner DA. Alzheimer's beta-amyloid: insights into fibril formation and structure from Congo red binding. *Subcell Biochem.* 2005 38:203-224.
- Jankowsky JL, Slunt HH, Ratovitski T, Jenkins NA, Copeland NG, Borchelt DR, Co-expression of multiple transgenes in mouse CNS: a comparison of strategies. *Biomol Eng.*, 2001;17:157-65.
- Kadish I, Van Groen T, Low levels of estrogen significantly diminish axonal sprouting after entorhinal cortex lesions in the mouse. *J Neurosci.*, 2002;22:4095-102.
- Klunk WE, Bacsikai BJ, Mathis CA, Kajdasz ST, McLellan ME, Frosch MP, Debnath ML, Holt DP, Wang Y, Hyman BT, Imaging Abeta plaques in living transgenic mice with

- multiphoton microscopy and methoxy-X04, a systemically administered Congo red derivative. *J Neuropathol Exp Neurol.*, 2002 61:797-805.
- Kumar-Singh S, Julliams A, Nuydens R, Ceuterick C, Labeur C, Serneels S, Vennekens K, Van Osta P, Geerts H, De Strooper B, Van Broeckhoven C. In vitro studies of Flemish, Dutch, and wild-type beta-amyloid provide evidence for two-staged neurotoxicity. *Neurobiol Dis.*, 2002;11:330-40.
- Lazarov O, Lee M, Peterson DA, Sisodia SS, Evidence that synaptically released beta-amyloid accumulates as extracellular deposits in the hippocampus of transgenic mice. *J Neurosci.*, 2002;22:9785-93.
- Lazarov O, Morfini GA, Lee EB, Farah MH, Szodorai A, DeBoer SR, Koliatsos VE, Kins S, Lee VM, Wong PC, Price DL, Brady ST, Sisodia SS, Axonal transport, amyloid precursor protein, kinesin-1, and the processing apparatus: revisited. *J Neurosci.* 2005 25:2386-2395.
- Lee VM. Amyloid binding ligands as Alzheimer's disease therapies. *Neurobiol Aging.* 2002 23:1039-1042.
- Masters CL, Beyreuther K, Alzheimer's centennial legacy: prospects for rational therapeutic intervention targeting the A β amyloid pathway. *Brain* 2006;29:2823-39.
- McGowan E, Eriksen J, Hutton M, A decade of modeling Alzheimer's disease in transgenic mice. *Trends Genet.* 2006 22:281-9.
- Monaco S, Zanusso G, Mazzucco S, Rizzuto N, Cerebral amyloidoses: molecular pathways and therapeutic challenges. *Curr Med Chem.* 2006 13:1903-1913.
- Morimatsu M, Hirai S, Muramatsu A, Yoshikawa M. Senile degenerative brain lesions and dementia. *J Am Geriatr Soc.* 1975 23:390-406.
- Nagele RG, D'Andrea MR, Anderson WJ, Wang HY, Intracellular accumulation of beta-amyloid(1-42) in neurons is facilitated by the alpha 7 nicotinic acetylcholine receptor in Alzheimer's disease. *Neuroscience.* 2002 110:199-211.
- Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang HY, Astrocytes accumulate A beta 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. *Brain Res.* 2003 971:197-209.
- Paresce DM, Ghosh RN, Maxfield FR, Microglial cells internalize aggregates of the Alzheimer's disease amyloid beta-protein via a scavenger receptor. *Neuron.* 1996 17:553-565.
- Radde R, Bolmont T, Kaeser SA, Coomaraswamy J, Lindau D, Stoltze L, Calhoun ME, Jaggi F, Wolburg H, Gengler S, Haass C, Ghetti B, Czech C, Holscher C, Mathews PM, Jucker M, Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep.* 2006 7:940-946.
- Rogers J, Lue LF, Microglial chemotaxis, activation, and phagocytosis of amyloid beta-peptide as linked phenomena in Alzheimer's disease. *Neurochem. Int.* 2001 39:333-340.
- Rogers J, Strohmeyer R, Kovelowski CJ, Li R, Microglia and inflammatory mechanisms in the clearance of amyloid beta peptide. *Glia.* 2002 40:260-269.
- Satpute-Krishnan P, DeGiorgis JA, Conley MP, Jang M, Bearer EL, A peptide zipcode sufficient for anterograde transport within amyloid precursor protein. *Proc Natl Acad Sci U S A.* 2006 103:16532-7.
- Selkoe DJ, Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* 2001 81:741-66.
- Sheng JG, Price DL, Koliatsos VE, Disruption of corticocortical connections ameliorates amyloid burden in terminal fields in a transgenic model of Abeta amyloidosis. *J Neurosci.* 2002 22:9794-9.

- Stalder M, Deller T, Staufenbiel M, Jucker M, 3D-Reconstruction of microglia and amyloid in APP23 transgenic mice: no evidence of intracellular amyloid. *Neurobiol. Aging*. 2001 22:427-434.
- Styren SD, Hamilton RL, Styren GC, Klunk WE. X-34, a fluorescent derivative of Congo red: a novel histochemical stain for Alzheimer's disease pathology. *J Histochem Cytochem*. 2000 48:1223-1232.
- Tsubuki S, Takaki Y, Saido TC, Dutch, Flemish, Italian, and Arctic mutations of APP and resistance of Abeta to physiologically relevant proteolytic degradation. *Lancet*. 2003 361:1957-1958.
- Uchihara T, Nakamura A, Yamazaki M, Mori O. Tau-positive neurons in corticobasal degeneration and Alzheimer's disease--distinction by thiazin red and silver impregnations. *Acta Neuropathol*. 2000 100:385-389.
- Vallet PG, Guntern R, Hof PR, Golaz J, Delacourte A, Robakis NK, Bouras C. A comparative study of histological and immunohistochemical methods for neurofibrillary tangles and senile plaques in Alzheimer's disease. *Acta Neuropathol*. 1992 83:170-178.
- van Groen T, Liu L, Ikonen S, Kadish I, Diffuse amyloid deposition, but not plaque number, is reduced in amyloid precursor protein/presenilin 1 double-transgenic mice by pathway lesions. *Neuroscience*. 2003 119:1185-97.
- van Groen T, Kiliaan AJ, Kadish I, Deposition of mouse amyloid beta in human APP/PS1 double and single AD model transgenic mice. *Neurobiol Dis*. 2006 23:653-62.
- van Groen T, Wiesehan K, Funke SA, Kadish I, Nagel-Steger L, Willbold D. Reduction of Alzheimer's disease amyloid plaque load in transgenic mice by D3, A D-enantiomeric peptide identified by mirror image phage display. *ChemMedChem*. 2008 3:1848-1852.
- van Groen T, Kadish I, Wiesehan K, Funke SA, Willbold D. In vitro and in vivo staining characteristics of small, fluorescent, Abeta42-binding D-enantiomeric peptides in transgenic AD mouse models. *ChemMedChem*. 2009 4:276-282.
- Van Nostrand WE, Melchor JP, Romanov G, Zeigler K, Davis J, Pathogenic effects of cerebral amyloid angiopathy mutations in the amyloid beta-protein precursor. *Ann N Y Acad Sci*. 2002 977:258-265.
- Van Regenmortel MH, Muller S, D-peptides as immunogens and diagnostic reagents. *Curr Opin Biotechnol*. 1998 9:377-382.
- Vetrivel KS, Thinakaran G, Amyloidogenic processing of beta-amyloid precursor protein in intracellular compartments. *Neurology*. 2006 66(Suppl 1):S69-73.
- Wang J, Tanila H, Puolivali J, Kadish I, van Groen T, Gender differences in the amount and deposition of amyloidbeta in APPswe and PS1 double transgenic mice. *Neurobiol Dis*. 2003 14:318-327.
- Watson DJ, Selkoe DJ, Teplow DB, Effects of the amyloid precursor protein Glu693-->Gln 'Dutch' mutation on the production and stability of amyloid beta-protein. *Biochem J*. 1999 340:703-709.
- Wiesehan K, Buder K, Linke RP, Patt S, Stoldt M, Unger E, Schmitt B, Bucci E, Willbold D, Selection of D-amino-acid peptides that bind to Alzheimer's disease amyloid peptide abeta1-42 by mirror image phage display *Chembiochem*. 2003 4:748-53.
- Wiesehan K, Willbold D, Mirror-image phage display: aiming at the mirror. *Chembiochem*. 2003 4:811-5.
- Wiesehan K, Stöhr J, Nagel-Steger L, van Groen T, Riesner D, Willbold D. Inhibition of cytotoxicity and amyloid fibril formation by a D-amino acid peptide that specifically binds to Alzheimer's disease amyloid peptide. *Protein Eng Des Sel*. 2008 21:241-246.



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Neuroimaging for clinicians sourced 19 chapters from some of the world's top brain-imaging researchers and clinicians to provide a timely review of the state of the art in neuroimaging, covering radiology, neurology, psychiatry, psychology, and geriatrics. Contributors from China, Brazil, France, Germany, Italy, Japan, Macedonia, Poland, Spain, South Africa, and the United States of America have collaborated enthusiastically and efficiently to create this reader-friendly but comprehensive work covering the diagnosis, pathophysiology, and effective treatment of several common health conditions, with many explanatory figures, tables and boxes to enhance legibility and make the book clinically useful. Countless hours have gone into writing these chapters, and our profound appreciation is in order for their consistent advice on the use of neuroimaging in diagnostic work-ups for conditions such as acute stroke, cell biology, ciliopathies, cognitive integration, dementia and other amnesic disorders, Post-Traumatic Stress Disorder, and many more

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