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AMYLOID PLAQUE (AND NOT DIFFUSE AMYLOID) IS A CONDITION FOR NEURONAL DYSFUNCTION

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There is no direct evidence that brain amyloid affects neuronal function. Here we studied hippocampal slices from nonmutated human amyloid precursor protein transgenic- and age-matched non-transgenic mice. We aimed to differentiate separate actions of the aged transgenic mice plaque-like amyloid and diffuse amyloid of the non-transgenic mice (verified by immunohistochemistry and Congo Red fluorescence) on synaptic plasticity. Extracellular recording of CA1 field EPSP in vitro revealed impairment of input/output characteristics, long-term potentiation, and the delay of few milliseconds in initial posttetanic traces in transgenic versus control mice. Our results indicate that amyloid plaque (and not diffuse amyloid) may cause synaptic dysfunction, and suggest importance factors other then amyloid in pre-plaque stages of Alzheimer's disease and in Down syndrome.

INTRODUCTION

Diffuse amyloid deposits and neuritic plaques of Alzheimer's disease patients are considered to be essential disease features.1 For this reason the prevention of amyloid formation from its precursor (APP) and inhibition of amyloid fibrillogenesis have been proposed as an important therapeutic targets for the disease cure.²⁻⁴ Nevertheless, there is no direct evidence that amyloid β (A β) has direct effect on neuronal dysfunction. An attempt to unravel this important issue was made in a report on transgenic mice expressing human amyloid precursor protein (APP₆₉₅) bearing the swedish mutation.5 These transgenic mice developed "elevated concentrations of $A\beta$ and significant amyloid deposits," and had impaired spatial and hippocampal long learning term potentiation (LTP), a long-lasting synaptic enhancement, the leading experimental model for the synaptic changes that underlie learning and memory.^{6WEB+} However, cited report⁵ as well as another earlier work on LTP deficit in transgenic mice overexpressing the carboxyterminal 104 aminoacids of APP,7 did not "determine whether the effects measured resulted from elevated concentrations of soluble A β , deposited A β or both".

Todate, it is not established whether the maturation of brain amyloid deposits, particularly the development of Congo Red positive neuritic plaques, is an essential event leading to neuronal dysfunction. Recent study by Naslund et al.⁸ attempted to correlate the amyloid load with the cognitive decline and the severity of dementia in Alzheimer disease patients. However, the latter report estimated

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55% and 40% of the study subjects without detectable plaques as the ones having readily detectable levels of " $A\beta(x\text{-}42)$ and $A\beta(x\text{-}40)$ ". In addition, recent reports have suggested the possible importance of factors other then brain amyloid in Alzheimer's neuronal abnormality $stress^{9WEB+}$ (oxidative or lipid metabolism^{10WEB+,11} disbalance, for example) and behavioral disturbances without amyloid deposits in mice overexpressing human APP with Flemish and Dutch mutations,12 keeping open the question on the role of neuritic plaques in the genesis of Alzheimer's neuronal dysfunction.

In our study, we utilized hippocampal slices of 16.5 and 25.5 month old transgenic mice expressing nonmutated human APP₆₉₅,¹³ and age-matched non-transgenic wild type control mice. We aimed to differentiate the separate actions of diffuse and plaque amyloid on synaptic plasticity hippocampal using immunohistochemical analysis, Congo Red staining, amyloid extraction and extracellular recording of CA1 field excitatory postsynaptic potentials (fEPSPs). The results indicate that amyloid plaque (and not diffuse amyloid) may represent one of the possible causes of neuronal dysfunction and synaptic plasticity failure.

EXPERIMENTAL PROCEDURES

Animal care and tissue collection

Transgenic and wild type mice of 16.5 and 25.5 months age groups (n=6) were maintained on the standard diet at the animal facility of the Weizmann Institute of Science, Rehovot, Israel. All experimental procedures were in accord with National Institutes of Health guide for the use of laboratory animals. Two or three hippocampal slices from each mouse were subjected to both electrophysiology and to immunohistochemistry.

Additionally, two transgenic and two wild type mice at the age of 16.5 months were subjected to transcardial perfusion, followed by thin sectioning of the brain and immunohistochemical analysis. In selected experiments, the hippocampal slices of 16.5 month old transgenic and wild type mice were subjected to the biochemical analysis of $A\beta$.

Ex-vivo hippocampal slices

Hippocampal slices were prepared and electrophysiological analysis was performed previously.10,14 essentially as described we Briefly, after mice decapitation the hippocampus was rapidly removed and placed into cold (2ºC) artificial cerebrospinal fluid (ACSF, pH 7.4 containing (in mM): 124 NaCl, 2.0 KCl, 1.24 KH₂PO₄, 2.0 MgSO₄, 2.5 CaCl₂, 26 NaHCO₃ and 10 D-glucose) saturated with 95 % O₂ / 5% CO₂ (flow rate 0.4 l/min) and adjusted with sucrose (7g per 600 ml of ACSF) to 320 mOsm osmolarity. The hippocampal slices (400 μ) were prepared with a McIlwain tissue slicer, USA. The slices were incubated in a recreation chamber at room temperature (25°C) for 1.5 h in ACSF.

Electrophysiology

The slices were transferred to a recording chamber (held at constant temperature of 32°C) and submerged slices were superfused with ACSF at a flow rate of ~1.5 ml/min. Extracellular electrodes (~4 M Ω , 0.75 mM NaCl) were guided by micromanipulator into stratum radiatum of CA1 (200 µ deep) under binocular. Bipolar A.R.K fabricated tungsten (50 μ wire size) stimulating electrode was also placed into CA1 stratum radiatum. The stimulations were delivered every 30 s at 50 us pulse duration yielding fEPSP waveforms. After stable baseline responses were established and input/output (I/O) curve values recorded, tetanus induced LTP was induced by delivering a 100 Hz, 1 sec stimuli train through the stimulation electrode at the baseline test stimulus intensity. For long term depression (LTD) study slices were stimulated every half a second (2 Hz) for 5 min at the test stimulus intensity.

Data were collected, stored and analyzed on a PC using Asyst 3.1 and GraphPad Prizm 2.0 data acquisition and analysis software. The I/O relationship, LTP and LTD were expressed as a fEPSP amplitude and slope change versus stimulus intensity and time, respectively. Data were normalized with respect to the steady baseline values and expressed as mean ± SEM. Non-parametric unpaired Mann-Whitney test was used for determining significant differences between potentiation/depression levels of trangenic and wild type slices at the indicated time points. A probability of 0.05 (one tailed) or less was accepted as statistically significant.

Immunohistochemistry and Congo Red staining for amyloid

The hippocampal slices from 25.5 month old mice used for synaptic plasticity study, were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS), pH=7.4 for 72 hrs.^{10,14} We also used hippocampal sections from 16.5 month old mice killed by transcardial perfusion with PBS and 4% paraformaldehyde in PBS.^{11,14} For immunohistochemistry, 400 μ -thick fixed slices were cutted with a microtome to a 40 μ sections. Free floating sections were washed in PBS (2x) and then incubated with 3% H₂O₂ (prepared on PBS, containing 10%

methanol) for 20 min to remove endogenous peroxidase activity. Sections were then washed (4x 10 min each) with PBS and blocked for 24 hrs at 4°C in 10% fetal calf serum and 1% glycine in PBS (blocking solution), followed by 14 hrs incubation with 4G8 or 6E10 (1:1000, Senetek, PLC.) monoclonal anti-A β antibodies in the blocking solution at 4°C. Tissue sections were washed (5x 40 min each) in blocking solution to remove unreacted primary antibodies. Secondary biotinylated goat antimouse antibody (1:750) were added for 1.5 h at 25°C, followed by washing with the blocking buffer (3x 40 min each). After washing, ABC solution (Vector Elite kit, 1:300 of reagents A and B in blocking solution) was added for 40 min, followed by section washing in blocking solution and then in PBS (2x 10 min each). For visualization, immunostained sections were 3',3-diaminobenzidine reacted with tetrahydrochloride (Sigma Tablet kit, USA) and washed in PBS.

For Congo Red staining thin sections were stained with 0.5% Congo Red in 50% alcohol for 30 min, followed by one minute treatment with 0.2% KOH in 80% alcohol and water. Sections were mounted on gelatinized slides, air dried, dehydrated in serially diluted ethanol (50, 70, 90, 95 and 100%), cleared with Xylenes, and coverslipped with cover glass and Permount.

To control the specificity of 4G8 and 6E10 immunostaining, antibody solutions were preadsorbed with the access of synthetic peptide $A\beta$ 1-40 (1 mg/0.5 ml) prior to the incubation with the sections.

Fluorescence of plaque-like amyloid labeled by Congo Red was obtained with 488 nm of excitation using a confocal microscope LSM 510 (Zeiss, Germany) equipped with an argon laser.

Amyloid extraction

Hippocampal slices were homogenized with the cold PBS (500 µl per 20 mg of tissue) containing protease inhibitors,15 and subjected to centrifugation in a Beckman TiL 100.2 rotor at 100,000 g for 3 hrs at 4°C. The supernatant fraction and the pellet were dialyzed against with 1,000 Da cut-off membrane water (Spectrum, USA), lyophilized and subjected to A β extraction with 20% and then with 80% acetonitrile in 0.1% trifluoroacetic acid.¹⁶ Both acetonitrile soluble fractions were combined, lyophilized and subjected to 13 % TRIS-Tricine SDS/PAGE^{17,18} and immunoblot analysis on Immobilon P membranes (Wattman, USA) with 6E10 and 4G8 anti-A β monoclonal antibodies and with the monoclonal antibody against APP

(Zymed, USA), followed by ECL (Amersham) essentially as previously reported.^{15,18}

RESULTS AND DISCUSSION

A. IMMUNOHISTOCHEMICAL ANALYSIS: AMYLOID AS ALZHEIMER'S HALLMARK

Immunohistochemistry of slices from aged animals (25.5 months) with 4G8 and 6E10 antibodies (anti-human/mouse-A β and antihuman $-A\beta$, respectively, see antibody specificity scheme, Figure 1G) revealed extracellular (staining with no triton) hippocampal immunoreactivity of mouse $A\beta$ in transgenic mice (Fig. 1A) and in wild types (Fig. 1B), and verified extracellular deposits of human $A\beta$ in the transgenic mice hippocampus (Fig. 1C). Essentially identical results were obtained under the condition of membrane permeabilization with 0.1% Triton X-100 in the blocking buffer (not shown). The specificity of immunostaining was confirmed by the preadsorption of antibodies with the access of synthetic peptide A β (1-40).

Staining for amyloid showed Congo Red fluorescence specifically in aged transgenic mice (and not in aged wild type mice) hippocampal sections (Fig. 1E). Congo red stains specifically amyloid plaques (but not diffuse amyloid) due to the binding to the β -pleated sheet secondary structure of A β protein in amyloid fibrils.^{1,2} The latter observation indicates that expression of non-mutated human A β in aged transgenic mice leads to a mature Alzheimer's plaque-like amyloid and that A β deposits in wild type mice have a nonmature diffuse nature.

In contrast to the 25.5 month aged animals, the 16.5 month old transgenic and wild type mice expressed neither human nor mouse $A\beta$ immunoreactivity (not shown). To confirm this observation we analyzed 16.5 month old transgenic and wild type mice for soluble and aggregated $A\beta$ by immunoblot analysis of the acetonitrile extracts of ultracentrifugation supernatantand pellet-fractions of hippocampal homogenates. We did not recover human transgenic (Fig. 1H) or mouse (not shown) $A\beta$ immunoreactivity in the transgenic and wild type hippocampus. However, antihuman A β antibody 6E10 labeled a protein of high molecular weight (in the range of 96 to 200 kDa) in the trangenic mice. This protein band was also stained with the antibody against APP aminoterminus (not shown) confirming human APP expression in the transgenic hippocampus.



Figure 1 (above). Immunochemical analysis of APP transgenic mice. (A-D), Comparison of extracellular $A\beta$ immunoreactivity in aged (25.5 months) human non-mutated APP695 transgenic mice and age-matched non-transgenic wild type control mice hippocampus. Immunohistochemistry was performed with no triton and 4G8 and 6E10 monoclonal antibodies (1:1000). The presented fields are CA1 areas of ex-vivo slices from the batches used for extracellular recording; Bar, 20 μ . Similar A β pattern was observed in the dentate gyrus (not shown). Alzheimer's-like plaque amyloid in transgenic mice (E), versus wild type mice (F), was visualized by confocal fluorescent microscopy of Congo Red stained sections; Bar, 100 μ . (H) 16.5 month old wild type (control, lanes 1 and 2) and transgenic mice (lanes 3 and 4) were analyzed for the soluble and/or aggregated human A β in the acetonitrile extracts of the supernatant (lanes 1 and 3) and pellet (lanes 2 and 4) fractions of the hippocampal homogenates, respectively, by immunoblot analysis with 6E10 and 4G8 (not shown) monoclonal antibody. While there were no detectable levels of A β present transgenic hippocampus, we recovered 6E10-positive high molecular weight human APP in immunoreactivity in the supernatant fraction (lane 3), and confirmed it by immunostaining with the antibody against APP aminoterminus. Lane 5, 5 ng of synthetic β -amyloid 1-40 (positive control). Molecular weight markers (in kDa) are shown on the left. Scheme (G) represents human and rodent A β 1-40 amino acid sequence differences and the sequence specificity of anti-A β monoclonal antibodies used in this study.



Fig 2 (left). Electrophysiological analysis of aged (25.5 months) APP transgenic mice. (A) Input/output (I/O) relationship in APP transgenic (squares) and wild type non-transgenic (WT, triangles) hippocampal slices. **(B)** Field synaptic responses obtained at the baseline (1) and high stimulus intensity (2)recordings well as as immediately after (3) and 3 (4) and 13 min after (5) the highfrequency train of stimuli. (C) Impairment of tetanic LTP in CA1 of ex-vivo slices from APP WТ transgenic versus hippocampus. In all cases n=9 and n = 10for APP slices WT transgenic and mice. respectively. indicates Arrow time of tetanus.

Clinical Medicine and Health Research, December, 2001, clinmed/2001110002v1



Figure 3 (above). Electrophysiological analysis of 16.5 month old APP transgenic mice. APP transgenic and wild type non-transgenic (WT) mice expressed similar tetanus (arrow) induced LTP (A) and were different in the amount of the long term depression (B). However, depression values probed 10 min after the low frequency stimulation sequence did not reach statistical significance (98.95 \pm 10.34%, n=7, and 79.3 \pm 12.8%, n=6 in the APP-TG and WT, respectively, p=0.0625, one-tailed).

Figure 4 (below). (A) Schematic matching of the positioning of the recording (rec) and stimulating (stim) electrodes for the employed in the study extracellular recording in the CA1 and the Congo Red fluorescence, observed in the APP transgenic hippocampal slice. (B) Individual fEPSPs recorded after the high frequency (100 Hz/1 s) train of stimuli revealed 1.5-2.0 msec delay in the onset of the evoked synaptic responses in the APP transgenic (arrow) compared to the wild type non-transgenic slices.



"Alzheimer's changes in neurochemistry of A β , tau, neuronal cytoskeleton, and oxidative stress reactions likely represent physiological transitory mechanisms aiming to compensate impaired brain cholesterol dynamics and/or associated neurotransmission and synaptic plasticity failure". For details log on to http://clinmed. netprints.org/cgi/content/full/2001100005v1 (Ref. 11) Our next article will experimentally address one of the above issues. Stay with us.

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B. ELECTROPHYSIOLOGICAL ANALYSIS: PLAQUE AMYLOID AND SYNAPTIC PLASTICITY

Electrophysiological analysis revealed that 25.5 month old transgenic mice had lower inputstimulus/output-response (I/O) characteristics (Figure 2A). It is generally accepted that in the CA1 area of the hippocampus EPSP consists of two major elements that depend on the activation of N-methyl-D-aspartate (NMDA) and non-NMDA (mainly AMPA) subtypes of ionotropic glutamate receptors. $^{19WEB\scriptscriptstyle+}$ The 50 μs stimulation pulse duration (employed here to evoke fEPSP) generates significant AMPA responses and it is thus possible that this particular component of the EPSP is responsible for lower I/O characteristics of aged transgenic mice.19WEB+

Aged transgenic mice were impaired in both the amount of initial post-tetanic potentiation (137.2±9.3%, n=9, against 201.8±20.8% of the wild type, n=10, p=0.014) and in the maintenance of the LTP, sampled 4 min $(99.53\pm9.78\%, n=9, and 148.6\pm15.01\%, n=10,$ p=0.0318) and 10 min (92.64±7.02%, n=9, and 133.2±9.29%, n=10, p=0.0357) after the tetanic There significant stimulation. were no (p=0.2403) differences in induction and maintenance (p=0.0649) of the LTP in wild type slices taken from 16.5 and 25.5 months old mice (Fig. 2 and Figure 3A) despite the development of diffuse mouse $A\beta$ deposits in wild type hippocampus over the indicated age (indicating the lack of importance of diffuse amyloid for synaptic plasticity failure). On the other hand, transgenic slices expressing plaque-like amyloid at age 25.5 months showed a significant decline in both induction (p=0.0047) and maintenance (p=0.0048) of the LTP compared to the 16.5 month old mice.

In contrast to the aged 25.5 month old mice, transgenic mice of the younger age (16.5 months) expressed no amyloid and did not differ from the wild type mice in the induction and maintenance of the LTP (Fig. 3A). There were, however, differences in the LTD (Fig. 3B), another important parameter of neuronal plasticity. It was shown previously20 that bath application of the soluble APP (100 nM, 1 h) adsorbed the ability of rodent hippocampal slices to maintain LTD. It is thus conceivable that mild modulation of the hippocampal LTD in adult transgenic mice (16.5 months) is due to the human APP expression in the transgenic 1H, mice hippocampus (Fig. see above). Another study by Larson et al.,²¹ however, suggests а more striking modulation of hippocampal physiology by human mutant (V717F)APP in transgenic mice at age 4-5

months. Although the experimental protocol of this report (specifically, maintaining slices at 36ºC; differences in the media recipe, particularly including ascorbate, known to modulate EPSP,22 in ACSF) does not match the one employed here and in the above cited report by Ishida et al.,²⁰ it indicates that APP mutations (yet representing very small cohort of all Alzheimer disease cases) may exacerbate additional abnormalities in synaptic and behavioral plasticity "prior to the formation of amyloid beta peptide deposits." The transgenic mice that we used in this study mild overexpress non-mutated human APP and in our view offer more relevant system to model non-mutated human APP expression.¹³

Two major components contributing to the tetanus induced LTP are NMDA LTP and non-NMDA (dependent on a rise in intracellular calcium concentration) LTP.^{23WEB+} Fast onset NMDA- and developing slow non- NMDA-LTP can be isolated by using a specific tetanic stimulation paradigm in the presence of 30 µM nifedepine, a blocker of voltage-gated calcium channels, and 25 µM D,L-2-amino-5phosphonovaleric acid, an NMDA antagonist, respectively.^{23WEB+,24} Moreover, another type of slow onset LTP was described, a muscarinic LTP, which can be evoked by the application of 0.25-2.0 µM carbachol in the absence of tetanic stimulation.^{23WEB+,24} Non-NMDA-LTP and muscarinic LTP share similar lack of expression in adult transgenic mice expressing human Cu/Zn-superoxide dismutase, SOD1,24 in very old (24-30 months) and in adult Wistar rat hippocampal slices treated with low (~30 μ M) dose of H_2O_2 .²³

Regardless of the deficit of specific receptor machinery hippocampal slices from aged transgenic mice may be different from wild type controls in their ability to regulate second messenger pathways and/or generate the action potential. Thus, transgenic mice may be impaired in the phosphorylation of the nuclear cAMP responsive element binding protein CREB), modulated (abbreviated as by micromolar concentrations of $A\beta^{25}$ and known as a necessary event in neuronal plasticity.26 Transgenic mice may be also impaired in metabotropic glutamate receptors (mGluR) and associated second messenger machinery, activation which was shown to be coupled to the APP processing²⁷ and is essential for the priming of the LTP.²⁸ It is also possible that in transgenic mice neurons are depolarized relative to the control slices, yielding reduction of their fEPSPs and impairment of their ability to express larger fEPSPs following tetanic stimulation.

Finally, have transgenic mice mav propagation deteriorated spike neuronal machinery due to the tunneling amyloid plaques (Figure 4A). This is supported by the study on the disruption of neural networks in Alzheimer disease.29 This modeled report the electrophysiological effect of the changed neuronal processes that cross through $A\beta$ plaque deposit and foretold the delay of several milliseconds over an average plaque. Our comparison of individual fEPSPs revealed this predicted change of few milliseconds in initial post-tetanic traces in transgenic versus wild type slices (Fig. 4B), confirming importance of plaque amyloid for neuronal spike propagation.

CONCLUSION

Our data provide evidence that one of the possible causes of Alzheimer's-like neuronal dysfunction and synaptic plasticity deficit is senile plaque (and not diffuse) amyloid. Our data are in dispute with the paper in JAMA8 and the accompanying commentary30 proposing that Aβ peptide not incorporated into histologically visible plaques is an early neurochemical hallmark of dementia. Our results also suggest that in Down syndrome (characterized by diffuse amyloid deposition in early life) and in pre-plaque stages of Alzhemer disease, the other factors (such as brain cholesterol and other lipid metabolism misregulation^{10WEB+,11} or oxidative stress disbalance9WEB+) may contribute to the neuronal dysfunction. The precise molecular mechanism of amyloid-plaque-mediated synaptic plasticity deficit, however, remains to be investigated

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REFERENCES

- Koudinova NV, Berezov TT, Koudinov AR. Amyloid beta: ALzheimer's disease and brain beta amyloidoses. *Biochemistry (Moscow)* 64, 752-757 (1999).
- 2. Koudinov AR, Koudinova NV, Berezov TT, Ivanov YD. HDL Phospholipid: a natural inhibitor of Alzheimer's amyloid β fibrillogenesis? *Clin Chem Lab M ed.* 37, 993-994 (1999).
- **3.** Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*, **400**, 173-177 (1999).
- 4. Soto C, Sigurdsson EM, Morelli L, Kumar RA, Castano EM, Frangione B. Beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for Alzheimer's therapy. *Nature M ed.* 4, 822-826 (1998).
- Chapman PF, White GL, Jones MW, et al. Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nature Neurosci.* 2, 271-276 (1999).
- 6. Rioult-Pedotti M-S, Friedman D, Donoghue JP. Learning-induced LTP in Neocortex. *Science* 290, 533-536 (2000).
- 7. Nalbantoglu J, Tirado-Santiago G, Lahsaini A, et al. Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein. *Nature* **387**, 500-505 (1997).
- Naslund J, Haroutunian V, Mohs R, et al. Correlation between elevated levels of amyloid βpeptide in the brain and cognitive decline. JAMA. 283, 1571-77 (2000).
- **9.** Perry G, Nunomura A, Hirai K, Takeda A, Aliev G, Smith MA. Oxidative damage in Alzheimer's disease: the metabolic dimension. *Int J Dev Neurosci.* **18**, 417-421 (2000).
- 10. Koudinov AR, Koudinova NV. Essential role for cholesterol in synaptic plasticity and neuronal degeneration. FASEB J. 15, 1858-1860, published online June 27, 2001, 10.1096/fj.00-0815fje (2001).
- 11. Koudinov AR, Koudinova NV. Brain Cholesterol Pathology is the Cause of Alzheimer's Disease. *Clin Med Health Res.* published online November 27, 2001, clinmed/2001100005, http://clinmed. netprints.org/cgi/content/full/2001100005v1
- **12.** Kumar-Singh S, Dewachter I, Moechars D, et al. Behavioral disturbances without amyloid deposits in mice overexpressing human amyloid precursor protein with Flemish (A692G) or Dutch (E693Q) mutations. *Neurobiol Dis.* **7**, 9-22 (2000).
- **13.** Lamb BT, Sisodia SS, Lawler AM, et al. Introduction and expression of the 400 kilobase *precursor amyloid protein* gene in transgenic mice. *Nature Genet.* **5**, 22-29 (1993).
- 14. Friedman LK, Koudinov AR. Unilateral GluR2(B) hippocampal knockdown: a possible novel partial seizure model in young rat. J Neurosci. 19, 9412-9425 (1999).
- **15.** Koudinov AR, Koudinova NV. Soluble amyloid beta protein is secreted by HepG2 cells as an

Clinical Medicine and Health Research, December, 2001, clinmed/2001110002v1

apolipoprotein. *Cell Biol Inter.* **25,** 265-271 (1997).

- 16. Kaplan B, Haroutunian V, Koudinov AR, Patael Y, Pras M, Gallo G. Biochemical assay for amyloid β deposits to distinguish Alzheimer's disease from other dementias. *Clin Chim Acta*, 80, 147-159 (1999).
- **17.** Koudinov AR, Berezov TT, Koudinova NV. The levels of soluble amyloid beta in different HDL subfractions distinguish Alzheimer's and normal aging CSF: implication for brain cholesterol pathology? *Neurosci. Lett.* **314**, 115-118 (2001).
- 18. Koudinov AR, Koudinova NV, Kumar A, Beavis R, Ghiso J. Biochemical characterization of Alzheimer's soluble amyloid beta protein in human cerebrospinal fluid: association with high density lipoproteins. *Biochem Biophys Res Commun.* 223, 592-597 (1996).
- 19. Astrelin AV, Sokolov MV, Behnisch T, Reymann KG, Voronin LL. Principal component analysis of minimal excitatory postsynaptic potentials. J Neurosci Meth. 79, 169-186 (1998).
- 20. Ishida A, Furukawa K, Keller J, Mattson MP. Secreted form of beta-amyloid precursor protein shifts the frequency dependency for induction of LTD, and enhances LTP in hippocampal slices. *Neuroreport* 8, 2133-2137 (1997).
- **21.** Larson J, Lynch G, Games D, Seubert P. Alterations in synaptic transmission and longterm potentiation in hippocampal slices from young and aged PDAPP mice. *Brain Res.* **840**, 23-35 (1999).
- **22.** Xie Z, Sastry BR. Induction of hippocampal long-term potentiation by alpha-tocopherol. *Brain Res.* **604**, 173-179 (1993).
- 23. Auerbach J. M. and Segal M. Peroxide modulation of slow onset potentiation in rat hippocampus. *J Neurosci.* 17, 8695-8701 (1997).
- 24. Koudinov AR, Groner Y, Segal M. Cu/Zn-SOD transgenic mice are impaired in slow onset, long term potentiation. *Neurosci Lett.* 51, S23 (1998).
- 25. Sato N, Kamino K, Tateishi K, et. al. Elevated amyloid β protein (1-40) level induces CREB phosphorylation at serine-133 via p44/42 MAP kinase (Erk1/2)-dependent pathway in rat pheochromacytome PC12 cells. *Biochem Biophys Res Commun.* 232, 637-642 (1997).
- 26. Segal M, Murphy DD. CREB activation mediates plasticity in cultured hippocampal neurons. Neural Plast. 6, 1-7 (1998).
- 27. Nitsch RM, Deng A, Wurtman RJ, Growdon JH. Metabotropic glutamate receptor subtype mGluR1alpha stimulates the secretion of the amyloid beta-protein precursor ectodomain. J Neurochem. 69, 704-712 (1997).
- 28. Cohen AS, Raymond CR, Abraham WC. Priming of long-term potentiation induced by activation of metabotropic glutamate receptors coupled to phospholipase. *Hippocampus* 8, 160-170 (1998).
- **29.** Knowles RB, Wyart C, Buldyrev SV, et al. Plaque-induced neurite abnormalities: implications for disruption of neural networks in Alzheimer's disease. *Proc Natl A ca Sci USA* **96**,

5274-5279 (1999).

30. Selkoe D. The origins of Alzheimer disease: a is for amyloid. *JAMA* **283**, 1615-1617 (2000).

Please note: While this paper was under submission another key contribution on this subject was published in December 2000: Chen G, Chen KS, Knox J, et al. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* **408**, 975-979 (2000).

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