

# Neurobiology of Lipids

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## AMYLOID BETA, NEURAL LIPIDS, CHOLESTEROL & ALZHEIMER'S DISEASE

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**T**o date great number of articles were devoted to cholesterol (chol) but only few articles studied the role for chol in neuron function/degeneration. For decades this molecule had been known to be important for atherosclerosis and heart disease. First indication of the involvement of chol in Alzheimer disease (AD), however, come from the mid 1990s. At that time it was shown that heart disease patients develop brain deposits of amyloid beta (A $\beta$ ), a major dogmatic molecule of AD; that apoE (a chol transport apolipoprotein) allele e4 is a major genetic risk factor for AD; and that lab animals fed a chol diet express brain amyloid.<sup>1</sup> These days it turns out that A $\beta$ , long thought to be exclusively a pathologic protein, is a normal and functional apolipoprotein constituent of high density lipoproteins in plasma and CSF. Thus, we and others showed that A $\beta$  modulates chol and phospholipid synthesis, and affects chol esterification.<sup>1</sup> Protection of lipoproteins and other biomolecules from oxidation may represent another important function of A $\beta$ .<sup>2</sup> We also discovered that neuronal chol homeostasis failure and the lack of chol supply to neurons by means of lipoprotein transport causes AD features, such as the failure of the neurotransmission and

synaptic plasticity, degeneration of neuronal cell processes, and tau protein pathology.<sup>3,4</sup>

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### POSING THE PROBLEM

Since 1966 more than one hundred thousand research papers were devoted to cholesterol and only few articles dealt with the role of cholesterol in neuronal function, synaptic plasticity and neurodegeneration (according to the PubMed and HighWire Press search engines). This mysterious molecule is accused in atherosclerosis and heart disease, but was largely understudied in relation to brain function and neural structural and functional (i.e. activity dependent) plasticity. Living cells (including neurons and glial cells) produce their own cholesterol and can receive or donate cholesterol via lipoprotein transit, an attested body lipid transportation system. This system operates a number of vehicle classes, including well-known (and considered to be "bad") LDLs and "good" HDLs.<sup>1,2</sup>

Apart from this whole-body-system stands distinct brain lipid transport authority that uses different subtypes of HDLs and normally does not maintain LDLs.<sup>3</sup> This vital service is in charge of cholesterol redistribution inside the brain and cholesterol export out of the brain border to liver for excretion. There is no reported cholesterol import to the brain, an issue that makes brain

cholesterol availability entirely dependent on local manufacturing. Brain lipid transportation must have good management and operating capacity, because the quantity of cholesterol in the brain is much higher than anywhere else in the rest of the body. Thus, having just two percent of the body weight, brain has a quarter of cholesterol present in the whole individual.

Conceivable, the break in any element of the harmonized system of brain/neuronal cholesterol transport (caused by genetic defects of one of the enzyme or receptor associated with cholesterol turnover; by pharmacological modulation or environmentally) may result in abnormal homeostasis of cholesterol in the brain and impair fine tuning of synaptic function (see online Refs. 2-4 for instant access to detailed bibliography).

### **SYNAPTIC FUNCTION BASIC**

Cholesterol (and phospholipids) is a building block of any cell membrane (the nervous system wrapping material, where neuronal information in the form of electric activity is generated and propagated) and specialized membrane structures, lipid rafts and synaptic vesicles. In the brain the information (coded as nerve impulse) is transmitted from presynaptic neuron-transmitter to a postsynaptic neuron-receiver at the tiny gap between cells called synapse. The first neuron output nerve ending (called axon) releases synaptic vesicles containing chemicals called neurotransmitters. The neurotransmitter molecules then bind to receptors of the neuron-receiver input processes, located in postsynaptic membrane functional domains, called lipid rafts. After the minute interaction with a receptor on the neuron-receiver, neurotransmitter molecules normally return back to the nerve ending (from where they were released) for recycling in order to be ready for the next act of neurotransmission. This transient neurotransmitter-receptor interaction represents the quantum of neurotransmission and synaptic function. It launches a number of chemical changes inside the postsynaptic neuron-receiver, essential for the nerve signal processing, synaptic amplification or modulation (also called synaptic plasticity), and for the formation of memory.<sup>2</sup>

### **CHOLESTEROL AND ALZHEIMER'S DISEASE BACKGROUND**

The turn of the century was marked by several reports that demonstrated Alzheimer's diseases features in neuronal cells, brain slices and laboratory animals that model cholesterol pathology (see Refs. 2, 4, 5 for details). Our related research

during past decade included the study of the interaction of lipid (particularly cholesterol) metabolism and amyloid  $\beta$  ( $A\beta$ ) protein (insoluble brain deposits of  $A\beta$  are widely believed to be Alzheimer's characteristic feature and the cause of the disease, see our another presentation at the 32nd Society for Neuroscience Annual Meeting 2002) as a "missing link in Alzheimer's puzzle" (for brief review also see Ref. 1). This relation became especially important recently because more than dozen studies showed that cholesterol modulation affects generation of  $A\beta$  and the processing of its precursor.<sup>4-6</sup>

In the past, however, just few reports implicated cholesterol in basic synaptic function, particularly in trafficking and recycling of synaptic vesicles, in receptor function, activity of accessory synaptic proteins, and in modulation of membrane biophysical properties (see bibliography in Refs. 2, 4).

### **CHOLESTEROL, PHOSPHOLIPIDS, SYNAPTIC PLASTICITY & NEURODEGENERATION**

In our recent study published in June 2001 as research article in *The FASEB Journal* and in March 2002 as letter in *Science magazine*<sup>1,2,7, fr1-fr3</sup> we attempted to gain basic knowledge on the role of cholesterol and phospholipids in neurotransmission, synaptic plasticity and nerve cells degeneration. To this end we prepared slices from the laboratory rat hippocampus, a part of the brain essential for learning and memory storage and having a lot of lipoprotein receptor molecules handling neuronal uptake of lipoproteins carrying cholesterol. Very thin slices (less than half-a-millimeter) of the hippocampus, retaining the hippocampal integrity and complex neurochemical and neuronal network architecture, were maintained live in a test tube. Then we set out to model cholesterol disbalance by acute increase of cholesterol turnover and inability of the hippocampus to redistribute cholesterol from one cell (astrocytes in particular) to another (neurons and their projections) via lipoprotein transport. To achieve this outcome the slices were subjected to biochemical increase of cholesterol removal with model and natural chemicals, methyl- $\beta$ -cyclodextrin or normal human CSF HDL. Simultaneously or immediately thereafter we evoked and recorded two different brain waveforms: so called paired pulse facilitation (PPF) and long term potentiation (LTP), indicative of neurotransmission and synaptic plasticity, respectively. We found that the lack of cholesterol supply of neurons via lipoprotein transport impaired

both PPF and LTP indicative of the neurotransmission and synaptic plasticity impairment. Moreover, we traced lipid synthesis with radioactive acetate (a precursor molecule which gets incorporated into all cellular lipids) after the generation of the LTP in the hippocampal slices. Based on the increase of the radioactive label incorporation into newly synthesized lipids specifically at the site of the LTP recording we concluded that this form of synaptic plasticity is associated with the increase of lipid synthesis. Such activity dependent lipid synthesis may represent the mechanism essential for structural plasticity of synapses at late stages of the LTP after the immediate access to lipids via lipoprotein transport during the LTP early stages.

In addition to the recording of neural waves we analyzed slices by immunofluorescence. We found that cholesterol disbalance causes neurofilament degeneration and the appearance of PHF-like phosphorylation of tau protein (confirmed in many studies by others, see Ref. 2, 4 for citations) in hippocampal axonal mossy fibers.<sup>2</sup> Excessive phosphorylation of tau is one of the key features of Alzheimer's disease that is also present in cells under genetic cholesterol homeostasis disorder called Niemann-Pick type.<sup>2,4</sup>

### **CHOLESTEROL IS THE POSSIBLE CAUSE OF NEURODEGENERATION IN ALZHEIMER'S DISEASE**

In a separate set of experiments by modifying rat cholesterol status with the diet containing 2 % cholesterol for several months we generated animal model, that expressed brain amyloid similar to amyloid deposition of Alzheimer's disease brain specimens.<sup>4,6, fr3-fr5</sup> We then prepared slices from the hippocampus, maintained them live *in vitro*, and subjected to the study of cholesterol synthesis by labeling with radioactive acetate. After labeling the lipids were extracted from slices, separated by thin layer chromatography, and quantitated by radioactivity counting. This methodology allowed us to quantitate the higher rate of cholesterol and phospholipids synthesis in rats fed a cholesterol diet, and to conclude that brain cholesterol synthesis upregulation is a possible cause (but NOT a consequence) of Alzheimer's-like brain amyloid. Most important, we also performed electrophysiological analysis of slices. We found that cholesterol-fed rats lack hippocampal LTP and thus have impaired synaptic plasticity.<sup>4,6</sup> It is notable that impaired LTP could be reversed by the reversal of a 2 % cholesterol diet to a regular diet for an extended period of time. It is in accord with the

## Supplement 1

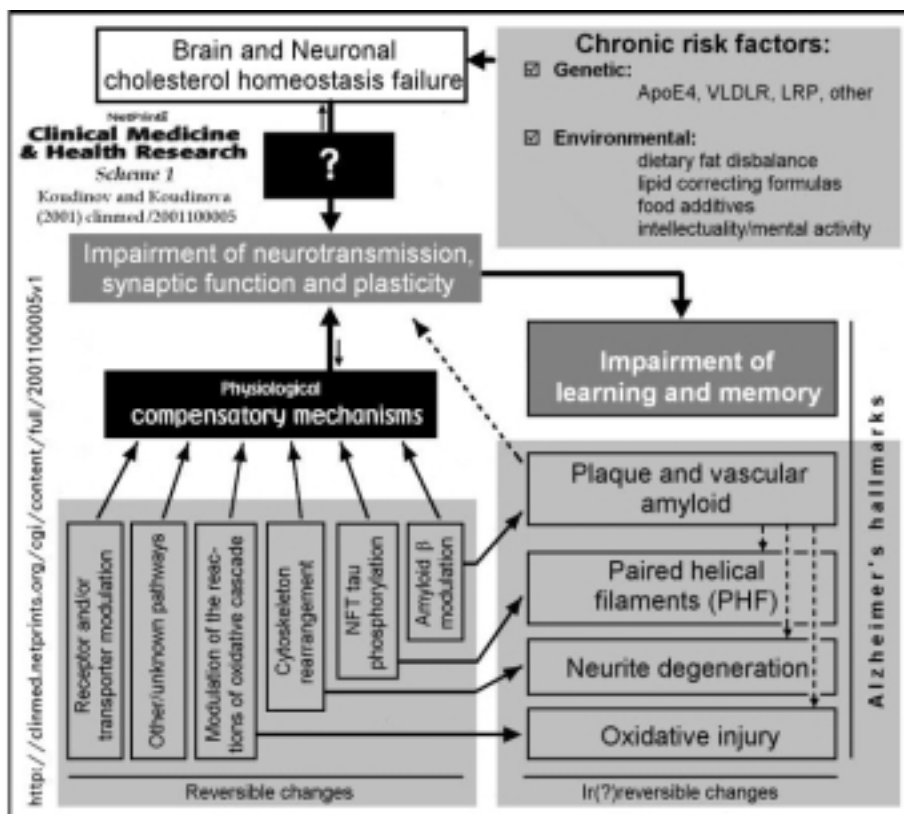
### **ALZHEIMER'S DISEASE, CHOLESTEROL AND AMYLOID $\beta$ : DOGMA PREVAILS**

**U**p to now, cholesterol's role in Alzheimer's disease was mainly explained in terms of the dogmatic view that a reduction of amyloid burden by lowering cholesterol is beneficial.<sup>5, fr8-10</sup> This viewpoint (that became questioned in October 2002 Neurology article<sup>fr9</sup> by Fassbender *et al.*) was based on the *in vitro* data on the importance of cholesterol in amyloid precursor protein processing and A $\beta$  generation.<sup>4,5</sup> Two recent articles further showed that cellular generation of A $\beta$  is modulated by cholesterol compartmentation and intracellular cholesteryl-ester levels.<sup>fr8</sup>

**The biochemical relation of cholesterol and A $\beta$ , however, is bidirectional.**

**Moreover, the modulation of neuronal cholesterol dynamics by A $\beta$  may have important functional consequences.**

Particularly, A $\beta$  modulates neuronal cholesterol esterification, influx, efflux, and thus may regulate neural cholesterol intracellular compartmentation and extracellular trafficking.<sup>4</sup> A $\beta$  also modulates neuronal physical property of membrane fluidity important for receptor function, and it is well possible that this effect is mediated by the peptide antioxidant properties (see next section). Additionally, A $\beta$  increases neural lipid synthesis, in contrast to the peptide inhibitory effect, observed in human hepatic HepG2 and in HEK293 cells, in fetal rat liver and in neuronal tissue under the condition of potassium-evoked depolarization and under oxidative stress. The latter results highlight the importance of developmental, tissue and neuronal functional specificity of A $\beta$ -cholesterol biochemical relation, which may vary in different brain regions and be of special importance in determining Alzheimer's specific areas of neurodegeneration. The latter data also suggest that A $\beta$  may serve a molecular messenger function and manage the crosstalk of hepatic, systemic and brain cholesterol, and thus maintain the tissue-specific coordinate regulation of cholesterol biosynthesis. Taken together, the above functional consideration and recent data on the importance of cholesterol compartmentation for A $\beta$  generation indicate feedback mechanism between cholesterol and A $\beta$  homeostasis, additionally supported by a dependency of amyloid precursor protein processing and A $\beta$  production on the site 2 processing of SREBP and associated inability of cells to upregulate the expression of several enzymes and proteins involved in cholesterol synthesis and turnover.<sup>4</sup> See Ref. 4 for detailed bibliography. Note that A $\beta$  also has the function as an antioxidant for lipoproteins (see below).



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## SCHEME 1. SCHEMATIC REPRESENTATION OF THE CASCADE OF THE COMMON SPORADIC FORMS OF ALZHEIMER'S DISEASE (AD).

Neuronal cholesterol dynamics misregulation causes the key Alzheimer's disease (AD) feature of learning and memory failure as a result of the impairment of neuronal function, neurotransmission and synaptic plasticity through the mechanisms precise molecular nature which remains to be identified. Cholesterol-mediated change in neurochemistry of amyloid beta, tau phosphorylation, neuronal cytoskeleton rearrangements and the modulation of physiological equilibrium of oxidative stress reactions could provide physiological transitory mechanisms aiming to compensate impaired brain cholesterol dynamics and neurotransmission and synaptic plasticity. The break in neuronal cholesterol homeostasis may require very long (i.e. chronic) onset time frame due to the physiologically slow turnover of the central nervous system (CNS) cholesterol. Such condition may be genetically set (right top) and be assisted environmentally by the long term dietary habits. While during the past 30 years the concept of healthy food has become synonymous with avoiding dietary cholesterol, the question of how this avoidance and its compensation affects brain cholesterol chemistry, learning and memory remained non-addressed for many years. Several basic reports, however, documented that brain cholesterol is a delicate substance very sensitive to many influences, ranging from lipid preparation diets and chemical delivery systems for drugs and food additives (cyclodextrins, for example) to learning process itself. It is thus possible that antifat lifestyle "soft science" doctrine contributed to the increase of dementia and Alzheimer's prevalence in industrialised countries during 1970s and 1980s. The indicated physiological compensatory changes may slowly invert when neuronal cholesterol dynamics is recovering slow to the initial physiological level. Such reversibility was proved experimentally (see Ref. 4) and certified by nature as an important mechanism of the CNS plasticity, as exemplified by high expression of PHF-phosphorylated tau during an ontogenic period of cholesterol-demanding intense neuritic outgrowth. General compensatory nature of amyloid and tau neurochemistry modulation was proposed previously and is illustrated by its change observed under related to cholesterol (but different from AD) cardiovascular and Niemann-Pick type C pathologies, as well as in normal cases and during aging. When neuronal cholesterol dynamics is not recovering compensatory mechanisms fail yielding (yet possible reversible) the development of conventional Alzheimer's disease hallmarks (right). These hallmarks, however, are not causative for the sporadic AD, and thus unlikely represent the proper target for the efficient AD therapy, as supported by the cognitive decline and dementia in AD patients without detectable lesions. Of these disease markers demonized amyloid beta pathology is the key enemy for the amyloid cascade hypothesis. Plaque amyloid may itself impair (dotted arrows) synaptic plasticity and learning, neural networks, protein phosphorylation and oxidative stress status. Therefore it may have separate pathogenetic significance for the familial forms of AD, caused by the mutations in amyloid precursor protein and presenilins genes. Similarly, oxidative stress independently disrupts synaptic plasticity and thus may have separate pathogenetic value for the Down syndrome (characterized by upregulation of the reactions of oxidative stress due to the possible overexpression of the enzyme Cu/Zn-superoxide dismutase (SOD1), a chromosome 21 gene product) and for the pre-plaque stages of AD. The hallmarks trigger third order events of microglia activation, astrocytosis, cytokine/acute-phase protein release and cell death (not shown). This may convert physiological compensation into the pathological final and lock the cascade and the disease irreversibility. Full text of this account is in free access at Ref. 4. Also see Refs. fr6, fr7. Scheme is reprinted by permission.<sup>4</sup>

notion that the rate of cholesterol turnover in the brain is very low, and thus requires long time for a disturbance (or a correction in case of the opposite order of events) to yield appreciable change. Our functional analysis data significantly extended several earlier histochemical reports that demonstrated amyloid buildup in rabbits and in amyloid precursor protein transgenic mice fed a cholesterol diet (see Refs. 4-6 for details).

## CHOLESTEROL, AMYLOID $\beta$ AND ALZHEIMER'S CSF-HDL

Our other study<sup>3</sup> showed that Alzheimer's patients have unique pattern of HDL distribution in the CSF, the lipoprotein fraction that is especially important in cholesterol transport in tissue environment and across the body, and that has soluble form of A $\beta$  as an apolipoprotein constituent.<sup>1</sup> Particularly we observed an increase in content of soluble A $\beta$  and selected apolipoproteins in the HDL subfraction called HDL<sub>1</sub>. Remembering a special role for HDL<sub>1</sub> in reverse cholesterol transport on one hand, and the role for A $\beta$  in cholesterol esterification (that causes HDL size change and the formation of HDL<sub>1</sub>, Ref. 3) we interpreted our data as an additional piece in Alzheimer's cholesterol puzzle.<sup>3,4</sup>

## AMYLOID $\beta$ IS A POTENTIAL PHYSIOLOGICAL ANTIOXIDANT FOR LIPOPROTEINS IN CEREBROSPINAL FLUID AND PLASMA

Increased oxidative stress is related to the Alzheimer's development, and amyloid beta protein (A $\beta$ ) is considered to be an important prooxidant in this process.<sup>8</sup> To induce oxidation, however, A $\beta$  must be present at high concentrations, typically in a micromolar range.<sup>9</sup> In addition, an A $\beta$  preparation must be 'aged' to yield A $\beta$  aggregates and fibrils.<sup>10</sup> In vitro, A $\beta$  is readily aggregated by transition metal ions;<sup>11</sup> in contrast, in the absence of metals A $\beta$  is monomeric. The presence of transition metals is not only required for A $\beta$  aggregation but also for its prooxidative activity.<sup>12</sup> Therefore, A $\beta$  toxicity is mediated by a direct interaction between A $\beta$  and transition metals with subsequent generation of reactive oxygen species (ROS).

The requirement of fibrillation and transition metals for the prooxidative activity of A $\beta$  can be understood taking into account its redox properties. In order to function as a prooxidant, A $\beta$  must first

bind metals to its metal-binding site(s) at His residues<sup>13</sup> and then reduce them in its metal-reducing site at Met35 residue<sup>14</sup> in order to produce ROS.

However, metals bind to the N-terminal hydrophilic part of A $\beta$ , whereas metal reduction occurs at its C-terminal part. Since metals must be placed in the vicinity of the reductant to be reduced, fibrillation is likely to fulfil this task by forming complexes where metal atoms bound to the N-terminal part of one molecule of A $\beta$ , at the same time might be available for the reductive Met35 residues belonging to other A $\beta$  molecules. The resulting reduced transition metal ions can participate in further redox reactions, generating various free radical species. Due to relatively slow reduction of metals by A $\beta$ , the above mechanism can only be operative at high (micromolar) concentrations of the A $\beta$  peptide.

In contrast to prooxidative properties, an antioxidative activity of A $\beta$  peptides (that contradicts the dogmatic view on A $\beta$  as toxic) has been barely studied. We have shown that at low-nanomolar concentrations (i.e., those of soluble A $\beta$  in CSF and plasma), exogenously added A $\beta$  inhibits metal-catalyzed oxidation of lipoproteins of human CSF and plasma.<sup>15,16</sup> The effect is observed at the peptide concentration reported for biological fluids (0.1-1.0 nM); at higher concentration of A $\beta$  its antioxidant action is abolished. In contrast, all A $\beta$  peptides are unable to considerably influence metal-independent lipoprotein oxidation, suggesting that the antioxidative activity of A $\beta$  is mainly mediated by chelating transition metal ions. Endogenous A $\beta$  present in CSF can also act as an antioxidant, as is suggested by the positive correlation between CSF resistance to oxidation and the CSF level of A $\beta$ .<sup>17</sup>

Our data were confirmed by Zou *et al.* who reported potent antioxidant activity of A $\beta$  in neuronal cells in the presence of transition metals.<sup>18</sup> A $\beta$  protected neurons against toxic action of copper and iron; the effect strictly depended on the aggregation state of the peptide. Monomeric A $\beta$  was protective even at micromolar concentrations, whereas aggregated A $\beta$  lost its antioxidant properties. These results are consistent with earlier observations of Whitson *et al.*, Yankner *et al.* and Koo *et al.* who showed that at low-nanomolar concentrations A $\beta$  is monomeric, non-toxic and exerts beneficial effect on neuron survival, axonal length and neurite outgrowth.<sup>19-21</sup> We propose that all these activities may be related to antioxidative properties of the peptide.

Mechanistically, antioxidative activity of A $\beta$  can be related to the fact that in lipoproteins, a metal-

binding region of A $\beta$  expresses greater hydrophilic properties and extends into the outer aqueous phase where it can bind transition metals and inhibit metal-catalysed oxidation. In this regard it is important to note that neuronal cell cultures secrete a high molecular weight product, presumably a lipoprotein complex, that possesses an antioxidative activity.<sup>22</sup>

As soon as A $\beta$  has antioxidative properties on one hand, and is secreted by cells as a part of lipoprotein complexes<sup>1,3,23</sup> on another, it is well possible that A $\beta$  is secreted by cells to serve as a natural antioxidant for lipoproteins. A $\beta$  can bind transition metal ions in inactive form and prevents them from catalyzing oxidation of lipoproteins and other biomolecular complexes. Amphiphilic properties of A $\beta$  may allow extracellular chelation of metal ions that escape binding by hydrophilic chelators.

### AMYLOID $\beta$ RESTORES HIPPOCAMPAL LONG TERM POTENTIATION: A CENTRAL ROLE FOR CHOLESTEROL

In our most recent study<sup>24</sup> we attempted to dissect out the role for A $\beta$  in the synaptic plasticity in brain slices from adult male rat hippocampus under the condition<sup>2</sup> that we characterised previously with regard to cholesterol and phospholipid synthesis. The prolonged maintenance of slices in a test tube for more than twenty hours in our experimental setup preserved synaptic function (input/output curve, a basic measure of synaptic function, for example) but abrogated synaptic plasticity (LTP). A $\beta$  protein of the 1-40 aminoacids' molecule length (representing the major form of soluble A $\beta$ ) rescued LTP while cholesterol synthesis inhibition with a statin abolished the LTP restoration by the peptide.

Our observation implies an intriguing perspective that A $\beta$  protein is a functional player in an activity-dependent cholesterol neurochemical pathways and in synaptic structure-functional plasticity.<sup>2-4,24,25</sup> The finding also supports our proposed hypothesis that the change in A $\beta$  biochemistry in Alzheimer's disease and related disorders is a functional (but NOT pathologic<sup>25,26,fr10</sup>) compensatory phenomenon aiming to counterbalance impaired cholesterol dynamics and associated neurotransmission and synaptic plasticity.<sup>3-5, 25</sup> Such cholesterol mediated failure of synaptic function and neural degeneration<sup>2-4</sup> in our view may represent the cause of the major sporadic form of Alzheimer's disease.

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### FURTHER READING\*

- fr1.** Interactive slide show of the poster presentation for: Koudinova NV, Koudinov AR. Essential Role for Cholesterol in Synaptic Plasticity and Neuronal Degeneration. *Soc Neurosci Abst.* **27**, 752.5 (2001). Available at: <http://anzwers.org/free/neurology/reports/presentations/slideshowsfn01/slideshowsfn01-01.html>
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