Chapter 15

# Ontogenetic and Phylogenetic Approaches for Studying the Mechanisms of Cognitive Dysfunctions

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**Abstract**

This chapter summarizes the phylogenetic and ontogenetic approaches for studying cognitive disorders such as Alzheimer's disease. It gives an extended example of evaluation of animal behavior and brain properties using an original model of prenatal hypoxia in rats by various physiological, behavioral, immunohistochemical, molecular biological, and biochemical techniques at different stages of postnatal development, which provide a better understanding of the pathological processes in the human brain during the development of neurodegeneration.

**Keywords:** ontogenesis, prenatal hypoxia, Alzheimer's disease, amyloid peptide, amyloid-degrading enzymes, animal models, cognitive dysfunctions, dendritic spines, synaptopodin, learning, memory, neuronal plasticity

## 1. Introduction

Developing the main postulates of evolutionary physiology, an eminent Russian scientist, Leon A. Orbeli, declared that for understanding the development of any functions, they should be studied from phylogenetic (using animals of different classes and species) and ontogenetic (studying organisms in development) points of view as well as under various pathological conditions and with the help of appropriate experimental models [1]. Cognitive functions represent the highest ability of the organisms to react to various stimuli from the internal and external environment by analyzing, memorizing, and storing them for immediate response or

future integration and planning of the actions. They underlie all aspects of perception, thinking, reasoning, and memory. Both in humans and animals, cognitive dysfunctions are manifested as impairment of these most complex processes in the brain. The data accumulated over decades indicate that the neural mechanisms of cognitive dysfunctions can be understood as impairment of specific neural circuits, and that their functions and dysfunctions can be influenced or altered by a variety of cognitive and pharmacological factors (for review see [2]). Cognitive dysfunctions can result from various pathological changes in the brain, including significant neuronal loss observed, in particular, in Alzheimer's disease (AD).

The effectiveness of the studies of the pathogenesis of AD and search for the strategies of its prevention and treatment depend on appropriate modeling of the pathological conditions in the brain leading to AD. Traditionally, the main focus on designing animal models of AD was related to the identification of brain areas and mediator systems related to memory. The most attention has been paid to the cholinergic system that undergoes the most significant changes in AD (for review, see [3]). Several experimental approaches were proposed to model AD using injections of muscarinic receptor antagonists [4]), disruption of the medial septal area [5], or nucleus basalis of Meynert [6]. All these manipulations resulted in reduced levels of cholinacetyltransferase (ChAT) and acetylcholinesterase (AChE) in animal brain cortex leading to impairment of learning [7]. Another model employed injections of a monoclonal antibody against growth factor receptor conjugated with saporin (192 IgG-saporin), which also resulted in the loss of cholinergic neurons and cognitive disorder [8]. Intracerebroventricular injections of streptozotocin (STZ), which inhibits insulin receptor function, were also shown to lead to cholinergic loss and neurodegeneration resulting in long-term and progressive deficits in learning, memory, and cognitive behavior [9, 10]. These studies have also provided an insight into the role of diabetes in development of the sporadic form of AD [11]. However, the data regarding the changes in the content of amyloid β peptide (Aβ), which is the major component of the senile plaques and causative molecule in AD pathogenesis [12, 13], are still contradictory [9, 14]. Also, these models do not always demonstrate properly the mnemonic deficits observed at the early stages of AD.

Another common approach for modeling AD has employed, that is, injections of Aβ into specific brain areas (for review see [15]). However, these models have serious limitations and do not always reproduce the symptoms of cognitive deficit characteristics for AD [16]. Although Aβ in rodents has a slightly different amino acid sequence compared to human and monkey peptides, it can form fibrils [17] indicating that the lack of evident amyloid deposits in rodent brain is not due directly to the specific changes in its sequence but to other factors. Because of that, most animal models of AD use transgenic mice and rats, which express human AD-related proteins such as amyloid precursor protein (APP), presenilins (PS1 and PS2), and tau protein (for review see [18]).

Studies in AD transgenic mice have provided deeper insight into the processes of Aβ formation and the role of soluble Aβ oligomers in its pathogenesis [19]. Thus, it was found that in APP transgenic mice, pathological and functional changes in the brain are observed well before the formation of amyloid plaques [20]. Although in transgenic mice expressing human APP and PS formation of amyloid plaques usually accelerates with aging compared to the wild type animals in many cases, they do not demonstrate another major feature of AD which

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is accumulation of neurofibrillary tangles composed of tau protein. To overcome this problem, a triple transgenic mouse model (3xTgAD) was designed expressing human $APP_{Sw}$ (containing the Swedish mutation leading to early onset AD), as well as mutated PS1 (M146 V) and tau protein (P301L) [21]. This model still is most often used in AD research. Apart from accelerated formation of amyloid plaques and neurofibrillary tangles, these mice demonstrate other pathological and behavioral feature characteristics of AD, for example, synaptic impairment and memory deterioration [22]. For elucidating the role of aging in development of sporadic AD, a model of senescence accelerated mice has been developed, which demonstrates Aβ deposits and cognitive decline as early as 6 months of age [23].

Although transgenic mouse models have prevailed over the last two decades, transgenic rat models are also becoming of more common use [24]. Despite significant physiological limitations of transgenic animals from lower phylogenetic species transgenic insects, for example, *Drosophila* [25] and worms, for example, *C. elegans* [26] have proved useful for deciphering the role of certain molecules in the development of neurodegeneration and testing various potential drugs. However, mammals still represent the main classes of animals for designing AD models since with aging some of them also develop features of neurodegeneration similar to humans [27], and recently, a transgenic mini-pig model has been approved [28].

Despite the significant data about pathogenesis of AD that have been obtained using various animal models, their major pitfall is related to the limited suitability for studying the molecular-cellular mechanisms of AD at the earliest stages of the disease when cognitive deficit is not yet accompanied by accumulation of amyloid plaques and massive cell death, but is determined by the first disruptive events in regulation of cellular interactions [29]. Also, they are mostly modeling rare genetic forms of AD, while most of the cases are sporadic late onset. Moreover, none of the therapeutic strategies based on these studies led to a successful anti-AD drug, although the amyloid cascade hypothesis underlying them has proved to be sound [30]. This can be partially explained by insufficient knowledge accumulated to date about the normal physiological role of APP and Aβ itself [31] as well as by the lack of studies modeling the early stages of AD pathology [32]. Studying mechanisms of cognitive deficit at early stages of the disease is of particular importance both from the diagnostics point of view and for design of preventive therapy. This dictates the necessity of appropriate zootropic models of experimental synaptopathies allowing to analyze the molecular and cellular bases of such conditions as mild cognitive disorder (MCI), which precedes the development of AD without modulating AD gene expression in experimental animals, and to study them at various stages of ontogenesis.

## 2. Cognitive functions after prenatal hypoxia

For modeling early pathologic changes of cognitive functions, we have developed a model of prenatal hypoxia in the period of the most active brain formation in rat embryogenesis. For this, Wistar female rats on the 14th day of pregnancy (E14) were placed for 3 h in a hypoxic normobaric chamber where oxygen content was gradually reduced to 7% by replacing it with helium for 10 min. The control rats were kept in the chamber for the same period of time but under normal oxygen content. The offspring of control and hypoxic rats were then subjected

to various behavioral tests at different stages of ontogenesis, and their brains were taken for morphological and biochemical analysis. The rats subjected to prenatal hypoxia demonstrated general retardation and delayed formation of innate motor reactions in the postnatal period, which become less pronounced compared to controls in the process of animal development [33, 34]. However, the impairment of cognitive functions in these rats was observed in various behavioral tests throughout their life span [35].

Thus, the rats subjected to prenatal hypoxia demonstrated reduced ability to learn a new instrumental reflex such as pushing a piston inside a narrow tube. On postnatal days 20–30, the number of rats capable of learning this reflex was 50% compared to 70% in the control group. At a more advanced age (3 months old), the number of hypoxic rats capable of pushing a piston with fixed duration was 46%, while in the control group, it was about 73% [36]. Analysis of long-term memory and retrieval of the instrumental reflex after a prolonged interval (5 weeks after initial training) demonstrated that control rats were able to remember the reflex with prolonged pushing of the piston, while the rats subjected to prenatal hypoxia returned to the level of performance before the initial testing. Further training of these animals allowed them to relearn the reflex and reach the level before the interval, while the rats from the control group were able to improve their performance further. These data testify to a significant memory deficit in rats subjected to prenatal hypoxia already at the age of 3 months.

Testing the short-term memory in a radial two-level maze has revealed that adult rats subjected to prenatal hypoxia also had a significantly lower number of correct visits to the arms with unchanged average time spent in them ($p < 0.01$), which testified to the impairment of their short-term memory. In the novel object recognition test, both young and adult rats subjected to prenatal hypoxia had impaired ability to discriminate the new and old objects, both 5–10 min after the first training (short-term memory) and 60 min or 24 h after it (long-term memory) [37].

All these data allowed us to conclude that the model of prenatal hypoxia provides well-reproducible changes of cognitive functions in the postnatal ontogenesis of rats. It allows studying at the molecular levels, changes in brain structure and functions, which accompany cognitive dysfunctions, and also testing the efficacy of various pharmacological agents [37, 38].

## 3. Effects of prenatal hypoxia on the development of cortical brain areas

It is well known that various pathological factors in certain periods of prenatal development can lead to structural-functional changes in the brain. Existence of the periods of higher sensitivity of the brain to the pathological factors is based on the heterogeneity of ontogenetic development of the nervous system [39]. Any unfavorable factor in these critical periods of embryonic development can lead to structural-functional changes at all levels of brain organization. Such factors as ionizing radiation [40] and ultrasound [41] were shown to affect the generation and migration of the neuroblasts into the cortical plate leading to disruption in the formation of the neocortex and accompanied by prolonged disturbance in the regulation of motor activity and cognitive functions.

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Using our model of prenatal hypoxia, we have also found that the underlying mechanism of the structural-functional changes observed by many authors in the postnatal development of animals subjected to hypoxia in various paradigms [42, 43] are related to the changes in generation and migration of neuroblasts caused by hypoxia in critical periods of embryonic development [44]. Using injections of 5'ethynyl-2'deoxyuridine to pregnant rats for labeling neurons generated on E14 or E18 in the fetuses, it was shown that in control rat pups, a majority of cells labeled on E14 were localized in the lower cortical layers V–VI, while the cells labeled on E18 were mainly found in the superficial cortical layers II–III. In postnatal development of rats subjected to prenatal hypoxia either on E14 or E18, we observed a certain degree of disruption in generation and migration of neuroblasts in the brain. However, hypoxia on these particular embryonic days affected different cell populations leading to specific patterns of cell labeling. Thus, hypoxia on E14, resulting in a decrease in the total number of labeled cells in the parietal cortex, led to an increase in the labeled neurons scattered in the superficial layers of the cortex of the pups. Although hypoxia on E18 also resulted in a decrease in the total number of labeled cells in the parietal cortex, the higher number of scattered labeled neurons was observed in the lower cortical layers. As a result, only rats subjected to hypoxia on E14, but not on E18, had impaired development of the whisker-placing reaction and reduced ability to learn reaching by a forepaw [44].

## 4. Changes in the structural-functional organization of the nervous tissue in postnatal ontogenesis after prenatal hypoxia

Using electron microscopy techniques, we have demonstrated that in early postnatal ontogenesis of rat pups subjected to prenatal hypoxia, there is a delay in formation of synaptic contacts in the neuropil, myelinization of nerve fibers, and differentiation of neurons at the ultrastructural levels both in the neocortex and basal ganglia [33, 34]. In particular, on postnatal days P10–30, we have observed a decrease in the total number of pyramidal neurons in layers II–III and V–VI of the brain cortex [45]. There were also changes in the ratio of pyramidal to nonpyramidal neurons in the first month of postnatal development of rats, which is characterized by intensive elimination of excessive cellular material in the brain and formation of intraneuronal contacts and new synapses in the cortical plate. The decrease in the number of pyramidal neurons has been observed only during the first month of postnatal development of rats, but not in adult animals, and also in the group of rats subjected to prenatal hypoxia on E14, but not on E18 [35, 44].

It is important to note the selective effect of prenatal hypoxia on different populations of cells in the brain cortex of rats. Thus, on P10–20, the rats subjected to prenatal hypoxia on E14 have a decreased number of large pyramidal neurons in layers V–VI of the neocortex due to impaired migration of neuroblasts, which forms this cell population. Application of hypoxia on E14 coincides with the period of generation of the first cells of the cortical plate, which later produce corticofugal afferents and serve as the basis for formation of cortical minicolumns. Impaired neuroblast migration in embryogenesis results in scattering of the majority of the pyramidal neurons, which should form layers V–VI of the cortex outside this area, and are

eliminated on P10–30 [44]. In the period of P20–30, in animals subjected to prenatal hypoxia on E14, we have also observed a decrease in the number of small pyramidal neurons in the layers I–III and of nonpyramidal cells (interneurons) along with the total decrease of cell density in the neocortex [45].

By P60, after elimination of excessive cellular material and temporal elements, such as subplates [46], there were no significant structural differences and cell composition between control rats and rats with compromised embryonic development. However, prenatal hypoxia in the period of formation of the first elements of the cortical plate (E14) impaired formation of cortical minicolumns in postnatal ontogenesis [44], which is important for the development of proper neuronal networks and motor reactions. Hypoxia applied to a later period (E18) is not that critical for the formation of brain cortex and does not induce significant alterations in its ultrastructure. Some changes in cell composition have also been observed in the dorsal hippocampus of animals, subjected to prenatal hypoxia. The CA1 area was characterized by a lower level of neurodegenerative changes such as a decrease in the number of neurons in the pyramidal layer and an increase in the number of neurons with retracted apical dendrites. Moreover, changes in the ratio of various cell types and their delayed death have been observed only on P20 [47].

## 5. Role of caspases in structure-function changes in the brain after prenatal hypoxia

There is a good reason to believe that the main cause of the changes in the total density of cell distribution and of their composition after prenatal hypoxia is related to the increase in the elimination of cells caused by impaired migration of neuroblasts during embryogenesis rather than direct cell death caused by hypoxia. The data of our studies testify to upregulated expression and activity of caspase-3 and an increased number of neurons with higher expression of proapoptotic protein p53 in the cortical brain areas in rats subjected to prenatal hypoxia [45, 48], which can be interpreted as induction of cell death via caspase-dependent apoptosis. Although the molecular mechanisms of apoptosis are complex and still far from being fully understood, it is well established that activated caspases affect various proteins in the cell cytoplasm, including cellular proteases which degrade structural and regulatory proteins at the very last stages of apoptosis (for review see [49]). There are data that caspase-3 and caspase-8 are activated in the brain after hypoxia or ischemia [50, 51]. It was suggested that activation of caspases (in particular, of caspase-3) in pre- and postsynaptic terminals leads to proteolysis of various synapse-associated proteins and impairment of neuronal plasticity [49]. Systemic impairment of neuronal contacts, including axo-dendritic, observed in many pathologies and in AD, are also believed to be related to the activation of caspases due to apoptosis induced by accumulation of Aβ [52]. This concept allowed us to consider a possibility of compensating the pathology by using various caspase inhibitors.

The data obtained in our studies demonstrate that caspase-3 regulation in early postnatal ontogenesis is different in animals with normal embryonic development and subjected to

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prenatal hypoxia [53]. Thus, i.v. administration of inhibitors (Z-DEVD-FMK or Ac-DEVD-CHO) on 18–25 days after birth inhibited caspase-3 enzyme activity in the brain cortical structures during 3 days after the injection. However, while in animals subjected to prenatal hypoxia and characterized by increased endogenous caspase-3 activity 1–3 days after the injections, its expression and activity reduced to the control levels in the group of control rats, and administration of the inhibitors led to an increase in caspase-3 activity and expression. One month after the injection of inhibitors, the activity of caspase-3 was found to be down to the initial level characteristics of each group of animals. Administration of Ac-DEVD-CHO to mature rats (P90) with normal embryonic development also led to a decrease in the activity of caspase-3 detected 3 h after the injection. However, there was no subsequent increase of this enzyme activity on days 1 and 3 after the injection, which we observed in young intact animals. Overall, our data suggest that in young and mature rats with normal development and subjected to prenatal hypoxia, the dynamics of caspase-3 activity and properties differ significantly, especially in the period of the most intensive development of the brain [53]. Administration of inhibitors also resulted in prolonged improvement of learning and short-term memory in rats subjected to prenatal hypoxia up to the levels of control animals when tested in the two-level maze even one and half month after the injections.

## 6. Synaptic plasticity as the basis of adaptive potential in neuronal networks

Literature data demonstrate that dendritic spines to a great extent determine the character of cellular interactions and can be considered as a major substrate of the neuronal plasticity. The most prolonged processes involved in memory are dependent on the formation of new dendritic spines, which can rather quickly form synaptic contacts and become active during several hours [54]. The synapses themselves also undergo fast (seconds and minutes) plastic changes that affect the efficacy of synaptic transmission [55] but the formation of memory involves both fast modulations of the synapses and more slow processes of reorganization [29]. In axonal-spine synapses, the spine apparatus is involved in the process of local synthesis, posttranslational modifications, and transport of numerous synapse-associated proteins [56].

One of the marker proteins of dendritic spines is an actin-associated protein synaptopodin. The short form of this protein is localized in the spine apparatus stabilizing the cytoskeleton in the spine neck and modulating actin-based shape and motility of dendritic spines [57]. Synaptopodin is also required for cytoskeletal remodeling of the spines (changing of their size and form), and transgenic mice lacking the synaptopodin gene demonstrate short-term memory impairment, reduction of LTP, and absence of developed spine apparatus in the hippocampal dendritic spines [58]. These observations suggest that synaptopodin participates in the plasticity of neuronal networks due to its ability to reorganize the properties and distribution of labile axon-spinal neuronal contacts [56] and consolidation of memory [59].

Although disruption of synaptogenesis and formation of spine apparatus are considered among the major factors affecting neuronal plasticity, cognitive deficit, and neuronal pathologies, there

are no studies evaluating the ratio of labile and stable axon-spine contacts in neuronal tissue after learning in control animals and with impaired memory. By comparing the number of labile axon-spine contacts in animals subjected to various experimental treatments, we have been able to demonstrate that both short- and long-term memory correlates with the number of synaptopodin-positive dendritic spines in the brain cortex [60]. We have shown that prenatal hypoxia in rats in the period of formation of the minicolumns in brain cortex (E14) results in a decrease in the number of synaptopodin-positive dendritic spines in the molecular layer of the neocortex and in the CA1 area of the hippocampus [61], which was accompanied by impairment of working memory. We suggest that the decrease in the number of labile synaptopodin-positive dendritic spines in the CA1 area of the hippocampus of rats subjected to prenatal hypoxia might be related to the changes in the entorhinal cortex which, in humans, is considered to be the earliest event in the development of AD [62]. According to our data, the reduction of the number of synaptopodin-positive spines along with decreased ability for learning is also observed in normally aging animals, which might be one of the reasons of cognitive dysfunctions related to advanced age, and in the sporadic form of AD [63].

## 7. Impairment of chemical neuronal interactions

Mechanisms of impairment of neuronal interactions caused by pathology in the embryonic period are more complex and not only involve changes in the plasticity of neuronal contacts. Literature data demonstrate that prenatal hypoxia can selectively cause disruption of various mediator systems in postnatal ontogenesis [42, 64, 65]. Using a vesicular acetylcholine transporter (VAChT) as a marker protein we have found that, in adult rats subjected to prenatal hypoxia on E14, the number of VAChT-positive cholinergic terminals, which form synapses on the bodies of the pyramidal neurons in the V–VI layers of the parietal cortex is decreased compared to control

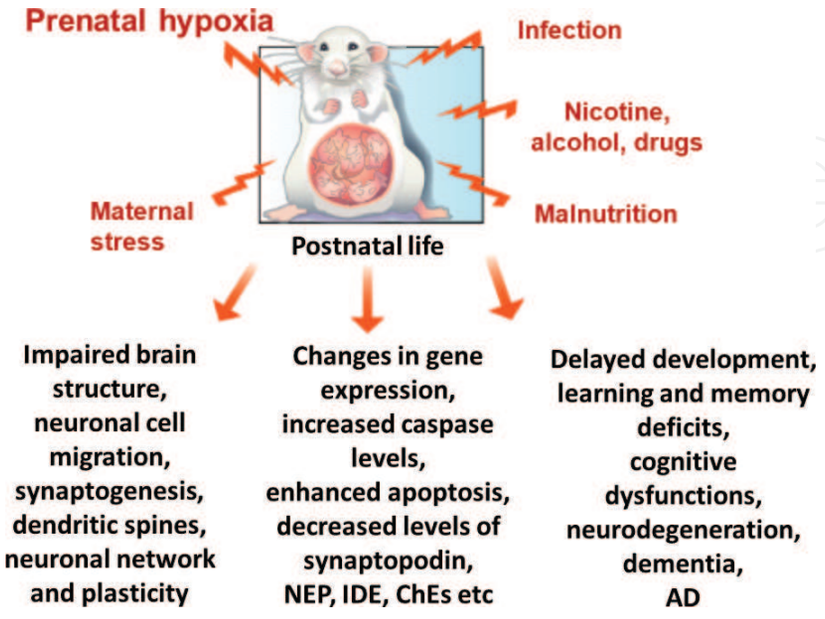


**Figure 1.** Effects of various types of prenatal pathology on the processes underlying development of organisms in postnatal life.

animals [66]. This also testifies to the changes in the cholinergic modulation of the cortical minicolumns in the brains of these animals, which might lead to the cognitive dysfunctions.

Comparative analysis by immunoblotting of the content of synaptophysin localized in the presynaptic terminals, independent on the nature of their mediator, as well as of the excitatory amino acid transporter (EAAT), as a marker of active glutamatergic terminals, has revealed that although in rats subjected to prenatal hypoxia synaptophysin content was not different from the controls, the EAAT levels were much higher [67]. This suggests that the intensity of glutamate release into the synaptic cleft in hypoxic animals is higher than in controls, which might provoke spontaneous epileptogenic activity and increase kindling in response to pharmacological agents and other external stimuli. These data also confirm more complex impairment in brain interconnections in postnatal ontogenesis of animals subjected to prenatal pathology. Our views on the processes underlying the changes observed in brain function after prenatal hypoxia are summarized in **Figure 1**.

## 8. Changes at the molecular and biochemical levels

Apart from the functional and structural changes induced by prenatal hypoxia in the nervous tissue of experimental animals, there were also significant alterations at the molecular and biochemical levels. Thus, in postnatal ontogenesis of hypoxic rats, the activity of acetyl- and butyrylcholinesterases (AChE and BChE) in the sensorimotor cortex had a significantly different dynamics compared to controls [68]. Apart from decreased activity of these enzymes during the first month of postnatal ontogenesis (and active formation of synaptic contacts), there were also significant changes in the distribution of the membrane-bound (involved in signal transduction) and soluble (participating in synaptogenesis [69]) forms of AChE. Moreover, with aging in rats subjected to prenatal hypoxia, there was an increase in the ratio of BChE in the total cholinesterase activity that could have a compensatory nature since this enzyme plays an important role in hydrolyzing various toxic agents, which might be produced by impaired brain tissue. On the other hand, increased activity of BChE in the brain is one of the characteristic features of AD and can be a marker of disruptions predisposing to neurodegeneration [70].

Analysis of the content of APP in the sensorimotor cortex also revealed different dynamics of expression of this protein in the postnatal ontogenesis of rats subjected to prenatal hypoxia [71]. While hypoxia led to an increase of the membrane bound APP at all analyzed stages of animal development, the production of its soluble forms (sAPP), which possess protective neuritogenic properties was decreased [72]. The most significant changes were observed on P10-P30 when a deficit of this neuritogenic factor might lead to disruption of formation of neuronal networks in the brain. Our data also testify that hypoxia significantly modifies the activity of $\alpha$-secretase, which is important in production of the main pool of soluble APP and prevention of the formation of Aβ. The deficit of this enzyme might also lead to a decreased production of soluble AChE [73]. In general, the increase of APP content in the brain and reduction in its nonamyloidogenic processing by $\alpha$-secretase after prenatal hypoxia could predispose to a shift in amyloid metabolism in the brain toward the processes initiating development of AD (**Figure 2**).

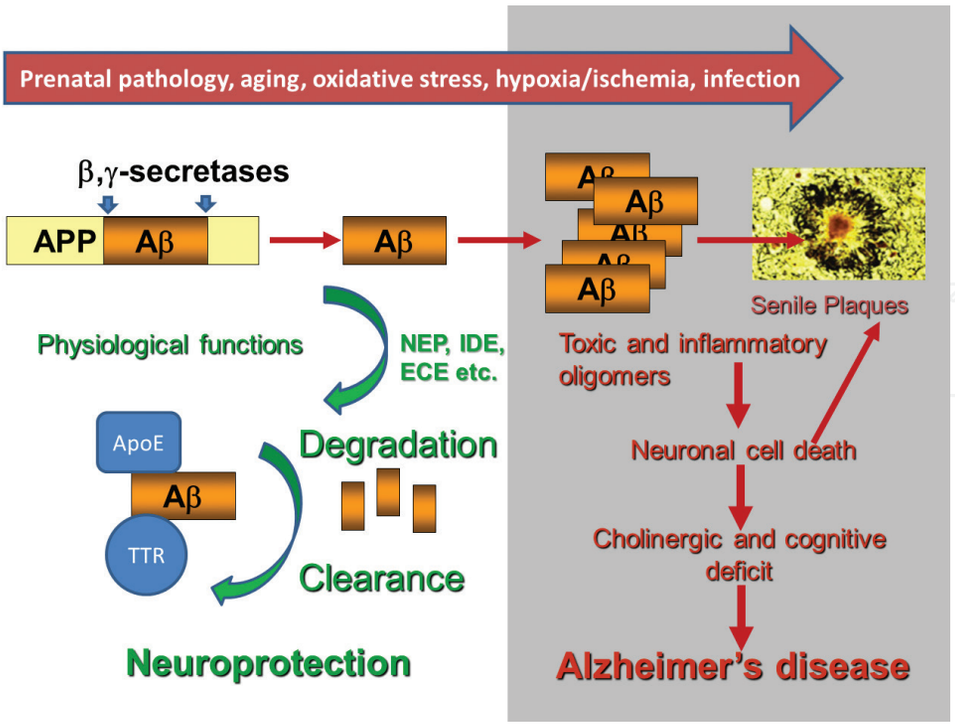


**Figure 2.** Schematic representation of the processes promoting the development of Alzheimer's disease. Amyloid peptide Aβ is produced from a large amyloid precursor protein (APP) after sequential cleavage by β- and γ-secretases. Aβ has a property to aggregate and form oligomers and fibrils which are toxic to the cells. In a complex with other proteins, it forms senile plaques. In the brain, there are various enzymes which can cleave Aβ, including neprilysin (NEP), insulin-degrading enzyme (IDE), and endothelin-converting enzyme (ECE). Transport proteins transthyretin (TTR) and apolipoprotein E (ApoE) are involved in Aβ clearance. Under pathological conditions, including prenatal hypoxia, and with aging, levels of NEP and IDE are reduced, and clearance of Aβ is compromised. Because hypoxia also leads to increased expression of APP, together with reduced Aβ clearance, it promotes Aβ production and accumulation resulting in the development of late onset Alzheimer's disease.

An important factor which leads to the accumulation of Aβ in the nervous tissue and causing development of the sporadic form of AD is a deficit in the activity of amyloid-degrading enzymes and impairment in the removal of this peptide from the brain (for review see [74]). According to our data, prenatal hypoxia leads to a decrease in expression of the activity of the major amyloid-degrading enzyme neprilysin (NEP) and its homolog endothelin-converting enzyme (ECE-1) [72], which could facilitate accumulation of Aβ and development of AD pathology and memory impairment. The levels of the NEP expression and activity in the brain also decrease with age in normal rats and humans as well as in AD patients [37, 72, 75]. Our experiments with administration of the NEP and ECE inhibitor phospharamidon in the cortex of intact adult rats ($2 \times 10^{-3}$ M, 0.25 μl per h during 28 days using a mini-pump, Alzet, USA) demonstrated a disruption of short-term memory in the radial maze and decrease in the average number of synaptopodin-positive spines in the molecular layer of brain cortex. These results were in good agreement with the data of our previous study with multiple single injections (6–8 times with one-day interval) of the NEP inhibitors phosphoramidon or thiorphan [76].

In animals subjected to prenatal hypoxia, the decrease in NEP activity was observed also in the hippocampus [37]. However, in blood plasma of such animals, we observed an increase in NEP activity compared to controls which testifies to the difference in regulation of NEP

expression in the brain and peripheral tissues. Moreover, the increase of NEP expression in plasma might be a result of compensatory changes to lower the levels of brain Aβ via maintaining the existing balance between its pools in the brain and blood [77].

Analysis of expression of another amyloid-degrading protease—insulin degrading enzyme (IDE), in brain structures after prenatal hypoxia revealed its decrease in different brain structures. During postnatal ontogenesis starting from P30 and in very old rats (P600), the content of this enzyme in the cortex was lower by 40–50% and in the striatum by 30% than in the controls. The most significant decrease in IDE expression (more than by 60%) was observed in hippocampus on P20. The decrease in IDE expression was also observed in animals during normal aging and in the case of experimental diabetes [78]. Since IDE also plays an important role in insulin metabolism its deficit after prenatal hypoxia might not only lead to the pathology caused by accumulation of Aβ but also to diabetes.

Searching for the means to increase the activity of amyloid-degrading enzymes, in particular of NEP, in the brain, it was found that NEP expression can be regulated via a feedback mechanism by the C-terminal fragment of APP named AICD, which is released together with Aβ [79, 80]. It was also shown that repression of the NEP gene is epigenetically regulated by histone deacetylases (HDAC) and their inhibition by valproic acid (VA) or trichostatin A results in a significant increase in NEP mRNA and protein content as well as of enzyme activity [80]. A similar mechanism of regulation by AICD and HDAC was also shown for a transport protein, transthyretin (TTR), which is involved in removal of Aβ from the brain (**Figure 3**) [81]. Antioxidants, in particular epigallocatechin gallate (EGCG), were also shown to increase NEP expression and improve neurological deficit in Parkinson's disease and AD [82, 83]. In our experiments with animals subjected to prenatal hypoxia, it was shown that *i.p.* injections of VA or *i.c.* injections of EGCG significantly increased expression of NEP in the cortex and hippocampus bringing it up to the level of control animals [37, 63]. The increase in NEP activity correlated with an improvement of animal performance in the radial maze and with restoration of the short- and long-term memory in the novel object recognition test. Moreover, there was also an increase in the number of the labile spines in the cortex after VA injections and in the hippocampus after EGCG injections compared to animals with normal embryogenesis or injected with saline.

In the studies of the effects of hypoxia on NEP expression in human neuroblastoma cells NB7, we found that hypoxia leads to a reduction of AICD content in the cells and its binding to the NEP promoter [84], which correlated with increased expression of a number of caspases, including caspase-3, which readily cleave AICD. Addition of a caspase-3 inhibitor Z-DEVD-FMK to the cells resulted in restoration of AICD levels and NEP expression and activity which correlated with reduced amount of Aβ secreted by the cells [84]. In the rats subjected to prenatal hypoxia, we have also observed a decrease of AICD content in the brain cortex, which correlated with reduced NEP expression [48]. These studies demonstrate a link between the changes in epigenetic regulation of neuronal genes caused by prenatal hypoxia, the level of neuronal plasticity and activation of caspases, which can degrade not only transcription factors like AICD but also cytoskeletal and synaptic proteins. Further studies using our model of prenatal hypoxia will allow us and others to get a deeper insight into the mechanisms of regulation of cognitive functions at the molecular levels, which could provide a basis for design of preventive measures and therapy of cognitive disorders.

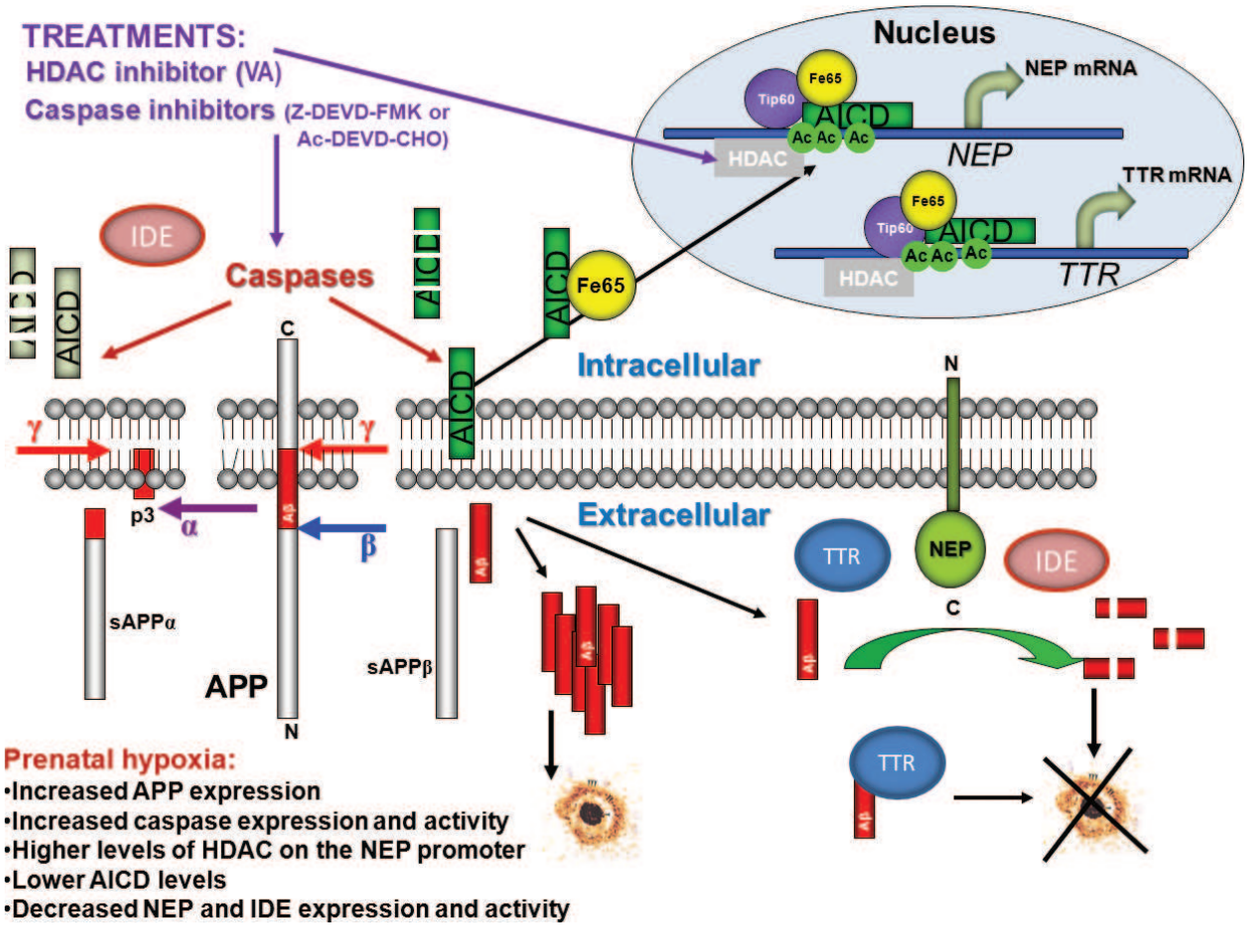


**Figure 3.** Effects of prenatal hypoxia on regulation of amyloid-clearing proteins and possible ways of their pharmacological up-regulation. APP is metabolized in cells via two distinct amyloidogenic and nonamyloidogenic pathways. In the amyloidogenic pathway, APP is first cleaved by β-secretase releasing a soluble ectodomain (sAPPβ) and the C-terminal fragment CTF99. The latter is cleaved by a multiprotein complex, γ-secretase, which includes presenilin-1, generating the transcriptional regulator known as the APP intracellular domain (AICD), and amyloid-β peptide (Aβ). In the nonamyloidogenic pathway, the APP molecule is first cleaved by an α-secretase within the Aβ-domain releasing a soluble ectodomain sAPPα and the C-terminal fragment CTF83. Proteolytic cleavage of CTF83 by γ-secretase also releases AICD and a short p3 fragment whose functions are still unknown. The soluble APP ectodomains, sAPPα and sAPPβ, have neuroprotective properties. AICD produced in the nonamyloidogenic pathway is cytoplasmic and readily cleaved by IDE, caspases or other proteolytic enzymes. It can also bind various proteins regulating their properties. The AICD fragment produced in the amyloidogenic pathway together with a stabilizing protein Fe65 and in a complex with other factors (including the histone acetyltransferase, Tip60) binds to an RNA polymerase mediator complex subunit Med12 acting as a transcription factor competing with histone deacetylases (HDAC) in regulation of variety of genes, including the amyloid-clearing proteins NEP and TTR allowing cells to control Aβ levels. After prenatal hypoxia in the brain, there is an increase in APP levels; however, the activation of caspases significantly reduces AICD content leading to decreased NEP expression and activity. It also results in higher levels of HDAC binding to the NEP promoter reducing its availability for AICD binding. Prenatal hypoxia also leads to reduced levels of IDE in the brain and to some changes in TTR expression as such shifting the amyloid balance toward accumulation of Aβ and neurodegeneration. Administration of an HDAC inhibitor valproic acid (VA) allows binding of AICD to the NEP promoter and restoration of NEP expression and activity. Caspase inhibitors also facilitate this process by restoring levels of AICD and protecting the brain from cognitive decline and neurodegeneration.

## 9. Concluding remarks

Despite the difference in the mechanisms of genesis of cognitive dysfunctions observed after prenatal pathology and development of such neurodegenerative disorders as AD, both these

pathologies have common features. In early postnatal ontogenesis after prenatal hypoxia and at the early stages of mild cognitive impairment, there is dysregulation of synapse-associated proteins which is accompanied by complex disruption of neuronal interactions and by a decrease in the plasticity and adaptive potential of the cortical areas of the brain. The increase in caspase expression and activity caused by prenatal hypoxia in early postnatal ontogenesis of animals subjected to prenatal hypoxia can also lead to excessive degradation of synapse-associated proteins and degeneration of the synapses, inducing cognitive dysfunctions in postnatal ontogenesis and with aging. Changes in the activity of the enzymes participating in production and catabolism of Aβ which is the major causative agent in AD can also lead to predisposition to Aβ accumulation with aging. In rats subjected to prenatal hypoxia, these changes can be observed at significantly earlier stages of development making them a useful tool for analyzing epigenetic and molecular mechanisms of brain development during postnatal ontogenesis and leading to cognitive dysfunctions. This model can also be used for testing various pharmacological agents for their efficacy to prevent or treat various neurodegenerative disorders. Overall, these studies prove that the postulate proposed by the famous physiologist and evolutionist Leon A. Orbeli about the importance of using experimental animal models at various stages of phylogenetic and ontogenetic development as the key instruments of comparative physiology is also perfectly true and effective for studying human pathologies.

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