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REVIEW

DNA Instability in Neurons: Lifespan Clock and Driver of Evolution

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Abstract—In the last ten years, the discovery of neuronal DNA postmitotic instability has changed the theoretical landscape in neuroscience and, more broadly, biology. In 2003, A. M. Olovnikov suggested that neuronal DNA is the "initial substrate of aging". Recent experimental data have significantly increased the likelihood of this hypothesis. How does neuronal DNA accumulate damage and in what genome regions? What factors contribute to this process and how are they associated with aging and lifespan? These questions will be discussed in the review. In the course of Metazoan evolution, the instability of neuronal DNA has been accompanied by searching for the pathways to reduce the biological cost of brain activity. Various processes and activities, such as sleep, evolutionary increase in the number of neurons in the vertebrate brain, adult neurogenesis, distribution of neuronal activity, somatic polyploidy, and RNA editing in cephalopods, can be reconsidered in the light of the trade-off between neuronal plasticity and DNA instability in neurons. This topic is of considerable importance for both fundamental neuroscience and translational medicine.

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INTRODUCTION

"A combination of various facts indicates that it is the brain that is the initial substrate of aging, and in a brain cell, this substrate is DNA."

A. M. Olovnikov, 2003

Alexey Matveyevich Olovnikov is widely known for his outstanding theoretical contribution to the field of biological chronometry and studies of mechanisms regulating life expectancy. He predicted the existence of telomeres, the "counters" of cell divisions, in proliferating cells even before their experimental discovery [1, 2]. After creating the telomere hypothesis of cell aging, A. M. Olovnikov has retained his interest in the problem of biological time [3]. In 2003, he publishes a large theoretical work on the redusome hypothesis of aging, in which he suggested the following: "A combination of various facts indicates that it is the brain that is the initial substrate of aging, and in a brain cell, this substrate is DNA". At that time, experimental data were still sparse and scattered; however, in recent years, the likelihood of this hypothesis has been increased by the results of numerous molecular neurobiology studies.

The aim of this article is to analyze experimental data published mostly during the last decade, that have shed light on the accumulation of damage by neuronal DNA and relation between this process and organism's aging and lifespan. We will discuss factors contributing to the accumulation of postmitotic DNA damage in neurons and specific genome regions, where this accumulation takes place. New data on a high instability of neuronal DNA have been changing our understanding of multiple processes in the nervous system, as well as of the evolution of nervous system itself.

Abbreviations: DSB, double-strand DNA break; indel, small insertion or deletion; NMDA, N-methyl-D-aspartate; Parp1, poly(ADP-ribose) polymerase 1; SNV, single nucleotide variant; SSB, single-strand DNA break; Topo IIβ, topoisomerase Iiβ.

IF NOT UNDERREPLICATION, THEN WHAT?

One of the problems with the existence of neuronal "clock" measuring biological time in neurons is terminal differentiation of these cells, i.e., the absence of mitotic division in their adult life [4, 5]. Therefore, the "clock" mechanism proposed by A. M. Olovnikov for dividing cells monitoring the shortening of specific DNA sequences due to the underreplication of chromosomal ends during mitosis [4] cannot be used for a postmitotic neuron. Olovnikov was sure that "for the sake of clock operation (in neurons – author's note), nature had to invent a mechanism that could work even in the absence of DNA replication" [4]. In his latest publication in 2022, Olovnikov proposed the idea of epigenetic labeling of specialized temporal DNA [3]. Long before that, he had suggested that such mechanism could be based on the formation of DNA breaks (e.g., during active transcription) and incomplete DNA repair, especially in the terminal sections of hypothetical "clock" DNA [4]. And only ten years after publication of his work [4], the first experimental data were obtained that demonstrated formation of single- and double-strand DNA breaks in neurons and their subsequent repair even in the course of normal physiological activity of these cells.

NORMAL PHYSIOLOGICAL ACTIVITY RESULTS IN THE FRAGMENTATION OF NEURONAL DNA

Year 2013 has not yet been recognized by the neuroscience community as a year of a very important and, perhaps, one of the most unexpected discoveries in neuroscience, the full consequences of which would be realized later. A group studying a mouse model of Alzheimer's disease discovered a higher content of double-strand DNA breaks (DSBs) in some brain regions after two hours of normal activity in an open field, which was observed not only in the diseased mice, but also in healthy control animals [6]. After 24 h, most DNA breaks in the control mice were repaired, while in animals with the Alzheimer's disease, both the number of neurons with fragmented DNA and the extent of DNA fragmentation (assessed by the length of fluorescent "tail" formed in the electromagnetic field by the nuclei with damaged DNA) remained significantly higher. To exclude the effect of stress caused by an open field the authors used adrenalectomised animals with implanted corticosterone pellets to hold their corticosterone level constant. This operation did not decrease the content of DSBs. Thus, stress seems not to account for the effect of novelty on DSBs content. Moreover, sensory stimulation in healthy animals led to the DNA fragmentation in the brain areas associated with sensory input. Thus, lateral optical stimulation increased the number of neurons DSBs in corresponding side of the visual cortex. Optogenetic stimulation of the striatum also increased the number of DSB-containing neurons [6].

Already two years later, it was demonstrated using next-generation whole-genome DNA sequencing that the electrical activity of neurons, their excitation, induce formation of DSBs [7]. Moreover, it is the DNA damage that links the electrical activity of neurons and transcription of neuronal immediate early genes (IEGs), such as *Fos*, *Npas4*, and *Egr1* (it should be noted that expression of these genes had been used as a marker of active neurons). Formation of DSBs in the promoter regions of IEGs was sufficient to activate their expression. It was suggested that the enzyme linking electrical activity of neurons and DSB formation is DNA topoisomerase IIβ (Topo IIβ). This enzyme introduces temporary breaks in DNA and then restores two DNA strands, leading to DNA demethylation in the promoters of early response genes and their transcription. The knockout of the Topo IIβ gene abolished formation of DSBs induced by the electrical activity of neurons and transcription of early response genes [7]. In 2022, Delint-Ramirez et al. [8] published an article that explained how cell excitation affects the activity of Topo IIβ [8]. Calcium influx in response to the activation of excitatory glutamate NMDA (N-methyl-D-aspartate) receptors activates the phosphatase calcineurin. Calcineurin dephosphorylates Topo IIβ at S1509 and S1511 residues, which stimulates its DNA cleavage activity and leads to the formation of DSBs. During the electrical activity of neurons, calcineurin interacts with Topo IIβ mostly at the nuclear periphery, where DNA breaks occur [8]. By showing that DNA breaks and their consequences took place in particular genome regions, the authors provided another important point in favor of the Olovnikov's concept. Beside involvement of calcium signaling in the formation of DSBs, we should mention the influence (although less specific) of intense neuronal metabolism, leading to the generation of free radicals and reactive oxygen species, which are commonly recognized as factors contributing to the instability of neuronal DNA [9].

The genome of neurons has been also searched for the hotspots of single-strand DNA breaks (SSBs), another type of DNA damage [10, 11]. It was found that postmitotic human neurons derived from pluripotent cells demonstrate unexpectedly high levels of SSBs in particular genome regions. Using genome-wide mapping, it was suggested that these breaks were located at or near CpG dinucleotides and sites of DNA demethylation within gene enhancers [10]. Similar conclusions were reached in 2021 by Reid et al. [12], who demonstrated that the processes of DNA repair (and hence the initial damage) often take place at the well-defined hotspots adjacent to important genes. These hotspots are enriched with histone γH2AX isoforms and RNA-binding

proteins and associated with evolutionarily conserved regulatory elements of the human genome. These stunning results required comprehension.

DNA BREAKS: A MECHANISM OF NEURONAL PLASTICITY OR A COST OF IT?

Year 2013 was signified by another development due to the publication of a theoretical work by the Russian scientist Alexei Krushinskii [13] (later, a revised version of this article was published in an open access journal [14]). Based on the Leon Brillouin's negentropy principle of information, Krushinskii suggested that to receive a new information, the brain should pay not only with energy, but also with a loss of original information. At present, this hypothesis looks as a remarkably beautiful description of events that molecular neuroscientists encounter in their studies of neuronal DNA, the "initial substrate" of intelligence and aging.

Madabhushi et al. [7], who were the first to link neuronal activity and transcription of early neuronal genes with the formation of DSBs, made a shocking conclusion that disruption of brain genome integrity is a natural physiological phenomenon essential for synaptic plasticity, learning, and memory, since these processes require participation of early response genes. "If you don't break DNA, you can't memorize" – this interpretation has been accepted by a number of researchers who have experimentally confirmed the need for temporary DNA damage for the formation of various types of memory and learning [15] or who have adhered to this concept in their review [16]. Some researchers were more wary of this new "tear, repair, remember" paradigm of memory formation at the DNA level [17-19]. After all, DNA repair is not a 100% efficient process, and if DNA breaks occur so often, the damage to the brain genome will inevitably accumulate in a form of both incomplete repair and errors (i.e., mutations). Do mutations accumulate?

ARE THERE MANY MUTATIONS IN THE GENOMES OF INDIVIDUAL NEURONS AND HOW ARE THEY DISTRIBUTED?

Sequencing of genomes of individual mouse neurons [20] revealed an unusually high frequency of postmitotic mutations in evolutionarily conserved genome regions, such as exons and promoters of genes with the highest expression levels. In other words, mutations accumulate at the hotspots, which are also the hotspot of DNA breaks. The most common type of mutation is a single nucleotide variant (SNV) [20] that might result from an error in the activity of repair DNA polymerases. A similar conclusion was reached in the analysis of mutations in human neurons [21-23]. Luquette et al. [23] used an improved primary template-directed amplification (PTA) method, which allowed to reduce the number of artifacts in detection of somatic mutations by DNA sequencing. Whole-genome sequencing data from fifty-two PTA-amplified single neurons were analyzed using SCAN2, a new genotyping method developed by the authors to identify SNVs and small insertions and deletions (indels). The results of analysis confirmed that the number of somatic mutations in individual human neurons increased with age and that mutations accumulated in functional genome regions, such as enhancers and promoters [18, 22, 24-26].

The table shows the data on the types of DNA damage in neurons occurring during normal physiological activity or induced by various physiological factors.

NEURONAL ACTIVITY REDUCES LIFESPAN

A strong argument in favor of the hypothesis that an organism pays for the neuronal activity with something truly significant was obtained in 2019 [27], when it was found that the level of neuronal excitation directly affects the lifespan. Zullo et al. [28] searched for the genes expressed in the human frontal cortex and associated with healthy longevity. Differential analysis of their expression was carried out in two groups of cognitively healthy people who died at the age of 70-80 and 85-100 years, respectively. Previously, the same group of researchers had found an increase in the content of protein encoded by the *REST* gene in centenarians [28]. The authors revealed an inverse correlation between the level of *REST* mRNA and the content of mRNAs of the genes associated with neuronal excitation [27]. The expression level of these genes, whose promoter regions contained the binding site for the repressor protein REST, was significantly lower in humans with extended longevity [27]. The authors also studied the relation between the *REST* expression and electrical activity of neurons in mice. Neurons of REST-deficient mice accumulated fluorodeoxyglucose (^{18}F-FDG), indicative of increased neural activity. Intermittent epileptiform discharges were significantly more frequent in REST-deficient animals vs. control mice [27]. These findings suggested a link between the neuronal excitation and lifespan in mammals, which was proven in a model very distant from mammals – the nematode *Caenorhabditis elegans*. These worms have an ortholog of mammalian *REST* gene, *spr-4*, the protein product of which protects cells from the damaging effects of reactive oxygen species and some other adverse factors. The authors used a broad arsenal of tools, from pharmacological agents that altered (promoted or inhibited) excitation to animals with suppressed or hyperactivated

DNA damage in neurons occurring during normal physiological activity or induced by various physiological factors

Type of DNA damage	Organism	Factor	Year	Source
DSBs	mice (wild-type)	exploratory behavior in open field	2013	[6]
DSBs	mice expressing hAPP (Alzheimer's disease model)	exploratory behavior in open field	2013	[6]
DSBs	mice (wild-type)	visual stimulation	2013	[6]
DSBs	mice (Adora2a-Cre)	optical stimulation of striatum	2013	[6]
DSBs	cultured mouse primary neurons	β-amyloid oligomers; NMDA-dependent effect	2013	[6]
DSBs	cultured mouse primary neurons	potassium chloride (KCl), NMDA, or bicuculline (promotion of neuronal excitation)	2015, 2023	[7, 19]
DSBs	mouse hippocampal slices	NMDA receptor agonist	2015, 2022	[7, 8]
DSBs	mouse hippocampus	memory reconsolidation	2020	[15]
DSBs	zebrafish *Danio rerio*	normal daily activity	2018	[32, 33]
DSBs	*Drosophila* larvae and imagoes	–	2020	[75]
SSBs	human neurons derived from pluripotent cells	–	2021	[10-12]
Mutations (SNVs, indels)	mouse neuron clones	–	2016	[20]
Mutations (SNVs, indels)	human neurons	–	2012, 2018, 2022	[21, 23, 24]

spr-4 gene (obtained using the CRISPR-Cas9 system), as well as with activated or repressed groups of excitatory or inhibitory neurons. The results clearly indicated that neuronal excitation decreased the lifespan, while suppression of excitation extended it. Why? It might seem surprising, but the authors of this discovery, which was published in the *Nature* journal, did not discuss the link between the neuronal excitation, disruption of genome integrity, and induction of DSBs! They did not even cite the reports of Suberbielle et al. [6] from 2013 and Madabhushi et al. [7] from 2015 that declared the role of neuronal excitation in the formation of DSBs in their titles.

A deeper insight into the mechanisms underlying the influence of neuronal excitation on the lifespan presents several possibilities. The first one suggests a direct effect of accumulated DNA damage. Thus, it is known that an incomplete repair of DSBs can trigger apoptosis in neurons (see review by Boutros et al. [16]). Another one is direct reduction of the overall viability of an organism by accumulated mutations [18, 26]. Indeed, there is an inverse correlation between the somatic mutation load and lifespan in *Drosophila melanogaster* [29] and mammals (however, in mammals, this association was described for the intestinal epithelium cells, as neurons were not assessed) [30]. Moreover, the effect of mutations on fitness can be greater than previously thought, since even synonymous mutations, earlier considered as mostly neutral, turned out to be strongly non-neutral because they change the level of gene expression and affect the organism [31].

The second option, which assumes an existence of a neuronal chronosome [4], would explain the influence of neuronal excitation on the rate of DNA shortening in such chronosome. Critical shortening leads to the neuronal death and, according to Olovnikov's hypothesis [4], has a hormonal effect on the entire organism.

Given that mitosis and excitation are the main factors of mutagenesis in dividing cells and neurons, respectively, the author of the current article proposed in 2020 [5] an existence of indirect neural mutation counter.

Similar to telomeres that "count" cell divisions in proliferating cells and indirectly determine the number of accumulated mutations, neurons might have a "counter" of excitations that estimates the duration of their history of excitation and determines the lifespan of a neuron. This counter could be a gene, whose expression gradually and irreversibly declines upon neuronal excitation and whose product acts as a repressor of apoptosis.

The report of Zada et al. [32, 33] indicated that the DNA repair enzymes themselves can activate various processes (e.g., sleep) at the organismal level. Thus, it was found that poly(ADP-ribose) polymerase 1 (Parp1), which initiates the repair of DSBs accumulated during the daytime activity, induces sleep in fish hatchlings and adult mice. The activity of Parp1 increases with sleep deprivation, while inhibition of this enzyme reduces the nighttime chromosome movement and suppresses repair of DNA damage accumulated during the daytime. These data suggest that the DNA repair system may also signal to the mutation counter.

The mechanisms underlying the influence of neuronal excitation on the lifespan remain to be elucidated, but the participation of neuronal DNA breaks in these mechanisms seems very likely. Interestingly, A. M. Olovnikov in his 2003 paper [4] referred to an older work showing that irradiation of the brain, leading to the DNA fragmentation in neurons, shortened the life of *Drosophila* larvae [34]. Unfortunately, at that time, it was still unknown that neuronal excitation affects both DNA stability and lifespan. However, Olovnikov's idea that "it is the brain that is the initial substrate of aging, and in a brain cell, this substrate is DNA" has been confirmed.

The finding that DNA of differentiated neurons is extremely unstable could significantly change many concepts of how the brain works and how it evolves. The following sections will provide examples how the well-known facts and recent discoveries can be reinterpreted when viewed from this perspective.

THE COST OF INTELLIGENCE: TRANSITION TO THE MOLECULAR LEVEL

It has been long known that although cognitive activity provides enormous benefits, it comes at a certain biological cost. For humans, this was discussed a century and a half ago in the famous work of Cesare Lombroso "*The man of genius*" [35], as well as in more modern works (for example, Gale et al. [36] and Smith et al. [37]). A new area of psychology has emerged, cognitive epidemiology, that studies the relationship between IQ and various physiological, genetic, and social factors in people [38]. In general, IQ positively correlates with health and longevity, which could be explained by a simple inability of a sick body to provide a high cognitive performance [38]. Obviously, social factors (e.g., access to better food, living conditions, and medicine) play an important role in ensuring this positive connection. People with an average and high IQ do not show an increased predisposition to mental illness, unlike people with either low or very high IQ that exhibit a higher predisposition to developing bipolar disorders [36]. In recent years, this research area has been significantly enriched by the data of genetic studies [39, 40]. It was shown that genes typical for people with pronounced creative abilities are also risk factors for various pathologies [39]. Later, a similar correlation with a predisposition to neuropathology was found for genes associated with the ability of individuals to obtain higher education [40].

Some negative consequences of cognitive activity have been described in mammals [13, 14, 41-45]. At the beginning of this century, the first experimental results were obtained indicating a high biological cost of learning, as well as selection for cognitive abilities in invertebrates (flies) [46-50]. "Smart" fruit flies had reduced stress resistance, fertility, and life expectancy. A similar correlation between the ability to learn and susceptibility to stress was found in two different populations of pond snails [51, 52]. However, methodological limitations and lack of understanding of where and at what level to look for the mechanisms providing the link between these functions have hindered the development of this interesting and important area of research. Partly, this was also due to a simple, falsely reassuring explanation: the brain consumes a lot of energy during cognitive activity, so that other organs or functional systems may suffer from the energy deficit [49, 50]. Now the situation has changed. Recent methods of genome sequencing and transcriptomic and epigenetic analysis of neurons have made it possible to study the molecular basis of the "cost of intelligence."

In previous sections, we discussed the influence of neuronal excitation on the formation of DNA breaks. However, there exists an epigenetic substrate of cognitive functions that can also increase the susceptibility of neuronal genome to the accumulated damage.

The statement that cognitive functions are based on the plasticity of expression of the neuronal genome appeared more than 15 years ago and has been confirmed experimentally in both vertebrates [53, 54] and invertebrates [55, 56]. As a rule, the open chromatin state and DNA demethylation correspond to higher cognitive levels [57-61]. Recently, additional evidence at the genome level has emerged indicating that not only learning and memory, but also a new environment which stimulates neurogenesis and learning, are associated with chromatin decondensation [62]. The same effects are caused by the motor activity (which also stimulates neurogenesis and memory) even in the first-generation progeny [63-65]. Finally, according to some data, prolonged excitation predisposes neuronal genome to DNA demethylation and/or heterochromatin decondensation [66-68].

Theoretically, both processes reduce the protection of DNA from possible mutations and increase the likelihood of transposable element insertion. The above-mentioned age-related accumulation of indels in the promoters of neuronal genes can be associated with the activity of transposable elements (see Dumitrache and McKinnon [69]) and not only with the repair of DNA breaks.

In other words, it is possible that brain "pays" for the acquisition of new information, thinking, and plasticity with the damage of its genetic information. Post-mitotic DNA damage in neurons is a relatively new topic (the first data were obtained only 10 years ago). From the evolutionary point of view, on the contrary, it is an ancient problem that might have appeared simultaneously with the appearance of first neurons (approximately 600 million years ago) and, one way or another, had been resolved in the course of evolution and, probably, differently in different taxa. The following sections of the review will discuss some possible evolutionary solutions to the problem of minimization of mutational cost of brain plasticity and its biological consequences.

EVOLUTIONARY SOLUTIONS TO REDUCE THE BIOLOGICAL COST OF BRAIN PLASTICITY

DNA repair systems in the brain. It is obvious that if neuronal activity poses an inherent risk to the genome stability, one way to adapt to it would be to improve the DNA repair systems in the brain. Indeed, the knockout of the DNA repair genes involved in the repair of SSBs caused multiple errors in the functionally significant genome regions, mainly in the enhancers [10]. The authors suggested that disruptions of this system could be associated with the development of human neurodegenerative diseases. Indeed, the risk of brain pathologies should increase with any DNA repair deficiency in neurons. Analysis of this relationship and studies of neuronal DNA repair systems have recently received considerable attention of researchers (for more information, see the review by Li et al. [25]).

The study published in February 2023 demonstrated that in the course of evolution, neurons have acquired specialized mechanisms for genome protection allowing them to withstand for decades the effects of various damaging factors during the periods of increased activity [19]. A DNA repair mechanism dependent on the neuronal activity has been identified that involved accumulation in the activated neurons of a novel form of NuA4-TIP60 chromatin modifier. This accumulation was observed in the regions also expressing the neuron-specific inducible transcription factor NPAS4. By examining the pattern of DSBs caused by the brain activity, the authors showed that NPAS4–NuA4 binds to the damaged regulatory elements in the genome. Disturbances in the NPAS4–NuA4 repair system result in multiple cellular defects, including changes in transcription, loss of the neuronal inhibition control, and genomic instability, which can shorten the lifespan of an organism. Therefore, the discovered neuron-specific complex directly links neuronal activity to the genome stability maintenance. These results are in good agreement with the hypothesis on the improvement of DNA repair systems as one of the important directions in the evolution of nervous system.

Minimization of synchronism between chromatin open state and electrical excitation? Although neuronal excitation is often followed by the upregulation of gene expression [66-68, 70, 71], it would be safer for the neuron to avoid simultaneous occurrence of electrical activity and epigenetic processes characterized by the open DNA state. In 1966, the first data showing that electrical stimulation of mollusk neurons causes a transient inhibition of mRNA synthesis with a strong rebound effect (activation of mRNA synthesis above the initial level) after cessation of electrical activity were obtained [72, 73], that were interpreted within the framework of hypothesis suggesting redistribution of metabolism and reduction of energy expenditures during cell excitation. However, these data take on a new meaning with current understanding of excitation as a threat to DNA stability. The transient shutdown of gene expression can be viewed as a mechanism protecting neuronal DNA.

Other data mentioned above indicate that the remodeling of active chromatin and DNA repair in fish motor neurons occurred during the sleep characterized by a shutdown of the electrical activity of these neurons [32]. Several recent reviews have summarized a current state of affairs in the studies of the gene expression dependence on the neuronal activity in mammals and model invertebrates [70, 71, 74], illustrating its complex and controversial nature.

Polyploidy of neurons. Accumulation of a large number of DNA mutations in the neurons of healthy people and animals indicates that DNA repair mechanisms are not efficient enough to ensure the stability of the neuronal genome. In this situation, an existence of "genetic safety net" could be another effective approach for reducing the biological cost of the nervous system activity. Since mutation is a random event, simply increasing the number of genome copies can be such safety mechanism. However, the number of genome copies can increase in different ways. Within a single neuron, it is a well-known phenomenon of somatic polyploidy. The number of neurons can be increased as well. Different animal taxa have implemented different solutions. Thus, vertebrates have an increased the overall number of cells, while some protostomes, for example, gastropods (but not only them), use somatic polyploidy [75, 76].

Giant neurons (up to 1 mm in diameter) of gastropods have been known for a long time. Most of cell body

in these neurons is occupied by the nucleus [77, 78]. It was found that this giant nucleus contains multiple genome copies (up to 600,000) [76, 78, 79] that are believed to be essential for the metabolism of giant cells and associated with neuronal gigantism. With the emergence of data on the DNA damage in neurons, it was logical to assume that neuronal polyploidy reflects one of the evolutionary solutions aimed to reduce the consequences of DNA damage. Experimental data in favor of this hypothesis have been obtained in the representatives of another taxon – insects, in which neuronal polyploidy also occurs by to a much lesser extent [75]. Several species-specific, as well as sex- and age-related differences in the number of polyploid neurons in the brains of three different *Drosophila* species were described in detail. On average, a fraction of polyploid (mostly tetraploid, 4 C) neurons was estimated as 10-15% of all neurons in the adult brain. The largest number of polyploid neurons was found in the optic lobes. It was shown that tetraploidy was not a result of cell fusion. Interestingly, the largest number of DSBs in the neurons has been identified in the optical lobes as well. Finally, using etoposide to induce DSBs, it was demonstrated that the brain response to this drug involved a significant increase in the proportion of polyploid neurons. And, perhaps, most importantly, polyploid neurons survived after induced DNA damage better than diploid neurons [75]. Therefore, this work confirmed two assumptions regarding the role of neuronal polyploidy. First, polyploidy, indeed, protects the cells in the event of DNA damage; second, neurons have a mechanism for activation of DNA endoreplication in response to DNA damage.

These data suggest a new look at the neuronal polyploidy in human brain. The association between neurodegenerative diseases and increase in the number of polyploid neurons had been demonstrated, and for some time, polyploidy had even been considered as a possible cause of these pathologies [80, 81]. However, it now seems more likely that polyploidy arises in response to DNA damage (which is apparently the main cause of neurodegeneration) as one of the protective mechanisms in neurons.

Altruistic neurons of vertebrates and glutamate. Out of the two approaches to increasing the number or genome copies (in the same cell or by increasing the number of cells), somatic polyploidy seems to be a less efficient solution. First, healthy copies of a damaged gene in a polyploid cell will play their protective role only until the "toxic" products encoded by the damaged gene and detrimental to the cell activity are synthesized. Second, it does not allow to sacrifice a damaged neuron without severe consequences for the rest of the brain. So, it seems that the vertebrate brain has chosen the second option. Neurons that accumulate mutations and DNA damage die, but their epigenetic and functional clones retain the information that remains unchanged. Such redundancy makes it also possible to distribute the activity between the neurons, which reduces mutational load on an individual cell and allows the body to maintain neurons longer. This assumption is in good agreement with numerous data showing that the same task is performed each time by slightly different populations of neurons [82, 83].

Looking at the evolution of vertebrate brain from this point of view, we can argue that the rapid increase in the number of neurons in the evolution of vertebrates has primarily served the purpose of protecting the brain and ensuring a sufficient lifespan of an organism. At the same time, it was also a pre-adaptation to the development of cognitive abilities. This assumption is in good agreement with the fact that the increase in the size of human brain in the evolution has not been accompanied by the development of more sophisticated tools over a period of millions of years [84]. As well as the pathogenesis of some neurodegenerative diseases (e.g., Parkinson's disease), characterized by the asymptomatic death of up to 30-70% of neurons in particular brain areas [85]. These data suggest an existence in human brain of a developed "safety net" in a form of multiple epigenetically similar and interchangeable neurons.

Adult neurogenesis might also be a subject of functional revision. Previously, its activation had been considered as a mechanism for promoting cognitive activity, i.e., an ability to learn and memorize information in a new environment [86]. Now, it can also be viewed as a protective mechanism triggered in anticipation of increased cognitive load.

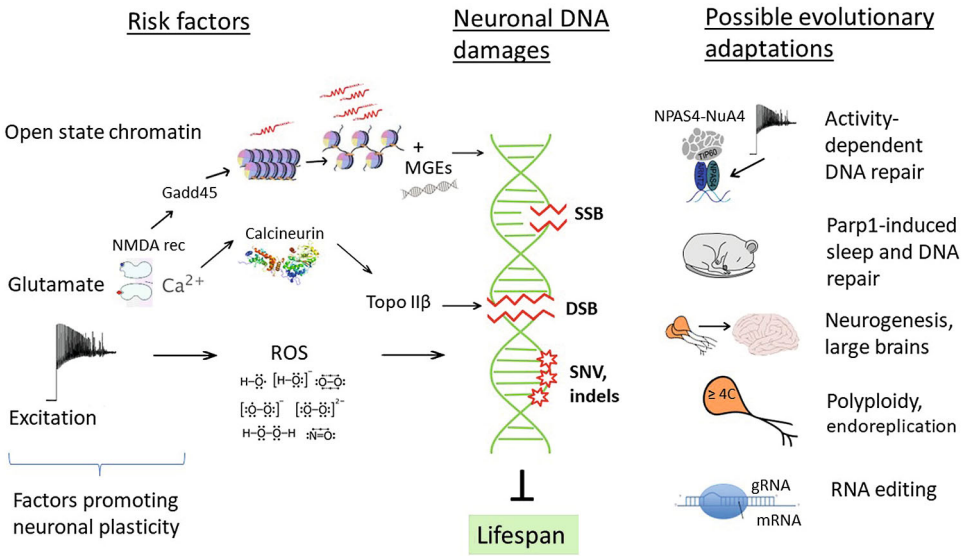
Recent data suggest that the increase in the number of neurons in the evolution of vertebrate brain has been achieved mainly due to the increase in the proportion of glutamate neurons [87] (compare 50% in rodents vs. 80% in humans). This seems paradoxical, since more complex systems tend to be more diverse. However, this suggestion makes sense if we assume that these additional neurons had originally served as a "safety net", a source of genome "guardians". Why glutamatergic neurons could be used for this purpose was explained in several recent publications by Moroz et al. [87-89]. To put it briefly, the development of glutamatergic phenotype is energetically cheap and epigenetically simple (only vesicular glutamate transporter has to be expressed, while glutamic acid is already present in all cells as their main metabolite). Another interesting finding is that vertebrates have lost the diversity of glutamate receptors compared to other groups and retained only the excitatory receptors [87]. It would be interesting to think about the benefits that might have been gained from this.

Excitability at the physiological level provides conditions for exit from a stable state and formation of new ensembles of neurons. Increasing the plasticity of the epigenome can achieve even greater plasticity and diversity. Indeed, there is an evidence of intracellular cascades

linking NMDA and AMPA glutamate receptors to the heterochromatin decompaction factor Gadd45 [90], i.e., excitatory glutamate appears to be associated with both functions that increase plasticity at the physiological and epigenetic levels. This can be another advantage of glutamatergic phenotype. However, glutamate, through ionotropic receptors, stimulates formation of DNA breaks and their repair [91], while excitation shortens the lifespan [27]. If this is the case, then the emphasis on the glutamate phenotype of neurons in the evolution of vertebrates could have and should have stimulated natural selection to promote an increase in the number of neurons in order to reduce the biological cost of DNA damage during glutamatergic signaling [92]. Positive feedback loop in the evolution of vertebrate brain?

Transition of plasticity to the RNA level in cephalopods. DNA instability in neurons could be a cause of another interesting phenomenon recently found in cephalopods, namely, modification of transcripts at the RNA level. RNA undergoes large-scale editing (or recoding) especially in the nervous system, where almost all transcripts are edited [93, 94]. Moreover, 65% of editing sites found in the coding sequences are nonsynonymous. Only one type of editing, replacement of adenosine with inosine (A-to-I), occurs at more than 70,000 sites in cephalopods vs. ~1000 sites in *Drosophila* and ~3000 in humans [95]. Moreover, unlike mammals, cephalopods demonstrate clear evolutionary selection for the increase in the number of RNA editing sites in functionally significant genes [93, 95], the editing of which affects the structure and function of encoded proteins [93]. In one case (recoding of potassium channel protein transcript in octopuses), RNA editing was found to provide an adaptation to different living temperatures [96].

However, the most important finding was a discovery of the protective role of RNA editing in the maintenance of genome stability that was announced in the title of the article "Trade-Off Between Transcriptome Plasticity and Genome Evolution in Cephalopods" [93]. The authors assessed the distribution of mutations (deletions, synonymous and nonsynonymous single nucleotide substitutions) and found that the DNA regions close to the RNA editing sites accumulated the least number of mutations. From 23 to 41% genome regions in different species of cephalopods were stabilized and these stabilized regions encoded RNAs that underwent editing. The mechanisms of such plasticity transfer remain to be explored. It can be assumed that the driver for the plasticity transition to the RNA level is protection of unstable DNA in neurons. Perhaps, this non-standard solution, combined with the increase in the number of neurons, ensures an outstanding cognitive evolution



The main risk factors for DNA stability in neurons, identified types of DNA damage, and possible evolutionary adaptations reducing the biological cost of neuronal DNA instability. The risk factors include high energy metabolism of neurons, formation of reactive oxygen species (ROS), neuronal excitation, and activation of NMDA receptors by glutamate. Activation of NMDA receptors leads to the formation of DSBs in the promoter regions of early response genes, which induces their expression and subsequent DNA repair. This mechanism involves an increase in the intracellular calcium concentration and activation of calcineurin, which phosphorylates Topo IIβ. Other risk factors include changes in the chromatin state and chromatin decondensation, which increases DNA susceptibility to various mutagens, e.g., mobile genetic elements (MGEs). Chromatin decondensation can also be induced by glutamate receptors via activation of Gadd45 heterochromatin. All these factors are necessary for normal functioning of the central nervous system to provide its plasticity and cognitive functions. Accumulation of neuronal DNA damage such as DSBs, SSBs, SNVs and indels is actively studied (table). These damages can directly or indirectly lead to the development of neurodegenerative diseases and might determine the lifespan. Possible evolutionary adaptations that reduce the cost of DNA instability in neurons include DNA repair dependent on the electrical activity of neurons (NPAS4–NuA4), Parp1-dependent repair of DSBs accumulated during the animal's daytime activity and activating sleep in vertebrates, neurogenesis and increase in the number of neurons in the central nervous system, somatic polyploidy (which is especially pronounced in gastropods), mRNA editing, and transfer of plasticity from the DNA to RNA level in cephalopods

of cephalopods, which rank first among protostomes in terms of their mental abilities.

CONCLUSION

The understanding that the post-mitotic DNA instability in neurons is a cost of their electrical activity and high genome plasticity is changing the theoretical landscape not only in neuroscience, but in biology as well. As Olovnikov suggested, neuronal DNA can be the "clock" determining the lifespan of an organism. Numerous accumulated data have significantly increased the likelihood of this hypothesis. In addition, the instability of neuronal DNA seems to have promoted the search for various ways to reduce the biological cost of brain functioning in the evolution of Metazoa and has become a driver of this evolution. Various phenomena, such as sleep, increase in the number of neurons in vertebrate evolution, adult neurogenesis, distribution of neuronal activity, somatic polyploidy, and RNA editing, have received a new meaning and new understanding when considered in the light of the resolution of the "plasticity vs. neuronal DNA instability" trade-off. The topic is very important not only for basic neuroscience, but also for translational medicine. The main risk factors, types of neuronal DNA damage, and possible evolutionary adaptations that reduce the biological cost of DNA instability are shown in the figure.

There are still many problems that require experimental verification and represent today's challenges in molecular neurobiology, which is especially true for the neurobiology of invertebrates as organisms that provide unique experimental opportunities. There are also other unsolved urgent problems.

(i) Verification of the assumption that neuronal excitation affects not only DSB formation, but also accumulation of DNA damage and mutations in neuronal DNA. This can be done in *C. elegance* and *D. melanogaster* using optogenetic approaches or by electrophysiological stimulation in gastropods whose giant neurons can be easily identified and isolated for electrophysiological studies with microelectrodes. For these species, DNA sequencing can be done for neurons with a significant baseline difference in their electrical activity or for the same neurons with and without electrical stimulation.

(ii) The search for specific genetic and epigenetic changes that predispose animals with higher cognitive abilities to shorter lifespans, reduced stress tolerance, and reduced fertility. The existing studies [46-52] should be continued at the level of neuronal DNA and epigenetic regulation of neuronal genome. In particular, do "smart" invertebrates express more actively the genes associated with neuronal excitation or chromatin open state? Do "smart" animals accumulate more mutations? Is the biological cost (stress intolerance, low fertility, and lifespan) in "smart" animals a direct consequence of accumulation of mutations?

(iii) It is easier to search for the chronomeres or other intracellular neuronal substrates responsible for the regulation of lifespan in invertebrates.

(iv) In the light of new data on the influence of neuronal activity on the damage of neuronal DNA, we cannot provide a convincing explanation or justification of the benefits of cognitive load on the brain health, which is now given so much importance. One can only assume a preconditioning for the cognitive load, similar to hypoxic or ischemic training. The exact mechanisms of this cognitive preconditioning still have to be discovered.

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