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REVIEW

Physiological and Pathological DNA Double-Strand Breaks in the Central Nervous System

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ABSTRACT: This review discusses the dual role of DNA double-strand breaks (DSBs) in the brain, where they can act as both physiological regulators of gene expression and contributors to neuronal dysfunction under pathological conditions. In post-mitotic neurons, which rely mainly on non-homologous end joining, the balance between DSB formation and repair appears to be especially important. Recent studies show that transient activity-induced DSBs, particularly at regulatory regions of immediate early genes (IEGs), support chromatin remodeling and transcriptional activation. In contrast, persistent DSBs associated with chronic stress, hyperexcitability, ageing, or neurodegenerative disorders are linked to impaired repair, prolonged DNA damage response signaling, neuroinflammation, and stable transcriptional alterations. Available findings also suggest that glial cells participate in DSB-related responses to stress, although these mechanisms remain less clearly understood than those in neurons. Overall, current evidence supports the view that the functional outcome of DSBs in the brain depends on their genomic context, duration, and efficiency of repair. The integration of DSB mapping with transcriptomic and epigenomic profiling may help to identify early transitions from adaptive to maladaptive DNA damage responses (DDR) in neurons and glia.

KEYWORDS: Physiological DNA damage; pathological DNA damage; neuronal genome stability; DNA double-strand breaks; genome integrity

1 Introduction

Genomic stability is crucial for homeostasis maintenance and the normal functioning of different cells of a multicellular organism. And DNA double-strand breaks (DSBs) can be viewed as the most severe type of DNA destabilization event [1]. DSBs are particularly important in the central nervous system (CNS) because most neurons are highly specialized, post-mitotic (non-dividing) cells that function throughout the organism's lifetime, often spanning several decades. Mammalian cells experience up to 50 DSB events per day [1] and have different mechanisms of detecting and repairing DNA DSBs, including homologous recombination (HR) and nonhomologous-end-joining (NHEJ). Unlike dividing cells, neurons are not considered to utilize homologous HR, as sister chromatids are not available for accurate DSB repair, and therefore rely primarily on NHEJ and other repair mechanisms [2].

With age, neurons accumulate signs of DNA breaks, and older animals consistently show elevated levels of phosphorylated histone H2AX (γH2AX) compared to younger ones [3,4]. This reflects a gradual shift in the balance between accumulation and repair of damage in cells that function for decades without division and are critically dependent on DNA integrity.

Over the past decade, it has become clear that not all DSBs in DNA in neurons are equally harmful. In addition to damage that accumulates with age, chronic stress, or disease, a class of temporary, tightly regulated DSBs has been identified that arises during gene expression activation and is rapidly repaired by the repair system [5]. Such "physiological" breaks are localized in regulatory regions and are being studied particularly actively in the context of learning and memory. In contrast, in pathological conditions, chronic stress, neurodegeneration—the balance is disrupted: DSBs become persistent, repair weakens, pro-inflammatory cascades and chromatin changes are triggered [6]. This review examines the molecular mechanisms of these processes, emphasizing the differences between adaptive and destabilizing DSB modes in the CNS.

2 DSB Sources in CNS Cells

2.1 Endogenous Sources: Oxidative Stress, Mitochondrial Dysfunction, Metabolic Factors

A cell's DNA is constantly exposed to the effects of metabolic products. One of the main endogenous sources of DNA damage is oxidative stress—an excess of reactive oxygen species (ROS) and nitrogen, which arise when the balance of cellular redox reactions is disturbed [7]. Neurons consume an extraordinary amount of oxygen and have an intense metabolism [8], which makes them vulnerable to the accumulation of free radicals. ROS can directly damage DNA strands, causing base modifications and strand breaks. Most often, the primary result of exposure to ROS is a single-strand break (SSB) or an oxidized base, which is eliminated by base excision repair (BER) [9,10]. However, if two SSBs occur opposite each other in complementary strands, or if an SSB appears against the background of replication (in a dividing cell), a DSB is formed.

Mitochondrial dysfunction is closely linked to oxidative stress, as mitochondria are the main source of ROS in neurons [7]. When the respiratory chain is disrupted, for example, due to mitochondrial gene mutations, accumulation of DNA damage, or substrate deficiency, cells accumulate excess superoxide, which diffuses into the cytoplasm and nucleus, damaging nuclear DNA. In addition, ATP deficiency due to mitochondrial dysfunction can impair the energy supply required for DNA repair. Neurons are particularly vulnerable to this "double hit": the level of DNA damage increases, while repair capacity declines [4].

Metabolic factors include excess neurotransmitters and impaired detoxification enzymes. For example, excessive stimulation of NMDA (N-methyl-D-aspartate) receptors by glutamate leads to overstimulation of NMDA receptors by glutamate and excessive calcium influx and activation of cascades that produce oxidant compounds (via NO synthase, phospholipase, and other enzymes) [3]. Hyperglycemia or deficiency of the antioxidant system (e.g., glutathione) can also increase oxidative DNA damage. Thus, endogenous metabolic disturbances often lead to an increased SSB load, which may be converted into DSBs when BER is impaired. It has been observed that the expression of some components of BER and related pathways decreases with age in the brain: for example, the levels of DNA polymerase β, FEN1, and PCNA fall in differentiated neurons [4].

Thus, age-related metabolic shifts (ROS accumulation, glycation of proteins, mitochondrial aging) lead to a progressive accumulation of poorly repaired lesions, including DSBs, which correlates with cognitive aging and increased risk of neurodegeneration.

2.2 Transcription-Associated Damage: R Structures, Topological Transcription Conflicts, Collapse of Transcription Complexes

Neurons are extremely active in transcribing certain genes related to neurotransmission, plasticity, etc. High levels of transcription itself can be a source of DNA damage. One mechanism is the formation of R-Loop structures during transcription. R-Loop is a hybrid triplet structure when newly synthesized RNA

heteroduplexes with matrix DNA, displacing the complementary DNA strand as a single-stranded loop [11]. Such structures are usually transient and are removed by specialized enzymes (e.g., RNase H). However, under intense transcription or deficiency of factors eliminating R-Loop, these hybrids stabilize, making DNA vulnerable: the displaced single-stranded region is prone to damage and breaks [12]. In addition, the cell itself can cut DNA in the region of stable R-Loop in an attempt to eliminate the "stuck" transcriptional complex. Thus, mutations in the gene senataxin (SETX), a spindle-shaped RNase H, lead to R-Loop accumulation and manifest as neurodegenerative diseases (ataxia with oculomotor apraxia type 2 (AOA2), a form of Amyotrophic Lateral Sclerosis type 4) due to genomic instability in motoneurons [13]. R-Loop levels are elevated in neurons with high transcriptional activity (e.g., motoneurons), increasing the risk of DSBs if these structures are not eliminated [14].

Another source of DSBs is associated with topological stress during transcription. As RNA polymerase moves along DNA, it generates supercoiling: overwound DNA accumulates ahead of the polymerase, while underwound—behind it [15]. Normally, these stresses are relieved by topoisomerases; however, when they are inhibited, mutations or high transcriptional load result in structural blocks [16]. Stalled transcriptional complexes can collapse; endonucleases (e.g., XPF/ERCC1 or MRE11) are thought to make cuts near RNA polymerase II, converting the obstruction to DSB, which is then repaired by standard pathways [17]. Thus, the cell seems to "sacrifice" DNA integrity to restart RNA synthesis or solve a topological problem.

In addition, conflicts between transcription and replication in dividing cells (e.g., glial cells) are a known source of DSBs: simultaneous operation of the replicative fork and RNA polymerase on the same DNA strand leads to collision and collapse of the fork, generating DSBs [18].

2.3 Programmed Breaks: Role of Topoisomerases

The eukaryotic cell is capable of deliberately inducing DSBs in certain situations by engaging specialized enzymes. A classic example is endonuclease Spo11, which forms dozens of breaks to initiate recombination in meiosis. Meiosis does not occur in neurons, but it has been shown that topoisomerases II (TopoII) can carry out programmed DSBs in somatic brain cells. Topoisomerase IIβ (TopoIIβ) is an enzyme that cuts both DNA strands to form a temporary DSB, through which it passes the other segment of the double helix, thus relieving torsional stress from DNA supercoiling [15]. In 2006, it was first shown that activation of ionotropic glutamate receptors correlates with the occurrence of TopoIIβ-mediated DSBs [19]. More direct confirmation of the functional role of this phenomenon came from work showing that neuronal depolarization induces DSBs in the promoters of a number of immediate early response (IEG) genes—such as *Fos*, *Npas4*, *Egr1* [5]. These genes encode key regulators of synaptic plasticity and learning, and are normally rapidly and transiently expressed at neuronal activity. Importantly, suppression of TopoIIβ (by siRNA or inhibitor) resulted in a decrease in the appearance of DSBs in promoters and the simultaneous weakening of the induction of these genes. Artificial targeting of DSB in the promoter region (using recombinant nuclease) activated *Fos* gene transcription even without stimulation [5].

TopoIIβ, in fact, acts as a molecular "switch on": upon receipt of a neuronal signal (for example, an influx of calcium), it locally cuts DNA in the promoter, thereby relaxing chromatin and allowing RNA polymerase II to quickly pass the initiation stage and begin mRNA elongation. Such DSBs are temporary—they appear in the first minutes after stimulation and are already repaired in ~2 h, coinciding in time with the transient expression of IEG genes [2]. Tus, TopoIIβ represents a key example of an enzyme that generates DSBs in a targeted manner in the regulatory regions of genes, thereby integrating chromatin mechanics with the regulation of transcription (the effects of DSBs on transcription are discussed in detail in Section 5).

2.4 Single-Strand Breaks (SSB) and Their Transition to DSB

An SSB is a disruption of the integrity of one strand of the DNA double helix, while the covalent continuity of the opposite strand is preserved. Tens of thousands of SSBs arise daily in each cell as a consequence of normal cellular metabolism, including oxidative damage and intermediates generated during BER [4]. Unlike DSB, single breaks are usually not fatal to the cell: the presence of an intact complementary strand provides a template for repair. SSB repair is carried out by a complex of enzymes, including PARP1, DNA polymerase β, ligase III, and XRCC1 protein, and the repair is usually completed in a matter of minutes [20].

However, the danger of SSBs is that, under certain conditions, they can develop into a double-stranded break. Such conversion scenarios may include: spatial convergence of two SSBs on complementary strands, the BER system, eliminating such dense damage, sometimes cuts out sections on both sides, leading to DSBs [10]. The collision of an SSB with the replication fork, this mechanism is relevant for proliferating brain cells (glia and progenitor cells). If, during S phase, a replication fork encounters an SSB on the template strand, the fork collapses, converting the SSB into a DSB at the ends of the daughter chromatid [18]. When the rapid SSB repair pathway is compromised (for example, through PARP1 inhibition), even isolated SSB may persist long enough to encounter a secondary destabilizing event, such as DNA replication or the formation of a second nearby SSB. In neurons, despite their post-mitotic state, PARP1 inhibition leads to the accumulation and prolonged persistence of SSBs, thereby increasing the likelihood of incidental DSB formation, for instance, through the spatial convergence of two unresolved SSBs [21].

Thus, although SSBs are a frequent and usually rapidly reversible event, their persistence increases the risk of DSB formation, threatening the stability of neurons.

3 DSB Recognition and Early DNA Damage Response (DDR) Signaling in Neurons and Glia

Although the core mechanisms of DSB formation, sensing, and repair are largely conserved across cell types, their operation in the central nervous system is shaped by cell-type-specific constraints. In mature post-mitotic neurons, the absence of DNA replication and the limited availability of homologous recombination make non-homologous end joining the predominant DSB repair route [22,23], whereas homologous recombination and ATR-dependent signaling remain more relevant in proliferating neural progenitors and dividing glial cells [24]. In addition, because neurons are long-lived and highly transcriptionally active, the functional consequences of DSB formation and signaling for transcriptional regulation and neural plasticity are especially important in these cells [2]. Thus, the specificity of DSB biology in the nervous system lies less in entirely unique pathways than in pathway usage, cellular context, and functional consequences.

Recognition of DSBs is the first step in a cell's response to genome damage. The main sensory complex in eukaryotic cells is the MRE11–RAD50–NBS1 (MRN) complex [25]. It binds to the ends of the broken DNA, holds them close to each other, and thereby prepares the damage for further processing. The key function of MRN is the recruitment and activation of ataxia telangiectasia mutated kinase (ATM), a central regulator of DSB response [26]. After binding, it activates and initiates phosphorylation of multiple proteins near the gap, including histone H2AX [25]. This leads to the formation of γH2AX-enriched domains around the lesion [3].

In neurons, most γH2AX loci colocalize with NBS1 (a DSB sensor) and p53-binding protein 1 (53BP1, a DSB signaling mediator), reflecting efficient detection of even single DSBs [27]. The clinical manifestations of ATM or NBS1 mutations (ataxia-telangiectasia, Nijmegen syndrome) emphasize the critical role of the MRN–ATM axis for neuronal survival [28].

The response to DSB is coordinated by three PI3K-like kinases: ATM, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and ATM- and Rad3-related (ATR) [29]. ATM is activated predominantly

in complex DSB and chromatin disorders, providing a widespread signaling response and chromatin remodeling [25]. DNA-PKcs, acting in complex with Ku70/Ku80, is a key enzyme of non-homologous end joining (NHEJ) and plays a crucial role in postmitotic neurons, where NHEJ is the main repair pathway [30,31]. ATR is activated by extended regions of single-stranded DNA generated during replication stress or end resection of DSBs [25]. Because mature neurons do not undergo DNA replication and exhibit limited end resection, ATR plays a minor role in DSB signaling in these cells, but remains important in neural progenitors and dividing glial populations.

ATM and DNA-PKcs signals are coordinated through common targets. Both kinases are capable of phosphorylating H2AX and the tripartite motif-containing 28, also known as transcriptional intermediary factor 1β and KRAB-associated protein-1 (TRIM28/KAP1) cofactor, which leads to local chromatin decondensation and, in some contexts, to transcription regulation [32,33]. In particular, joint ATM/DNA-PK—dependent phosphorylation of KAP1 is necessary to break the pause of RNA polymerase II and activate immediate early genes in neurons [34]. Thus, ATM and DNA-PKcs do not act in isolation, but in cooperation, with partial functional overlap.

The early DSB response is accompanied by the formation of characteristic molecular markers. γH2AX serves as a platform for the assembly of a signal repair focus through the involvement of mediator of DNA Damage Checkpoint 1 (MDC1), E3-ubiquitin ligases RING Finger Proteins (RNF8/RNF168), and subsequent recruitment of 53BP1 or Breast Cancer gene 1 (BRCA1) [3,35]. 53BP1 restricts end resection and promotes NHEJ, which is especially important in G0-phase neurons, whereas BRCA1 is associated with resection and homologous recombination, mainly in dividing cells. 53BP1/BRCA1 imbalance is associated with the accumulation of repair errors and neurodegeneration; in particular, a decrease in BRCA1 has been observed in non-neuronal models of aging [36] and in Alzheimer's disease brain tissue, where altered BRCA1 levels have been associated with tau pathology and cognitive impairment [37].

Thus, early DDR in neurons is an integrated network of signals linking DSB recognition, repair path selection, chromatin status, and transcriptional outcomes, which is crucial for distinguishing between physiological and pathological DSB modes.

The importance of intact DDR pathways for the nervous system is also illustrated by inherited neurological disorders caused by mutations in DNA repair genes. Pathogenic variants in ATM cause ataxia-telangiectasia, a multisystem disorder with prominent progressive cerebellar ataxia and other neurological symptoms [38]. Defects in NBN (nibrin), a component of the MRN complex, cause Nijmegen breakage syndrome, characterized by microcephaly, developmental abnormalities, and genome instability [39]. Autosomal recessive mutation of tyrosyl DNA phosphodiesterase 1 (TDP1) causes the neurodegenerative syndrome spinocerebellar ataxia with axonal neuropathy 1 (SCAN1) [40]. Together, these disorders indicate that defective DNA damage signaling and repair can itself be a primary driver of nervous system pathology.

4 DSB Repair in Mature Neurons

The classical non-homologous end joining (NHEJ) is the main, and in postmitotic neurons, it is practically the only effective mechanism for repairing DSBs [41]. Unlike homologous recombination, NHEJ does not require a template and provides direct crosslinking of DNA ends. DSB recognition, as described above, begins with the binding of the Ku70/Ku80 heterodimer, which stabilizes the ends and serves as a platform for the recruitment of DNA-dependent protein kinase (DNA-PKcs) [31]. The resulting DNA-PK complex coordinates the processing of the ends and their subsequent ligation. With compatible ends, the gap can be directly repaired by the IV–XRCC4 ligase complex with the participation of XLF [42]. If the

ends are damaged or incompatible, processing enzymes are activated, including the Artemis endonuclease and specialized polymerases (Pol μ, Pol λ), which allow minimal preparation of the ends for crosslinking.

NHEJ is a fast pathway: most DSBs in neurons are eliminated within hours [5]. Genetic data confirm the key role of NHEJ: XRCC4 or ligase IV knockouts lead to massive neuron death, microcephaly, and mortality, and mutations of NHEJ components in humans cause severe neuroimmune syndromes [43,44].

Although NHEJ is considered to be less accurate, its mutagenicity is usually limited to microarrays (1–4 nucleotides) at the break site [23]. In the context of the neural genome, such changes are usually less dangerous than an unstitched gap. Thus, for neurons, NHEJ is a compromise between speed and accuracy necessary to maintain viability.

NHEJ limitations are manifested in complex breaks (covalently bound proteins, long single-stranded tails) or in multiple simultaneous DSBs, when the risk of misalignment of the ends and chromosomal rearrangements increases [42]. Nevertheless, under physiological conditions, NHEJ remains the most reliable and preferred mechanism of DSB repair in mature neurons [45]. At the level of interspecific comparisons, the expression of NHEJ pathway genes demonstrates a closer relationship with encephalization indices than with longevity [46], which is interpreted as a possible coevolution of NHEJ components with an increase in the relative size of the mammalian brain.

Alternative end joining (alt-EJ), including the micro-homologous junction (MMEJ), is activated when classical NHEJ is disrupted, for example, with Ku70/Ku80 or ligase IV deficiency, or with complex types of damage [47]. These pathways require resection of the DSB ends and use short micro-homologies (2–6 nucleotides), which inevitably leads to sequence loss [48].

Alt-EJ involves PARP1, XRCC1, and ligase III, as well as resection factors (MRE11, CtIP) [49]. As a result, deletions and structural rearrangements characteristic of mutagenic repair are formed [50]. Normally, alt-EJ is suppressed in neurons due to the effective binding of the ends by Ku proteins, and increased expression of Ku70 in such models reduces the level of DSB and increases the survival of neurons [51]. Evidence obtained in G1-arrested progenitor B cells showed that loss of Ku permits Alt-EJ-mediated joining of DSBs, leading the authors to suggest that a similar mechanism may help explain why Ku deficiency can partially rescue the neuronal developmental phenotype of ligase IV-deficient mice [52]. Thus, weakening of Ku-dependent end protection may create conditions for more error-prone end-joining outcomes when classical NHEJ is compromised.

Homologous recombination (HR) requires the presence of a sister chromatid and actively functions in the S/G2 phases of the cell cycle. In mature neurons located in G0, HR is practically inaccessible due to the lack of a matrix and low expression of key HR proteins (RAD51, BRCA2, etc.) [2]. During differentiation, neurons purposefully suppress HR and switch to using NHEJ [53].

HR retains its importance in dividing populations of the brain—neural progenitors and glial cells [54]. Thus, the choice of the repair pathway in the central nervous system is tightly linked to the cell cycle: dividing cells use HR, while mature neurons rely almost entirely on NHEJ and its alternatives.

DSB repair in neurons is rarely completely neutral. The most common consequences are microdeletions and microinserts, and in the case of erroneous stitching, structural variants (deletions, duplications, translocations). The combination of such changes leads to somatic mosaicism of the brain, in which the genomes of individual neurons differ [55].

Somatic mosaicism is viewed in two ways. On the one hand, the accumulation of mutations can contribute to neurodegeneration and cognitive decline. On the other hand, there is a hypothesis that the limited genomic diversity of neurons may contribute to the functional heterogeneity and plasticity of neural networks. So far,

the prevailing opinion is that excessive mosaicism is harmful, but a moderate level is an inevitable consequence of the use of DSB in the physiological processes of transcription regulation and plasticity [31,56].

In general, DSB repair in neurons represents a balance between maintaining the integrity of the genome and allowing small local changes. This balance underlies both the resilience of neurons and their vulnerability to aging and brain diseases.

5 Functional DSB in the Regulatory Regions of Activated Genes

One of the fundamentally new concepts that has emerged in recent years is the understanding that DSBs can act not only as damage, but also as a regulatory tool of neurons embedded in activity-dependent transcription programs. In Section 2.3, the role of TopoIIß and programmable DSBs in promoters of IEGs has already been discussed. Next, the focus shifts from the molecular mechanism to the functional level: how DSBs are involved in transcriptional, synaptic, and behavioral plasticity, and where the boundary between adaptation and pathology lies.

Physiological DSBs in neurons are not randomly distributed across the genome but are preferentially localized to regulatory regions involved in activity-dependent transcription. Increased neuronal activity leads to elevated levels of DSBs and SSBs in the neuronal genome, accompanied by up-regulation of IEGs and late-response genes (LRGs) [57]. Recurrent DSB clusters have been identified within genes involved in synaptic function and cell–cell adhesion [57]. DSBs are also enriched in promoters and enhancers of activity-induced genes, including classical IEGs such as *Fos*, *Egr1*, and *Npas4* [5].

The genome in the neuronal nucleus is organized into topologically associating domains (TADs), within which chromatin interactions are frequent, whereas contacts between neighboring domains are constrained by boundaries enriched in CTCF and cohesin [58]. This organization is critical for gene regulation, as enhancers typically interact with promoters within the same TAD and are insulated from genes in adjacent domains [59].

Importantly, IEG promoters are often located in open chromatin regions enriched in H3K27ac and frequently positioned near TAD boundaries or chromatin loop anchors. Studies demonstrated that sites of TopoIIβ—induced breaks often coincide with CTCF/cohesin loop anchors [59]. In this context, DSB formation may transiently relax local chromatin topology, alleviating topological constraints that normally limit enhancer–promoter communication. Although this model remains partly hypothetical in neurons, it is consistent with observations that DSBs are enriched at strong CTCF sites and within transcriptionally active (A-compartment) euchromatin, while being largely excluded from constitutive heterochromatin [60].

Thus, neuronal genomes appear to “sacrifice” local DNA integrity in a controlled manner to enable rapid transcriptional responses. Similar principles operate in immune cells, where programmed DSBs occur at defined loci (e.g., immunoglobulin genes) [61].

Traditionally, DSBs have been associated with transcriptional repression in cis, as transcription is halted near DNA lesions to allow repair [62]. However, in neurons, activity-induced DSBs in promoter regions represent a distinct regulatory mode. Many inducible genes, including IEGs, harbor RNA polymerase II (RNAPII) stalled in a promoter-proximal pause. This poised state enables rapid activation but requires active pause release.

It has been shown that γH2AX and phosphorylated TRIM28/KAP1 accumulate near serum- or activity-induced genes and that this process is regulated by DNA-dependent protein kinase (DNA-PKcs) and ATM [32]. Acting together, these kinases control RNAPII pause release and productive transcriptional elongation. Inhibition of DNA-PKcs or ATM prevents efficient elongation despite transcriptional stimuli, indicating that DDR signaling is functionally coupled to transcription activation rather than merely responding to damage.

IEGs encode proteins that are critical for synaptic plasticity, learning, and memory [63]. Some IEGs encode transcription factors, such as AP-1 (c-Jun/c-Fos) and c-Myc, which subsequently regulate downstream LRGs, including *bdnf*, *homer1*, and *fgf1* [64–66].

Multiple pieces of evidence demonstrate that DSB formation is an integral part of IEG regulation (Fig. 1). DSBs form in the promoter regions of Fos, Egr1, and Npas4 during neuronal activation [5]. In neurons of the CA1 region of the hippocampus, DSBs occur in the Npas4 promoter during fear memory reconsolidation, and inhibition of TopoIIβ reduces both DSB formation and memory performance [67]. Exposure to a novel environment induces transient DSBs in dentate gyrus neurons, which are efficiently repaired within 24 h, while sensory and optogenetic stimulation similarly increase DSB levels in activated neural networks [3].

Genomic events are rapidly translated into changes at the synapse level. IEG products trigger a cascade of structural rearrangements: the growth of new appendages, the strengthening of existing synaptic contacts, and changes in the receptor composition of synapses. If DSB formation and subsequent synthesis of such key proteins are blocked, the neuron will not be able to rebuild its synaptic network adequately to new stimuli [67]. As a result, long-term memory, learning, and cognitive flexibility suffer [68].

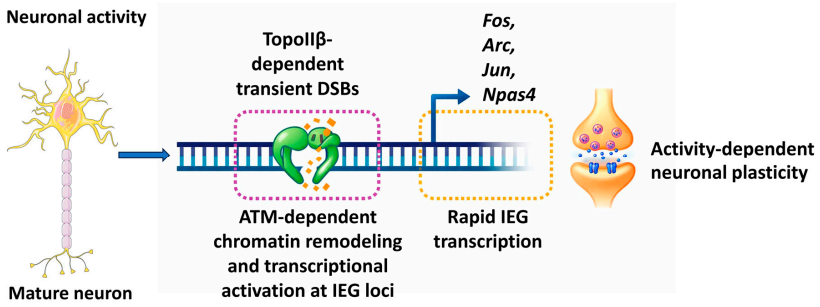

Figure 1: Topoisomerase IIβ-dependent DSB formation at activity-dependent gene promoters. Adapted from [69]. In mature post-mitotic neurons, neuronal activity is proposed to trigger topoisomerase IIβ (TopoIIβ)-dependent transient DNA double-strand breaks (DSBs) at regulatory regions of immediate early genes (IEGs). These breaks are thought to facilitate local ataxia telangiectasia mutated (ATM) kinase-dependent chromatin remodeling and transcriptional activation, thereby enabling rapid induction of IEGs such as *Fos*, *Arc*, *Jun*, and *Npas4*.

DSB, being a powerful signal, attracts a number of chromatin remodeling factors to the rupture site. The binding of 53BP1, BRCA1, and other proteins changes the local architecture of DNA. It has been shown that during neuronal activity, DSBs on the promoter can facilitate contacts between the enhancer and the gene promoter, enhancing transcription [5]. In addition, a DDR response is initiated at the ends of the gap, including acetylation/ubiquitination of histones, which can affect neighboring genes [70,71]. Thus, a single programmed break can change the epigenetic state of the locus, making it more suitable for active transcription or, conversely, after repair, transfer it to a new stable state.

An interesting hypothesis is that DNA fragments excised during the repair of such breaks can be preserved as extrachromosomal ring DNA and serve as a kind of long-term marker that the neuron was active [72,73]. Such rings have been found in some cases [74,75] and contain promoter sequences of activated genes. Although this idea requires further confirmation, it highlights how non-trivial the consequences of a seemingly simple breakup of a molecule can be.

In contrast to IEGs, LRG activation is more strongly associated with oxidative damage–mediated SSBs at promoter regions rather than direct DSBs [58]. Ligand-induced gene activation can involve SSBs and secondary DSBs generated during chromatin demethylation at histones and CpG sites, with repair being required for successful transcription [76]. Thus, neurons appear to deploy distinct types of DNA lesions for

different temporal layers of transcriptional programs: DSBs for rapid IEG induction and SSBs/oxidative lesions for longer-lasting transcriptional remodeling.

Physiological DSBs are characterized by their transience, spatial restriction, and efficient repair. Activity-induced γH2AX foci appear within minutes and typically resolve within hours, rarely persisting beyond 24 h under normal conditions [3]. These breaks are few in number, localized to specific neuronal populations and genomic regions, and do not trigger global DDR responses such as widespread tumor protein p53 (p53) activation or apoptosis.

Recent studies show that glial cells (astrocytes, oligodendrocytes, and microglia) are capable of forming "adaptive" DSBs under certain physiological stimuli. In experiments on mice, contextual fear learning (associated with stress stimulation) caused an increase in DSB (γH2AX) markers in brain glial cells, accompanied by activation of transcription of multiple genes. In particular, glial cells showed a pronounced response to stress hormones (glucocorticoids)—many genes that are strongly expressed in astroglia, microglia, and oligodendrocytes after fear training coincide with the sites of DSB occurrence [77]. These data suggest that the mechanisms of activity-induced DSBs can affect not only neurons, but also all major types of glial cells in the brain. However, under normal conditions (without strong stimulation or stress), physiological DSBs in glia have not yet been practically described. Rather, glial DSBs are discussed in the context of CNS pathologies.

In contrast (See Table 1), pathological DSBs persist, accumulate, and are associated with chronic DDR signaling, epigenetic alterations, and neuronal dysfunction. The ability to rapidly repair programmed DSBs, therefore, defines a threshold between physiological plasticity and pathology. Ageing or disease-related declines in repair efficiency may shift normally tolerable levels of activity-induced DSBs into a pathogenic range.

Table 1: Key differences between physiological and pathological DNA double-strand breaks in the brain.

Feature	Physiological DSBs	Pathological DSBs
Conditions of occurrence	Normal neuronal activity, including sensory stimulation, novelty exposure, learning, and memory formation; acute stress within adaptive limits [3,5,67]	Chronic stress, sustained hyperexcitability, metabolic and oxidative imbalance; age-related accumulation; exposure to neurotoxic factors (e.g., amyloid-β) [6,7]
Genomic localization	Spatially restricted DSBs in regulatory regions, predominantly in promoters of IEGs such as *Fos*, *Npas4*, and *Egr1*, facilitating rapid transcriptional activation [5]	Broader and less organized genomic distribution, including non-regulatory regions [6]
Duration and repair kinetics	Transient lesions that are rapidly detected and efficiently repaired within hours to a day, without long-term accumulation [2,3,5]	Persistent and recurrent breaks with delayed or incomplete repair, leading to progressive accumulation [6,53]
Cellular consequences	Chromatin remodeling, activation of plasticity-related transcriptional programs; not associated with neuronal loss [6,63]	Chronic activation of DNA damage response pathways (e.g., ATM–p53, NF-kB), transcriptional dysregulation, cellular senescence or apoptosis, and induction of neuroinflammatory processes [78,79]
Cell types involved	Predominantly active neurons within engaged neural circuits [3,5]	Increased DSB markers detected in neurons and astrocytes, with secondary activation of microglia [6,78]
Representative outcomes	Activity-induced DSBs during learning and memory reconsolidation; interference with Topoisomerase IIβ activity impairs IEG induction and long-term memory formation [5,68,69]	Neurodegenerative and stress-related conditions: accumulation of DSBs correlates with cognitive decline; neurotoxic stimuli exacerbate DNA damage and impair repair mechanisms [80,81]

Note: DSBs, DNA double-strand breaks; IEGs, immediate early genes; ATM, ataxia telangiectasia mutated; p53, tumor protein p53; NF-kB, nuclear factor kappa B; *Fos*, Fos proto-oncogene; *Npas4*, neuronal PAS domain protein 4; *Egr1*, early growth response 1.

6 Transition to Pathological DSB Modes in the Central Nervous System

Prolonged/repetitive stress, hyperexcitation (epileptiform activity), inflammation and oxidative imbalance increase the frequency of DSB while reducing the effectiveness of their elimination (Fig. 2). In conditions of severe hyperexcitability, the threshold of "physiology" is quickly exceeded: after a kainate-induced seizure, multiple γH2AX foci form in the hippocampus [19,82] and early neuron death is noted, which corresponds to a massive, poorly repaired DSB response.

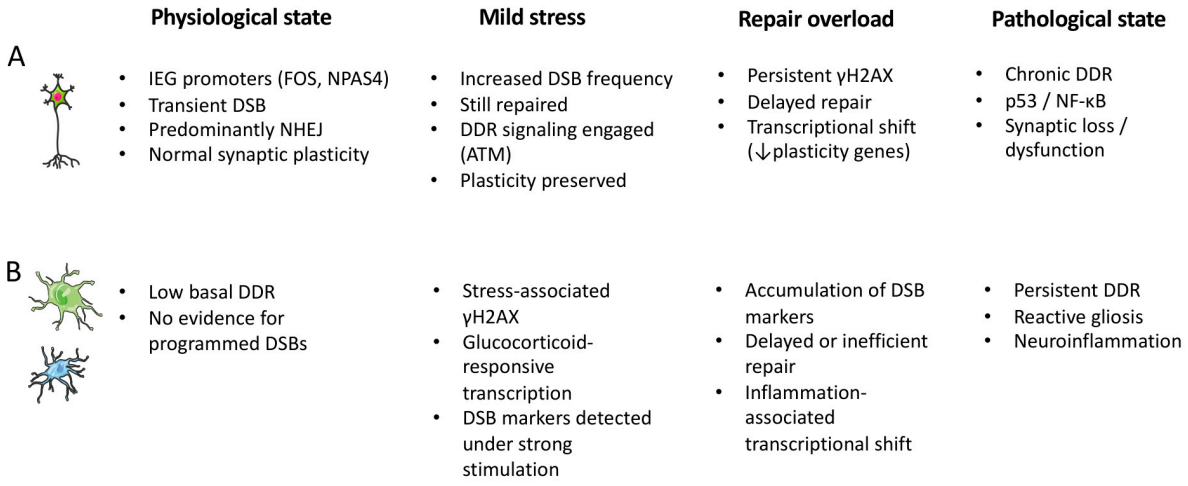

Figure 2: Continuum of DSB states in neurons and glia. In (**A**) neurons, temporary DSBs can occur under physiological conditions and, with effective repair, contribute to activity-dependent gene expression and synaptic plasticity. Under moderate stress, DSB formation and signaling in response to DNA damage (DDR) are enhanced, but the repair ability remains sufficient to maintain genomic integrity. Under conditions of repair overload, persistent DSBS, prolonged DDR activation, and incomplete restoration of DNA integrity can lead to altered transcription and impaired neuron function. In pathological conditions, DSB accumulation and chronic DDR signaling can contribute to synaptic dysfunction, genome instability, and neurodegeneration. No well-programmed DSBs have been demonstrated in glial cells (**B**); however, stress and inflammatory stimuli can increase the level of DSB markers and activate repair-related pathways. As the severity of damage increases, the constant transmission of DSB-related signals in glia can lead to changes in gene expression towards reactive and maladaptive states, which ultimately contribute to chronic inflammation, aging, or loss of supportive functions. Abbreviations: IEG, immediate early gene; DSB, DNA double-strand break; NHEJ, non-homologous end joining; DDR, DNA damage response; ATM, ataxia telangiectasia mutated; γH2AX, phosphorylated histone H2AX; NF-kB, nuclear factor kappa B. Created by the authors in Microsoft PowerPoint using elements adapted from Servier Medical Art (Smart.servier.com), licensed under CC BY 4.0.

In AD (Alzheimer's disease) models (e.g., hAPP mice), hyperactivity of neural networks is associated with an increased level of DSB, and pharmacological suppression of hyperactivity reduces DSB load and improves cognitive performance [3]. Psychological chronic stress is also associated with increased markers of DNA damage and DDR response in the brain [83,84]. Perhaps chronic stress can perpetuate pathology through DDR (loop stress-DSB/DDR-increased arousal → new DSBs). If DSBs persist, DDR ceases to be local: prolonged ATM/DNA-PK activity can involve NF-kB and form a stable pro-inflammatory transcription regime [85–87].

With aging, the basic level of γH2AX and other DSB markers in the brain increases even without obvious pathology [86,88]; with cognitive decline, these markers are higher [89,90]. The reasons include: a decrease in repair power (NHEJ/BER, etc.), an increase in metabolic/oxidative pressure, a change in

chromatin, and activation of repeats, which can increase the R-loop/DSB load [91]. An age-related decrease in NHEJ components (Ku70/Ku80, DNA-PKcs) [92] and a decrease in BER [93] have been reported, which increases the flow of SSB and secondary DSB.

Functionally, chronic DDR shifts expression during aging: synapse/plasticity-related programs decrease, stress and immune programs (NF-kB/p53-dependent) increase. This is accompanied by epigenetic changes and senescence-like states of postmitotic cells, including variants of neuronal aging with proinflammatory secretion (SASP—senescence-associated secretory phenotype) [78]. Additionally, long-lived neurons accumulate somatic variants (mosaicism; see Section 4), which can contribute to functional decline.

Neurodegenerations are characterized by an increased background of DNA damage and chronic DDR activation [2]. For AD, DSB accumulation is considered an early event [79]. The effect of tau on the level of damage has also been shown (in some studies, a decrease in tau reduces the DSB background) [3]. The pathways Aß-$Ca2^{+}$/ROS-DSB and the possible inhibition of NHEJ/DDR components are discussed [80,81].

Similar associations of DDR failures have been described for other diseases: Parkinson's disorder [94,95], ALS (amyotrophic lateral sclerosis) [2,96], HD (interactions of mutant huntingtin with NHEJ components; effects of Ku70 enhancement) [97]. In sum, neurodegeneration can be considered a condition where chronic DSB stress and damage resolution defects support pathological circles of dysfunction and inflammation.

7 Increased Repairs of DSBs in Brain Cells: Conditions and Mechanisms

Increased DSB repair activity has also been described in the literature. Regular physical activity can have a positive effect on DNA repair in the brain. For example, middle-aged people who actively engage in sports have been found to have increased expression of certain proteins that protect the genome, including DSB repair components in particular, telomeric repeat-binding factor 2 (TRF2), and the Ku70/Ku80 complex compared with sedentary peers [98].

In addition, exercise increases the level of neurotrophic factors in the brain, such as brain-derived neurotrophic factor (BDNF), which, in turn, increases the expression of DNA repair enzymes [99]. For example, BDNF has been shown to protect neurons from death caused by oxidative DNA damage by enhancing repair: through activation of the cAMP response element-binding protein (CREB) pathway, it increases the expression of apurinic/apyrimidinic endonuclease 1 (APE1), a key enzyme of the basic repair pathway [100]. Although APE1 is primarily involved in the elimination of oxidized bases and SSB repair, this enhancement of basic repair prevents single-strand damage from developing into DSBs. Taken together, these findings suggest that physical activity may support neuronal genome maintenance indirectly through neurotrophic signaling and enhanced DNA repair pathways, thereby potentially limiting DSB accumulation.

A mild stress stimulus can "train" neurons and glia, increasing their resistance to subsequent damage, including by strengthening DNA repair systems. A typical example is ischemic preconditioning, when short–term sublethal ischemia makes the brain tolerant to subsequent severe stroke. It was found that such a preconditioning ischemic episode induces pronounced changes in the DSB repair system in vulnerable hippocampal neurons. In particular, mature CA1 neurons normally almost do not express Ku70/Ku80 proteins involved in NHEJ, but 1–3 days after undergoing ischemic preconditioning, they show noticeable Ku70 expression, and this is closely correlated with the survival of neurons [101]. Other studies using proteomic approaches confirm that repeated episodes of ischemia/reperfusion lead to increased expression of a number of proteins involved in DNA repair, including the Ku70/Ku80 complex [102].

Studies on human astrocyte cultures have shown that changes in the functional state of the cell affect the nature and effectiveness of DSB repair. Thus, the induction of a "reactive" phenotype in astrocytes

leads to increased repair activity: after irradiation in reactive astrocytes, the restoration of DNA breaks in actively transcribed regions of the genome is faster than in “calm” cells [103].

There is evidence that targeted interventions, from dietary restrictions to changes in the expression of individual genes, can enhance the repair system of double–stranded breaks in the brain. One of the most striking examples is short–term calorie restriction. A four-week calorie-reduction diet in young mice resulted in a significant increase in the effectiveness of NHEJ in a number of tissues, including brain cells (compared with the control group on full nutrition) [104].

Genetic factors that enhance DSB repair have been identified in both an evolutionary and a population context. Comparative studies of 18 rodent species with different life spans have shown that long-lived species have more powerful mechanisms for repairing double-stranded breaks, while repairing less serious injuries (for example, nucleotide excision repair, NER) is not so different, and this enhanced DSB repair in long-lived species is associated with increased SIRT6 activity [105]. Genetic variations affecting DSB repair have also been found in the human population. Thus, a rare allele of the SIRT6 gene, identified in centenarians (centenarians), is associated with enhanced control of mobile LINE-1 elements and increased efficiency of repair of DSBs [106]. These examples confirm that the genetic enhancement of DSB repair pathways contributes to the preservation of the genomic integrity of the brain. Moreover, they indicate potential targets (SIRT6, DNA-PK, Ku70, etc.) for pharmacological strategies aimed at maintaining the effectiveness of DNA repair in neurons and glia during aging and neurodegenerative conditions.

8 Methods for DSB Detection and Mapping

Methods used to detect DSBs differ in what they measure: some assays report indirect chromatin or DDR markers at the cell or tissue level, whereas others directly map DNA ends genome-wide. Because this distinction is especially important in brain tissue, where material may be limited and cell-type heterogeneity is high, we summarize below (Table 2) the main features, strengths, and limitations of commonly used DSB-detection approaches, including whether they have already been applied to neural cells or CNS material.

Table 2: Major approaches for detecting DNA DSBs.

Method	Main Principle	Resolution	Typical Input	Applied to the Brain/CNS	Limitations
γH2AX immunostaining/IHC/IF	Indirect DSB-associated chromatin mark; useful for cell- and region-level detection of DSB-associated foci [3]	Low–moderate; cellular/subnuclear foci, not nucleotide-level [107]	Fixed cells or tissue sections	Yes: mouse brain neurons after exploration and stimulation [3]; human brain tissue [108]	Indirect; signal can extend over broad chromatin domains and is not always equivalent to real DSB ends [107]
53BP1 immunostaining/IF	Indirect DSB-response foci; often used with γH2AX to increase confidence that focal signal reflects DSB-associated repair sites [3]	Low–moderate; cellular/subnuclear foci [109]	Fixed cells or tissue sections	Yes: validated in mouse brain together with γH2AX [3]; also used in AD brain tissue [110]	Indirect; reports DDR focus formation rather than direct break ends [109]
Neutral comet assay	Physical migration of fragmented DNA under neutral conditions; more specific for DSBs than alkaline comet [111]	Low; single-cell bulk damage, not locus-specific [3]	Isolated nuclei/cells embedded in agarose; requires homogenization	Yes: mouse [3] and rat brain [84]	No genomic localization; sensitive to sample handling and dissociation; best as validation rather than mapping [111]
γH2AX ChIP-seq	Genome-wide mapping of chromatin regions enriched for γH2AX [107]	Moderate; genome-wide but not nucleotide resolution because γH2AX spreads over broad domains	Crosslinked chromatin; typically requires substantial material/pooled tissue or sorted nuclei [77]	Yes: used in the mouse medial prefrontal cortex and hippocampus after contextual fear conditioning [77]	Indirect; broad domains reduce positional precision; signal reflects DDR-associated chromatin rather than direct DNA ends [107]
BLISS/sBLISS	Direct *in situ* labeling and sequencing of DSB ends; genome-wide DSB mapping [107]	High; genome-wide, near nucleotide-level break mapping	BLISS: fixed cells or tissue sections, relatively low input; sBLISS: suspension-based format compatible with cultured neural cells	Yes: sBLISS was applied to human neuroepithelial stem cells, neural progenitor cells, and post-mitotic neural cells during neurogenesis [112]	Requires careful control of background and end processing; semi-quantitative rather than absolute [107]
END-seq	Direct ligation-based mapping of DSB ends in agarose plugs; highly sensitive genome-wide detection of DSBs [113]	High; genome-wide, near nucleotide-level mapping	Purified cells/nuclei; higher input than BLISS and requires a specialized workflow with agarose plugs [107]	Not yet a standard brain tissue method; widely used in other systems	Technically demanding

Note: IHC, Immunohistochemistry; IF, Immunofluorescence; ChIP-seq, Chromatin Immunoprecipitation Sequencing; BLISS, Breaks Labeling *In Situ* and Sequencing; sBLISS, in-suspension breaks labeling *in situ* and sequencing; END-seq, DNA Breaks and End Resection Measured Genome-wide by End Sequencing; γH2AX, phosphorylated H2AX; 53BP1, p53-binding protein 1; DSBs, DNA double-strand breaks; DDR, DNA damage response; CNS, Central Nervous System; AD, Alzheimer's disease.

9 Conclusion

Taken together, the available evidence suggests that DSBs are sensitive indicators of the functional state of neurons, ranging from normal physiological activity (for example, during the expression of IEGs) to stress and early pathological changes. Neurons and glial cells go through a number of "molecular states"—from healthy (homeostatic) to senescent or dysfunctional, and each of these stages is characterized by a DSB/DDR profile and concomitant transcriptomic-epigenetic shifts. In this regard, it becomes necessary to integrate DSB mapping with transcriptome and epigenome analysis in order to more fully characterize the current state of neurons and glia and trace the trajectory of molecular changes [114]. This approach allows not only to detect the presence of DNA damage, but also to reconstruct the trajectories of the shift of brain cells from adaptive DSBs and DDR modes to states in which the transience of breaks and pathological programs are being launched. Such an approach may be particularly informative for identifying pre-symptomatic prior to overt neurodegeneration or cell loss.

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