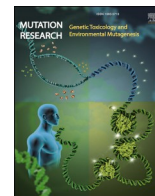


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Relationship between DNA damage measured by the comet-assay and cognitive function

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ABSTRACT

Recent studies exploring the relationship between DNA damage measured by the comet assay (single-cell gel electrophoresis) and cognitive function in both animal models and humans are reviewed and summarized. This manuscript provides an overview of studies exploring cognitive dysfunction related to DNA damage due to biological ageing process, cancer treatment, adverse environmental or occupational exposures, and prenatal genotoxic exposure. The review confirms the potential of comet assay to further explore the link between DNA damage, as indicative of genomic instability, and cognitive impairment in different research and clinical areas. Analysed studies support, in fact, the significant relationship between DNA damage and cognitive impairment, mainly affecting attention, working memory and executive functions. These cognitive domains are crucial to daily functioning and occupational performance, with important clinical implications. Although evidence support the relationship between DNA damage measured by the comet assay and cognitive function in different settings, further longitudinal research is needed to disentangle the temporal relationship between them over time, and to explore the potential of comet assay-detected DNA lesions to predict response to interventions.

1. Introduction

The comet assay (also known as single cell gel electrophoresis) is a simple, sensitive and rapid technique for detecting DNA damage and repair at the level of individual cells [1]. It can be applied to a wide variety of cell samples, in fact, any eukaryotic cell type that can be obtained as a single cell or nuclear suspension can be amenable to comet assay analysis. It is extensively used in genotoxicity testing – both in *in vitro* and *in vivo* studies – in human biomonitoring studies to examine the effects on DNA of environmental or occupational exposures to

potentially hazardous agents, and in clinical settings to study factors contributing to disease [2]. Breaks present in the DNA relax the supercoiling of DNA loops, which can migrate under electrophoresis to form comet-like images. Alkaline conditions used in the standard version of the assay favour transformation of additional lesions (abasic sites and other alkali sensitive sites) into breaks, so that they can be detected in the assay as well. DNA damage is determined by the intensity of the comet tail relative to the head [3]. Essential advantages of the comet assay are the low cost, speed, simplicity, need for relatively low number of cells without requirement for cell culture, and wide versatility [3,4].

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Indeed, modifications of this technique allow the measurement of different types of alterations in the DNA structure (e.g., oxidation, alkylation, cross-links, etc.) [5,6] and DNA repair capacity [7]. A recent analysis suggests a new role for the comet assay as a tool to risk biomarker, strengthening the evidence that the level of DNA damage in circulating leucocytes may be predictive of the risk of chronic diseases, including cancer, and mortality of healthy individuals [8]. These findings provide epidemiological evidence encouraging the implementation of the comet assay technique in preventive public health strategies for non-communicable diseases.

While age is the strongest known risk factor for declining cognitive function, other risk factors across the lifespan include education level, brain injury, environmental exposure to potentially hazardous contaminants (e.g., pesticides), frailty, cognitive and physical inactivity, unhealthy diets, toxic habits (such as drug or alcohol abuse), depression, social isolation, and chronic medical conditions or pharmacological treatments [9]. Since cognitive impairment is costly and invalidating, the identification of novel and sensitive techniques to better understand factors involved in its development and mechanisms of action is especially important. Considering that risk factors of cognitive impairment have been associated with DNA damage [e.g., age [10], alcohol abuse, unhealthy dietary components and environmental or occupational exposures to contaminants (reviewed in [11]), neuropsychiatric disorders (reviewed in [12]), chronic cardiovascular, metabolic and pulmonary diseases (reviewed in [13])], the promising potential of comet assay to further explore the link between DNA damage and cognitive dysfunction in different research and clinical settings was examined.

In this review, studies exploring the relationship between DNA damage measured by the comet assay in any of its variants (strand breaks, oxidative damage, incision repair activity) and cognitive function in both animal models and humans were analysed. Their main features are described in Tables 1 and 2, respectively.

2. Search strategy

Studies included in this review were identified by a bibliographic search using the Web of Science (WoS, all databases, all collections) (<https://www.webofscience.com>) and Scopus (<http://www.scopus.com>) databases, updated to March 2022. The search strategy comprised two terms that were intersected using the Boolean term “AND”. The search term included as first descriptor was “comet assay”, and the second one included descriptors related to cognitive performance (“cognit*”). Initial screening was focused on Topic (including title, abstract, author keywords, and Keywords Plus) in WoS, and on Title/Abstract/Keywords in Scopus. Language was not a criterion of the research. Nevertheless, in all articles found English language was present. Reviews, conference papers and case reports were excluded. The initial search retrieved a total of 77 manuscripts. After a thorough revision, 63 of them were discarded due to: (i) they were reviews, (ii) they were in vitro studies, (iii) the presence of the search terms was limited to the References section, (iv) no cognitive parameter was included, or (v) no comet assay analysis was conducted. Eventually, 14 manuscripts could be included in this review.

3. DNA damage and cognitive impairment among older adults

The rapid ageing of populations around the world has imposed a huge health impact on the society. A longer lifespan might translate into additional years living with neurocognitive disorders, such as cognitive impairment and dementia [9], leading to a reduced quality of life and increasing demands for health and social care services. Literature provides evidence that increased oxidative DNA damage levels contribute to brain ageing and neurodegenerative diseases [14]. Indeed, DNA damage from oxidation is considered to be a key event in ageing per se [15,16], as well as an early pathogenic event in many neurodegenerative disorders, including Alzheimer’s disease (AD) [17,18]. Furthermore,

lymphocytes of patients with AD showed an altered DNA repair kinetics under in vitro treatment with hydrogen peroxide (evaluated by the challenge-comet assay, also known as cellular repair assay) compared to age-matched healthy controls, suggesting that repair pathways may be compromised in these patients [19]. Furthermore, conclusions from a recent review on DNA damage and repair in neuropsychiatric disorders (including age-related diseases such as AD and Parkinson’s) indicate that DNA mutations and damage, as well as disruptions in repair pathways, are likely to contribute to the onset and progression of neurodegenerative disorders [12].

Maintaining a healthy and active lifestyle (e.g., balanced diet, regular physical exercise, etc.) has been suggested as a protective factor to prevent DNA damage [20] and decline in the cognitive function [21] related to the ageing process. In a study conducted among Austrian institutionalized older adults (n = 105, 65–98 years old), it was reported that a 6-month cognitive training (consisting of memory training and finger dexterity coordinative exercises) as well as a supervised progressive resistance training (consisting of exercises for the main muscle groups, progressively increasing in intensity and volume from the fifth week on) decreased DNA damage induced in peripheral blood lymphocytes by hydrogen peroxide (evaluated by standard alkaline comet assay), suggesting that lifestyle interventions, both cognitive and physical, might have provided the subjects with a better defence system against H₂O₂-induced oxidative stress, confirmed also by a general tendency to increase in antioxidant enzyme activity [22].

Frailty has emerged as a reliable measure of the ageing process and has been related to cognitive impairment [23]. It is a multidimensional geriatric syndrome characterized by higher vulnerability to stressors, with an increased risk of adverse health outcomes such as morbidity, disability, hospitalization, institutionalization, and mortality [24]. However, the relationship between frailty and DNA damage is not clear. Only a few studies were done using the comet assay and, so far, no association of primary DNA damage (single and double strand breaks assessed by the standard alkaline comet assay) or oxidative DNA damage (determined by the fpg- or hOGG1-modified comet assay) was found [25–27]. Nevertheless, frailty has been positively related to DNA double strand breaks, evaluated by the γ H2AX assay, in peripheral blood mononuclear leucocytes [27], as well as in circulating hematopoietic progenitor stem cells [28]. And significantly higher frequencies of micronuclei in lymphocytes were observed in frail vs. non-frail individuals [29]. Moreover, a clear tendency to decline in repair capacity (assessed by the bleomycin challenge-comet assay) with increasing frailty status (i.e., non-frail > pre-frail > frail) was also observed, although statistical significance was not reached. Based on these results, the potential link between physical frailty syndrome and DNA damage related to cognitive impairment deserves further investigation.

Cumulative DNA damage and a reduced capacity of DNA repair in the ageing brain may result in neuronal dysfunction and contribute to cognitive impairment [30]. Supporting this hypothesis, in cross-sectional studies using the comet assay to evaluate the level of DNA damage, it has been demonstrated that two different cohorts of Malaysian older adults with cognitive impairment show significantly higher levels of DNA damage in peripheral blood lymphocytes as compared to older adults with normal cognitive function [31,32]. Furthermore, cognitive impairment was associated in multivariate binary logistic regression analysis with poor serum folate concentration and DNA damage (n = 232) [31], and with the level of trace elements, namely lead (Pb) and copper (Cu), in toenails (n = 317) [32], suggesting that nutritional factors, such as folate deficiency, and environmental exposures to contaminants, such as heavy metals, may have important adverse effects on the nervous system in later life.

Folate (found in a wide variety of food including green leafy vegetables, cereals, beans, fruit, and liver) is essential for the synthesis and repair of DNA; it acts in normal cellular metabolism to maintain genomic stability through the provision of nucleotides for DNA replication and DNA repair, and by regulating DNA methylation and gene expression

Table 1
Main features of animal studies exploring the relationship between comet assay-evaluated DNA damage and cognitive function.

Category	Study	Experimental group	Comet assay version and measurements	Cognitive function parameter	Main results
Age-related neurodegeneration	Borai et al. (2017) [45]	Male rats with aluminium chloride-induced neurodegenerative features of AD	Alkaline comet assay. Basal DNA damage	T-maze test	Aluminium treatment resulted in impaired cognitive function and increase in DNA fragmentation in brain cells. Treatment of AD-rats with VLP or RIVA improved neurobehavioral changes and produced a reduction in DNA damage
Chemotherapy	Krynetskiy et al. (2013) [52]	Male Swiss-Webster mice treated with 5FU	Alkaline comet assay. Basal DNA damage	Behavioural functioning (learning and memory) in an autoshaping-operant procedure	Significant increase in DNA damage in brain cells was observed in 5FU-treated mice vs. controls. Positive correlations were obtained between increased response rates and increased rate of errors, and DNA damage on day 1
	Fouad et al. (2021) [53]	Wistar rats treated with DOX	Alkaline comet assay. Basal DNA damage	Home cage observations of unusual behaviours: spinning, convulsions, decreased physical activities, or lethargy	DOX treatment resulted in remarkable signs of neurotoxicity (ataxia, lethargy, decreased physical activities), rats did not respond to behavioural tests (T-maze) due to cognitive dysfunction, and showed significant elevation in comet parameters. Co-treatment with BEB showed no clinical signs of neurotoxicity and significantly counteracted DOX-induced genotoxicity.
Stimulant use	Frenzilli et al. (2007) [69]	Male C57 black mice treated with MDMA hydrochloride	Alkaline comet assay. Basal DNA damage	Locomotor activity, EEG evaluation and kainic acid-induced limbic seizure evaluation	MDMA administration selectively produced DNA damage in the hippocampus soon after the last injection and persisted 5 days later, in the absence of striatal DNA damage. Changes in DNA integrity persisted and accompanied the onset of behavioral sensitization, slowed EEG activity and persistent reduced threshold to convulsive limbic seizures
	Wojtas et al. (2021) [70]	Male Wistar-Han rats treated with 25B-NBOMe	Alkaline comet assay. Basal DNA damage	Hallucinogenic activity: head and body twitch response. Cognitive functions (short-term memory): NOR test. Locomotor activity: OF test. Anxiogenic/anxiolytic effect: LDB test	25B-NBOMe induced hallucinogenic activity, lowered the recognition index vs. control in the NOR test, decreased locomotor activity, and increased the time spent in the dark zone in the LDB test dose-dependently. 25B-NBOMe treatment produced a minor DNA damage in the rat frontal cortex cells
Prenatal DNA damage	Zabrodina et al. (2016) [75]	60-day-old offspring of streptozotocin-induced diabetic albino rats	Alkaline comet assay. Basal DNA damage	Food seeking behaviour under the conditions of free choice in a 6-arm maze	The formation of the food-procuring skill was significantly delayed in the offspring of diabetic rats. Administration of afobazole and betaine significantly decreased DNA damage and improved formation of a food-procuring skill. Correlation analysis showed a strong relationship between DNA damage in cells of the embryo and placenta during intrauterine development and cognitive dysfunction in the postnatal offspring of animals with streptozotocin-induced diabetes

Abbreviations: 25B-NBOMe, 4-bromo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine; 5FU, 5-fluorouracil; AD, Alzheimer's disease; BEB, berberine; DOX, doxorubicin; EEG, electroencephalogram; LDB, light/dark box; MDMA, 3,4-methylenedioxymethamphetamine; NOR, novel object recognition; OF, open field; RIVA, rivastigmine; VLP, Vitis vinifera leaves polyphenolic extract.

Table 2

Main features of human studies exploring the relationship between comet assay-evaluated DNA damage and cognitive function.

Category	Study	Experimental group	Comet assay version and measurements	Cognitive function parameter	Main results
Older adults	Leandro et al. (2013) [18]	AD patients (n = 8) vs. elderly matched controls (n = 8)	Alkaline and hOGG1-modified challenge-comet assay. H ₂ O ₂ -induced DNA damage and repair kinetics	MMSE, CDR	Induction of DNA damage by H ₂ O ₂ treatment was higher in the AD group. AD patient cells showed an altered DNA repair kinetics
	Franzke et al. (2015) [21]	Institutionalized older adults (n = 105) subjected to a 6-month progressive resistance training or cognitive training	Alkaline and fpg-modified comet assay. Basal DNA damage and H ₂ O ₂ -induced DNA damage	Cognitive training consisted of memory training and finger dexterity exercises in sitting position.	%DNA in tail decreased significantly in cells exposed to H ₂ O ₂ in the resistance and cognitive training groups. No intervention effects were detected for fpg-sensitive sites
	Lee et al. (2009) [30]	Older adults with cognitive impairment (n = 51) vs. normal cognition (n = 181)	Alkaline comet assay. Basal DNA damage	Elderly Cognitive Assessment Questionnaire	Cognitive impairment was significantly associated with DNA damage (linear correlation). Multivariate binary logistic analysis demonstrated poor serum folate concentration and DNA damage were associated with cognitive impairment in both sexes
	Meramat et al. (2017) [31]	Older adults with cognitive impairment (n = 197) vs. normal cognition (n = 120)	Alkaline comet assay. Basal DNA damage	Montreal Cognitive Assessment	Subjects with cognitive impairment had significantly higher levels of DNA damage as compared to the group with normal cognitive function. Multiple logistic regression revealed DNA damage and level of trace elements in toenails (lead and copper) as predictors for cognitive impairment
Chemotherapy	Carroll et al. (2019) [49]	Middle-aged women survivors of breast cancer (n = 94)	Alkaline comet assay. Basal DNA damage	Neuropsychological test battery assessing learning, memory, attention, visuospatial, executive function and motor speed. Functional Assessment of Cancer Therapy-Cognitive Function	Higher DNA damage was found to be statistically significantly related to lower executive function scores adjusting for age, body mass index, race, years from treatment, and intelligence score.
	Root et al. (2021) [50]	Breast cancer patients (n = 23) vs. controls (n = 18). Evaluations prior to initiation of chemotherapy (baseline) and following completion of treatment (follow-up)		Functional magnetic resonance imaging during a working memory n-back task.	Working memory performance indicated a significant improvement in the controls at follow-up, and no change in cases. Oxidative DNA damage levels were elevated in the cases at follow-up compared to controls, but no associations were found between the comet assay variables and functional imaging at either time-point or group.
Occupational exposures	Bortolotto et al. (2021) [64]	Hospital nurses (n = 78)	Alkaline comet assay. Basal DNA damage	Selective attention: Stroop test. Declarative and working memory: digit and word span tests	Increased levels of DNA damage were associated with decreased scores in selective attention and declarative and working memory. Cortisol levels on waking up were associated positively with the DNA damage
Stimulant use	Winhusen et al. (2013) [72]	Methamphetamine-dependent (n = 45) and cocaine-dependent (n = 120) users	Fpg-modified comet assay. Oxidative DNA damage	Executive function: Frontal Systems Behaviour Scale	Oxidative DNA damage was significantly greater in methamphetamine-dependent participants with, than without, executive dysfunction. Executive dysfunction was a significant mediator of oxidative DNA damage and stimulant use during active treatment. Contrary to prediction, oxidative DNA damage was not significantly greater in cocaine-dependent participants with, than without, significant executive dysfunction

Abbreviations: AD, Alzheimer's disease; fpg, formamidopyrimidine DNA glycosylase; hOGG1, human 8-oxoguanine DNA glycosylase, MMSE, mini-mental state examination.

[33]. Effects of folate depletion on DNA stability in human lymphocytes in vitro have been reported using the comet assay. They showed increased DNA strand breakage in a time- and concentration-dependent manner after lymphocytes were cultured with decreasing amounts of folic acid, as well as inability of folate-deprived cells to efficiently repair

oxidative DNA damage induced by hydrogen peroxide, determined by the challenge-comet assay using endonuclease III (endoIII) to detect oxidized pyrimidines [34]. Similarly, genomic instability (micronucleus test) induced by bile acids in human colon NCM460 and liver L-02 cells was also recently shown to be exacerbated by folate deficiency, with

folate supplementation acting as an efficient protective factor against genotoxicity [35]. Folic acid-deficient B-lymphoblastoid WIL2-NS cells were more susceptible to micronuclei formation by glucose and/or methylglyoxal [36]. Using a combined proteomics and biochemical approach, folate deficiency was also shown to differentially alter activity and expression of proteins involved in DNA repair [e.g., XRCC5, MSH2] in human colonocytes [37]. Moreover, DNA breakage related to folate deficiency has been suggested to contribute to the increased risk of cognitive dysfunction in humans [38].

Regarding studies on heavy metals, Pb is a well-studied toxicant because of its detrimental effects on a broad range of physiological, biochemical, and behavioural functions, affecting almost all tissues and organ systems [39]. It causes genotoxicity, mainly through indirect mechanisms such as inhibition of DNA repair or production of free radicals [40]. Human exposure to Pb can lead to significant neuropsychological and functional decline, including difficulties in intelligence, memory, executive functioning, attention, processing speed, language, visuospatial skills, motor skills, and affect/mood [41]. While Cu is an essential nutrient for humans, it can pose risks to human health with elevated exposure [42]. Cu toxicity typically results from the production of reactive oxygen species (ROS) during redox reactions involving excess free or ionic Cu forms, and from sufficient accumulation to overwhelm protein-binding capacity [43]. Exposure to heavy metals from environment has long been debated as a potential environmental risk factor for neurodegenerative disorders, such as AD and Parkinson's disease (reviewed in [44,45]). In fact, neurodegenerative features of AD were produced in an aluminium-intoxicated rat model (male rats treated with AlCl₃ orally daily for 4 successive weeks) [46]. Rats showed behavioural alterations, assessed by the rewarded T-maze test, indicating neurocognitive decline (AD group showed increased elapsed time to receive the reward displaying decreased working memory and learning), and increase in DNA fragmentation as evidenced by the alkaline comet assay was observed in brain cells. Further, grape (*Vitis vinifera*) leaves polyphenols extract was effective in reversing the aluminium-induced neurotoxicity in the experimental rats, by reducing brain DNA damage and ameliorating the functional outcome, as shown in behavioural T-maze test, and confirmed by the comet assay.

Results obtained in the previously mentioned studies in Malaysian older adults [31,32] suggest that folate deficiency and/or increased levels of heavy metals are associated with cognitive impairment, and that this association might be mediated by DNA damage production. However, in both studies only a measure of general cognitive functioning was adopted to assess cognition, namely the Elderly Cognitive Assessment Questionnaire (ECAQ) [47], a 10-item scale derived from items in the Mini-Mental State Examination (MMSE) and Geriatric Mental State Schedule (GMS), specific for quantitative assessment of cognitive impairment among older adults living in developing countries. In this context, future longitudinal studies including comprehensive neuropsychological testing and neuroimaging techniques are needed to further explore the relationship between DNA damage and cognitive impairment, and the influence of genetic and environmental factors on their relationship.

4. Cognitive function and chemotherapy-induced DNA damage

The majority of cancer treatments (radio- and chemotherapy) are not tumour-specific and can cause systemic toxicity and vast amounts of DNA damage in otherwise healthy tissue, increasing the risk of late and long-term sequelae in cancer-survivors. Often these individuals develop medical conditions similar to patients with deficiencies in DNA repair processes, who show a greater burden of DNA damage and accelerated ageing. Thus, cancer survivors treated with radiation and chemotherapy age several decades faster than individuals not exposed to these genotoxic agents (reviewed in [48]), exhibiting manifestations of cognitive dysfunction like those observed during biological ageing. Furthermore, infant patients requiring therapy targeted to the central nervous system

to treat childhood brain tumours experience neurocognitive decline much earlier than their siblings [49].

In this line, a higher DNA damage determined using the comet assay has been moderately related to lower standardized executive function scores in middle-aged survivors of breast cancer (n = 94) [50], paralleling the age-related cognitive changes and suggesting a significant association between measures of biological ageing (DNA damage) and objective measures of cognitive performance (executive function) in these subjects. However, the significance was not observed after correction for multiple tests using the false discovery rate.

A recent neuroimaging prospective study by Root et al. (2021) [51] revealed a persistent task-induced deactivation in prefrontal regions during the execution of a working memory n-back task [52] in breast cancer patients (n = 23) from baseline (immediately prior to the start of treatment) to follow-up (after completion of treatment), compared to controls with no cancer history (n = 18), who exhibited reduced task-induced deactivation and improved working memory performance. The authors interpret the lack of behavioural improvement and persistent task-induced deactivation in cancer patients as a failure to benefit from previous exposure to the scanner environment and cognitive task, as opposed to controls who showed both reduced task-induced deactivation and improved performance at follow-up. Oxidative DNA damage levels, evaluated by endoIII and formamidopyrimidine DNA glycosylase (fpg)-modified comet assay, in peripheral lymphocytes were also increased in the cancer patients at follow-up as compared to baseline and to controls at follow-up. Still, no associations were found between the comet assay values and functional imaging activations or deactivations at either group or time-point that survived comparison for multiple corrections. Nevertheless, the finding of greater oxidative DNA damage levels may suggest a mechanistic explanation for alterations to brain activity seen in chemotherapy treated breast cancer patients [51].

In a rodent model, DNA damage induced by the antineoplastic chemical 5-fluorouracil in brain cells, assessed by the standard alkaline comet assay, has been associated with behavioural changes (learning and memory deficits) [53], supporting the hypothesis that chemotherapy-induced DNA damage of murine brain cells is related to behavioural changes. Specifically, the 5-fluorouracil-treated rats failed to demonstrate the same degree of acquisition and retention of a response task for food reinforcement than the saline-treated group. The authors concluded that DNA damage might change the gene expression status and functioning of neural cells, providing a hypothetical mechanism for cognitive impairment after pharmacological treatment of cancer.

Another study demonstrated remarkable signs of neurotoxicity in rats treated with the chemotherapeutic agent doxorubicin, and showed that they did not respond to behavioural tests such as T-maze, due to cognitive dysfunction [54]. In fact, rats in the experimental group showed exposure-related unusual behaviours such as ataxia, lethargy, and decreased physical activity as compared to control rats. Importantly, co-treatment of the rats with doxorubicin and berberine (chemical isolated from barberry (*Berberis spp.*), a well-known ancient medicinal plant used in traditional Chinese and Ayurvedic medicine [55]) demonstrated genoprotective potential against doxorubicin-induced DNA damage, counteracting oxidative stress and neuroinflammation, with no clinical signs of neurotoxicity.

In summary, although significant evidence of a direct relationship between DNA damage induced by chemotherapeutic treatment in cancer patients and alterations in cognitive performance could not be obtained thus far, plausibility for this association mainly based on evidence obtained from rodent studies support future studies conducted in larger populations.

5. DNA damage due to hazardous exposures and cognitive function

Exposure to genotoxic agents in environmental or occupational

settings may induce DNA damage. Common examples include exposure to ionizing radiation [56], pesticides [57], heavy metals such as Pb [58], organic solvents such as formaldehyde [59], or fuel oils [60]. Besides, exposures to chemical or physical agents may also cause distinct types of alterations in the cognitive performance. In fact, it has been demonstrated that environmental exposure to chemicals is related to neurological impairments (including neuropathies, cognitive, motor, and sensory impairments), neurodevelopmental disorders (including autism and attention deficit hyperactivity disorder), and neurodegenerative diseases (including AD, Parkinson's disease, and amyotrophic lateral sclerosis) [61].

Nevertheless, to our knowledge, the existence of a possible relationship between DNA damage (as evaluated by the comet assay) induced by a particular exposure and the manifestation of cognitive dysfunction in the exposed subjects was only addressed so far in hospital nurses and in cases of experimental administration or use of illicit stimulant substances. These studies are described in the two following sections.

5.1. Occupational factors

Hospital nurses are exposed to several occupational stressors including chemical (e.g., antineoplastic drugs, ethylene oxide, anaesthetic gases, formaldehyde), physical (e.g., ionizing radiation), biological (e.g., bacterial or virus infections), and psychosocial (e.g., stress, shift work, violence in the workplace) agents. Occupational stress, which is common among hospital nursing teams, may affect specific brain regions (mainly the frontostriatal circuits) and cognitive functioning [62,63], as well as induce DNA damage [64].

Indeed, in a cross-sectional study conducted in Brazilian nurses (n = 78) facing stressful and adverse occupational exposures at a University Hospital [65], increased levels of DNA damage were associated with decreased scores in cognitive tests assessing selective attention (Stroop test) and declarative and working memory (digit and word span). Occupational exposures were not specifically determined, although over half of the participants worked in contact with ionizing radiation (at doses below the annual levels allowed by law, according to personal dosimeters). Mean (\pm standard deviation) duration of work at the institution was 16 (\pm 10.8) years. Furthermore, cortisol level on waking up, an indicator of psychophysiological stress, was positively associated with DNA damage. Given the above, the authors suggested that the DNA damage, measured by the comet assay, could be an indicator of systemic adverse effects of chronic stress, which can also affect memory, evaluated by the digit span score. This finding has important clinical implications since both attention and memory functions are essential cognitive abilities for the nursing staff professional activities, hence alterations in these abilities can lead to lower productivity, errors in clinical settings, and lack of concern in handling patients.

5.2. Stimulant use

Illicit stimulant use, including cocaine and amphetamines, increases the formation of ROS causing oxidative stress [66,67] and has been associated with cognitive deficits and brain changes. Evidence from preclinical research suggests that oxidative stress play a role in the neurotoxic effects related to consumption of illicit stimulants, specifically amphetamines and cocaine [67–69].

According to this evidence, systemic administration to mice of low doses of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), producing hippocampal hyperexcitability and comparable with those self-administered by humans, produced acute oxidative stress (measured by glutathione content and superoxide dismutase activity) and DNA single and double-strand breaks (assessed by the comet assay) in hippocampal cells [70]. Changes in DNA integrity persisted and accompanied the onset of behavioural sensitization, slowed electroencephalographic (EEG) activity and persistent reduced

threshold to convulsive limbic seizures. The authors concluded that administration of MDMA produces selective hippocampal alterations (including DNA damage) which may underlie cognitive impairment and seizure susceptibility. In contrast, administration of 4-bromo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25B-NBOMe, a hallucinogen exhibiting high binding affinity for 5-HT_{2A/C} serotonin receptors) to rats in other study showed only minor damaging effect on DNA in frontal cortex cells, although impacts in short-term memory (novel object recognition test), locomotion (open field test), and anxiogenesis (light/dark box) were reported in the treated animals [71]. Interestingly, MDMA, used as the reference drug, did induce potent DNA damage in the cells.

In a study enrolling illicit stimulant abusers, oxidative DNA damage (as evaluated by the fpg-modified comet assay) was significantly increased in methamphetamine-dependent patients with executive dysfunction (deficits in memory, attention and problem-solving (assessed with the Frontal Systems Behaviour Scale (FrSBe) [72]), as compared to those without executive dysfunction [73]. Moreover, executive dysfunction was a significant mediator of oxidative DNA damage and self-reported stimulant use during the active treatment. This was consistent with previous results documenting the neurotoxicity of methamphetamine across a range of species [74]. Unexpectedly, no differences in oxidative DNA damage were observed between cocaine-dependent participants with and without executive dysfunction, although cocaine was found not neurotoxic to dopamine and serotonin neurons [75]. These results provide support for the hypothesis that methamphetamine use causes oxidative DNA damage, which results in executive dysfunction that in turn increases vulnerability to future stimulant use.

6. Relationship between prenatal DNA damage and manifestations of cognitive dysfunction in the postnatal period

Genotoxic insults to placental and embryonic cells might play a key role in the prenatal development and postnatal manifestations of cognitive disturbances. To evaluate this hypothesis, Zabrodina et al. [76] used a murine model of diabetes, induced by treatment with streptozotocin. The hyperglycemia characteristic of diabetes causes oxidative stress, which in turn can result in genotoxic stress, DNA damage, metabolic alterations, and subsequently perturbed embryogenesis [77]. Zabrodina et al. [76] assessed the genotoxic damage (by means of the comet assay) to the placenta and embryos in the offspring of streptozotocin-induced diabetic rats, and studied changes in cognitive behaviour of the adult offspring. The results obtained showed that the offspring of streptozotocin-induced diabetes rats is characterized by cognitive dysfunction, manifested in motor retardation during food-seeking behaviour and long latency of learning. Furthermore, the increased levels of DNA damage found in embryonic samples were positively correlated with a rise in the time-to-obtain food reinforcement, and negatively correlated with the frequency of food-taking episodes. The levels of DNA damage and severity of cognitive dysfunction symptoms decreased significantly and dose-dependently after administration of compounds with antimutagenic properties (afobazole and betaine), supporting the involvement of genotoxic events in the prenatal period in the development of postnatal manifestations of cognitive disorders.

A schematic diagram showing the relationships between DNA damage due to different biological and exogenous factors and cognitive impairment is represented in Fig. 1.

7. Conclusions

Before drawing conclusions on the review conducted, it is important to note that the most common objectives in published human studies employing comet assay have been monitoring exposure to exogenous or endogenous mutagens, or checking levels of oxidative stress in

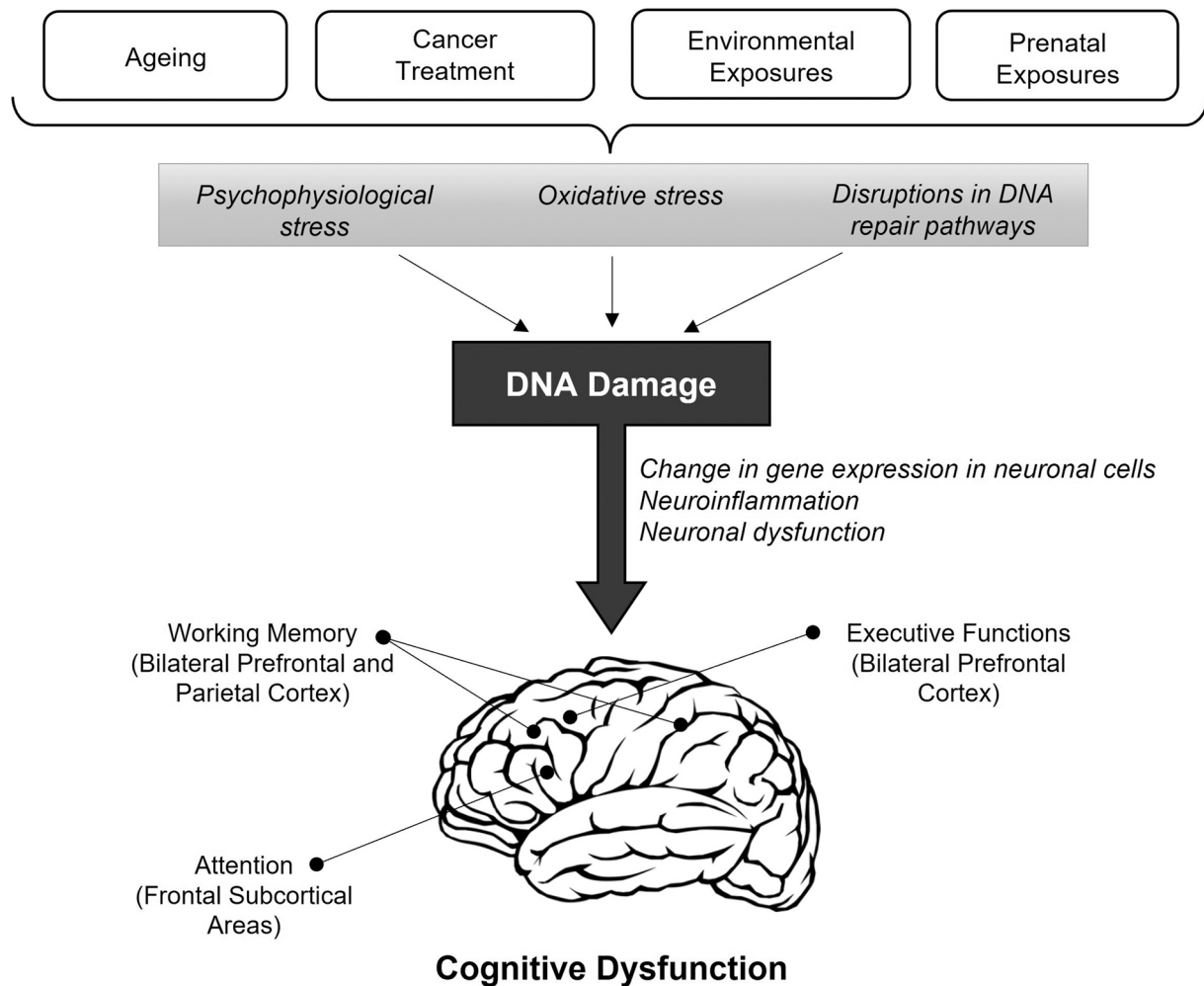


Fig. 1. Diagram representing biological and exogenous factors associated with DNA damage that, in turn, lead to cognitive impairment, affecting mainly attention, working memory and executive functions.

individuals affected by different diseases or clinical conditions. This is the case in most studies included in the present review, in which cognitive dysfunction is secondary to the exposure or to the medical conditions. In this scenario, direct relationship between comet assay and cognitive function cannot be completely explored and encourages further research. Thus, the promising potential of the comet assay technique to further expand our understanding of the link between DNA damage and cognitive impairment should be explored in depth in the near future.

Literature suggest that DNA damage may play a role in cognitive dysfunction by eliciting neuroinflammation or neurodegeneration. However, we still have limited understanding of the precise molecular mechanisms underlying cognitive dysfunction associated with DNA damage. Both rodent and human studies support this association due to several factors, affecting attention, memory, and executive functions mainly dependent on the structural and functional integrity of the prefrontal cortex. These higher-level cognitive domains are critical to daily functioning and occupational performance and productivity, with important clinical implications. However, future longitudinal research is needed to disentangle the relationship between DNA damage and cognitive function over time, and to explore the potential of comet assay-detected DNA lesions to predict response to interventions for the prevention and/or the delay of progression of cognitive decline.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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the OECD list of recommended assays for genotoxicity testing (Test No. 489: In Vivo Mammalian Alkaline Comet Assay). For all of this, we deeply thank Andrew and are very honoured to have worked with you.

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