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Dynamic regulation of cerebral DNA repair genes by psychological stress

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ABSTRACT

Neuronal genotoxic insults from oxidative stress constitute a putative molecular link between stress and depression on the one hand, and cognitive dysfunction and dementia risk on the other. Oxidative modifications to DNA are repaired by specific enzymes; a process that plays a critical role for maintaining genomic integrity. The aim of the present study was to characterize the pattern of cerebral DNA repair enzyme regulation after stress through the quantification of a targeted range of gene products involved in different types of DNA repair. 72 male Sprague–Dawley rats were subjected to either restraint stress (6 h/day) or daily handling (controls), and sacrificed after 1, 7 or 21 stress sessions. The mRNA expression of seven genes (*Ogg1, Ape1, Ung1, Neil1, Xrcc1, Ercc1, Nudt1*) involved in the repair of oxidatively damaged DNA was determined by quantitative real time polymerase chain reaction in the prefrontal cortex (PFC) and hippocampus (HC). DNA repair gene expression in PFC exhibited a general trend towards an induction after acute stress and a decrease after subchronic exposure compared to control animals. After chronic stress, a normalization towards control levels was observed. A similar pattern was seen in HC, but with overall smaller effects and without the induction after acute stress. Nuclear DNA damage from oxidation as measured by the comet assay was unaffected by stress in both regions. We conclude that psychological stress have a dynamic influence on brain DNA repair gene expression; however, since we were unable to identify concurrent changes in DNA damage from oxidation, the down-stream consequences of this regulation, if any, remains unclear.

1. Introduction

According to the World Health Organization, major depression is the leading cause of disability worldwide and a major contributor to the global burden of disease [1]. A very harmful aspect of recurrent depression, both from an individual and a socioeconomic perspective, is that the cumulative exposure to depression is associated with the development of cognitive dysfunction in the euthymic state [2,3], and an increased risk for out-right dementia [4,5]. The cognitive deterioration occuring with each depressive episode may render the individual more susceptible to new episodes [6,7].

There is compelling evidence that depression and neurocognitive dysfunction are interconnected through the neurohormonal stress response, in particular a prolonged activation of the

Abbreviations: HPA-axis, Hypothalamic-pituitary-adrenal axis; ROS, reactive oxygen species; BER, base excision repair; NER, nucleotide excision repair; SSBR, single strand break repair; Ogg1, 8-oxoguanine glycosylase 1; Neil1, endonuclease VIII-like 1; Ung1, uracil DNA N-glycosylase 1(Ung1); Ape1, apurinic/apyrimidinic endonuclease 1; Xrcc1, X-ray repair cross-complementing protein 1; Ercc1, excision repair cross-complementing rodent repair deficiency, complementation group 1; Nudix, (nucleoside diphosphate linked moiety X)-type motif 1; PFC, prefrontal cortex; HC, hippocampus; RT-PC, Rreverse transcriptase polymerase chain reaction.

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HPA-axis, which results in the systemic release of glucocorticoids [7]. In rodents, both experimental stress and glucocorticoid administration cause a reduced dendritic branching in the frontal cortex and the hippocampus, with corresponding impairments in cognitive function [8–10]. In humans, analogous reductions of hippocampal volume, cognitive deficiencies and increased dementia risk occur after prolonged stress exposure and in association to elevated glucocorticoid levels [11,12].

DNA damage from oxidation is considered to be a key event in aging per se [13,14], as well as an early pathogenic event in many neurodegenerative disorders, including Alzheimer's disease [15,16]. Previous studies have found increased levels of oxidatively generated DNA damage in the brain after acute and subchronic stress [17,18]. Correspondingly, both in vitro and in vivo studies in rodents and humans have linked increased glucocorticoid levels to increased levels of DNA damage [19–21]. DNA damage activates signaling cascades which are known to regulate neuronal survival and synaptic plasticity, including apoptosis [22], and thereby have down-stream effects that plausibly connect depression with neuronal degeneration and dysfunction. In line with this, a recent post-mortem study of gene expression profiles in the frontal cortex of depressed individuals found evidence of inflammation as well as oxidative stress and apoptosis [23]. Thus, neuronal genotoxic insults from oxidative stress is a plausible molecular link between stress and depression on the one hand, and cognitive dysfunction and dementia on the other.

Oxidatively damaged DNA is repaired by specific enzymes, and therefore, the steady-state DNA lesions reflect a balance between the ROS-induced damage and repair activity. Consequently, the cellular toxicity of genomic stress from oxidation is not solely determined by the load of ROS-induced insults, but also by the ability to repair such insults. Specific phenotypes of accelerated aging and neurodegeneration have been described in rodents and humans deficient in enzymatic DNA repair [24], and reduction in cerebral DNA repair capacity has been established in the early phases of Alzheimer's disease [25,26]. In neurons, the predominating repair pathway for oxidized DNA lesions is BER, which detects and repairs single-base damage. However, other repair pathways with different substrate specificities, such as NER or SSBR, are also active in neurons [27].

The aim of the present study was to characterize the pattern of DNA repair enzyme regulation in the brain after experimental psychological stress (restraint stress) through the quantification of a targeted range of gene products involved in different types of DNA repair. The gene expression levels were assessed in brain regions relevant to the cognitive domains affected by clinical depression (e.g., attention and memory) [2,28] and abundant in glucocorticoid receptors [9], namely PFC and HC. The genes selected for mRNA quantification *Ogg1*, *Neil1*, *Ung1*, *Ape1*, and *X-ray repair cross-complementing protein 1* (all involved in various steps of BER and SSBR); *Ercc1* (involved in NER); and *Nudt1* (involved in the removal of oxidatively damaged nucleotides from to nucleotide pool) [27,29]. All genes have previously been implicated in neuroprotection and/or found to be inducible by stress paradigms [30–37]. In parallel to the gene expression measurements, we determined the nuclear levels of oxidatively damaged DNA in the same brain regions. Both parameters were determined after acute stress (AS), subchronic stress (SCS) and chronic stress (CS).

Based on findings from a previous study [37], and the hypothesis that genomic protection is part of the brain's adaptation to stress, we hypothesized that experimentally induced psychological stress would increase the expression of genes involved in DNA and nucleotide pool repair. To our knowledge, the regulation of cerebral DNA repair as an allostatic adaptation to stress has not previously been specifically addressed.

2. Materials and methods

2.1. Animal model

Male Sprague–Dawley rats (Charles River, $n = 72$, initial weight: 220–230 g) were pair housed (Scanbur type III high Techniplast) and acclimatized 10 days prior to the experiment. Room temperature was $21 \pm 1\,^{\circ}C$, 12:12 light/dark cycle between 7AM and 7PM. The animals were fed Altromin 1319 (Brogaarden, Gentofte, Denmark) and had access to acidified water ad libitum except during restraint sessions. All animals were weighed every third day and immediately prior to sacrificing.

The animals were matched by weight and randomly distributed into 6 groups ($n = 12$ per group), treated either as control (daily handling) or with restraint stress for 6 h/day during 1, 7 or 21 consecutive days, representing AS, SCS, and CS, respectively. Chronic restraint stress is a widely validated animal model of depression, which reliably induces depressive behavior, which in turn is reversed by antidepressant treatments [10,38,39]. It further induces dendritic retractions in the hippocampus and prefrontal cortex [8,10,40]. The stress groups were restrained in their home cage daily from 9AM–3PM, using a fitted wire mesh restrainer, designed to allow natural breathing and to avoid problems of overheating, as previously described [10,37]. To avoid sonic and ultrasonic influences between the groups, stress and control groups were housed in separate rooms.

Animals were sacrificed by decapitation immediately after the first restraint stress session (AS group) or in the morning following the last session (SCS and CS groups). This procedure was applied to avoid recording acute effects of the last session in the SCS and CS groups. Animals from the stress and control groups were sacrificed in random order, and decapitation took place within <30 seconds after removal from the home cage to avoid induction of a corticosterone response. A sample of trunk blood was collected in 10 ml EDTA tubes (BD Vacutainer, K2E 18,0 mg. Plymouth. PL6 7BP. UK), followed by manual bilateral dissection and weighing of the adrenal glands. Trunk blood was stored at 4 °C until centrifugation at 3000 rcf for 15 min at 4 °C. Plasma was removed and stored at −80 °C until assayed for corticosterone, by a commercially available ELISA kit as previously described [41]. Immediately upon blood sampling, the PFC and HC were dissected mannually on an ice-cold metalic plate. Both regions were instantly frozen in liquid nitrogen for storing at −80 °C. The left brain-area was used for comet assays and the right for RT-PCR-analysis.

2.2. Gene expression analysis

The relative change in mRNA expression of specific target genes was determined by RT-PCR as described previously [42], using primers from Life Technology; Ogg1(Rn00578409_m1), Ung1 (Rn01499386_m1), Neil1(Rn01422336_g1), Ape1(Rn00821186_g1), Xrcc1(Rn01457689_m1), Nudt1(Rn00589097_m1) and Ercc1(Rn01498472_m1). Endogenous control gene was eukaryotic 18 S rRNA (P/N 4352930E) [43]. Isolation of total RNA was performed with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After dilution of samples in RNase-free water, 1:10, purity of RNA was assesed and concentration of sample-RNA was calculated with a spectrophotometer (Eppendorf BioPhotometer, 8.5 mm light center hight). The purity (A_{260}/A_{280}) of the mRNA was 1.60 ± 0.12 and 1.73 ± 0.07 in the PCF and HC samples, respectively. To avoid DNA contamination, DNase treatment of the purified RNA samples was performed using the RQ1 RNase-Free DNase kit (Promega, Madison, WI, USA). Thereafter the samples were stored at −20 °C. RT-PCR reactions were run with PFC, HC and a Standard (St) in 3 different sets, with a minus RT-sample as control each time, using the High-Capacity cDNA transcription Kit (Applied Biosystems,

Calsbad, CA, USA). The incubation period for the cDNA synthesis encompassed 4 steps of 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min and finally at 4 °C until storing of the reaction product at −20 °C. PCR reactions were run with TaqMan Fast Universal PCR Master Mix (Applied Biosystems) on an Applied Biosystems 7900HT Fast Real-Time PCR system, using a quantitative standard setting with 45 repeats. The level of target cDNA was calculated as the difference between the mean-value of the triplicate for each sample and enzyme, and the mean-value of the corresponding 18 S sample. The relative gene-expression normalized to 18 S was calculated as $2^{-\Delta Ct}$ [44]. For each day of mRNA level analysis, we assessed two samples from each group (12 samples per experiment) to have a fully balanced experimental design.

2.3. Oxidatively damaged DNA

Levels of oxidatively generated DNA damage were determined by the single cell gel electropheresis (comet) assay. Frozen samples of brain tissue (stored at −80 °C) were homogenized through a metallic cylinder (0.5 cm in diameter, mesh size 0.4 mm) placed in 500 μl ice cold Merchant buffer [0,14 M NaCl, 147 mM KH2PO4, 27 mM KCl, 81 mM Na2HPO4, 10 mM Na2EDTA, pH 7,4] and filtered in to a new tube on ice. 275 μl of the suspension was added to 1800 μl 0.75% low melting point agarose diluted in phosphate-buffered saline (PBS) kept in a heating block (37 °C). The agarose embedded cells (120 μl) were applied to GelBond® films (Lonza Copenhagen ApS, Vallensbæk Strand, Denmark). We had reference control samples in each experiment (corresponding to each electrophoresis) that included undamaged or photosensitizer Ro19-8022 and white light exposed peripheral blood mononuclear cells, which generates OGG1- and FPG sensitive sites. The Ro19-8022 photosensitizer was a kind gift from F. Hoffmann-La Roche (Basel, Switzerland). The GelBond films were kept at 4 °C for at least 5 min, followed by covering with lysis-buffer (1% Triton X-100, 2.5 mM NaCl, 100 mM Na_2EDTA, pH 10) for minimum 2 h. The GelBonds were washed 3 × 5 min in enzyme-free buffer (40 mM HEPES, 0.1 M KCl, 0.5 mM Na_2EDTA, 0.2 mg/ml BSA, pH 8). After brief air drying, 60 μl OGG1 (0.16 U/gel, BioNordika, Glostrup, Denmark), formamidopyrimidine DNA glycosylase (FPG) (1 mg/ml, gift from professor Andrew Collins, University of Oslo, Norway) or enzyme-free buffer was applied separately to the GelBonds, covered with cover slips and incubated for 45 min at 37 °C. OGG1 excises the 8-oxoguanine lesion, whereas FPG is a DNA glycosylase with AP-lyase activity that removes ring-opened formamidopyrimidine as well as 8-oxoguanine. After incubation, the cover slips were removed and GelBonds were transferred to electrophoresis buffer (300 mM NaOH, 1 mM Na_2EDTA, pH > 13) and incubated for 40 min at 4 °C to unwind the DNA. After unwinding in electrophoresis buffer, the nuclei were subjected to electrophoresis at 25 V (0.83 V/cm across the electrophoresis platform), 300 mA for 20 min at 4 °C, with circulation of electrophoresis buffer (3.6 l/h). After completed electrophoresis, the GelBonds were washed 15 min in Tris buffer (0.4 M Tris-Cl, pH 7.5) and placed in 96% ethanol over night. The nuclei were air dried and stained with 50 μl of YOYO-1 iodide (P/N 491/509, Molecular Probes, The Netherlands) in PBS and visualized under a Olympus fluorescence microscope with 40 times magnification. As previously described [45], 100 randomly chosen comets per gel were counted and scored for DNA damage, using a five category rating scale, by an observer blinded to the origin of the gel. Independent studies have shown a strong correlation between a visual scoring and computerized image analysis systems for measurement of DNA damage in the comet assay [46]. In addition, we have shown that one individual observer has a high consistency in scoring, whereas there is difference between different observers [47,48]. For this reason, it was the same observer who scored all comets in the present study. Specific enzyme-sensitive sites were calculated by subtraction of non-enzyme treated samples from each of the enzyme-treated samples (OGG1 and FPG, respectively) and converted to lesions per 10^6 base pairs using a calibration curve, where one arbitrary unit in a scale of 0–100 arbitrary units corresponds to 0.0273 lesions/10^6 bp, as described elsewhere [49]. Enzyme-treated samples exhibiting negative values after subtraction of non-enzyme treated samples were set to zero. The reference controls (Ro19-8022 irradiated with white light) had increased number of hOGG1 (22.0 ± 5.3 arbitrary units, corresponding to 0.24 ± 0.06 lesions/10^6 bp) and FPG sensitive sites (59.0 ± 9.7 arbitrary units, corresponding to 0.62 ± 0.11 lesions/10^6 bp), whereas the basal number of DNA lesions was (14.1 ± 2.6 arbitrary units, corresponding to 0.16 ± 0.04 lesions/10^6 bp (mean ± SEM)).

2.4. Ethics

The animal experiments performed in this study were approved by the Animal Experiments Inspectorate under the Danish Ministry of Justice (license number 2007/561-1309). All procedures were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the Guide for the Care and Use of Laboratory Animals (2011) in a fully AAALAC accredited facility. All efforts were made to minimize pain or discomfort as well as the number of animals used during the experiment.

2.5. Statistics

Data are presented as means (±SEM) if not otherwise stated. Data were analyzed with two-way analysis of variance (ANOVA) with time (acute, subchronic or chronic stress) and stress condition (stress vs. control) as fixed factors. In the case of a significant time × condition interaction, post-hoc comparisons of stress vs. control animals were performed by independent samples *t*-test for each time point. The corticosterone and comet assay data deviated from a normal distribution and were analyzed with a Kruskal–Wallis test followed by a Mann–Whitney test for each time point. For the RT-PCR data, all gene expression data sets deviating from a normal distribution were log-transformed, which resulted in normal distribution of the data. One PFC sample exhibited extreme values (>3 times the interquartile range of the data) across several genes and was excluded from analysis. Statistical analyses were carried out in the Statistical Package for Social Sciences (SPSS) software version 20.0 (IBM Corporation, NY, USA). The significance level was a priori set at $p < 0.05$.

3. Results

3.1. Stress validation

Restraint stress was associated with significantly lower body weight after SCS and CS compared to controls (SCS $p < 0.001$, CS $p < 0.001$) (Fig. 1A). Absolute adrenal gland weight showed a trend towards larger adrenals after CS (two-way ANOVA stress × time interaction: $F_{(2,71)} = 2.651$; $p = 0.078$) (Fig. 1B). As expected, relative adrenal gland weights (as percent of total body weight) in stressed and control animals sacrificed immediately after acute stress (AS) showed no significant difference. However, SCS and CS animals had substantially larger relative adrenal gland weights than controls ($p = 0.001$ and $p < 0.001$, respectively) (Fig. 1C). Compared to controls, stressed animals had a higher level of plasma corticosterone in general (Kruskal–Wallis $H = 31.817$, $p < 0.001$), which was significant after AS and CS in post-hoc comparisons ($p < 0.001$ and $p = 0.001$, respectively) (Fig. 1D). Collectively, these four variables document a robust stress induction in the restrained animals.

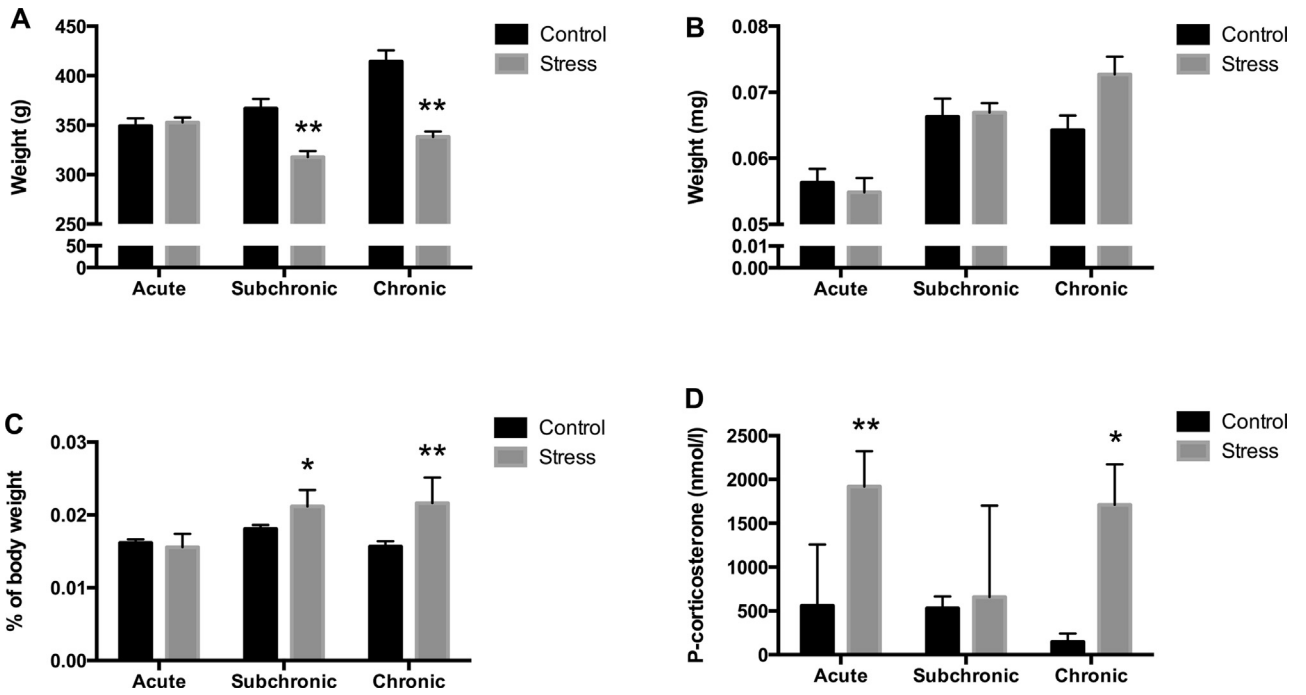

Fig. 1. Biological markers of psychological stress induction after acute (6 h), subchronic (7 days) and chronic (21 days) restraint stress.
Note that the acute stress group were sacrificed immediately after one restraint session, whereas the subchronic and chronic stress groups were sacrificed on the morning following their last session. **A**: Total body weight at the time of sacrifice. **B**: Total adrenal gland weight. **C**: Relative adrenal gland weight. **D**: Plasma corticosterone concentration in trunk blood obtained immediately upon sacrifice. Data are presented as means (±SEM) (A–C) or median (interquartile range) (D) and analyzed with two-way ANOVA (A–C) or Kruskal–Wallis test (D) followed by post-hoc comparisons of the control vs. stress groups at each time point by Student's *t*-tests or Mann–Whitney test, as appropiate. $^{*}p = 0.001$, $^{**}p < 0.001$.

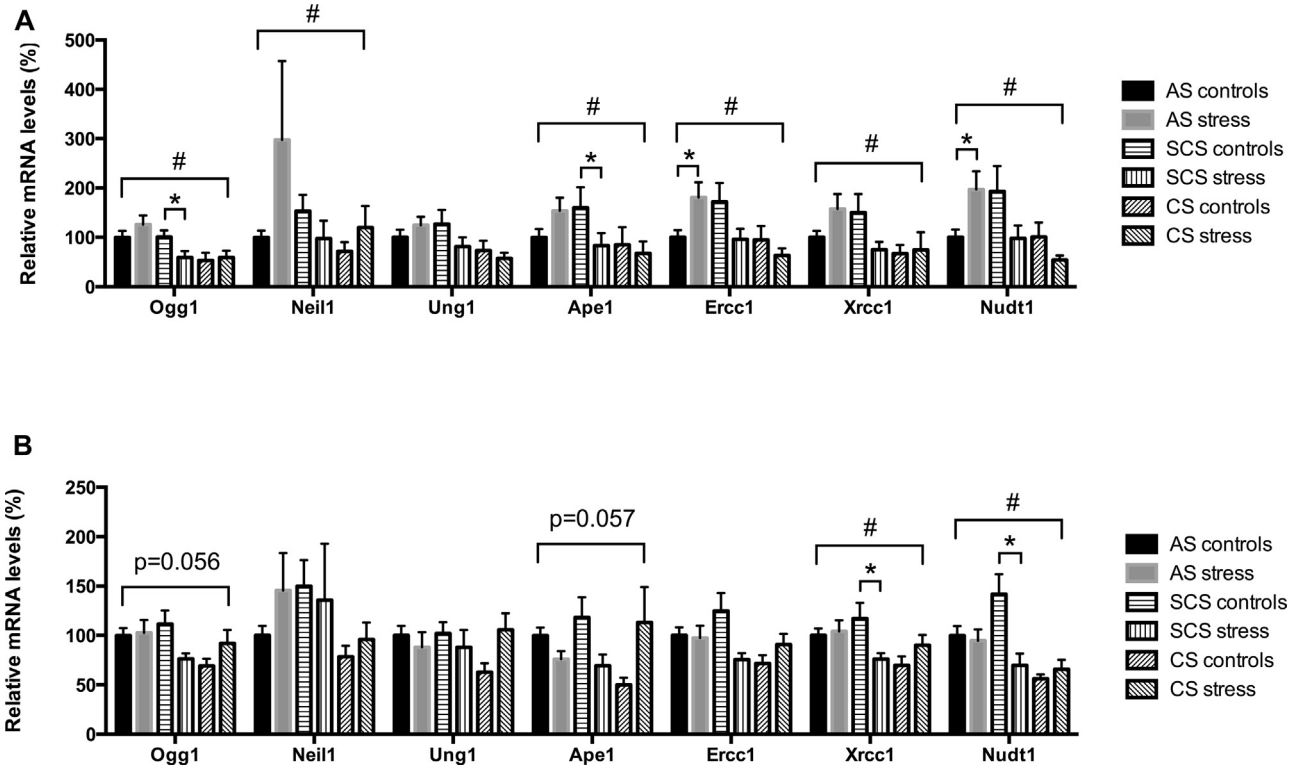

Fig. 2. Expression of DNA repair genes in the prefrontal cortex (A) and hippocampus (B) after acute (6 hours, AS), subchronic (7 days, SCS), and chronic (21 days, CS) restraint stress.
For clarity, data are presented as deviation from the AS control group level (100%) for each gene (means (±SEM)). All analyses were performed on raw or log-transformed data by two-way ANOVA, followed by post-hoc comparisons of the control vs. stress groups at each time point by Student's *t*-tests in the case of a significant stress × time interaction in the ANOVA. Ogg1: 8-oxoguanine glycosylase 1. Neil1: Endonuclease VIII-like 1. Ung1: Uracil DNA N-glycosylase 1. Ape1: Apurinic/apyrimidinic endonuclease 1. Ercc1: Excision repair cross-complementing rodent repair deficiency, complementation group 1. Xrcc1: X-ray repair cross-complementing protein 1. Nudt1: Nudix (nucleoside diphosphate linked moiety X)-type motif 1. $^{\#}p < 0.05$ for the interaction between stress condition and time point. $^{*}p < 0.05$, post-hoc comparison of stress vs. controls.

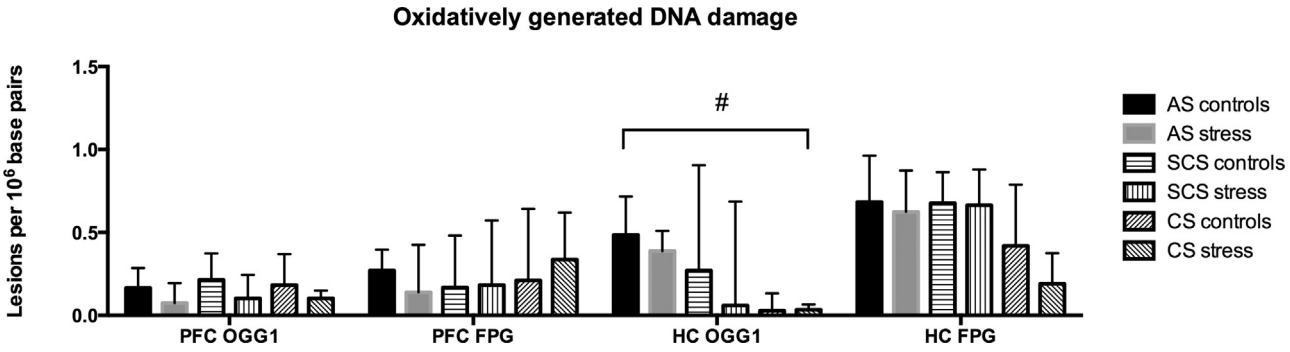

Fig. 3. Oxidatively damaged nuclear DNA in the prefrontal cortex (PFC) and hippocampus (HC) after acute (6 h, AS), subchronic (7 days, SCS), and chronic (21 days, CS) restraint stress (comet assay).
Data are presented as median (error bars indicate the upper level of the interquartile range) and analyzed with Kruskal–Wallis test followed by post-hoc comparisons of the control vs. stress groups at each time point by Mann–Whitney test. OGG1: 8-oxoguanine glycosylase 1-treated slides. FPG: formamidopyrimidine DNA glycosylase-treated slides. $^{\#}p = 0.001$ (none of the post-hoc comparisons of stress vs. controls at each individual time point were significant).

3.2. Gene expression analysis

Results are summarized in Fig. 2A and B. There was an overall pattern of increased levels of the target genes after acute stress, decreased levels after subchronic stress, and no difference after chronic stress compared to control level at each time point. This was particularly the case in the PFC. Specifically, for PFC samples, there was a significant stress × time interaction for *Ogg1*, *Ape1*, *Neil1*, *Ercc1*, *Xrcc1* and *Nudt1* (all *p*-values <0.05). Post-hoc comparisons of stress vs. control animals showed a significant increase for *Nudt1* and *Ercc1* after AS ($p < 0.05$), and a significant decrease of *Ogg1* and *Ape1* after SCS ($p < 0.05$). *Ercc1* and *Nudt1* showed borderline significant decreases ($p = 0.07$ and 0.09, respectively). No genes differed after CS. In HC, we found a significant stress × time interaction for *Xrcc1* and *Nudt1* ($p < 0.05$), and a borderline significant stress × time interaction for *Ogg1* ($p = 0.056$) and *Ape1* ($p = 0.057$). Post-hoc comparison of stress vs. controls was significant after SCS for *Xrcc1* ($p < 0.05$) and *Nudt1* ($p < 0.01$). Again, we found no significant differences between the treatment groups after CS in the HC.

We found a significant effect of time in PFC for *Ogg1* ($p < 0.001$) and *Ape1*, *Ung1*, *Ercc1*, *Xrcc1* and *Nudt1* ($p < 0.05$) and in HC for *Ogg1*, *Ape1* and *Nudt1* ($p < 0.01$), and *Ung1*, *Ercc1* and *Xrcc1* ($p < 0.05$), reflecting generally higher levels in the controls of the subchronic stress group.

3.3. Oxidatively damaged DNA

Results are summarized in Fig. 3. In Kruskal–Wallis test, we found an overall difference in the OGG1-treated slides of HC ($H = 22.043$, $p = 0.001$), but with no significant differences between stress vs. control animals at each individual timepoint in post-hoc comparisons. For all other region and enzyme combinations, we found no significant differences.

4. Discussion

The overall finding of the study was that experimentally induced psychological stress dynamically regulates the expression of DNA repair genes in brain regions that are key to the cognitive domains affected by recurrent depression, namely the prefrontal cortex and the hippocampus. Specifically, acute stress caused an increase in PFC DNA repair gene expression compared to non-stressed controls, whereas subchronic exposure generally was associated with reduced expression. After chronic stress, a normalization towards control levels was observed. This pattern was also present in HC, but with overall smaller effects and without the induction of gene expression after acute stress.

The finding is consistent with the concepts of allostasis and allostatic load, which state that short term stress activates adaptive biological systems (such as the HPA-axis), whereas the prolonged and uninterrupted activation of the same biological systems after more chonic stress exposure – such as a depressive episode – can be maladaptive and lead to, for example neuronal damage and dysfunction [50]. In the context of the present study, an activation of DNA repair after acute stress may offer a protection of the brain against stress-induced damage in the short term, whereas a dysregulation of DNA defense mechanisms after medium-term stress exposure may leave open a “window of opportunity” for neuronal damage to persist. This may particularly be the case because chronic stress and mental disease is often associated with unhealthy lifestyle choices that increase oxidative stress, such as lack of exercise, high-fat diets, and smoking [51].

If DNA repair mechanisms are protecting the brain from neurodegeneration during periods of neurohormonal stress, then naturally occuring variations in repair capacity would be expected to influence the cognitive decline of normal aging in humans. A recent large longitudinal study of healthy Norwegian subjects found evidence that this is in fact the case [52]. Hence, an intriguing clinical implication of the present finding is that genetic variations in DNA repair capacity may influence the risk for developing cognitive dysfunction or dementia after recurrent depressive episodes.

The pattern of gene expression regulation was non-specific to various subtypes of DNA repair pathways, involving both genes of BER, NER and removal of oxidized nucleobases from the nucleotide pool. This points to the underlying mechanism being a non-specific regulator of gene expression, such as epigenetic modifications. Interestingly, a recent study found that histone H3 acetylation, an epigentic mechanism that increases transcriptional activity, exhibits a transient increase, followed by a sustained decrease, after chronic psychosocial stress in the HC of mice [53].

We did not detect any significant changes in levels of nuclear DNA damage as measured by the comet assay. This is in contrast to findings of increased DNA damage as well as other markers of oxidative stress in the the brain after experimentally induced stress [18,54], but in line with our own previous finding [37]. The fact that we did not find changes in the levels of oxidatively generated DNA damage brings into question to what extent the observed gene expression changes have downstream effects on the genomic protection against oxidative stress. It could be speculated that DNA damage may follow a different time course than changes in DNA repair gene expression. Hence, in the study by Consiglio et al. [18], DNA damage in the hippocampus was found immediately and seven days after only one session of 30 min of restraint. Alternatively, it may be that the comet assay is not sufficiently sensitive to

detect the relatively subtle changes in DNA damage levels expected to occur after psychological stress exposure. Nevertheless, if repair of oxidatively damaged DNA is increased and the levels of damage in DNA are unchanged it must follow that the rate of damage is increased and given that repair is error-prone this can have effects in terms of increased mutations [55].

We observed that PFC was more sensitive than HC to the effects of stress on DNA repair gene expression. A plausible mediator of transcriptional regulation after stress is corticosterone, which is known to exert a feed-back influence on the brain through glucocorticoid (GR) and mineralocorticoid (MR) receptor binding in both PFC, HC and the hypothalamus [9]. However, in the rodent brain, while both GRs and MRs are relatively abundant in HC, PFC has a relative absence of MR's [56]. Accordingly, the region-specific differences observed (i.e., more pronounced differences in gene expression in PFC vs. HC) could perhaps be explained by different distributions of corticosteroid receptors in the rat brain, with hippocampal MR's playing an attenuating role on DNA repair transcriptional regulation by stress. Another possible mechanism underlying the observed gene expression changes, in particular the increased gene expression in PFC after acute stress, is stimulation of PFC and HC neurons through activation of the basolateral amygdala, which is activated immediately upon acute stress and sends projections to both brain regions [57].

We found significant effects of time point in most PFC and some HC genes assessed, with overall higher levels in the SCS controls compared to the AS and CS controls (Fig. 2). Recently, NER in the mammalian brain has been found to show diurnal variation [58]. Furthermore, DNA repair activity in the brain has been suggested to be age-dependent [59]. In our study, the time of sacrifice differed between the groups, i.e., at approximately 4PM in the AS group and 9AM in the SCS and CS groups, and inherently, the CS animals were older at the time of sacrifice than the animals in the AS and SCS groups. Thus, a combination of circadian clock regulation of DNA repair gene expression and age differences could have influenced overall gene expression levels at the three different time points.

Some limitations of the study should be mentioned. We did not determine DNA repair at the protein and/or enzyme activity level, and the observed changes in gene expression are not necessarily associated with changes on these levels. We used homogenized brain samples, and therefore neither RT-PCR nor comet assay data are specific to cell types or subregions of the brain areas investigated. Finally, the study provides no causal explanation for how stress influences the cerebral DNA repair gene expression.

5. Conclusion

In conclusion, we found that acute restraint stress caused an overall increase in the expression of genes involved in DNA repair in PFC. In contrast, subchronic stress exposure was associated with an overall reduction in the expression of DNA repair genes in both the PFC and HC. After chronic stress, levels did not differ from controls in neither brain region. The study suggests that cerebral DNA repair is a part of the allostatic adaptation to stress, which could play a role for the cognitive deterioration ocurring with repeated depressive episodes. However, given that we were unable to detect changes in the levels of nuclear DNA damage, the significance of stress-induced DNA repair regulation for neuronal viability remains unclear. To our knowledge, this is the first study to specifically address the involvement of cerebral DNA repair in psychological stress.

Role of the funding source

The funders had no role in the study design process; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Conflict of interest

The authors declare that there are no conflicts of interest.

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