

Effects of psychotherapy on DNA strand break accumulation originating from traumatic stress

Running title: Effects of psychotherapy on DNA breakage

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Abstract

**Background:** Previous research reveals an association between traumatic stress and an increased risk for numerous diseases, including cancer. At the molecular level, stress may increase carcinogenesis via increased DNA damage and impaired DNA repair mechanisms. We assessed DNA breakage in peripheral blood mononuclear cells (PBMCs) from individuals with posttraumatic stress disorder (PTSD) and measured the cellular capacity to repair single-strand breaks after exposure to ionizing x-radiation. We also investigated the effect of psychotherapy on both DNA breakage and DNA repair.

**Methods:** In a first study we investigated DNA breakage and repair in 34 individuals with PTSD and 31 controls. Controls were subdivided into 11 trauma-exposed and 20 individuals without trauma exposure. In a second study, we analyzed the effect of psychotherapy (Narrative Exposure Therapy) on DNA breakage and repair. 38 individuals with PTSD were randomly assigned to either a treatment or a waitlist control condition. Post-tests were performed four months and one year after therapy.

**Results:** In Study 1 we found higher levels of basal DNA breakage in individuals with PTSD and trauma-exposed subjects than in controls, indicating that traumatic stress is associated with DNA breakage. However, single-strand break repair was unimpaired in individuals with PTSD. In Study 2, we found that psychotherapy reversed not only PTSD symptoms, but also DNA strand break accumulation.

**Conclusion:** Our results show - for the first time *in vivo* - an association between traumatic stress and DNA breakage, and moreover demonstrate changes at the molecular level, *i.e.* the integrity of DNA, after psychotherapeutic interventions.

Traumatic life events can lead to post-traumatic stress disorder (PTSD), which is characterized by intrusive recollections of the traumatic event, hyperarousal, and avoidance of stimuli associated with the trauma [1]. In the new DSM-5 [2], the avoidance symptom cluster was divided into persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness and the new symptom cluster negative alterations in cognition and mood. Traumatic experiences and PTSD are associated with a premature aging of immune system cells [3-5], blood plasma [6] and DNA extracted from buccal swabs [7], leading to greater physical morbidity [8], and even higher mortality from numerous diseases [9, 10] including cancer [11].

A well-established pathway regulates DNA damage through  $\beta_2$ -adrenoreceptors and  $\beta$ -arrestin-1, stimulated by  $\beta$ -adrenergic catecholamines [12]. Stress may increase DNA damage and impair DNA repair mechanisms [13] via dysregulation of catecholamines and glucocorticoids, as observed in individuals with PTSD [14, 15]. In human leukocytes, for example, epinephrine induces DNA strand breaks [16], and high levels of urinary cortisol were associated with increased oxidative DNA damage in the elderly [17]. Furthermore, *in vitro* exposure of murine 3T3 fibroblasts to cortisol, epinephrine, or norepinephrine led to a five-fold increase in DNA damage and interferes with the repair of DNA damage [13]. In addition, individuals with PTSD show an increased inflammatory status [18, 19]. Similarly, depression has been linked to increased oxidative DNA damage [20]. Pro-inflammatory cytokines are associated with an excessive production of nitric oxide and reactive oxygen species (ROS), causing DNA damage and inhibiting DNA repair [21]. In summary, stress hormones and pro-inflammatory cytokines may induce DNA damage and alter DNA repair in individuals with PTSD.

DNA damage and genomic instability are not only important driving forces for carcinogenesis but are also associated with ageing of cells and organisms [22]. This may account for the fact that chronic inflammation has been associated with an increased risk for mutations, carcinogenesis and pathological aging [23].

Trauma-focused treatment is effective in reducing psychological symptoms of PTSD [24, 25]. Narrative Exposure Therapy (NET) is based on principles of current neurocognitive theories of PTSD, aimed at treating victims of organized and domestic violence with severe forms of PTSD [26]. In NET, the patient constructs a chronological narrative of his or her life with the assistance of the therapist, focusing on his or her traumatic experiences. The aim of this procedure is to transform the generally fragmented reports of the implicitly coded traumatic experiences [27] into a coherent narrative *i.e.*, verbally accessible autobiographic memory. The efficacy of NET on improving PTSD symptomatology has been shown in a series of randomized controlled trials [28-30].

However, only little is known about the impact of psychotherapeutic treatment on altered biological parameters in PTSD. A few studies have focused on endocrinological changes; however, results remain inconsistent and both an increase of cortisol and DHEA [31] as well as a decrease of cortisol [32] has been found after psychotherapeutic treatment. Additionally, a systematic review demonstrated a decrease in heart rate and blood pressure as well as changes in activity of frontal brain structures and the amygdala through trauma-focused treatment [33]. Nevertheless, nothing is known about how psychotherapy changes altered levels of catecholamines and cytokines or in DNA damage and DNA repair in PTSD.

The aims of this study were to investigate the effects of traumatic stress and psychotherapy on DNA damage and repair (primary endpoint), possibly with therapy-induced changes in PTSD symptoms (secondary endpoint) as a mediating effect.

• Methods Study 1

Participants

In Study 1 (baseline study), DNA breakage and DNA repair were analyzed in 65 participants: 34 individuals with PTSD and 31 controls. The control group was subdivided into 11 persons with trauma-exposure but without PTSD, and 20 control subjects without substantial trauma exposure.

Individuals with PTSD (23 male and 11 female) were refugees (18 Africa, 2 the Balkans, 14 Middle East and Afghanistan) with a history of war and torture experiences. The median length of residence in Germany was 2.2 years (range: 2 months – 18 years). 82% of the individuals with PTSD fulfilled criteria for co-morbid Major Depressive Disorder according to DSM-IV-TR [1] (Table 1). Individuals with PTSD were recruited through the Center of Excellence for Psychotraumatology, University of Konstanz.

The eleven trauma-exposed individuals (6 male, 5 female) were also refugees (7 Africa, 1 the Balkans, 3 Middle East and Afghanistan), but did not fulfill DSM-IV-TR criteria for current PTSD (45% of them fulfilled criteria for Major Depressive Disorder). The median length of residence in Germany of trauma-exposed individuals was 2.8 years (range: 5 months – 18 years). Trauma-exposed individuals were also recruited through the Center of Excellence for Psychotraumatology, University of Konstanz.

Twenty control subjects without substantial trauma exposure (8 male, 12 female) were matched for ethnicity (8 Africa, 2 the Balkans, 10 Middle East and Afghanistan). Controls were recruited through advertisements in the town and at the university. Controls were living in Germany for 13.9 years on median (range: 1.8 years – 36 years). The three groups did not differ significantly with respect to age (Table 1), ethnicity and gender.

The inclusion criterion for the PTSD group was a diagnosis of current PTSD according to DSM-IV-TR [1] in the aftermath of war and torture experiences. Inclusion criteria for the group of trauma-exposed individuals were substantial exposure to traumatic stress, but no diagnosis of PTSD. Control subjects had to be free of any current psychiatric disorder.

Exclusion criteria for all groups were psychotic disorders and chronic inflammatory diseases (such as acute infections, Hepatitis, HIV, rheumatoid arthritis, osteoarthritis, bronchitis, or asthma). Psychotropic medication was taken by 20 individuals with PTSD (4 hypnotics, 3 anxiolytics, 10 antidepressants, 2 neuroleptics, 1 antimanics), 3 trauma-exposed individuals (1 hypnotics, 2 antidepressants), and 2 controls (2 hypnotics).

The study was conducted at the Center of Excellence for Psychotraumatology and at the Molecular Toxicology Laboratory, both at University of Konstanz, Germany. The Ethics Committee of the University of Konstanz had approved the study. All study participants provided written informed consent after detailed information about the procedures and the background of the study. Participants received 30 € as compensation. Clinical diagnostic interview

Psycho-diagnostic interviews were conducted by trained psychologists specialized in the field of trauma, with the help of trained interpreters if necessary. All participants underwent the same psycho-diagnostic interview. Traumatic events, PTSD diagnosis, and symptom severity were assessed with the Clinician Administered PTSD Scale (CAPS) [34]. The Vivo checklist of war, detention and torture events [35] was used to identify war and torture experiences. Depressive symptoms were quantified with the Hamilton Depression Rating Scale (HAM-D) [36, 37]. Other potential mental disorders were assessed with the Mini International Neuropsychiatric Interview (M.I.N.I.) [38].

Analysis of damage to and repair of DNA

Blood samples were collected at 10 a.m. A complete blood count and tests for hepatitis and HIV infections were done in an independent routine clinical chemistry laboratory in Konstanz. For analysis of DNA breakage and DNA repair, 10 ml blood was taken using Coagulation 9 NC/ 10 ml Monovettes® (Sarstedt, Germany). All blood samples were coded before they were transferred to the Molecular Toxicology laboratory to guarantee blinding of all laboratory staff involved.

An automated version of the Fluorimetric Detection of Alkaline DNA Unwinding (FADU) method [39, 40] was used for analyzing formation and repair of DNA strand breaks in living cells [41]. This method is characterized by high reproducibility and high throughput. Furthermore, the automation highly contributes to standardization minimizing bias, which makes it eligible for its application in human studies. The steps of the automated FADU are described in detail elsewhere [40]. Briefly, cells were lysed and DNA breaks present in the cell lysate (as well as the ends of the chromosomes) are starting points for DNA unwinding due to the presence of limiting concentrations of alkali. This time-dependent process of alkaline unwinding is stopped after incubation for a certain time period at a defined temperature, and the amount of DNA remaining double stranded is measured via Sybr®Green fluorescence. Therefore, a decrease in the fluorescence intensity of Sybr®Green indicates an increase of DNA unwinding and, consequently, a higher number of DNA strand breaks.

Human PBMCs were isolated from whole blood according to the density gradient principle using Biocoll® (Biochrom AG, 12247 Berlin, Germany), counted using a cell counting device (Casy® counter), pelleted (5 min, 200 g), and resuspended in RPMI-1640 medium (Invitrogen) containing 100 U/ml penicillin (Invitrogen) and 100 mg/ml streptomycin (Invitrogen) at  $5 \times 10^5$  cells per ml. Then several aliquots of 100  $\mu$ l cell suspension were irradiated on ice with 3.8 Gy, (dose rate 1.9 Gy/min, x-ray radiation time 2 min) using an x-ray generator (CHF Müller, Hamburg, Germany, 70KeV, 1mm Al-filter). To allow DNA repair, cells were incubated in a CO<sub>2</sub> incubator at 37°C for various periods of time and subsequently transferred to the pipetting robot for the FADU assay. DNA repair was analyzed by the FADU assay every 10 minutes over a time span of 90 minutes.

Power analysis

We conducted a pilot study with 4 individuals with PTSD and 4 healthy volunteers and calculated a power analysis based on the observed differences in these pilot data. The differences in DNA repair in individuals with PTSD vs. healthy controls had an effect size of Cohen's d = 1.46 [42] for the main effect of Group. Note that this is a clinically relevant difference: the DNA breakage we detected in our stress patients is comparable to that of atomic bomb survivors exposed to a dose of 1 Gy that led to a substantial life-time increase in cancer formation [43]. Monte Carlo analysis showed that this effect size was large enough to be detected at only n = 10 per group at = .05 and = .80. However, we preferred to be on the safe side by using at least n = 20 individuals with PTSD.

Statistical analysis

Statistical analysis was performed using R 2.11.0 [44]. Since residuals in the model of age, PTSD symptom severity scores (CAPS scores), DNA breakage after x-ray irradiation and DNA breakage after 90 min of repair were not normally distributed, group differences were analyzed using non-parametric statistics (Kruskal-Wallis and Wilcoxon-Mann-Whitney test). An alpha level of 0.05 was used. Group differences in DNA breakage were analyzed using ANOVAs. In case of significant effects, post-hoc tests (t-tests) were calculated. DNA repair was analyzed using a linear mixed model with group  $\times$  time (3 groups  $\times$  9 repeated measurements) as factors. Data of fluorescence signals were logarithmized [40]. However, model fit estimates (AIC, Akaike information criterion) [45] clearly favored the economical model without covariates. Differences in time effects between PTSD, trauma-exposed, and control participants were analyzed using simultaneous tests for general linear hypotheses [46]. Possible mediation effects of therapy-induced changes in PTSD symptoms were examined using Sobel's test [47, 48]. Significance was assessed by bootstrapping the indirect effect's empirical distribution 10,000 times [49, 50].

• Results Study 1

Basal DNA breakage differed significantly between groups ( $F_{(2,62)} = 3.95; p = .02$ ), with more DNA breakage in individuals with PTSD ( $t_{(39,1)} = 2.69; p = .005$  one-sided) and trauma-exposed individuals ( $t_{(18,1)} = 1.94; p = .04$  one-sided) compared to controls (Table 1, Figure 1a).

There was a group  $\times$  time interaction in DNA repair ( $F_{(18,556)} = 1.72; p = .03$ ): there was more DNA repair over 90 minutes in the PTSD and the trauma-exposed group than in controls (both  $z = 3.60, p < .001$ ), whereas

there was no difference between the PTSD and trauma-exposed group ( $z = -.97, p = .60$ ; Figure 1b). To control for smoking behavior we repeated the analysis only with non-smokers (PTSD:  $n = 24$ ; trauma-exposed  $n = 7$ ; controls  $n = 17$ ) and found higher DNA breakage also in this subgroup of individuals with PTSD ( $F_{(2,45)} = 4.99; p = .01$ ). Including gender, age, psychotropic medication or smoking behavior as covariates in the model did not alter results.

In a follow-up experiment to assess whether more DNA damage, as observed above in the PTSD and trauma-exposed group, goes along with higher levels of DNA repair, we recruited 4 additional healthy young volunteers who were not participants in Study 1 or 2. We irradiated PBMCs *ex vivo* with increasing doses of x-rays (2 Gy, 5 Gy, 10 Gy, 20 Gy) to simulate the increased DNA breakage in individuals with PTSD. We found that DNA repair occurred also in highly irradiated samples with more DNA breakage and that higher initial DNA breakage was even associated with accelerated DNA repair. The ANOVA with factors Amount of Irradiation (2 Gy, 5 Gy, 10 Gy, 20 Gy) and Time (10 min, 20 min, ..., 90 min) showed a significant interaction of Amount of Irradiation  $\times$  Time,  $F_{(27,108)} = 3.38, p < .0001$  (Supplementary Figure 1).

• **Methods Study 2**

**Procedure and Participants.** The impact of psychotherapy on the breakage and repair of DNA was investigated in 38 individuals with PTSD (34 individuals participated also in Study 1, 4 individuals with PTSD were additionally recruited). Individuals with PTSD were randomly assigned to either a treatment condition (Narrative Exposure Therapy [NET] group:  $n = 19$ ) or a waiting condition (Waitlist Control [WLC] group:  $n = 19$ ). Participant flow is shown in Supplementary Figure 2.

**Power Analysis.** If we anticipate that NET reduces the effect size of PTSD on DNA repair found in the pilot data described above by 0.5 (from 1.46, as found in the pilot data, to an assumed remaining difference of  $1.46 - 0.5 = 0.96$ ), a power calculation shows that again  $n = 10$  participants per group would be sufficient to detect this effect at  $\alpha = 0.5$  with a power of 0.8, using a one-sided test. A larger sample size was chosen to again be on the safe side.

The Ethics Committee of the University of Konstanz approved the study. All participants of the treatment study signed a second informed consent. The trial was registered at clinicaltrials.org, NCT 01206790.

**Treatment**

An independent person randomly assigned individuals with PTSD to either a treatment condition (NET) or a waitlist control (WLC) group using permuted blocks of variable length. The capacity of the therapists who carried out Narrative Exposure Therapy was the criterion for the lengths of the blocks – that is, if the next therapist to be sent patients had k free therapy slots, then k of the next 2k participants would be randomly assigned to the NET group and referred to this therapist, while the other k participants would be assigned to the WLC group, using a shuffled set of envelopes which were opened only after a new participant was included in the study. Diagnosticians were not aware of which participants were allocated to which group. Blinded diagnosticians conducted post-test interviews.

The treatment intervention comprised 12 sessions of Narrative Exposure Therapy (NET) lasting approximately 4 months. Therapists were 12 clinical psychologists employed by the Center of Excellence in Psychotraumatology in Konstanz, who are specialized in trauma therapy and exclusively work with NET. During NET therapy sessions, therapists recorded narratives [26] and took minutes. Both narratives and minutes were taken in writing and reviewed by JM to ensure adherence to the NET protocol. Therapists relied on trained interpreters if necessary. The waiting period in the WLC group was 8 months, during which time period no psychotherapeutic intervention took place. The first post-test was conducted 4 months after the end of therapy in the NET group, respectively 8 months after the baseline assessment in the WLC group. Individuals with PTSD in the NET group were invited 1 year after the end of therapy for a second follow-up interview. Individuals with PTSD of the WLC group received psychotherapy after the waiting period for ethical reasons, and were therefore not available for a corresponding follow-up.

**Measures**

Measures of outcome were changes in DNA breakage and repair four months and one year after the end of treatment with NET (primary endpoint) and the diagnosis of PTSD and the change of its severity score according to CAPS (secondary endpoint).

**Statistical analysis**

Linear mixed models were calculated to analyze the primary and secondary outcomes of changes in DNA breakage and repair as well as changes in PTSD symptom severity (CAPS score). Differences in time effects between the NET and the WLC group were analyzed using simultaneous tests for general linear hypotheses [46]. Paired t-tests were calculated to analyze differences from pre to post-test within groups. Within and between treatment effect sizes were calculated by Cohen's  $d$  [42].

• **Results Study 2**

Baseline socio-demographic and clinical characteristics from the NET and the WLC group are presented in Table 2. Groups presented with very similar socio-demographic and clinical characteristics prior to treatment.

**PTSD symptom severity.** Pre-therapy PTSD symptom severity (CAPS score) did not differ between the NET and the WLC group (Table 2). Treatment led to a significant reduction of PTSD symptoms in the NET group (pre to 4-months post-test:  $t_{(14)} = -5.21; p = .0001$ ; within treatment  $d = -1.72$ , Table 3). There was also a reduction in the CAPS sum score in the WLC group after 8 months of waiting time ( $t_{(13)} = -2.36; p = .03$ ), but symptom reduction was significantly higher in the NET group ( $z = -4.96; p < .0001$ , Table 3).

Mean change scores of PTSD symptom severity (CAPS score) were significantly greater in the NET than in the WLC group ( $t_{(21)} = -3.10; p = .005$ ; between treatment  $d = -1.14$ ; Table 3). Moreover, a mixed models analysis revealed a significant Group  $\times$  Time interaction ( $F_{(1,27)} = 10.34, p = .003$ ). At one year follow-up, symptom levels had further decreased relative to the 4-months post-test ( $d = -0.32$ ; see Table 3).

**Diagnosis of PTSD.** Pre-treatment all study participants of the NET ( $n = 19$ ) and all study participants of the WLC ( $n = 19$ ) fulfilled diagnosis of current PTSD. Four months after treatment,  $n = 8$  participants of the NET, but only  $n = 1$  participant of the WLC group had recovered from PTSD. At the one-year follow up  $n = 6$  participants of the NET group still fulfilled criteria for current PTSD, but were clinically improved.

**DNA breakage.** Before therapy, groups did not differ in basal DNA strand breaks (Table 2). Parallel to a reduction in symptoms of PTSD, we observed a significant reduction of DNA strand breakage in NET-treated individuals (pre to post-test:  $t_{(9)} = 3.08; p = .01$ ) with a large effect size of  $d = 0.99$ , but not in the WLC group (pre to post-test:  $t_{(12)} = 0.97; p = .35$ , see Table 3). Mean change scores of basal DNA breakage were significantly greater in the NET group, compared to the WLC group ( $t_{(17)} = 2.46; p = .02$ ), with a between treatment effect size of  $d = 1.04$ . A mixed models analysis revealed a significant Group  $\times$  Time interaction in DNA breakage ( $F_{(1,21)} = 4.44, p = .05$ , Supplementary Figure 3). However, we found no evidence for a mediator effect of the change in PTSD symptoms on baseline DNA strand breakage (indirect effect  $-3.67$ , bootstrapped 95% CI  $-9.38$  to  $3.18$ ). Most importantly, 1 year after the end of treatment, reversion of DNA breakage not only remained stable, but was even more pronounced ( $d = 0.32$ ) compared to 4 months post-test (Table 3).

**DNA repair.** The course of DNA repair in individuals with PTSD who received NET therapy returned to the pattern seen in controls without substantial trauma exposure. Mixed models analysis showed a significant interaction Group  $\times$  Time, i.e. pre- vs. post-therapy ( $F_{(1,525)} = 6.45; p = .01$ ; Supplementary Figure 4).

• **Discussion**

We found that both individuals with PTSD and trauma-exposed individuals presented significantly higher levels of endogenous DNA strand breaks in PBMCs, which could, by itself, have serious implications for physical health, in particular for carcinogenesis. Indeed, apart from PTSD, depression, which is often comorbid with PTSD [51], has been associated with increased oxidative DNA damage and has been linked to a possibly increased risk for cancer development [20].

After x-ray irradiation of PBMCs *ex vivo*, individuals with PTSD and trauma-exposed subjects displayed significantly higher exogenously induced DNA breakage, and the progression of DNA repair over 90 minutes showed a significant Time  $\times$  Group interaction. While the latter might at first sight suggest improved DNA strand break repair capacity in trauma or individuals with PTSD, it is more likely to result from a higher level of initial DNA breaks as investigated in the additional follow-up experiment with PBMCs of 4 healthy young volunteers: higher initial DNA breakage was associated not with impaired, but with accelerated DNA repair. Therefore, our data indicate that DNA repair as such is not impaired by traumatic stress; yet specific DNA repair processes might be altered. In line with this interpretation, students during a high-stress exam period showed increased nucleotide excision repair in lymphocytes 2 hours after UV-light induced DNA damage compared to a lower stress period [52]. Highly distressed patients suffering from mental disorders, showed reduced DNA double-strand break repair in lymphocytes 5 hours, but not 2 hours, after x-ray irradiation of samples [53]. These apparent discrepancies may be explained by the different repair pathways analyzed (nucleotide excision repair dealing with UV-induced damage vs. repair of double-strand breaks induced by x-rays). In addition, sample size and statistical power may contribute to controversial results.

The finding that psychotherapy (here: NET) is able to reverse the increased levels of endogenous DNA breakage in individuals with PTSD to a normal level is intriguing. Several other studies have already reported PTSD symptom reduction after NET [28–30]; however, the positive impact of psychotherapy on a molecular parameter with potential long-term impact on physical health, i.e. DNA strand breakage, has not been demonstrated before. Although our sampling of tissue in the present study was restricted to PBMCs, we have presented a proof-of-principle for the reversibility of DNA strand breakage, an established risk factor in genomic instability and carcinogenesis, in somatic cells of individuals with PTSD after successful psychotherapy.

The mediation analysis found no evidence for a mediation effect of the reduction in PTSD symptoms on baseline DNA strand breakage. It may be that the effect of therapy on DNA damage is not mediated by the reduction in PTSD symptoms as such, but by more elementary biological pathways, which may influence both PTSD symptomatology and DNA damage. This is a promising avenue of further research, especially as our understanding of DNA damage and repair continues to improve.

This study has implications regarding the necessity to promptly treat PTSD and possibly stress-related mental disorders in general. Damage to DNA, including DNA breakage, is a well-known mechanism of irreversible tumor initiation [54], which may precede clinically manifest tumor formation by decades. The reversibility of DNA breakage in individuals with PTSD via psychotherapy described here clearly indicates that there is indeed a possibility not only to reduce the psychological burden of PTSD but also the long-term, and potentially lethal, somatic effects of this mental disorder.

Our study has some important limitations: we do not have information on PTSD symptoms and DNA damage in the WLC group at the time point of the 1-year follow up. For ethical reasons we wanted to offer these highly traumatized individuals psychotherapy after the first post-test of the treatment group. In addition, we found strong effects of NET treatment on DNA breakage, but their clinical relevance and the effects on actual health outcomes are yet unclear and can probably only be investigated using long-term prospective studies assessing the incidence of age-related diseases including cancer in PTSD patients with and without treatment. Our mediator analysis did not identify PTSD symptomatology improvement as a mediator for the effects of psychotherapy on DNA damage but may have been underpowered, or other (biological) mediators may be involved and should be assessed, guided by accumulating knowledge about DNA damage and repair. In particular, future studies should investigate whether results such as ours are due to differential susceptibility of different PBMC subpopulations to PTSD-induced DNA damage, which may lead to changes in the composition of PBMCs, and possibly a reversal of such changes through psychotherapy. In addition, other mechanisms than traumatic stress, for example, nutrition or physical exercise, could have an additional effect on DNA damage and/or repair and should be investigated in future studies. Similarly, given that depression has been associated with DNA damage [20] and is highly comorbid with PTSD, an additional group of non-traumatized depressed individuals would yield information about the differences between PTSD with comorbid depression and depression without PTSD in DNA damage – and a therapeutic intervention in the depression group would allow a comparison in terms of reduced DNA damage. Finally, future studies with larger sample size would lead to more precise results.

In summary, our results reveal that exposure to traumatic life events, especially when sufficiently severe to result in a diagnosis of PTSD, is associated with higher levels of DNA damage in PBMCs. The underlying mechanism might be an increased endogenous production of reactive oxygen species. If maintained for extended periods of time, this may represent an increased risk for age-related diseases including malignant tumors. This high-risk state can be reversed by effective psychotherapeutic intervention.

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**Conflict of Interest.** The authors declare no conflict of interest.

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Table 1. Clinical characteristics and DNA breakage in control subjects, trauma-exposed and individuals with PTSD.						
Variables mean (SD)	Controls (n= 20)	Trauma-exposed (n= 11)	PTSD (n= 34)	Statistics	p	Effect size
Age <sup>a</sup> median (range)	31.0 (19 - 61)	21.0 (15 - 51)	30.0 (15 - 46)	$\chi^2 = 2.45$	.29	$\eta^2 = .03$
Controls vs. Trauma-exposed				$W = 148.5$	.12	$d = .43$
Controls vs. PTSD				$W = 389.0$	.38	$d = .35$
Trauma-exposed vs. PTSD				$W = 221.5$	.37	$d = .18$
Traumatic event load (CAPS event list) mean (SD)	4.40 (2.41)	6.82 (2.09)	8.03 (2.08)	$F(2,62) = 17.31$	$\leq .001$	$\eta^2 = .36$
Controls vs. Trauma-exposed				$t(23.4) = -2.91$	.008	$d = -1.05$
Controls vs. PTSD				$t(35.4) = -5.61$	$\leq .001$	$d = -1.64$
Trauma-exposed vs. PTSD				$t(16.9) = 1.67$	.11	$d = .58$
PTSD symptom load (CAPS) <sup>a</sup> median (range)	0.00 (0 - 13)	35.00 (0 - 58)	91.00 (63 - 114)	$\chi^2 = 51.36$	$\leq .001$	$\eta^2 = .89$
Controls vs. Trauma-exposed				$W = 36$	$\leq .001$	$d = 2.14$
Controls vs. PTSD				$W = 0$	$\leq .001$	$d = 6.93$
Trauma-exposed vs. PTSD				$W = 374$	$\leq .001$	$d = 3.41$
Basal DNA breakage (% fluorescence) mean (SD)	79.42 (10.52)	70.88 (12.33)	71.52 (10.24)	$F(2,62) = 3.95$	.02	$\eta^2 = .11$
Controls vs. Trauma-exposed				$t(18.1) = 1.94$	.07	$d = .76$
Controls vs. PTSD				$t(39.1) = 2.69$	.01	$d = .76$
Trauma-exposed vs. PTSD				$t(14.7) = 0.15$	.88	$d = .06$
DNA breakage after x-ray irradiation (% fluorescence) median (range)	38.42 (31.3 - 64.1)	31.74 (22.8 - 48.1)	34.7 (16.8 - 56.9)	$\chi^2 = 9.18$	.01	$\eta^2 = .12$
Controls vs. Trauma-exposed				$W = 172$	.009	$d = 1.04$
Controls vs. PTSD				$W = 478$	.01	$d = .64$
Trauma-exposed vs. PTSD				$W = 231$	.25	$d = .32$
DNA breakage after 90 min of repair (% fluorescence) median (range)	65.62 (40.1 - 110.2)	61.06 (46.8 - 89.1)	67.9 (44.3 - 119.9)	$\chi^2 = .52$	.77	$\eta^2 = .01$
Abbreviations: CAPS (Clinical Administered PTSD Scale). <sup>a</sup> data were not normally distributed. Statistics: SD (standard deviation), $\chi^2$ (Kruskal-Wallis test), $W$ (Wilcoxon-Mann-Whitney test). All tests were calculated on an alpha level of 0.05 (two-sided).						

Table 2. Baseline characteristics of the Narrative Exposure Therapy (NET) and the Waitlist Control (WLC) group before treatment.

Characteristics	NET (n = 19)	WLC (n = 19)	Statistics	P	Effect Size
Female sex (No, %)	6 (31.6 %)	6 (31.6 %)	$\chi^2 = 0.12$	.73	
Smoking (No, %)	7 (36.8 %)	4 (21.1 %)	$\chi^2 = 0.51$	.74	
Asylum status insecure (No, %)	18 (94.7 %)	18 (94.7 %)	$\chi^2 = 0.53$	.47	
Comorbid depression (No, %)	15 (78.9 %)	15 (78.9 %)	$\chi^2 = 0.16$	.69	
Psychotropic medication (No, %)	5 (26.3%)	8 (42.1%)	$\chi^2 = 0.47$	.49	
Age Mean (SD)	28.7 (9.54)	30.1 (8.21)	$t(35.2) = -0.47$	.64	$d = .15$
Traumatic event load (CAPS) Mean (SD)	7.7 (2.6)	8.4 (1.5)	$t(29.5) = -1.0$	.33	$d = .32$
PTSD symptom load (CAPS) Mean (SD)	92.4 (14.2)	86.5 (15.5)	$t(35.7) = 1.21$	.23	$d = .39$
Basal DNA breakage Mean (SD)	73.9 (9.5)	70.6 (10.3)	$t(29.3) = 0.94$	.35	$d = .33$
Abbreviations: CAPS (Clinical Administered PTSD Scale). Statistics: SD (standard deviation), $\chi^2$ Chi-squared-test. All tests were calculated on an alpha level of 0.05 (two-sided).					

Table 3. Means and standard deviations (SD) of PTSD symptom severity (CAPS score) and basal DNA breakage in Narrative Exposure Therapy (NET) group and Waitlist Control (WLC) group.

Variables	Pre-test mean (SD)	4-months post-test mean (SD)	1-year post-test mean (SD)
CAPS score: NET	92.37 (14.16)	55.07 (27.01)	42.73 (28.20)
CAPS score: WLC	86.53 (15.46)	76.86 (17.14)	...

<b>Basal DNA breakage: NET</b>	73.91 (9.50)	85.09 (12.73)	88.53 (8.05)
<b>Basal DNA breakage: WLC</b>	70.65 (10.28)	73.18 (9.71)	-- <sup>a</sup>
Abbreviations: CAPS (Clinical Administered PTSD Scale), <i>SD</i> (standard deviation)			
<sup>a</sup> There was no 1-year follow-up in the WLC group.			

**Figure Legends**

**Figure 1.** DNA breakage and DNA repair in PBMCs from control ( $n = 20$ ), trauma-exposed ( $n = 11$ ) and PTSD subjects ( $n = 34$ ). **(a) DNA breakage.** Percentage of SybrGreen fluorescence intensities of DNA that had remained double-stranded during the alkaline pH phase of the FADU assay<sup>22</sup>. Lower values indicate higher numbers of strand breaks. PTSD and trauma-exposed demonstrated higher DNA breakage compared to controls. **(b) DNA repair.** Following x-radiation (3.8 Gy) on ice, PBMCs were incubated at 37 °C for the indicated periods to allow DNA repair. Error bars represent standard errors of means.