Immunological and Molecular Alterations in Posttraumatic Stress Disorder and the Reversibility through Psychotherapy

Dissertation zur Erlangung des

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Julia Morath

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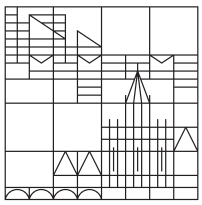

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1. Referent: Prof. Dr. Iris-Tatjana Kolassa
2. Referent: Prof. Dr. Thomas Elbert

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Summary

The experience of traumatic life events has severe consequences on both psychological and physiological health. In particular, there is an augmented probability for the development of a posttraumatic stress disorder (PTSD) (Kolassa, Ertl, et al., 2010; Neuner, Schauer, Karunakara, et al., 2004), as well as for numerous of physical diseases (Felitti et al., 1998), with a cumulative number of traumatic life events. Therefore, individuals with PTSD present an increased risk for cardiovascular, infectious-, autoimmune diseases and cancer (Boscarino, 2004; Glaesmer et al., 2011). Previous research indicated extensive biological alterations concerning the endocrine- and the immune system in individuals with PTSD (Pace & Heim, 2011). Since many of these biological alterations are age-dependent and normally occur in people in old age (Ershler & Keller, 2000; Fagnoni et al., 2000), this might point towards an association between psychological stress and premature biological aging (Bosch et al., 2009; Epel, 2009; Graham et al., 2006). Furthermore, the question remains if the biological alterations found in individuals with PTSD are specific for the diagnosis of PTSD, or if also individuals who experienced traumatic events, but did not develop a PTSD show stress-induced biological alterations. In this way, there might be a cumulative effect of traumatic load on biological alterations.

The aim of the present thesis is to investigate, in a first step, the impact of traumatic load on biological alterations in individuals with PTSD, and to analyze in a second step the exertion of influence through psychotherapy. Immunological and molecular alterations were analyzed in severely traumatized refugees with multiple trauma exposure through war and organized violence. The control group included persons with a background of migration, but without traumatic load and without psychiatric diseases. To test the hypothesis of a dose-response effect of traumatic load on biological alterations, all three studies included an additional group with substantial trauma-exposure, but without the diagnosis of PTSD.

In **study 1** of this thesis, the findings of a reduced proportion in naïve cytotoxic ($CD8^+$) and regulatory T cells, as well as an increase in the proportion of memory $CD8^+$ T cells, were approved in an expansion of the sample from Sommershof et al. (2009) of 34 individuals with PTSD, 24 trauma-exposed and 19 control subjects. Moreover, there was evidence for a dose-response effect of traumatic load on the alterations in T Lymphocyte distribution. In a second step, it was investigated if effective psychotherapeutic treatment can cause a normalization of the altered T Lymphocyte distribution in individuals with PTSD. Psychotherapeutic treatment

included 12 sessions of Narrative Exposure Therapy (NET), which has been verified as an effective trauma-focused treatment approach in several studies (Robjant & Fazel, 2010). To analyze the effectiveness of NET on psychological and biological changes, 34 individuals with PTSD were randomly assigned to either a treatment group or a waitlist control group. Four months after the end of treatment, PTSD symptom severity and somatic complaints improved significantly in the NET group, and even improved further in the one-year follow-up. One year after the end of treatment, there was also a significant increase in the proportion of regulatory T cells, however the altered proportions of naïve and memory $CD8^+$ T cells did not change through psychotherapeutic treatment. The results indicate not only an improvement in psychological symptoms, but also a normalization of the altered regulatory T Lymphocyte distribution through psychotherapeutic treatment.

In **study 2**, damage and repair of the DNA has been investigated in 34 individuals with PTSD, 11 trauma-exposed and 20 control subjects. It was demonstrated that individuals with PTSD showed a significantly accelerated amount of DNA damage compared to controls, with a cumulative effect of trauma load on DNA damage. Against expectations, the repair of damaged DNA was not impaired in individuals with PTSD, but rather was improved in capacity. The influence of psychotherapeutic treatment with NET on DNA damage and repair was investigated in a sample of 38 individuals with PTSD who were randomly assigned to either a treatment or a waitlist control group. Four months after the end of treatment, there was significant improvement not only in PTSD symptom severity but also in DNA damage in the NET treatment group, however not in the waitlist control group. DNA repair capacity changed analogue with the amount of DNA damage and normalized after the end of treatment. The results demonstrate that psychotherapeutic treatment is effective even on a molecular level and, since carcinogenesis is considerably related to DNA damage, the reduction of DNA damage is a relevant factor to prevent the physical health of individuals with PTSD.

Both the shift in the proportion of cytotoxic T cells, as well as the increase in DNA damage are characteristic for age-dependent alterations that normally occur in old people (Fagnoni et al., 2000; Lombard et al., 2005). Therefore, in **study 3** the hypothesis of premature biological aging in individuals with PTSD was tested with the GlycoAge test. The GlycoAge test describes an age-dependent profile of N-glycosylation that has been identified as a marker for physiological aging by Vanhooren et al., (2010). Therefore, the *N*-glycosylation profile of 13 individuals with PTSD, 9 trauma-exposed and 10 control subjects was analyzed with the

GlycoAge test. Individuals with PTSD differed significantly from controls in their *N*-glycosylation profile and presented a shift in the GlycoAge test, which would be representative for individuals about 15 years older. Moreover, also with respect to the *N*-glycosylation profile, a dose-response effect of traumatic load appeared.

In conclusion, the findings of the three studies demonstrate a cumulative effect of traumatic load on immunological and molecular alterations as well as a process of premature biological aging in individuals with PTSD. Moreover, and most importantly it has been shown that effective trauma-focused psychotherapeutic treatment not only improved psychological health, but also contributed to a normalization of the immunological and molecular alterations in individuals with PTSD.

Zusammenfassung

Das Erleben von traumatischen Lebensereignissen hat weitreichende Folgen sowohl für die psychische als auch für die körperliche Gesundheit. Insbesondere besteht durch eine Anhäufung von unterschiedlichen traumatischen Erlebnissen ein zunehmendes Risiko für die Entwicklung einer Posttraumatischen Belastungsstörung (PTBS) (Kolassa, Kolassa, Ertl, Papassotiropoulos, & De Quervain, 2010; Neuner, Schauer, Karunakara, et al., 2004), sowie für eine Vielzahl von körperlichen Erkrankungen (Felitti et al., 1998). Hierbei zeigen Personen mit PTBS insbesondere ein erhöhtes Risiko für Herz-Kreislauf Erkrankungen, Infektionskrankheiten, Autoimmunerkrankungen und Krebs (Boscarino, 2004; Glaesmer, Brähler, Gündel, & Riedel-Heller, 2011). Bisherige Untersuchungen bei Personen mit PTBS deuten auf weitgreifende biologische Veränderungen, die sowohl das Endokrine- als auch das Immunsystem betreffen (Pace & Heim, 2011). Da viele der biologischen Veränderungen, die bei Personen mit PTBS beobachtet werden können, altersabhängige Veränderungen darstellen und normalerweise erst bei alten Menschen auftreten (Ershler & Keller, 2000; Fagnoni et al., 2000), könnte dies ein Hinweis für einen möglichen Zusammenhang zwischen psychologischem Streß und vorzeitiger biologischer Alterung darstellen (Bosch, Fischer, & Fischer, 2009; Epel, 2009; Graham, Christian, & Kiecolt-Glaser, 2006). Darüber hinaus bleibt die Frage bestehen, ob die beobachteten biologischen Veränderungen bei Personen mit PTBS spezifisch sind für die Diagnose einer PTBS, oder ob auch Personen, die nach dem Erleben von traumatischen Ereignissen keine PTBS entwickeln, Stress bedingte biologische Veränderungen aufweisen. In diesem Sinne wäre es denkbar, daß ein kumulativer Effekt der traumatischen Belastung auf die Veränderbarkeit biologischer Parameter besteht.

Das Anliegen dieser Arbeit besteht darin, in einem ersten Schritt den Einfluß von traumatischer Belastung auf biologische Veränderungen bei Personen mit PTBS zu untersuchen, und in einem zweiten Schritt die mögliche Einflußnahme durch Psychotherapie näher zu beleuchten. Hierfür wurden sowohl immunologische als auch molekularbiologische Veränderungen bei schwer traumatisierten Flüchtlingen mit multiplen traumatischen Erlebnissen durch Krieg und organisierte Gewalt erhoben. Die Vergleichsgruppe bestand aus Personen mit entsprechendem Migrationshintergrund, jedoch ohne traumatische Belastung und ohne psychische Erkrankung. Um einen möglichen Dosis-Wirkungseffekt von traumatischen Lebensereignissen auf immunologische und molekularbiologische

Veränderungen untersuchen zu können, wurde in allen drei Studien zusätzlich eine Gruppe von Personen mit traumatischer Belastung, jedoch ohne PTBS aufgenommen.

In **Studie 1** konnten zunächst die Befunde von Sommershof et al., (2009) hinsichtlich einer Reduktion der naiven zytotoxischen ($CD8^+$) und der regulatorischen T-Zellen, sowie eine proportionale Zunahme der Gedächtnis $CD8^+$ T-Zellen, in einer Erweiterung der Stichprobe auf 34 Personen mit PTBS, 24 Trauma-exponierten und 19 Kontrollpersonen, bestätigt werden. Zusätzlich zeigte sich ein Dosis-Wirkungseffekt der traumatischen Belastung auf die Veränderung der T-Lymphozyten-Verteilung. In einem zweiten Schritt wurde die Einflußnahme von effektiver Psychotherapie auf eine Normalisierung der veränderten T-Lymphozyten-Verteilung bei Personen mit PTBS untersucht. Die psychotherapeutische Behandlung beinhaltete 12 Sitzungen Narrative Expositionstherapie (NET), welches ein Trauma-fokussiertes Expositionsverfahren darstellt, dessen Effektivität bereits in etlichen Studien gezeigt werden konnte (Robjant & Fazel, 2010). Zur Überprüfung der Therapieeffektivität wurden 34 Personen mit PTBS entweder einer Therapie- oder einer Wartegruppe randomisiert zugeteilt. Vier Monate nach Ende der Therapie zeigte sich eine signifikante Verbesserung der PTBS Symptomatik sowie der somatischen Beschwerden in der NET Gruppe, die sich im Laufe eines Jahres sogar noch weiter verbesserten. Ein Jahr nach Ende der Therapie konnte ebenfalls ein signifikanter Anstieg der regulatorischen T-Zellen in der Therapiegruppe beobachtet werden, wohingegen die Verschiebung in den Proportionen der naiven und der Gedächtnis $CD8^+$ T-Zellen nicht durch Psychotherapie veränderbar war. Die Ergebnisse legen nahe, daß durch Psychotherapie nicht nur eine Verbesserung der psychischen Symptomatik, sondern auch eine Normalisierung der veränderten Verteilung der regulatorischen T-Lymphozyten erreicht werden kann.

In **Studie 2** wurde die Schädigung und Reparatur der DNA bei 34 Personen mit PTBS, 11 Trauma-exponierten und 20 Kontrollpersonen untersucht. Es zeigte sich ein signifikant höheres Ausmaß basaler DNA Schädigung bei Personen mit PTBS im Vergleich zu Kontrollpersonen sowie ein kumulativer Effekt der traumatischen Belastung auf die DNA Schädigung. Entgegen der Erwartung erwies sich die DNA Reparatur bei Personen mit PTBS nicht als beeinträchtigt, sondern zeigte vielmehr noch eine Zunahme in der Reparaturkapazität. Die Einflußnahme einer psychotherapeutischen Behandlung mit NET auf die Schädigung und Reparatur der DNA wurde an einer Stichprobe von 38 Personen mit PTBS untersucht, die randomisiert entweder einer Therapie- oder einer Wartegruppe zugeteilt wurden. Vier Monate nach Ende der Therapie zeigte sich nicht nur ein signifikanter Rückgang

der PTBS Symptomatik, sondern auch eine signifikante Reduktion der basalen DNA-Schädigung in der Therapie-, nicht jedoch in der Wartegruppe. Die DNA Reparaturleistung verhielt sich entsprechend dem Ausmaß der Schädigung und normalisierte sich nach Ende der Therapie. Die Ergebnisse demonstrieren, dass durch effektive Psychotherapie selbst Veränderungen auf molekularer Ebene erzielt werden können und, da Krebserkrankungen in direktem Zusammenhang mit Schäden an der DNA stehen (Khansari, Shakiba, & Mahmoudi, 2009), eine Reduktion der DNA Schädigung einen wichtigen Beitrag zur Erhaltung der körperlichen Gesundheit bei Personen mit PTBS darstellt.

Sowohl Verschiebungen in den Proportionen der zytotoxischen T-Zellen, als auch vermehrte DNA Schädigung gelten als charakteristisch für altersabhängige biologische Veränderungen, die sich normalerweise erst bei alten Menschen zeigen (Fagnoni et al., 2000; Lombard et al., 2005). Zur Überprüfung der Hypothese einer vorzeitigen biologischen Alterung bei Personen mit PTBS, wurde in **Studie 3** das *N*-Glykosilierungsprofil von 13 Personen mit PTBS, 9 Trauma-exponierten Personen und 10 Kontrollpersonen anhand des GlycoAge Test analysiert (Vanhooren et al., 2010). Der GlycoAge Test erfaßt ein spezifisches alterungsbedingtes Profil der *N*-Glykosilierung und wurde von Vanhooren et al. (2010) als Marker für physiologische Alterung etabliert. Das *N*-Glykosilierungsprofil von Personen mit PTBS unterschied sich signifikant von dem der Kontrollgruppe und entsprach einer Veränderung im GlycoAge test, welche normalerweise bei Personen beobachtet wird, die etwa 15 Jahre älter sind. Darüber hinaus bestätigte sich auch hinsichtlich der *N*-Glykosilierung ein Dosis-Wirkungseffekt der traumatischen Belastung.

Schlußfolgernd, verdeutlichen die drei durchgeführten Studien einen kumulativen Effekt traumatischer Belastung auf immunologische und molekularbiologische Veränderungen, sowie einen vorzeitigen biologischen Alterungsprozess bei Personen mit PTBS. Darüber hinaus konnte erstmals gezeigt werden, daß effektive Trauma-fokussierte Psychotherapie nicht nur zu einer Verbesserung der psychischen Gesundheit, sondern auch zu einer Normalisierung von immunologischen und molekularbiologischen Veränderungen bei Personen mit PTBS beitragen kann.

Abbreviations

ACTH	Adrenocorticotropic hormone
AIC	Akaikes Information Criterion
BER	Base excision repair
CAPS	Clinician administered PTSD scale
$CD3^+$	T lymphocytes (include $CD8^+$ and $CD4^+$ T cells)
$CD4^+$	T helper cells
$CD8^+$	Cytotoxic T cells
CRH	Corticotropin releasing hormone
DNA	Deoxyribonucleic acid
DEX	Dexamethasone
DSM	Diagnostic and statistical manual of mental disorders
F	F-statistic
FADU	Fluorimetric detection of alkaline DNA unwinding
HAM-D	Hamilton depression rating scale
HPA	Hypothalamic-pituitary-adrenal axis
HR	Homologous recombination
IL	Interleukin
MDE	Major depression episode
MINI	Mini international neuropsychiatric interview
min	Minutes
MMR	Mismatch repair
NA2F	Fucosylated glycan (peak 6)
NER	Nucleotid excision repair
NET	Narrative Exposure Therapy

NGA2F	Galactosylated glycan (peak 1)
N-Glycan	N-linked oligosachharide
NHEJ	Non-homologous end joining
NK	Natural killer cells
O-Glycan	O-linked oligosachharide
PBMC	Peripheral blood mononuclear cells
PTSD	Posttraumatic stress disorder
r	Correlation coefficient
ROS	Reactive oxygen species
SNS	Sympathetic nervous system
SOMS	Screening for somatoform symptoms
t	T-statistik
Th1	Cellular immune response
Th2	Humoral immune response
TNF	Tumor necrosis factor
T_{reg}	Regulatory T cells
W	Wilcoxon-Mann-Whitney-Test
WLC	Waitlist control
χ^2	Kruskal-Wallis test (Chi-square distribution)

Submitted Articles and Research Contributions

The studies in this thesis were realized with the support of a number of colleagues. In the following, the three articles are listed with my independent research contributions.

STUDY A: Does effective trauma-focused therapy change the altered T cell distribution in individuals with PTSD? Evidence from a randomized controlled trial.

Authors: Julia Morath[*], Hannah Gola[*], Annette Sommershof[*], Gilava Hamuni, Stephan Kolassa, Claudia Catani, Hannah Adenauer, Martina Ruf-Leuschner, Maggie Schauer, Thomas Elbert, Marcus Groettrup, Iris-Tatjana Kolassa[#]

Submitted

I coordinated the study jointly together with Hannah Gola, recruited study participants and carried out a large number of clinical interviews and psychotherapies. I performed the statistical analysis and drafted the manuscript.

STUDY B: Effects of psychotherapy on DNA strand break accumulation originating from traumatic stress.

Authors: Julia Morath[*], Maria Moreno-Villanueva[*], Gilava Hamuni, Stephan Kolassa, Martina Ruf-Leuschner, Maggie Schauer, Thomas Elbert, Alexander Bürkle[#], Iris-Tatjana Kolassa[#]

Submitted

I coordinated the study, recruited study participants and carried out a large number of clinical interviews and psychotherapies. I performed the statistical analysis under the supervision of Stephan Kolassa and I drafted the manuscript.

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* Equally contributing, # Corresponding Author

STUDY C: *N*-Glycosylation profiling of plasma provides evidence for accelerated physiological aging in Posttraumatic Stress Disorder

Authors: Julia Morath[*], Maria Moreno-Villanueva[*], Valerie Vanhooren, Thomas Elbert, Stephan Kolassa, Claude Libert, Alexander Bürkle[#], Iris-Tatjana Kolassa[#]

Accepted in 'Translational Psychiatry – Nature'

I coordinated the study, recruited study participants and carried out a large number of clinical interviews. I performed the statistical analysis and drafted the manuscript.

1 General Introduction

1.1 The impact of stress on immunological and molecular alterations

The experiences of traumatic life events -- such as natural disasters, war, organized violence, physical attacks or sexual abuse -- have expansive negative effects for psychological and physical health. The severe consequences of traumatic stress become apparent in individuals with Posttraumatic Stress Disorder (PTSD), who are adversely affected by unwanted recollections of the traumatic event, avoidance of remembering the traumatic event and a permanent state of physiological hyper arousal (American Psychiatric Association, 2000). In addition to the psychological strain, individuals with PTSD present poor physical health with an increased risk for numerous physical diseases, including cardiovascular-, infectious- and autoimmune diseases, chronic pain conditions and cancer (Boscarino, 2004; Sareen et al., 2007). Both, the risk for the development of PTSD (Kolassa, Ertl, et al., 2010; Neuner, Schauer, Karunakara, et al., 2004), as well as the risk for physical illness (Felitti et al., 1998) increases with the accumulation of traumatic load.

The experience of a traumatic life event, characterized by a life-threatening situation with subjective feelings of extreme fear, helplessness or horror (American Psychiatric Association, 2000), is always attended by a physiological alarm response that enables the organism for a rapid fight-or-flight response (Elbert, Rockstroh, Kolassa, Schauer, & Neuner, 2006). Within seconds, the sympathetic nervous system (SNS) is activated by the hypothalamus and causes the distribution of the catecholamines epinephrine and norepinephrine. In a second step, the hypothalamic activation leads to the release of cortisol by the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The release of stress hormones has far-reaching consequences and causes alterations in the immune response. Both catecholamines and cortisol have in general down-regulating effects on the immune system (Elenkov et al., 2000; Elenkov et al., 2008) by inhibiting the expression of pro-inflammatory cytokines and stimulating the expression of anti-inflammatory cytokines (Elenkov & Chrousos, 2002; Sternberg, 2006; Webster, Tonelli, & Sternberg, 2002). Thereby, a shift from a cellular (Th1) immune response, that is mainly regulated by pro-inflammatory cytokines, to a humoral (Th2) immune response, mainly regulated through anti-inflammatory cytokines, is induced. While the expression of macrophages, natural killer cells (NK cells) and cytotoxic T cells is part of Th1 immune response, the production of eosinophils, mast cells and B cells

pertain to the Th2 immune response (Elenkov, Wilder, Chrousos, & Vizi, 2000; Webster et al., 2002).

Yet, stress hormones affect not only the function and the quantity, but also the molecular structure of immune cells. Since the chemical structure of the Deoxyribonucleic Acid (DNA) is rather instable, DNA damage can be caused by numerous different endogenous and exogenous factors (Lindahl, 1993; Lombard et al., 2005). Besides well-known damaging factors such as reactive oxygen species (ROS), alkylating chemicals or ultraviolet (UV) and ionizing radiation (Knippers, 2005), psychological stress has also been associated with DNA damage (Joergensen et al., 2011). Evidence for the damaging effects of stress hormones has been given by *in vitro* experiments that confirmed a five-fold increase in DNA damage through epinephrine, norepinephrine and cortisol (Flint, Baum, Chambers, & Jenkins, 2007). Furthermore, inflammatory cytokines also induce DNA damage via a demanding production of nitric oxids and ROS (Jaiswal, LaRusso, & Burgart, 2000). Since there are thousands of DNA damages in each cell at every day, effective DNA repair is essential for the survival of the cell. Due to the different damaging factors, the kinds of DNA damage are also variable and include the modification of bases, single strand breaks or double strand breaks. Accordingly to the different types of DNA damage, there also exist a number of divergent DNA repair mechanisms. Whereas the repair of DNA single strand breaks include base excision repair (BER), nucleotid excision repair (NER) or mismatch repair (MMR), the repair of double strand breaks include homologous recombination (HR) or non-homologous end joining (NHEJ) (Knippers, 2005). Most importantly, stress hormones and inflammatory cytokines also impair DNA repair mechanisms (Flint et al., 2007; Jaiswal et al., 2000).

Individuals with PTSD show remarkable deregulations of the endocrine system, however, where the levels of catecholamines are increased (Pervanidou, 2008; Pervanidou & Chrousos, 2012; Yehuda, Southwick, Giller, Ma, & Mason, 1992; Young & Breslau, 2004), the findings regarding the levels of cortisol are more heterogeneous. The majority of studies reported decreased cortisol concentrations in the plasma and saliva in individuals with PTSD (Bauer, Wieck, Lopes, Teixeira, & Grassi-Oliveira, 2010; Heim & Nemeroff, 2009; Morris, Compas, & Garber, 2012; Pace & Heim, 2011), but considering the cortisol concentration in the hair (Steudte et al., 2011) and the cerebrospinal fluid concentration (Baker et al., 2005) increased cortisol concentrations were found in individuals with PTSD. Since there are close interactions between the endocrine and the immune systems, it is not surprising that

individuals with PTSD also show alterations in the immune system. Accordingly to the low levels of cortisol in individuals with PTSD, pro-inflammatory cytokines are increased. Especially, the expression of Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and Tumor necrosis factor-α (TNF-α) are enhanced (Baker et al., 2001; Gola et al., 2013; Maes et al., 1999; Spivak et al., 1997; Tucker et al., 2004; von Känel et al., 2007). With regard to a potential shift from cellular to humoral immune response in individuals with PTSD, no alterations are reported in B cell counts (Boscarino & Chang, 1999; de Kloet et al., 2007), nor in B cell reactivity (Krnic et al., 2007), indicating that the humoral immune response is not impaired in individuals with PTSD. With respect to the cellular immune response, T lymphocyte proliferation is highly controversial in the literature: while some studies report higher T cell counts (Boscarino & Chang, 1999; Boscarino, 2004), other studies report lower T cell counts (Kawamura, Kim, & Asukai, 2001) and some studies report no alterations at all (Vidović et al., 2007; Sommershof et al., 2009). However, with respect to T cell subpopulations, a reduced proportion of naïve and an increased proportion of memory cytotoxic $CD8^+$ T cells have been found in individuals with PTSD. Moreover, regulatory T cells have also been reported to decrease about 50% in individuals with PTSD (Sommershof et al., 2009).

Many of the observed immunological alterations in individuals with PTSD, such as an increase in pro-inflammatory cytokines (Ershler & Keller, 2000), a shift in the proportion of naïve and memory cytotoxic T cells (Fagnoni et al., 2000) and a heightened DNA damage, can be also seen in old people. With respect to age-dependent alterations in individuals with PTSD, traumatic stress might promote a process of premature aging (Epel, 2009). Vanhooren et al., (2010) identified an age-dependent shift in the *N*-glycosylation profile as a biomarker for physiological aging. Moreover, this change in *N*-glycosylation has been also associated with inflammaging[1] (Dall'olio et al., 2012). Glycosylation describes a post-translational modification of proteins through the attachment of glycans. The attachment of glycans give stability and structure to the protein and as glycans are not directly encoded in the genome, their structure is highly variable and can be influenced by environmental conditions (Varki et al., 2009). Glycosylation is essential for numerous immune functions, since most of the proteins and immunoglobulines that are involved in the immune response are glycosylated (Raman, Raguram, Venkataraman, Paulson, & Sasisekharan, 2005; Rudd, Elliott, Cresswell, Wilson, & Dwek, 2001). Structural alterations in the glycosylation of glycoproteins and

1 "Inflammaging" is used as a term, that describes a status of low-grade inflammation characteristically for elderly

glycolipids are associated with a number of different diseases such as cancer, inflammatory- and autoimmune diseases (Callewaert et al., 2003; Durand & Seta, 2000; Reis, Osorio, Silva, Gomes, & David, 2010; Van Beneden et al., 2009), on that individuals with PTSD are at a higher risk for (Boscarino, 2004).

There is strong evidence for stress induced immunological alterations in individuals with PTSD (Pace & Heim, 2011), but only little is known about the reversibility of deviant immunological alterations. Therefore, most importantly, the question remains: What could be done to improve the physical health in individuals with PTSD? Since the immunological alterations in individuals with PTSD seem to be stress-dependent, there might be the chance that a reduction of the perceived psychological stress might also lead to a normalization of immunological changes. Could an improvement in psychological health through effective psychotherapeutic treatment also promote a strengthening of the immune system? Until now, most of psychotherapeutic research that has investigated the impact on immune functions has focused on cancer or HIV patients (Kiecolt-Glaser & Glaser, 1992; G. E. Miller & Cohen, 2001). However, little is known about the influence of psychotherapeutic treatment on the immune system in psychiatric patients. A few studies have focused on endocrine alterations after psychotherapeutic treatment in individuals with PTSD, but yet, results remain inconsistent (Gerardi, Rothbaum, Astin, & Kelley, 2010; Heber, Kellner, & Yehuda, 2002; Olff, de Vries, Güzelcan, Assies, & Gersons, 2007).

1.2 Aim of the present thesis

The aim of the present thesis was two-fold and included, in a first step, the investigation of baseline biological alterations in individuals with PTSD, and in a second step the reversibility through psychotherapeutic treatment. To research the cumulative effect of traumatic load on biological alterations, a group with substantial trauma exposure, but without diagnosis of PTSD, was included in all of the three studies.

In **study 1** of this thesis, T lymphocyte distributions were analyzed to reappraise the findings of a shift in the proportion of naïve and memory cytotoxic T cells and a reduction in the proportion of regulatory T cells in individuals with PTSD (Sommershof et al., 2009). Afterwards, the impact of psychotherapeutic treatment with Narrative Exposure Therapy (NET) on T lymphocyte distributions, in individuals with PTSD, was investigated. It was

hypothesized that the reduced proportion of naïve cytotoxic and regulatory T cells should increase with a reduction in PTSD symptom severity after effective psychotherapeutic treatment. **Study 2** focused on the quantification of DNA damage and DNA repair in individuals with PTSD and the reversibility through psychotherapy. Both endogenous DNA damage and exogenous DNA damage after x-ray irradiation were analyzed, and the process of DNA repair has been observed over 90 minutes. In a second step, the reversibility of DNA damage and a possibly normalization of DNA repair through psychotherapeutic treatment with NET were examined. It was hypothesized that individuals with PTSD would present an increase in DNA damage and an inhibition in DNA repair. Due to effective psychotherapeutic treatment, a decrease of DNA damage and a normalization of DNA repair have been expected. Since many of the observed biological alterations in individuals with PTSD are also characteristic of people in old age, the hypothesis of premature physiological aging in individuals with PTSD was tested in the third study. Therefore, in **Study 3**, the profile of *N*-glycosylation in individuals with PTSD was analyzed by the GlycoAgeTest, which has been established as a biological marker for physiological aging (Vanhooren et al., 2010). It has been hypothesized that there would be a shift in the *N*-glycosylation profile of individuals with PTSD that would be in accordance with the age-dependent shift characteristic in old people.

2 STUDY A: Does effective trauma-focused therapy change the altered T cell distribution in individuals with PTSD? Evidence from a randomized controlled trial

2.1 Abstract

Posttraumatic stress disorder (PTSD) has been associated with a reduced ratio of naïve cytotoxic T lymphocytes, an increased ratio of memory cytotoxic T lymphocytes, and a reduced proportion of $FoxP3^+$ regulatory T lymphocytes. This study aimed to investigate whether these immunological alterations in individuals with PTSD are reversible through an evidence-based psychotherapeutic treatment. Therefore, 34 individuals with PTSD were randomly assigned to either a treatment condition of 12 sessions Narrative Exposure Therapy (NET) or a waitlist control (WLC) group. Post-tests were conducted four months and one year after the end of therapy. PTSD symptoms were significantly reduced in the NET group, but not in the WLC group after four months (effect size: Hedges' g = -1.61). At the one-year follow-up, PTSD symptoms were improved even further in the NET group compared to baseline (Hedges' g = -1.96). This symptom improvement was mirrored in an increase in the originally reduced proportion of regulatory T cells (T_{regs}) in the NET group at the one-year follow-up, when comparing subgroups matched for baseline T_{reg} numbers. However, no changes were found for the initially reduced proportion of $CD45RA^+CCR7^+$ naïve T lymphocytes, or the enhanced proportion of $CD45RA^-$ memory T lymphocytes. In conclusion, NET was effective in reducing trauma-related PTSD symptoms and had a positive effect on the proportion of T_{regs} cells, thus demonstrating an effect of psychotherapy on an immunological level. Yet, the shift in the proportion of naïve and memory T lymphocytes in individuals with PTSD, characteristic for an aging immune system, was not reversible and thus might render individuals with PTSD permanently more susceptible for infectious diseases.

2.2 Introduction

The probability of developing Posttraumatic Stress Disorder (PTSD) in the aftermath of psychological trauma increases with the number of traumatic event types experienced (Kolassa, Ertl, et al., 2010; Neuner, Schauer, Karunakara, et al., 2004). Likewise, a clear dose-response effect of trauma exposure has been demonstrated for the development of physical health problems (Felitti et al., 1998) and an increased risk for somatic diseases like

chronic pain, cancer, cardiovascular, respiratory, gastrointestinal, and autoimmune diseases in individuals with PTSD has been reported (Boscarino et al., 2010; Boscarino, 2004; Sareen et al., 2007), whereby the poor physical health found in individuals with PTSD might be moderated by deviant immune functions (Pace & Heim, 2011).

However, the aim to link PTSD to alterations of bulk T cell populations, representing a major branch of adaptive immunity, has been controversial: Whereas the number of circulating $CD8^+$ cytotoxic T cells in individuals with PTSD has been found to be mostly lower (Ironson et al., 1997; Kawamura et al., 2001; Sommershof et al., 2009) or unchanged (Altemus, Dhabhar, & Yang, 2006; Laudenslager et al., 1998; Vidović et al., 2007; Wilson, van der Kolk, Burbridge, Fisler, & Kradin, 1999), the number of circulating $CD3^+$ T-lymphocytes or $CD4^+$ T helper cells has been found to be lower, unchanged or even higher (Boscarino & Chang, 1999; Boscarino, 2004; Ironson et al., 1997; Kawamura et al., 2001; Laudenslager et al., 1998; Sommershof et al., 2009; Vidović et al., 2007; Wilson et al., 1999). As peripheral T lymphocytes consist of a range of functionally different subpopulations, one reason for these inconsistent findings might be that changes in PTSD might be specific to certain T lymphocyte activation and differentiation states. Sommershof et al. (2009) investigated this further differentiation of $CD4^+$ T helper and $CD8^+$ cytotoxic T cells in naïve, memory and effector cells, applying a differentiation model of T cells defined by changes in the expression of the lineage markers CD45RA and CCR7 (Hamann, Roos, & Van Lier, 1999; Sallusto, Lenig, Förster, Lipp, & Lanzavecchia, 1999). They found a decreased ratio of ($CD45RA^+CCR7^+$) naïve $CD8^+$ T cells and an increased proportion of ($CD45RA^-$) memory $CD8^+$ T cells in individuals with PTSD (Sommershof et al., 2009). As a shrinking repertoire of naïve T cells may correlate with an enhanced susceptibility to infectious diseases (Fagnoni et al., 2000; Shen, Kim, & Weksler, 1999), this reduction in naïve T cells represents a possible explanation for the enhanced risk of infectious diseases in individuals with PTSD (Sommershof et al., 2009). Furthermore, Sommershof et al. (2009) observed a 50% decrease in the proportion of $CD4^+CD25^+FoxP3^+$ regulatory T cells (T_{reg}) in individuals with PTSD. T_{reg} cells are critical for maintaining balance in the immune system, regulating the immune response, and preventing autoimmune diseases (Vignali, Collison, & Workman, 2008). Decreased counts of $CD4^+CD25^+FOXP3^+$ T_{reg} cells have been associated with autoimmune diseases like diabetes, multiple sclerosis, rheumatoid arthritis, psoriasis, anemia and eczema (Bennett et al., 2001; Buckner, 2010; Wildin, Smyk-Pearson, & Filipovich, 2002), conditions for which individuals with PTSD show an increased risk (Boscarino et al., 2010; Boscarino, 2004; Weisberg et al., 2002).

Given the considerable prevalence of traumatic stress, and in particular the high prevalence of PTSD in populations affected by conflict, terror and combat (Neuner & Elbert, 2007; Neuner, Schauer, Karunakara, et al., 2004), a highly relevant question in the context of traumatic stress and physical disease is: Can effective treatment reverse the effects of traumatic stress not only on a psychological but also on an immunological level? A considerable body of clinical research has revealed that a variety of psychotherapeutic interventions may effectively reduce trauma-related mental suffering (National Institute of Clinical Excellence, 2005). Moreover, it was demonstrated that successful psychotherapeutic treatment also significantly reduced cough, diarrhea, and fever (Neuner et al., 2008). Yet, to our knowledge there is no study, investigating the effect of psychotherapy on immune functions in individuals with PTSD. So far, the impact of psychological interventions on T lymphocyte populations (which are the focus of our paper) has mainly been examined in patients with cancer and human immunodeficiency virus (HIV), yielding mixed results: E.g. Mindfulness-based stress reduction and disclosure interventions have been associated with a stabilization of $CD4^+$ T lymphocytes in HIV patients (Creswell, Myers, Cole, & Irwin, 2009; Petrie, Keith, Fontanilla, Thomas, Booth, & Pennebaker, 2004; Sherman, Bonanno, Wiener, & Battles, 2000). However, $CD4^+$ and $CD8^+$ T lymphocytes were not affected by cognitive behavioral stress management, that effectively decreased HIV viral load (Antoni et al., 2006) and herpesvirus IgG antibody titers (Carrico et al., 2005) in HIV infected men. Also, a structured psychiatric intervention program that effectively improved psychological symptoms did not affect $CD3^+$, $CD4^+$ or $CD8^+$ T lymphocyte cell counts in cancer patients (Hosaka, Tokuda, Sugiyama, Hirai, & Okuyama, 2000).

In a recent study we observed a decrease in DNA strand breaks in individuals with PTSD after trauma-focused treatment with Narrative Exposure Therapy (Morath et al., submitted), verifying the effectiveness of psychotherapy on a biological level in a PTSD sample. Narrative Exposure Therapy (NET) is a trauma-focused treatment approach (as recommended by the clinical guidelines [National Institute of Clinical Excellence, 2005]) specifically developed for survivors of war and torture experiences with a diagnosis of PTSD (Schauer, Neuner, & Elbert, 2011). The efficacy of NET has been proven in a number of randomized controlled trials in post-conflict regions (Ertl, Pfeiffer, Schauer, Elbert, & Neuner, 2011; Neuner, Schauer, Klaschik, Karunakara, & Elbert, 2004) and in European Countries (Hensel-Dittmann et al., 2011; Robjant & Fazel, 2010).

The aim of the present study was two-fold: 1) to extend the findings by Sommershof et al. (2009) in a larger sample of individuals with PTSD, trauma-exposed non-PTSD subjects and controls and 2) to investigate whether the altered T cell distribution in individuals with PTSD can be reversed by psychotherapeutic treatment with NET. Individuals with PTSD were investigated before treatment and four and 12 months after the end of therapy and T cell differentiation subsets were analyzed. We hypothesized that the NET treatment group would show an increase in the proportions of $CD45RA^{+}CCR7^{+}$ naïve $CD8^{+}$ as well as in the proportion of $CD4^{+}CD25^{+}FOXP3^{+}$ T_{reg} cells.

2.3 Methods

Participants

Thirty-four individuals with PTSD and 43 non-PTSD controls participated in this study. Subjects were recruited through the Center of Excellence for Psychotraumatology, University of Konstanz, and public advertisements. Sixteen subjects with PTSD and 27 controls were also participants in a previous study by Sommershof et al. (2009). After the initial screening, individuals with PTSD (age range: 16 to 47 years) – refugees (13 Africa, 21 Middle East) with a history of war and torture experiences – were randomly assigned to either a treatment (NET group: n = 17) or a waitlist control condition (WLC group: n = 17). The non-PTSD control group (age range: 16 to 50 years), recruited for baseline comparison, consisted of refugees and immigrants (9 Africa, 13 Balkan, 21 Middle East) without a diagnosis of PTSD and varying traumatic load (0 to 9 traumatic event types). As the number of traumatic events experienced influences T cell distribution in a cumulative way (Sommershof et al., 2009), we further divided the control group into a group with substantial trauma exposure (trauma-exposed, n = 24) and a control group with no or little trauma exposure (non-trauma-exposed, n = 19) by median split of a traumatic load index[2].

[2] Traumatic load index = [(number of traumatic event types on the CAPS event list/items on the CAPS event list) + (number of war experiences on the vivo checklist/items on the war checklist) + (number of torture experiences on the vivo checklist/items on the torture checklist)].

Table 2.1. *Sociodemographic and clinical characteristics of individuals with PTSD, who were assigned to the Narrative Exposure Therapy (NET) and the Waitlist Control (WLC) group, of Trauma-exposed individuals and control subjects.*

Variables	PTSD		Trauma-exposed	Controls	*p*
	NET (n = 17)	WLC (n = 17)	(n = 24)	(n = 19)	
Age[b] (years)	28 (16-47)		32 (16-50)	25 (19-49)	.35
	28 (16-43)	31 (17-47)			.40
Sex (female/ male)[c]	14/20*		13/11*	16/3	.01
	8/9	6/11			.36
Region of Origin (%)[c]					.004
Africa	*38.2*		*29.2*	*10.5*	
Balkan	-		*25.0*	*36.8*	
Middle East	*61.8*		*45.8*	*52.6*	
Smokers (%)[c]	20.6		25.0	5.3	.22
	17.6	23.5			.68
Medication (%)[c]	41.2*#		16.7*	-	.002
	41.2	41.2			1.0
Hypnotics	*5.9*	*11.8*	*8.3*	-	
Anxiolytics	*5.9*	*11.8*	-	-	
Antidepressives	*35.3*	*23.5*	*8.3*	-	
Neuroleptics	*11.8*	-	-	-	
Traumatic load index[b]	1.3 (0.3-2.2)*#		0.8 (0.3-2.2)*	0.1 (0-0.3)	< .0001
	1.3 (0.3-2.1)	1.3 (0.6-2.2)			.71
War/ torture events[b]	9 (0-22)*#		3 (0-20)*	0 (0-1)	< .0001
	9 (0-22)	8 (0-19)			.79
CAPS events[a]	7.0 ± 2.0*#		5.6 ± 1.9	2.2 ± 1.2	< .0001
	6.8 ± 2.3	7.2 ± 1.7			.62
CAPS Score[b]	88 (56-114)*#		0.5 (0-57)*	0 (0-10)	< .0001
	91 (63-114)	75 (56-106)			.01
HAM-D Score[b]	27.5 (0-44)*#		7 (0-31)*	0 (1-5)	< .0001
	27 (0-44)	28 (15-39)			.63
SOMS-7 Score[b]	21 (3-58)*#		6 (0-41)*	1 (0-3)	< .0001
	21 (13-48)	21 (3-58)			.56

[a]Group comparisons in continous variables were performed with ANOVA. Data are presented as mean ± standard deviation. [b]When residuals of the model were not normally distributed, non-parametric testing in continuous variables was done with the Kruskal-Wallis test (χ^2). Data are presented as median and range.[c]Group comparisons in categorical variables were performed with chi-squared tests (χ^2). CAPS, Clinician Administered PTSD Scale; HAM-D, Hamilton Depression Rating Scale; SOMS-7, Screening for Somatoform Symptoms-7. * significantly different from non-exposed controls. # significantly different from trauma-exposed controls.

Exclusion criteria were acute infections or chronic somatic illnesses (e.g. Hepatitis, HIV, osteoarthrosis) and glucocorticoid medication. In addition, non-trauma-exposed control group subjects were excluded if they met the criteria for any mental disorder according to DSM-IV or reported intake of psychotropic medication. Individuals with PTSD and trauma-exposed controls were excluded if they met the criteria for comorbid alcohol or substance abuse and dependence or a current or past history of a psychosis according to DSM-IV.

Individuals with PTSD showed no significant group differences from trauma-exposed and non-trauma-exposed controls with respect to age and smoking behavior, but groups differed significantly with respect to gender and intake of psychotropic medication (for sociodemographic and clinical characteristics of the sample see Table 2.1). Moreover, individuals with PTSD had experienced significantly more different traumatic event types, assessed by the event list of the Clinician Administered PTSD Scale (CAPS; Blake et al., 1995), significantly more war and torture events, assessed by the Vivo checklist (Schauer et al., 2011b), and showed higher symptom scores in the CAPS, the Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960) and the screening for somatoform symptoms (SOMS-7; Rief, Schäfer, & Fichter, 1992), than trauma-exposed and non-exposed controls. In addition, trauma-exposed individuals differed significantly from non-exposed controls, with respect to CAPS, HAM-D and SOMS-7 scores (see Table 2.1). In accord with the building block effect of traumatic stress (Kolassa, Kolassa, Ertl, Papassotiropoulos, & de Quervain, 2010; Neuner, Schauer, Karunakara, et al., 2004), the traumatic load index correlated positively with PTSD symptom severity ($r = .65$; $p < .0001$).

Concerning the treatment study: the NET group and the WLC did not differ significantly with respect to age, sex, ethnicity, smoking behavior or intake of psychotropic medication, number of traumatic event types experienced, HAM-D score, or SOMS-7 score. However, the NET group showed a significantly higher PTSD symptom score (see Table 2.1).

Procedure

Baseline screening: All participants were screened with a clinical diagnostic interview applied by trained clinical psychologists from the Center of Excellence for Psychotraumatology, always starting at 10 a.m. If participants were not fluent in English or German, diagnostic interviews were completed with the help of trained interpreters. The interview started with socio-demographic as well as health-related (e.g. smoking behavior, use of psychotropic medication, physical disorders) data collection. In the second part of the interview, the

number of different traumatic event types experienced and PTSD symptom severity were assessed using the CAPS (Blake et al., 1995). In addition, the Vivo Checklist of War, Detention, and Torture Events (Schauer et al., 2011b) were administered to assess of war and torture experiences in more detail. Depressive symptoms were quantified by the HAM-D (Hamilton, 1960) and somatic complaints by a short version of the SOMS-7 (Rief, Schäfer, & Fichter, 1992). Comorbid psychiatric disorders were assessed using the Mini International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). The same clinical diagnostic interview was repeated at the four and 12 months post-test.

Treatment study: The trial was conducted in an ambulant setting at the Center of Excellence for Psychotraumatology, University of Konstanz, Germany. Therapists were clinical psychologists specialized in the field of trauma and experts for Narrative Exposure Therapy (NET). If participants were not fluent in English or German, treatments were completed with the help of trained interpreters.

The 34 individuals with PTSD were randomly assigned to either the NET group or a WLC group using permuted blocks of variable lengths. The NET group received 12 treatment sessions of 90 minutes, on a weekly or bi-weekly basis. For a detailed description of the treatment procedure, please see Schauer et al. (2011a). Treatment adherence was monitored by means of regular supervision. The WLC group waited for about eight months without any standardized intervention. Post-tests were conducted four months (t_1), and one year (t_2) after the end of treatment in the NET group. For the participants in the WLC group, the time spans between pre- and post-tests were individually matched with the NET group. For ethical reasons, the WLC group received treatment with NET after the first post-test, therefore only the NET group was invited to the one-year follow-up. The diagram of participant flow is shown in Figure 2.1 with the numbers of participants who were randomly assigned, received treatment, and were analyzed. Diagnosticians were blind with regard to group belongingness of participants at baseline and at both post-tests.

The ethics committee of the University of Konstanz approved the study and all participants provided written informed consent before study participation. Participants received 30 € remuneration for each blood drawing. Treatment with NET was provided without any costs for the PTSD group. The study was registered at clinicaltrials.gov (identifier: NCT01206790).

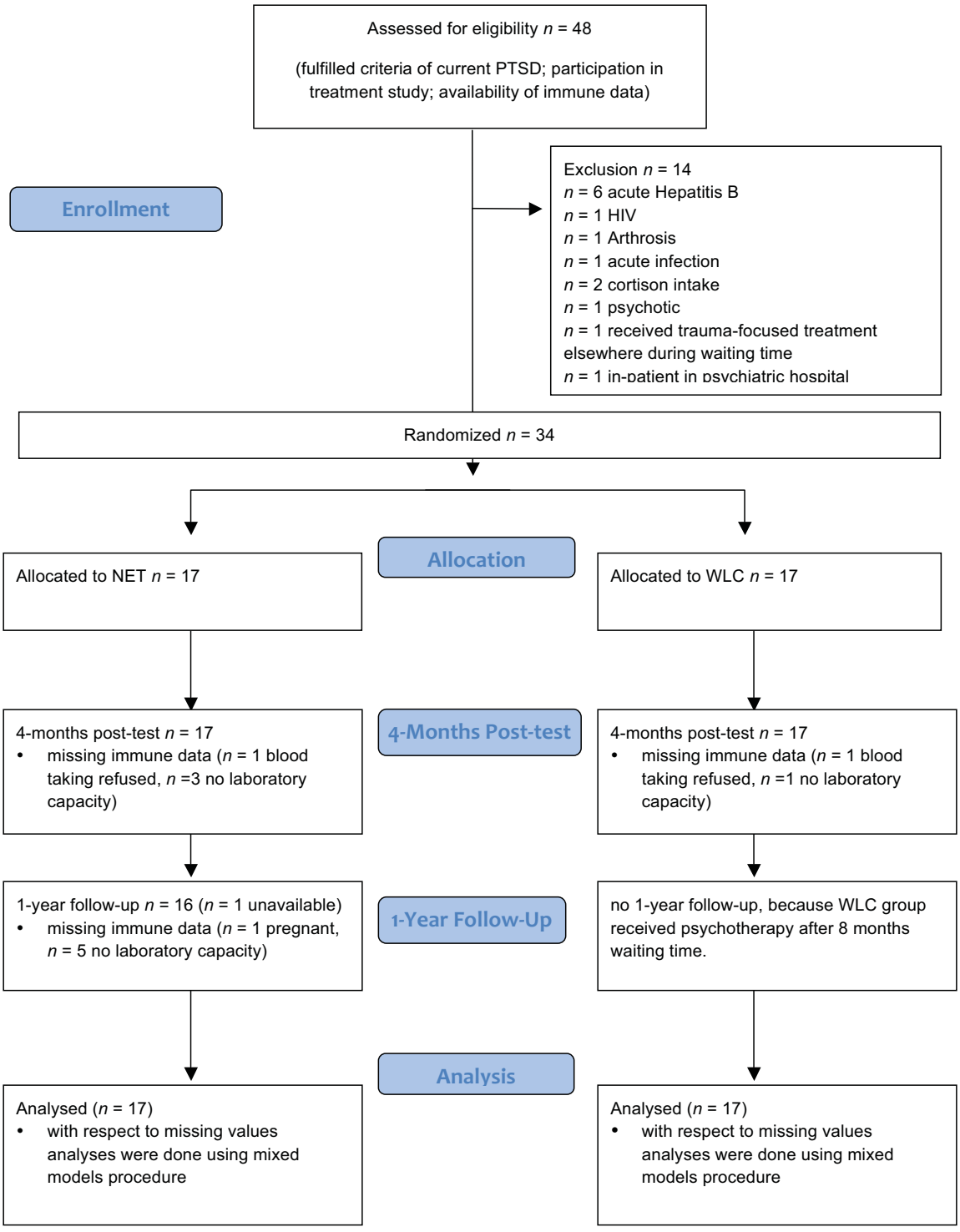

Figure 2.1. Flow of participants through the study. PTSD indicates Posttraumatic Stress Disorder; NET indicates Narrative Exposure Therapy and WLC indicates waitlist control group.

Blood collection and Lymphocyte phenotyping

Blood drawings and lymphocyte phenotyping were performed before treatment started (t_0), four months post-treatment (t_1), and one year after the end of treatment (t_2). Blood was collected always at 10:00 a.m. in EDTA-treated tubes and the fresh blood was sent within one hour to the Laboratory of Immunology, University of Konstanz, Germany, where the samples were processed. Blood samples were coded to guarantee blinding of the laboratory staff involved. The method used for lymphocyte phenotyping was stringently equal to the method used by Sommershof et al. (2009).

In a first step, the total number of monocytes, granulocytes, and lymphocytes were counted. Lymphocytes were further subdivided into B cells ($CD19^+$), NK cells ($CD16^+$ $CD56^+$), $CD3^+$ T cells, $CD3^+CD4^+$ T helper cells, $CD3^+CD8^+$ cytotoxic T cells, and $CD4^+CD25^+FoxP3^+$ T_{reg} cells. The surface molecules CD45RA and CCR7 were used to characterise distinct T cell maturation subsets: naïve ($CD45RA^+$ $CCR7^+$), central memory T_{CM} ($CD45RA^-$ $CCR7^+$), effector memory T_{EM} ($CD45RA^-$ $CCR7^-$) and CD45RA-positive effector memory cells (T_{EMRA} $CD45RA^+CCR7^-$). Absolute numbers of lymphocytes were obtained using an automated hematology analyzer (XT-2000i, Sysmex, Horgen, Switzerland).

Outcomes

Outcome measures were changes in PTSD symptom severity (CAPS score), changes in depressive symptoms (HAM-D score), changes in somatic complaints (SOMS-7 score) and changes in ($CD45RA^+CCD7^+$) naïve and ($CD45RA^-$) memory subsets of $CD3^+$, $CD4^+$, and $CD8^+$ T cells as well as changes in $CD4^+CD25^+FoxP3^+$ T_{reg} cells four months and one year after the end of treatment.

Statistics

Group differences study: Differences between groups (PTSD, trauma-exposed controls, non-exposed controls) in clinical characteristics and T cell distributions were analyzed by ANOVA. As age influences T cell distribution (Fagnoni et al., 2000; Hong, Dan, Choi, & Kang, 2004), age was included as a covariate in the models as also suggested by model fit estimates (Akaike's Information Criterion, AIC; Burnham & Anderson, 2002). Including smoking and gender as additional factors into the model did not alter results. Furthermore,

results remained also stable when participants with psychotropic medication intake were excluded.

The non-parametric Kruskal-Wallis test (χ^2) was used to analyze group differences when residuals of the model (ANOVA) were not normally distributed. As we had specific hypotheses for the direction of T cell changes (reduction in the proportion of $CD8^+$ naïve and T_{reg} cells), one-sided independent t-tests or the non-parametric Wilcoxon-Mann-Whitney test (W) were used for post-hoc analysis for these variables. Correlations were analyzed with the Kendall tau rank correlation.

Treatment study: Linear mixed models were used to analyze changes in clinical characteristics and lymphocyte differentiations from baseline (t_0) to the four months post-test (t_1). Age was included as covariate for T cell analysis. Since residuals in the model of $CD3^+$ total T cells and $CD8^+$ memory T cells were not normally distributed, data were retested with a logarithmized data set and results remained stable. Changes from pre treatment (t_0) to the four months post-test (t_1) and the one-year follow-up (t_2) within the NET group were analyzed by linear mixed models and paired *t*-tests for post-hoc comparisons. Treatment effect sizes were calculated by Hedges' g (Hedges, 1981).

2.4 Results

The effect of traumatic stress and PTSD on T cell distribution

There was no difference in the absolute cell number of lymphocytes (χ^2 = 0.72; p = .70) between individuals with PTSD (median = 1939; range: 1249 – 2800), trauma-exposed individuals (median = 1960; range: 1301 – 2938), and non-traumatized controls (median = 1815; range: 1308 – 2965), but the percentage of total $CD3^+$ T cells was significantly reduced in individuals with PTSD compared to non-traumatized controls (see Table 2.2).

Extending the results of Sommershof et al. (2009), we found a significant main effect for Group with respect to the percentage of naïve $CD8^+$ T cells ($F_{(2,68)}$ = 3.72; p = .03), with post-hoc tests revealing significant group differences between individuals with PTSD and non-traumatized controls ($t_{(27.0)}$ = 2.04; p = .04; one-sided) and between trauma-exposed subjects and controls ($t_{(31.5)}$ = 1.87; p = .03; one-sided), but not between individuals with PTSD and trauma-exposed controls ($t_{(38.9)}$ = -0.04; p = .49; one-sided; see Table 2.2). Again, there were

significantly increased percentages of memory $CD8^+$ T cells in individuals with PTSD compared to non-PTSD controls ($\chi^2 = 8.65$; $p = .01$). Post-hoc tests revealed significant group differences between individuals with PTSD and non-traumatized controls ($W = 181.5$; $p = .005$; one-sided) and between individuals with PTSD and trauma-exposed subjects ($W = 456.0$; $p = .01$; one-sided), but not between trauma-exposed subjects and controls ($W = 171$; $p = .31$; one-sided).

Variables (%)	**PTSD** ($n = 34$)		**Trauma-exposed** ($n = 24$)		**Controls** ($n = 19$)		**Statistics**	***p***
	M	*SD*	*M*	*SD*	*M*	*SD*		
$CD3^+$								
Total[a]	68.6*	36.5 – 79.6	66.2*	40.0 – 78.3	73.9	51.4 – 78.1	$\chi^2 = 7.03$	.03
Naïve[b]	34.9	11.4	37.1	9.6	41.0	12.8	$F_{(2,72)} = 1.89$	.16
CD45RA− memory[b]	41.5*	9.9	39.0	8.1	34.5	9.4	$F_{(2,72)} = 3.70$	.03
T_{EMRA}[a]	21.9	8.5 – 40.3	23.4	3.9 – 48.5	20.2	8.4 – 53.5	$\chi^2 = 0.17$	.92
$CD8^+$								
Total[b]	22.0	5.3	23.1	5.3	25.4	5.1	$F_{(2,70)} = 2.64$	.08
Naïve[b]	30.8*	12.7	30.9*	13.2	41.0	19.5	$F_{(2,68)} = 3.72$	.03
CD45RA− memory[a]	31.7*#	12.2 – 61.4	24.1	10.4 – 52.3	23.5	10.1 – 46.8	$\chi^2 = 8.65$	.01
T_{EMRA}[b]	36.6	13.2	43.4	15.5	34.9	15.5	$F_{(2,68)} = 1.99$	.14
$CD4^+$								
Total[b]	35.9	7.9	39.2	7.0	39.1	8.1	$F_{(2,72)} = 1.68$	.19
Naïve[b]	43.6	13.7	43.6	12.3	48.6	11.9	$F_{(2,70)} = 1.07$	.35
CD45RA− memory[b]	53.7	13.1	52.7	12.4	46.9	11.8	$F_{(2,70)} = 1.84$	.17
T_{EMRA}[a]	1.7	0.5 – 12.1	2.8	0.2 – 11.8	1.9	0.6 – 17.9	$\chi^2 = 2.89$	.24
$T_{regulatory}$[a] ($CD4^+CD25^+$ $FoxP3^+$)	1.4*#	0.5 – 5.0	2.7	1.2 – 4.0	2.4	1.0 – 3.5	$\chi^2 = 9.79$	.007

[a]Residuals of the model were not normally distributed; median and range are displayed; non-parametric testing was done with the Kruskal-Wallis test (χ^2). [b] Analysis were done with age as covariate using ANCOVA; means and standard deviations *(SD)* are displayed.* significantly different from non-exposed controls. # significantly different from trauma-exposed controls.

Table 2.2. *T Lymphocyte distribution of individuals with PTSD, of Trauma-exposed individuals and of control subjects, at baseline (t_0).*

Furthermore, we confirmed the reduction in the percentage of T_{reg} cells in individuals with PTSD ($\chi^2 = 9.79$; $p = .007$): T_{reg} cells were significantly reduced in the PTSD compared to the non-traumatized control group ($W = 140.5$; $Z = -1.9$; $p = .03$; one-sided); similarly,

individuals with PTSD showed a larger reduction than trauma-exposed controls (W = 157.5; Z = -2.9; p = .0015; one-sided); there was no significant difference between trauma-exposed subjects and the control group (W = 111; Z = -1.0; p = .15; one-sided; see Table 2.2). No significant group differences were found for the percentage of total $CD8^+$ or $CD8^+$ T_{EMRA} cells as well as for the percentage of total $CD4^+$, $CD4^+$ naïve, memory and T_{EMRA} cells (see Table 2.2).

The number of different traumatic event types experienced correlated negatively with the percentage of naïve $CD8^+$ T cells (r = -0.24; p = .04), i.e. higher values in traumatic load as measured by the traumatic load index were associated with a stronger reduction in naïve $CD8^+$ T cells.

Effects of trauma-focused PTSD treatment on T cell distribution

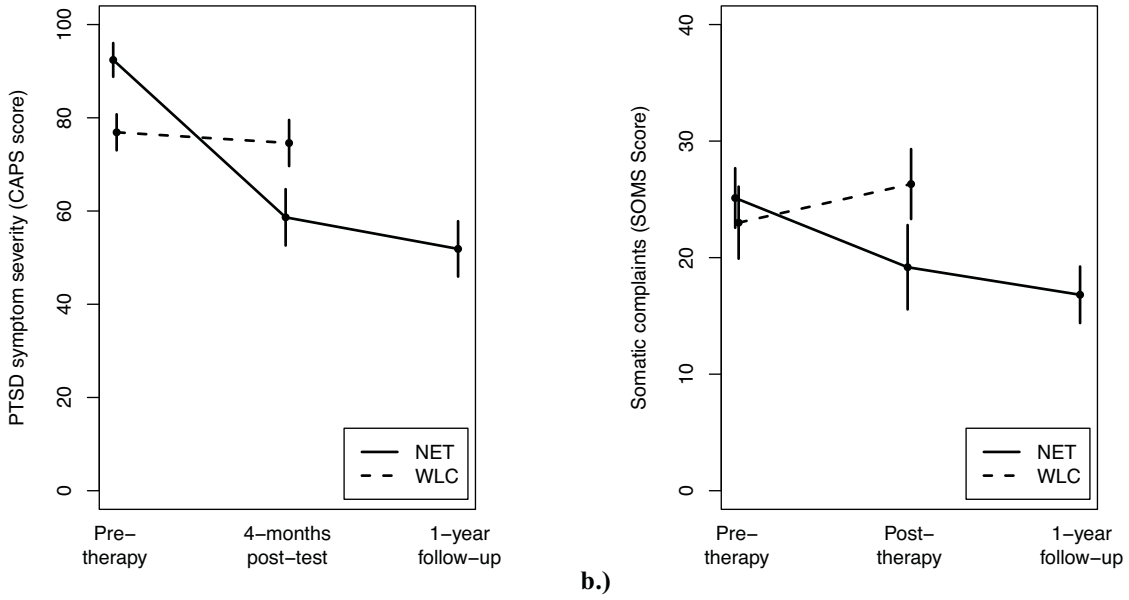

Figure 2.2. (a) PTSD symptom severity (CAPS score) and **(b)** somatic complaints (SOMS-7 score) in the Narrative Exposure Therapy (NET) group and the Waitlist Control (WLC) group pre therapy (t_0), at 4-months post-test (t_1) and in the 1-year follow-up (t_2).

Post-hoc tests showed a significant decline in PTSD symptom severity in the NET group from t_0 to t_1 ($t_{(16)}$ = -5.99; p < .0001), whereas PTSD symptom severity remained stable in the WLC group. At t_2, the NET group showed an even greater decline in PTSD symptoms (Time $F_{(1,32)}$ = 32.04; p = .0001) with an effect size of Hedges' g = -1.96 from t_0 to t_2 (see Figure 2.2a). NET also improved somatic symptoms, as measured with the SOMS-7, in the treatment but not in the WLC group (Time × Treatment, $F_{(1,31)}$ = 6.19; p = .02; Figure 2.2b).

Table 2.3. *Changes in clinical characteristics and T cell distribution in the Narrative Exposure Therapy (NET) and the Waitlist Control (WLC) group.*

Variables	**Group**	**Pre-therapy** *M (SD)*	**4-months post** *M (SD)*	**1-year post** *M (SD)*	**Statistics** (Treatment x Time)	***p***
CAPS score	NET	92.41* (14.95)	58.65 (24.93)	51.88 (24.52)	$F_{(1,32)} = 16.90$	.00
	WLC	76.88 (15.95)	74.59 (20.42)	--		
HAM-D score	NET	22.82 (11.73)	17.00 (9.81)	17.63 (9.84)	$F_{(1,32)} = 0.89$	.35
	WLC	25.94 (6.55)	24.18 (9.21)	--		
SOMS score	NET	25.12 (10.55)	19.18 (14.93)	16.81 (10.00)	$F_{(1,31)} = 6.19$	.02
	WLC	23.00 (12.75)	26.31 (12.38)	--		
% CD3 total[a]	NET	65.10 (11.18)	67.65 (12.57)	69.05 (6.04)	$F_{(1,25)} = 0.17$	.68
	WLC	65.53 (7.54)	65.25 (11.45)	--		
% CD3 naïve	NET	35.11 (11.69)	38.36 (11.38)	35.61 (12.31)	$F_{(1,26)} = 0.00$	.98
	WLC	34.71 (12.05)	36.73 (12.59)	--		
%CD3 memory	NET	40.15 (9.01)	40.19 (9.23)	40.15 (11.03)	$F_{(1,26)} = 0.08$	.78
	WLC	42.94 (10.15)	44.73 (12.11)	--		
% CD8 total	NET	23.96* (5.55)	23.64 (4.70)	23.72 (6.89)	$F_{(1,26)} = 0.34$	.56
	WLC	20.17 (4.52)	20.99 (5.40)	--		
% CD8 naive	NET	29.06 (13.42)	34.91 (13.42)	34.24 (15.93)	$F_{(1,25)} = 0.07$	.80
	WLC	30.63 (12.33)	33.82 (14.21)	--		
% CD8 memory[a]	NET	30.55 (10.58)	27.41 (7.16)	25.26 (7.80)	$F_{(1,25)} = 0.60$	.45
	WLC	34.53 (14.42)	32.61 (13.67)	--		
% CD4 total	NET	34.54 (7.13)	38.23 (5.49)	39.03 (6.52)	$F_{(1,25)} = 0.05$	.83
	WLC	38.02 (8.71)	42.04 (6.52)	--		
% CD4 naïve	NET	43.29 (16.39)	42.28 (15.99)	43.17 (15.72)	$F_{(1,25)} = 0.29$	.61
	WLC	42.06 (13.54)	38.01 (14.57)	--		
% CD4 memory	NET	53.84 (15.26)	55.99 (15.03)	55.11 (15.29)	$F_{(1,25)} = 0.05$	.82
	WLC	55.28 (13.37)	59.37 (14.42)	--		
% T_{reg}	NET	2.50* (1.73)	2.42 (1.02)	3.54 (1.25)	$F_{(1,23)} = 3.06$	.09
	WLC	1.46 (0.81)	2.35 (1.04)	--		

Abbreviations: CAPS: Clinical Administered PTSD Scale; Statistics: *M* (mean), *SD* (standard deviation). Age was included as covariate into the models of T cell analysis. [a]Since residuals of the model were not normally distributed, data were retested with a logarithmized data set and results remained stable. * Significantly different from WLC at t_0.

However, depressive symptoms (HAM-D) were not significantly improved in the NET compared to the WLC group (Time x Treatment, $F_{(1,32)} = 0.89$; $p = .35$, see Table 2.3).

Against our hypothesis, there was no treatment specific increase in the percentage of naïve $CD8^+$ T cells from t_0 to t_1 (Time × Treatment $F_{(1,25)} = 0.05$; $p = .82$, see Table 2.3 and Figure

2.3a), or from t_0 to t_2 within the PTSD group (Time $F_{(1,21)} = 2.17$; $p = .16$). Furthermore, there were no treatment specific alterations in the percentage of memory $CD8^+$ T cells in the NET, compared to the WLC group (Time × Treatment $F_{(1,25)} = 0.60$; $p = .45$) and no significant changes in the percentage of memory $CD8^+$ T cells over time (Time $F_{(1,21)} = 0.28$; $p = .60$) within the NET group (see Figure 2.3b).

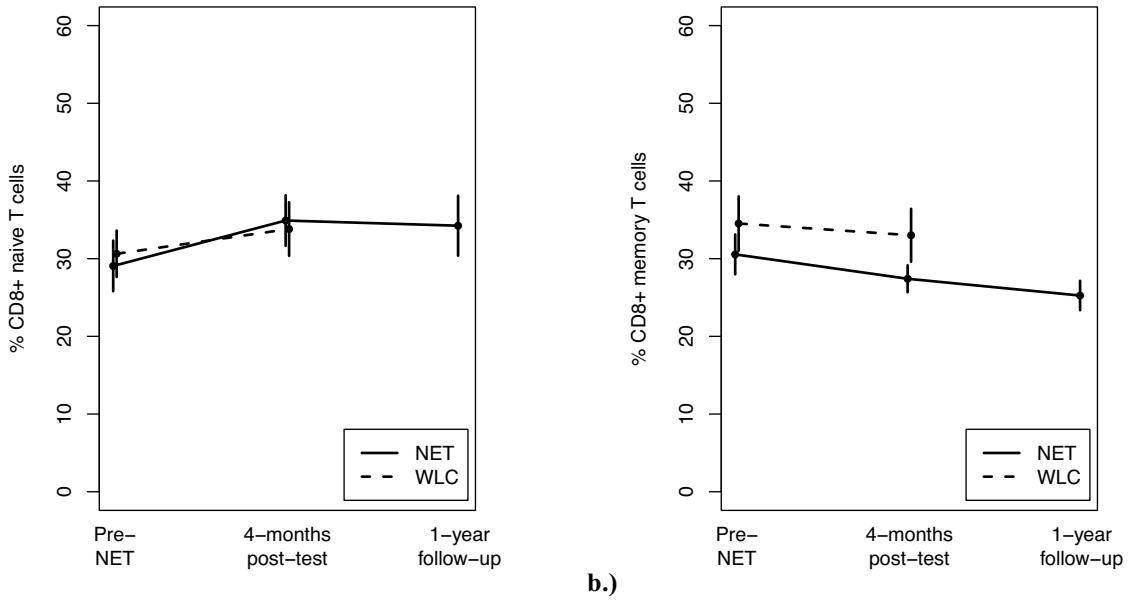

Figure 2.3 (a) Percentages of ($CD45RA^+CCR7^+$) naïve $CD8^+$ T cells and **(b)** percentages of ($CD45RA^-$) memory $CD8^+$ T cells in the Narrative Exposure Therapy (NET) group and the Waitlist Control (WLC) group pre therapy (t_0), at 4-months post-test (t_1) and in the 1-year follow-up (t_2).

With respect to the percentage of T_{reg} cells, we found no Time × Treatment interaction ($F_{(1,23)} = 3.06$; $p = .09$) from t_0 to t_1 (Table 2.3), and no significant increase in the percentage of T_{reg} over time (Time $F_{(1,20)} = 3.40$; $p = .08$) within the NET group. However there was a significant time effect from t_1 to t_2 within the NET group ($t_{(6)} = 2.37$; $p = .05$). Since the NET and the WLC group differed significantly at t_0 in T_{reg} cell counts ($t_{(22.7)} = 2.24$; $p = .04$), we performed an additional analysis excluding the two subjects with the highest percentage of T_{regs} in the NET and the two subjects with the lowest percentage of T_{regs} in the WLC group thus parallelizing groups with respect to T_{reg} cell counts ($t_{(20.6)} = 1.32$; $p = .20$). Again, there was no Time × Treatment interaction from t_0 to t_1 ($F_{(1,19)} = 0.85$; $p = .37$), but the NET group showed a significant increase in T_{regs} over time (Time $F_{(1,17)} = 8.06$; $p = .01$; Figure 2.4). Post hoc tests revealed a significant increase from t_0 to t_2 ($t_{(7)} = 1.45$; $p = .05$) and from t_1 to t_2 ($t_{(5)} = 2.56$; $p = .05$), but not from t_0 to t_1. Furthermore, there was a marginally significant but

large positive association between PTSD symptom reduction from t_0 to t_2 and the increase of T_{regs} from t_0 to t_2 (r = .75; p = .09; Supplementary Figure 2.1).

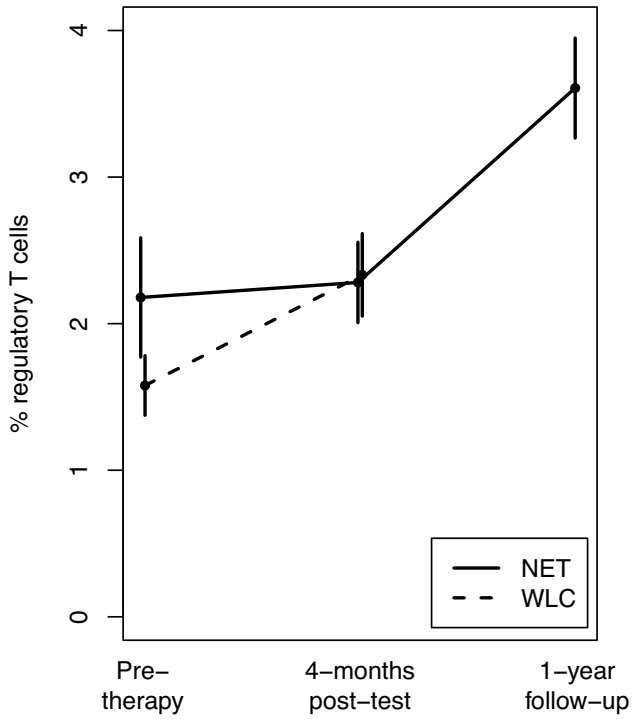

Figure 2.4 Percentages of ($CD4^+CD25^+FOXP3^+$) regulatory T cells in the Narrative Exposure Therapy (NET) group and the Waitlist Control (WLC) subgroups parallelized for baseline T_{reg} numbers, pre therapy (t_0), at 4-months post-test (t_1) and in the 1-year follow-up (t_2).

No treatment specific alterations were found for the other T cell subtypes investigated (see Table 2.3) except for the total percentage of $CD4^+$ T cells, increasing significantly from t_0 to t_2 within the NET group ($F_{(1,22)}$ = 6.53; p = .02).

Results did not change when smoking and gender were included as additional factors in the model.

2.5 Discussion

In the present study we investigated 1) T cell subpopulations in a sample of severely traumatized individuals with PTSD compared to trauma-exposed non-PTSD subjects and controls with no or few trauma exposure; and 2) the reversibility of the altered T cell distribution in individuals with PTSD following Narrative Exposure Therapy (NET).

Extending the findings of Sommershof et al. (2009) in a larger sample, we found a decreased proportion of ($CD45RA^+CCR7^+$) naïve $CD8^+$ T cells, an increased proportion of ($CD45RA^-$) memory $CD8^+$ T cells, and a decreased proportion of $CD4^+CD25^+FOXP3^+$ T_{reg} cells at baseline in individuals with PTSD compared to controls with no or few trauma exposure. Moreover, trauma-exposed non-PTSD subjects showed a significantly lower proportion of naïve $CD8^+$ T cells than non-exposed controls, and the number of traumatic event types experienced was significantly negatively correlated with the percentage of naïve $CD8^+$ T cells, indicating a cumulative effect of exposure to traumatic stressors. As we found no differences between non-PTSD subjects with or without trauma exposure with respect to T_{reg} cells, we propose, that the reduction of naïve $CD8^+$ T cells is a consequence of trauma burden rather than an explicit feature of PTSD, whereas alterations in regulatory T cells seem to be specifically associated with the chronic stress experienced by individuals with PTSD. The decrease in naïve and the increase in memory $CD8^+$ T cells in individuals with PTSD are in accordance with a T lymphocyte distribution that is typical for old people (Dorshkind, Montecino-Rodriguez, & Signer, 2009; Fagnoni et al., 2000; Hong et al., 2004). Therefore, the alterations in T lymphocyte distributions in individuals with PTSD might indicate a process of premature immunosenescence, which is in line with previous findings, showing that psychological stress is associated with immunological aging (Bosch et al., 2009; Epel et al., 2004; Kiecolt-Glaser et al., 2003).

Concerning the treatment study, as expected, the NET treatment group showed significantly reduced PTSD symptoms four months after treatment, and a further symptom reduction one year after treatment, while PTSD symptom severity in the WLC control group remained stable over measuring points. Consistent with the reported benefits of NET on physical health conditions (Neuner et al., 2008), NET also effectively improved somatic complaints such as headache, diarrhea, nausea or unspecific chronic pain conditions in the treatment group. However, no effect of NET was found on depressive symptoms. With respect to our immunological dependent variables: We found no therapy-relevant changes for the initially reduced proportion of $CD8^+$ $CD45RA^+CCR7^+$ naïve or the initially enhanced proportion of $CD45RA^-$ memory T lymphocytes at the four months or one-year follow up, suggesting that the shift in the proportion of naïve and memory T lymphocytes in individuals with PTSD, was not reversible and thus might render individuals with PTSD permanently more susceptible for infectious diseases (Fagnoni et al., 2000; Shen et al., 1999).

However, with respect to the population of $CD4^+CD25^+FOXP3^+$ T_{reg} cells, the symptom improvement in the NET group was mirrored in a significant increase of the originally reduced proportion of T_{regs} at the one-year follow-up, when comparing subgroups matched for baseline cell numbers. No changes were found for total counts or in naïve and memory subsets of $CD3^+$, $CD4^+$, or $CD8^+$ T cells four months after therapy as well as at the one-year follow-up, except for the total percentage of $CD4^+$ T cells, which increased significantly during therapy within the NET group and reached levels of non-traumatized controls at the one-year follow-up.

To our knowledge, there is currently no study, investigating changes in T cell maturation subsets following psychotherapeutic treatment, thus not allowing us easily to integrate the present results in the body of previous findings. Yet, in accordance with our outcomes, $CD8^+$ T lymphocytes have been reported to be stable over time and not affected by psychotherapeutic interventions in cancer or HIV patients (Carrico et al., 2005; Hosaka et al., 2000), while $CD4^+$T lymphocyte populations seem to react more favorably to psychotherapeutic interventions (Creswell et al., 2009; Petrie, Keith et al., 2004; Sherman et al., 2000). With respect to regulatory T cells, the administration of antidepressant medication has been shown to increase T_{reg} ($CD4^+$ $CD25^{hi}$) cells counts and to decrease interleukin-1β levels, as well as depressive symptoms in patients suffering from a depressive episode in a pharmacological treatment study (Himmerich et al., 2010). Moreover, autoimmune diseases seem to react favorably to psychological interventions (Carlson, 2012). To reduce the augmented risk for autoimmune diseases in individuals with PTSD, a future perspective might be the combination of a trauma-focused psychotherapeutic treatment approach and an antidepressant medication treatment.

Limiting factors for the interpretation of our data are: 1) the lack of a one-year follow-up in the WLC group 2) Missing information about T cell distributions in healthy controls over time. Yet, in a study with healthy university students T cell subpopulations remained stable over a period of 3-months free of examinations (Hamuni et al., in prep); 3) The intake of psychotropic medication in about 40% of the individuals with PTSD, which was, however, at least equally distributed across groups and thus should not be able to be the factor accounting for the observed effects; 4) The relatively small sample size; and 5) The varied ethnicities of study participants. However, since treatment with NET has been shown to be effective in various populations all over the world (Robjant & Fazel, 2010), we can assume that there is no cultural difference in the biological processing of traumatic stress; 5) We investigated a

severely traumatized PTSD sample with a high symptom score, allowing us to study consequences of extreme stress. However, treatment with NET in such an affected sample resulted only in a significant reduction of symptoms, but not in a full recovery from PTSD (58.8 % of the NET group still fulfilled PTSD criteria at the four months post-test). Moreover, individuals with PTSD continued to live under stressful life circumstances (e.g. 88.2 % of the individuals with PTSD have an insecure asylum status and fear deportation). These factors might have precluded stronger therapy effects concerning T cell maturations subsets and might also explain the persistence of depressive symptoms.

In conclusion, our results suggest that some biological consequences of severe trauma seem to be irreversible, while others do react favorably to effective psychotherapeutic treatment (Morath et al., submitted; Olff et al., 2007), thus further underlining the need of effective treatment of PTSD in trauma-affected populations in order to prevent the manifestation of secondary physical diseases.

2.6 Acknowledgements

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3 STUDY B: Effects of psychotherapy on DNA strand break accumulation originating from traumatic stress

3.1 Abstract

Traumatic stress is associated with numerous diseases, including an increased risk of 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 evidence-based psychotherapy on both DNA strand breakage and DNA repair. In a first study we investigated DNA breakage and DNA repair in 34 individuals with PTSD and 31 control subjects. In a second study, we analyzed the effect of trauma-focused psychotherapy (here: Narrative Exposure Therapy) on DNA breakage and repair. Thirty-eight individuals with PTSD were randomly assigned to either a treatment (N = 19) or a waitlist control condition (N = 19). Post-tests were performed four months and one year after therapy. In Study 1 we found higher levels of basal DNA strand breaks in individuals with PTSD than in controls (p = .02), 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 (p = .003), but also DNA strand break accumulation (p = .05). 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.

3.2 Introduction

Childhood physical abuse (Felitti et al., 1998; Fuller-Thomson & Brennenstuhl, 2009) and the experience of traumatic life events (Glaesmer et al., 2011) are not only associated with mental suffering, but also with numerous diseases, including an increased risk of cancer. At the molecular level, stress may increase carcinogenesis via increased DNA damage and impaired DNA repair mechanisms (Flint et al., 2007). DNA damage and genomic instability are not only known as an important driving force for carcinogenesis but are also associated with

ageing of cells and organisms (Beneke & Bürkle, 2007; Bürkle et al., 2007). In human leukocytes, for example, epinephrine induced DNA strand breaks (Crespo & Bicho, 1995), and high levels of urinary cortisol were associated with increased oxidative DNA damage in the elderly (Joergensen et al., 2011). Furthermore, in vitro exposure of murine 3T3 fibroblasts to cortisol, epinephrine, or norepinephrine led to a five-fold increase in DNA damage, and both cortisol and norepinephrine interfered with the repair of DNA damage in cells exposed to UV radiation (Flint et al., 2007). A well-established pathway regulates DNA damage through β_2-adrenoreceptors and β-arrestin-1, stimulated by β-adrenergic catecholamines (Hara et al., 2011). The immune system is directly influenced by glucocorticoids and catecholamines (Elenkov et al., 2000), both of which are dysregulated in individuals with PTSD (Pace & Heim, 2011), who in the aftermath of traumatic experiences suffer from intrusive recollections of the traumatic event, avoidance of stimuli associated with the trauma, and hyperarousal (Elbert & Schauer, 2002). A number of studies reported hypocortisolism in individuals with PTSD (Gill & Page, 2008; Rohleder, Joksimovic, Wolf, & Kirschbaum, 2004; Yehuda, Southwick, Nussbaum, & Wahby, 1990), but considering the cortisol concentration in the hair (Steudte et al., 2011) and the cerebrospinal fluid concentration (Baker et al., 2005) increased cortisol concentrations were found in individuals with PTSD. In addition catecholamine concentration is reported to be increased in individuals with PTSD (Young & Breslau, 2004). Since the toxic effects of glucocorticoids and catecholamines on the structure of the DNA were demonstrated in *in vitro* experiments (Flint et al., 2007), the high state of cortisol (Baker et al., 2005; Steudte et al., 2011) and catecholamines (Young & Breslau, 2004) in individuals with PTSD might cause DNA damage also *in vivo*.

Individuals with PTSD have been reported to show an increased inflammatory status (Gill, Saligan, Woods, & Page, 2009), a reduction of naïve and regulatory T-cells (Sommershof et al., 2009), and an enhanced production of pro-inflammatory cytokines by peripheral blood mononuclear cells (PBMCs) (Gola et al., 2013). Pro-inflammatory cytokines can lead to excessive production of nitric oxide and reactive oxygen species (ROS), causing DNA damage (Jaiswal et al., 2000). In summary, stress hormones and pro-inflammatory cytokines may induce DNA damage and alter DNA repair in PTSD.

Systematic reviews and meta-analyses show strong evidence for the clinical efficacy of psychotherapy in individuals with PTSD, especially for trauma-focused treatments (National Institute of Clinical Excellence, 2005). 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 (Schauer et al., 2011a). The efficacy of NET has been shown in a series of randomized controlled trials (Ertl et al., 2011; Robjant & Fazel, 2010). The aim of the present study was to investigate the effects of traumatic stress at the molecular level by investigating DNA damage and repair response after 3.8 Gy of x-irradiation administered ex vivo to freshly obtained immune cells of highly stressed individuals with PTSD compared to trauma-exposed individuals without PTSD as well as healthy controls. Moreover, we investigated whether psychotherapy not only improves mental health but also physical health by improving DNA integrity and DNA repair mechanisms.

3.3 Methods Study 1 (Baseline Study)

Participants

In Study 1 (baseline study), DNA breakage and DNA repair were analyzed in a total number of 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 traumatic experiences. The three groups did not differ significantly with respect to age (Table 3.1).

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 depression according to DSM-IV-TR (American Psychiatric Association, 2000) (Table 3.1). Individuals with PTSD were recruited through the Center of Excellence for Psychotraumatology, University of Konstanz.

The eleven trauma-exposed individuals were also refugees (7 Africa, 1 the Balkans, 3 Middle East and Afghanistan), but they did not fulfill criteria for current PTSD (45% of them exhibited depressive symptoms). The median length of residence in Germany of trauma-exposed individuals (6 male, 5 female) 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.

Table 3.1. *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***
Age [a] *median (range)*	31.0 (19 - 61)	21.0 (15 - 51)	30.0 (15 - 46)	$\chi^2 = 2.45$	.29
Controls vs. Trauma-exposed				$t_{(17.3)} = 1.06$	.30
Controls vs. PTSD				$t_{(32.5)} = 1.15$	.26
Trauma-exposed vs. PTSD				$t_{(12.6)} = 0.42$	.68
Traumatic event load (CAPS event list) *mean (SD)*	4.40 (2.42)	6.82 (2.09)	8.03 (2.08)	$F_{(2,62)} = 17.31$	$\leq$.001
Controls vs. Trauma-exposed				$t_{(23.4)} = -2.91$	.008
Controls vs. PTSD				$t_{(35.4)} = -5.61$	$\leq$.001
Trauma-exposed vs. PTSD				$t_{(16.9)} = 1.67$	.11
PTSD symptom load (CAPS) [a] *median (range)*	0.00 (0 - 13)	35.00 (0 - 58)	91.00 (63 - 114)	$\chi^2 = 51.36$	$\leq$.001
Controls vs. Trauma-exposed				$z = -3.48$	$\leq$.001
Controls vs. PTSD				$z = -6.17$	$\leq$.001
Trauma-exposed vs. PTSD				$z = -4.94$	$\leq$.001
Basal DNA breakage (% fluorescence) *mean (SD)*	79.42 (10.52)	70.88 (12.33)	71.52 (10.24)	$F_{(2,62)} = 3.95$	.02
Controls vs. Trauma-exposed				$t_{(18.1)} = 1.94$	.07
Controls vs. PTSD				$t_{(39.1)} = 2.69$	.01
Trauma-exposed vs. PTSD				$t_{(14.7)} = 0.15$	.88
DNA breakage after x-ray irradiation (% fluorescence) *mean (SD)*	41.28 (8.42)	32.82 (7.66)	35.63 (9.02)	$F_{(2,62)} = 4.18$	.02
Controls vs. Trauma-exposed				$t_{(22.5)} = 2.84$	.009
Controls vs. PTSD				$t_{(42.2)} = 2.32$	.03
Trauma-exposed vs. PTSD				$t_{(19.8)} = 1.01$	.33
DNA breakage after 90 min of repair (% fluorescence) *mean (SD)*	69.16 (15.94)	66.02 (14.21)	69.64 (15.66)	$F_{(2,61)} = 0.21$	.81

Abbreviations: CAPS (Clinical Administered PTSD Scale). [a] data were not normally distributed. Statistics: *SD* (standard deviation), χ^2 (Kruskal-Wallis test), *z* (Mann-Whitney U test). All tests were calculated on an alpha level of 0.05 (two-sided).

Twenty control subjects (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 inclusion criterion for the PTSD group was a diagnosis of current PTSD, according to DSM-IV-TR (American Psychiatric Association, 2000), 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). 20 individuals with PTSD (4 hypnotics, 3 anxiolytics, 10 antidepressants, 2 neuroleptics, 1 antimaniacs), 3 trauma-exposed individuals (1 hypnotics, 2 antidepressants), and 2 control subjects (2 hypnotics) reported psychotropic medication intake. All results remained stable when including psychotropic medication as a covariate.

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; Blake et al., 1995). The Vivo checklist of war, detention and torture events (Schauer et al., 2011b) was used to identify war and torture experiences. Depressive symptoms were quantified with the Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960). Other potential mental disorders were assessed with the Mini International Neuropsychiatric Interview (M.I.N.I; Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, 1998).

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 (Maria Moreno-Villanueva et al., 2011; Maria Moreno-Villanueva et al., 2009) was used for analyzing formation and repair of DNA strand breaks in living cells (Birnboim & Jevcak, 1981). 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 (Maria Moreno-Villanueva et al., 2009). 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 5×10^5 cells per ml. Then several aliquots of 100 µl cell suspension were irradiated on ice with 3.8 Gy, (dose rate1.9 Gy/min, x-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_2-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.

Statistical analysis

Statistical analysis was performed using R 2.11.0 (R Development Core Team, 2010). Since residuals in the model of age and PTSD symptom severity scores (CAPS scores) were not

normally distributed, group differences were analyzed using non-parametric statistics (Kruskal-Wallis and Mann-Whitney-U 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 x time (3 groups x 9 repeated measurements) as factors. Data of fluorescence signals were logarithmized (Maria Moreno-Villanueva et al., 2009). Inclusion of gender, age, medication and smoking in the model did not alter results. However, model fit estimates (AIC, Akaike information criterion; Burnham & Anderson, 2002) 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 (Hothorn, Bretz, & Westfall, 2008).

3.4 Results Study 1 (Baseline Study)

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 3.1, Figure 3.1a). There was a group × 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 3.1b). This apparent effect, however, is likely to result from a higher level of initial DNA breaks. We recruited in a follow-up experiment 4 healthy young volunteers who were not participants in Study 1 or 2. We x-irradiated PBMCs *ex vivo* with increasing doses of x-rays to simulate the increased DNA breakage in individuals with PTSD. We found that DNA repair was not impaired, but rather apparently accelerated in the case of higher initial DNA breakage (for results see Supplementary Figure 3.1). This suggests that higher initial DNA-breakage stimulates DNA repair.

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 ($F_{(2,45)} = 4.99$; $p = .01$).

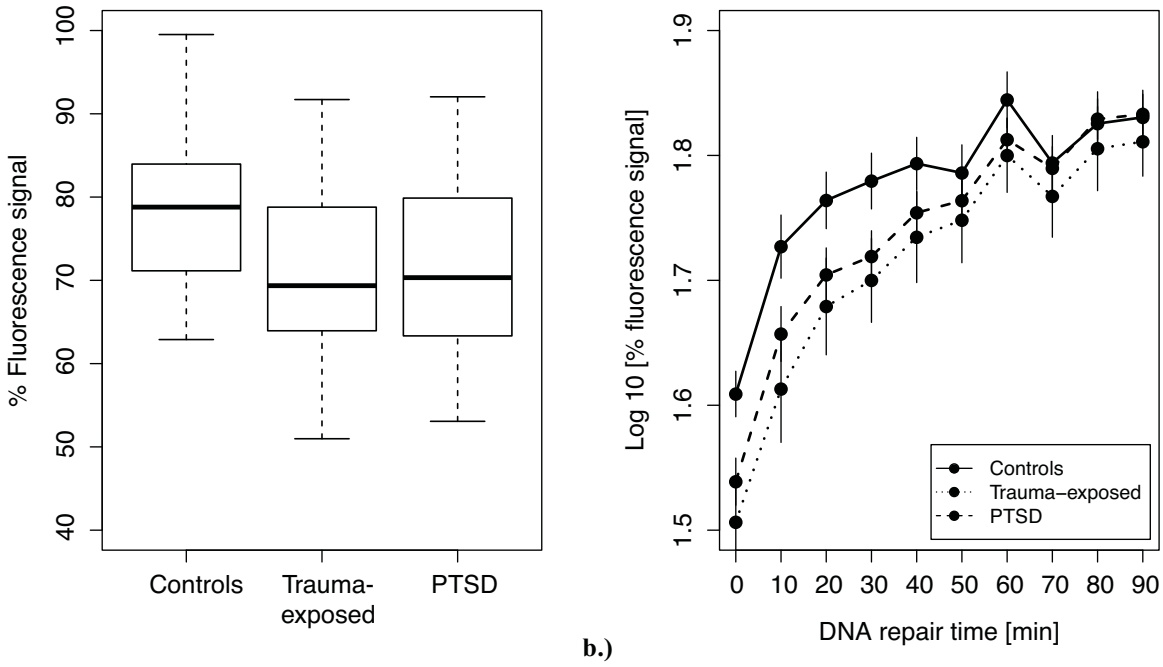

Figure 3.1. (a.) DNA strand breaks (means and standard error of the mean [SEM]) in PBMCs from control (*n* = 20), trauma-exposed (*n* = 11) and PTSD subjects (*n* = 34). Percentage of SybrGreen fluorescence intensities of DNA that had remained double-stranded during the alkaline pH phase of the FADU assay. Lower values indicate higher numbers of strand breaks. PTSD and trauma-exposed demonstrated higher DNA breakage compared to controls. **(b.)** DNA repair in PBMCs from control (*n* = 20), trauma-exposed (*n* = 11) and PTSD subjects (*n* = 34). 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.

3.5 Methods Study 2 (Treatment Study)

Procedure and Participants

This was a one-center study in an ambulant setting at the University of Konstanz, Germany. Participant flow is shown in Figure 3.2. 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, four 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). Exclusion criteria were psychotic disorders and chronic inflammatory diseases (such as infections, Hepatitis, HIV, rheumatoid arthritis, osteoarthritis, bronchitis, or asthma).

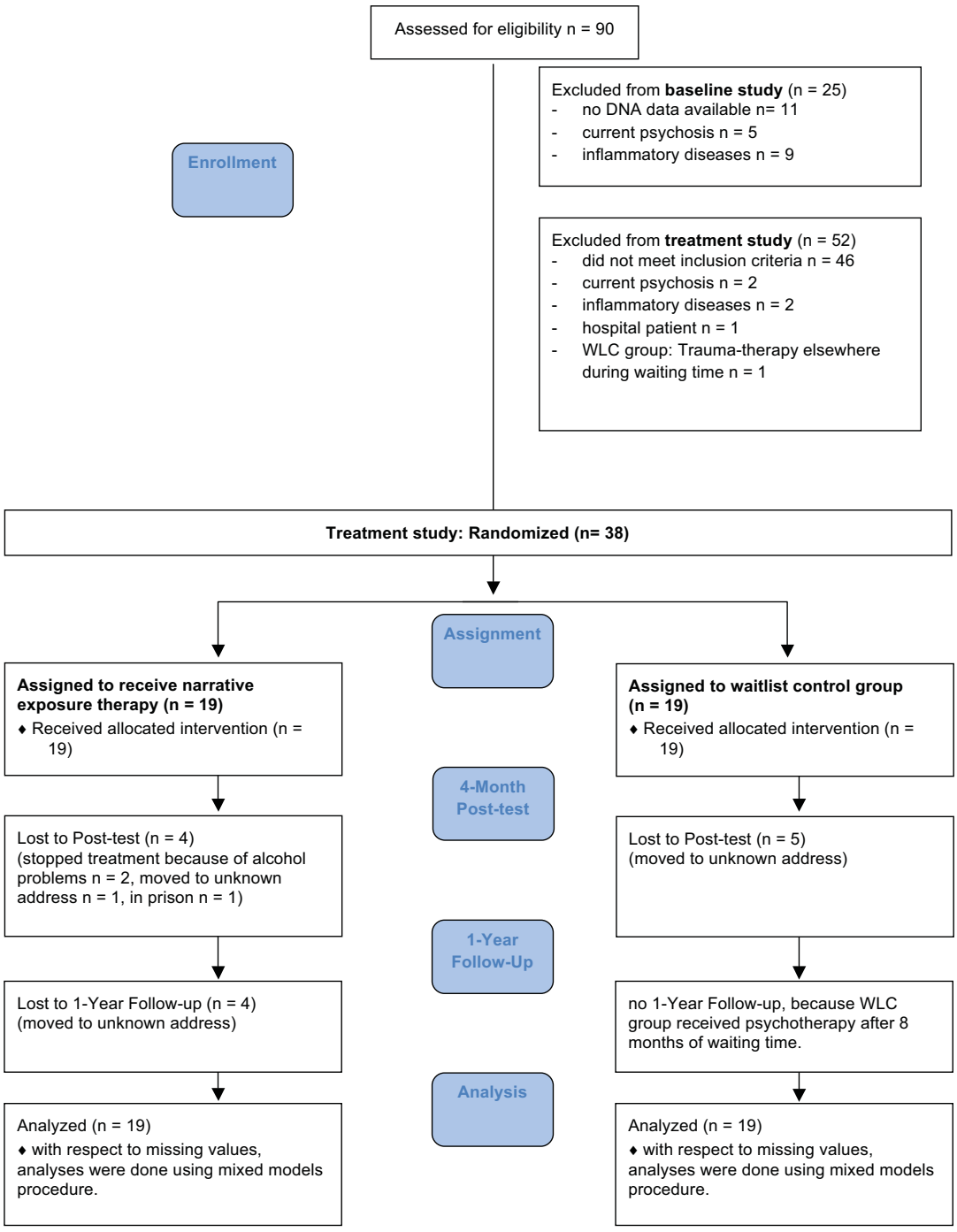

Figure 3.2. Flow of participants through the study. PTSD indicates Posttraumatic Stress Disorder; NET indicates Narrative Exposure Therapy and WLC indicates waitlist control group.

The first post-test was conducted 4 months after the end of therapy. Individuals with PTSD of the NET group were invited 1 year after the end of therapy for a second follow-up interview. PTSD patients of the WLC group received psychotherapy after the waiting period for ethical reasons, and were therefore not available for a corresponding follow-up.

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

The treatment intervention comprised 12 sessions of Narrative Exposure Therapy (NET) lasting approximately 4 months. Therapists were clinical psychologists with specializations in trauma therapy, and relied on the help of trained interpreters if necessary. The waiting period in the WLC group was 8 months, during which time period no psychotherapeutic intervention was offered. In NET (Schauer et al., 2011a) 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 (Elbert & Schauer, 2002) into a coherent narrative i.e., verbally accessible autobiographic memory.

Measures

The primary measure of outcome was the diagnosis of PTSD and the change of its severity score according to the CAPS, at 4 months and 1 year after treatment was completed. The secondary measures of outcome were changes in DNA breakage and repair. 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. Diagnosticians were not aware of which participants were allocated to which group. Blinded diagnosticians conducted post-test interviews.

Statistical analysis

Group differences in age were analyzed using non-parametric statistics (Mann-Whitney-U test), since residuals in the model were not normally distributed. Linear mixed models were calculated to analyze the primary and secondary outcomes of changes in PTSD symptom severity (CAPS score) and changes in DNA breakage and repair. Differences in time effects

between the NET and the WLC group were analyzed using simultaneous tests for general linear hypotheses (Hothorn et al., 2008). 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* (Cohen, 1988).

3.6 Results Study 2 (Treatment Study)

Baseline socio-demographic and clinical characteristics from the NET and the WLC group are presented in Table 3.2. Groups presented with very similar socio-demographic and clinical characteristics prior to treatment.

Table 3.2. *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***
Female sex *(No, %)*	6 (31.6 %)	6 (31.6 %)	$\chi^2 = 0.00$	.10
Smoking *(No, %)*	7 (36.8 %)	4 (21.1 %)	$\chi^2 = 1.15$	.28
Asylum status unsecure *(No, %)*	18 (94.7 %)	18 (94.7 %)	$\chi^2 = 0.00$	.10
comorbid depression *(No, %)*	15 (78.9 %)	15 (78.9 %)	$\chi^2 = 0.00$	.10
Psychotropic medication *(No, %)*	5 (26.3%)	8 (42.1%)	$\chi^2 = 1.05$	.31
Age [a] *median (range)*	29.0 (15 - 46)	32.0 (17 - 45)	$z = -0.64$	.52
Traumatic event load (CAPS) *mean (SD)*	7.74 (2.56)	8.42 (1.54)	$t_{(36)} = -0.99$	.33
PTSD symptom load (CAPS) *mean (SD)*	92.37 (14.16)	86.53 (15.47)	$t_{(36)} = 1.22$	.23
Basal DNA breakage *mean (SD)*	73.91 (9.50)	70.65 (10.28)	$t_{(31)} = 0.93$	.36

Abbreviations: CAPS (Clinical Administered PTSD Scale). [a] data were not normally distributed. Statistics: *SD* (standard deviation), χ^2 Chi-Quadrat-Test, *z* (Mann-Whitney U test). All tests were calculated on an alpha level of 0.05 (two-sided).

Primary outcomes. Pre-therapy PTSD symptom severity (CAPS score) did not differ between the NET and the WLC group (Table 3.2). Treatment with NET led to a significant reduction of PTSD symptoms in the NET group (pre to 4-months post-test: $t_{(14)} = -5.21$; $p = .0001$),

with a large within treatment effect size of d = -1.72. 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).

Mean change scores of PTSD symptom severity (CAPS score) were significantly greater in the NET group ($t_{(21)}$ = -3.10; p = .005), with a between treatment effect size of d = -1.14 (Figure 3.3a). Moreover, a mixed models analysis revealed a significant group × time interaction ($F_{(1,27)}$ = 10.34, p = .003). Improvements in PTSD symptom reduction not only remained stable 1 year after the end of therapy, but also were higher compared to 4-months post-test (effect size: d = -0.32; Table 3.3).

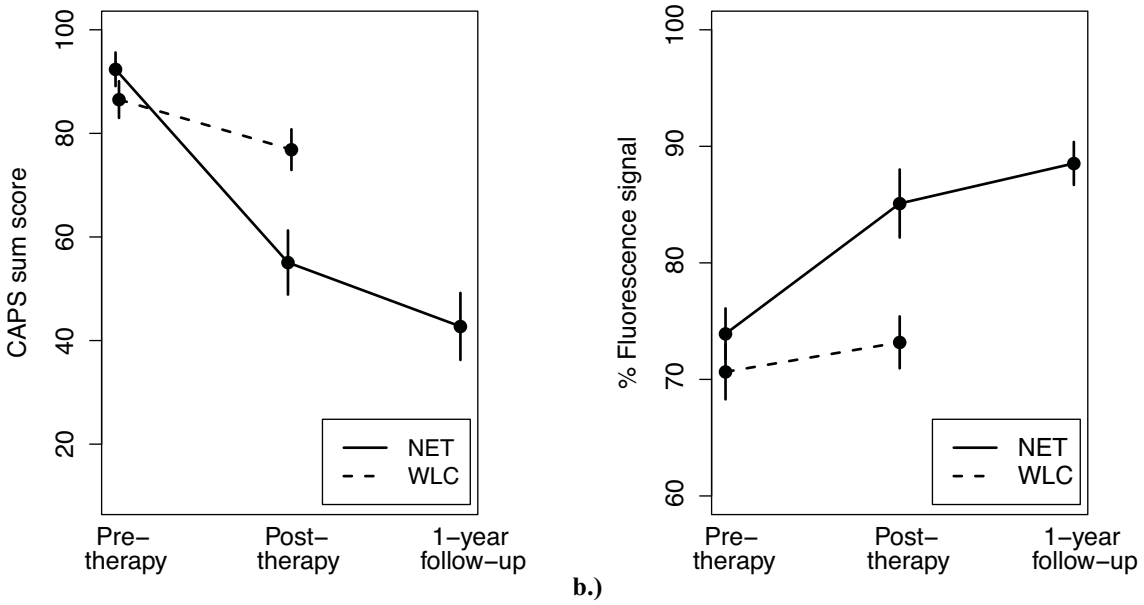

Figure 3.3 (a.) PTSD symptom severity (means and SEM) in the Narrative Exposure Therapy (NET) group (n = 19) and the Waitlist Control (WLC) group (n = 19). PTSD symptom severity (CAPS score) was analyzed pre-therapy, 4 months post-therapy and at the 1-year follow-up. There was a significant group × time interaction, with significant reduction of PTSD symptom severity in the Narrative Exposure Therapy (NET) group. **(b.)** DNA integrity (means and SEM) under basal condition in the Narrative Exposure Therapy (NET) group (n = 19) and the Waitlist Control (WLC) group (n = 19). DNA breakage was analyzed pre-therapy,4 months post-therapy and at the 1-year follow-up. There was a significant group x time interaction with significant apparent decline of DNA breakage in the Narrative Exposure Therapy (NET), but not the Waitlist Control (WLC) group.

Secondary outcomes. Pre-therapy groups did not differ in basal DNA strand breaks (Table 3.2). 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). 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 × time interaction in DNA breakage ($F_{(1,21)} = 4.44$, $p = .05$, Figure 3.3b). 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 3.3).

Table 3.3. *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)	-- [a]
Basal DNA breakage: NET	73.91 (9.50)	85.09 (12.73)	88.53 (8.05)
Basal DNA breakage: WLC	70.65 (10.28)	73.18 (9.71)	-- [a]

Abbreviations: CAPS (Clinical Administered PTSD Scale). [a] There was no 1-year follow-up in the WLC group. Statistics: *SD* (standard deviation),

The course of DNA repair in individuals with PTSD who received NET therapy returned to the pattern seen in healthy controls, presumably baseline DNA breakage was reduced after therapy. Mixed models analysis showed a significant interaction between group and time, i.e. pre- vs. post-therapy ($F_{(1,525)} = 6.45$; $p = .01$; Supplementary Figure 3.2).

3.7 Discussion

We found that PTSD, a stress-induced mental illness, is associated with significantly higher levels of endogenous DNA strand breaks in PBMCs, which should, by itself, have serious implications for physical health, in particular for carcinogenesis. Indeed, apart from PTSD, adverse childhood experiences have been associated with an increased risk for cancer (Brown et al., 2010; Felitti et al., 1998; Fuller-Thomson & Brennenstuhl, 2009) and premature mortality (Brown et al., 2009). Childhood maltreatment and severe life events during the past year were found to predict the immune response in patients with basal cell carcinoma (Fagundes et al., 2012) and epidemiological studies demonstrate an increased risk of cancer in populations with a history of war-related traumatic stress (Glaesmer et al., 2011; Keinan-Boker et al., 2009). In addition, a recent meta-analysis also supports the association between an increased prevalence of cancer and psychosocial stress (Chida, Hamer, Wardle, & Steptoe,

2008). Finally, depression, which is often comorbid with PTSD (Robjant & Fazel, 2010), has also been linked with increased damage to DNA (Irie, Miyata, & Kasai, 2005). At the molecular level, the link between psychosocial, psychological, and traumatic stress and cancer might be increased DNA damage, induced via a pathway involving β-adrenergic receptors (Hara et al., 2011) and enhanced cytokine-dependent ROS production (Khansari et al., 2009), leading to an increased risk for mutations, carcinogenesis, and pathological aging. The association of stress-induced deregulation of the immune system with an enhanced risk for developing cancer may provide an additional explanation (Reiche, Morimoto, & Nunes, 2005). After x-radiation of PBMCs *ex vivo*, individuals with PTSD and trauma-exposed individuals displayed significantly higher exogenously induced DNA breakage and the progression of DNA repair over 90 minutes showed a significant time × 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 an additional follow-up experiment with PBMCs of healthy controls irradiated with increasing doses of x-rays. Here we showed that higher DNA breakage was associated with faster DNA repair (Supplementary Figure 3.1). Therefore, our data indicate that neither exposure to trauma nor PTSD interferes with DNA strand break repair. The relevant data from the literature appear fragmentary and controversial. On the one hand, it has been reported that nucleotide excision repair in lymphocytes of students during a high-stress exam period was increased 2 hours after UV-light induced DNA damage compared to a lower stress period (Forlenza, Latimer, & Baum, 2000). On the other hand, DNA double-strand break repair in lymphocytes of highly distressed psychiatric patients was found to be reduced 5 hours after DNA damage induced via x-radiation, whereas 2 hours after induced DNA damage there was no difference in DNA repair between the sub-groups that were subjected to high and low levels of stress (Kiecolt-Glaser, Stephens, Lipetz, Speicher, & Glaser, 1985). 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).

The finding that effective psychotherapy (here: NET) is able to reverse the increased levels of endogenous DNA breakage in individuals with PTSD to a normal level is intriguing. On the one hand, several other studies have already reported PTSD symptom reduction after NET therapy (Robjant & Fazel, 2010); however, the positive impact of psychotherapy on a physical health parameter with long-term impact at the molecular level, *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. On the other hand, our 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 (Friedberg, 2003), which may precede clinically manifest tumor formation by decades. The swift reversibility of DNA breakage in individuals with PTSD via psychotherapy described here is a clear indication that there is indeed a therapeutic window not only to revert the psychological burden of the disease PTSD but also the long-term, and potentially lethal, somatic effects of this mental disorder.

Our study has some important limitations: since PBMCs were analyzed for DNA breakage and repair, it is not possible to give evidence that all types of cells included in PBMCs (e.g. lymphocyte populations, monocytes) reacted in the same manner. Another limitation is that 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.

Although sample size was limited, effect sizes revealed strong effects for both reducing the severity of the PTSD symptoms and improving DNA breakage after treatment with NET. The change in risk for health of the observed effects in baseline DNA damage on the one hand and the amelioration of DNA damage through psychotherapy on the other hand, however, remains to be quantified.

One strength of this study is the diversity of participants who came from numerous different war and conflict zones. This is further evidence that effectiveness of NET to reduce the severity of PTSD symptoms holds for different cultures and countries (Ertl et al., 2011; Robjant & Fazel, 2010); therefore, it can be assumed that the effects of decreasing DNA breakage after treatment with NET could be generalized as well.

In summary, our results reveal that exposure to traumatic life events, especially when sufficiently severe to result in a diagnosis of PTSD, are associated with higher levels of DNA damage in PBMCs, assessed as DNA breakage. The mechanism behind might be an increased endogenous production of reactive oxygen species. If maintained for extended periods of time, this may represent an increased risk for carcinogenesis and pathological aging. It is encouraging to see that we have means to reverse such a high-risk state, as demonstrated by

the decrease in stress-induced endogenous DNA damage after effective psychotherapeutic intervention in individuals with PTSD.

3.8 Acknowledgements

We thank the German Research Foundation (DFG; grant number Ko 3895/1) and the European Refugee Fund for financial support. We thank Heike Riedke for coordination and medical assistance and Monika Schulz and Judy Salzwedel for technical support.

4 STUDY C: *N*-Glycosylation profiling of plasma provides evidence for accelerated physiological aging in Posttraumatic Stress Disorder

4.1 Abstract

The prevalence of age-related diseases is increased in individuals with Posttraumatic Stress Disorder (PTSD). However, the underlying biological mechanisms are still unclear. *N*-Glycosylation is an age-dependent process, identified as a biomarker for physiological aging (GlycoAge Test). To investigate whether traumatic stress accelerates the aging process, we analyzed the *N*-glycosylation profile in $n = 13$ individuals with PTSD, $n = 9$ trauma-exposed individuals and in $n = 10$ low-stress control subjects. Individuals with PTSD and trauma-exposed individuals presented an upward shift in the GlycoAge Test, equivalent to an advancement of the aging process by 15 additional years. Trauma-exposed individuals presented an intermediate *N*-glycosylation profile positioned between severely traumatized individuals with PTSD and low-stress control subjects. In conclusion, our data suggest that cumulative exposure to traumatic stressors accelerates the process of physiological aging.

4.2 Introduction

In the aftermath of traumatic events, the probability of developing Posttraumatic Stress Disorder (PTSD), which is characterized by a cluster of intrusive symptoms, avoidance behavior, and hyperarousal (American Psychiatric Association, 2000) increases with the number of different traumatic event types experienced (Kolassa, Kolassa, et al., 2010; Neuner, Schauer, Karunakara, et al., 2004). This cumulative effect of traumatic load is presumably based on the development of a neuronal fear network: the connectivity between nodes is strengthened and the network is enlarged by every additional traumatic event experienced (Kolassa & Elbert, 2007).

In addition to psychological suffering, traumatic stress also increases the risk of developing somatic illnesses, such as cardiovascular, inflammatory, and autoimmune diseases (Boscarino, 2004). As a result, trauma exposure is associated with premature mortality and an increased risk for carcinogenesis (Brown et al., 2010; Fagundes et al., 2012; Glaesmer et al., 2011). One underlying biological mechanism might be that chronic stress is associated with an accelerating process of cellular aging (Epel, 2009). Individuals with PTSD not only show

typical age-related diseases (Boscarino et al., 2010; Sareen et al., 2007) and report an older subjective age than controls (Solomon, Helvitz, & Zerach, 2009a; Solomon & Ohry, 2010), but they even show characteristics indicative of accelerated aging. For instance, naïve T lymphocytes decrease with age and are lowest in centenarians (Fagnoni et al., 2000) – and individuals with PTSD also show a reduction of naïve T lymphocytes (Sommershof et al., 2009). Next, the proportion of memory T lymphocytes increases with normal aging (Effros, 1998; R. Miller, 1994) – and individuals with PTSD also display a higher proportion of memory T lymphocytes compared to age-matched controls (Sommershof et al., 2009). Gene expression of the pro-inflammatory cytokine interleukin-6 is increased after menopause and andropause in elderly adults (Ershler & Keller, 2000) – and the concentration (Gill & Page, 2008) and spontaneous production (Gola et al., 2013) of interleukin-6 is enhanced in individuals with PTSD. C-reactive protein, which is a marker for low-grade inflammation, is elevated in women over the age of 60 years (Paik et al., 2012) – and also in individuals with PTSD (Spitzer et al., 2010). On a bio-molecular level, DNA damage increases with age (Humphreys et al., 2007) – and DNA damage, detected as DNA single-strand breaks, also accumulates in individuals with PTSD (Morath et al., submitted). Contrary to expectations, elevated levels of DNA damage are not related to impaired DNA repair, but rather seem to accelerate DNA repair capacity, since neither old people (Humphreys et al., 2007) nor individuals with PTSD show deficits in DNA repair capacity (Morath et al., submitted). Telomeres, which stabilize the end of chromosomes, gradually shortened by advancing age in many cell types (Frenck, Blackburn, & Shannon, 1998) – and telomere shortening is again also associated with childhood maltreatment (Price, Kao, Burgers, Carpenter, & Tyrka, 2012) and PTSD (O'Donovan et al., 2011).

N-Glycosylation is an enzymatic process that produces sugar chains covalently linked to macromolecules like proteins and lipids. *N*-Glycans are oligosaccharides covalently attached to protein at asparagine (Asn) residues by an *N*-glycosidic bond mediating numerous biological functions. In contrast to proteins, *N*-glycans are not controlled by the genome, but are secondary gene products and can be altered by environmental influences (Varki et al., 2009). Previous studies identified nine prominent *N*-glycan structures in human plasma, named peak 1 through peak 9 (Liu et al., 2007). The concentration of *N*-glycans in human plasma varies with age (Knezevic et al., 2010) and while *N*-glycan peak 1 (agalactosylated core-α-1,6-fucosylated biantennary; NGA2F) increases with age, *N*-glycan peak 6 (bigalactosylated core-α-1,6-fucosylated biantennary; NA2F) decreases (Vanhooren et al., 2007). The log ratio of these two *N*-glycans [log10 (peak1/peak6)], called GlycoAge Test, is a

biomarker for physiological aging and gives information beyond chronological age (Vanhooren et al., 2010). Interestingly, juvenile patients with Cockayne syndrome, a progeroid disease that is due to a genetic deficiency in transcription-coupled nucleotide excision repair (Hoeijmakers, 2009) show high values in the GlycoAge Test (Vanhooren et al., 2010).

We hypothesize that trauma exposure might lead to accelerated aging. We examined the *N*-glycosylation profile in the plasma of individuals with PTSD, of trauma-exposed individuals that were not diagnosed with PTSD, and of a low-stress control group. We hypothesized that individuals with PTSD would present higher values in the GlycoAge Test, compared to the low-stress control group. Furthermore we hypothesized that trauma-exposed individuals should be positioned between individuals with PTSD and low-stress controls.

4.3 Methods

Participants

We analyzed the *N*-glycosylation profile of 13 individuals with PTSD (DSM IV-TR; American Psychiatric Association, 2000) in comparison with a high-stress (trauma-exposed individuals: $n = 9$) and a low-stress ($n = 10$) control group. Individuals with PTSD were refugees living in Germany (4 from Africa, 1 Balkan, 8 Middle East and Afghanistan) with a history of severe war and torture experiences. Survivors of multiple traumata commonly also exhibit depressive symptoms. In individuals with PTSD the average scores on the Hamilton rating scale (Hamilton, 1960) ranged from 0 to 39, with 7 of the 13 patients reaching a threshold of >26 (Berrios & Bulbena, 1990).

Non-PTSD subjects were matched for ethnicity (6 from Africa, 4 Balkan, 9 Middle East and Afghanistan) and age (Table 4.1). Since non-PTSD subjects differed substantially in the number of traumatic experiences, we divided this group by median split into a high-stress (Trauma-exposed) group with substantial trauma exposure (traumatic load: $M = 1.18$, $SD = 0.63$) and a low-stress group without substantial trauma-exposure (traumatic load: $M = 0.23$, $SD = 0.09$). The traumatic load in individuals with PTSD was significantly higher (traumatic load: $M = 1.61$, $SD = 0.49$; $F_{(2,29)} = 25.88$; $p < .0001$). Traumatic load was specified by the proportion of specific war and torture experiences, assessed by the Vivo checklist of war,

detention, and torture events (Schauer et al., 2011a) and general traumatic event types, assessed by the Clinical Administered PTSD Scale (CAPS; Blake et al., 1995).

Table 4.1. *Mean and standard deviations of clinical characteristics, in n = 10 low-stress control subjects, n = 9 trauma-exposed individuals and n = 13 individuals with PTSD.*

Variables	**low-stress Controls** *M (SD)*	**Trauma-exposed** *M (SD)*	**PTSD** *M (SD)*	**statistics**	***p***
Age	30.20 (8.94)	38.67 (14.05)	34.62 (6.80)	$F_{(2,29)} = 1.73$	.19
CAPS	0.5 (1.58)	26.00 (23.54)	89.62 (18.20)	$F_{(2,29)} = 84.11$	<.0001
Event types (CAPS)	3.2 (1.69)	7.56 (3.36)	8.85 (1.99)	$F_{(2,29)} = 16.76$	<.0001
Traumatic load	0.23 (0.09)	1.18 (0.63)	1.61 (0.49)	$F_{(2,29)} = 25.88$	<.0001
HAM-D score	2.60 (6.13)	9.00 (12.16)	26.62 (10.53)	$F_{(2,29)} = 18.14$	<.0001

Abbreviations: CAPS (Clinical Administered PTSD Scale)**;** Statistics: *M* (mean), *SD* (standard deviation), all tests were calculated on an alpha level of 0.05 (two-sided).

Twelve subjects reported taking psychotropic medication: Eight individuals with PTSD (2 hypnotics, 5 antidepressants, 1 neuroleptics, 1 lithium), 3 trauma-exposed individuals (1 benzodiazepines, 2 antidepressants) and 1 control subject (hypnotics). To control for possible effects of medication, we compared the *N*-glycosylation profile of individuals with PTSD with (n = 7) and without medication (n = 6). Individuals with PTSD were recruited by the Center of Excellence for Psychotraumatology. Control subjects were recruited through advertisements, which were posted in town and university.

Exclusion criteria were co-morbid psychological disorders (other than Major Depression Episode [MDE]), acute infections, and chronic inflammatory diseases. MDE was not used as an exclusion criterion because the comorbidity of MDE in individuals with PTSD is high (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995) and MDE symptomatology overlaps with PTSD pathology in a wide range (*e.g.* diminished interest, restricted range of affect, difficulty with falling or staying asleep, lack of concentration).

Psycho-diagnostic interviews took place at the Center of Excellence for Psychotraumatology, University of Konstanz, Germany. Blood analyses were performed in the Laboratory for Molecular Toxicology, University of Konstanz, Germany and the Department for Molecular Biomedical Research, Ghent University, Belgium. The Ethics Committee of the University of

Konstanz approved the study. All study subjects signed an informed consent and received a remuneration of 30 €.

Psycho-diagnostic Interview

Trained clinical psychologists with a specialization in the field of trauma conducted psycho-diagnostic interviews. Whenever participants were not fluent in German or English, interviews were conducted with the help of trained interpreters. Traumatic events, PTSD diagnosis, and PTSD symptom severity were assessed by using the CAPS (Blake et al., 1995). Traumatic experiences were assessed by using the Vivo Checklist of War, Detention, and Torture Experiences (Schauer et al., 2011a). The Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960) was used for quantification of depressive symptoms. To exclude potential co-morbid psychiatric disorders, the Mini International Neuropsychiatric Interview (M.I.N.I.; Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, 1998) was applied.

Clinical characteristics

Groups did not differ with respect to age. Individuals with PTSD experienced significantly more different traumatic event types and showed higher traumatic load compared to low-stress controls. PTSD symptom severity (CAPS score) and depressive symptoms (HAM-D score) were highest in individuals with PTSD. Trauma-exposed individuals were between the two groups (Table 4.1).

Analysis of N-glycosylation

To standardize the time of blood collection, blood was always collected at 10 a.m. using Coagulation 9 NC Monovettes® (Sarstedt, Germany). Blood samples were coded to guarantee blinding of the laboratory staff. In a first step, blood plasma was isolated and quick-frozen at -80°C at the Laboratory for Molecular Toxicology, University of Konstanz. In a second step, frozen plasma samples were shipped on dry ice to the Department for Molecular Biomedical Research, Ghent University, Belgium for *N*-glycosylation analysis. *N*-Glycosylation was analyzed in 2 µl of plasma.

Nine *N*-glycan structures (peak 1 – peak 9), which were identified to be most prominent in previous studies,(Liu et al., 2007) and the GlycoAge Test [$\log_{10}$ (peak1/peak6)] (Vanhooren

et al., 2010), were measured using DNA sequencing equipment, Fluorophore Assisted Carbohydrate Electrophoresis (DSA-FACE) (Vanhooren, Laroy, Libert, & Chen, 2008).

Statistical Analysis

Statistical analyses were performed using R 2.11.0 (R Development Core Team, 2010), using an alpha level of .05. Group differences in clinical characteristics and in the *N*-glycosylation profile were analyzed using ANOVAs. Age, use of tobacco, psychotropic medication and gender were included separately as covariates into the model. The Akaike Information Criterion (AIC) was used for evaluating the appropriateness of models (Burnham & Anderson, 2002). The model without covariates was favored overall. In the case of significant effects, *t*-tests were calculated for post-hoc comparison. Residuals in all models were tested for deviations from the normal distribution. Residuals in the models of the GlycoAge Test, the *N*-glycan peak 2, the *N*-glycan peak 8 and the *N*-glycan peak 9 were not normally distributed; therefore these variables were re-analyzed using non-parametric statistics (Kruskal-Wallis test; for post-hoc comparison Mann-Whitney-U test). Correlations were analyzed using the Kendall Tau rank correlation.

4.4 Results

Individuals with PTSD and trauma-exposed individuals presented higher values in the GlycoAge Test compared to low-stress controls (χ^2 = 7.00; p = .03; Table 4.2). There were significant group differences between the low-stress control group and individuals with PTSD ($W = 25$; p = .01). Trauma-exposed individuals differed neither significantly from individuals with PTSD ($W = 70$; p = .47) nor from controls ($W = 22$; p = .06), but were positioned in-between these two groups. Interestingly, traumatic load was positively correlated with the GlycoAge Test (r = .41; p = .02, Figure 4.1).

Individuals with PTSD with psychotropic medication [n = 7; median = -0.33; range = -0.53 – (-0.23)] did not differ significantly in the GlycoAge Test (W = 22.0; p = .41) from individuals with PTSD without medication [n = 6; median = -0.51; range = -0.62 – (-0.34)].

Table 4.2. *N-Glycans in PTSD, trauma-exposed and low-stress control subjects.*

Variables	**low-stress controls** (*n* = 10) *M (SD)*	**Trauma-exposed** (*n* = 9) *M (SD)*	**PTSD** (*n* = 13) *M (SD)*	**statistics**	***p***
Peak 1	6.46 (2.72)	8.28 (2.35)	8.48 (2.03)	$F_{(2,29)} = 2.36$	.11
Peak 2 [a] *(median, range)*	0.79 (0.63 – 2.21)	1.23 (0.87 – 1.81)	1.32 (0.86 – 2.37)	$\chi^2 = 6.04$	.05
Peak 3	6.99 (1.78)	7.17 (1.88)	7.69 (2.20)	$F_{(2,29)} = 0.28$	.69
Peak 4	6.19 (1.43)	6.41 (1.31)	6.13 (1.42)	$F_{(2,29)} = 0.11$	.89
Peak 5	40.31 (5.24)	40.80 (5.37)	40.35 (4.72)	$F_{(2,29)} = 0.03$	.97
Peak 6	22.77 (1.78)	20.30 (2.87)	20.49 (3.35)	$F_{(2,29)} = 2.44$	.11
Peak 7	6.39 (1.58)	5.63 (1.26)	4.91 (0.73)	$F_{(2,29)} = 4.35$	.02
Peak 8 [a] *(median, range)*	6.75 (4.57 – 9.67)	5.94 (4.09 – 11.10)	5.91 (4.78 – 11.77)	$\chi^2 = 0.52$	.77
Peak 9 [a] *(median, range)*	1.48 (0.71 – 2.93)	2.21 (1.49 – 5.26)	1.31 (0.51 – 4.06)	$\chi^2 = 2.47$	.29
GlycoAge Test [a] *(median, range)*	-0.54 (-0.99 – -0.33)	-0.38 (-0.56 – -0.18)	-0.36 (-0.62 – -0.23)	$\chi^2 = 7.00$	.03

Statistics: *M* (mean), *SD* (standard deviation), χ^2 (Kruskal-Wallis test), all tests were calculated on an alpha level of 0.05 (two-sided).[a] Data were not normally distributed.

In addition to the effect on GlycoAge Test, also in the *N*-glycan peak 2 individuals with PTSD presented significantly higher values ($\chi^2 = 6.04$; $p = .05$). In post-hoc tests, individuals with PTSD ($W = 30$; $p = .03$) and trauma-exposed individuals ($W = 20$; $p = .04$) differed significantly from low-stress controls. There were no significant differences between PTSD and trauma-exposed individuals ($W = 65.5$; $p = .66$; Table 4.2 and Figure 4.2a).

Furthermore, individuals with PTSD, surprisingly, presented reduced values in *N*-glycan peak 7 ($F_{(2,29)} = 4.35$; $p = .02$). Individuals with PTSD differed significantly from the low-stress control group ($t_{(11.9)} = 2.75$; $p = .02$), and trauma-exposed individuals were positioned in-between, but did not differ significantly from the PTSD or the low-stress control group (Table 4.2 and Figure 4.2b).

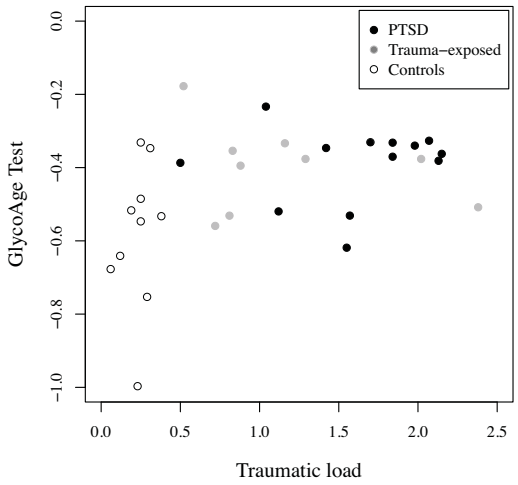

Figure 4.1. Scatterplot between the GlycoAge Test and Traumatic load in n = 10 low-stress controls, n = 9 trauma-exposed and n = 13 PTSD subjects. Higher values in the GlycoAge Test were positively correlated with Traumatic load.

However, since we did not have *a priori* hypotheses for the *N*-glycans peak 2 and peak 7, but rather made explorative analysis, we corrected these results for multiple comparisons. After a stepwise Holm correction (Holm, 1979), group differences in the *N*-glycan peak 2 and in the *N*-glycan peak 7 did not remain significant.

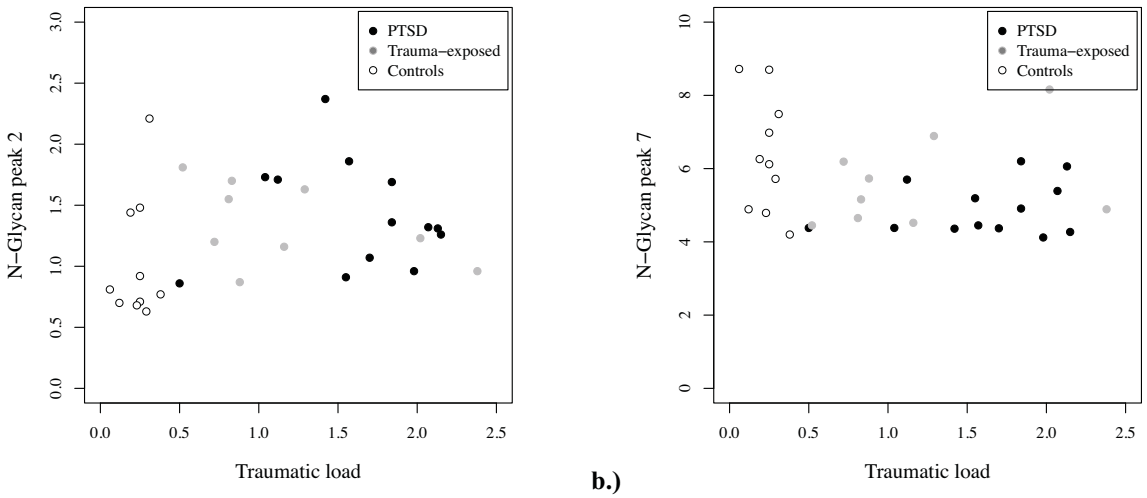

Figure 4.2. Scatterplots in the *N*-glycan peak 2 and the *N*-glycan peak 7 from n = 10 low-stress controls, n = 9 trauma-exposed and n = 13 PTSD subjects. **(a.)** Individuals with PTSD demonstrated significant higher values in the *N*-glycan peak 2, compared to low-stress controls. Trauma-exposed individuals were positioned between PTSD and the low-stress control group. **(b)** Individuals with PTSD demonstrated significant lower values in the *N*-glycan peak 7, compared to low-stress controls. Trauma-exposed individuals were positioned between PTSD and the low-stress control group.

The *N*-glycosylation of peak 1,3,4,5,6,8 and 9 did not differ significantly between individuals with PTSD and low- or high-stress controls (Table 4.2).

4.5 Discussion

Individuals with PTSD and trauma-exposed individuals showed increased values in the GlycoAge Test, which is a biomarker for physiological aging (Vanhooren et al., 2010). In the general population, values in the GlycoAge Test are stable until 40 years of age and then increase continuously, with the highest values being observed at the age of 90 to 99 years (Vanhooren et al., 2010). The GlycoAge Test profile in individuals with PTSD and trauma-exposed individuals with a mean age of 35 years is similar to the GlycoAge Test profile in people with a mean age of about 50 years, in a Belgian population (Vanhooren et al., 2010). This shift in *N*-glycosylation strongly indicates that traumatic stress may accelerate the process of physiological aging.

Given that there are interactive effects between stress, age, and immune functions (Graham et al., 2006), it is noteworthy that the *N*-glycosylation profile in older people is associated with a state of IgG-mediated low-grade inflammation (Dall'olio et al., 2012). Low-grade inflammation and changes in the immunoglobulin regulation, in turn, have been reported in individuals with PTSD as well (Boscarino, 2004; Gill et al., 2009). Alterations in *N*-glycosylation may therefore not only be a marker of physiological aging, but might actually contribute to the pathogenesis of aging itself (Dall'olio et al., 2012) via inflammation processes, which can be caused by traumatic stress (O'Donovan, Neylan, Metzler, & Cohen, 2012).

Furthermore, individuals with PTSD presented higher values in the *N*-glycan peak 2, although these results did not remain significant after statistical corrections. The *N*-glycans peak 1 and peak 2 are reported to increase gradually with age, while the *N*-glycan peak 6 is reported to decrease with age (Vanhooren et al., 2007). We see the same picture in individuals with PTSD, even though group differences in the *N*-glycan peak 1 and peak 6 were not statistically significant (Table 4.2).

Interestingly, we found a reduction in the *N*-glycan peak 7 in individuals with PTSD, although this effect did not withstand statistical corrections. However, we report this effect because this

finding might be interesting for future studies. To the best of our knowledge, there has been only one study on the *N*-glycan peak 7 so far, which was found to be decreased in patients with hepatocellular carcinoma compared to liver cirrhosis patients, with a negative correlation between the *N*-glycan peak 7 and tumor development (Liu et al., 2007). At the present state of knowledge it is difficult to pinpoint a mechanistic link that could explain the concordant *N*-glycan peak 7 effects in these two quite diverse disease conditions.

In conclusion, we present evidence that traumatic stress leads to an accelerated process of physiological aging, as revealed by a shift in the *N*-glycosylation profile that is typical of persons with higher age, which is possibly mediated by a state of low-grade inflammation.

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5 Conclusion and Future Directions

In light of the numerous conflicts settings throughout the world, and the subsequent high prevalence of PTSD observed as a consequence in post-conflict regions (Neuner & Elbert, 2007), it is imperative that we gain a better understanding of the impact of traumatic stress for mental and physical health. Study 1 and 2 examined potential underlying biological mechanisms for the high risk of infectious diseases, autoimmune diseases and cancer development in individuals with PTSD. Specifically, they suggested that these effects are potentially attributable to altered T lymphocyte distribution and enhanced DNA damage. Remarkably, given the immunological and molecular alterations in individuals with PTSD, psychotherapeutic treatment with NET not only alleviated mental suffering, but was even effective on an immunological and molecular level.

The shift in the proportion of naïve and memory cytotoxic $CD8^+$ T cells in individuals with PTSD is characteristic of an aging immune system, and does not normally start until the sixth decade of life (Dorshkind et al., 2009). With a reduction in naïve $CD8^+$ T cells, the ability for an effective immune response against pathogens is limited and the risk for infectious diseases increases (Fagnoni et al., 2000). In the elderly, an involution of the thymus has been identified as a contributing factor for the reduced proportion of naïve $CD8^+$ T lymphocytes (Dorshkind et al., 2009). However, until now very little is known about the underlying biological mechanisms responsible for the reduced proportion of naïve $CD8^+$ and regulatory T cells in individuals with PTSD. It has been postulated that deregulations of the endocrine system influence cellular immunity (Elenkov & Chrousos, 2000). However, since the findings of cortisol in individuals with PTSD are highly inconsistent, including decreased levels of cortisol in the saliva and in the blood (Bauer et al., 2010; Heim & Nemeroff, 2009; Morris et al., 2012; Pace & Heim, 2011), but increased levels of cortisol in the cerebrospinal fluid (Baker et al., 2005) and in the hair (Steudte et al., 2011), further research is needed for a better understanding of the interrelation between the endocrine and the immune system in individuals with PTSD. Moreover, even though catecholamines show in general suppressing effects upon cellular immunity, they may on the other hand also stimulate the production of pro-inflammatory cytokines such as IL-1, TNF-α and IL-8 in local immune responses (Elenkov & Chrousos, 2000). Most importantly, the interrelation between the endocrine and the immune system is very complex and it is unlikely that they are related by only “one

pathway". It can be hypothesize that there is in fact an expansive infiltration of the immune system by stress hormones in individuals with PTSD.

The reduced proportion of regulatory T cells has been associated with an increased risk for autoimmune diseases in individuals with PTSD (Sommershof et al., 2009). Regulatory T cells show strong immunomodulating effects and an increase in the proportion of regulatory T cells has been associated with a decline in pro-inflammatory cytokines (Himmerich et al., 2010). Therefore, since the proportion of regulatory T cells rose one-year after the end of treatment with NET, it is possible that psychotherapy plays a role in diminishing the pro-inflammatory cytokines in individuals with PTSD via an increase in regulatory T cells. Pro-inflammatory cytokines are part of chronic inflammation that is associated with a number of age-related diseases such as diabetes, cardiovascular diseases, autoimmune diseases and cancer (Khansari et al., 2009; Marx, 2004). Moreover, pro-inflammatory cytokines produce ROS and induce DNA damage causing cancer development (Khansari et al., 2009) and thus, the decline of pro-inflammatory cytokines may play a major role in the prevention of physical diseases in individuals with PTSD. Himmerich et al., (2010) demonstrated that the administration of antidepressive medication in depressive patients initiated an increase in regulatory T cells that was associated with a reduction in the pro-inflammatory cytokines IL-1β and IL-6, both of which are also enhanced in individuals with PTSD (Gola et al., 2013). In this way, future studies are needed to focus on the interrelation between regulatory T cells and pro-inflammatory cytokines, and the distinct effects of antidepressive medication and psychotherapeutic treatment on regulatory T cell proliferation in individuals with PTSD.

Furthermore, it is possible that there is also an interrelation between the altered proportions of T lymphocyte distribution and the heightened DNA damage in individuals with PTSD. However, nothing is known about the specific toxic effects of catecholamines, cortisol and pro-inflammatory cytokines on the different kinds of T lymphocyte subsets, such as naïve and memory $CD8^+$ or $CD4^+$ and regulatory T cells. Future studies should investigate whether naïve $CD8^+$ and regulatory T cells show accelerated amounts of DNA damage leading to apoptosis and thereby causing a decline in the proportion of naïve $CD8^+$ and regulatory T cells in individuals with PTSD.

Most importantly, although we know that psychotherapeutic treatment has an effect upon the immune system, it is still not clear precisely how this works. This needs to be investigated in

future research. However, we can still postulate that the improvement in mental health and the release of psychological stress may result in a stabilization of the endocrine system that may provide a normalization of the immune system and a diminishment of toxicity on the DNA.

Moreover, all of the three studies provided evidence for a dose-response effect of traumatic load on immunological and molecular alterations. Since a dose-response effect of traumatic load also occurred in the shift of N-glycosylation, the accumulation of traumatic stress might promote the process of physiological aging and in this way might cause age-dependent biological alterations, such as the shift in cytotoxic T cell distribution and the increase in DNA damage. Since individuals with PTSD not only present a state of premature biological aging, but also report an older subjective age (Solomon, Helvitz, & Zerach, 2009b), it would be of great interest to investigate biological parallels as well as biological differences between "stress-induced" and "normal" physiological aging in individuals with PTSD compared to old people.

In conclusion, the findings suggest that psychotherapeutic treatment is not only effective in improving mental health, but also promotes a normalization of immunological and molecular alterations and therefore may contribute to an improvement in physical health in individuals with PTSD.

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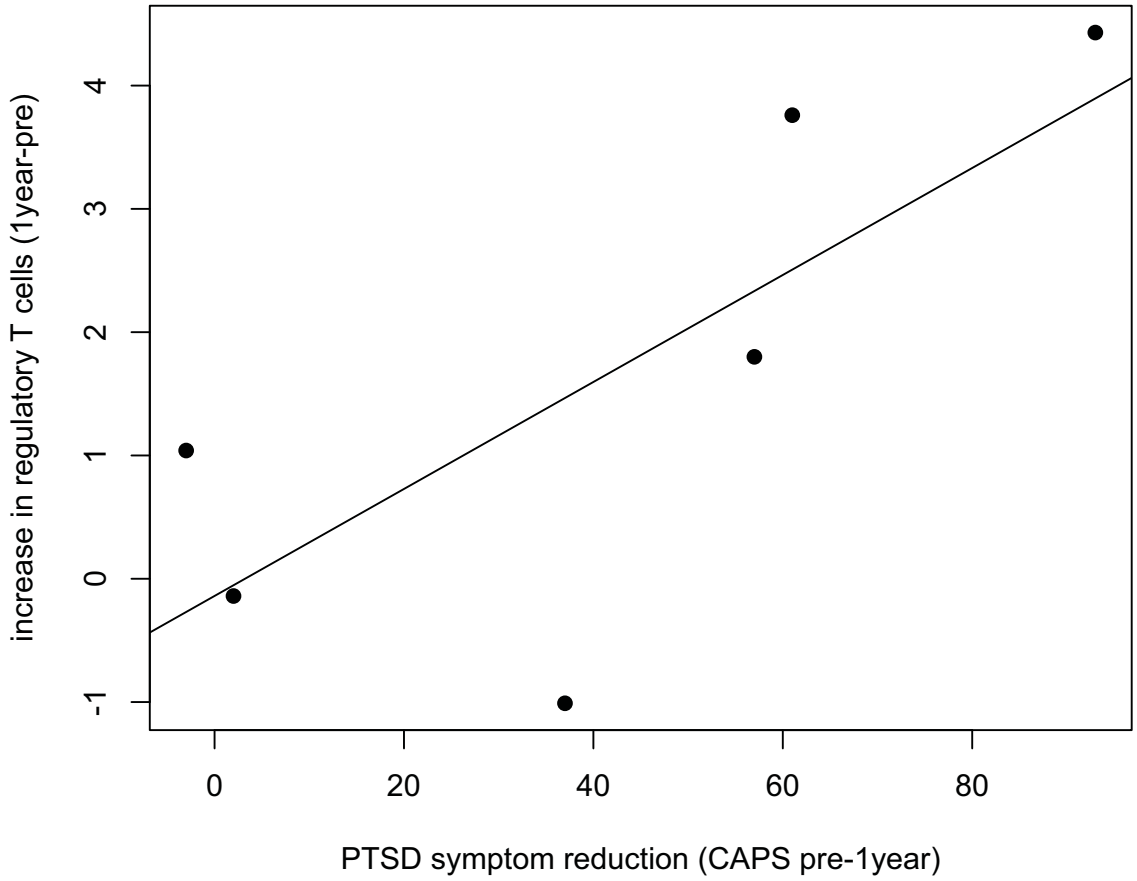
7 Appendix

7.1 Supplementary Figures Study A

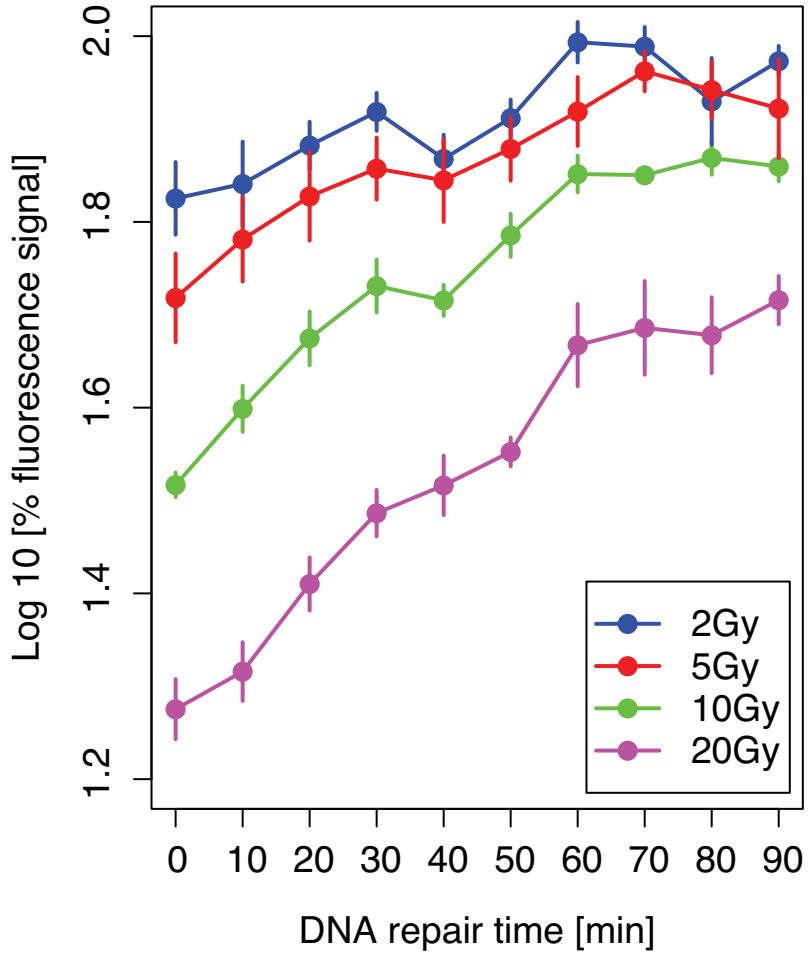
7.1.1 Supplementary Figure 2.1



Supplementary Figure 2.1. Correlation between the PTSD symptom reduction from t_0 (pre therapy) to t_2 (one-year follow-up) and the increase in regulatory T cells from t_0 to t_2.

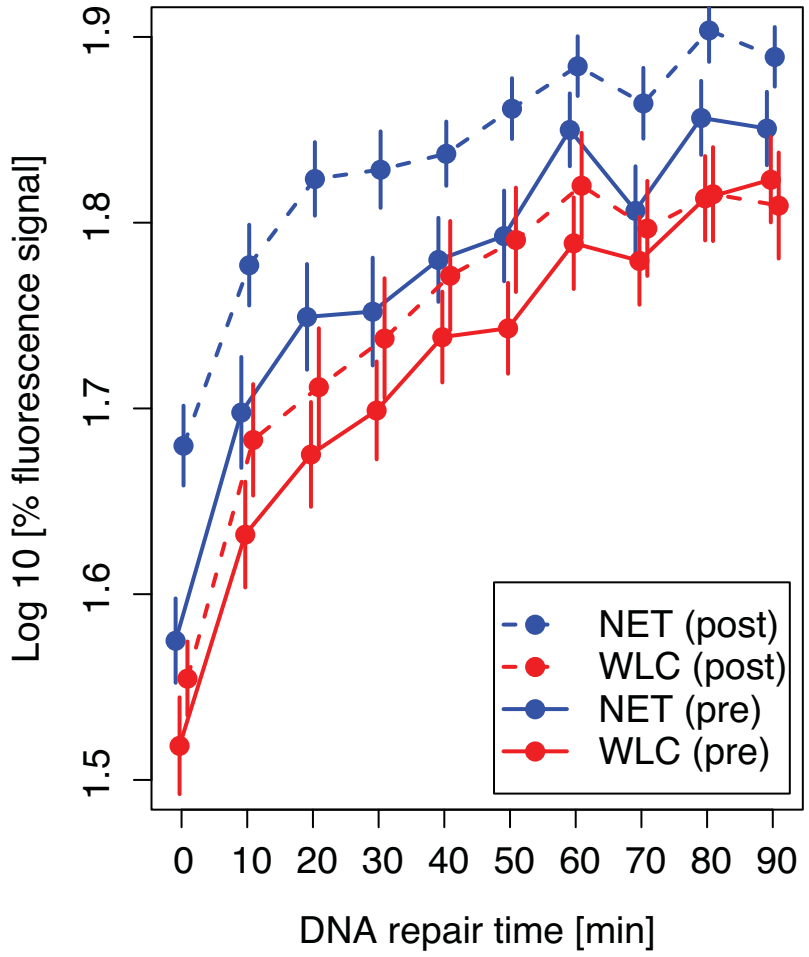
7.2 Supplementary Figures Study B

7.2.1 Supplementary Figure 3.1



Supplementary Figure 3.1. DNA repair from PBMCs of 4 healthy donors, x-irradiated with 2, 5, 10 or 20 Gy respectively. PBMCs from four healthy volunteers were isolated as described before. Following x-irradiation (2, 5, 10 or 20 Gy) on ice, PBMCs were incubated at 37°C for the time periods indicated in order to allow DNA repair. Fluorescence intensity is expressed as the percentage of total SybrGreen fluorescence, *i.e.* the fluorescence intensity obtained in the absence of any DNA unwinding in parallel samples. Mixed model analysis showed a significant interaction between Group and Time ($F_{(27,108)} = 3.38$; $p < .0001$). Error bars represent standard errors.

7.2.2 Supplementary Figure 3.2



Supplementary Figure 3.2. DNA repair pre-treatment and 4-month post-treatment in the NET and the WLC group. Time course of repair of DNA strand breaks after x-irradiation *ex vivo* of PBMCs from individuals with PTSD either receiving psychotherapy (NET) or being on the waiting list (WLC), before ("pre") and after ("post") therapy or waiting, respectively. Mixed model analysis showed significant interaction between group and the two measurement time points pre therapy and post therapy ($F_{(1.525)} = 6.45$; $p = .01$). Error bars represent standard errors. $N = 38$ individuals with PTSD (19 NET and 19 WLC).