Acute War Stress in Ukrainian Refugees: Neurobiological and Psychological Impact

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Abstract

Purpose: We raised the question whether the neurobiological and psychological impact of war stress could be observed in Ukrainian refugees during an ongoing war. Ukrainian refugee women (UG, n=43) from the war-torn regions were investigated and were compared to Czech women control group without stress (CG, n=21). Men were not allowed to leave Ukraine due to the war conditions. Method: Psychological testing was performed using tests available in Ukrainian language: Posttraumatic stress disorder Check List; Zung Self- Rating Depression Scale; The State-Trait Anxiety Inventory; Test of Intellectual Potential and Digit Span - WAIS III. Magnetic Resonance Imaging included voxel-based morphometry, functional MR, seed-based connectivity. Finding: UG scored significantly higher than CG in stress (p<0.001), depression (p=0.019) and anxiety (p=0.001) and lower than CG in an abstract reasoning task (p<0.030). The groups did not differ significantly in memory and intellectual tasks. MRI showed an enlargement of the posterior and central parts of the thalamus that were related to the frontal orbital gyrus and insula indicating a connectivity with limbic system regions. Conclusion: The data show the impact of acute and ongoing war-related stress on psychological features as well as on the thalamus and connectivity of stress-related cortical areas. Acute and ongoing war stress had a significant neurobiological impact on refugees from war-torn Ukraine.

Introduction

The conflict between Russia and Ukraine started with the annexation of Donbas, Luhansk, and Crimea in 2014; the actual war started in February 2022. Fighting has occurred mostly in the eastern and southern part of the country, but bombing has been widespread in most Ukrainian regions. There have been increases in civilian fatalities and injuries since the Russian invasion of Ukraine on February 24, 2022, and a long-term impact of stress on the health of generations of Ukrainians is expected [1]. Many Ukrainian citizens have left the country. Based on 43,571 interviews with refugees from Ukraine conducted between May and November 2022, most of these refugees are women with higher levels of education (46% with university or higher degrees) and with diverse professional experience (UNHCR [2]). Between March 28 and April 4, 2022, the international research agency 4Service, a collective member of the Ukrainian Association of Contact Centers (UACC [3]), conducted a pan-European sociological survey of the mood of Ukrainians who were forced to leave their homes because of the war. The study covered 3,355 respondents in 36 European countries, including Poland, Germany, Czech Republic, Slovakia,
Hungary, Romania, Austria, Italy, Spain, and Moldova. According to the survey, most refugees are women (92%). Moreover, 60.4% of the refugees left to save their children, and 19% left with their parents. In 97% of respondents, relatives and friends remained in Ukraine [3]. More than 7.8 million refugees from Ukraine were recorded across Europe; 472,000 Ukrainian refugees were registered in the Czech Republic. The refugees received help from the Czech government and population; however, they remained under stress due to the continuation of the war and in most cases because family members stayed in Ukraine, and the husbands of the refugees often enlisted in the military.

We focused on the neurobiological and psychological impact of war-related stress in Ukrainian women. We investigated only women as most men were not allowed to leave the country due to the war conditions. Our study explored the impact of acute war stress on cerebral structure and connectivity imaging (Magnetic Resonance Imaging - MRI) and mental health during the ongoing conflict. We analyzed the relationship between war exposure, psychological symptoms, brain morphometry and connectivity in Ukrainian refugees. In the interview we explored refugees’ experience with the war, including exposure to traumatic events. The study is based on our previous research on the lifelong impact of extreme stress of Holocaust survivors. Psychological testing and MRI showed a lifelong neurobiological effect of extreme stress on brain structures and connectivity in three generation and of Holocaust survivors 70-75 years after the war [4-9].

In this study we questioned whether the neurobiological (MRI) and psychological impact of acute war-related stress could be observed in refugees during an ongoing war. We investigated Ukrainian women who had left Ukraine during wartime and who were living in the Czech Republic as war refugees.

Methods

Participants and procedure

The study was conducted at the Central European Institute of Technology (CEITEC), Centre for Neuroscience at Masaryk University in Brno. Ukrainian participants were recruited in cooperation with Masaryk University, and with the help of Ukrainian psychologists working with our team, and through social networks.

We compared two groups: Ukrainian refugees (UG) and Czech controls (CG). All participants were women. All but one respondent in the UG was born in Ukraine, and they all had Ukrainian citizenship. The participants of the Ukrainian group were civilians who had lived in different regions of Ukraine with an impact of war (Donetsk, Dniprop, Zaporizhzhia, Kyiv, Poltava, Sumy, Kharkiv, and Kherson regions) and who experienced psychological trauma from the war in Ukraine. They were hidden in basements during air raids and had lived in occupied cities or under systematic bombing, without water, electricity, or communication. In the interview, all UG respondents reported having had a personal experience of the war in Ukraine. The Ukrainian refugees were under war event between several weeks and 2 months. After that they left Ukraine and arrived at Czech Republic or other countries. In the UG group, 19% had experienced bombing, 26% had experienced shooting, 79% had seen people dead or wounded, and 88% had direct experience of fighting. Based on the exposure to the described traumatic events (bombing, shooting, fighting, etc), we presumed that they could be probably psychologically and biologically affected. Based on interview with all participants it is evident that all UG were stressed.

The median age of the Ukrainian refugee group (UG) (n=43) was 38 years (age range: 18 to 60). Participants who did not meet our quality standards were excluded from the study. Czech control group (CG) participants (n=21) were gender-matched Czech civilian volunteers with a median age of 35 years (age range: 21 to 58). The inviting of CG was published in press and in Masaryk University. None of the CG had participated in any military action and they were not under direct life-threatening danger. The age difference between UG and CG was not significant.

The inclusion criteria for the UG were female gender, residency in Ukraine during the war (February 2022 onwards), being a refugee, obtaining refugee status in the Czech Republic, and willingness to participate in the study. Only healthy Ukrainian participants were accepted in this study. They were interviewed by Ukrainian psychologists before the testing and their health status was considered by neurologist. The UG had no psychiatric disease and did not report any acute health problems.

The exclusion criterion for the UG and CG was any kind of severe brain impairment (brain injury, tumors, neurodegenerative diseases, cognitive decline, psychiatric drug treatment or alcoholism). Contraindications for MRI were metal implants, pacemakers, and claustrophobia.

The research was approved by the ethics committee at Masaryk University and informed written consent was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

We explored refugees’ experience in the war with exposure to traumatic events. We analysed the relationship between war exposure, psychological symptoms, brain morphometry and connectivity in Ukrainian refugees compared to Czech controls.

Psychological testing

The interview and psychological testing were performed by two Ukrainian psychologists (Sofia Berezka and Tetyana Evmenova – co-authors) who were also war refugees. They were integrated into Czech society or Masaryk University and coordinated with
an experienced Czech psychologist (Marek Preiss) who had participated in our previous studies and published papers based on our study on the impact of the Holocaust on survivors and two generations of descendants [5,6]. A Ukrainian-language version of the test was used [10].


The PTSD Check List – Civilian Version (PCL-5) is a 17-item measure of the DSM-5 symptoms of PTSD derived from the PCL-Military Version (PCL-M). The PCL-5 is a self-administered screening instrument that asks respondents to consider a “list of problems and complaints that people sometimes have in response to stressful experiences” and to indicate how much they “have been bothered by each problem in the past month” on a scale of 1 (not at all) to 5 (extremely). A higher score is associated with a greater level of PTSD symptoms. The lower bound of internal consistency as measured by Cronbach’s alpha reached .874 for PCL-5. A cut-off raw score is 3821. A Ukrainian-language version of the instrument was used [10].

• Zung Self-Rating Depression Scale [16-18] (SDS)

The Zung Self-Rating Depression Scale is a short, self-administered survey to quantify the depressed status of a patient in the previous weeks. There are 20 items on the scale that rate the four common characteristics of depression: the pervasive effect, the physiological equivalents, other disturbances, and psychomotor activities. Each question is scored on a scale of 1 to 4 from “none or a little of the time,” “some of the time,” “a good part of the time,” and “most or all of the time.” A higher score is associated with a greater level of depressive symptoms. The total score ranges from 25-100. An alpha coefficient of 0.82 was reported by DeJonghe & Baneke [17] and by Leung et al [18].

The State-Trait Anxiety Inventory [19] (STAI)

The STAI is a commonly used measure of trait and state anxiety. Trait anxiety describes anxiety as a part of the personality (stable and persistent) and/or way of seeing the world. State anxiety describes anxiety that occurs in response to stressful situations. The STAI consists of 40 self-report items on a 4-point Likert scale. A higher score is associated with a greater level of anxiety symptoms. The total score ranges from 20 to 80. Internal consistency coefficients for the scale have ranged from .86 to .95; test-retest reliability coefficients have ranged from .65 to .75 over a 2-month interval. A Ukrainian-language version of the inventory was used [10].

• Abstract reasoning task - Test of intellectual potential (TIP) [20,21]

One of four subtests, the Test of Intellectual Potential contains 29 picture puzzles with six possible solutions of which only one is correct. The test provides a relatively language-independent measure of abstract reasoning. A higher score is associated with a higher level of abstract reasoning. The total score is calculated as the sum of the correct solutions in 12 minutes. The total raw score was used.

• Verbal memory task - Digit Span from WAIS-III [20]

A digit-span task is used to measure verbal short-term memory and working memory’s number storage capacity and the capacity of attention/concentration. The task requires subjects to repeat series of digits of increasing length. Digit span forward is a measure of simple attention, and most healthy individuals perform within the “seven plus or minus two” span of apprehension. Digit Span Forward and Digit Span Backward tasks were used, and the overall score was calculated.

Interview

Participants were asked about demographic data, information about close people, events preceding the war, their experience with the war in Ukraine, the effect of the war, psychological difficulties, the most stressful traumatic events, the experience of emigrating to the Czech Republic, their health and medical conditions, and the overall assessment of their own life in the professional and personal sphere. The interviews lasted about 60 to 90 minutes. The Ukrainian women experienced terrible psycho-traumatic events, such as being under fire for several days or weeks or living in occupied cities (these cities were later liberated by the Ukrainian army). They were often cut off from communication, without access to food and water, and had seen their homes burn or lost a loved one (parent, spouse, close friend). During the interviews, 74% of the interviewed women said that the war had changed them, making them more emotionally unstable and anxious. The refugees were still under stress because many of them have relatives in Ukraine, including their parents and their husbands, who are at risk or who are actively involved in the military operations. The Ukrainian refugee group in our study experienced constant anxiety and fear that a loved one could die at any moment.

Statistics

Group differences in psychological parameters PCL-5, abstract reasoning task, verbal memory task, STAI – state anxiety, STAI - trait anxiety, and SDS were assessed using a two-sample t-test; the effect of age and education was corrected using general linear model.

Neuroimaging

MR examinations were performed on a Siemens Prisma 3T scanner using a 64-channel head coil.

Morphometry

The MRI protocol for voxel-based morphometry included 3D T1-weighted magnetization prepared rapid gradient echo (MPRAGE)
sequence with TR = 2.3 s, TE = 2.33 ms, TI = 0.9 s, flip angle = 8, isometric voxel size 1 mm in FOV 224 × 224 mm and 240 slices. Anatomical MRI data were analysed using SPM12 (www.fil.ion.ucl.ac.uk) and CAT12 toolbox (www.neuro.uni-jena.de/cat) running in Matlab R2020a.

Individual data were adjusted for spatial inhomogeneity with an intensity normalization filter and then denoised with the Non-Local Means (SANLM) denoising filter. High resolution data were then segmented into gray matter (GM) using the SPM Tissue Probability Map (TPM) and registered into common MNI space using shooting template IXI555_MNI152_GS. Finally, spatially normalized, and modulated GM maps were smoothed with a 6 mm FWHM isotropic Gaussian kernel.

Group statistics for stress effects were calculated with a second-level model using SPM12. The modulated GM images were multiplicatively corrected with total intracranial volume and then analysed. A two-sample t-test comparison of gray matter volume files between UG and the CG groups was performed; age was included as a nuisance variable. Group-level results were evaluated with cluster-level inference for non-stationary conditions with family-wise error (FWE) correction at p (FWE) < 0.05 with initial cut-off threshold of p (uncorrected) = 0.005.

**Functional MRI data**

The functional protocol used a multi-echo multiband T2 echoplanar imaging sequence with TR = 0.85 s, TEs = (15, 36, and 58) ms, flip angle = 25, isometric voxel size 2.5 mm in FOV 200 × 200 mm, 60 slices, and 700 scans.

Data were processed in SPM12, accompanied with in-house Matlab scripts. Preprocessing consisted of realign (motion correction), CNR-weighted combination of individual echoes [22], RETROICOR procedure to suppress physiological artifacts (resting state only), co-registration of anatomical scans to BOLD data, spatial normalization using MNI template, and spatial smoothing using a Gaussian kernel with FWHM of 6 mm. Subsequently, very low frequencies were removed from BOLD data using a high-pass filter with a cut-off of 128 s; other nuisance effects were regressed out of BOLD data, specifically white matter and CSF signals, and 24 movement parameters obtained from the realign procedure.

**Seed-based connectivity**

In the resting-state imaging, seed-based connectivity maps from the left and right thalamus (based on morphological data showing the impact on the thalamus) were analysed. Individual connectivity was calculated based on correlations between the ROI signal (first principal component) and all other voxels in the brain. Group statistics for stress effects were calculated with a second-level model using SPM12. A two-sample t-test comparison of the individual connectivity maps of the UG and CG was performed; age was included as a nuisance variable. Group-level results were evaluated with cluster-level inference for non-stationary conditions [13] with family-wise error (FWE) correction at p < 0.05 with initial cut-off threshold of p (uncorrected) = 0.005.

**Data quality**

Data from all participants were manually checked for artifacts and pathology. Participants who did not meet our quality standards were excluded from the study. Furthermore, we automatically checked data for spatial abnormalities (such as dropouts, failures in normalization) with the Mask Explorer tool [23] and for the presence of excessive movement using frame wise displacement (FD) with the criterion of FD<1.5mm in any scan. Scans with FD>1.5mm were excluded from the analysis.

**Results**

**Psychological testing**

Psychological testing was performed in Ukrainian language in two Ukrainian psychologists working in our team. UG scored significantly higher than CG in PCL-5 [13,15] (t=3.907, df=61, p<0.001), in STAI – trait anxiety (t=3.466, df=61, p=0.001), and in depression (t=2.402, df=61, p=0.019). UG scored significantly lower than CG in an abstract reasoning task (t=-2.216, df=61, p=0.030). The groups did not differ significantly in a verbal memory task (t=-1.888, df=61, p=0.064), or in STAI – state anxiety (t=-0.928, df=61, p=0.357), 0.928, df=61, p=0.357) [11,12, 16-21]. (Table 1).
Table 1: Differences between Ukrainian refugees and control group (corrected for age and education).

### Neuroimaging

#### Morphometry

We compared gray matter volume in the UG with that of the CG. Both the right and left thalamus were significantly larger in the UG than in the CG (Figure 1). The largest changes were in the posterior and central part of the thalamus ([18 -25 12] mm, 12 385 mm³, p(corrected) = 0.001); by contrast, the anterior part was not affected.

![Figure 1: Slice view of two-sample t-test comparing gray matter volume between the UG and the CG. The UG had a larger volume of the posterior and central thalamus. A small decrease in volume in the right parietal lobe was also observed.](image-url)
Seed-based connectivity. We found stronger significant connectivity between the left thalamus and right frontal orbital gyrus and right insula ([38 23 -15] mm, 1 531 mm³) in the UG as compared to the CG (Figure 2). Seed connectivity from the right thalamus was not statistically significant.

Figure 2: Slice view of two-sample t-test comparing left thalamus seed connectivity between the UG and the CG. Connectivity is significantly stronger in the UG in the right frontal orbital gyrus and right insula.

Discussion
The psychological and neurobiological impact of acute and ongoing war stress was the target of this study. We questioned whether acute war stress impaired the psychological impact and the brain in healthy Ukrainian people who survived the war as compared to a Czech control group (CG) without stress. The study analysed the impact of recent acute and ongoing war stress on Ukrainian women who left Ukraine during the invasion that started in February 2022. Based on interview all of them experienced severe psychological trauma (bombing, hostility raids, occupied cities, etc.); leaving the country was also dangerous, with serious risks. The difference between acute and chronic trauma stress in Ukrainian refugees is difficult to distinguish. Acute trauma was certain during their presence in Ukraine during the war; however, even after they came to the Czech Republic, the war in their home country continues. The refugees received support from the Czech government and population; however, they all still experience stress as their family members and friends remained in the war-torn country. Some of their family members or friends were killed. In these stressed groups, there are people who lost a loved one (parent, spouse, close friend), who were in the war, who saw their homes burn, or who were under bombardment in the Ukrainian cities. Based on the interviews, it was evident that all Ukrainian refugees were stressed by the ongoing war. During the interviews, 74% of the UG said that the war had changed them by making them more emotionally unstable. The psychological tests showed that Ukrainian women experienced significantly more traumatic stress symptoms, depression, and anxiety than the control group. Higher levels of depression might accompany traumatic stress. The psychological data of the Ukrainian refugees were compared with data from a Czech control group who lived without war or extreme stress. The stress, anxiety and depression difference between Ukrainian refugees and Czech control group was significant. The first paper on Ukrainian stress was already published [24].

In the Magnetic Resonance Imaging (MRI) study, we performed a structural imaging of the brain using voxel-based morphometry, functional data processing and seed-based connectivity [13,14,22,23]. Our data show that acute and ongoing stress leads to the modification of the thalamus and of its connectivity to stress-related cortical areas (Figure 1, Figure 2). An enlargement of the bilateral posterior and central part of the thalamus was observed. A recent study noted abnormalities in the thalamus in patients with post-traumatic stress disorder (PTSD) [13]. The thalamic dysfunction in patients with PTSD may be related to the disorder’s psychopathology [19,25-31]. The thalamus modulates activity in the limbic circuitry. The right thalamus connectivity was present...
but non-significant with limbic system however the left thalamus was a seed with significant connectivity with the right frontal orbital gyrus and insula. Both structures are clearly involved in stress. This connectivity confirms the involvement of the thalamus in stress-related circuitry. Connectivity with limbic circuitry was visible also in our Holocaust survivor research, 70 years after the extreme stress [4,32].

The mechanisms affecting the human brain through traumatic stress are not fully known. Posttraumatic stress disorder is not a single homogeneous disorder. It is based on various subcategories that may lead to different presentations and pathophysiological mechanisms [26]. The stress may lead to autonomic nervous system dysfunction, altered concentrations of stress hormones, or noradrenergic hyper-reactivity linked with altered function of the hypothalamic-pituitary adrenal axis. The hypersensitivity of glucocorticoid receptors is modified by levels of cortisol [27,28]. Evidence from previous studies suggest that traumatic stress affects the brain by altering the connections between neurons. Acute stress could promote the formation of new synapses or increase the existing synapses [26]; this might explain the acute enlargement of the thalamus in our study.

Psychological modifications [29] have been reported in several other stress-related disorders [26]. The involvement of the thalamus in anxiety has also been observed, in association with changes in the thalamic-limbic circuit [30]. Several factors may activate this circuitry; however, it appears that the fear and stress that was present in the Ukrainian refugees had the inputs to the thalamus [19] and limbic circuitry. Processing of the thalamic-limbic circuitry could contribute to multiple aspects of posttraumatic psychopathology [26].

In a previous study, we aimed to assess the lifelong impact of extreme stress on people who survived the Holocaust. We researched Holocaust survivors 70 to 75 years after the extreme stress of murder and war [4-8,31] and their two generation descendants. We were able to show a neurobiological impact on the brain structures in survivors and MRI connectivity differences compared with control groups in the second and third generations [5,6]. Areas with reduced gray matter correspond to the map of the impact of stress on the brain structure: bilateral insula, anterior cingulate, the sub genu cingulate/orbitofrontal cortex, temporal pole, prefrontal cortex, and angular gyrus, however. The thalamus was not significantly involved [4]. It is possible that in the future, the changes in the brains of Ukrainian refugees could be similar; however, the conditions are different as the Ukrainian refugees are being tested while suffering acute stress.

Acute war stress was studied also in Dutch soldiers before and after deployment to a combat zone in Afghanistan [33,34]. It was found that combat stress increased amygdala and insula reactivity to biologically salient stimuli across the group of combat exposed individuals. PTSD was not present in this group. The amygdala increase due to a dangerous environment was normalized in a relatively safe setting within 1.5 years. Volume decreases of the amygdala and hippocampus were present in a Holocaust prenatal stress group, (children born by the end of the war), 70 to 75 years after the war [32]. The volume modification present in the Dutch soldiers [33] was not present in the Ukrainian refugees in our study, but the stress impact was probably different between soldiers and Ukrainian war victims. It is probable that different types of stress (Holocaust survivors, Holocaust prenatal stress, Dutch soldiers, Ukrainian refugees) may have variable impacts on brain and psychology.

**Conclusion**

Acute and ongoing war stress has a significant neurobiological impact on refugees from war-torn Ukraine. This study reveals significant impact on psychology, cerebral structures (thalamus) and connectivity as compared with Czech control group. The psychological tests in Ukrainian refugees showed significant stress, depression, and anxiety. The influence of ongoing stress is present in the posterior and central part of thalamus, connected with parts of the limbic circuitry. The lesion in the thalamus compared to Czech control group is significant. The modification of the limbic system observed in long-term stress is also involved in acute and ongoing Ukrainian stress. The data we obtained in Ukrainian refugees show the impact of war-related stress on psychological features as well as on cerebral structures in the thalamus and connectivity with stress-related cortical areas. The structural and connectivity data in this study reveal the characteristics of acute and ongoing stress in Ukrainian refugees.

**Limitations**

The sample of respondents included representatives of different regions of Ukraine and different social status and marital status; however, the entire UG was stressed in comparison to the control group and stress-related changes in MRI were present. Most men were not allowed to travel outside Ukraine at the time of our study; therefore, the study does not consider the gender aspect of stress.

The interview and psychological testing were performed by two Ukrainian psychologists (Sofia Berezka and Tetyana Evmenova – co-authors) who were also war refugees. The psychological investigation was limited as we could use only psychological tests that were available in Ukrainian language. We performed approx. 45 to 60 minutes of MRI, up to 90–120 minutes of testing the psychological features, and 60 to 90 minutes of interviewing. The refugees provided subjective information about their psycho-emotional and physical state, life experiences, and experience of the war. This approach was subjective; however, all participants provided information about living through the stress of war. Sleep was not investigated; however, major sleep disturbances were not
reported by the refugees.

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**Data availability:** The datasets generated and analyzed during the current study are available upon request at the Repository of CEITEC Masaryk University, MAFIL CF. Data are available from the authors upon reasonable request. Contact: Prof. Dr. Ivan Rektor, ian.rektor@fnusa.cz

**Code availability (mandatory):** code spm12; code cat; grant AZV NU22-04-00661

**Author contributions:** Ivan Rektor (corresponding author, neuroscientist) – Conceptualization, Supervision, Writing – Original Draft, Resources, Data Curation; Monika Fňašková (neuroscientist) - Project Administration, Investigation, Resources, Data Curation, Visualization; Sofiia Berezka (Ukrainian psychologist) - Investigation, Formal Analysis; Martin Gajdoš (biomedical engineer) - Methodology, Software, Formal Analysis, Investigation, Visualization; Pavel Riha (biomedical engineer) - Methodology, Software, Formal Analysis, Investigation, Visualization; Tetjana Evmenova (Ukrainian psychologist) - Investigation, Formal Analysis; Marek Preiss (Czech psychologist) - Conceptualization, Methodology, Validation.

**Competing interests:** The authors declare no competing interests.

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