Trauma Exposure Rather than Posttraumatic Stress Disorder Is Associated with Reduced Baseline Plasma Neuropeptide-Y Levels

Charles A. Morgan III, Ann M. Rasmusson, Brendon Winters, Richard L. Hauger, Jeffrey Morgan, Gary Hazlett, and Steven Southwick

Background: *Exposure to uncontrollable stress reduces baseline plasma neuropeptide-Y levels in animals. We previously reported that baseline plasma neuropeptide-Y levels, as well as neuropeptide-Y responses to yohimbine, were lower in combat veterans with posttraumatic stress disorder, but we were unable to determine whether this was attributable to posttraumatic stress disorder or trauma exposure. The current report addresses this issue.*

Methods: *A) Baseline plasma neuropeptide-Y levels were measured in 8 healthy combat veterans compared to 18 combat veterans with posttraumatic stress disorder and 8 healthy nontraumatized subjects; and B) Baseline plasma neuropeptide-Y levels, trauma exposure, and posttraumatic stress disorder symptoms were assessed in 41 active military personnel.*

Results: *Plasma neuropeptide-Y was negatively associated with trauma exposure but not posttraumatic stress disorder symptoms in active duty personnel. Baseline neuropeptide-Y was reduced in combat veterans with and without posttraumatic stress disorder.*

Conclusions: *Trauma exposure rather than posttraumatic stress disorder is associated with reduced baseline plasma neuropeptide-Y levels. Future studies must determine if neuropeptide-Y reactivity differentiates trauma-exposed individuals with and without posttraumatic stress disorder.* Biol Psychiatry 2003;54:1087–1091 © *2003 Society of Biological Psychiatry*

Key Words: Military stress, resilience, stress vulnerability, peptides, military active duty, veterans

Introduction

Neuropeptide-Y (NPY) is a 36 amino acid peptide neurotransmitter co-localized with norepinephrine in sympathetic nerve fibers and present in noradrenergic perivascular, enteric, cardiac nonsympathetic, and parasympathetic nerves (Wahlestedt and Reis 1993). In the brain, NPY is highly expressed in the locus coeruleus, amygdala, cortex, hippocampus, and periaqueductal gray, where NPY receptor signaling may play an important role in the mammalian stress response (Heilig and Widerlov 1990). Moreover, NPY has been shown to exert an anxiolytic-like action in a variety of animal behavioral paradigms (Heilig et al 1989, 1992, 1993; Heilig 1995; Wahlestedt et al 1993; Ehlers et al 1997; Britton et al 1997, 2000). In several human studies, a negative relationship between plasma or cerebrospinal fluid NPY levels and psychological responses to stress has been observed, consistent with the hypothesis that this neuropeptide may contribute to stress adaptation (Widerlov et al 1988; Rasmusson et al 2000; Morgan et al 2000, 2001). In a pharmacologic challenge study evaluating the effect of administration of the $\alpha 2$ receptor antagonist, yohimbine, on plasma levels of NPY, Rasmusson et al (2000) reported significantly lower baseline plasma levels of NPY and a blunted plasma NPY response to intravenous (IV) yohimbine in combat veterans with posttraumatic stress disorder (PTSD) compared to healthy control subjects. Due to the absence of combat control subjects in this study, it was not possible to assess whether the reduced NPY levels were the result of the condition of PTSD or whether they reflected the impact of trauma exposure.

Preclinical data show that repeated exposure to stress results in a lowering of baseline plasma NPY levels in rats (Corder et al 1992). These data are consistent with the possibility that in humans, trauma exposure rather than PTSD (or depression) may account for the reported lower levels of NPY. To address this question, we investigated the relationship between baseline plasma NPY levels, trauma exposure, and the presence of PTSD symptomatol-

From the National Center for PTSD (CAM, AMR, SS), VA Connecticut Healthcare Systems, Department of Psychiatry (CAM, AMR, SS), and Physician Associate Program (BW), Yale University School of Medicine, New Haven, Connecticut; VA San Diego Heathcare Systems and Department of Psychiatry (RLH), University of California, San Diego, California; Special Forces Underwater Warfare Operations (JM), Flemming Key, Key West, Florida; and JFK Special Warfare Training Center and School (GH), Fort Bragg, North Carolina.

Address reprint requests to Charles A. Morgan III, M.D., M.A., National Center for PTSD, 116A, VA Connecticut Healthcare Systems, West Haven CT 06516.

Received August 13, 2002; revised April 1, 2003; accepted April 15, 2003.

0006-3223/03/$30.00
doi:10.1016/S0006-3223(03)00433-5

ogy in two separate studies: 1) Study A, a study involving combat veterans with and without PTSD and healthy nontraumatized civilian comparison subjects; and 2) Study B, a study involving active duty military personnel. The two studies were independent of one another and were conducted at different times and in different localities. The two studies are presented together in this article to convey to the reader a more comprehensive perspective on the relationship between NPY and trauma exposure. Based on the preclinical data of Corder et al (1992), we hypothesized that in Study A, baseline plasma NPY would be significantly lower in individuals exposed to life-threatening trauma independent of the diagnosis of PTSD and that in Study B, baseline plasma NPY would be significantly lower in military subjects with greater levels of trauma exposure and independent of severity of PTSD symptoms.

Methods and Materials

Study A

Study A was a completely separate investigation from Study B. All subjects provided written informed consent to participate in a placebo-controlled yohimbine challenge study approved by the West Haven Connecticut Veterans Administration Medical Center Human Studies Subcommittee and conducted by Southwick et al (1993, 1997). Subjects included in the current analyses had sufficient plasma available for the measurement of baseline NPY levels. Baseline plasma NPY levels were measured in 8 healthy combat veterans without PTSD or other current psychiatric disorders, 18 combat veterans with PTSD, and 8 healthy nontraumatized subjects. All subjects were free of significant medical illness and had abstained from drug and alcohol use for a minimum of 4 weeks before the study. Combat veterans scored above 12 on a Combat Exposure Scale score (Keane et al 1989). The PTSD subjects were evaluated using the Structured Clinical Interview for DSM-III-R. Eight (44%) met criteria for current major depression and 4 (22%) met criteria for current panic disorder; none had a psychotic disorder. The combat control and healthy nontraumatized subjects were screened for current and past psychiatric disorders using a Structured Psychiatric Interview for healthy subjects. Baseline plasma NPY levels were drawn 1 hour and 1 hour 15 minutes after placement of an intravenous line.

Study B

Study B was a completely separate study from Study A. Forty-one male active duty special operations personnel participating in the Special Forces Underwater Warfare Operations Combat Diver Qualification Course (CDQC) were the subjects of this study. Subjects were recruited by the principal investigator (CAM) and provided written, informed consent to participate in the protocol that was jointly approved by Yale University and the John F. Kennedy Special Warfare Training Center and School (Womac Army Hospital). All subjects had received medical and psychiatric clearance to participate in CDQC and were free of illicit substances as determined by urine toxicology screens. The mean age, height, and weight of the subjects were 27 ± 4.3 years, 70 ± 2.2 in, and 177 ± 20 lb, respectively. No subject was taking prescribed medications during CDQC.

Table 1. History of Exposure to Trauma and to Traumatic Events in Military Personnel (Study B: $n = 41$)

Type of Potentially Traumatic Event	Number of Subjects Endorsing Exposure to Event (%)
War Zone or Peacekeeping	11 (27.5)
Accident	16 (40)
Natural Disaster	19 (47.5)
Life-Threatening Illness	1 (2.5)
Childhood Physical Abuse	6 (15)
Childhood Sexual Abuse	0 (0)
Mugging or Assault	18 (45)
Other Type of Fearful Situation	16 (40)
Death of Family Member	6 (15)
Witnessing Traumatic Events	18 (45)
Number of Subjects with Exposure to Trauma	36 (90)
Average Number of Potentially Traumatic Events	2.7 (SD = 1.9)
Traumatic Stress Exposure	17 (43)
One's Life in Danger During Event	17 (43)
Physical Injury Sustained During Event	10 (25)[a]

Note: An event is coded as a traumatic event on the Brief Trauma Questionnaire (BTQ) if the subject also endorses one or both BTQ items related to DSM-IV criterion A (Life in danger; physical injury sustained).

[a]All subjects who endorsed the experience of physical injury also endorsed the "life in danger" probe.

Subjects completed a valid, reliable self-report instrument designed to assess exposure to 10 potentially traumatic experiences (Brief Trauma Questionnaire [BTQ]) (Schnurr et al 1999) (Table 1). Within the design of the Brief Trauma Questionnaire, when a subject has endorsed exposure to a potentially traumatic event, the subject is prompted to respond to two additional questions: "Was there a realistic threat to your life/a sense of fear for your life?" and "Were you physically injured?" Subjects who responded in the affirmative to the first of these questions (threat to life) were considered to meet DSM-IV criterion A2 for exposure to a traumatic event, due to the fact that such an endorsement signals the presence of both the A1 and A2 components (i.e., the person was exposed to a realistic threat and experienced a sense of subjective distress). Subjects who endorsed having been only physically injured were not considered to have met the A2 criterion, since they did not necessarily experience a sense of subjective fear, distress, or horror. Thus, subjects who endorsed threat to life (with or without an endorsement of physical injury) were considered to have met DSM-IV criteria for a traumatic event exposure (American Psychiatric Association 1994).

In addition to trauma assessment, PTSD symptoms were assessed using the Impact of Events Scale, Revised (IES-R) (Zilberg et al 1982), which queries criterion B, C, and D symptoms of PTSD along a Likert-like scale (Weiss and Marmar 1997). Responses to the items on the IES-R can be scored to reflect the B, C, and D clusters of the DSM-IV for PTSD. The subscales of the IES-R are summed to convey a total IES-R score. Subjects provided a blood specimen approximately 5 minutes after completing the questionnaires.

Sample Processing Method

Plasma samples were placed on ice, spun in a refrigerated centrifuge, pipetted into microtubules, and frozen at −70°C within 40 minutes of venipuncture for the study of active military personnel and immediately in the case of subjects in the studies by Southwick et al (1993, 1997). For both studies, plasma samples were thawed only once, immediately before the NPY levels were measured, according to the methods previously described (Rasmusson et al 2000).

Data Analysis

STUDY A. The average of the two baseline plasma NPY values was calculated. A one-way analysis of variance (ANOVA) with post hoc Scheffé contrasts was used to evaluate differences in baseline plasma NPY among the Combat Control, PTSD, and Healthy Comparison subject groups. The level of significance was set at $p < .05$. Age was not included as a factor or covariate in the ANOVA, for two reasons: plasma NPY levels in this sample of subjects was not significantly associated with age; and second, previous studies of NPY in humans have not found NPY to vary across the age ranges characteristic of subjects included in this study (Rasmusson et al 2000; Dotsch et al 1997; Southwick et al 1999).

STUDY B. Pearson correlation analyses were performed to evaluate the relationship between the number of traumatic events to which a person had been exposed, PTSD symptoms (as indicated by the IES-R total score), and plasma NPY. Due to the differences in scale magnitude, the data were transformed into standardized scores before performing analyses (SPSS.11.5; SPSS Inc., Chicago, Illinois). Stepwise linear regression analyses were used to evaluate whether or not the independent variables age, height, weight, or branch of the military were associated with baseline NPY levels. The level of significance was set at $p < .05$.

Results

Study A

There were significant differences in baseline plasma NPY levels among the combat control, PTSD, and healthy nontraumatized subjects: $F(2,31) = 9.22$, $p < .001$. As shown in Figure 1, the mean age differed significantly between the three groups of subjects [healthy subjects = 26.2 (SD = 7.2, range 28–42), PTSD subjects = 42.8 (SD = 2.1, range 39–48), combat control subjects = 47.3 (SD = 5.0, range 40–55); $F(2,29) = 51.3$; $p < .001$]. Baseline plasma NPY levels were comparable between the combat control and PTSD groups ($p > .98$) and were approximately 30% lower compared to those of the healthy nontraumatized control group ($p < .01$ in both cases) (Figure 2). Weight did not differ significantly between the three groups of subjects [healthy subjects = 87.9 kg (SD = 13.4), PTSD subjects 88.8 kg (SD = 13.8), combat control subjects = 84.4 kg (SD = 14.1); $F(2,31) = 0.28$; $p = .76$].

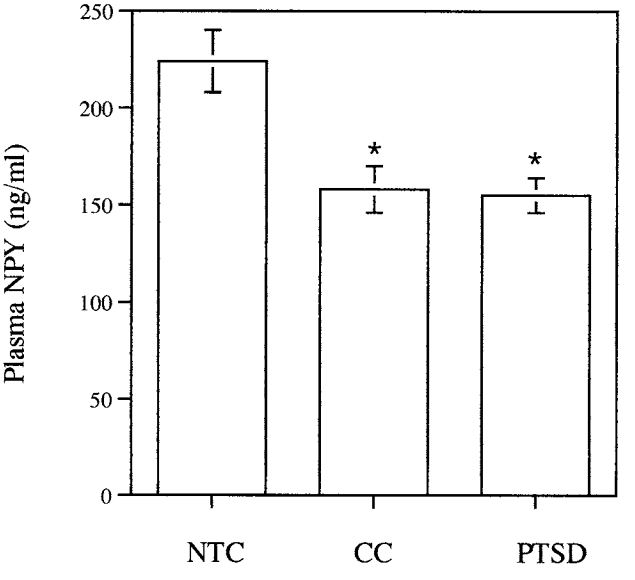

Figure 1. Study A: Baseline plasma NPY levels in 8 healthy nontraumatized (NTC), 8 combat-exposed (CC), and 18 combat posttraumatic stress disorder (PTSD) subjects. Values represent the mean ± SEM. *$p < .01$ versus the NTC group. NPY, neuropeptide-Y.

Study B

The variables age, weight, height, and group (Army Green Berets; Air Force Combat Control Technicians) were not significantly associated with the test variables baseline NPY levels, trauma exposure, the three subscales of the IES-R (intrusion [mean: 12, SD = 7], avoidance [mean: 7, SD = 6], hyperarousal [mean 3, SD = 1]), or the IES-R

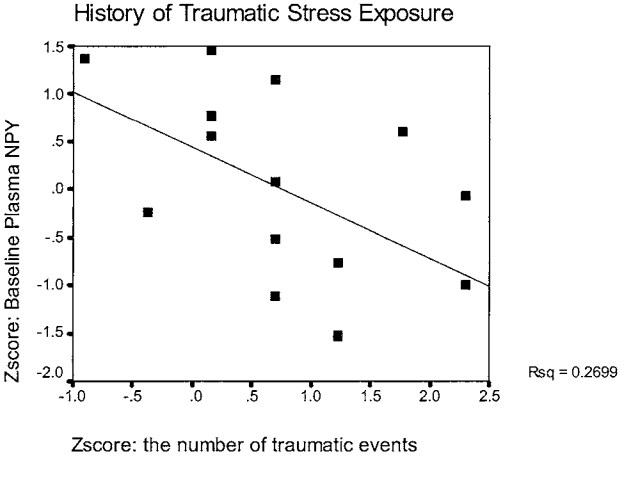

Figure 2. Study B: Baseline plasma NPY levels in healthy active duty military personnel is negatively correlated with traumatic stress exposure. Note: The apparent mismatch between the number of subjects depicted and the number in the calculation is due to the fact that a number of subjects had identical values of NPY and are represented as a single point. NPY, neuropeptide-Y.

total score (sum of the three subscales; mean: 21, SD = 11). Table 1 shows the number of subjects endorsing each type of potentially traumatic event queried by the BTQ and the number of subjects who were considered to be exposed to a traumatic stressor by virtue of endorsing that during their exposure to the trauma they experienced a fear for their life. Thirty-six individuals were exposed to one or more potentially traumatic events, while 17 were exposed to one or more criterion A traumatic events (subjective response of fear for life in the presence of realistic threat). No significant relationship was observed between the number of potentially traumatic events experienced and baseline plasma NPY ($r = .002$; $p < .9$); however, as shown in Figure 2, there was a significant, negative relationship between the number of traumatic, life-threatening events experienced and baseline NPY levels among the individuals exposed to traumatic stress ($r = -0.52$; $p < .04$). No significant relationship was detected between baseline NPY and the IES-R scores for the group as a whole or for the group of subjects endorsing exposure to traumatic stress ($r = .12$, $p < .6$; $r = -0.28$, $p < .3$, respectively).

Discussion

The current studies suggest that repeated exposure to traumatic stress, rather than the presence of PTSD or PTSD-type symptoms, is associated with a reduction in baseline plasma NPY levels in humans. In Study A, baseline NPY levels were significantly lower in combat veterans with or without PTSD compared to healthy control subjects, and in Study B, there was a negative relationship between baseline NPY and the number of times a person had been exposed to traumatic stress. In these active duty subjects, there was no observable relationship between NPY and reported PTSD-type symptoms, as measured on the IES-R. Taken together, the results of these two studies are consistent with the preclinical work by Corder et al (1992), demonstrating a reduction in baseline plasma NPY in rats exposed to repeated restraint stress.

It is possible that stress-induced reductions in NPY may reflect an adaptation to stress on the part of the organism. Since extraneuronal NPY is known to inhibit the release of norepinephrine from sympathetic neurons, a reduction in plasma NPY may promote a more rapid and robust sympathetic fight/flight response and thus may constitute a normal and positive adaptation to life-threatening stress; however, it is also possible that low baseline NPY levels provide a necessary but not sufficient mechanism for the development of PTSD and other stress-related psychiatric disorders. For instance, we have observed a blunting of plasma NPY responses to yohimbine, as well as a reduction in baseline plasma NPY levels, in combat veterans with PTSD compared to healthy nontraumatized subjects (Rasmusson et al 2000). Blunted NPY release in the company of a readily triggered and intense sympathetic noradrenergic response (Southwick et al 1999) may not be adaptive.

Recent preclinical data have demonstrated that mice with a homozygous deletion of the neuropeptide-Y gene exhibit anxiety-like behavior and also a sensitized acoustic startle response (Bannon et al 2000), behaviors potentially analogous to those seen in humans with PTSD who exhibit exaggerated startle, anxiety, heightened arousal in response to a mildly stressful stimuli, and central and peripheral noradrenergic hyperactivity. Similarly, Morgan et al (2000, 2001, 2002) found that lower plasma NPY levels were correlated with greater psychological distress, increased symptoms of dissociation, and poorer performance among active duty personnel.

The data from these two studies suggest that exposure to life threatening trauma is related to lower baseline levels of NPY. The clinical implications of lowered baseline NPY are currently not well understood. In a previous study of military personnel waiting to participate in survival school training, Morgan et al (2002) reported that low baseline NPY was predictive of a blunted NPY response, increased symptoms of dissociation, and poorer military performance, suggesting that low baseline NPY may not be adaptive; however, in the present study, baseline NPY levels were not related to psychopathology (i.e., PTSD) but instead to trauma exposure. There was not enough available plasma from the combat control subjects participating in Study A to permit an analysis as to whether NPY responses to yohimbine, rather than baseline NPY levels, would differentiate combat veterans without PTSD from those with PTSD. It will be important for future studies to determine whether variability in the stress-induced release of NPY, rather than variability in baseline levels of NPY, plays a role in the development of maladaptive responses to stress.

We thank Colonel Jeffrey Cairns, Sgt. William Barchers, Stephen Garrett, P.A., Jeremy Cordova, M.A., Willie Ford, and the staff of the Special Forces Underwater Warfare Operations Facility. Their support and work significantly facilitated the completion of this project. In addition, we thank Alan Turken for performing the NPY assays. Funding for this project was provided by a grant from the Center for Naval Analyses (CAM) and from the United States Army Medical Research Institute of Infectious Diseases (CAM). RLH is supported by a VA Merit Review Grant and the VA Mental Illness Research, Education and Clinical Center of VISN22.

The opinions reflected in this paper are not to be considered policy or guidance of the United States Navy or Army or the Department of Defense but reflect solely the opinions of the authors.

References

American Psychiatric Association (1994): *Diagnostic and Statistical Manual of Mental Disorders,* 4th ed. Washington, DC: American Psychiatric Association.

Bannon AW, Seda J, Carmouche M, Francis JM, Norman MH, Karbon B, et al (2000): Behavioral characterization of neuropeptide Y knockout mice. *Brain Res* 868:79–87.

Britton KT, Akwa Y, Southerland S, Koob GF (2000): Neuropeptide Y blocks the "anxiogenic-like" behavioral action of corticotropin-releasing factor. *Peptides* 21:37–44.

Britton KT, Southerland S, Van Uden E, Kirby D, Rivier J, Koob G (1997): Anxiolytic activity of NPY receptor agonists in the conflict test. *Psychopharmacology (Berl)* 132:6–13.

Corder R, Castagne V, Rivet J-M, Mormede P, Gaillard RC (1992): Central and peripheral effects of repeated stress and high NaCl diet on neuropeptide Y. *Physiol Behav* 52:205–210.

Dotsch AJ, Englaro P, Dotsch A, Hanze J, Blum WF, Kiess W, et al (1997): Relation of leptin and neuropeptide-Y in human blood and cerebrospinal fluid. *J Neurol Sci* 151:185–188.

Ehlers CL, Somes C, Seifritz E, Rivier JE (1997): CRF/NPY interactions: A potential role in sleep dysregulation in depression and anxiety. *Depress Anxiety* 6:1–9.

Heilig M (1995): Antisense inhibition of neuropeptide Y (NPY)-Y1 receptor expression blocks the anxiolytic-like action of NPY in amygdala and paradoxically increases feeding. *Regul Pept* 59:201–205.

Heilig M, Koob GF, Britton KT (1992): Anxiolytic-like effect of neuropeptide Y (NPY), but not other peptides, in an operant conflict test. *Regul Pept* 41:65–69.

Heilig M, McLeod S, Brot M, Heinrichs S, Menzaghi F, Koob G, et al (1993): Anxiolytic-like action of neuropeptide Y: Mediation by Y1 receptors in amygdala, and dissociation from food intake effects. *Neuropsychomarmacology* 8:357–363.

Heilig M, Soderpalm B, Engel J, Widerlov E (1989): Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacology (Berl)* 98:524–529.

Heilig M, Widerlov E (1990): Neuropeptide Y: An overview of central distribution, functional aspects, and possible involvement in neuropsychiatric illnesses. *Acta Psychiatr Scand* 82:95–114.

Keane TM, Fairbank JA, Caddell JM, Zimering RT, Taylor KL, Mora CA (1989): Clinical evaluation of a measure to assess combat exposure. *Psychol Assess* 1:53–55.

Morgan CA III, Rassmuson A, Wang S, Hoyt G, Hauger R, Hazlett G (2002): NPY, cortisol and subjective distress in humans exposed to acute stress: Replication and extension of previous report. *Biol Psychiatry* 52:136–142.

Morgan CA III, Wang S, Rassmuson A, Hazlett G, Anderson G, Charney DS (2001): Relationship among cortisol, catecholamines, neuropeptide-Y and human performance during exposure to uncontrollable stress. *Psychosom Med* 63:412–422.

Morgan CA III, Wang S, Southwick SM, Rasmusson A, Hauger R, Charney DS (2000): Plasma neuropeptide-Y in humans exposed to acute uncontrollable stress. *Biol Psychiatry* 47:902–909.

Rasmusson A, Hauger RL, Morgan CA, Bremner JD, Charney DS, Southwick SM (2000): Low baseline and yohimbine-stimulated plasma neuropeptide Y (NPY) levels in combat-Related posttraumatic stress disorder. *Biol Psychiatry* 47:526–539.

Schnurr PP, Vieilhauer MJ, Weathers F, Findler M (1999): *The Brief Trauma Questionnaire.* White River Junction, VT: National Center for PTSD.

Southwick SM, Bremner JD, Rasmusson A, Morgan CA III, Arnsten A, Charney DS (1999): Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol Psychiatry* 46:1192–1204.

Southwick SM, Krystal JH, Bremner JD, Morgan CA III, Nicolaou AL, Nagy LM, et al (1997): Noradrenergic and serotonergic function in posttraumatic stress disorder. *Arch Gen Psychiatry* 54:749–758.

Southwick SM, Krystal JH, Morgan CA, Johnson DR, Nagy LM, Nicolaou A, et al (1993): Abnormal noradrenergic function in posttraumatic stress disorder. *Arch Gen Psychiatry* 50:266–274.

Wahlestedt C, Pich EM, Koob GF, Yee F, Heilig M (1993): Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligodeoxynucleotides. *Science* 259:528–530.

Wahlestedt C, Reis D (1993): Neuropeptide Y-related peptides and their receptors—are the receptors potential therapeutic drug targets? *Annu Rev Pharmacol Toxicol* 32:309–352.

Weiss D, Marmar C (1997): The Impact of Event Scale-Revised. In: Wilson J, Keane T, editors. *Assessing Psychological Trauma and PTSD.* New York, NY: Guilford, 399–411.

Widerlov D, Lindstrom LH, Wahlestedt C, Ekman R (1988): Neuropeptide Y and peptide YY as possible cerebrospinal markers for major depression and schizophrenia, respectively. *J Psychiatr Res* 22:69–79.