High dose hydrocortisone immediately after trauma may alter the trajectory of PTSD: Interplay between clinical and animal studies

Joseph Zohar, Hila Yahaloma, Nitsan Kozlovsky, Shlomit Cwikel-Hamzany, Michael A. Matar, Zeev Kaplan, Rachel Yehuda, Hagit Cohen

Division of Psychiatry, The State of Israel Ministry of Health, The Chaim Sheba Medical Center, Sackler Medical School, Tel-Aviv University, Tel Hashomer, Israel
Ministry of Health, Anxiety and Stress Research Unit, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
Department of Psychiatry and Neurobiology, Mount Sinai School of Medicine, New York, NY, USA

Received 23 February 2011; received in revised form 12 May 2011; accepted 5 June 2011

KEYWORDS
Posttraumatic stress disorder;
Animal model;
Hydrocortisone;
Secondary prevention;
Dendritic arborization;
Brain-derived neurotrophic factor (BDNF);
Postsynaptic density-95 (PSD-95)

Abstract

High-dose corticosteroids have been reported to reduce symptoms of acute stress and post-traumatic stress in polytrauma patients and in animal studies. The underlying mechanism of action remains largely unclear. These issues were addressed in parallel in the clinical and preclinical studies below. In this preliminary study, 25 patients with acute stress symptoms were administered a single intravenous bolus of high-dose hydrocortisone (100–140 mg) or placebo within 6 h of a traumatic event in a prospective, randomized, double-blind, placebo-controlled pilot study. Early single high-dose hydrocortisone intervention attenuated the core symptoms of both the acute stress and of subsequent PTSD in patients. High-dose hydrocortisone treatment given in the first few hours after a traumatic experience was associated with significant favorable changes in the trajectory of exposure to trauma, as expressed by the reduced risk of the development of PTSD post-trauma. In parallel, a comparative study of morphological arborization in dentate gyrus and its modulating molecules was performed in stress-exposed animals treated with high-dose hydrocortisone. Steroid-treated stressed animals displayed significantly increased dendritic growth and spine density, with increased levels of brain-derived neurotrophic factor (BDNF) and obtunded postsynaptic density-95 (PSD-95) levels. The animal study provided insights into the potential mechanism of this intervention, as it identified...
1. Introduction

Glucocorticoids (GCs) play a major role in orchestrating the complex physiological and behavioral reactions essential for the maintenance of homeostasis (McEwen, 2002). These compounds enable the organism to prepare for, respond to, and cope with the acute demands of physical and emotional stressors. The appropriate GC release, commensurate with stressor severity, enables the body to properly contain stress responses so as to promote recovery by rapidly restoring homeostasis (Yehuda et al., 1998). Indeed, inadequate GC release following stress not only delays recovery by disrupting biological homeostasis in the short run but can also interfere with the processing or interpretation of stressful information that results in long-term disruptions in memory integration (McEwen, 2002). A salient example of such an impaired post-traumatic process in the clinic is exemplified in post-traumatic stress disorder (PTSD) (DSM-IV-TR, American Psychiatric Association, 2000).

While conventional wisdom holds that people who develop PTSD following exposure to extreme trauma might have sustained elevations in GCs, several studies have reported that lower cortisol levels in the acute aftermath of trauma are predictors for subsequent PTSD symptoms (Delahanty et al., 2000; McFarlane et al., 1997; McFarlane, 2000; Witteveen et al., 2010). Therefore, it is possible that the administration of cortisol immediately after exposure to a trauma might alter the trajectory of trauma exposure by promoting recovery. To date, however, information on the effect of cortisol injection on trauma recovery is limited, although a series of naturalistic studies have demonstrated that administration of cortisol following septic shock reduced the incidence of PTSD (Schelling et al., 1999, 2001, 2003). Several studies have reported that exogenously administered cortisol reduces PTSD symptoms in patients with chronic PTSD (Aerni et al., 2004; Miller et al., 2011; Suris et al., 2010).

Our group has initiated a series of studies examining the role of GCs in susceptibility to “PTSD-like behaviors” in a well-validated animal model for PTSD (Cohen et al., 2003, 2005). In keeping with the little data that is available on traumatized people, these studies demonstrated a greater susceptibility to experimentally induced PTSD-like behavioral changes in rats with a hypoactive and hypo-reactive hypothalamic–pituitary–adrenal (HPA) axis, i.e., Lewis strain, compared to a rat strain with a hyper-responsive HPA-axis, i.e., Fischer rats. Exogenous administration of cortisol to Lewis rats prior to the stressor effectively decreased the prevalence of subsequent extreme behavioral disruption (Cohen et al., 2006a). Further animal studies examined the effect of the single intervention with high-dose corticosterone immediately after exposure to a stressor (Cohen et al., 2008b). A controlled prospective study showed a significant reduction in the incidence of PTSD-like behaviors and improved resilience to subsequent trauma (Cohen et al., 2008b). However, corticosterone administration 14 days following stress-exposure and immediately after memory reactivation had no effect on the behavior of the rats (unpublished data). These findings suggest that a disruption in the initial adaptive endogenous response of the HPA-axis unfavorably alters the trajectory of trauma exposure. To the extent that findings from animal models “translate” to humans, treatment with GCs could provide a possible avenue for early pharmacotherapeutic intervention in the acute phase, aimed at prevention of chronic stress-related disorders, such as PTSD.

The goal of this study was twofold: a) to evaluate the therapeutic effects of a single dose of hydrocortisone in acutely traumatized persons, and b) to explore the morphological and molecular changes in brain tissue of animals “treated” with hydrocortisone immediately after exposure to trauma. The first goal was accomplished in the context of a randomized, prospective, double-blind, placebo-controlled trial, and the second, by evaluating dendritic arborization in Golgi-impregnated neurons in dentate gyrus (DG) granule cells of stress-exposed animals and the impact of these changes on the expression of brain-derived neurotrophic factor (BDNF) and postsynaptic density-95 (PSD-95) in this region.

We hypothesized that trauma patients in a hospital emergency room (ER) treated with a single injection of hydrocortisone would have an altered trajectory of PTSD, as measured at one and three months, in a favorable way, as compared to those given a placebo. In parallel, we hypothesized that animals receiving a single high-dose of hydrocortisone immediately after exposure would exhibit increased synaptic plasticity, synaptic strength and dendritic complexity with a concomitant attenuation of behavioral stress responses (less prevalence of PTSD-like response).

2. Materials and methods

2.1. Clinical trial

2.1.1. Participants

Seventy consecutive patients who were exposed to a traumatic event, experienced either acute stress reaction or sub-threshold acute stress reaction, and met the DSM-IV PTSD A.1 (stressor) and A.2 (response) criteria (fulfilling criteria A, 2 of the symptoms in criteria B, 3 out of 4 of criteria C, D, E, and F, and meeting criterion H of the ASD criteria set out in DSM-IV) were recruited from the emergency department at the Chaim Sheba Medical Center. Exclusion criteria included serious physical injury (a score of 3 or above on the Abbreviated Injury Scale), brain trauma, substance abuse disorders, cardiac pacemaker implant, a history of epilepsy, neurosurgery, chronic medical conditions of any sort. Medication-specific exclusion criteria included hypersensitivity to hydrocortisone, pregnancy, or treatment for asthma. After receiving full explanation of the procedures, only subjects signing a written informed consent 25 out of 70 approved by the Helsinki Ethics Committee of The Chaim Sheba Medical Center were recruited. The subject group consisted of 14 men and 11 women, with a mean age of 35.16 (±S.D. 12.62) years, range 20–62. The types of trauma were: traffic accidents = 20, work accident = 4, and snake bite = 1.
2.1.2. Dropouts
Of the 25 subjects who provided consent to participate, only 19 returned for the 2-week follow-up, 15 returned for the 1-month follow-up and 17 for the 3-month follow-up. We currently have data on 17 patients who completed a course of the study (Fig. 1).

2.1.3. Procedure
A senior psychiatrist (HY, SC-H) determined medical eligibility and completed informed consent procedures. The participants were randomized by a predetermined program, and entered in a double blind, placebo-controlled design. Hydrocortisone or placebo was given between 1.5 and 5.5 h following the traumatic event. Patients received hydrocortisone intravenously in a single bolus at a dose ranging from 100 to 140 mg based on body weight: 100 mg for weights of 60–69 kg, 120 mg for weights of 70–89 kg, and 140 mg for weights of 90–99 kg. The patients were assessed by senior psychiatrists (HY, SC-H) at five time points — before intervention, one day after the intervention by telephone, two weeks, 1 and 3 months after the intervention. Ratings of ASD and PTSD symptoms, anxiety, and depression were carried out at 4 time points — before the intervention, at 2 weeks, 1 month and 3 months after the trauma — by an expert investigator who was blind to the treatment condition. All patients had free access to the senior psychiatrist.

2.1.4. Demographics and background questionnaire
The questionnaire included age, marital status, date and place of birth, education, place of residence, and the type of trauma that led them to the ER.

2.1.5. Visual analog scales for anxiety
Visual analog scales for anxiety (VAS-A), depression (VAS-D), panic distress and dissociation were assessed at baseline (McCormack et al., 1988). These ratings are designed to assess the responder’s anxiety and depression severity from a subjective point of view by indicating feelings on a scale ranging from 0 to 100. VAS-A and VAS-D scores were obtained at each of the 4 visits.

2.1.6. Statistical methods
Psychopathology ratings were entered into two-way repeated measures analysis of variance with covariance for baseline (ANCOVA), with the group factor being treatment group and the within-subjects factor being time. A repeated-measure ANOVA was used in order to compare the CAPS scores of the treatment group, as well as to estimate the effect of time. Pearson chi-square analyses were conducted to examine treatment group differences in diagnostic status.

2.2. Animal model
In parallel to the clinical trial, the animal studies were designed with the idea that exogenous hydrocortisone administration at the time of trauma would promote the restoration of homeostasis by constraining CNS activity. With this objective in mind, we became interested in the outcome of dendritic arborization of the hippocampal DG granule cells rather than symptoms. The hippocampal dentate gyrus (DG) was chosen as a target in this study for four reasons: 1) The DG is a unique structure in that it is one of the few telencephalic brain areas that reliably produce new neurons well into adulthood (Redila and Christie, 2006). 2) The DG is also highly sensitive to stress (Kavushansky et al., 2006). 3) DG granule cells play a critical role in the function of the entorhinal–hippocampal circuitry in health and disease. 4) DG granule cells

---

**Figure 1** Participant screen, randomization, and retention.
are situated to regulate the flow of information into the hippocampus.

2.2.1. Animals

Adult male Sprague–Dawley rats weighing 150–200 g were employed (N=121). The animals were housed four per cage in a vivarium with stable temperature and a reversed 12-hour light/dark cycle (lights off: 08:00 a.m.) with unlimited access to food and water.

Three schedules were used in separate experiments: 1) The dendritic profile of Golgi-stained granule cells in the DG was evaluated in harvested brains from animals exposed to predator scent-stress (PSS) and classified according to CB at day 8 post-PSS exposure. The animals were subdivided into groups reflecting magnitude of response according to the CBC, focusing selectively on EBR, PBR and MBR. 2) The behavioral effects of hydrocortisone/vehicle (25 mg/kg) injected immediately after stress exposure were evaluated 7 days later. The rats were sacrificed within 24 h (8 days post exposure) and their brains collected for measurement of morphology. 3) The effects of hydrocortisone/vehicle injected immediately after stress exposure were evaluated 2 h, 24 h, and 8 days post-exposure. The rats were sacrificed and their brains collected for measurement BDNF and PSD-95 immunoreactive expression.

2.2.1.1. Predator scent stress. Stress consisted of placing the test animals on well-soiled cat litter (in use by the cat for 2 days, sifted for stools) in a plastic cage placed on a yard paving stone, for 10 min in a closed environment. Control animals were exposed to fresh, unused litter for the same amount of time and in the same conditions.

2.2.1.2. Corticosterone injections. Animals were injected with 25.0 mg/kg of hydrocortisone (Sigma-Aldrich, Israel), 1 h after PSS/sham exposure. Control groups were given 0.9% saline solution at the same time and in the same manner. The corticosterone dose was determined according to our previous study (Cohen et al., 2008a).

2.2.1.3. Behavioral measurements. Behavioral responses were assessed in the elevated plus-maze (EPM) and the acoustic startle response (ASR) paradigms as previously described (Cohen et al., 2003; Cohen and Zohar, 2004; Cohen et al., 2004, 2005). Behavioral tests were recorded and analyzed using an Etho-Vision automated tracking system (Noldus Information Technology, The Netherlands).

2.2.1.4. The cut-off behavioral criteria (CBC) model. Recently, we developed an animal model that assesses the magnitude of individual stress responses rather than of global populations. The classification of individuals according to the degree to which their individual behavior was affected by a stressor is based on the premise that extremely compromised behavior in response to the priming trigger is not conducive to survival and is thus inadequate and maladaptive, representing a pathological degree of response (Cohen et al., 2003, 2006b; Cohen and Zohar, 2004; Cohen et al., 2004, 2005, 2006c).

2.3. Golgi–Cox staining

2.3.1. Tissue preparation

24 h after the behavioral tests, between 12:00 and 14:00, animals were deeply anesthetized and perfused intracardially with 0.9% saline. The brains were immediately dissected and processed as described below. Tissue was prepared by using the rapid Golgi kit (FD Neurotechnologies, USA) according to manufacturer’s instructions.

2.3.1.1. Analysis of neuronal morphology. In order to obtain accurate measurements of dendritic parameters, strict criteria were adopted for the selection of the filled neurons before quantitative analysis: 1) Only well-impregnated neurons were chosen for the histological analysis. 2) Granule cells were included in this analysis only if the cell body and primary dendrites were clearly stained and easily distinguishable from those of neighboring cell bodies and their dendrites. 3) Granule cells were sampled from the suprapyramidal blades (SPB) of the DG, in both the right and left sides of the brain. 4) Granule cells from the inner granule zone (IGZ) were included in this analysis (because the dendritic morphology of hippocampal DG cells varies with their position in the granule cell layer (Green and Juraska, 1985)). A cell was classified as belonging to the IGZ if the entire soma was positioned in the inner half of the granule cells layer (GCL). Granule cells whose soma was intersected by the midline of the GCL, in the outer granule zone (OGZ), or in the subgranular zone were not included in any analysis.

We performed an analysis to characterize the extent that dendrites branched out from both somal and dendritic sites. Primary dendrites were defined as direct extensions from the soma of at least 10 μm in length. Only DGs with at least one primary dendrite >10 μm in total length were analyzed. When a primary dendrite bifurcated at a branch point, the dendrites extending from that branch point were classified as secondary dendrites. We extended this analysis to include tertiary (3), quaternary (4), quinary (5) and senary (6) order dendrites. This procedure provides an additional measure of the pattern of dendritic arborization, allowing a more comprehensive analysis of differences in the branch patterns of the dendrites themselves. We also performed a Sholl analysis (Sholl, 1956). A series of concentric rings, spaced 25 μm apart, was placed over the neuron and centered on the cell body, and the number of dendrite crossings as a function of distance was recorded.

All slides were coded and the analysis was performed with the experimenter blinded as to the origin of the slides. Dendritic morphology was observed by epifluorescent microscopy (Leica, Germany). A 0.5 μm interval z-series was captured throughout the extent of the dendritic arbor of the DG with a CCD camera (Leica, Germany) controlled by LAS software.

2.3.2. Spine density

The spines on the dendrites of the granule cells were counted using a 100× oil immersion objective lens (NA, 0.8; working distance, 0.66 mm). To count the spines, straight branches that provided clear resolution of spines were preferred, and spine density was calculated as the number of spines per 10 μm of dendrite for six segments per cell and five cells per animal. For each animal, cell values were averaged to derive a single value per variable.

2.4. Immunofluorescence

2.4.1. Tissue preparation

2 h, 24 h and 7 days after the behavioral tests, animals were deeply anesthetized (ketamine and xylazine mixture) and perfused transcardially with cold 0.9% physiological saline followed by 4% paraformaldehyde (PFA) (Sigma-Aldrich) in 0.1 M phosphate buffer (pH 7.4). Brains were quickly removed, postfixed in the same fixative for 12 h at 4 °C, and were cryoprotected overnight in 30% sucrose in 0.1 M phosphate buffer at 4 °C. Brains were frozen on dry ice and stored at −80 °C. Serial coronal sections (10 μm) at the level of the dorsal hippocampus (distance to the bregma: −3.80 mm, Plate 33) corresponding to the rat brain atlas of Paxinos and Watson (2005) were collected for each animal, using a cryostat (Leica CM 1850), and mounted on coated slides. After being dried, the sections were stored at −80 °C.

Sliced sections were air dried and incubated in frozen methanol (2 min) and in 4% paraformaldehyde (4 min). After three washes in phosphate buffer saline (PBS) containing Tween 20 (PBS/T) (Sigma-Aldrich), the sections were incubated for 60 min in a blocking solution in normal goat or horse serum in PBS) and then overnight at 4 °C with the primary antibodies against p50, p65, IκBα, p38, and...
phospho-p38 (1:250 each; Santa Cruz Biotechnology). After three washes in PBS/T, sections were incubated in Dylight-488 labeled goat-anti-rabbit IgG or Dylight-594 goat anti-mouse IgG (1:250; KPL, MD, USA) in PBS containing 2% normal goat or house serum for 2 h. Sections were washed, mounted with mounting medium (Vectorstain Vector laboratories, USA). Control staining was performed in the absence of the primary antibodies. Additionally, secondary fluorescent labels were swapped to check cross-reactivity and sections were incubated without any primary antibodies to check for any nonspecific binding of the secondary antibodies.

2.4.2. Quantification

Six sections from each animal were examined. The sections were processed in batches (which included all groups or treatment conditions) and analyzed by two observers blinded to the treatment protocol, and the results were averaged. Hippocampal DG regions of all sections were the main focus for study. Quantitative measurements of the immunoreactivity for BDNF and PSD-95 were performed by using NIH 1.61 Image software. Standard technique was used to estimate the number of PSD-95 and BDNF cells profiles per unit area for each investigated hippocampal structure. Photomicrographs were acquired using a Leica DFC340FX camera.

2.4.2.1. Statistical analyses. Behavioral, molecular and morphological data were analyzed using two-way ANOVA and the post-hoc Bonferroni test for multiple comparisons. Total dendritic length and total dendritic number in study 2 were analyzed using one-way ANOVA. The prevalence of affected rats as a function of rat group was tested using cross-tabulation and nonparametric Chi-squared tests.

3. Results

3.1. Clinical trial

The hydrocortisone and placebo groups did not differ significantly on any of the demographic measures such as age, gender, marital status, education or on any clinical characteristics (Table 1).

3.1.1. Outcome

Using the CAPS total scores, we found that participants treated with hydrocortisone exhibited significantly lower total CAPS score than those treated with placebo at the 2-week and 3-month follow-up assessments (post-hoc Bonferroni: \( p < 0.025 \), \( p < 0.02 \), respectively) (Fig. 2A–C). No statistically significant differences were observed at the 1-month assessment between participants treated with placebo and hydrocortisone (\( p = 0.081 \)). Moreover, at the 2-week follow-up assessment, 6 (66.7%) participants in the placebo treatment group were diagnosed with acute stress reaction, as opposed to only 2 participants from the hydrocortisone treatment group (20.0%) (Fig. 2D). Of the 8 subjects in the placebo treatment group, 3 (37.5%) had developed PTSD, according to criteria, by either the 1- or 3-month assessment period. One subject from the hydrocortisone treatment group had developed PTSD at the 1-month follow-up assessment period. No subjects in the hydrocortisone group were diagnosed with PTSD at the 3-month assessment.

Participants treated with hydrocortisone exhibited significantly lower VAS-anxiety (Fig. 3A–C) and VAS-depression (Fig. 3D–F) scores at all follow-up assessment time points than those treated with vehicle (post-hoc Bonferroni: 2-weeks: \( p < 0.025 \), \( p < 0.015 \); 1-month: \( p < 0.04 \), \( p < 0.006 \); 3-month: \( p < 0.0095 \), \( p < 0.0045 \), respectively).

3.1.2. Side effects

The treatment was well tolerated with no noticeable side effects.

3.2. Animal study

3.2.1. Morphometric results

Eight days after PSS-exposure, the total dendritic length (Fig. 4A) and the total dendritic number (Fig. 4B) were significantly lower in EBR animals compared to unexposed controls (\( p < 0.0035 \), \( p < 0.02 \), respectively) and MBR animals (\( p < 0.0025 \), \( p < 0.0004 \), respectively). No differences were detected between MBR, PBR and unexposed controls in both parameters.

The number of Sholl intersections, points where dendrites cross the virtual Sholl shells, is a parameter that reflects the complexity of the dendritic tree. In response to PSS, neurons from EBR animals had significantly fewer dendritic intersections with each sphere at Sholl radii of 10–185 \( \mu \text{m} \) than did neurons from unexposed controls and MBR animals (Bonferroni post-hoc \( p < 0.05 \)) (Fig. 4C). No differences were detected at any radii between MBR animals and unexposed controls. PBR rats exhibited a trend with fewer intersections than unexposed control and MBR animals, and more intersections than EBR animals.

Quantitative analysis of spine density per 10 \( \mu \text{m} \) of dendrite revealed that there were significantly fewer spines along the dentate granule cells in EBR animals as compared to unexposed controls (\( p < 0.045 \)) and MBR animals (\( p < 0.0005 \)) (Fig. 4D). No differences were found in the soma circumference for deep or superficial neurons.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic data and clinic characteristic of patients (baseline).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Placebo</td>
</tr>
<tr>
<td>N=8</td>
<td>N=9</td>
</tr>
<tr>
<td>Gender (N)</td>
<td>Male/female</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Family status</td>
<td>Married (N)</td>
</tr>
<tr>
<td></td>
<td>Unmarried (N)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Type of trauma (N)</td>
<td>Work accident</td>
</tr>
<tr>
<td></td>
<td>Motor vehicle accident</td>
</tr>
<tr>
<td></td>
<td>Snake bite</td>
</tr>
<tr>
<td>Time elapse since trauma (h)</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>VAS anxiety</td>
<td>Mean</td>
</tr>
<tr>
<td>VAS depression</td>
<td>Mean</td>
</tr>
<tr>
<td>VAS panic</td>
<td>Mean</td>
</tr>
<tr>
<td>VAS distress</td>
<td>Mean</td>
</tr>
<tr>
<td>VAS dissociation</td>
<td>Mean</td>
</tr>
</tbody>
</table>

Results are expressed as mean and std. dev.
3.2.1.1. High-dose hydrocortisone immediately post-exposure attenuated anxiety-like behavioral responses. Comparison between groups (not comparing individuals within each group) revealed that exposed group treated with vehicle exhibited a significant decrease in overall time spent in the open arms (Fig. 5B), a significantly increased mean startle amplitude (Fig. 5C) and a significant deficit in startle habituation (Fig. 5D) compared to unexposed controls (p < 0.0001 for all) and to exposed rats treated with corticosterone (p < 0.0001 for all) (two-way ANOVA).

3.2.1.1.1. Relative prevalence rates according to CBC. The prevalence of EBR individuals among PSS-exposed rats injected with vehicle was 30% of the total population, a marked difference from the rates observed in the unexposed (0%) and in the PSS-exposed groups treated with hydrocortisone (χ² = 4.33, p < 0.04). There were no significant differences in the prevalence of either MBR or PBR among groups.

3.2.2. Morphometric results

Exposed animals treated with hydrocortisone exhibited significantly greater total dendritic length (Fig. 6A) and total dendritic number (Figs. 6, 5B) as compared to exposed animals treated with vehicle (p < 0.001 for both parameters) or to unexposed animals treated with hydrocortisone (p < 0.02 for both parameters).

Neurons from exposed animals treated with hydrocortisone had significantly more dendritic intersections with each sphere at Sholl radii of 85–135 μm and 210–260 μm than did neurons from animals of all other groups (Bonferroni post-hoc p < 0.05) (Fig. 6C). No differences were detected at any radii between unexposed animals treated with hydrocortisone or vehicle or the exposed group treated with vehicle.

Quantitative analysis of spine density per 10-μm of dendrite revealed that there were significantly fewer spines along the DG cells in the exposed animals as compared to unexposed controls (p < 0.03) (Fig. 4D). The spine density along the dentate granule cells was significantly increased in animals treated with hydrocortisone as compared to exposed animals treated with vehicle (Bonferroni post-hoc: p < 0.001) (Fig. 6D).

The lack of effect on total dendritic length and numbers, dendritic intersections and spine density between the PSS vehicle treated group and unexposed groups is a statistical result of whole group analysis. Thus, it is important to distinguish between affected and unaffected subjects within each group (as shown in Fig. 5).

3.2.2.1. High-dose hydrocortisone immediately post-exposure changed expression of DG PSD-95 and BDNF. Administration of hydrocortisone immediately after PSS exposure significantly

Figure 2  Effects of hydrocortisone/placebo treatment administered immediately after trauma on total CAPS-2 core and PTSD symptoms: Panel A presents the 2-week, 1- and 3-month clinician-administered, posttraumatic stress disorder scale (CAPS) total scores for the hydrocortisone-treated and placebo groups. Two-way repeated measures ANOVA showed a significant effect of treatment (F(1,13) = 20.8, p < 0.000055). No effects were observed for time or for treatment–time interaction. Post-exposure hydrocortisone treatment significantly decreased the total CAPS scores at 2-week and 3-month follow-up assessments, as compared to placebo treatment. Panel B presents the 2-week, 1- and 3-month CAPS total scores for the placebo-treated group. Panel C presents the 2-week, 1- and 3-month CAPS total scores for the hydrocortisone-treated group. (D) Categorical PTSD (right axis) and acute stress disorder (left axis) outcomes. Results displayed as mean ± S.E.M.
increased BDNF immunoreactive cells when assessed 2 h (p < 0.04), 24 h (p < 0.0001) or 7 days (p < 0.0001) after the exposure as compared to exposed animals treated with vehicle (Bonferroni post-hoc) (Fig. 7A). In contrast, hydrocortisone treatment significantly decreased PSD-95 IR cells when assessed 24 h (p < 0.0001) and 7 days (p < 0.04) after the exposure as compared to exposed animals treated with vehicle (Fig. 7B) (Bonferroni post-hoc).

4. Discussion

The studies described herein provide supportive evidence that the use of high-dose hydrocortisone in trauma care may be protective against the subsequent development of PTSD after traumatic experience. The study highlights two major concepts. The first is that of "golden hours", i.e. focusing on a defined and limited "window of opportunity", namely the first 6 hours after the trauma exposure, and the second is secondary prevention via a single high-dose of hydrocortisone, a procedure that aims at recalibration of the HPA axis in order to ensure proper reaction of the HPA axis. The study also provides some insights regarding the potential mechanism of this treatment, as it identifies relevant cytostructure of dentate gyrus granule cells as well as biochemical correlates of the observed pilot clinical observations.

4.1. Prospective clinical study

Currently, the administration of hydrocortisone following trauma is indicated clinically only in the treatment of patients with significant physical illnesses or poly-trauma, although numerous reports indicate that treatment with hydrocortisone is associated with reduced symptoms of PTSD (Aerni et al., 2004; Miller et al., 2011; Schelling, 2002; Schelling et al., 1999, 2001, 2003, 2004; Suris et al., 2010; Weis et al., 2006). In this study, a single intravenous bolus of high-dose hydrocortisone (100–140 mg) or placebo was administered to patients with acute stress disorder (including sub-threshold patients) within the first 6 h after a traumatic experience. The findings demonstrated attenuating effects on subsequent acute stress reaction and acute PTSD. Follow-up assessments at one and three months after the trauma revealed statistically significant reductions in total CAPS, VAS-anxiety and VAS-depression scores in steroid-treated participants as compared to placebo-treated controls. Although the statistical significance of prevalence rates of PTSD and/or ASR is severely curbed by the small size of the population sample, the attenuating effect on the severity of PTSD core symptoms was clearly demonstrated.

Since a single administration of intravenous hydrocortisone is safe and well tolerated — causing no serious complications —
it provides a viable option for secondary prevention. A single, high dose of hydrocortisone immediately after a traumatic experience seems to assist in "recalibrating" the HPA-axis, thereby facilitating those processes required for a return to homeostasis and hence for post-stress recovery. Additionally, the exogenous hydrocortisone may act indirectly to prevent PTSD by reducing the norepinephrine requirement (McReynolds et al., 2010; Sun et al., 2010). The possible therapeutic benefits implied by the results of this prospective pilot study warrant further investigation on a larger scale and may call for reconsideration of the currently accepted clinical indications for steroid treatment in trauma patients.

4.2. Arborization/cytostructure of dentate gyrus granule cells

Concurrently to the clinical pilot study, we launched a study using the animal model to examine factors affecting neural/dendritic synaptic connectivity in response to interventions with high-dose steroids.
Dendritic arborization is a prime factor in determining a variety of neural functions (Claiborne et al., 1990; Feria-Velasco et al., 2002; Peng et al., 2009; Rahimi and Claiborne, 2007). The morphological characteristics of granule cells in the DG subarea of the hippocampus were examined by using Golgi–Cox methods in the above animal model. The results
showed that PSS exposure altered the morphology of the dendrites of dentate granule cells selectively in individuals whose behavior was extremely disrupted (EBR) in response to the exposure, whereas animals whose behavior was less severely affected displayed no significant changes in DG morphology. Extreme responders clearly demonstrated...
significantly lower dendritic complexity, lower total dendritic length, fewer branches and lower spine density along DG dendrites eight days after exposure, as compared to naïve unexposed individuals and to the PBR and MBR groups. Since the dendritic arbor is responsible for receiving and consolidating neuronal information input (Sorra and Harris, 2000; Vessey and Karra, 2007), the reduced dendritic arbor in the EBR animals can have considerable consequences for the functional properties of cells and neuronal circuitry, including decreased synaptic plasticity and synaptic strength, and impaired stabilization of synaptic connectivity, which may in turn lead to vulnerability to psychopathology. Thus, these results suggest that PSS exposure leads to dendritic atrophy of dentate granule cells, and this probably decreases the amount of information that neurons can obtain from the environment. The mechanism underlying this is not completely understood. Stress-related changes in cyto-architecture have been reported quite extensively for both acute and chronic stress paradigms (Alfarez et al., 2009; Gould et al., 1990, 1991; Magarinos and McEwen, 1995a; Magarinos and McEwen, 1995b; Magarinos et al., 1996, 1997). The changes in the cyto-architecture of the DG induced by hydrocortisone might underlie the enhancement of hippocampal long-term potentiation, structural neuronal plasticity and connectivity and thus the behavioral responses observed and the innate increased resilience of MBR animals.

4.3. Effect of high-dose hydrocortisone immediately post-exposure

We demonstrate that early post-stressor intervention with high-dose hydrocortisone, which attenuates posttraumatic stress response, was associated with a dramatic increase in the number of dendrites of dentate granule cells, total dendritic length, and dendritic spine density, as compared to vehicle controls. The increased dendritic complexity and spine density cannot be attributed to additional parameters of the stress response nor can it be related to stress that may accompany...
exposure to the maze, to motor activity within the maze or to intense activation of the olfactory system by odors, as no difference was observed between neurons from naive unexposed rats and from unaffected (MBR) rats. Additionally, the differences in dendritic complexity observed were not caused by a sampling bias with respect to location of the soma: in all groups, cells were quite evenly distributed over the extent of the inner granule cell layer.

The precise timing at which this increase in dendritic complexity and spine density occurs, and the time-course in which it may fade away is of great interest, and is the subject of an ongoing study. It should be noted that activity-induced dendritic morphogenesis can occur within tens of minutes (Maletic-Savatic et al., 1999). In the present study we examined modifications in dendritic complexity and spine density 8 days after stress exposure, as physiological modifications are most pronounced at this point in time.

4.4. Expression of DG PSD-95 and BDNF

Although the precise molecular mechanisms underlying the factors that regulate the orientation, morphology, and elaboration of dendritic processes are largely unknown, there is now compelling evidence that outgrowth and morphogenesis of the dendritic arbor depends on the coordinated action of brain-derived neurotrophic factor (BDNF), calcium/calmodulin-dependent protein kinase II, the small GTPases RhoA, Rac1, and Cdc42, glutamate receptor-interacting protein (GRIP), and a calcium-responsive transactivator (Aizawa et al., 2004; Fink et al., 2003; Hoogenraad et al., 2005; Li et al., 2000; Threadgill et al., 1997). As with most growth processes, signals that promote growth must be balanced by cues that subsequently limit and regulate that growth (McFarlane, 2000). Charych et al. (2006) demonstrated that postsynaptic density-95 (PSD-95), a postsynaptic scaffolding protein, acts as a negative regulator of dendritic branching in the hippocampus. The authors reported that PSD-95 acts as a stop signal for branching and that overexpression of PSD-95 results in decreased secondary dendrite number because of a decreased proportion of primary dendrites that branch. Decreasing PSD-95 expression results in increased secondary dendrite number. The idea that PSD-95 acts as a brake for dendrite branching is quite interesting because the majority of studies have focused on the role of PSD-95 as a scaffolding protein at postsynaptic sites (Kim and Sheng, 2004). However, PSD-95 has been previously shown to be enhanced in rat hippocampi following stress (Chertkow-Deutsher et al., 2010; Hu et al., 2009; Yang et al., 2008) and following chronic elevation of corticosterone (Liu et al., 2006). Given the importance of BDNF and PSD-95 in neuronal function and plasticity, the expression of these factors in the DG was examined at 2 h, 24 h, and 8 days post-exposure and compared between hydrocortisone- and vehicle-treated groups. The results showed that stress-exposed animals treated with high-dose hydrocortisone displayed significantly higher levels of BDNF than vehicle-treated animals at all time periods, with a peak at 24 h. Animals treated with high-dose hydrocortisone had levels of PSD-95 that were stable and significantly lower than vehicle-treated animals at 24 h and 7 days.

Taking into account the known functional roles of BDNF and PSD-95 in cytoskeletal dynamics, these results are in keeping with the findings of the morphological data mentioned in the previous section. In other words, treatment with steroids results in high BDNF and obtunded PSD-95 levels, which enhance and facilitate dendritic growth and increased spine density, with a concomitant attenuation of behavioral stress responses, i.e. increased stress resilience.

High levels of corticosterone and its glucocorticoids are considered to be the main factors in the dendritic atrophy that occurs following repeated stress exposure or pharmacological manipulations (Romeo et al., 2004). Thus, considerable interest was generated in response to the reports that prolonged adrenalectomy damages hippocampal DG neurons (Conrad and Roy, 1993; Sapolsky et al., 1991; Wossink et al., 2001). Wossink et al. (2001) examined the dendritic trees of dentate granule cells 7 days after adrenalectomy or sham operation. They reported that granule cells from animals with extremely low corticosterone levels (from undetectable to 0.3 μg/100 ml) had dendritic trees with reduced total length and fewer branches. In the absence of corticosterone, dendritic trees of dentate granule cells display atrophy (Conrad and Roy, 1993; Sapolsky et al., 1991). Thus, both prolonged severe underexposure and overexposure of glucocorticoids can be deleterious to a number of hippocampal neuron types.

To summarize, the results of these studies highlight the importance of an initial bolus of endogenous corticosteroids in the normative response to stress as a key to a return to homeostasis. Aberrations in the normative response play an equally pivotal role in determining long-term cyto-architecture, and also localized brain bio-molecular and overall neuro-hormonal disruptions underlying the PTSD-like behavioral responses in animals, and may be related to the emotional and behavioral symptoms of PTSD in patients.

The data provides initial evidence that a single dose of hydrocortisone administered in the acute aftermath of trauma promotes recovery while promoting enhanced synaptic plasticity and connectivity in the secondary prevention of PTSD. We suggested that exogenous hydrocortisone, if given during the “window of opportunity” — right after the exposure and before consolidation of the traumatic memory — may promote the restoration of homeostasis by constraining central nervous system activity. We suggest that the cumulative findings of these and prior studies warrant evaluation of single, high-dose hydrocortisone treatment as a potential option for early secondary prevention of stress-related clinical disorders in large-scale prospective clinical studies.

Role of the funding source

The National Institute for Psychobiology in Israel, funded by Charles E. Smith Family, The Israel Academy of Science and Humanities (grant #416/09), and the Ministry of Health (grant #3-0000-6086) given to H.C.

Contributors

Joseph Zohar designed the clinical and animal studies, participated in the data analysis and writing of the paper.

Hila Yahalom participated in the clinical study.

Nitsan Kozlovsky participated in the lab and data analysis.

Shlomit Cwikel-Hamzany participated in the clinical study.

Michael Matar participated in the animal study’s planning and assisted in the writing of the paper.
Rachel Yehuda designed the clinical study and writing of the paper.
Zeev Kaplan participated in the animal study’s planning and assisted in the writing of the paper.
Hagit Cohen designed the animal study, wrote the behavioral protocols, and participated in the data analysis and writing of the paper.
All authors contributed to the study and have approved the final draft of the manuscript.

Conflict of interest
There is no conflict of interest for any of the authors.

Acknowledgment
We are grateful for funding from the National Institute for Psychobiology in Israel, funded by Charles E. Smith Family, the Israel Academy of Science and Humanities grant (416/09) and the Ministry of Health (3-0000-6086) grant to H.C.

References
Hu, S., Ying, Z., Gomez-Pinilla, F., Frautschy, S., 2009. Exercise can increase small heat shock proteins (sHSP) and pre- and post-synaptic proteins in the hippocampus. Brain Res. 16, 191–201.
High dose hydrocortisone immediately after trauma may alter the trajectory of PTSD


