Physical stress differs from psychosocial stress in the pattern and time-course of behavioral responses, serum corticosterone and expression of plasticity-related genes in the rat

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Abstract
Stressors differ in their physiological and behavioral outcomes. One of the major mechanisms by which stressors affect the brain and behavior is alteration in neuronal plasticity. We investigated in the rat the effects of a single exposure to psychophysical (electrical foot shock) vs. psychological (social defeat) stressors on anxiety- and depression-related behaviors, serum levels of corticosterone and the expression of plasticity-related genes CAM-L1, CREB, GAP-43, and laminin in the prefrontal cortex (PFC), the amygdala and the hippocampus. Rats were examined for 24 h or 1 week after the exposure to stress. Footshocks enhanced anxiety-related behaviors, whereas social defeat induced depression-related behaviors at both time points and less pronounced anxiety 1 week post-exposure. Serum corticosterone concentrations were enhanced 24 h after shocks, but only 1 week after exposure to the social stressor. Moreover, the shock-stressed rats exhibited decreased CAM-L1 protein level in the hippocampus 24 h post-exposure and decreased GAP-43 protein level in the PFC 1 week post-exposure. By contrast, the social stressor enhanced expression of the plasticity-related proteins in the amygdala and the hippocampus, mostly 1 week after the exposure. These results indicate stressor-specific time-dependent changes in different neuronal pathways, and suggest consideration of a cause-specific approach to the treatment of stress-related disorders.

Keywords: Anxiety, depression-related behavior, corticosterone, psychophysical stress, psychosocial stress, plasticity-related genes

Introduction
Stress plays an important role in the pathogenesis of various chronic disorders, such as anxiety and other affective disorders (Pacak and Palkovits 2001). Stress has initially been defined as a universal, non-specific response of the organism to various environmental demands called "stressors" (Selye 1974). However, different stressors differ in their physiological and behavioral outcomes (Zafar et al. 1997; Pacak and Palkovits 2001). For example, activity of the hypothalamus–pituitary–adrenal (HPA) axis, regarded as a key mediator of the stress response (Keller-Wood and Dallman 1984; Charmandari et al. 2005), could increase, decrease, or remain unchanged in response to different stressors (Mason 1971, 1975; Pacák 2000). It seems that adaptive responses to stressors are stressor-specific, and involve distinct neurobiological pathways. Several classifications of stressors have been proposed (Dayas et al. 2001; Pacak and Palkovits 2001; Herman et al. 2003; Anisman and Matheson 2005). Stressors can be acute or prolonged, and differ qualitatively. Dayas et al. (2001) have proposed that the brain categorizes stressors as physical (such as...
haemorrhage) and psychological (such as restraint), which are processed by different groups of brain neurons. Most stressors encountered by humans or animals in everyday life and those applied in experimental procedures involve a mixture of physical and psychological components. It is not clear yet how psychophysical and psychological stressors are processed in the brain.

Brain areas implicated in stress responses and stress-related psychopathologies include the prefrontal cortex (PFC), especially its medial part (mPFC), the hippocampus and the amygdala (Bremner 2006, 2007). These brain regions, which are anatomically and functionally interconnected, appear to differentially react to stress. The PFC, responsible for filtering out unessential stimuli and inhibiting unwanted responses, shows decreased volume and hypofunction following exposure to traumatic experiences of different types (Bremner 2007). Both physical and psychological stressors have been shown to impair the structure and function of the PFC (Inoue et al. 1994; Radley et al. 2005; Czéh et al. 2007), although some studies have demonstrated that the reaction of the mPFC to stress may be stressor-specific (Jedema et al. 1999; Herman et al. 2005).

Stress has been shown to induce degenerative changes in the hippocampus as well (Sapolsky et al. 1985; Woolley et al. 1990; Uno et al. 1994; Sapolsky 1996). For example, magnetic resonance imaging studies in adult humans report reduced hippocampal volume in stress-related pathologies, such as anxiety and affective disorders (Kaufman and Charney 2001).

In contrast to the PFC and the hippocampus, the amygdala usually shows enhanced reactivity and thriving morphology following trauma-related stimuli, possibly due to a failure of PFC inhibition on amygdalar activity (Vyas et al. 2002; Hendler et al. 2003; Vouimba et al. 2004; Akirav and Maroun 2007). Nevertheless, some animal studies have shown decreased plasticity in the amygdala following stress (Pizarro et al. 2004; Karst and Joels 2005; Kavushansky et al. 2006).

One of the major ways by which stress influences the brain is by modifications in neuronal plasticity (Duman 2002). Stress-induced neural plasticity includes alterations in cell morphology and cell survival and adaptations of intracellular signal transduction pathways and gene expression (Duman et al. 2000). Neuronal plasticity-related genes CAM-L1, CREB, GAP-43, and laminin have been demonstrated to be oppositely expressed in the brain following stress and antidepressant treatment (Laifenfeld et al. 2002, 2005). Several interactions between these proteins have been reported, among them cooperation between CAM-L1 and laminin in promotion of neurite outgrowth (Hall et al. 1997; Berardi et al. 1999), and synergistic enhancement of axonal sprouting and growth by Gap-43 and CAM-L1 (Zhang et al. 2005). Therefore, these molecules may act as one functional group to promote differentiation and neuroplasticity (Laifenfeld et al. 2005). However, the exact mechanisms by which specific stressful conditions modulate the expression of plasticity-related genes have not been directly studied.

The aim of the present study was to investigate in the rat whether the time course and the pattern of behavioral and endocrine changes induced by a single acute exposure to psychophysical stressor differ from those induced by exposure to psychosocial stressor. Moreover, we were specifically interested to examine whether the differences between the two stressors are reflected at the level of molecular and neuroanatomical alterations. Twenty-four hours or 1 week following a single short-term exposure to psychophysical (electrical foot shock; hereinafter—“shock”) or psychosocial stressor (social defeat; hereinafter—“social”) rats were subjected to tests of anxiety-like and depression-like behaviors, and the subjects’ serum levels of the stress hormone corticosterone, as well as expression of CREB, CAM-L1, GAP-43, and laminin in specific brain regions were assessed. The following brain regions were examined: (1) mPFC (prelimbic and infralimbic parts); (2) the amygdala (the basolateral part; BLA); and (3) the hippocampus (the dorsal CA1 area).

Materials and methods

Animals

For the behavioral experiments male Sprague–Dawley rats (Harlan Laboratories, Jerusalem, Israel) weighing 200–250 g (N = 85) were used. For the social stressor, male rats weighting over 500 g were used as “residents” (n = 24). The “residents” were individually housed in Plexiglas cages for 2 weeks prior to the beginning of the experiments. The subjects were housed in Plexiglas cages (five rats per cage) in a temperature-controlled (23 ± 1°C) animal quarters and maintained on a free-feeding regimen with a 12:12 h light/dark schedule (lights on at 07:00 am). Following 5 days of acclimation to their new environment, rats were handled once a day for three consecutive days and then taken on the fourth day for the stress procedures. Naïve rats were handled similarly to the stress groups, but did not undergo the stress exposure. Following the stress procedures the rats were returned to their home cages until examination. Twenty-four hours (N = 26; naïve, n = 9, shock, n = 9, social, n = 8) or 1 week (N = 38; naïve, n = 13, shock, n = 12, social, n = 13) after the stress procedures the rats were subjected to behavioral tests, which were always done in the same order (first—the open-field test, second—the elevated platform test, and finally—the forced swim test (FST)) and then killed for further analyses.
These behavioral tests have been shown to be stressors and to increase plasma corticosterone levels (Rodgers et al. 1999; Hall et al. 2001). In order to verify that the hormonal effects of the two stressors did not stem solely from exposure of the rats to the behavioral tests, additional groups of rats ($N = 21; 7$ in each experimental group) were subjected to the same stress procedures (social group exposed to the same residents) and killed in parallel with the 1-week groups, but without behavioral testing.

**Ethical approval**

Experiments were approved by the institutional Animal Care and Use Committee and were carried out in accordance with the Guide for the care and use of laboratory animals as adopted and promulgated by the National Institute of Health. Efforts were made to minimize the suffering of the rats and the number of the rats used.

**Foot shock stressor**

The shocker consisted of a Plexiglas box ($30 \times 26 \times 28.5$ cm) placed in a ventilated sound-proof dimly lighted container. Scrambled foot shocks were delivered through the grid floor, each bar of which was connected to a delivering box via a microchip. The cage was cleaned with 1% acetic acid before and after each session. Each rat received six 2-s shocks of 1.5 mA intensity, which is considered traumatic (Cordero et al. 2002).

**Social stressor**

This procedure is a modification of the standard resident-intruder stress used in rats (Marini et al. 2006). One day before the stress procedures each resident was exposed to a naïve rat ("tester") in order to assess the level of territoriality. Residents that did not defeat the tester rat were excluded from the experiment. The subject intruder rat ($200–250$ g) was introduced into the home cage of the resident, and the ensuing social encounter was videotaped for 30 min. Social defeat was considered to be achieved when the intruder rat was put on its back by the resident, was mounted by the resident, or led to a corner of the cage without a possibility to get out. No intruder was bitten or wounded by a resident. Each resident was used only once per day and for no more than four consecutive days.

**Open-field (OF) test**

A mini-OF with a wooden platform ($50 \times 50$ cm) divided into 25 squares ($10 \times 10$ cm) and a wall $38.5$ cm high was used, placed in a sound-proof ventilated container in a dimly lit room (Jacobson-Pick et al. 2008). At the beginning of the trial, the rat was placed in one of the corners. Each trial lasted 3 min, during which the rat path was videotaped and then analyzed off-line. Two areas of the maze were defined: the external (squares along the walls) and the internal (the nine central squares). The number of line crossings in these two areas was counted.

**Elevated plus-maze test (EPM)**

The EPM was made of Plexiglas, with two open arms ($60 \times 10$ cm) and two closed arms of the same dimensions but with the addition of $40$ cm high opaque walls. The arms were connected to a central arena ($20 \times 20$ cm) and the maze was elevated 60 cm above the floor. Each arm of the maze was divided into six equal parts by lines drawn on the floor. The rat was put in the central arena and its path was videotaped for 3 min and then analyzed off-line. The number of line crossing in different areas (the closed and the open arms) was counted.

Two aspects of behavior are examined by the OF and EPM: (1) The level of anxiety—indicated by less line crossings in the central area of the OF, or in the open arms of the EPM. (2) Total activity—The total number of line crossings in the OF/ EPM.

**Forced swim test (FST)**

This test is a modification of the Porsolt swim task, which is considered a standard procedure for assessing depression-like behaviors in rodents (Porsolt et al. 1977). The rat was placed for 6 min in a transparent cylindrical container ($48$ cm deep and $19.2$ cm in diameter), filled with $36$ cm of water of $24^\circ$C. The behavior was videotaped and then analyzed off-line using a stopwatch. The amount of time that the rat spent struggling or immobile and latency to the first time that it became immobile were calculated. Struggling was defined as movement of the forelimbs and hindlimbs, in which the front paws broke the surface of the water or scratched the sides of the container. Rats were considered to be immobile when they were floating in a vertical position in the absence of any movement other than necessary to keep the head and nose above the water. Two independent observers, blind to the experimental procedures, coded this test, and between-raters reliability was verified.

**Assessment of serum corticosterone levels**

Rats were decapitated immediately after the behavioral procedures (no anesthetic was used in order to avoid the possible effects of anesthesia on hormonal and molecular processes). Their brains were quickly extracted and frozen on dry ice and stored at $−80^\circ$C.
until the analysis. Trunk blood was collected into plastic tubes, left at room temperature for 1 h, and then centrifuged for 10 min (1600g at 4°C). Collected serum was stored at -80°C until the analysis. Serum corticosterone levels were assessed using the DSL-10-81000 ELISA kit (DSL, Webster, TX, USA). The sensitivity of the assay was 1.6 ng/ml. Intra-assay variation was <3% at 51 ng/ml, inter assay variation was <7% at 51 ng/ml.

**Brain samples collection**

Coronal sections (1 or 1.5 mm) were cut at -25°C using a LEICA cryostat, according to the atlas of Paxinos and Watson (1998). Tissues were dissected bilaterally from these sections by punching with a 14 G needle, always in the same order. The mPFC (coordinates from bregma in mm: 3.7–2.2 anterior; 0–1 lateral; 3.5–5 ventral) was dissected first, then the amygdala, mostly the BLA part (coordinates from bregma in mm: 2.3–3.3 posterior; 4.2–5.2 lateral; 7.5–9 ventral), then the hippocampus, the dorsal part, aiming at the CA1 region (coordinates from bregma in mm: 2.3–3.3 posterior; 0.2–2 lateral; 2.2–3.5 ventral) and finally the OCC, V1 and V2 (coordinates from bregma in mm: 5.8–7.3 posterior; 3–5 lateral; 1–2.5 ventral).

**Western blot analysis**

Tissue samples were suspended in lysis buffer containing 10 mM Tris (pH 7.4), 250 mM sucrose, 5 mM EDTA, 0.5% NP-40, and protease and phosphatase inhibitors (Complete™ protease inhibitor cocktail tablets; Roche Diagnostics, Mannheim, Germany; Phosphatase inhibitor cocktails I and II; Sigma-Aldrich, St Louis, MO, USA). Protein concentration was assessed by the Bradford method using the Bio-Rad protein assay. Approximately 350 µg protein were obtained from each region. The concentration of protein for gel running was determined by previous experiments (Laifenfeld et al. 2005) and validated again here (Figure 1). Protein (60 µg) was prepared with 2 x sample buffer (Sigma-Aldrich), run on a 10% SDS-PAGE and transferred to a nitrocellulose membrane. Quality of transfer was assayed by the Ponceau staining (Sigma-Aldrich). Membranes were blocked in 5% non-fat milk (Bio-Rad, Hercules, CA, USA) for 2 h at room temperature and then incubated overnight at 4°C with the appropriate primary antibody. Following washes, the membranes were incubated with the corresponding secondary antibody for 1 h at room temperature. Membranes were developed with enhanced chemiluminescence (ECL) reagents (Amersham Biosciences, UK).

Figure 1. Films representing the linear range of the antibodies for pCREB, CREB, CAM-1, GAP-43 laminin, and β-actin.
Amersham, UK) and exposed to XLS Fuji film. Films were analyzed by densitometer (Vilber-Lourmat, France), using β-actin levels for normalization and a single batch of protein of rat brain as a positive control and for normalization between gels. Because extracts from two to three different batches of rats, each consisting of representatives of the three experimental groups, were pooled, the molecular data were additionally normalized to the levels of the naïve group in each batch. Primary antibodies were: laminin γ-1 (H-190, 1:250), NCAM L-1 (C-20, 1:300), CREB-1 (C-21, 1:500), GAP-43 (H-100, 1:3,000) all purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), β-actin (1:10,000) purchased from Sigma-Aldrich and pCREB (Ser 133, 1:500), purchased from Cell Signaling Technologies (Danvers, MA, USA). Corresponding secondary antibodies (anti-rabbit, anti-mouse, or anti-goat IgGs) were purchased from Santa Cruz Biotechnology.

**Data analysis**

The results were analyzed with SPSS software (version 14.0), using one-way ANOVA to compare between the three groups, followed by Tukey’s honestly significant differences (HSD) post hoc test.

**Results**

**Short-term (24 h) effects of electrical foot shocks vs. social defeat stressor**

**Behavioral tests.** Shock significantly reduced activity in the OF as measured by overall line crossing, compared to that of the naïve and of the socially stressed rats \((F(2, 23) = 7.68, p < 0.003; \text{post hoc test: } p < 0.004 \text{ shock vs. naïve and } p < 0.012 \text{ shock vs. social}). By contrast, social defeat significantly enhanced immobility \((F(2, 23) = 4.53; p < 0.022; \text{post hoc test: } p < 0.017 \text{ social vs. naïve}) and decreased latency to immobility in the FST \((F(2, 23) = 3.57; p < 0.045; \text{post hoc test: } p < 0.036 \text{ social vs. naïve}). There was no difference between the groups in the EPM test (Figure 2).

**Serum corticosterone levels.** The ELISA revealed a significant difference between the groups \((F(2, 19) = 6.17; p < 0.009), the shock group exhibiting the highest concentrations of serum corticosterone \((p < 0.038 \text{ shock vs. naïve and } p < 0.011 \text{ shock vs. social}; Figure 3).

**Molecular alterations in different brain regions.** In the amygdala the social stressor enhanced the expression of CAM-L1 \((F(2, 21) = 3.75; p < 0.041; \text{post hoc: } p < 0.05 \text{ social defeat vs. naïve}). In the hippocampus, the shock-stressed group exhibited a significant reduction in the expression of CAM-L1 compared to that of the naïve rats \((F(2, 21) = 3.2; p < 0.05; \text{post hoc: } p < 0.05 \text{ shock vs. naïve}). There was no significant difference between the groups in the expression of the four proteins in the mPFC and OCC (Figure 4).
Figure 4. The short-term effects of foot shock and social defeat stressors on the expression of the plasticity markers in the four brain areas. The results are presented as optical density (OD) standardized to reference in case of the ratio of pCREB/CREB, to reference and to β-actin in the case of CAM-L1, GAP-43 and laminin, and then normalized to the levels of the naïve group. Upper panel of each graph shows representative blots for: (a) the corresponding antibody and (b) for CREB in case of pCREB/CREB and β-actin in case of the other genes. The white bars represent the naïve group, the black bars—the shock group, and the gray bars—the social group. In the amygdala (BLA), the social stressor enhanced the expression of CAM-L1. In the hippocampus (CA1), shock reduced the expression of CAM-L1 (*p < 0.05).
Long-term (1 week) effects of shock vs. social stressor

Behavioral tests. One week post-exposure to the stressors, the shock-stressed group made significantly fewer line crossings in the central arena of the OF \((F(2, 34) = 3.87, p < 0.031; \text{post hoc test: } p < 0.023, \text{ shock vs. naı̈ves})\) and in the open arms of the EPM \((F(2, 34) = 6.38, p < 0.004; \text{post hoc test: } p < 0.003 \text{ shock vs. naı̈ves}), \) compared to the naı̈ve group. In addition, shock-stressed rats showed a significant decrease in the total number of line crossings in the OF \((F(2, 34) = 7.29, p < 0.002; \text{post hoc test: } p < 0.002, \text{ shock vs. naı̈ves})\) and in the EPM \((F(2, 34) = 9.85, p < 0.0001; \text{post hoc test: } p < 0.0001 \text{ shock vs. naı̈ve and } p < 0.011 \text{ shock vs. social}). The social defeat group exhibited decreased total line crossing in the OF \((p < 0.036, \text{ social vs. naı̈ve})\) and decreased latency to immobility in the FST \((F(2, 30) = 3.68, p < 0.037; \text{post hoc test: } p < 0.041 \text{ social vs. naı̈ve}; \text{ Figure 5}).

Serum corticosterone concentrations. One week post-exposure serum corticosterone concentrations were significantly increased in the social defeat group compared to the naı̈ve group \((F(2, 30) = 3.77; p < 0.036; \text{post hoc: } p < 0.027 \text{ social vs. naı̈ve}; \text{ Figure 6(a)).}

The additional group of rats subjected to the stress procedures/handling, but without the behavioral testing, exhibited lower serum concentrations of corticosterone than the behaviorally tested rats, and a similar, however not significant, trend of effects of the stress procedures on serum corticosterone levels (Figure 6(b)).

Molecular alterations in different brain regions. In the mPFC the shock-stressed group exhibited reduced expression of GAP-43 \((F(2, 33) = 3.314; p < 0.05; \text{post hoc test: } p < 0.05 \text{ shock vs. naı̈ve}), \) a similar, though not statistically significant, trend was observed in the social defeat group. In the amygdala, social defeat increased the ratio of pCREB/CREB \((F(2, 32) = 3.38; p < 0.047; \text{post hoc test: } p < 0.036 \text{ social vs. naı̈ves})\) and the expression of GAP-43 \((F(2, 34) = 4.3; p < 0.022; \text{post hoc: } p < 0.020 \text{ social vs. naı̈ve}). \) In the hippocampus, the social defeat group exhibited enhanced expression of CAM-L1 \((F(2, 31) = 5.5; p < 0.009; \text{post hoc test: } p < 0.046 \text{ social vs. naı̈ve and } p < 0.011 \text{ social vs. naı̈ve}).

Figure 5. The long-term behavioral effects of the foot shock and social defeat stressors. Shock increased anxiety behavior and decreased activity in both the OF and EPM. The social stressor increased anxiety behavior in the OF and decreased latency to immobility in the FST \((^*p < 0.05; ^{*}* p < 0.005; ^{*}**p < 0.0001)\).

Figure 6. The effects of the foot shock and social defeat stressors on serum corticosterone concentrations 1 week post-stress exposure. (a) The social defeat group exhibited enhanced serum concentrations of corticosterone compared to the naı̈ve rats in the behaviorally tested groups. (b) A similar, but not significant trend was observed in stressed rats that were not behaviorally tested \((^*p < 0.05)\).
shock) and GAP-43 ($F(2, 33) = 4.1, p < 0.03; \textit{post hoc}: p < 0.039 \text{social vs. shock}$). Finally, in the OCC there was a non-significant trend to an increase in the ratio of pCREB/CREB in the social defeat group (Figure 7).

**Discussion**

The main finding of the present study is that psychophysical and psychosocial stressors differ in a time-dependent manner in their effects on anxiety/depression-related behaviors, serum corticosterone levels and the neuroanatomical pattern of expression of the neuronal plasticity-related genes, CAM-L1, laminin GAP-43, and CREB (Table I for summary of the results).

Shock-stressed rats exhibited decreased activity in the OF at both time points and in the EPM at 1-week after testing. In addition, shock-stressed rats showed enhanced anxiety in both anxiety tests 1 week post-exposure, which is in concordance with previous reports in a similar paradigm (Pijlman and van Ree 2002). By contrast, social defeat induced short- and long-term enhancement in depression-related behavior (as measured by immobility and latency to immobility in the FST), and less pronounced anxiety responses, which were apparent 1 week after the stress exposure.

Both stressors in our study consist of a mixture of physical and psychological stimuli, and this could explain the similar pattern of responses observed in some of the tests. Yet, the subtle differences in the composition of psychological and physical aspects of each stressor may be responsible for driving the behavioral manifestations towards a more anxiety-related profile in the shock group \textit{vs.} a more depression-related profile in the social defeat group.

Although both stressors have been reported to enhance plasma corticosterone levels immediately after the exposure (Cordero et al. 1998; Marini et al. 2006), in our study, subsequent corticosterone responses demonstrated a stressor-specific time course. In shock-stressed rats serum corticosterone levels were enhanced 24 h (Cordero et al. 1998), but not 1 week post-exposure (Louvart et al. 2006). In contrast to shock-stressed rats, in the social defeat group the possible initial peak of serum corticosterone was not seen at 24 h (Koolhaas et al. 1997), but was evident 1 week later. This suggests diversity in the course of reorganizational changes involved in the hormonal regulation of stress response systems in the two groups. One possible explanation is the ratio between mineraloid glucocorticoid receptors (MR and GR) in the hippocampus. Gesing et al. (2001) have demonstrated that acute stress increases the MR-mediated inhibitory tonus on HPA-axis activity, preserving plasma corticosterone levels at a normal range 24 h, but not 1 week post-exposure. Interestingly, these effects were constrained to psychological stressors and did not occur following acute physical (cold) stress. In an additional study, 3 weeks post-social defeat ligand binding to hippocampal MR was significantly decreased, while binding to GR was increased (Buwalda et al. 2005). These findings suggest a delayed release of the HPA-axis from MR inhibitory control and may explain the enhancement of serum corticosterone 1 week post-exposure in the social defeat, but not shock-stressed rats in our study. Future studies will investigate the time-dependent stressor-specific activation of hippocampal corticoid receptors and its relation to hormonal regulation.

In order to discern the stressogenic effects of behavioral tests \textit{per se} on hormonal alterations induced by the two stressors, an additional group of rats was subjected to the stress procedures and killed 1 week later without behavioral testing. In this group significantly lower levels of serum corticosterone were observed, compared to those exhibited by rats subjected to behavioral tests (Figure 6) confirming the findings of previous studies, which show that exposure to behavioral tests in itself increases the level of plasma corticosterone (Hall et al. 1999). Interestingly, these rats exhibited a similar trend of hormonal changes as in those that were behaviorally tested. Serum corticosterone levels were 3.6 times higher in the social defeat group compared to naïve rats. The reason that the difference between the groups did not attain significance can be attributed to the small number of rats in each group and the large within-group variability.

The differences in the behavioral and hormonal responses induced by the two stressors were paralleled by a dissimilar pattern of expression of plasticity-related genes in the amygdala and hippocampus, but not in the mPFC.

In the mPFC, shock reduced the expression of GAP-43 at 1 week post-exposure. Although not significant, a similar pattern of reduced expression of GAP-43 was evident in the socially stressed rats (50% reduction at 24 h week inspection). This apparent lack of difference between the stressors in their influences on the mPFC is consistent with the role of this brain region in conducting a wide range of both anxiety- and depression-related behaviors (Jinks and McGregor 1997; Hrdina et al. 1998; Drevets 2000; Shah and Treit 2003; Weiss 2007). GAP-43 was the only protein studied that was altered in the mPFC in our acutely stressed rats, while in chronic variable stress the expression of CAM-L1, laminin, and pCREB has been found to be decreased as well (Laifenfeld et al. 2002, 2005; Kuipers et al. 2003). This may suggest that in our model the expression of GAP-43 in the mPFC is a more sensitive marker of a stress response. The expression of GAP-43 was suggested to reflect the level of modifiability of neural connections (Hrdina et al. 1998), growth and retraction of presynaptic terminals (Weickert et al. 2001), experience-dependent plasticity and long-term potentiation (Nelson and
Routtenberg 1985; Routtenberg 1985; Lovinger et al. 1986; Bliss and Collingridge 1993). Impairment in these processes has been associated with anxiety- and affective disorders (Diamond et al. 2004), suggesting a link between the reduction in mPFC GAP-43 and the stress-induced behavioral alterations observed in this study.

In contrast to the mPFC, the amygdala (BLA) exhibited increased protein levels of the plasticity-related genes in the socially stressed group, in line with the reported increased activation of the amygdala following stress (Bilang-Bleuel et al. 2002; Rodriguez Manzanares et al. 2005; Vyas et al. 2006). Many studies support the participation of the BLA in fear conditioning, involving exposure of the rat to foot shocks (Bailey et al. 1999; Stanciu et al. 2001; Rumpel et al. 2005). Recently Chen and Sara (2007) demonstrated that foot shock-induced activation of the locus coeruleus resulted in inhibition of amygdala neurons, and a decrease in BLA neuronal firing. However, in our experiments foot shock did not influence plasticity markers in the BLA at 24 h, or at 1 week. It is possible that the examined time-points or plasticity markers are not the targets of the specific shock paradigm used.

The social stressor enhanced amygdalar levels of CAM-L1 24 h post-exposure, and those of GAP43 and pCREB/CREB 1 week after the exposure. CAM-L1 has been associated with stimulation of neurite outgrowth (Skaper et al. 2001), and the BLA has been shown to undergo increases in dendritic length and branching following psychological, but not variable stress (Vyas et al. 2002; Vyas and Chattarji 2004). Moreover, it was shown that a delayed induction of BLA spinogenesis was paralleled by a gradual development of anxiety-like behavior in the EPM 10 days after psychological stress (Mitra et al. 2005). Taken together, these findings may indicate that the increased CAM-L1 levels in the amygdala 24 h after social stress represent an initial stage in a complex network of dynamic reorganization of the amygdalar structure, leading to the development of anxiety-related behaviors 1 week later (It is also possible that alteration of amygdalar CAM-L1 expression in early stages of stress-induced dynamics decays over time, to be replaced by changes in other genes.). In the same way, the enhanced expression of GAP-43 may indicate reorganization of BLA axonal pathfinding and sprouting (Benowitz and Routtenberg 1997). Finally, the elevation of pCREB/CREB ratio in the BLA specific to psychological stress, is in concordance with previous studies (Bilang-Bleuel et al. 2002; Shen et al. 2004; Reagan et al. 2007), and may indicate that psychosocial stress activates gradual hormonal and molecular processes, which may over time reorganize the structure and function of the BLA, preparing it to react to specific stimuli in future.

The hippocampus exhibited a distinct pattern of change, with opposite alterations in the plasticity-related proteins following the two stressors. In accordance with numerous studies showing a reduction in CA1 plasticity after shock stress (Yang et al. 2004; Li et al. 2005), foot shock in our study decreased the expression of hippocampal CAM-L1 24 h post-exposure. In contrast, the socially stressed rats exhibited enhancement of CAM-L1 and GAP-43 at 1 week after stress (*p < 0.05).

Table I. Summary of the results.

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<td><strong>CORT</strong></td>
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CORT, corticosterone.
stress has been previously reported to enhance plasticity-related processes in the hippocampus (Pardon et al. 2005; Artola et al. 2006; but see Pizarro et al. 2004; Reagan et al. 2007). However, the concomitant elevation of serum corticosterone at 1-week in these rats was unexpected, since augmentation of stress hormones is usually associated with decreased CA1 plasticity (Kavushansky et al. 2006; Krugers et al. 2006; but see Karst and Joëls 2005). One possible mechanism for the parallel enhancement of plasticity markers and serum corticosterone is the corticosterone-induced activation of hippocampal voltage-dependent calcium channels (VDCC). Upon binding to hippocampal MRs and GRs, corticosteroids become potent modulators of intracellular calcium levels via N-methyl-D-aspartate receptors (NMDARs) and via VDCC, which in turn regulate synaptic plasticity (Grover and Teyler 1990; Karst et al. 2000; Freir and Herron 2003; Krugers et al. 2005). Impairment in synaptic efficacy caused by elevated GR levels is usually associated with activation of NMDA receptors (Diamond et al. 1992; Alfarez et al. 2002). However, corticosterone-induced activation of VDCC induces NMDA-independent plasticity, and the final outcome depends on the balance between these two mechanisms (Krugers et al. 2005). It was suggested that this balance is regulated by a net influence of many factors, such as the age of the rat and the recent history of local synaptic innervation (Bienenstock et al. 1982; Norris et al. 1998; Shankar et al. 1998; Krugers et al. 2005). The different processes evoked by different stressors may be an additional factor determining this balance. Interestingly, CAM-L1, GAP-43, and laminin have all been shown to interact with VDCC and exert their impact on plasticity via VDCC (Meiri et al. 1991; Ohbayashi et al. 1998; Archer et al. 1999; Nishimune et al. 2004). It possible that various stressors differentially affect the complex interplay between NMDARs/VDCC activation, corticosterone and plasticity-related proteins in the hippocampus. Future studies should assess the effects of different stressors on the activation patterns of these receptors.

Our results are limited to male rats of one (Sprague–Dawley) strain, and more studies are required in order to assess the generalizability of our findings. In our study, the impact of the social stressor was more pronounced than that of the shock stressor at the level of plasticity-related changes and resulted in a more assorted behavioral manifestation. This may be related to the complex nature of the psychosocial stressor compared to the shock stressor (Blanchard et al. 2001; Sgoifo et al. 2005). Alternatively, it is possible that the brain areas examined in the present study are more relevant to the multimodal psychosocial stress (Yaniv et al. 2004), suggesting that psychophysical and psychological stressors activate different brain pathways. It is conceivable that the specific mosaic of central reactions induced by each experience may determine the time-course and development as well as the pattern of the behavioral manifestations. This suggests the importance of considering cause-specific approaches to treatment of different stress-related disorders.

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