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Enhanced fear recall and emotional arousal in rats recovering from chronic variable stress

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Abstract

Emergence of posttraumatic-like behaviors following chronic trauma is of interest given the rising prevalence of combat-related posttraumatic stress disorder (PTSD). Stress associated with combat usually involves chronic traumatization, composed of multiple, single episode events occurring in an unpredictable fashion. In this study, we investigated whether rats recovering from repeated trauma in the form of chronic variable stress (CVS) express posttraumatic stress-like behaviors and dysregulated neuroendocrine responses. Cohorts of Long Evans rats underwent a 7d CVS paradigm followed by behavioral and neuroendocrine testing during early (16 hr post CVS) and delayed (7d) recovery time points. A fear conditioning-extinction-reminder shock paradigm revealed that CVS induces exaggerated fear recall to reminder shock, suggestive of potentiated fear memory. Rats with CVS experience also expressed a delayed expression of fearful arousal under aversive context, however, social anxiety was not affected during post-CVS recovery. Persistent sensitization of the hypothalamic-pituitary-adrenocorticotropic response to a novel acute stressor was observed in CVS exposed rats. Collectively, our data are consistent with the constellation of symptoms associated with posttraumatic stress syndrome, such as re-experiencing, and arousal to fearful contexts. The CVS-recovery paradigm may be useful to simulate trauma outcomes following chronic traumatization that is often associated with repeated combat stress.

Keywords

PTSD; chronic variable stress; fear memory; arousal; anxiety; HPA

Introduction

Posttraumatic stress disorder (PTSD) is a stress-linked disorder that affects a substantial proportion of the population, and is particularly prevalent in combat veterans. PTSD can be triggered by acute or chronic exposure to traumatic events, with intensity and duration of trauma considered important determinants in the trajectory of posttraumatic symptoms [1]

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There are several animals models of PTSD in the literature, including acute predator exposure (odor and physical presence of a predator), electric tails shocks, single prolonged stress, and inescapable stress, among others (reviewed in [2]; and references within). However, efforts to develop paradigms that model chronic stress-induced emergence of posttraumatic stress-like behaviors have been limited [3–5]. Since combat-related PTSD usually follows exposure to chronic and unpredictable trauma, we are interested in modeling the emergence of PTSD-like pathophysiology following exposure to an unpredictable stress regimen (chronic variable stress-recovery) (CVS-R). The CVS-R model is based on generation of deficits arising after repeated exposure to multiple, single event, variable stressors and is pertinent to chronic trauma exposure such as that found in combat operations. Previous work reported a delayed emergence of neuroendocrine deficits in rats following CVS [6], suggesting that the model can induce enduring changes that persist after stress cessation. The current study investigates whether exposure to CVS invokes the expression of potentiated fear memories, anxiety to aversive and social encounters, and dysregulation of HPA function. Our results indicate that CVS exposure produces a delayed and selective expression of exaggerated fear memory recall as well as context-dependent enhancement of fear behaviors. These behavioral sequelae are accompanied by persistent sensitization of neuroendocrine stress responses. Our data are consistent with chronic stress induction of late emerging and persistent physiological and behavioral deficits characteristic of PTSD

Methods

Subjects

A total of 168 male Long-Evans rats (275–300g) were used for all experiments. Animals were purchased from Harlan (Indianapolis, IN) and singly housed in a climate-controlled vivarium on a 12-12 light dark cycle (lights on 6 a.m.). Except for brief periods during the chronic stress procedure, all animals had *ad libitum* access to food and water. All experiments and chronic variable stress protocols were conducted during the lights-on period. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati. To investigate the effects of CVS on specific outcomes and avoid cross sensitization and conditioning effects between different measures, separate cohorts of animals were used for each behavioral endpoint and for neuroendocrine outcomes.

Experimental Plan

Figure 1 illustrates the temporal layout of the experimental plan. After a one week acclimation period animals were either exposed to CVS or handled as controls for seven days. All testing was performed at early (16 h post CVS) and delayed (7d) recovery stages, except for fear conditioning and extinction studies where only the 7d delayed recovery time point was assessed, due to temporal overlap of 16 h extinction and 7 d conditioning experiments that needed to be performed within the same context.

Chronic variable stress (CVS) Paradigm

The chronic variable stress was a modification of that described previously [6]. Subjects were randomly assigned to handled control and chronic stress groups. CVS animals underwent twice daily, morning and afternoon exposure to alternating stressors for seven days. Additionally, experimental animals were housed in mouse cages overnight on two occasions during this period. Morning and afternoon stressors were administered between 0900 – 1100 h and 1400 – 1600 h, respectively. Overnight stressors began immediately after cessation of afternoon stressors and terminated at the initiation of the next day's morning stressor. Stressors were administered in a randomized order with each stressor represented

an equivalent number of times except restraint and cold room exposures that were conducted thrice. Stressors included i) cold swim (10m, 16–18°C), ii) warm swim (20m, 30–32°C), iii) hypoxia (30m, 8%O_2), iv) 1h shaker (100rpm), v) 1h cold room (~ 8–10 °C) and vi) 1h restraint. Cold and warm swim consisted of trials of 5–8 animals per trial. For hypoxia, all animals were tested at the same time in hypoxia chambers divided in the center, with 5–8 animals on a side. During the shaker stress, animals were grouped 5–8 per cage in neutral cages. For the cold room animals were paired in cages without bedding. For the restraint stressor, the animals' home cages were moved out of the housing room and the animals were placed in restrainers and returned to their home cage.

Physiological Measures

To determine the efficacy of the CVS paradigm several physiological measures were assessed. Body weight was recorded at the initiation of the experiment prior to CVS, at the early recovery (16h) and delayed recovery (7d). Thymus and adrenal glands were removed and weighed for comparison with unstressed group. Physiological measures from separate cohorts exposed to behavioral testing were not different from each other, so these data were pooled.

Fear conditioning

Animals were allowed to recover from CVS for 7d, and were exposed to fear conditioning paradigm thereafter (see Fig 2A for schematic). All animals underwent a contextual conditioning paradigm to investigate three phases of training: fear conditioning, extinction and post extinction recall of fear memory. All of these phases were conducted in the same context. Exposure to a post extinction re-shock was chosen to investigate whether re-exposure to mild trauma reminder post extinction can produce sensitized fear responses and recall, a phenomenon observed in individuals with PTSD [7]. On day 1, each animal was placed in the Gemini Shock apparatus (San Diego Instruments) and acclimated to the chamber for 3 minutes, then received 3 shocks of 1mA intensity, 1s duration administered 1 minute apart. Animals were recorded for post shock freezing for 3 minutes. The animals were placed in the chamber the next five days and recorded for 5 minutes without shocks to measure fear conditioning and extinction. Seven days after the initial shock training, the animals were placed in the same context and after 1 minute received a single, 1mA, 1s shock. Behavior was recorded for 3 minutes post shock. Post shock freezing after initial shock, freezing in the absence of shocks (conditioned fear and extinction), and fear memory recall (freezing following reminder shock) was measured. Freezing was defined as the absence of all movement except that necessary for respiration [8].

Anxiety-Associated Behaviors

Elevated Plus Maze—The EPM test was based on previous studies [9]. To study the expression of anxiety and innate fear we assessed behavior on the EPM under two conditions, a "low threat" minimal light setting (6 lux) and an "aversive" bright light setting (650 lux). CVS and controls were tested on the EPM apparatus at early (16 hr) or delayed (7 d) recovery time points post chronic variable stress. The apparatus comprised of a PVC maze with two open (40 × 10) and two enclosed (40 × 10 × 20) arms. The arms radiated from a 10cm central square. The entire apparatus is elevated 60cm off the floor. For testing, each animal was placed on the center square of the maze facing the same open arm. Behavior was recorded from an overhead ceiling camera for 5 minutes. Video files were captured and saved for later scoring. A number of behavioral measures were analyzed, which included standard measures of exploration and anxiety-like behavior as well as additional measures of fear and arousal. Parameters were scored manually and using the Topscan program CleverSys (CleverSys Inc. Reston, VA) for certain endpoints such as

locomotor activity. Several parameters were scored; arm time (open and closed) arm entries (open and closed), rearing, grooming, freezing, stretch-attend posture (SAP)/risk assessment, head dips, and general locomotor activity.

Social Interaction

The Social Interaction test procedure was based on previous studies [10], with modifications. CVS and handled controls animals were tested at early and delayed recovery time intervals (n=12/group). In addition to the experimental cohorts neutral "interactor" animals were used for providing social experience to control and CVS animals. For acclimation, all the animals were brought into the testing room and handled daily during the stress and recovery period prior to testing. Handling consisted of lifting the animals and allowing them to rest on a gloved hand for several seconds. The testing arena consisted of a standard rat shoebox cage without bedding. On the day before testing, all animals were weighed and experimental animals were weight matched to an interactor to within 5% of body weight. For identification purposes, the interactor animals were marked on their white sides with a non-toxic black marker. All animals were habituated individually to freely explore the arena for 10 min. The following day, experimental animals were placed in the test arena and the interactor animal was introduced immediately thereafter. Test cages were videotaped for 10 minutes. Interactions were scored by two observers blinded to experimental conditions. Active and total interaction, grooming, freezing, rearing and fighting was scored. Active interaction was determined when the experimental animal (CVS or control) was actively engaged with the interactor, sniffing, following, climbing over or fighting.

Hypothalamic Pituitary Adrenocortical Axis (HPA) response to acute stressor

Corticosterone responses were measured following an unexpected acoustic stimulus which served as an acute stressor. Acute stress and blood sampling occurred between 8:00 am and 2:00 pm. The stimulus was administered using the SR-LAB system (San Diego Instruments, San Diego, CA). The apparatus included ventilated, soundproof chambers measuring approximately 52cm × 52cm 76cm and contained an enclosure of approximately 12.5cm diameter. The enclosure was of sufficient size to restrict but not restrain the animal and allow it to turn around. The chambers and enclosures were cleaned between animals with 0.1% acetic acid. Background noise in the chamber was maintained at 68 dB. After habituation for 5min, each animal was exposed to acoustic stimulus that comprised of a 40ms, 108 dB burst of white noise emitted at random intervals for 200 ms determined semi-randomly by computer. After exposure to stimulus, animals were returned to their home cages and moved to a separate room. Tail blood was collected in unrestrained animals by tail vein nick at 30 minutes, 60 minutes and 120 minutes from the time the animals were exposed to the initial stimulus. These timepoints were selected to measure peak or near peak levels of corticosterone (30 minutes) as well as the termination of the response by feedback inhibition. Plasma corticosterone was measured using the ImmuChem Double Antibody Corticosterone ^{125}I RIA kit (MP Biomedicals, Orangeburg, NY) according to the manufacturer's protocol. Corticosterone concentration was calculated using AssayZap software (Biosoft, Cambridge, UK).

Statistical Analysis

Data are shown as mean ± SEM and were analyzed by two-factorial ANOVA, student t test, or non parametric test where applicable. Physiological data are reported as percent changes or adjusted (normalized to body weight) data. These were analyzed by two-way ANOVA with *Stress* (Control, CVS) and *Recovery Time* (Early, Delayed) as between-subject factors. Fear conditioning data expressed as percent freezing were analyzed by two factorial ANOVA using stress and recovery time as between subject variables. EPM and social

interaction data were analyzed by non parametric Wilcoxon Rank Sum test for treatment group differences. Time course of corticosterone responses were analyzed by repeated measures two-way ANOVA with stress as between subject factor and time as a within-subject factor. Statistical significance was taken as $p<0.05$

Results

Effects of chronic variable stress on physiological measures

The efficacy of the CVS paradigm was verified by assessment of several physiological parameters (Table 1). Body weight gain was significantly lower in CVS exposed animals compared to controls [stress, $F_{(1, 94)}= 16.12$; $p<0.05$] during recovery. An overall effect of recovery time was found on body weights [time, $F_{(2,94)} = 44.88$; $p<0.05$]. There was also a significant stress × time interaction [stress × time, $F_{(2,94)} = 4.507$; $p<0.05$]. *Post hoc* analyses revealed that the CVS animals displayed lower body weight gain at both 16 hr and 7 d recovery time points ($p<0.05$).

As previously noted, CVS increased adjusted adrenal weight (stress, $F_{(1,30)} = 8.85$; $p<0.05$) and decreased thymus weight (stress, $F_{(1,35)} = 10.71$; $p<0.05$), consistent with stress-induced adrenal hypertrophy and thymic atrophy. Significant stress by recovery time interactions were observed for both organs (adrenal: $F_{(1,30)} = 6.25$; $p<0.05$, thymus: $F_{(1,35)} = 5.926$; $p<0.05$). Post hoc analysis revealed that CVS-induced adrenal hypertrophy was significant at early 16h recovery ($p<0.05$) point but was normalized at the 7d time interval, consistent with post-stress withdrawal of HPA axis drive.

Sensitized fear recall in CVS animals

A contextual fear conditioning paradigm was used to assess the impact of chronic variable stress on fear memory related behaviors (see Fig 2A for schematic). We compared post shock freezing following initial conditioning and reminder shocks to gauge fear memory recall in control and CVS animals. Freezing to context was also measured on five consecutive days following conditioning (Test 1–4) to assess conditioned fear (Test 1) and extinction (Test 2–4). Conditioned fear measured 24 hr post shock (Test1) revealed significant increase in freezing in CVS animals during the first two minutes (Fig 2B). Significant effects of stress [$F_{(1,55)} =6.66$; $p<0.05$] and time [$F_{(4,55)} =6.37$; $p<0.05$] were observed, with no significant stress × time interaction. Post hoc analysis revealed significantly increased freezing at the 2 minute interval (Fig 2B). To assess extinction of contextually conditioned fear, freezing time in the chamber was measured in the absence of shock for consecutive days (Fig 2C). Two way repeated measures ANOVA revealed an overall significant main effect of stress [$F_{(1,30)} = 5.909$; $p<0.05$] and time [$F_{(3,30)} = 26.76$; $p<0.05$]; however, no stress × time interaction was noted. Post hoc analysis revealed statistically significant differences between CVS and control animals only on Test 4 ($p<0.05$). Interestingly, following extinction, rats with CVS experience exhibited an exaggerated response to a mild reminder shock as compared to the control group (Fig 2D). Two way analysis of variance revealed a significant main effect of stress [stress, $F_{(1,22)} = 5.924$; $p<0.05$]; with no significant effect of time or an interaction between stress and time. Post hoc analysis revealed significantly increased freezing in the CVS group after the reminder shock as compared to the control group ($t= 2.756$; $p<0.05$). It should be noted that the frequency of reminder shock was lower than the initial (single versus three shocks, respectively). However, the magnitude of freezing response to the weak reminder was as high as the initial shocks in CVS animals. On the other hand control animals showed reduced freezing to the reminder shock. No significant differences in freezing were observed between the two groups after the initial conditioning shocks.

Delayed emergence of fearful arousal in CVS animals on elevated plus maze

CVS and control animals were tested on the EPM at early and delayed recovery period (Fig 3A). Testing was performed under low and bright light conditions to investigate effects on anxiety-like behaviors in contexts that are mildly or highly aversive in nature. All indicators of anxiety and arousal were scored and assessed between groups at early and delayed recovery time points. We observed striking differences in behavioral outcomes between the two testing conditions. Under aversive bright light conditions a delayed emergence of behaviors representing fear associated arousal was observed in CVS animals with 7d recovery. As shown in Fig 3B, an increase in open arm time was observed which trended towards statistical significance ($p= 0.08$). This was accompanied by a significant increase in freezing ($p=0.049$), motor activity ($p=0.023$) and rearing ($p=0.055$) as determined by the Wilcoxon non parametric statistical analysis (see Fig 3C). Grooming was significantly increased in CVS animals at early recovery ($p=0.004$); however, open arm time was not significantly different between CVS and control animals at this time point.

In contrast to bright light conditions, testing on the EPM under low light produced increased open time times at early recovery ($p<0.05$), accompanied by a significant increase in grooming ($p<0.05$, data not shown); these effects were normalized after 7d recovery. However, other parameters (motor activity and rearing) were not significantly different between CVS and control cohorts (data not shown). Animals did not show any freezing responses under low light conditions. Risk assessment behaviors were not different between groups at either recovery time intervals (data not shown).

CVS does not induce anxiety associated with social interaction

Exposure of CVS animals to a novel conspecific produced no significant effect on active interaction as compared to handled control animals at either the early or delayed recovery times. CVS animals spent 95.833 ± 5.681 mean ± SEM time (s) in active social interaction while control animals spent 98.417 ± 9.77 mean ± SEM time at 16 h. After 7 d recovery, active interaction time was 105.33 ± 11.772 (CVS) and 117.10 ± 6.897 (control). Two-way ANOVA revealed no significant effects of stress or recovery time or a stress × time interaction. In addition to active interaction, time spent grooming and in aggressive encounters (fighting) was assessed and found to be similar between CVS and control groups (Fig 4). Interestingly, a significant increase in rearing frequency was observed in the CVS group only at the delayed recovery interval ($p= 0.032$).

Novel acute stressor induced neuroendocrine responses show persistent sensitization in CVS animals during recovery

Time course of plasma CORT response to an acoustic stimulus revealed that rats exposed to CVS exhibited a significantly sensitized peak CORT response as compared to control animals both one day and seven days after stress cessation (Fig 5B). For early post CVS recovery, two way ANOVA analysis revealed a significant main effect of time from the onset of stressor [Time, $F_{(2, 59)} = 98.22$; $p<0.05$], with trends for effects of stress [Stress, $F_{(1,59)} = 3.581$; $p=0.06$] and stress by time interaction [Stress × Time, $F_{(2,59)} = 3.07$; $p=0.0537$]. *Post hoc* analysis indicated significantly elevated plasma CORT levels at the 30-min time point in CVS animals ($p<0.05$). CVS rats tested at 7d recovery showed a significant main effect of time [Time, $F_{(2, 55)} = 48.57$; $p<0.05$] and a trend for significance for stress × time [Stress × Time, $F_{(2,55)} = 2.986$; $p=0.0587$]. *Post hoc* analysis indicated significantly elevated plasma CORT levels at the 30-min time point in CVS animals ($p<0.05$). The significance of peak CORT responses at 30 min was also verified by unpaired t tests. CORT responses at 60 and 120 min following stress initiation were not significantly different between control and CVS group, suggesting that stress termination was not affected by the CVS exposure.

Discussion

The primary finding of these experiments is that rats exposed to repeated unpredictable stressors exhibit emergence of behavioral responses consistent with enhanced fear reactivity. Importantly, behavioral impairments are not be expressed during the early "peri-traumatic" recovery time point, but rather emerge at long latencies after cessation of stress. The data suggest that pronounced behavioral plasticity is precipitated by prolonged exposure to unpredictable stress.

Sensitization of fear memory and recall by CVS

Although stress-evoked potentiation of conditioned fear has been investigated by previous paradigms [11–15], there has been limited work on chronic stress effects on fear extinction [14;15], while reinstatement of fear following extinction has not been investigated to our knowledge. We included the testing of fear reinstatement by a reminder shock to simulate re-exposure to unconditioned stimuli post extinction, which is pertinent to chronic traumatization situations. Our data on fear conditioning, extinction and reminder shock-induced reinstatement of extinguished fear revealed significantly sensitized fear responses in animals recovering from CVS. Increased conditioned fear observed by us is similar to that reported by other stress paradigms testing PTSD-like behaviors [11;13–15]. Extinction trials over the next few days indicated an overall compromised extinction in the CVS group, although significant differences were only evident during late phase. Most interestingly, significant potentiation of fear to a low intensity reminder shock was observed in the CVS group. Fear reinstatement in the CVS group may be contributed by different processes. Since reminder shock followed extinction training, it is possible that the exaggerated fear reaction in CVS rats to a reminder is a result of impaired recall of extinguished fear or to a deficit in extinction retention. Significantly lower freezing in the unstressed control group after reminder shock may indicate a recall of extinction learning resulting in a desensitized fear response as compared to the initial unconditioned response. Interestingly, a previous study reported impaired recall of extinction in rats exposed to chronic homotypic stress [16]. Another possibility might be that the CVS animals learned the context-shock association better than the control group that may have resulted in higher magnitude of freezing. Collectively, our data suggest that repeated exposure to repeated trauma may influence fear regulatory pathways, especially those controlling extinction and recall. It is important to note that CVS induced fear memory deficits are observed after 7 days of recovery, consistent with late-emerging changes in fear response dispositions.

CVS exposure induces delayed expression of fearful arousal

Since context and environment may play an important role in the expression of sensitized behavior, we controlled the degree of aversiveness of the EPM experience by testing under low and bright light conditions. Interestingly, a delayed emergence of behaviors representing "fear arousal" was observed in the CVS group only under aversive bright light condition. These behaviors are distinct from the usual stress-evoked anxiogenic responses reported in previous paradigms [2]. After 7 days of recovery, animals with prior CVS experience displayed increased freezing, motor activity and rearing behaviors. A trend towards increased open arm time was also observed. Importantly, these effects were not observed at the early recovery timepoint. The presence of enhanced freezing with increased locomotion and open arm time suggests that in this case, increased open arm time in CVS animals is not completely consistent with anxiolysis [17]. It is important to note that this phenomenon was not evident early after CVS in our case, whereas hyperlocomotion associated arousal and anxiolysis was observed immediately following chronic stress cessation in other studies [17–19]. Because these behaviors only emerged at a delayed time point we believe that CVS exposed animals do not exhibit non-specific hyperactivity associated with CVS exposure. In

addition, increased freezing behavior in CVS animals at delayed recovery suggests that they were not less anxious but more fearful. Note that freezing at early recovery is similar in both groups suggesting a delayed emergence of fear in the CVS animals. Finally, increased freezing was also accompanied by an increase in rearing behavior. Rearing frequency is a measure of the stereotypical response to a change in the environment signifying exploration and emotionality [20;21]. Increased rearing in rats has also been found to be associated with anxiogenic behaviors [22]. Our observations of increased rearing accompanied by fear and arousal may represent a fear associated hyperaroused state in the CVS group that shows a delayed emergence.

Under low light illumination, CVS animals elicited increased open arm time as compared to controls only at early recovery, an effect that did not persist after 7d recovery and is therefore not pertinent to posttraumatic phenomenon. Previous reports on chronic stress effects on anxiety-like behavior tested on the elevated plus maze are contradictory. Studies have reported an increased incidence of anxiety-like behavior (reduced open arm time) [23], decreased anxiety-like behavior (increased open arm time) response [18;24] or no effect [25]. The interpretation of these reports is further complicated by the duration, type of stressors (homotypic versus heterotypic), illumination intensity during testing and strain of experimental animals. Most studies on chronic variable stress exposure and anxiety were performed early after stress cessation and correspond to the early recovery time-point (e.g., 16 h) in this study. There are limited studies on the effects of chronic stress exposure on anxiety outcomes at delayed time points following chronic unpredictable stress cessation. In agreement with our data, one study tested rats at 1, 7 and 14 days following chronic unpredictable stress on the EPM under low intensity light but found no significant effects on anxiety-like behavior [25]. Degree of aversiveness of the EPM context (light) was found to be crucial for trajectory of long term outcomes of chronic stress at least in our model.

We also tested the emergence of social anxiety in animals exposed to CVS. No significant differences in active or passive social interaction, grooming or aggressive encounters were observed between control and CVS animals at either early or delayed recovery. Our data are in agreement with previous studies where social interaction was unchanged following emotional stress [26], but contrast with other studies where reduced social interaction is reported following exposure to a single predator [27]or footshock [14;28] exposure. It is possible that differences in duration and modality of stressor may induce different outcomes in the SI test. Presence of an interactor animal led to significant increases in rearing behavior in CVS exposed animals which was observed only at delayed recovery. Although the exact explanation for the delayed increase in rearing is unclear, the results parallel changes in rearing seen on the EPM under bright illumination, and may be associated with enhanced arousal in the novel social situation. The CVS paradigm does not appear to invoke or social anxiety or avoidance associated behaviors. It should be noted that reduced social functioning is often observed in PTSD [29], but is not identified as a consistent feature or defining symptom of the disorder.

Persistent sensitization of Hypothalamic Pituitary Adrenotropic responses during recovery from CVS

To investigate whether the sensitivity of neuroendocrine responses is altered by CVS trauma, we analyzed CORT response to an acute stressor at early and delayed recovery. Rats exposed to CVS had significantly elevated CORT levels 30 min following an acoustic stressor at both early and delayed recovery. This observation is in agreement with previous studies with other chronic stress paradigms [30;31], supporting that exposure to chronic stress enhances the capacity of the HPA axis to respond to further challenges [32]. Sensitization was not dependent on the nature of acute stressor, since exposure to novel environment also led to HPA sensitization in animals exposed to CVS (data not shown). Our

current data does not agree with previous findings of reduced HPA response to an acute stressor in CVS animals [6]. Although an exact explanation for these contrasting responses cannot be provided, these differences may be attributed to strain differences (the current study used Long-Evans while Sprague-Dawley rats were employed in the previous study). This is supported by differential CVS induced physiological changes such as thymic involution between these strains as shown in studies from our group. While significant CVS induced thymic involution is observed in Long-Evans rats [31, and our results], this effect is not always observed observed in Sprague-Dawley rats [6]. Conversely, adrenal hypertrophy is more pronounced in Sprague-Dawley rats than in the Long-Evans strain [6,31, our results). Between-strain differences in peak circulating CORT have also been reported in similar chronic stress paradigms [33,34] and in other stress models [35]. Thus, the literature suggests that there may be strain differences in HPA axis responses to stress that are dependent on genetic background.

In agreement with our observations, facilitation of HPA response was also observed following predator stress in conjunction with chronic social instability [5]. Notably, previous models of posttraumatic stress-like behaviors have reported sensitized [3;36] as well as blunted CORT responses to acute stress. Understanding the long term effects of stress on the HPA between these studies may often be complicated by the use of homotypic versus heterotypic acute stressors, the duration and intensity of initial stressor, and most importantly whether HPA response is measured during or after the acute stressor (discussed in [37]). The finding that elevated corticosterone responses persist well after the cessation of CVS insult indicates long-lasting reorganization of stress pathways. These data are consistent with findings in PTSD patients, where cortisol responses are enhanced in situations provoking anticipatory anxiety [38] as well as trauma-related stimuli [39].

CVS-R paradigm as a model of chronic traumatization?

In development of models pertinent to combat stress, it is necessary to simulate the phenomenon of chronic traumatization that is associated with combat exposure [40]. Moreover, it is essential to include multiple, single-incident traumatic events within the traumatization period that may be experienced in an unpredictable fashion [41]. Within our paradigm we included stressors such as cold swim and hypoxia that would simulate “an event that involved actual threat of serious injury or threat to physical intergrity” a criterion for a traumatic event. Previous models of posttraumatic stress-like behaviors have ranged from acute stressors to subchronic models [2]. Acuteness or brevity of stressors has been considered as an important feature in animal models for the induction of PTSD-like patho-physiology [42]. However, it is important to simulate the prolonged, repeated exposure, chronicity, as well as unpredictability, all of which are relevant to combat stress. Important features of combat stress are unpredictability and lack of control. These are major contributing factors in the development and expression of posttraumatic stress-like behaviors [43,44]. In our model, exposure of animal to variable stressors in a repeated and unpredictable manner satisfies these criteria and induced the emergence of altered fear memory and arousal. It is important to note that certain behavioral outcomes appear to be unique to the CVS-recovery paradigm. In contrast to the “avoidant” anxiety behaviors observed in acute stress associated models (reviewed in [2]), we observed the delayed emergence of “aroused” behavior associated with fear.

Summary and Conclusions

This study attempted to investigate the early and delayed expression of behavioral and neuroendocrine outcomes of chronic variable stress exposure, a potential model for chronic traumatization. The spectrum of data collected in this study point to a pronounced negative impact of CVS on later processing of emotional information, manifest as impaired

extinction, behavioral hyper-responsiveness to a reminder stimulus, fearful arousal in response to intense (but not mild) emotional stimuli, and persistent sensitization of physiological stress responses. Collectively, these measurements are consistent with the constellation of symptoms associated with posttraumatic stress syndrome, such as re-experiencing, and arousal to fearful contexts. We propose that the CVS-recovery paradigm may be useful to simulate trauma outcomes following chronic traumatization that is often associated with repeated combat stress.

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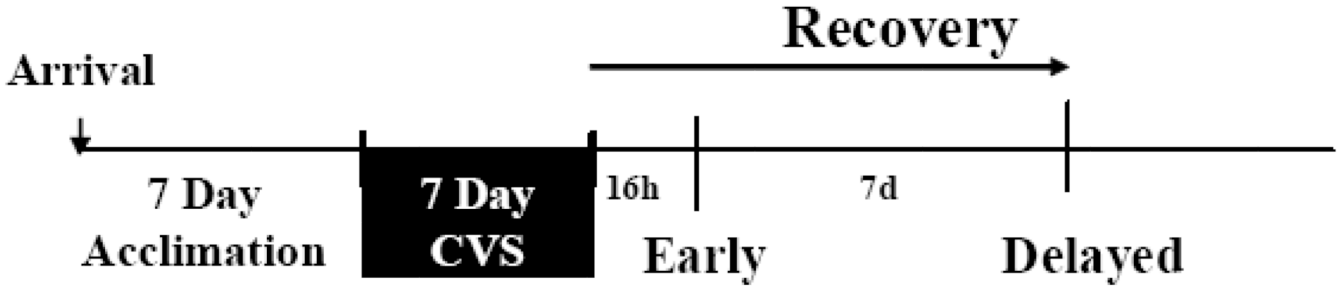

Fig. 1.

Schematic of experimental timeline. Rats were acclimated to the vivarium for one week after arrival. Rats were weighed and assigned to CVS or control groups. Rats were exposed to a one week CVS paradigm or were gently handled during the period of CVS exposure. Independent cohorts of control and CVS exposed animals were tested for various behavioral tests or neuroendocrine response at either early (16 hr) or delayed (7d) post CVS recovery.

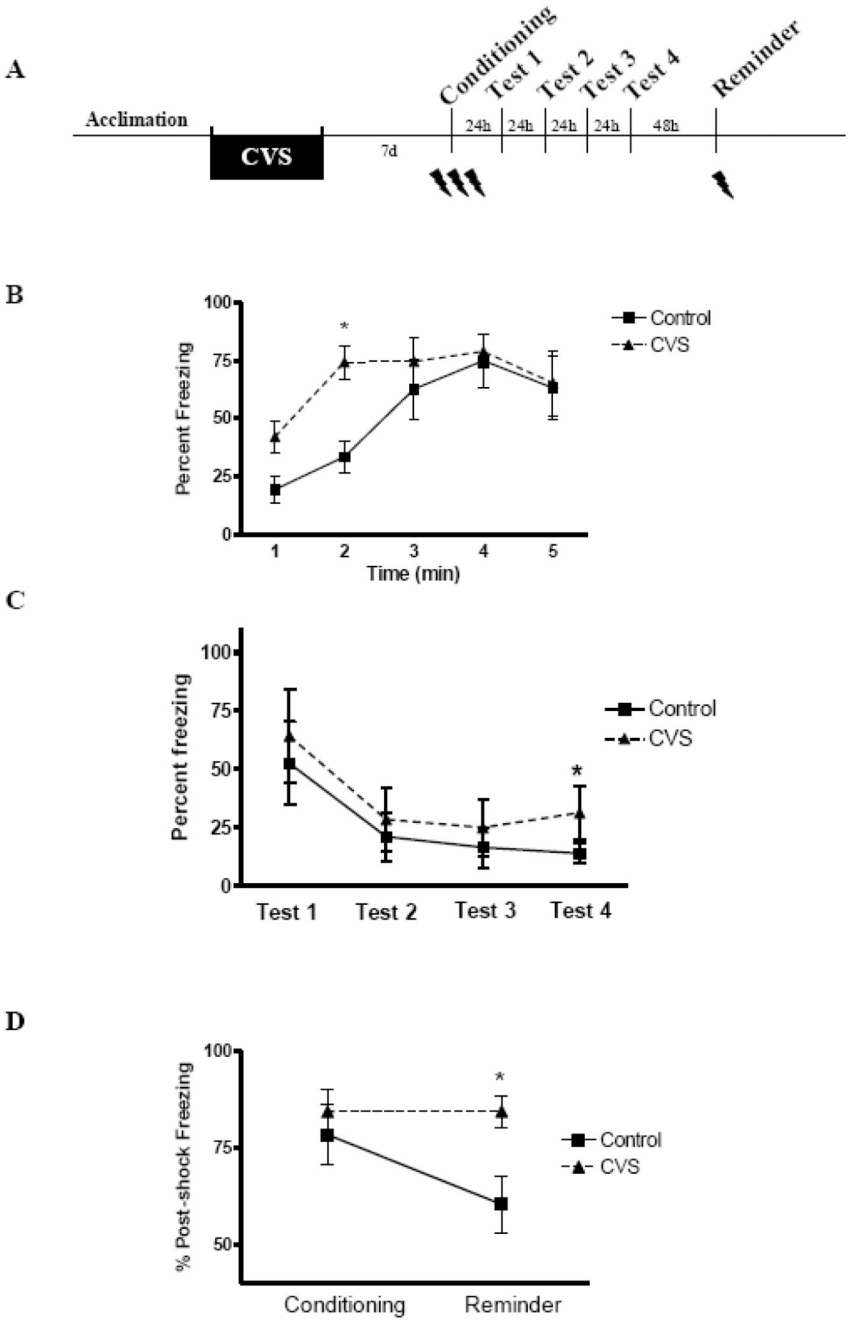

Fig. 2.

CVS animals express sensitization of conditioned fear and fear memory recall as well as impaired extinction. **(A)** Fear conditioning, extinction and reinstatement training and testing schedule. At 7 days post CVS rats were administered three shocks for conditioning followed by measurement of conditioned fear and extinction (Test 1–Test 4). To assess fear memory recall, a single reminder shock was administered 48 hr after the last extinction test. Post shock freezing and contextual freezing in the absence of shock was measured for 5 min. **(B)** Percent freezing ± SEM per minute in groups of control and CVS rats exposed to context 24 hr after conditioning. **(C)** Extinction of conditioned fear in groups of control and CVS rats between Test 1 to Test 4. Mean percent freezing ± SEM over five minutes was measured.

(D) Mean post shock percent freezing ± SEM following initial conditioning shocks and reminder shock in control and CVS rats. * $p<0.05$ versus control responses (n= 6–7 per group)

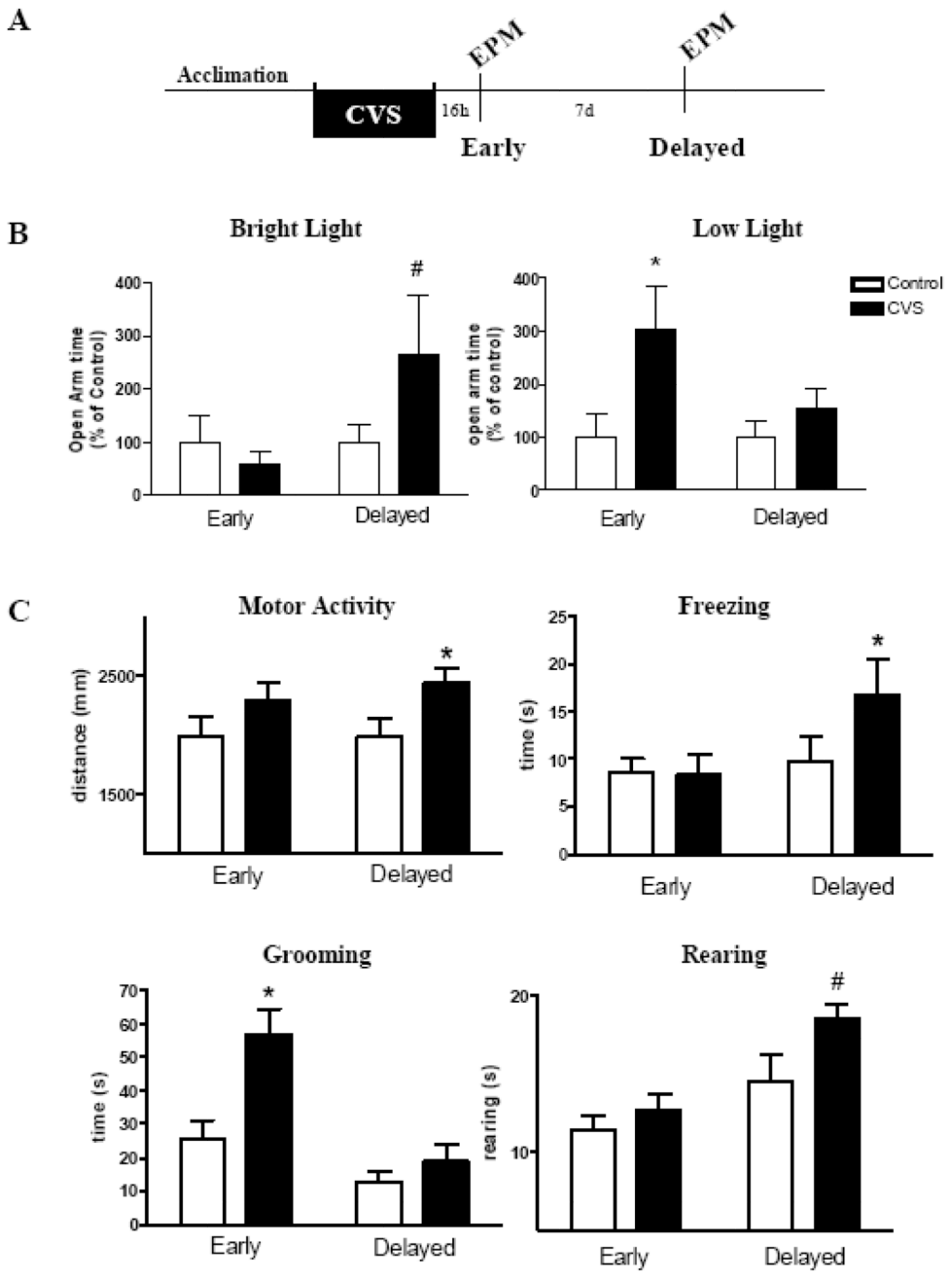

Fig. 3.

Elevated Plus Maze testing reveals delayed expression of fear associated arousal in rats exposed to CVS. **(A)** EPM testing schedule schematic. Testing at early and delayed recovery was conducted under bright light or low light conditions using separate cohorts of CVS and control animals for each condition. **(B)** Open arm time in CVS and Control groups tested under bright (left) and low (right) light conditions. Data as shown as mean ± SEM represented as percentage of control. **(C)** Panel shows motor activity, freezing, grooming and rearing outcomes measured for bright light conditions in CVS and control rats at early and delayed recovery. Data are represented as mean ± SEM values

* $p<0.05$ versus control; # trends for significance $p=0.08$ (open arm time) and $p=0.053$ (rearing) n = 10–12 animals/group.

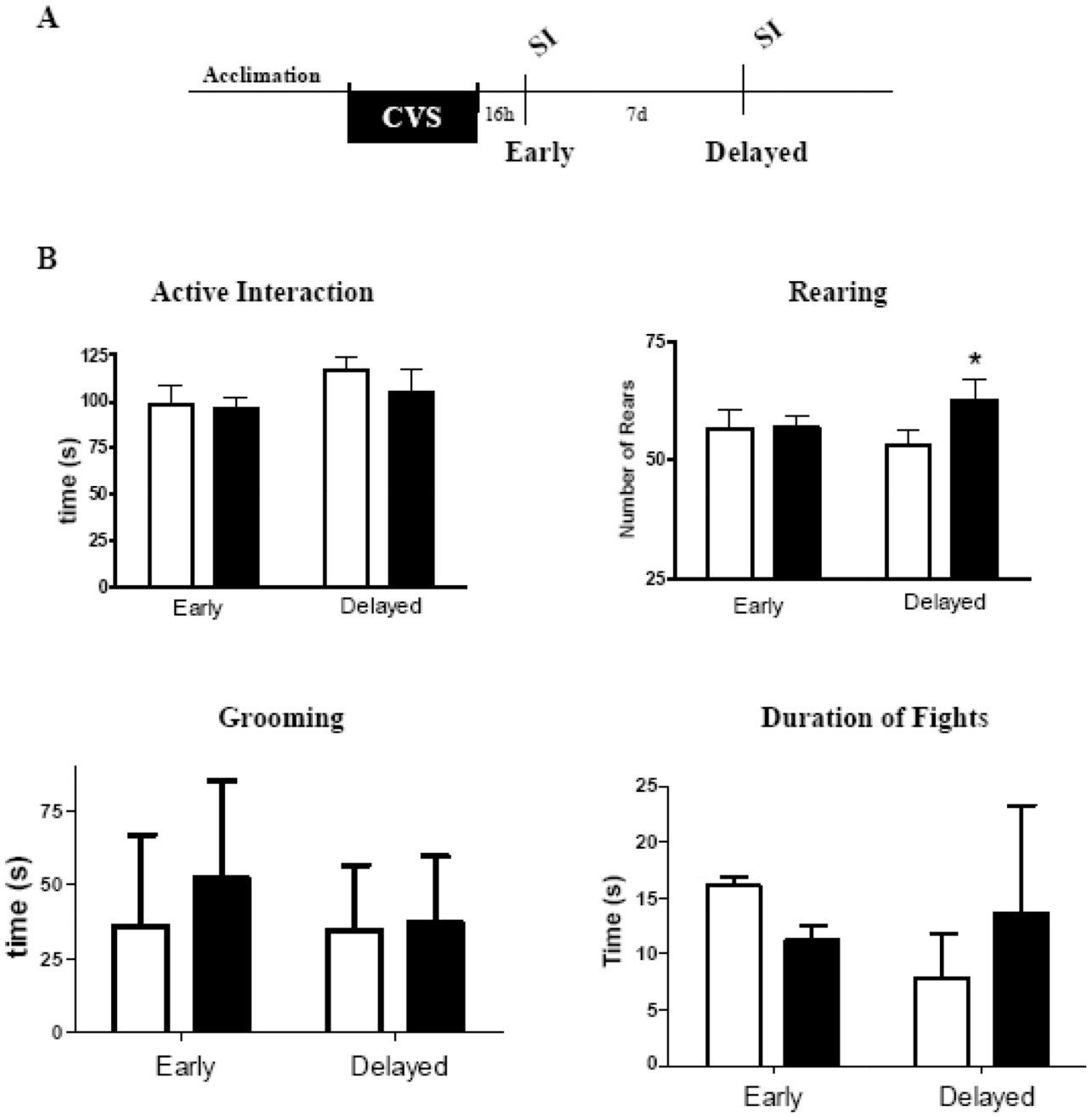

Fig. 4.

Social interaction is not affected by CVS exposure. **(A)** Social Interaction testing schedule schematic. **(B)** Panel shows active interaction, rearing, grooming and duration of fights in CVS and control rats at early and delayed recovery. Data are represented as mean ± SEM values; n = 12 animals/group, * $p<0.05$ versus control

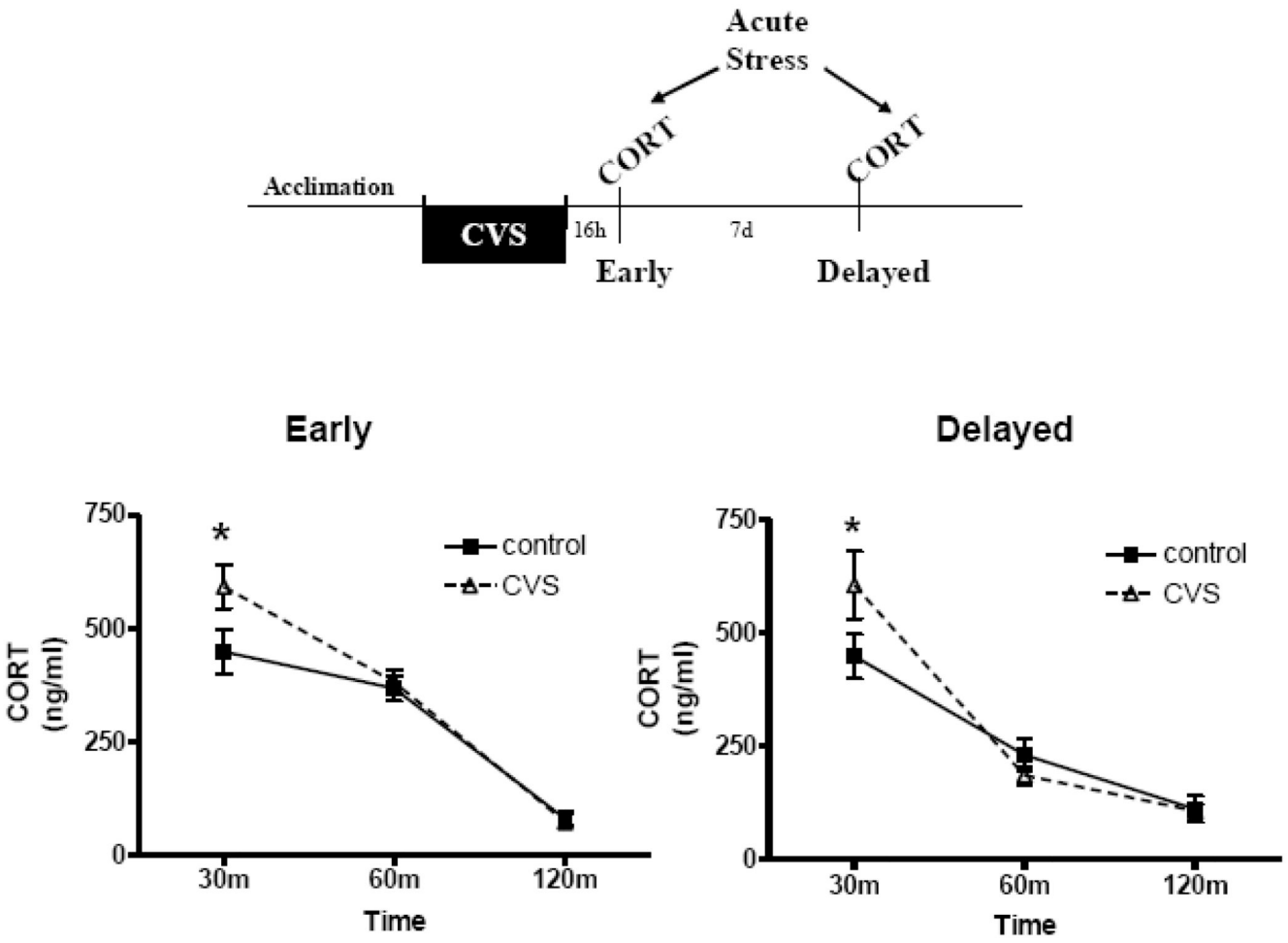

Fig. 5.

Rats exposed to chronic variable stress exhibit sensitized plasma corticosterone response to a novel acute acoustic stressor. **(A)** Acute stress response testing schedule schematic **(B)** Plasma corticosterone levels at 30, 60 and 120 min after initiation of stressor at early (left panel) or delayed (right panel) recovery post CVS. Values represent mean ± SEM; n=10–12 animals per group, * $p < 0.05$ versus control group.

Table 1

Physiological measures during recovery from CVS.

	Recovery	
	Early (16 hr)	**Delayed (7d)**
BW change (%)		
Control	6.0 ± 0.74	13.65 ± 1.65
CVS	0.215 ± 0.08 *	8.40 ± 0.85 *
Thymus Wt (mg/gm BW)		
Control	111.23±8.6	97.42±8.40
CVS	86.9±8.40 *	96.69±9.49
Adrenal Wt (mg/gm BW)		
Control	17.27±0.76	16.30±1.74
CVS	19.40±1.05 *	16.45±1.49

Data represent mean ± SEM from 12–20 rats per group. Rats were exposed to CVS for 7 day or were unstressed controls. At each recovery point, body weights were determined prior to any experimental manipulation. Time points reflect the length of time after cessation of stress.

* CVS is significantly different from corresponding control group ($p<0.05$)