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Intracerebroventricular D-Pen^2, D-Pen^5-enkephalin administration soon after stressor imposition influences behavioral responsivity to a subsequent stressor encounter in CD-1 mice

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Abstract

Endogenous opioid peptide systems diminish stress-induced autonomic nervous system, neuroendocrine (hypothalamic-pituitary-adrenal axis) and behavioral responses, attenuating a collection of physiological symptoms basic to emotional and affective states. Neurogenic stressors may incite specific central changes in opioid peptide availability as well as changes in mu and delta-opioid receptor function. The present investigation evaluated the proactive influence of an intracerebroventricular injection of the opioid receptor agonist D-Pen^2, D-Pen^5-enkephalin (DPDPE) (0 μg, 0.005 μg, 1.0 μg or 2.5 μg) on locomotor behavior of mice following uncontrollable footshock (Shock) or novel shock chamber exposure (No Shock). It was expected that DPDPE administration following Shock on Day 1 would restore locomotor activity up to 1 week and prevent shock-associated behavior of mice encountering a brief session of footshock 18 days later. Exposure to Shock reduced horizontal locomotor and vertical locomotor (rearing) activity of mice while 2.5 μg DPDPE restored behavior. Eighteen days following Shock and DPDPE challenge, mice were exposed to either an abbreviated session of footshock (Mild Stress) or the shock chamber (Cues). Mice in the No Shock and Shock groups administered 2.5 μg DPDPE on Day 1 did not exhibit any locomotor deficits in response to Mild Stress on Day 18. Mice in the Shock group administered 0.005 μg DPDPE on Day 1, did not exhibit exaggerated rearing deficits following ensuing Mild Stressor encounter relative to mice reexposed to Cues on Day 18. Taken together, these data show that (a) footshock differentially affects rearing and locomotor activity, (b) DPDPE administration increases locomotor activity for up to 1 week following footshock and DPDPE administration, (c) reexposure to Mild Stress affects rearing and locomotor performance differently depending on previous stressor history and DPDPE dose, (d) DPDPE affords long-lasting protection to previously non-stressed mice against the deleterious effects of subsequent mild stress on locomotor activity, while a low dose of DPDE is sufficient to prevent shock-induced sensitization of rearing deficits, 18 days following original stressor and drug presentation. Finally, our investigation demonstrates that DPDPE administration alters the behavioral impact of future stressful encounters and emphasizes the importance of investigating opioid mechanisms in chronic stress disorders.

Keywords: Opioid; Footshock; Horizontal locomotion; Rearing; Dose; DPDPE; Contextual learning; Cues; Memory; Sensitization

1. Introduction

Experiential variables influence organismic responsivity to aversive life events and pharmacological challenge (Antelman et al., 1989; Kuribara, 1996; Ohmori et al., 1995). The nature and severity of stressors induce brain region specific neurochemical release, influence profiles of behavioral alteration and contribute to the enduring neurochemical responses following stressor exposure (Pierce and Kalivas, 1997). Neurogenic (i.e.,

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footshock) and psychogenic (i.e., novelty) stressors provide behavioral and neurochemical indices of affective and motivational deficits attending depression and anxiety (Blanchard et al., 1993; Zacharko et al., 1998), following acute (Adamec and Shallow, 1993; Van Dijken et al., 1992) or chronic stressor challenge (Blanchard et al., 1993; Prasad et al., 1995). Stressor-induced alterations in central neurotransmitter and putative neurotransmitter activity, including dopamine (DA) (Bjijou et al., 1996) and enkephalin (Kalivas, 1985; Kalivas et al., 1985) among others (Cador et al., 1993; Kim and Vezina, 1998), are well documented and underlie stressor-associated alterations of shuttle escape (Kokkinidis et al., 1986), intracranial self-stimulation (Zacharko et al., 1998), startle (Gifkins et al., 2002) and horizontal locomotor activity (Vezina et al., 1989). In this respect, prospective pharmacological interventions, which ameliorate stressor-associated behavioral and neurochemical deficits in animals may attenuate the neuronal processes that underlie psychopathology in humans.

There is sufficient evidence to suggest involvement of endogenous opioids in the pathophysiology of depression, anxiety and post-traumatic stress disorder (PTSD). Indeed, endogenous opioids contribute to various neurobiological processes following stressor imposition including fear, anxiety, memory, ambulation (locomotion) and mood, as well as the modulation of hormonal, respiratory, cardiovascular, autonomic and immune function (Olson et al., 1998). Individuals with severe, chronic anxiety disorders display perturbations in cerebrospinal fluid (CSF) β-endorphin levels coinciding with hypothalamic dysfunction (Baker et al., 1997; Darko et al., 1992; Eriksson et al., 1989; Goodwin et al., 1993). Among clinically stable combat veterans with PTSD, mean CSF β-endorphin levels were significantly greater relative to normal control subjects. However, β-endorphin CSF and serum levels were decreased in patients displaying intrusive and avoidant symptoms of PTSD. Taken together, fluctuations in central and peripheral β-endorphin levels among individuals with PTSD suggest that while low chronic endogenous opioid levels may perpetuate the pathogenesis of the disorder, hypersecretion of opioids in the central nervous system may serve to blunt emotional disturbances provoked by stressor exposure and serve as an adaptive response following severe traumatic life events (Baker et al., 1997; Hoffman et al., 1989). Furthermore, exposure of human subjects to emotionally salient images has been associated with enhanced vascular flow in the prefrontal cortex and the lateral amygdaloid nucleus and reduced μ-receptor binding in these central identical sites (Liberzon et al., 2002). Delta (δ)-opioid receptor deficient mice or preproenkephalin gene-deficient mice demonstrate increased anxiety in the light–dark test and an animal profile reminiscent of depression in the forced swim paradigm relative to mu (μ) opioid receptor knock-out mice (Filliol et al., 2000; Gaveriaux-Ruff and Kieffer, 2002). Clearly, central μ- and δ-opioid receptors contribute to the immediate and long-term repercussion(s) of initial and ensuing stressor exposure(s).

Exposure of animals to severe stressors, including inescapable footshock, may promote the manifestation of behavioral features characteristic of severe anxiety conditions. Investigators have proposed that the long-lasting behavioral changes observed in animals following repeated exposures to situational reminders of the initial intense traumatic event serves as a model for conditioned fear (Louvart et al., 2005; Pynoos et al., 1996). Exposure to uncontrollable footshock is associated with a 40–50% reduction of met- and leu-enkephalin levels in whole rat brain (McGivern et al., 1983) and a comparable met-enkephalin reduction in the ventral tegmental area (VTA) (Kalivas et al., 1988). In mice, systemic administration of the enkephalinase-inhibitor, RB 101, attenuates immobility to shock associated environmental cues (Baamonde et al., 1992). In rats, pretreatment with the opioid antagonist naloxone attenuates ambulation, rearing and sniffing among rats previously exposed to the visual, auditory and olfactory cues of conspecifics receiving footshock (Van den Berg et al., 1998). Indeed, behavioral responsivity of mice following uncontrollable footshock presentation may be modulated by manipulation of central enkephalin availability (Mendella and Zacharko, 1996; Zacharko et al., 1998). The present experiment determined whether central administration of the opioid receptor agonist, D-Pen^2, D-Pen^5-enkephalin (DPDPE), immediately following the termination of uncontrollable footshock would prevent chronic behavioral deficits of CD-1 mice to subsequent milder stressor experiences (e.g., situational reminders) encountered at protracted intervals. While previous investigations in this laboratory demonstrated that the acute behavioral events associated with DPDPE administration immediately following stressor imposition are dose and time dependent (Mendella and Zacharko, 1996), the present investigation sought to extend these findings and to determine whether DPDPE, effective for therapeutic restoration of acute stressor-induced deficits, was also an effective prophylactic agent able to avert subsequent stressor-induced pathology.

2. Materials and methods

2.1. Subjects

Naive, male, CD-1 mice ($n=96$) were obtained from Charles River, Canada (St. Constant, Quebec) at 5 weeks of age, group housed ($n=5$ per cage) with free access to food and water and maintained on a 12-h light–dark schedule (lights on 7 am; lights off 7 pm). The genetically diverse CD-1 outbred strain of mice has been routinely employed in this laboratory. Moreover, the behavioral heterogeneity (diversity) of these mice has been argued to make these animals suitable for studies reminiscent of human psychopathology (Matzel et al., 2003). The particulars of the experimental protocol were approved by the Carleton University Animal Care Committee in accordance with regulations pertaining to animal use detailed by the Canada Council on Animal Care.

2.2. Surgery

Mice had achieved an appropriate surgical weight of 35 g at 12 weeks of age. At that time, mice were anesthetized with sodium pentobarbital (Somnotol) and stereotaxically implanted

with a cannula (23-gauge hypodermic needle) in the lateral ventricle (A.P. +0.8 mm from Bregma, L. +0.7 mm from midline and V. −2.7 to −2.9 mm from a flat skull surface) secured with (3) jeweler screws and dental cement. Cannulae were fitted with a 30-gauge stylette protruding 0.5 mm from the guide cannula tip. Postoperatively, animals were individually housed, placed on a warm heating pad and supplemented with a wet mash diet for at least 3 days. Following postoperative recovery, mice were returned to the main animal area for 10 days prior to behavioral testing.

2.3. Apparatus

Horizontal and vertical activity was measured in black aluminum tubs (28 cm diameter × 32.5 cm high) (Carleton University Technology Center). Horizontal activity was recorded by interruption of photobeams mounted 0.5 cm above the floor of the tub. Vertical activity (rearing) was recorded by an independent series of photobeams positioned 7 cm above the tub floor. A Macintosh computer recorded behavioral indices on a Tub Monitor program (Schnabel Electronics, Saskatoon, Saskatchewan).

Footshock was administered in black, Plexiglas boxes (30 × 40 × 15 cm) with the floor of each box consisting of 0.32 cm stainless steel rods spaced 1 cm apart, connected in series by neon bulbs. The end walls of each box were lined with stainless steel plates connected in series with the grid floor. Footshock (150 μA, 60 × 6 s, 59 s inter-trial interval) was delivered by a microcomputer controlled 3000-V source (Carleton University Science Technology Center). The footshock parameters were chosen based on previous investigations in this laboratory demonstrating that exposure of mice to acute footshock of this intensity was associated with (a) mesocorticolimbic alterations in dopamine, norepinephrine and serotonin levels (Shanks et al., 1990) and (b) perturbations in reward (Hebb and Zacharko, 2003a), anxiety (MacNeil et al., 1997) and locomotor activity (Hebb and Zacharko, 2003b). Moreover, exposure of mice to an acute session of footshock of relative severity will maximize conditioned fear to environmental context-shock pairings following a single pre-exposure to footshock 18 days earlier (Rau et al., 2005).

2.4. Behavioral testing

Following postoperative recovery, all mice were introduced to activity chambers and baseline horizontal locomotor activity and rearing scores were established. Behavioral measures were recorded at 3-min intervals over a 30-min test session. In particular, animals were tested for 15 min (15-min test session) and then briefly removed from the tub and handled according to a protocol that simulated intracerebroventricular drug administration. Mice were manually restrained, stylettes removed, and an injector, albeit no drug was administered, was inserted in the cannula. Animals were then reintroduced to the activity chambers and behavioral assessment lasted for an additional 15-min (30-min test session) trial block. This protocol was followed for 3 consecutive days and the data derived on Days 2 and 3 were averaged and employed as baseline measures.

Following the establishment of baseline activity, independent groups of mice were exposed to footshock (Shock, $n=48$) or were placed in the shock apparatus for an equivalent period of time (i.e., 66 min) but footshock was withheld (No Shock, $n=48$). Following Shock or No Shock treatment, mice were immediately placed in the activity chambers and horizontal locomotor activity and rearing scores were assessed over 15 min. Mice were subsequently removed from the testing chambers and injected intracerebroventricularly ($n=12$/treatment cell) with physiological saline (0 μg DPDPE) or DPDPE (0.005 μg, 1.0 μg or 2.5 μg). Intracerebroventricular injections were delivered in a 1 μl volume of sterile saline over a 1-min period employing a 5 μl Hamilton syringe connected to a 30-gauge injector by polyethylene tubing. Following DPDPE administration, the microinjector was left in place for an additional minute to facilitate drug diffusion. The stylette was replaced and each mouse was returned to the activity chamber for an additional 15 min (30-min test session). Behavioral measures were re-assessed for 30 min in accordance with a protocol employed to simulate Day 1 intracerebroventricular drug administration, 24 h (Day 2) and 168 h (Day 7) following initial stressor (Shock or No Shock) and DPDPE administration. All mice were returned to the animal housing area between behavioral tests. Eighteen days following the initial stressor (Shock or No Shock) and central DPDPE administration (i.e., 11 days following the 168 h behavioral test), mice ($n=6$/treatment cell) were exposed (i.e., Reexposure condition) to either a brief session of footshock (Mild Stress) (consisting of 6 × 6 s, 150 μA, 59 s inter-trial interval footshock presentations) or merely exposed to the shock apparatus (Cues, 6 min), immediately prior to a 15-min horizontal locomotor activity and rearing evaluation. Animals were then briefly removed from the tubs and handled in a manner simulating intracerebroventricular drug administration on Day 1, although no drug was administered. Behavioral measures were then re-assessed for an ensuing 15 min (30-min test session). Horizontal activity and rearing were re-evaluated 24 (Day 19) and 168 h (Day 24) following reexposure of animals to a Mild Stressor or Cues previously associated with footshock for two concurrent 15-min test sessions in accordance with the protocol simulating intracerebroventricular drug administration on Day 1. The intervals for stressor imposition and DPDPE administration were based on previous investigations in this laboratory (Hebb and Zacharko, 2003a,b).

2.5. Histology

All mice were overdosed with sodium pentobarbital (Somnotol) and perfused intracardially with physiological saline followed by a 10% formalin solution. Brains were excised from the cranial cavity and placed in formalin for at least 1 week prior to histological assessment. Brains were frozen, blocked in a rostral-caudal plane and sectioned on a microtome. Coronal sections (40 μm) were stained with cresyl violet and ventricular cannula placement was verified.

2.6. Statistical analyses

The horizontal locomotor activity and rearing data corresponding to the average baseline as well as the post-baseline test sessions were subjected to independent analyses of variance (ANOVAs) for horizontal locomotor activity or rearing as a 2 (Stressor: Shock or No Shock) × 4 (Drug: Saline, 0.005 μg DPDPE, 1.0 μg DPDPE or 2.5 μg DPDPE) × 2 (Reexposure Condition: Mild Stressor or Cues) design with repeated measures over Day; i.e., Immediate (Day 1), 24 h (Day 2) and 168 h (Day 7); Reexposure at Immediate (Day 18), 24 h (Day19) and 168 h (Day 24), with repeated measures over Test Session (15 min or 30 min). The inclusion of the 15-min and 30-min test sessions on subsequent test days (Days 2, 7,

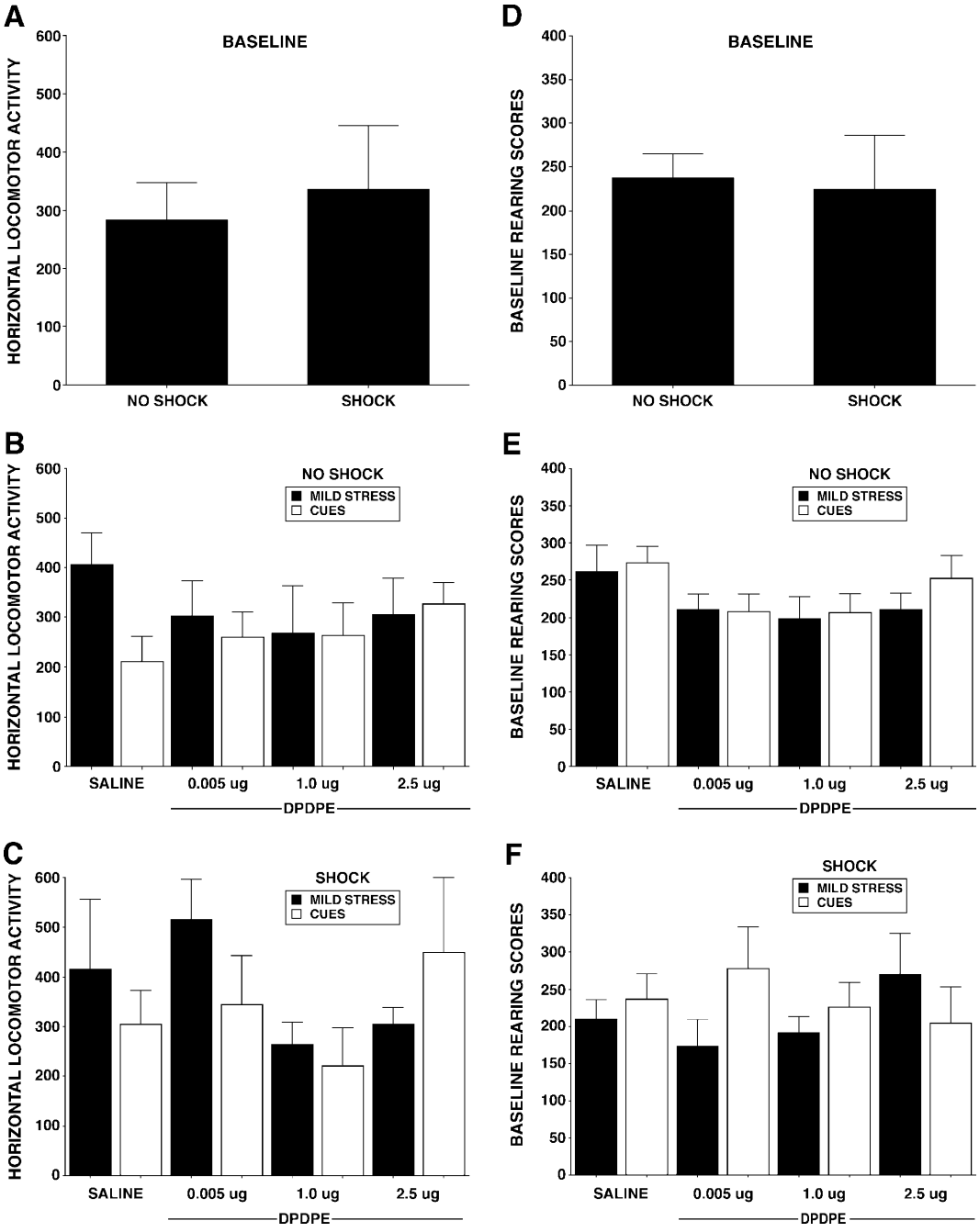


Fig. 1. Mean (±SEM) basal horizontal locomotor activity (A, B and C) and rearing (D, E and F) scores during the first 15 min of the behavioral test among mice prior to experimental group assignment. (A) Mean (±SEM) baseline horizontal locomotor activity scores among mice in No Shock ($n=48$) and Shock ($n=48$) groups prior to experimental treatment. (B) Mean (±SEM) baseline horizontal locomotor activity scores among mice in the No Shock groups prior to experimental treatment ($n=6$ per treatment cell). (C) Mean (±SEM) baseline horizontal locomotor activity scores among mice in the Shock groups prior to experimental treatment ($n=6$ per treatment cell). (D) Mean (±SEM) baseline rearing scores among mice in No Shock ($n=48$) and Shock ($n=48$) groups prior to experimental treatment. (E) Mean (±SEM) baseline rearing scores among mice in the No Shock groups prior to experimental treatment ($n=6$ per treatment cell). (F) Mean (±SEM) rearing scores among mice in the Shock groups prior to experimental treatment ($n=6$ per treatment cell). There were no statistically significant differences among groups prior to experimental manipulations.

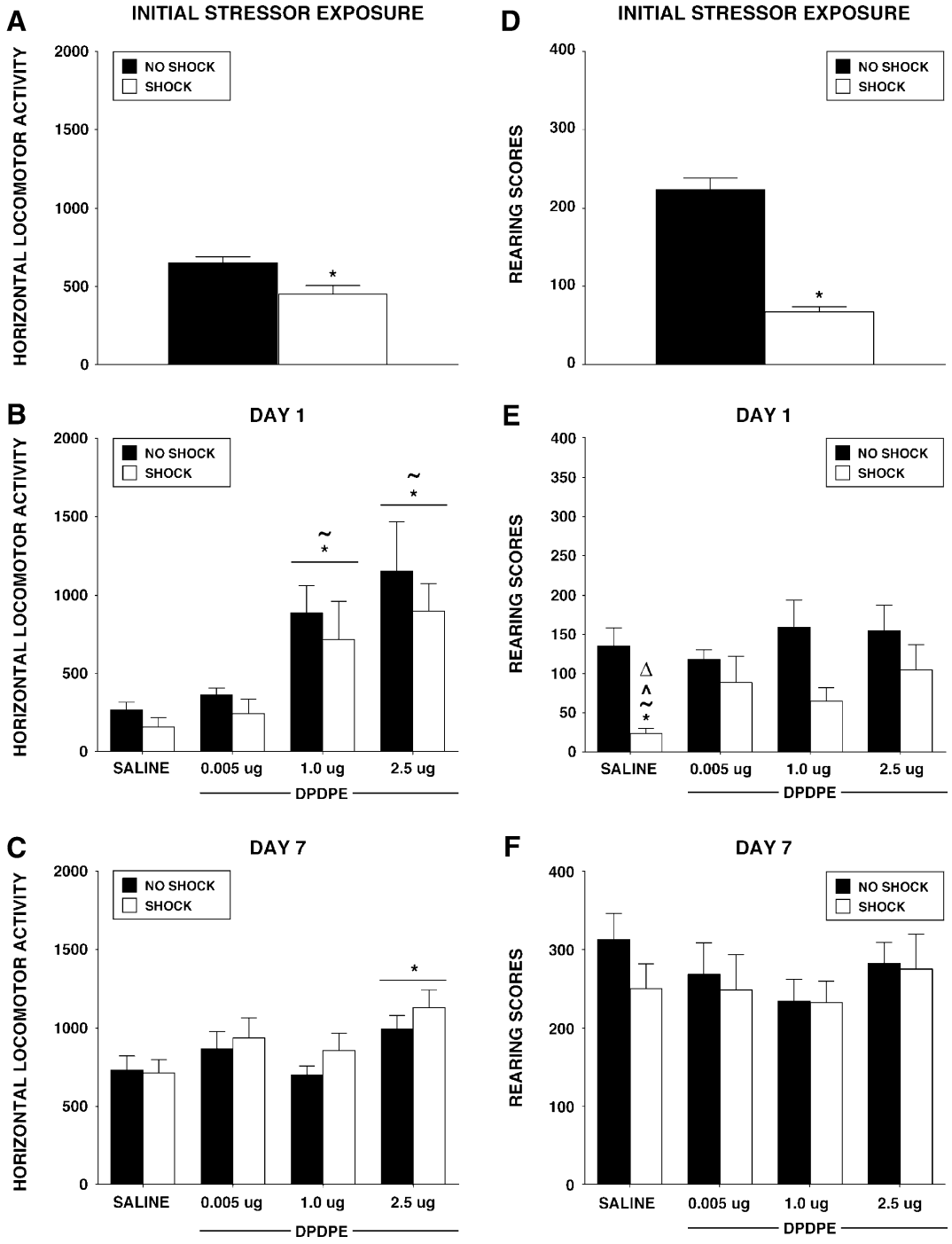


Fig. 2. Mean (±SEM) horizontal locomotor activity and rearing scores following stressor and DPDPE administration during the immediate 15-min behavioral test session. (A) Horizontal locomotor activity was reduced in mice immediately following footshock (Shock) relative to horizontal locomotor activity of mice in the No Shock condition (*$p<0.05$) during the immediate 15-min test session following the termination of the Stressor. (B) DPDPE administration dose-dependently increased horizontal locomotor activity among mice in the No Shock and Shock conditions during the 15-min test session immediately following drug administration on Day 1. Among Saline treated animals, the decrease in locomotor activity following Shock relative to No Shock animals has dissipated. The * and ~ indicate a statistically significant increase in horizontal locomotor activity of mice treated with 1.0 μg and 2.5 μg DPDPE relative to saline and 0.005 μg DPDPE, respectively. (C) Mice administered 2.5 μg DPDPE demonstrated enhanced horizontal locomotor activity during the initial 15-min test session (shown) and subsequent 30-min tests session (not shown) following a 5-day period of acquiescence (Day 7) relative to saline treated mice (*$p<0.05$). (D) Rearing was reduced in mice following footshock (Shock) relative to rearing of mice in the No Shock condition (*$p<0.05$) during the immediate 15-min test session. (E) Rearing was decreased in Shock/Saline mice relative to No Shock/Saline (*$p<0.05$), Shock/0.005 μg DPDPE ($^{\wedge}p<0.05$), Shock/1.0 μg DPDPE ($^{\sim}p<0.05$) and Shock/2.5 μg DPDPE ($^{\Delta}p<0.05$) treated mice on Day 1 during the immediate 15-min test session following DPDPE administration. Unlike horizontal locomotor activity (see panel B), the effect of footshock was still apparent on rearing performance in saline treated mice 30 min later following Shock relative to mice previously exposed to No Shock conditions. (F) There were no differences in rearing performance attributable to experimental manipulations 168 h (Day 7) following stressor and drug execution. The data presented represent the first 15-min test session.

18, 19 and 24) was employed to identify relevant temporal intervals that may incite contextual conditioning specific to the temporal presentation of Stressor and Drug treatments on Day 1. ANOVA of the horizontal locomotor activity or rearing scores (Stressor × Drug: Days 1, 2 and 7; Stressor × Drug × Reexposure: Days 18, 19 and 24) with repeated measures over Test Session were also conducted at each post-stressor test interval. Significant ANOVA results were further analyzed using Tukey's honestly significant different (HSD) multiple comparisons ($\alpha = 0.05$) where appropriate.

3. Results

3.1. Histology

Histological analyses verified cannula position in the lateral ventricle of all animals included in statistical analyses of locomotor and rearing behavior following experimental conditions ($n = 96$). Mice were not included in the statistical analyses due to cannulae positioned outside of the lateral ventricle ($n = 5$), severe tissue necrosis ($n = 1$) or drug diffusion ($n = 1$).

3.1.1. Baseline data

Mice assigned to No Shock ($n = 48$) or Shock ($n = 48$) conditions on Day 1 did not differ on mean baseline measures of horizontal locomotor activity (Fig. 1A) or rearing (Fig. 1D) during the 15-min test session, F's < 1. However, horizontal locomotor activity and rearing were reduced during the 30-min test session relative to the initial 15-min test session, indicative of habituation, [$F_{1, 80} = 32.48$, $p < 0.001$] and [$F_{1, 80} = 230.5$, $p < 0.001$], respectively (data not shown). Mice which were subdivided to the various Stressor, Drug and Reexposure conditions ($n = 6$ per 16 treatment cells) did not differ on baseline horizontal locomotor measures (Fig. 1B, C) or rearing (Fig. 1E and F, [$p > 0.05$]). However, the partitioning of subjects from 2 (Shock and No Shock) into 16 ($2 \times 4 \times 2$) independent groups induced some variability among baseline horizontal locomotor activity (Matzel et al., 2003) (Fig. 1B and C) and rearing (Fig. 2E and F). Group variability scores did not produce fluctuations consistent with a violation of homogeneity of variance arguments. Documented locomotor activity scores following neurogenic stressor imposition and opioid manipulation among CD-1 mice accordingly provided cogent evidence favoring opioid modulation of stressor responsivity.

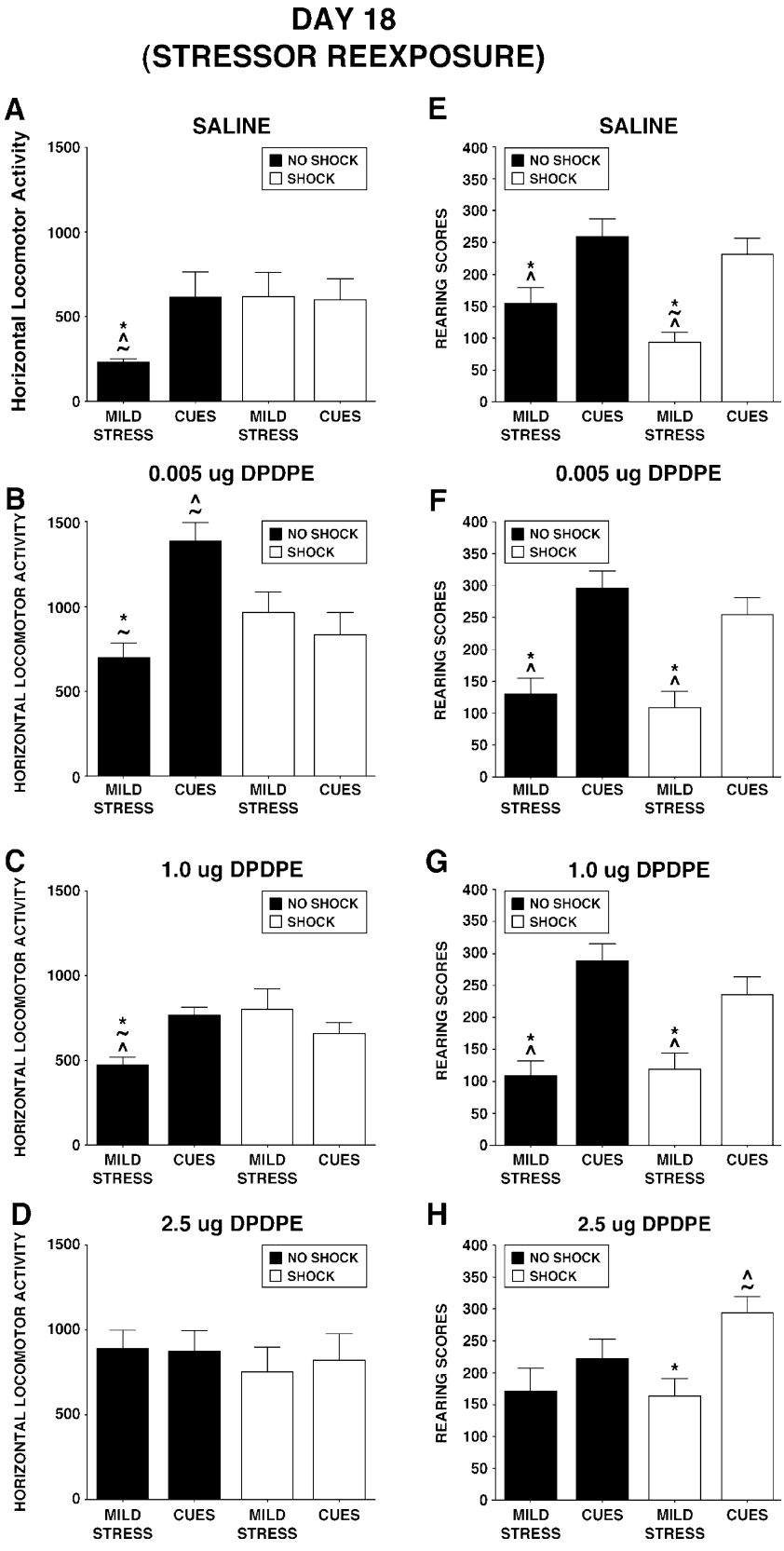
3.1.2. Experimental data

3.1.2.1. Days 1, 2 and 7. Mice in the Shock condition exhibited lower horizontal locomotor scores relative to mice in the No Shock condition immediately post-stressor on Day 1 during the 15-min test session [Stress $F_{1, 94} = 8.74$, $p < 0.01$] (see Fig. 2A). Shocked mice also displayed reduced rearing behavior during the immediate 15 min following shock relative to non-shocked mice [Stressor $F_{1, 94} = 91.93$, $p < 0.001$] (see Fig. 2D). DPDPE increased horizontal locomotor activity among shocked and non-shocked mice immediately following drug administration on Day 1. The performance of mice treated with either 1.0 μg or 2.5 μg DPDPE was elevated relative to the behavioral scores of Saline or 0.005 μg DPDPE-challenged mice immediately post-drug administration [Drug, $F_{3, 88} = 10.41$, $p < 0.001$] (see Fig. 2B). Shocked mice challenged with saline continued to display reduced rearing scores relative to non-shocked mice [Stressor $F_{1, 88} = 11.24$, $p < 0.001$]. Shocked mice treated with 0.005 μg DPDPE, 1.0 μg DPDPE or 2.5 μg DPDPE exhibited elevated rearing scores relative to saline treated shocked mice. There were no significant

Fig. 3. Mean (±SEM) horizontal activity and rearing scores among mice with prior stressor (Shock or No Shock) and DPDPE (saline, 0.005 μg, 1.0 μg or 2.5 μg) experience 18 days following initial stressor encounter and opioid receptor activation. Mice were exposed to Mild Stress (6 min of uncontrollable footshock) or merely reexposed to the shock apparatus in which the stressor was originally imposed (Cues) and horizontal locomotor activity and rearing was immediately assessed for 15 min. (A) Horizontal locomotor activity of No Shock/Saline/Mild Stress mice was depressed relative to horizontal locomotor activity of mice in the No Shock/Saline/Cues (*$p < 0.05$), Shock/Saline/Mild Stress (˜$p < 0.05$) and Shock/Saline/Cues (^$p < 0.05$) conditions. (B) Horizontal locomotor activity of No Shock/0.005 μg DPDPE/Mild Stress mice was depressed relative to horizontal locomotor activity of mice in the No Shock/0.005 μg DPDPE/Cues (*$p < 0.05$), and Shock/0.005 μg DPDPE/Mild Stress (˜$p < 0.05$) conditions, while horizontal locomotor activity of mice in the No Shock/0.005 μg DPDPE/Cues condition was elevated relative to horizontal locomotor activity of mice in the Shock/0.005 μg DPDPE/Cues (*$p < 0.05$) and Shock/0.005 μg DPDPE/Mild Stress (^$p < 0.05$) conditions. (C) Horizontal locomotor activity of No Shock/1.0 μg/Mild Stress mice was depressed relative to horizontal locomotor activity of mice in the No Shock/1.0 μg/Cues (*$p < 0.05$), Shock/1.0 μg/Mild Stress (˜$p < 0.05$) and Shock/1.0 μg/Cues (^$p < 0.05$) treatment conditions. (D). There were no differences in horizontal locomotor activity among mice administered 2.5 μg DPDPE attributable to Stressor or Reexposure conditions. (A–D) Examination of horizontal locomotor scores of No Shock mice administered 0 μg, 0.005 μg or 1.0 μg DPDPE and subsequently exposed to Mild Stress revealed an effect of Mild Stress in previously Shock naïve mice, which was exacerbated in saline treated mice and not observed in mice treated with the highest dose of DPDPE. Indeed, 2.5 μg DPDPE prevented the deficits associated with Mild Stress presentation in previously shock naïve animals. (E) Rearing of Shock/Saline/Mild Stress mice was depressed relative to rearing of mice in the No Shock/Saline/Mild Stress (˜$p < 0.05$), Shock/Saline/Cues (*$p < 0.05$) and No Shock/Saline/Cues (^$p < 0.05$) treatment conditions. Rearing of mice in the No Shock/Saline/Mild Stress group was decreased relative to rearing of mice in the No Shock/Saline/Cues (*$p < 0.05$) and Shock/Saline/Cues (^$p < 0.05$) conditions. (F) Rearing of mice in the No Shock/0.005 μg DPDPE/Mild Stress group was decreased relative to rearing of mice in the No Shock/0.005 μg DPDPE/Cues (*$p < 0.05$) and Shock/0.005 μg DPDPE/Cues (^$p < 0.05$) conditions. Rearing of mice in the Shock/0.005 μg DPDPE/Mild Stress group was decreased relative to rearing of mice in the Shock/0.005 μg DPDPE/Cues (*$p < 0.05$) and No Shock/0.005 μg DPDPE/Cues (^$p < 0.05$) conditions. (G) Rearing of mice in the No Shock/1.0 μg DPDPE/Mild Stress group was decreased relative to rearing of mice in the No Shock/1.0 μg DPDPE/Cues (*$p < 0.05$) and Shock/1.0 μg DPDPE/Cues (^$p < 0.05$) conditions. Rearing of mice in the Shock/1.0 μg DPDPE/Mild Stress group was decreased relative to rearing of mice in the Shock/1.0 μg DPDPE/Cues (*$p < 0.05$) and No Shock/1.0 μg DPDPE/Cues (^$p < 0.05$) conditions. (H) Rearing of mice in the Shock/2.5 μg DPDPE/Cues was elevated relative to rearing of mice in the Shock/2.5 μg DPDPE/Mild Stress (*$p < 0.05$), No Shock/2.5 μg DPDPE/Mild Stress (^$p < 0.05$) and No Shock/2.5 μg DPDPE/Cues (˜$p < 0.05$). All horizontal and rearing scores were obtained from the initial 15-min behavioral test. (E–H) Examination of rearing scores of No Shock mice administered 0 μg, 0.005 μg or 1.0 μg DPDPE and subsequently exposed to Mild Stress revealed an effect of Mild Stress in previously Shock naïve mice, irrespective of DPDPE dose, an effect not observed in mice treated with the highest dose of DPDPE. Indeed, 2.5 μg DPDPE prevented the rearing deficits associated with Mild Stress presentation in previously shock naïve animals.

differences in the rearing scores of non-shocked and shocked mice challenged with 0.005 μg DPDPE, 1.0 μg DPDPE or 2.5 μg DPDPE during the immediate test session on Day 1 (see Fig. 2E).

Twenty-four hours following stressor and DPDPE administration there were no differences in horizontal or vertical locomotor scores of mice (data not shown). However, an increase in horizontal locomotor activity among previously



non-shocked and shocked mice administered 2.5 μg DPDPE reemerged 168 h (Day 7) following stressor and drug application [Drug, $F_{3,\ 88}=3.22$, $p<0.05$)]. The horizontal locomotor performance of non-shocked and shocked mice treated with 2.5 μg DPDPE was elevated during the 15-min (see Fig. 2C) and 30-min (data not shown) test sessions relative to the behavioral scores of saline challenged animals. There were no significant differences in rearing attributable to experimental manipulation on this subsequent 168-h (Day 7 (see Fig. 2F) test session.

3.1.2.2. Days 18, 19 and 24. On Day 18, during the initial 15-min test session, horizontal locomotor activity of No Shock/Saline treated animals was depressed following reexposure to Mild Stress relative to No Shock/Saline mice reexposed to Cues, as well as relative to Shock/Saline mice reexposed to either the Mild Stressor or Cues previously associated with the neurogenic stressor (see Fig. 3A). The horizontal activity of No Shock/0.005 μg DPDPE/Mild Stressor treated mice was increased relative to No Shock/Saline/Mild Stressor treated mice (see Fig. 3A and B). The horizontal activity of No Shock/0.005 μg DPDPE-treated mice reexposed to stressor-associated Cues was elevated relative to that of No Shock/0.005 μg DPDPE mice exposed to the Mild Stressor and the Shock/0.005 μg DPDPE treatment group (see Fig. 3B). Among No Shock/Saline (see Fig. 3A), No Shock/0.005 μg DPDPE (see Fig. 3B), and No Shock/1.0 μg DPDPE-treated mice (see Fig. 3C), reexposure to the Mild Stressor decreased horizontal activity within 15 min relative to mice in the Cue reexposure treatment condition and given the identical DPDPE dose. Horizontal locomotor activity of No Shock/Saline, No Shock/0.005 μg DPDPE and No Shock/1.0 μg DPDPE-treated mice reexposed to Mild Stress was also decreased relative to shocked mice reexposed to either Cues or the Mild Stressor in the identical drug condition. In contrast, there were no significant differences among No Shock or Shock animals previously challenged with 2.5 μg DPDPE on Day 1 (see Fig. 3D) [Drug, $F_{3,\ 80}=3.65$, $p<0.05$; Stressor × Reexposure, $F_{1,\ 80}=4.48$, $p<0.05$]. Closer examination of the horizontal locomotor scores of mice in the No Shock stressor treatment group following Reexposure to either the Mild Stressor or the Cues previously associated with the stressor on Day 18 revealed intriguing data. Administration of 2.5 μg DPDPE immediately following No Shock treatment on Day 1 protected mice against the deleterious effect of the Mild Stressor on Day 18 [Drug, $F_{3,40}=5.63$, $p<0.01$, Reexposure $F_{1,\ 40}=8.57$, $p<0.01$] (see Fig. 3D). In comparison, mice administered Shock on Day 1 did not demonstrate any locomotor deficits on Day 18, relative to mice in the No Shock/Saline/Cues treatment condition, irrespective of the DPDPE dose (see Fig. 3). In all instances, mice displayed reduced horizontal and vertical activity during the second 15-min test session (30 min) relative to the initial 15-min test session indicative of habituation of the locomotor response to the test environment [Test Session, $F_{1,\ 80}=102.2$, $p<0.001$ and $F_{1,\ 80}=132.5$, $p<0.001$, respectively] (data not shown).

Among the No Shock and Shock mice administered saline on Day 1, reexposure to the Mild Stressor on Day 18 reduced rearing relative to No Shock/Saline and Shock/Saline mice reexposed to the stressor associated Cues. Moreover, Shock/Saline/Mild Stressor exposed mice displayed an exaggerated reduction in rearing relative to No Shock/Saline/Mild Stress treated mice (see Fig. 3E). No Shock and Shock exposed mice administered 0.005 μg DPDPE on Day 1 and reexposed to the Mild Stressor on Day 18 demonstrated reduced rearing relative to No Shock/0.005 μg DPDPE and Shock/0.005 μg DPDPE treated mice reexposed to the stressor associated Cues. However, there were no significant differences in rearing of the No Shock/

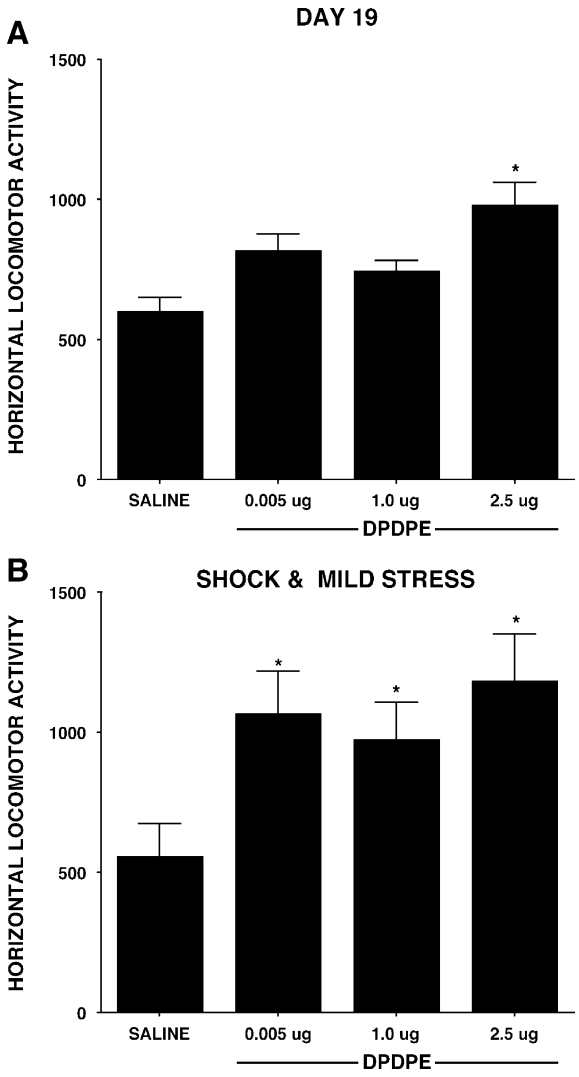


Fig. 4. Horizontal locomotor activity of mice during the initial 15-min test session 24 h (Day 19) following reexposure to Mild Stress or Cues. (A) Mice treated with 2.5 μg DPDPE displayed elevated horizontal locomotor activity relative to saline, 0.005 μg, or 1.0 μg DPDPE (*$p<0.05$) treated mice irrespective of their past history of Shock or No Shock stressor treatment 24 h (Day 19) post-stressor reexposure during the immediate 15-min test session. Data is collapsed across No Shock and Shock treatments as well as collapsed across the reexposure conditions of Mild Stress or Cues. (B) Among previously shocked mice, DPDPE administration was associated with enhanced horizontal locomotor activity of mice relative to activity of mice treated with saline, during the initial 15-min test session 24 h following reexposure to Mild Stress (*$p<0.05$).

0.005 μg DPDPE/Mild Stressor and the Shock/0.005 μg DPDPE/Mild Stressor treated mice. Taken together these data suggest that the lowest dose of DPDPE was able to block expression of the exaggerated stressor-induced rearing deficits among previously shocked mice reexposed to the Mild Stressor (see Fig. 3F). Among No Shock and Shock mice administered 1.0 μg DPDPE on Day 1, reexposure to the Mild Stressor on Day 18 was associated with reduced rearing of mice relative to No Shock/1.0 μg DPDPE and Shock/1.0 μg DPDPE-treated mice reexposed to the stressor associated cues. Again, there were no significant differences in rearing among the No Shock/1.0 μg DPDPE/Mild Stressor and Shock/1.0 μg DPDPE/Mild Stressor-treated mice (see Fig. 3G). In contrast to the performance of saline, 0.005 μg DPDPE and 1.0 μg DPDPE-treated mice, administration of 2.5 μg DPDPE following No Shock on Day 1 preserved rearing performance of mice in the face of the Mild Stressor on Day 18. Moreover, administration of 2.5 μg DPDPE following Shock on Day 1 was associated with enhanced rearing of mice immediately following reexposure to stressor-associated Cues, relative to the 3 other 2.5 μg DPDPE groups, 18 days following initial stressor and drug manipulations (see Fig. 3H) [Reexposure, $F_{1, 80}=32.51$, $p<0.001$].

Mice administered 2.5 μg DPDPE displayed elevated horizontal activity independent of prior Stressor or Reexposure conditions relative to saline treated mice on Day 19, during the first 15-min test session (see Fig. 4A). The elevated activity scores among CD-1 mice were no longer in evidence by the end of the 30-min test session (data not shown). Mice in the

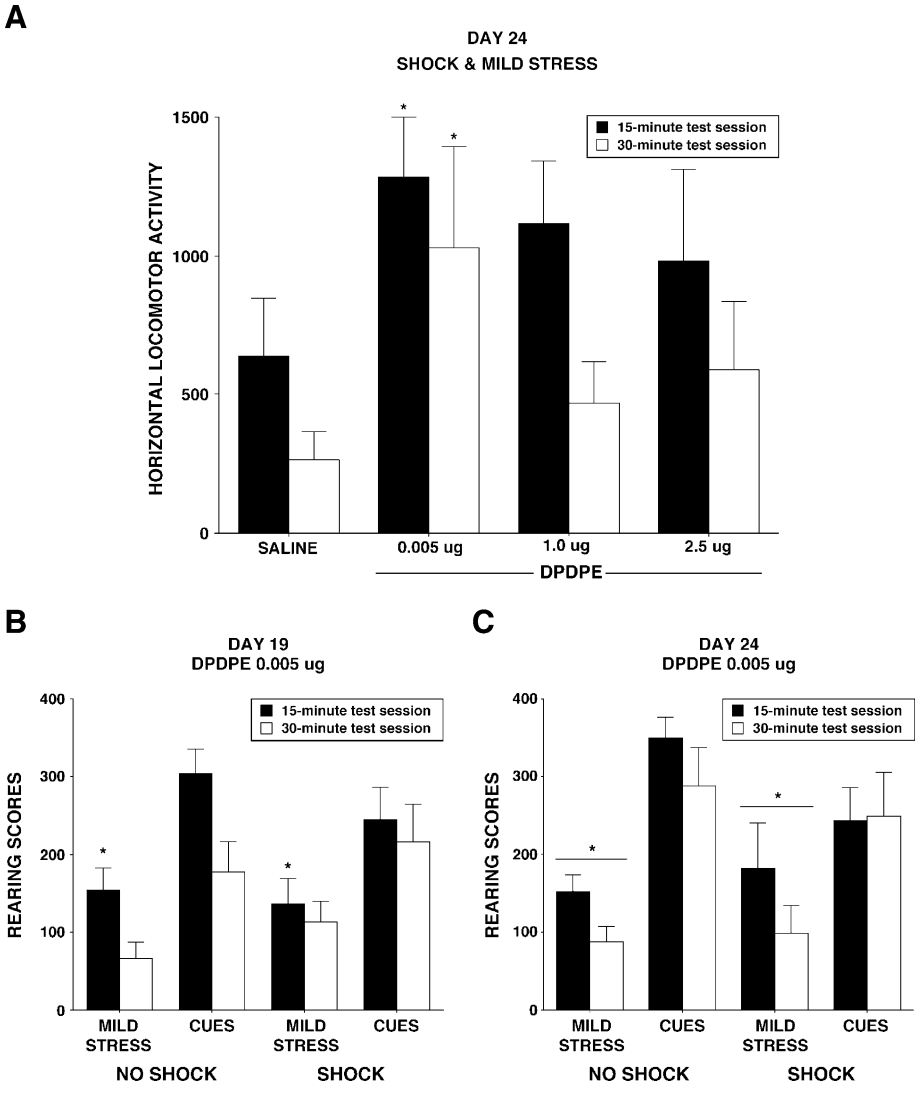


Fig. 5. Horizontal locomotor activity and rearing of mice during the initial 15-min and subsequent 30-min behavioral test session 19 and 24 days after the initial Stressor and DPDPE challenge on Day 1, and following exposure to Mild Stress/Cues on Day 18. (A) Previously stressed mice treated with 0.005 μg DPDPE displayed elevated horizontal locomotor activity during both test sessions on Day 24 relative to saline treated mice 7 days following reexposure to Mild Stress (*$p<0.05$). (B) Rearing performance of mice in the No Shock/0.005 μg DPDPE/Mild Stress treatment condition displayed reduced rearing relative to mice in the No Shock/0.005 μg DPDPE/Cues treatment condition. Mice in the Shock/0.005 μg DPDPE/Mild Stress treatment condition displayed reduced rearing relative to mice in the Shock/0.005 μg DPDPE/Cues treatment condition. These effects were evident during the initial 15-min test session occurring 24 h following stressor reexposure on Day 19 (*$p<0.05$). (C) Mice in the No Shock/0.005 μg DPDPE/Mild Stress treatment condition displayed reduced rearing relative to mice in the No Shock/0.005 μg DPDPE/Cues treatment condition. Mice in the Shock/0.005 μg DPDPE/Mild Stress treatment condition displayed reduced rearing relative to mice in the Shock/0.005 μg DPDPE/Cues treatment condition. These effects were evident during both 15-min test sessions occurring on Day 24 (*$p<0.05$).

Shock/0.005 μg DPDPE, Shock/1.0 μg DPDPE, and Shock/2.5 μg DPDPE groups displayed enhanced horizontal locomotor activity relative to Shock/Saline mice on Day 19 during the immediate 15-min test session, 24 h following reexposure to Mild Stress [Drug, $F_{3,40}=2.78$, $p<0.05$; Test Session, $F_{1,\ 80}=188.62$, $p<0.001$] (see Fig. 4B).

Among No Shock and Shock mice previously administered 0.005 μg DPDPE on Day 1, rearing was depressed 24 h (Day 19) following reexposure to the Mild Stressor relative to the rearing scores of mice exposed to stressor-associated Cues. These data are reminiscent of rearing performance on Day 18 (see Fig. 3F) and were confined to the 15-min test session [Reexposure, $F_{1,\ 80}=6.05$, $p<0.05$; Reexposure × Test Session interaction, $F_{1,\ 80}=5.03$, $p<0.05$] (see Fig. 5B). There were no differences in rearing among No Shock and Shock mice previously administered 0 μg, 1.0 μg or 2.5 μg DPDPE (data not shown) 24 h (Day 19) following reexposure to Mild Stress or Cues previously associated with the stressor.

There was an increase in horizontal locomotor activity among shocked mice previously administered 0.005 μg DPDPE 168 h (Day 24) following reexposure to the Mild Stressor relative to Shock/Saline/Mild Stressor treated mice (see Fig. 5A) during the initial 15-min test session [Test Session, $F_{1,\ 80}=254.98$, $p<0.001$, Drug × Test Session, $F_{3,\ 80}=3.68$, $p<0.05$, and Stressor × Drug × Test Session, $F_{3,\ 80}=2.62$, $p<0.05$], and No Shock mice in this identical drug and reexposure condition (data not shown). Shock/1.0 μg DPDPE/Mild Stress-treated mice exhibited enhanced horizontal locomotor activity relative to No Shock exposed mice (data not shown) in this identical drug and reexposure condition. There were no significant differences among mice in the No Shock and Shock/2.5 μg DPDPE conditions 168 h (Day 24) following reexposure to Mild Stressor or Cues associated with the stressor (data not shown).

Among 0.005 μg DPDPE-treated mice, 168 h following reexposure to Mild Stress, decreased rearing was evident relative to No Shock and Shock counterparts reexposed to stressor related Cues. This effect was evident during both the 15-min and 30-min test sessions [Drug × Test Session, $F_{3,\ 80}=2.69$, $p<0.05$; Drug × Reexposure, $F_{3,\ 80}=3.23$, $p<0.05$], an effect that appeared on Day 18 and persisted 168 h following Mild Stress reexposure (see Fig. 5C). Among mice in the No Shock/1.0 μg DPDPE, Shock/1.0 μg DPDPE, No Shock/2.5 μg DPDPE and Shock/2.5 μg DPDPE (data not shown) treatment conditions, there was no influence of reexposure treatments evident 168 h post-stressor reexposure.

4. Discussion

The present experiment established the enduring influence of stress and acute intracerebroventricular DPDPE administration on horizontal locomotor activity and rearing in mice. These data reveal that (a) footshock differentially affects horizontal locomotor activity and rearing, (b) DPDPE administration increases locomotor activity for at least 1 week, (c) reexposure to a brief but identical stressor episode affects horizontal and vertical activity depending on the previous shock and DPDPE history, (d) DPDPE affords protracted protection against the deleterious effects of a Mild Stressor on horizontal locomotor activity, and (e) a low dose of DPDPE 18 days earlier following initial stressor presentation is sufficient to prevent shock-induced sensitization of rearing deficits.

4.1. Neural mechanisms underlying the immediate and protracted effects of DPDPE on stress-induced behaviors

The observation that horizontal locomotor activity and rearing were reduced in CD-1 mice immediately following exposure to uncontrollable footshock and that DPDPE administration dose-dependently enhanced the performance of shocked and non-shocked animals during the immediate post-stressor interval is consistent with data previously collected in this (Mendella and Zacharko, 1996) and other laboratories (Katoh et al., 1990; Meyer et al., 1995; Meyer and Meyer, 1993). In the present investigation we have demonstrated that footshock and DPDPE administration differentially affects horizontal and vertical locomotor activity. Indeed, while DPDPE attenuated stressor-induced deficits in rearing, an effect that was independent of dose, DPDPE had no effect on rearing of non-shocked mice. The lack of a DPDPE influence on rearing among non-shocked mice is in accordance with data previously reported from this (Mendella and Zacharko, 1996), and other laboratories (Meyer and Meyer, 1993; Mickley et al., 1990). While there is an immediate decrease in horizontal and vertical locomotor activity within the first 15-min test session consistent with the decreases shown in the immediate 3-min stressor period previously reported from our laboratory (Mendella and Zacharko, 1996), the shock-induced deficits in vertical locomotor activity remain for 30 min following stressor imposition while horizontal locomotor activity is restored to pre-shock levels at the same temporal interval. DPDPE administration dose-dependently increased horizontal locomotor activity in both non-shocked and shocked animals. The dose-dependent increase in horizontal locomotor activity was not observed in rearing of non-shocked mice, with all doses restoring rearing behavior to pre-shock levels. The differential effect of shock and DPDPE on horizontal and rearing behavior suggests that these two measures are supported by differential central mechanisms, and not simply measures of a generalized pattern of motor activity. While horizontal locomotion may be a measure of motor activity in response to a novel environment supported by dopaminergic mechanisms in the forebrain, rearing may serve as indices of anxiety and fear, supported by non-dopaminergic mechanisms in the amygdala, hippocampus and cortex (Giovanni et al., 2001; Thiel et al., 1999). While the pharmacological actions of DPDPE on dopaminergic activity have been well-documented in previous investigations (Michael-Titus et al., 1989; Mickley et al., 1990) it should be considered that the excitatory influence of 1.0 μg and 2.5 μg DPDPE on horizontal locomotor activity among stressed and non-stressed mice on Day 1 may have been a result of enhanced mesocorticolimbic

dopamine (DA) activity. Indeed, in the rat intracerebroventricular administration of DPDPE induced an increase in DOPAC and DOPA concentrations in the nucleus accumbens (Manzanares et al., 1993). At this juncture it is not clear of the exact neurobiological underpinnings of the effect of footshock and novelty on the differential patterns of rearing and horizontal locomotor activity observed, although there is evidence to suggest that DA, GABA, glutamate and acetylcholine activity in the frontal cortex, hippocampus, amygdala and midbrain may play distinct roles. Moreover, activation of the VTA, nucleus accumbens and ventral palladium by DPDPE may contribute to increased horizontal locomotor activity while other brain areas would support rearing (Hooks and Kalivas, 1995). A role for enkephalin in modulating DA turnover in the nucleus accumbens following opioid peptide release in the VTA has been well documented (Kalivas, 1993; Kalivas and Abhold, 1987; Steiner and Gerfen, 1998; Suzuki et al., 1997). Notwithstanding, the contributions of separate neurochemical systems on horizontal and vertical locomotion are brief as the increase in horizontal locomotion subsides 24 h following stress and DPDPE administration.

In addition to replicating our previous findings showing that shock decreases horizontal and vertical locomotor activity and DPDPE dose-dependently increases horizontal locomotor activity in both shocked and non-shocked mice with no dose-dependent increase in rearing, we have demonstrated in this investigation enhanced horizontal locomotor activity following a 5-day respite period from behavioral testing at the 168-h test interval among 2.5 μg DPDPE treated mice irrespective of shock history. Data collected from some laboratories suggest that the neural mechanisms activated by stressor exposure contribute to the induction and maintenance of long-term behavioral change following subsequent exposure to motivationally relevant stimuli (Kalivas and Stewart, 1991; Le Moal and Simon, 1991; Vezina, 1996). Indeed, the enhanced locomotor response of mice challenged with 2.5 μg DPDPE following a period of acquiescence may be due in part to a reactivation of DA neurons. Indeed, Puglisi-Allegra et al. (1991) demonstrated that release from restraint stress resulted in enhanced mesolimbic DA levels, as well as DOPAC and HVA metabolite concentrations in rats displaying stress-reduced DA and DA metabolite levels, as measured by microdialysis. The authors attributed the elevation in DA activity to emotional arousal prompted by environmental change. It is conceivable that in the present investigation reintroduction of mice to the testing chambers reinstated DA activation previously encoded by 2.5 μg DPDPE. Such a neural memory effected a re-mergence of enhanced locomotor activity at the 168-h interval. However, the 168-h rearing scores of saline and DPDPE-treated mice were comparable regardless of prior stressor history. The failure of DPDPE to sustain elevations in rearing suggests that the transient increase in rearing among stressed mice following DPDPE on Day 1 did not reach a critical threshold (cf. DALA: Kalivas et al., 1985), favorable to the expression of conditioned behavior on Day 7 and rearing and locomotor activity may be supported by activation of distinct neural pathways.

Robust increases in locomotor activity and rearing are observed in mice following initial exposure to a novel environment relative to mice that have been habituated through repeated environmental exposures to the same environment (Hooks and Kalivas, 1995). In the present investigation, motor activity was higher during the first 15 min of exploration while exploration habituation was evident during the second 15-min test session for both vertical and horizontal locomotor activity, as evidenced by reduced locomotor and rearing scores. However, animals that received 2.5 μg of DPDPE displayed increased locomotor activity during the second 15-min test session. In other investigations, rats sacrificed following a 5-day habituation exposure to a novel environment displayed decreased neuronal activation in the striatum and cortex, as measured by Fos immunoreactivity relative to animals sacrificed in their home cage or following an initial novel environmental manipulation (Struthers et al., 2005). Moreover, rats exposed to a novel environment previously associated with footshock displayed conspicuous freezing as well as increased Fos immunoreactivity within the lateral amygdala (Rosen et al., 1998). Medial prefrontal cortical administration of c-*fos* antisense oligonucleotide was associated with decreased locomotor activity of rats in a novel environment (Persico et al., 1998). Taken together, mesocorticolimbic c-*fos* expression may underlie the neurobiological changes associated with increased locomotor activity in response to physical stressors and stress associated with novel environmental experiences.

Protooncogenes or immediate early genes (IEGs) such as c-*fos* are expressed immediately (e.g., 5–10 min) in response to extracellular stimuli and play a fundamental role in signal transduction and transcriptional regulation of neuronal cells. Fos forms heterodimers with Jun proteins (i.e., Fos–Jun complex) known as AP-1 and binds to the AP-1 binding sites of target genes, including preproenkephalin (ppENK) among others, up-regulating or down-regulating the expression of enkephalin precursor peptides (Herrera and Robertson, 1996; Hope et al., 1994). Fos is believed to induce transduction cascades coupling external stimuli to the long-term responses of neurons necessary for long-term memory and integration of fear and anxiety to emotionally relevant environmental stimuli (Bruijzeel et al., 1999; Herdegen and Leah, 1998). Fos and fos-related antigens are expressed rapidly and transiently in response to a variety of stimuli (Herdegen and Leah, 1998; Morgan and Curran, 1996), habituate with repeated exposure (Ryabinin et al., 1999), and the number of Fos expressing cells is dependent on the intensity of the experience (Campeau and Watson, 1997). The results that Fos is down-regulated following repeated exposure to the same stimuli (Ryabinin et al., 1999), are contrasted with observations that Fos is up-regulated following exposure to a conditioned stimulus (Campeau et al., 1991) that has acquired emotional significance.

The maintenance of the locomotor stimulating properties of 2.5 μg DPDPE following a 5-day respite from behavioral testing may help the animal prepare for future stressor encounters. Indeed, endogenous δ-receptor activity, promoted in part by leu-enkephalin release in the hippocampus, may be

conducive to vigilance or preparedness by modulating attentional variables attending environmental stimuli (Bramham and Sarvey, 1996; Hernandez and Watson, 1997; Tang et al., 1999). Rats trained in a spatial learning task displayed increased mRNA encoding the δ-opioid receptor, glutamate and acetylcholine in the hippocampus, relative to non-trained animals (Robles et al., 2003). In effect, DPDPE-induced opioid receptor activation may dose-dependently attenuate the habituating influence of enduring exposure to novel environments. However, it must be considered that because DPDPE was administered intracerebroventricularly, the injected peptide was expected to reach all periventricular structures, including the brainstem. The locus coeruleus (LC) plays a key role in the regulation or attention, vigilance, learning and memory in response to environmental cues (Luque et al., 1995). LC activation improves memory and produces a general release of norepinephrine in target structures including the hippocampus and the amygdala (Clayton and Williams, 2000), brain areas whose roles in memory and the development and expression of conditioned fear responses have been clearly established (Davis et al., 1994; Kjelstrup et al., 2002; Trivedi and Coover, 2004). In rats and non-human primates, noradrenergic neurons of the LC are activated by environmental stimuli that demand attention, provoking an orienting response to the target stimulus in a visual discrimination task (Aston-Jones et al., 1991). Environmental stimuli, including footshock or opiates, increase the excitability of LC neurons, through a cyclic AMP (cAMP)-pathway (Nestler et al., 1999) modulating norepinephrine release and autoregulation of endogenous enkephalin peptide release (Van Bockstaele et al., 1997). The cAMP-dependent signaling pathway is involved in the up-regulation of the ppENK gene. For example, the cAMP-activated signaling pathway induces this gene via an enhancer that is composed of multiple protein binding elements, including the cAMP response element-2 binding protein (CREB), which in its phosphorylated state, appears to be required for functional activity of the ppENK gene (Bilecki et al., 2000; Kobierski et al., 1999). The regulation of ppENK gene transcription involves a variety of transcription factors and binding sites. The most important portion of the promoter region appears to be a stretch of nucleotides that contains the cAMP response elements CRE-1 and CRE-2, in addition to an AP-1 response element (Borsook and Hyman, 1995). It has been shown that the CRE-2 site, which is capable of binding CREB and CREB-like proteins, is a critical regulatory site on the ppENK gene (Borsook and Hyman, 1995; Kobierski et al., 1999). CREB, a key protein in the induction of a wide variety of genes, including ppENK, requires phosphorylation at serine-133 for activity (Kobierski et al., 1999). Taken together, 2.5 μg DPDPE administration may sufficiently activate LC neurons inducing a central neurochemical cascade prompting increased attention to environmental detail and underlying the increased behavioral output 7 days following opioid administration.

At this juncture it should be considered that although DPDPE is considered a prototypical δ-opioid receptor agonist in vitro (Mansour et al., 1986; Mulder et al., 1989) it also exhibits weak affinity for μ and kappa (κ)-receptors. In vivo, evidence suggests that μ- and δ-receptors participate in mediating motivation and reward (Jenck et al., 1987; Piepponen et al., 1999) while κ-receptors mediate aversion (Svingos et al., 1999). Enkephalin-containing varicosities, as well as μ-, δ- and κ-receptor subtypes, have been detected in sub-areas of the VTA (Dits and Kalivas, 1989, 1990; German et al., 1993; Speciale et al., 1993) and the shell of the nucleus accumbens (Lu et al., 1998; Svingos et al., 1999; Voorn and Docter, 1992). The opposing motivational effects of μ/δ- and κ-opioid receptor activation are paralleled by an increase or a decrease of DA release from the VTA and shell of the nucleus accumbens, respectively (Cooper, 1991; Kalivas and Abhold, 1987; Mansour et al., 1995; Schoffelmeer et al., 1997; Spanagel et al., 1990; Svingos et al., 1999). In particular, μ- and δ-opioid receptors are involved in the regulation of DA activity in the nucleus accumbens (Piepponen et al., 1999; Churchill et al., 1995). For example, peripheral administration of the μ-agonist etonitazene (2.5 μg/kg) promoted place preference and was associated with increased rat accumbal DA metabolism as measured by in vivo microdialysis. The μ-opioid receptor antagonist, naloxonazine (15 mg/kg), attenuated accumbal extracellular DA concentrations as well as the conditioned place preference induced by etonitazene (Piepponen et al., 1999). Furthermore, central administration of the δ_1-agonist DPDPE (15 nmol i.c.v.) but not the δ_2-agonist [D-Ala^2]deltorphin II (DELT) (5nmol i.c.v.), at pharmacologically rewarding doses (e.g., place preference), increased DA turnover in the nucleus accumbens, an effect which was antagonized by administration of the δ_1-antagonist 7-benzylidenenaltrexone (BNTX; 0.05 mg/kg, s.c.) and not the δ_2-antagonist naltriben (0.05 mg/kg, s.c.). Neither DPDPE nor DELT increased DA turnover in the striatum (Suzuki et al., 1997). These data support a selective role of opioid receptor subtypes in stressor-mediated behavior and suggest that μ- and δ-receptors may modulate neuronal activity in mesolimbic sites. However, although DPDPE and DELT are considered protypical δ-opioid receptor agonists in vitro, in vivo these agonists retain activity in δ-opioid receptor knock-out mice in measures of thermal analgesia; effects that are subsequently averted with administration of selective μ-opioid receptor antagonists. Moreover, DPDPE potency to nocioceptive-elicited stimuli is reduced in μ-opioid receptor knock-out mice (Guo et al., 2003). These data provide evidence that despite δ-opioid receptor selectivity in vitro, in vivo DPDPE and DELT activate μ-opioid receptors under specific experimental conditions (Scherer et al., 2004). Moreover, administration of DPDPE intracerebroventricularly likely results in sufficient concentrations to activate μ-opioid receptors, which are highly abundant in periventricular areas including the LC (Mansour et al., 1995). The presence of δ-opioid receptors on presynaptic axon terminals within the LC, however, suggests that δ-opioid receptor activation may also modulate the release of both inhibitory and excitatory neurotransmitters within this brain area (Van Bockstaele et al., 1997). Accordingly, the current results do not preclude the combined involvement of both μ- and δ-opioid receptor subtypes on the impact of DPDPE administration on immediate and future stressful encounters.

4.2. Reexposure to mild stressor or stressor-associated cues 18 days following initial stressor and DPDPE challenge: evidence for behavioral sensitization

Behavioral sensitization refers to an augmentation of behavior among animals challenged with a low psychostimulant dose (Ohmori et al., 1995; Kuribara, 1996) or brief footshock (Robinson et al., 1987) following acute or chronic D-amphetamine treatment and/or acute or chronic neurogenic stressor exposure. Behavioral change among animals exposed to footshock, D-amphetamine or cocaine may appear within 24 h and may persist for 1 year following initial stressor presentation and ensuing psychostimulant or stressor reexposure (Antelman et al., 1980; Jackson and Nutt, 1993; Paulson et al., 1991; Post et al., 1992; Robinson and Becker, 1986; Robinson, 1988; Steketee et al., 1992; Sorg and Kalivas, 1991). Likewise, stereotypy and locomotor activity may be augmented within 24 h, as well as 1 month following acute or chronic maintenance doses of D-amphetamine or cocaine (Battisti et al., 1999). In the present investigation, acute central DPDPE administration was insufficient to promote eventual increases in horizontal locomotor activity exceeding the behavioral expression evident on Day 1. Yet, among previously shocked mice, DPDPE administration was associated with enhanced horizontal locomotor activity of mice relative to activity of mice treated with saline, during the initial 15-min test session 24 h and again 168 h following reexposure to Mild Stress. Central enkephalin activity has been observed to vary with experimental protocols that promote behavioral sensitization, including footshock. For example, rats subjected to 5 days of footshock demonstrated enhanced activity to DALA challenge 10 days later relative to rats that were merely exposed to the shock apparatus (Kalivas et al., 1986). In our study, an acute intracerebroventricular injection of DPDPE was sufficient to alter the course of stressor-induced behavior. The low dose of DPDPE (i.e., 0.005 μg) among mice previously exposed to No Shock conditions was associated with augmented activity pursuant to apparatus reexposure 18 days following the initial encounter. Yet, the 0.005 μg dose of DPDPE was previously ineffective in producing activity increments among non-shocked mice (i.e., Day 1). Although footshock was never initiated in these chambers, the initial sortie among mice of this treatment regimen was experientially novel. Novel environments provide a mild stressor experience (Badiani et al., 1998; Day et al., 2001), the predictability or saliency of which was likely altered by DPDPE soon after the initial experience. Moreover, No Shock/2.5 μg DPDPE-treated mice did not display shock-induced locomotor deficits when exposed to the Mild Stressor on Day 18. It should be considered that as the disparity between the characteristics attending successive stressor presentations is increased (i.e., No Shock versus Mild Stressor), the DPDPE dose sufficient to mimic endogenous neurochemical activity conducive to prophylactic treatment of behavioral pathology is also increased. It is likely that as the discrepancy between the intensity of the initial and ensuing stressor experience increases, the central neurochemical coding of that encounter is progressively modified. Nevertheless, the magnitude of this behavioral result habituated with progressive behavioral testing.

In contrast to horizontal locomotor activity, deficits in rearing of Shock/Saline mice relative to No Shock/Saline mice exposed to the Mild Stressor were exacerbated, indicative of sensitization to the shock-associated deficits. Mild stressor reexposure also affected behavior of mice previously exposed to footshock or initially confined in a novel environment followed by saline, 0.005 μg DPDPE or 1.0 μg DPDPE. Curiously, while there was no persistent influence of the Mild Stressor on rearing among Saline, 1.0 μg DPDPE or 2.5 μg DPDPE treated mice, No Shock or Shock/0.005 μg DPDPE-treated mice reexposed to the Mild Stressor on Day 18 continued to display reduced rearing scores 24 and 168 h later relative to the remaining treatment conditions. It appears that prior central administration of a very low dose of DPDPE preserved the Mild Stressor experience. Indeed, δ-agonist and leu-enkephalin administration facilitate memory formation in chicks (Freeman and Young, 2000), while leu-enkephalin and DA D_2 receptor activation promoted memory retention in mice (Dubrovins and Ilyutchenok, 1996) and rats (Janak et al., 1994) in passive avoidance tasks. These prolonged rearing deficits evident among mice in the No Shock or Shock/0.005 μg DPDPE/Mild Stressor treatment groups are inconsistent with the increased locomotor response observed among Shock/0.005 μg DPDPE/Mild Stressor treated mice relative to No Shock/0.005 μg DPDPE/Mild Stressor treated mice and mice in the Shock/Saline/Mild Stressor treatment condition. In essence, the neural mechanisms which influence rearing following stressor and DPDPE administration are not congruent with those subserving horizontal locomotor activity in mice and may involve differential and site-specific recruitment of specific neurochemical systems (Starr and Starr, 1987; Swanson et al., 1997; Ukai et al., 1989), as previously discussed.

Consideration of the behavioral data pertaining to the proactive influence of DPDPE on stressor associated activity would be remiss in the absence of some discussion of central mechanisms contributing to such an outcome and the contribution of attending neurochemical systems. In addition to DA mechanisms, there is considerable evidence to implicate glutamate, NMDA receptor activation and long-term potentiation (LTP) in the protracted behavioral and neurochemical change following stressor imposition. Overton et al. (1999) demonstrated that excitatory amino acid (EAA) synapses on DA neurons in midbrain slices exhibit LTP. Moreover, LTP was reduced in amplitude by the AMPA/kainate type EAA-receptor antagonist CNQX and the NMDA-type EAA receptor antagonists, AP-7, CPP and MK-801. Interestingly, administration of NMDA receptor antagonists, MK-301, AP7 or CPP, 30 min prior to cat exposure, prevent increased anxiety in rats in the elevated plus maze for 3 weeks following initial predatory exposure. Morrow et al. (1999) demonstrated that intra-VTA administration of the NMDA receptor agonist, R(+) HA-966, prevented stressor-induced increases in mesocortical DA metabolism and decreased freezing to apparatus cues previously associated with footshock. These data suggest that the

neural mechanisms subserving behavioral sensitization may evoke LTP of DA neurons. Indeed, some laboratories argued that LTP, which underlies memory and learning, contributes to the expression of stressor-induced behaviors. In this respect, enhanced sensitivity to previously neutral stimuli and NMDA receptor activation may heighten neuronal sensitivity and behavioral activation (Bonci and Malenka, 1999; Overton et al., 1999; Vezina and Kim, 1999; Wu et al., 1993). In particular, Shors et al. (1997) demonstrated that stressors activate NMDA receptors in the basolateral amygdaloid (BLA) nucleus and promote protein kinase activity inciting protracted behavioral change (i.e., associative learning, LTP). Amygdaloid protein kinase activity was reinduced soon after (e.g., 5 days) among rats merely reexposed to the chambers in which the stressor was previously applied. Consistent with these findings, Shors (1999) verified that BLA unit activity was suppressed by restraint and diminished electrophysiological activity was reinduced following limited stressor reexposure. The mesolimbic neurocircuitry defining contextual conditioning, LTP and behavioral sensitization has been linked to the hippocampus (Commons and Milner, 1995; Commons and Milner, 1996), striatum (Angulo and McEwen, 1994), nucleus accumbens (Riedel et al., 1997) and VTA (Nader and LeDoux, 1999). Ordinarily, activation of μ- and δ-receptors positioned on GABA inter-neurons (Commons and Milner, 1995, 1996) prompted by stressor imposition disinhibits DA activity favoring augmented neurochemical and behavioral activity conducive to coping. Yet, uncontrollable stressors, if sufficiently protracted, affect sensitization and may reintroduce pathology.

5. Conclusion

The data of the present investigation suggest that central DPDPE administration modifies neural signals that ordinarily prompt stressor reactivity. Specifically, an acute, intracerebroventricular injection of 2.5 μg DPDPE immediately following exposure of mice to either a novel environment (No Shock) or Shock on Day 1, prevented a reduction in horizontal locomotor activity and rearing of mice following subsequent mild stressor (i.e., brief footshock) presentations for at least 3 weeks. The data of the present experiment also revealed that DPDPE intervention enhanced behavioral output among mice in a contextually dose-dependent manner. It would seem appropriate to target pharmacological manipulations which affect such neurochemical change to the treatment of psychological disturbance, including PTSD, in which recurring stressor encounter and enhanced stressor sensitivity are prominent features of the disorder.

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