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ORIGINAL INVESTIGATION

Social defeat stress-induced sensitization and escalated cocaine self-administration: the role of ERK signaling in the rat ventral tegmental area

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Abstract
Rationale Intermittent social defeat stress can induce neuroadaptations that promote compulsive drug taking. Within the mesocorticolimbic circuit, repeated cocaine administration activates extracellular signal-regulated kinase (ERK).
Objective The present experiments examine whether changes in ERK phosphorylation are necessary for the behavioral and neural adaptations that occur as a consequence of intermittent defeat stress.
Materials and methods Rats were exposed to four brief intermittent defeats over the course of 10 days. Ten days after the last defeat, rats were challenged with cocaine (10 mg/kg, i.p.) or saline, and ERK activity was examined in mesocorticolimbic regions. To determine the role of ERK in defeat stress-induced behavioral sensitization, we bilaterally microinjected the MAPK/ERK kinase inhibitor U0126 (1 μg/side) or vehicle (20 % DMSO) into the ventral tegmental area (VTA) prior to each of four defeats. Ten days following the last defeat, locomotor activity was assessed for the expression of behavioral cross-sensitization to cocaine (10 mg/kg, i.p.). Thereafter, rats self-administered cocaine under fixed and progressive ratio schedules of reinforcement, including a 24-h continuous access "binge" (0.3 mg/kg/infusion).
Results We found that repeated defeat stress increased ERK phosphorylation in the VTA. Inhibition of VTA ERK prior to each social defeat attenuated the development of stress-induced sensitization and prevented stress-induced enhancement of cocaine self-administration during a continuous access binge.
Conclusions These results suggest that enhanced activation of ERK in the VTA due to brief defeats is critical in the induction of sensitization and escalated cocaine taking.

Keywords Stress · Sensitization · Cocaine · Self-administration · Ventral tegmental area · ERK

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Introduction

Social defeat is an ethologically valid form of stress that can produce long-lasting changes in immediate early gene expression in mesocorticolimbic structures (Covington et al. 2005; Nikulina et al. 2004; Nikulina et al. 2008) that may contribute to intense drug taking (Anstrom et al. 2009; Berton et al. 2006; Covington et al. 2005, 2008; Covington and Miczek 2001; Fanous et al. 2010; Krishnan et al. 2007; Nikulina et al. 2004; Razzoli et al. 2011; Miczek et al. 2011). Rats exposed to repeated intermittent defeat show behavioral cross-sensitization to the locomotor-stimulating effects of psychostimulants (Covington and Miczek 2001; Miczek et al. 1999; Nikulina et al. 1998; Yap et al. 2005; Yap and Miczek 2007) and acquire cocaine self-administration more readily compared to nonstressed controls (Kabbaj et al. 2001; Tidey and Miczek 1996). Both drugs of abuse and aversive stress experiences activate the mesocorticolimbic dopamine system, suggesting that this is a common neural substrate in

which changes in gene expression can increase susceptibility to escalated drug self-administration (Hernandez and Hoebel 1988; Kelly and Iversen 1976; Miczek et al. 2008; Roberts et al. 1977; Sorg and Kalivas 1991; Sorg and Kalivas 1993).

Cocaine activates extracellular signal-regulated kinase (ERK) within the mesocorticolimbic pathway (Valjent et al. 2000; Valjent et al. 2004; Berhow et al. 1996), and ERK can subsequently activate the transcription factor cAMP response element binding protein (CREB) (Carlezon et al. 2005) and increase expression of the transcription factors *c-fos* and *zif268* (Graybiel et al. 1990; Konradi et al. 1994; Sgambato et al. 1998). This cascade of immediate early gene activation has been shown to regulate behavioral responses to psychostimulants (Shilling et al. 2006; Lu et al. 2006). *Zif268* is a transcription regulatory factor that is expressed at high levels in brain neurons. Like *c-fos*, *zif268* is markedly activated in striatum by cocaine and amphetamine, and this *zif268* activation involves the dopamine system, particularly the activation of D1 and D2 receptors (Bhat et al. 1992; Gerfen et al. 1995; Keefe and Gerfen 1995). Intermittent social defeat also elevates expression of *c-fos* and *zif268* in the mesocorticolimbic dopamine system, and these changes can persist for up to 60 days after the last defeat experience (Covington et al. 2005; Nikulina et al. 2004). Systemic (Valjent et al. 2006) or intra-ventral tegmental area (VTA) (Pierce et al. 1999) inhibition of ERK prior to daily cocaine injections attenuates cocaine-induced locomotor sensitization. Both acute and chronic unpredictable stress increase ERK signaling within the VTA, and overexpression of ERK2 within the VTA increases susceptibility to stress (Iñiguez et al. 2010). While acute stress can increase ERK activity in the VTA, we are interested in how repeated intermittent defeat can produce long-lasting, enduring changes in the VTA that may lead to compulsive cocaine intake. The present study examines how ERK signaling in the mesocorticolimbic dopamine system, particularly in the VTA, affects social defeat stress-induced sensitization and cocaine self-administration, using the measurement of pERK levels as an indicator of the activation of the ERK pathway and the MEK inhibitor, U0126, to block ERK phosphorylation. We hypothesize that the cascade of intracellular events occurring in the VTA in response to brief repeated defeat episodes can modulate subsequent intravenous cocaine-taking behavior, particularly during a 24-h "binge."

Materials and methods

Experiment 1: effects of intermittent stress on CREB and ERK protein levels in the mesocorticolimbic dopamine system

Male Long-Evans rats (Charles River Laboratories, Wilmington, MA) weighed 225–250 g upon arrival to the facility. Rats were placed in one of two groups (n=6–8 per group): four intermittent defeats (defeats on days 1, 4, 7, and 10) or nonstressed control group (handling on days 1, 4, 7, and 10). These groups were further divided into saline- or cocaine-challenged rats. Intermittently defeated rats and their contemporary controls were individually housed in custom-built acrylic chambers (30×30.5×24.5 cm^3). Removable panels on each wall of the home cage were fitted with wire mesh (0.5 cm^2 mesh holes). The floor of each cage was lined with Cellu-Dri paper pellet bedding (Shepherd Specialty Papers, Kalamazoo, MI) and maintained on a reversed light/dark cycle (lights on 20:00–08:00), controlled temperature (21±1 °C), and humidity (35–40 %) with unlimited access to food (Purina laboratory chow) and water. All experimental procedures were approved by the Tufts Institutional Animal Care and Use Committee, following the principles of the Guide for the Care and Use of Laboratory Animals. Stimulus residents (500–700 g male Long-Evans rats) were housed with females in large stainless steel cages (45.7×71.1×45.7 cm). Each resident was selected based on its consistent display of aggressive behavior during regularly scheduled confrontations with an intruder rat (Miczek 1979).

Social defeat procedure

Intermittently defeated rats were subjected to a defeat episode as described previously (Covington et al. 2005; Covington and Miczek 2001; Tornatzky and Miczek 1993) on days 1, 4, 7, and 10. Specifically, each episode of social defeat took place in an adjacent room to the housing location, while control animals remained in their home cages in the vivarium. Before each defeat, rats in both the experimental and control groups were weighed and handled. Intermittently stressed rats were then placed inside the home cage of an aggressive resident male rat which served as stimulus. Following 10 min of being threatened behind the protective mesh of the experimentally stressed rat's home cage, the protective home cage was removed, and the resident stimulus rat attacked the experimental rat. Defeat was defined as the display of 5 s of the supine posture or a maximum of ten bites within a maximum of 5 min, whichever appeared first (Covington et al. 2005; Covington and Miczek 2001). Each experimental intruder rat was exposed to a different resident over the course of experimental days 1, 4, 7, and 10. During each defeat experience, the attack latency, number of bites received, and duration of each aggressive encounter were recorded. Immediately after each defeat, the experimental rat was again placed back in its own protective home cage and again placed back inside the resident's home cage for an additional 10 min. At the completion of this final threat period, each stressed rat was moved back to the vivarium.

Test for expression of sensitization

Ten days after the last stress or handling experience, the rats were assessed for behavioral sensitization with a challenge injection of cocaine (10 mg/kg, i.p.). While remaining in the home cage, a previously stressed rat and a control rat were each moved into an adjacent procedure room. Each rat was then briefly removed from its cage in order to be weighed and injected with saline. Following the saline injection, rats were placed back into their home cages and locomotor behavior was monitored for 10 min following injection. Both animals were then injected with cocaine or saline, and locomotor behavior was monitored for 20 min following the challenge injection. To determine behavioral sensitization to social defeat stress, the walking frequency per minute during each 20-min increment of behavioral analysis—before and after challenge injection of either saline or cocaine—was analyzed using repeated measures two-way ANOVA (stress × drug injection). Post hoc analysis of all pairwise comparisons was performed using *t* tests with Bonferroni correction.

Behavioral observations

A trained observer analyzed each video record using a custom keyboard and commercial software (The Observer Video-Pro version 5.0, Noldus Information Technology, Wageningen, Netherlands) without knowledge of the treatment conditions. The frequency and duration of rearing, walking, sniffing, grooming, digging, and inactivity were recorded. Five-minute samples were analyzed 5–10 min following saline baseline injection and 5–10 and 10–15 min after cocaine challenge. Mean walking frequency per minute was calculated.

Tissue preparation and Western blotting procedure

Twenty minutes after saline or cocaine injection (immediately after locomotor activity analysis), animals were killed by rapid decapitation and brains were immediately frozen in 2-methylbutane over dry ice. It has been previously shown that pERK expression peaks 20-min after cocaine injection (Mattson et al. 2005). Brains were stored at −80 °C until tissue extraction using 15- or 21-gauge tissue punches. Bilateral punches (1 mm thick) of the nucleus accumbens (NAC) (15 gauge; 1.7 mm from bregma) and VTA (21 gauge; −4.8 mm from bregma) and a single midline punch of the medial prefrontal cortex (PFC) (15 gauge; 2.2 mm from bregma) were taken from brains in a −20 °C cryostat, and tissue punches were immediately placed on dry ice. After removal of the tissue cores, coronal sections (30 μM) of the exposed face of the brain were taken and Nissl stained with cresyl violet for histological analysis of placements. Only the rats in which the tissue punches were targeted appropriately were included in analyses. Given the small size of the VTA, punch placements were first optimized by taking tissue sections from a formaldehyde-perfused rat brain and processing them for immunohistochemistry as described (Chartoff et al. 2008), with an antibody directed against tyrosine hydroxylase (TH; rabbit anti-TH, Chemicon, 1:5000) to visualize the extent of the VTA (Fig. 2e). All tissue samples were stored at −80 °C until tissue sonication. Tissue was sonicated in 1 % sodium dodecyl sulfate (SDS) to dissociate membranes. Total protein concentrations in samples were determined using the Bio-Rad (Hercules, CA) DC Protein Assay kit, and the concentration of each sample was adjusted to 2.0 mg/ml protein. Cell lysates were heated to 70 °C for 10 min before polyacrylamide gel electrophoresis. Twenty micrograms of each sample were loaded onto NuPAGE Novex 4–12 % Bis-Tris gels (Invitrogen) for electrophoresis. Proteins were transferred to polyvinylidene fluoride membranes (PerkinElmer Life Sciences, Boston, MA), and nonspecific binding sites were blocked for 2 h at room temperature in 5 % nonfat dry milk in phosphate-buffered saline and 0.1 % Tween 20 (PBS-T). Membranes were then probed with either monoclonal anti-pERK 1/2 (1:4000; Cell Signaling Technology, Beverly, MA) or monoclonal anti-pCREB (1:4000; Cell Signaling Technology) in PBS-T, shaking, overnight at 4 °C. Blots were washed 4×15 min each in PBS-T and then incubated in secondary antibody (1:5000 horseradish peroxidase-linked goat anti-mouse IgG; Vector Laboratories, Burlingame, CA) for 2 h at room temperature. Blots were washed 4×15 min each in PBS-T, followed by colorimetric detection with Chemiluminescnce Reagent Plus (PerkinElmer Life and Analytical Sciences). Blots were then stripped by incubating in stripping buffer (62.5 mM Tris, 2 % SDS, and 100 mM β-mercaptoethanol, pH 6.8) for 15 min at 50 °C and subsequently reblocked. Blots were then probed with either 1:6000 polyclonal anti-ERK2 (Cell Signaling Technology) or 1:4000 polyclonal anti-CREB (Upstate Biotechnology, Lake Placid, NY), shaking, overnight at 4 °C, and incubated in secondary antibody (1:6000 horseradish peroxidase-linked goat anti-rabbit IgG; Vector Laboratories) for 2 h at room temperature. Blots were processed, and proteins were visualized in the same manner stated above. Blots were stripped a final time and incubated with 1:12,000 monoclonal anti-β-tubulin (Sigma) to serve as a loading control. SeeBlue Plus2 (Invitrogen) pre-stained standards were run on each gel for molecular weight estimation. pCREB and CREB bands were detected at 43/42 kDa; pERK2 was detected at 42 kDa; ERK2 was detected at 42 kDa; and β-tubulin was detected at approximately 51 kDa.

Protein immunoblots were analyzed using Kodak 1D Image Analysis software (Carestream Health, Rochester, NY). Mean optical densities were determined for each band of interest. To control for loading differences of protein, the optical density of each band was normalized with the

corresponding optical density of β-tubulin. To allow for comparisons of blots from independent dissections, data were normalized to the saline-treated unstressed controls. Additionally, pERK2 data in the VTA are also expressed as means (±SEM) of the optical density ratios of pERK2/ERK2. This was done in order to visualize ERK2 activation relative to the amount of total ERK2 present (i.e., pERK2/ERK2). Data were analyzed using two-way ANOVA (stress × drug). Post hoc analysis of all pairwise comparisons was performed using *t* tests with Bonferroni correction.

Experiment 2: the role of VTA ERK phosphorylation in the development of social defeat stress-induced sensitization and increased cocaine self-administration

Animals

Male Long-Evans rats (Charles River Laboratories, Wilmington, MA) weighed 225–250 g upon arrival to the laboratory. Rats were placed in one of four groups (n=15 per group): no stress + vehicle, no stress + U0126, intermittent stress + vehicle, and intermittent stress + U0126. Intermittently defeated rats and their respective controls were individually housed in the same manner as in experiment 1. Stimulus residents (500–700 g male Long-Evans rats) were also housed and screened just as in experiment 1. One week following introduction to the vivarium, rats were implanted with guide cannulae into the VTA. Intermittent defeat rats were subjected to a defeat episode as described previously in experiment 1 on days 1, 4, 7, and 10.

Intra-VTA cannula placements

While under ketamine (100 mg/kg) and xylazine (6 mg/kg) anesthesia, rats were surgically implanted with bilateral guide cannulae aimed 1 mm above the VTA. Specifically, microinjection cannulae (26-gauge stainless steel, 11 mm beyond plastic pedestal; Plastics One, Roanoke, VA) were placed: AP −5.2; ML +1.8; DV −7.5, at a 10° angle relative to bregma (Paxinos and Watson 1997) in order to avoid impinging upon the midsagittal sinus. Cannulae were cemented by affixing dental acrylic to three stainless steel screws anchored into the skull. Patency of the cannulae was maintained by the insertion of 33-gauge obturators protruding 1 mm beyond the tip of the guide cannulae. Following a 1-week recovery period, rats received four microinjections of U0126 (1 μg per side over 3 min in a volume of 0.5 μl) or vehicle (20 % dimethyl sulfoxide (DMSO) in artificial cerebrospinal fluid (aCSF)) 20 min before the stress experience or in the absence of social stress (control). Stereotaxic coordinates, dose and time of injection of U0126 were chosen based on previous reports (Lu et al. 2004; Lu et al. 2005; Mattson et al. 2005). Infusion of the drug was confirmed by movement of an air bubble in the injection tubing. Injectors remained in place for 1 min to allow diffusion away from the injector and prevent backflow, and then obturators were replaced.

Test for expression of sensitization

Procedures used to induce sensitization and assess the expression of sensitization are the same as previously described in experiment 1. Motor behavior was analyzed and measured as in experiment 1.

Intravenous catheter implantation

Following the test for the expression of sensitization on day 20, rats were permanently implanted with indwelling catheters (Silastic® silicon tubing, ID 0.63 mm, OD 1.17 mm) into the right jugular vein (Covington and Miczek 2001) under a combination of ketamine (100 mg/kg) and xylazine (6 mg/kg) anesthesia. The catheter was passed subcutaneously to the rat's back, where it exited through a small incision and affixed to a plastic pedestal (Plastics One, Roanoke, VA) mounted inside a harness system (Instech Laboratories, Plymouth Meeting, PA). After catheter surgery, each rat was allowed to recover for at least 5 days.

Intravenous cocaine self-administration procedures

Following recovery from catheter surgery, rats remained in their home cage, and this cage was moved into a procedure room equipped for intaveneous (i.v.) self-administration experiments (Miczek and Mutschler 1996). Catheters were flushed with heparinized saline (20 IU/ml) each morning. During the light phase, 0.17 ml pulses of saline were delivered every 30 min.

1. Acquisition and fixed ratio performance during limited access

   Rats were allowed to self-administer cocaine (0.75 mg/kg/infusion) without a priming infusion according to a fixed ratio 1 (FR1) schedule of reinforcement with a 30-s time-out period following each infusion of cocaine. Each daily session terminated after the delivery of 15 infusions or after 5 h of access. After reliable self-administration was verified (two consecutive days of 15 infusions within 5 h), the FR schedule progressively increased to FR5, over three to five additional days. If rats did not achieve this requirement within the first 2 days of cocaine access, they were behaviorally shaped on the third day. Behavioral shaping consisted of placing female urine or palatable food on the active lever to attract the animals. Levers were wiped clean, and behavioral shaping was terminated once the rats reliably self-administered cocaine. The purpose of shaping the animals was to take

advantage of the catheter life throughout the next phases of the protocol (i.e., FR, PR, and binge). Due to shaping, differences in acquisition rates cannot be assessed. Rats were maintained on an FR5 schedule of limited access for at least five consecutive days (i.e., 15 infusions/day).

2. Progressive ratio performance

   After the acquisition phase, rats self-administered cocaine (0.3 mg/kg/infusion) according to a progressive ratio (PR) schedule of reinforcement for 3 days. This dose of cocaine was chosen to reveal more subtle effects of sensitization to the reinforcing effects of a low dose of i.v. cocaine (Flory and Woods 2003). Between each PR session, rats self-administered the dose used during acquisition (0.75 mg/kg/infusion) on an FR5 schedule. The algorithm used for the progression was previously derived by Richardson and Roberts (1996). The ratio progression was as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, and 178. The last completed ratio, which resulted in the final infusion, was defined as the "breaking point" (Hodos 1961). The average number of completed infusions for each group of rats was calculated as the dependent measure.

3. Fixed ratio performance during a 24-h binge

   After the final PR session, each rat was allowed one additional day of limited access to cocaine (0.75 mg/kg/infusion, FR5, 15 infusions). The very next day, a prolonged access binge protocol was implemented starting at approximately 10:00 a.m. (i.e., 2 h after the start of the dark phase). Each rat was allowed continuous access to cocaine (0.3 mg/kg/infusion, FR5) for 24 h. The amount of cocaine administered and the pattern of responding were recorded.

Histological confirmation of cannula placements

After the 24-h binge, rat brains were histologically examined to confirm placement of the cannulae in the VTA. Rats were anesthetized with an overdose of sodium pentobarbital (100 mg/kg, i.p.). Brains were removed and immediately frozen in 2-methylbutane over dry ice. Brains were stored at −80 °C until sectioning in a cryostat at −20 °C. Sections surrounding the cannula tracts were cut in the coronal plane to 50-μm thickness, mounted on slides, and subsequently stained with cresyl violet.

Experiment 3: effect of U0126 on pERK after a single defeat

Male Long-Evans rats (Charles River Laboratories, Wilmington, MA) weighed 225–250 g upon arrival to the facility. One week later, rats were implanted with cannulae in the VTA as previously described for experiment 2. Following a 1-week recovery period, rats received a single microinjection of U0126 (1 μg per side over 3 min in a volume of 0.5 μl) or vehicle (20 % DMSO in aCSF) bilaterally into the VTA 20 min before the single stress experience or in the absence of social stress (control). The stress procedure was the same as described for experiments 1 and 2. Brains were collected 20 min following the stress (or no stress) experience, and the VTA was punched for analysis of pERK expression using Western immunoblotting as in experiment 1. Blots were also probed for tyrosine hydroxylase (TH) expression using a rabbit polyclonal antibody (Millipore; 1:10,000) as an additional control for quality of VTA punches and to determine whether defeat stress or U0126 alters TH expression. To control for protein loading differences on the SDS-PAGE gels, blots were stripped and reprobed for β-actin (Sigma; 1:20,000). Data were analyzed using two-way ANOVA (stress × microinjection treatment). In addition, we conducted a *t* test between the vehicle + stress and U0126 + stress groups because this was a planned comparison. Cannula placements within the VTA and tissue punch locations were verified as previously described in experiments 2 and 1, respectively.

Drugs

Cocaine hydrochloride was obtained from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD) and was dissolved in sterile 0.9 % saline. U0126 is highly selective for MEK-1 and MEK-2, and U0126 shows little, if any, effect on the kinase activities of protein kinase C, Abl, Raf, MEKK, ERK, JNK, MKK-3, MKK-4/SEK, MKK-6, Cdk2, or Cdk4 (Dudley et al. 1995; Favata et al. 1998). U0126 inhibits MEK, thereby inhibiting ERK phosphorylation.

U0126 exhibits a short half-life of ca. 2 h in vivo (London and Clayton 2008). U0126 was purchased from LC Laboratories (Woburn, MA) and dissolved in 20 % DMSO in aCSF (147 mM NaCl, 1.3 mM anhydrous CaCl2, 0.9 mM anhydrous MgCl2, 4.0 mM KCl, pH=7).

Statistical analyses

Behavioral data from defeat experiences were analyzed using a chi-squared test for the analysis of the proportion of defeats ending in supine and one-way repeated measures ANOVAs for the analyses of attack latency, attack bites, and defeat duration. To determine behavioral sensitization following social defeat stress, the walking frequency per minute during each 20-min increment of behavioral analysis—before and after psychostimulant injections—was analyzed using two-way ANOVA (stress × microinjection treatment). Post hoc analysis of all pairwise comparisons was performed using *t* tests with Bonferroni correction. Acquisition data were calculated as the proportion of rats attaining the maximal 15

infusions during the first two self-administration sessions prior to behavioral shaping. No other analysis of acquisition was performed due to the shaping. PR data obtained during i.v. self-administration were analyzed according to the average number of accumulated reinforcements over each session. The average number of infusions delivered (corresponding to a specific ratio criterion) for all rats was averaged for each group and compared to all other groups by two-way ANOVA (stress × microinjection treatment). Total binge intake (expressed as total number of infusions during 24 h) for each rat contributed to the group's average and was compared across groups by two-way ANOVA (stress × microinjection treatment). Cumulative number of infusions during each 8-h time block during the binge was also calculated for each animal, and group differences were analyzed using repeated measures three-way ANOVA (stress × microinjection treatment × time). Bonferroni post hoc analyses for all pairwise comparisons were made for PR and binge intake data, when appropriate.

Results

Effects of intermittent stress on CREB and ERK protein levels in the mesocorticolimbic dopamine system following cocaine or saline challenge

Day 20 expression test—cocaine or saline challenge

There was a significant main effect of intermittent stress ($F_{1,20}$=25.411, p<0.001) and a significant main effect of cocaine injection ($F_{2,20}$=16.111, p<0.001) on locomotor activity (as reflected by frequency of walking behavior). There was also a significant interaction between stress and drug injection ($F_{2,20}$=19.819, p<0.001). Episodically stressed rats showed an augmented locomotor response to cocaine (p<0.001) compared to their baseline locomotion and compared to stressed rats challenged with a second saline injection (Fig. 1). Nonstressed control animals showed significantly decreased locomotor activity following a second saline injection (p=0.034).

Effects of intermittent stress and cocaine on total and phosphorylated CREB protein levels

Experience with intermittent social defeat stress tended to result in decreased CREB and pCREB levels in the mesolimbic system, regardless of cocaine treatment. Specifically, defeat stress resulted in significantly decreased CREB protein levels ($F_{1,20}$=5.580, p=0.028; Table 1), and nonsignificant decreases in pCREB levels, in the PFC (Table 1). There was no main effect of stress or drug on CREB protein expression in the NAC (Table 1), while pCREB was

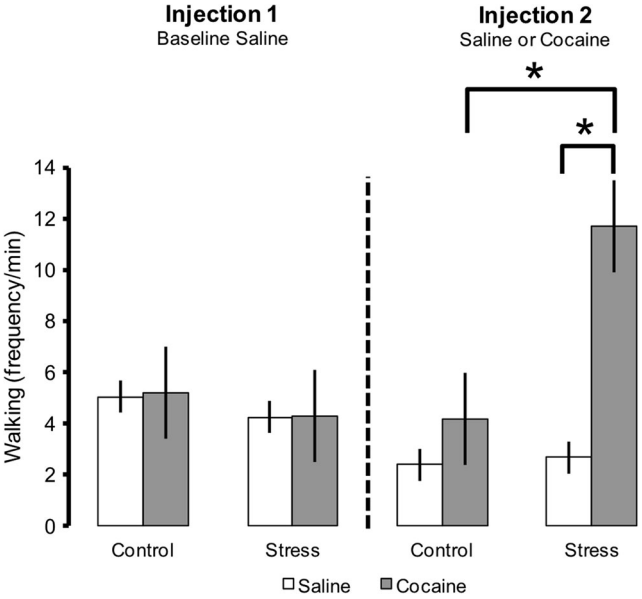

Fig. 1 Locomotor activity following day 20 saline or cocaine challenge in intermittently stressed (four defeats) or nonstressed control rats. Effects of intermittent stress on the later behavioral response to saline or cocaine (10 mg/kg, i.p.). There are six subjects (n=6) per group. Data are expressed as mean frequency of walking behavior per minute (±SEM) during a 20-min time period. *Open bars* represent saline-challenged rats (injection 2). *Filled bars* represent cocaine-challenged rats. *p<0.05

decreased due to stress ($F_{1,20}$=4.504, p=0.0405). Post hoc analyses reveal that stressed rats that received a cocaine challenge showed significantly decreased pCREB in the NAC compared to nonstressed rats that received cocaine (p=0.045). In the VTA, there was no significant effect of stress or cocaine on levels of pCREB or CREB, although there was a trend for stress to decrease protein levels (Table 1).

Effects of intermittent stress and cocaine on total and phosphorylated ERK protein levels

In contrast to CREB regulation, intermittent social defeat stress tended to increase ERK and pERK levels in the PFC and NAC and decrease ERK in the VTA. Specifically, there was no significant effect of intermittent stress or cocaine on pERK2 or total ERK2 in the PFC (Table 1), although there was a trend for both to be increased. In the NAC, there was a significant increase in total ERK2 levels as a result of defeat ($F_{1,20}$=7.480; p=0.013; Table 1), but only a nonsignificant trend for pERK2 to be increased. In the VTA, there was a significant overall stress effect on pERK2 ($F_{1,20}$=4.278; p=0.049; Table 1; Fig. 2a). This stress effect was robust, particularly when the optical density ratios of pERK2/ERK2 were examined in the VTA ($F_{1,20}$=7.564; p=0.003; Fig. 2c). Calculation of the optical density ratios of pERK2/ERK2 allowed for the visualization of how stress-induced reductions in total ERK2 mean that ERK2 activation relative to the amount of total ERK2 present (i.e., pERK2/ERK2) is greater than ERK2

Table 1 Effect of intermittent defeat on CREB and ERK activity in the PFC, NAC, and VTA

		pCREB		CREB (total CREB)		pERK2 (42-kDa band)		ERK2 (total ERK2)	
		Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine
PFC	Control	1.0±0.273	0.20±0.113	1.0±0.194	1.059±0.203	1.0±0.288	0.959±0.330	1.0±0.062	1.407±0.184
	Stress	0.447±0.158	0.269±0.107	***0.588±0.226***	***0.547±0.152***	1.514±0.452	1.229±0.275	1.502±0.239	1.664±0.212
NAC	Control	1.0±0.353	1.374±0.161	1.0±0.164	1.175±0.098	1.0±0.174	1.659±0.473	1.0±0.116	1.052±0.231
	Stress	***0.819±0.126***	***0.501±0.120****	0.612±0.160	0.768±0.194	1.575±0.289	1.319±0.154	***2.030±0.335***	***2.142±0.484***
VTA	Control	1.0±0.170	1.091±0.217	1.0±0.188	1.320±0.251	1.0±0.232	0.982±0.293	1.0±0.108	1.134±0.103
	Stress	0.940±0.703	0.521±0.229	0.704±0.297	0.709±0.302	***2.024±0.558***	***1.519±0.401***	***0.612±0.187***	***0.616±0.140***

Data are expressed as mean fold induction over tubulin (±SEM) normalized to saline nonstressed control group. There are six subjects (n=6) per group. Bolded values indicate significant effect of $p<0.05$. Bolded, italicized values indicates significance at $p<0.05$ compared to nonstressed rats (i.e., main effect of stress).

*$p<0.05$ compared to cocaine nonstressed rats

activation in the volume of tissue punched (i.e., pERK2/tubulin). There was a significant main effect of defeat stress on total ERK2 in the VTA ($F_{1,20}$=11.818; p=0.010; Table 1; Fig. 2b), with ERK2 being lower in stressed compared to nonstressed animals. Figure 2d, e depicts location of VTA punches.

The effect of U0126 on pERK after a single defeat

To test whether VTA ERK is necessary for social defeat stress-induced sensitization and increased cocaine self-administration, we blocked ERK activation in the VTA with the pharmacological inhibitor U0126. This compound is one of the most potent and selective ERK inhibitors and acts to prevent activation of mitogen-activated protein kinase kinase (MKK1) (Davies et al. 2000). To validate its effects on stress-induced ERK activation, U0126 was microinjected into the VTA 20 min prior to a single defeat stress and rats killed 20 min after the stress (experiment 3). Histological analysis revealed that VTA punches in 2 out of 25 rats were off-target. Data from these rats were excluded, and pERK2 and actin values were replaced with their respective group means. A pre-planned comparison between stressed rats with intra-VTA vehicle and stressed rats with intra-VTA U0126 showed that pERK levels were significantly lower in U0126 treated rats (Fig. 3; $p<0.05$; unpaired t test). For comparison, we show that treatment has no effect on TH levels in the VTA (Fig. 4).

The role of VTA ERK phosphorylation in the development of social defeat stress-induced sensitization and increased cocaine self-administration

Repeated social defeat

Fifteen rats were repeatedly stressed, made it through the 24-h continuous cocaine binge, and had accurate VTA cannulae placements. Eight of those rats received intra-VTA injections of vehicle prior to each defeat, and seven received microinjections of U0126 in the VTA prior to defeat. There was no difference between treatment groups (U0126 vs vehicle) in the proportion of animals that ended the defeat by holding the supine posture for 5 s and those that did not (chi-squared (1, N=15)=1.151, p=0.283). There was no effect of drug treatment on attack latency ($F_{1,39}$=0.773, p=0.395) or attack bites ($F_{1,39}$=3.759, p=0.075). There was an effect of microinjection treatment on defeat duration ($F_{1,39}$=6.535, p=0.024). On average, rats that received U0126 had shorter fight durations compared to the vehicle group (163 vs 94.821 s, respectively).

Cocaine challenge

On experimental day 20 (i.e., 10 days following the last defeat episode), no significant differences were found between groups with regard to the frequency of walking behavior recorded after an initial saline injection. However, the subsequent cocaine challenge increased locomotor activity in the rats given microinjections of vehicle (20 % DMSO in aCSF) prior to each defeat (Fig. 5a). After cocaine injection, a main effect of stress was found on frequency of walking behavior in intermittently stressed rats compared to controls ($F_{1,55}$=8.141, p=0.006). There was also a main effect of U0126 microinjection ($F_{1,55}$=7.554, p=0.008) and a significant interaction between stress and microinjection treatment ($F_{1,55}$=11.038, p=0.002). Among vehicle-treated animals, episodic social defeat stress resulted in significantly higher walking frequency compared to nonstressed controls ($p<0.001$). Post hoc analysis revealed a significant difference between stressed rats that received U0126 or vehicle ($p<0.001$), showing that U0126 microinjections prevented the induction of behavioral sensitization.

Acquisition, progressive ratio, and 24-h binge

The proportion of animals acquiring cocaine self-administration during the first 10 h (i.e., before shaping is

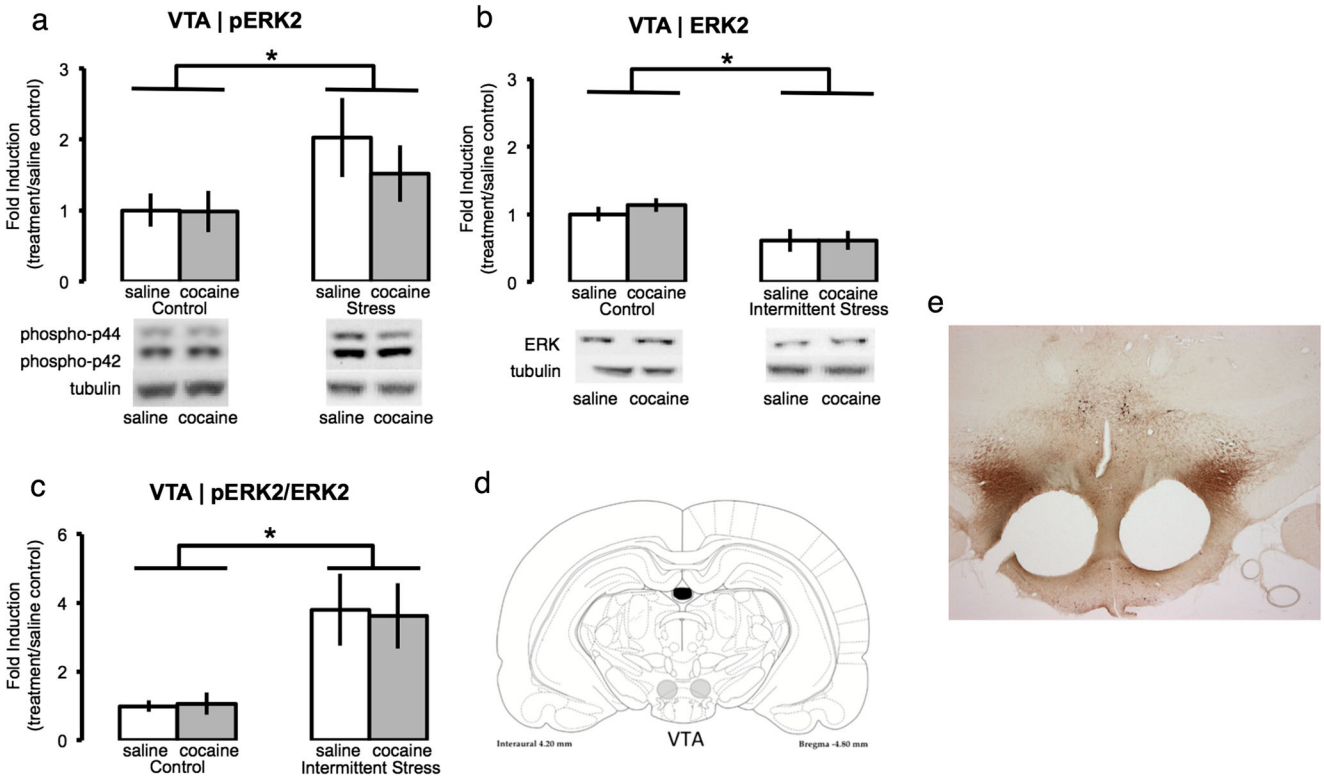

Fig. 2 **a** ERK2 phosphorylation in the VTA following cocaine (10 mg/kg, i.p.) or saline challenge. There are six subjects (n=6) per group. Data are expressed as the mean fold induction of pERK2/tubulin (±SEM) compared to saline-challenged nonstressed controls. *Open bars* represent saline-challenged rats. *Filled bars* represent cocaine-challenged rats. *p<0.05. **b** Effect of intermittent stress on ERK protein levels in the VTA following cocaine (10 mg/kg, i.p.) or saline challenge. There are six subjects (n=6) per group. Data are expressed as the mean fold induction (±SEM) of ERK/tubulin (normalized to the saline nonstressed control group). *Open bars* represent saline-challenged rats. *Filled bars* represent cocaine-challenged rats. *p<0.05. **c** Optical density ratios of pERK2/ERK2 in the VTA following cocaine (10 mg/kg, i.p.) or saline challenge. There are six subjects (n=6) per group. Data are expressed as the mean fold induction of pERK2/ERK2 (±SEM) compared to saline-challenged nonstressed controls. *Open bars* represent saline-challenged rats. *Filled bars* represent cocaine-challenged rats. *p<0.05. **d** Location of VTA punches. All punches were 1 mm thick. VTA punches were taken using a 21-gauge metal tube. Diagram of coronal section was taken from Paxinos and Watson (1997). **e** Representative photomicrograph indicating location of VTA punches. This is a representative coronal brain section processed for tyrosine hydroxylase immunohistochemistry

initiated) is similar across treatment groups ($F_{1,29}$=0.0573, p=0.813; data not shown). There were no significant differences between groups on cocaine intake on a PR schedule of reinforcement (Fig. 5b). During the binge, total intake was highest in vehicle-treated stressed rats (Fig. 5c), which contributed to a significant interaction between stress and microinjection treatment ($F_{1,29}$=9.776, p=0.004). Vehicle-treated intermittently stressed rats had significantly higher cocaine intake compared to vehicle-treated nonstressed controls (p=0.038), and U0126 prevented this escalated cocaine intake due to stress (p=0.008). Three-way ANOVA for cumulative number of infusions per every 8 h revealed a significant interaction between stress and microinjection treatment ($F_{1,58}$=12.251, p=0.0015) and a significant time × stress × microinjection treatment interaction ($F_{2,58}$=5.247, p=0.0080). Post hoc analyses revealed a significant effect of stress in vehicle-treated animals compared to nonstressed vehicle controls at hours 8, 16, and 24 of the binge (p=0.0256, p=0.0132, p=0.0395, respectively; Fig. 5d). While control animals that received repeated U0126 seem to show increased cocaine taking compared to control animals that received repeated vehicle, there were no significant differences between these two groups at any of the 8-h time points (p=0.1863, p=0.1580, p=0.1573, respectively). There were no significant differences between treatment groups on inactive lever pressing on the PR schedule or during the 24-h binge (data not shown).

VTA cannula placements

Sixty rats were implanted with bilateral cannulae directed at the VTA. Forty-four of these rats were implanted with indwelling catheters, and 37 rats successfully completed all cocaine self-administration phases (due to development of infections or loss of catheter patency). Four of those 37 rats were excluded due to placements outside of the VTA, and the 33 rats that completed the binge were confirmed to have placements located within the VTA (Fig. 6a, b). These 33 rats were in the following groups: stress vehicle (n=8), stress

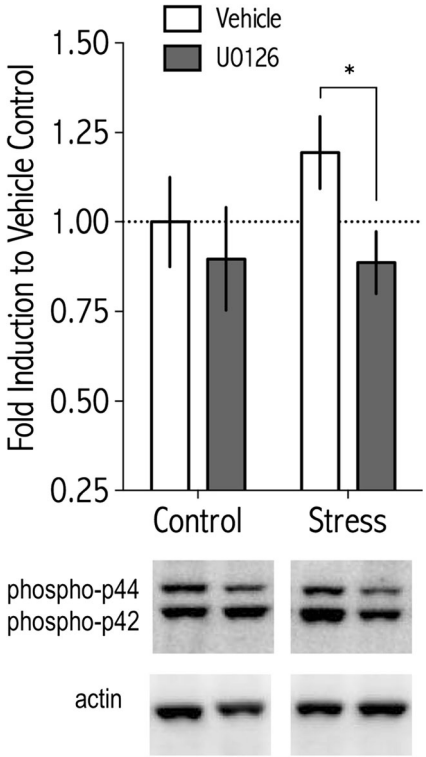

Fig. 3 Effect of VTA microinjection of U0126 or vehicle prior to a single defeat episode on ERK2 phosphorylation in the VTA. There are six subjects (n=6) per group. Data are expressed as the mean fold induction of pERK2/actin (±SEM) compared to nonstressed controls microinjected with vehicle into the VTA. *Open bars* represent vehicle-microinjected rats. *Filled bars* represent U0126-microinjected rats. *p<0.05

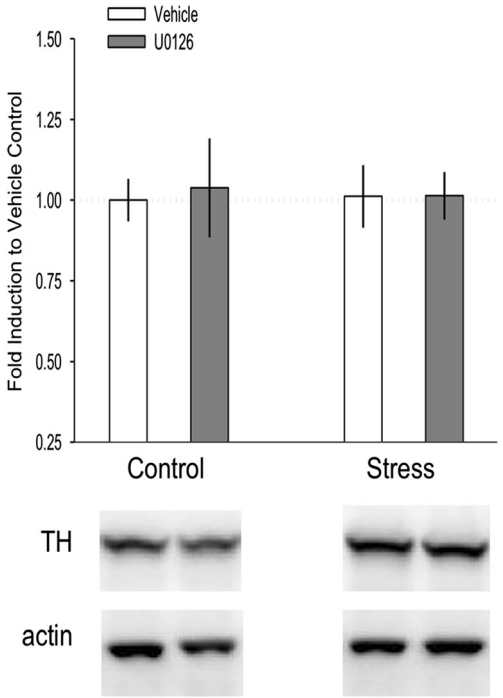

Fig. 4 Effect of VTA microinjection of U0126 or vehicle prior to a single defeat episode on tyrosine hydroxylase (TH) levels in the VTA. There are six subjects (n=6) per group. Data are expressed as the mean fold induction of TH/actin (±SEM) compared to nonstressed controls microinjected with vehicle into the VTA. *Open bars* represent vehicle-microinjected rats. *Filled bars* represent U0126-microinjected rats

U0126 (n=7), control vehicle (n=8), and control U0126 (n=10).

Discussion

Changes in intracellular signaling in the VTA, NAC, and PFC following episodic social defeat

Intermittent social defeat produced profound changes in ERK regulation in the VTA, which were necessary for defeat stress-induced behavioral sensitization to cocaine and elevated cocaine intake during binge self-administration. Intermittent social defeat decreased total ERK2, yet increased pERK2 levels in the VTA. Stress-induced increases in pERK2 are consistent with a prior study utilizing unpredictable stress (Iñiguez et al. 2010). Acute cocaine in nonstressed rats did not significantly increase pERK2, consistent with previous findings (Berhow et al. 1996; Valjent et al. 2004). A cocaine challenge failed to further increase pERK2 levels in socially defeated rats, suggesting that intermittent social defeat stress mimics cocaine's effects on ERK signaling in the VTA and occluded the effects of cocaine on ERK activation in the current study. In support of this, we found that microinjection of the MEK inhibitor U0126 into the VTA prior to each social defeat prevented the development of behavioral sensitization to cocaine and the escalation in cocaine intake during a 24-h unlimited access session.

There was a significant decrease in total CREB in the PFC due to stress. While not significant, there was a trend toward stress decreasing pCREB in the PFC. We have previously shown that repeated social defeat leads to decreased basal *zif268* mRNA expression in the medial prefrontal cortex, and this long-lasting effect is seen 60 days following the last defeat experience (Covington et al. 2005). Reduced pCREB in PFC may reflect stress-induced decreases in PFC activity, which could in turn activate compulsive drug taking (Sorg and Kalivas 1993; Chen et al. 2013). Repeated defeat stress results in increased dopamine in the PFC (Perez-Jaranay and Vives 1991; Tidey and Miczek 1996) and decreased *zif268* immediate early gene expression in the PFC (Covington et al. 2005). Using another form of stress, footshock, it has been shown that repeated footshock sessions (five daily 20-min sessions) result in a significantly reduced dopamine response to acute cocaine (216 % of baseline dopamine levels) compared to rats that received daily sham stress (five daily 20-min session of being in an identical apparatus as the stress group except no stress is administered) and are challenged with acute

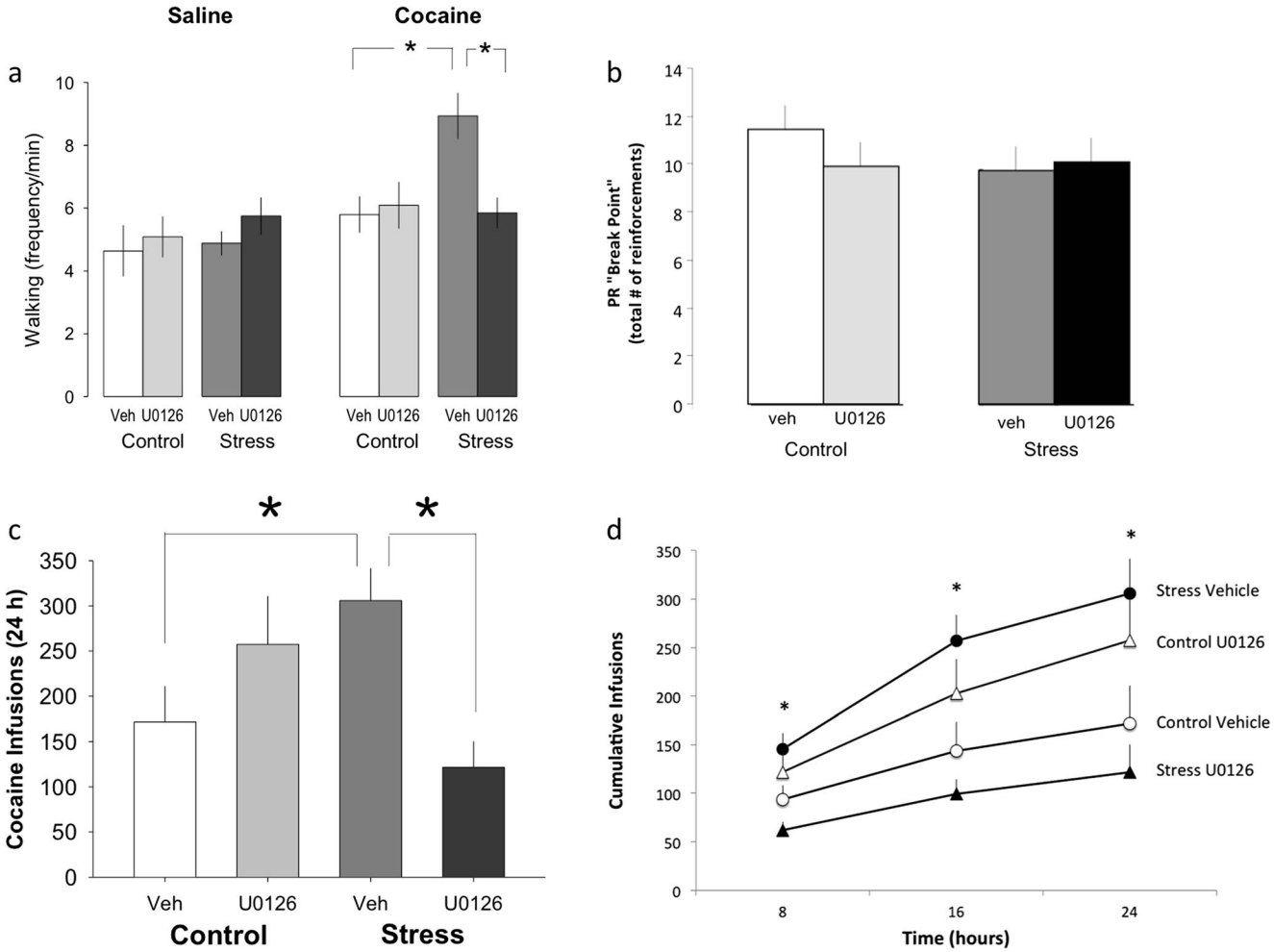

Fig. 5 **a** Day 20 cocaine challenge for rats microinjected with either U0126 or vehicle in the VTA prior to each defeat. Effects of intra-VTA microinjection of either U0126 (1 μg/side) or vehicle (20 % DMSO) prior to each defeat experience on the later behavioral response to cocaine (10 mg/kg, i.p.). There are four groups: control + vehicle (n=15), control + U0126 (n=14), stress + vehicle (n=15), and stress + U0126 (n=15). Data are expressed as mean frequency of walking behavior per minute (±SEM) during a 20-min time period. *p<0.001. **b** Cocaine intake during a progressive ratio schedule of reinforcement. Effects of intra-VTA microinjection of either U0126 (1 μg/side) or vehicle (20 % DMSO) prior to each defeat experience on subsequent cocaine self-administration during a PR schedule. There are four groups: control + vehicle (n=15), control + U0126 (n=14), stress + vehicle (n=15), and stress + U0126 (n=15). Data are expressed as mean total number of cocaine infusions (±SEM.) achieved. **c** Total cocaine intake during a 24-h binge. Effects of intra-VTA infusions of either U0126 (1 μg/side) or vehicle (20 % DMSO) prior to each defeat experience on subsequent cocaine self-administration during a 24-h binge. There are four groups: control + vehicle (n=8), control + U0126 (n=10), stress + vehicle (n=8), and stress + U0126 (n=7). Data are expressed as mean total number of cocaine infusions (±SEM). *p<0.05. **d** Cumulative cocaine infusions plotted across 24 h. Effects of intra-VTA infusions of either U0126 (1 μg/side) or vehicle (20 % DMSO) prior to each defeat experience on subsequent cocaine self-administration during a 24-h binge. There are four groups: control + vehicle (n=8), control + U0126 (n=10), stress + vehicle (n=8), and stress + U0126 (n=7). Each data point represents the mean (±SEM) cumulative cocaine infusions across 24 h (per 8-h time bin). *p<0.05 compared to nonstressed control vehicle group

cocaine (500 % of baseline; Sorg and Kalivas 1993). Animals expressing the strongest drug-seeking behaviors exhibit a prolonged reduction in activity in the deeper layers of the prelimbic cortex, a part of the brain thought to be associated with compulsive drug seeking (Chen et al. 2013). Correcting this hypoactivity using optogenetic strategies prevents cocaine-seeking behaviors, and additionally, optogenetic inhibition of prelimbic activity is sufficient to drive compulsive drug seeking (Chen et al. 2013). The neuroadaptive changes that mediate behavioral sensitization to the effects of abused drugs have been postulated as a possible mechanism contributing to increased drug use (Robinson and Berridge 1993, 2000).

Repeated defeat significantly decreased pCREB in the NAC. Viral vector-mediated elevations in CREB within the NAC reduce the rewarding effects of cocaine, morphine, and sucrose (Carlezon et al. 1998; Pliakas et al. 2001; Barrot et al. 2002), which indicates that a sustained elevation of CREB activity in the NAC produces an anhedonia-like profile, whereas reductions in CREB activity in the NAC through viral vector-mediated expression of the dominant-negative mutant mCREB increases the rewarding effects of cocaine,

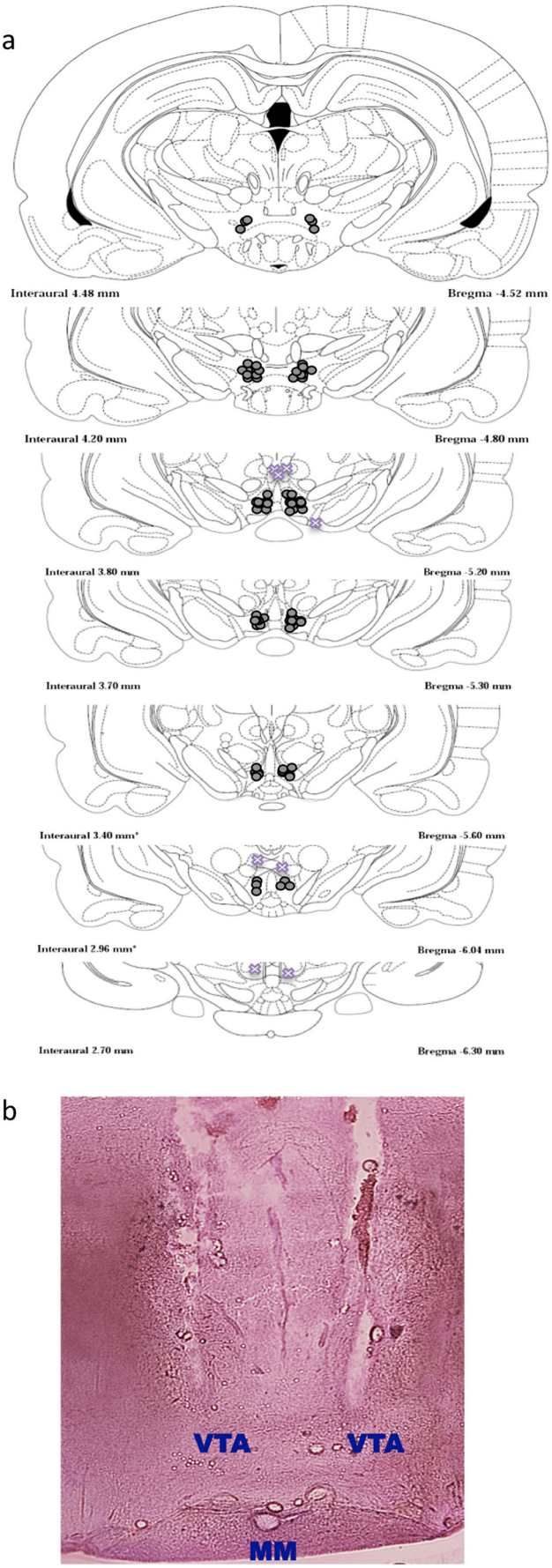

Fig. 6 **a** Schematic portrayal of accurately placed intra-VTA injection sites. The figures correspond to coronal sections of the rat brain at −4.52 to −6.30 mm from bregma. *Filled circles* represent the average location of each pair of bilateral injector tips. The injection sites of the four rats with inaccurate placements are shown as *open x's*. Thirty-three out of 37 rats had accurately placed intra-VTA injection sites. **b** Photomicrograph of representative intra-VTA cannula placements. *MM* medial mammillary nucleus

morphine, and sucrose (Barrot et al. 2002; Carlezon et al. 1998; Dinieri et al. 2009) and produces antidepressant-like effects in the forced swim test (Pliakas et al. 2001) and learned helplessness paradigm (i.e., escape behavior from footshock) (Newton et al. 2002). Decreases in CREB function in the NAC due to repeated defeat may increase the rewarding effects of cocaine and contribute to compulsive cocaine self-administration during the 24-h binge. It should be noted that chronic subordination stress and intermittent defeat lead to divergent behavioral phenotypes, with chronic stress leading to depressive-like behaviors and while intermittent stress can be activating in terms of cocaine and sucrose self-administration (Miczek et al. 2011). Repeated social defeat also significantly increased total ERK2 in the NAC. Although not significant, there was a trend toward stress increasing pERK2 in the NAC. Cocaine-induced ERK activity is enhanced in the NAC following repeated cocaine administration (Mattson et al. 2005), and repeated defeat may affect accumbens ERK activity similarly. Finally, it has been shown in vitro that persistent ERK phosphorylation negatively regulates CREB activity by increasing association of CREB binding protein (CBP) to CREB kinase $pp90^{RSK}$, leading to $pp90^{RSK}$ hypophosphorylation followed by suppression of CREB transcriptional activity (Wang et al. 2003). This ERK phosphorylation-induced inhibition of CREB phosphorylation is antagonized by the mitogen-activated protein kinase kinase inhibitors PD98059 and U0126 (Wang et al. 2003). Taken together, this may explain why decreased pCREB was observed under conditions of elevated ERK2 activity.

Surprisingly, we did not find an increase in ERK and CREB phosphorylation in the NAC following acute cocaine administration in our nonstressed control animals. While it failed to reach significance, there was a strong trend for cocaine to increase pERK2 in the NAC. Factors such as handling, housing conditions (i.e., novel versus conditioned drug context and single versus pair housing) and drug dose can all influence DA activity and thus, ERK and CREB function. It may be that our control rats were handled significantly more than the rats in previous studies (Berhow et al. 1996; Valjent et al. 2004). Stress itself can activate CREB and ERK in the NAC (Pliakas et al. 2001; Bruchas et al. 2008), which may be additive with cocaine's effects. Thus, ameliorating handling stress might decrease the total effect of cocaine injection on ERK and CREB activation. Additionally, the dose of cocaine used in our study (10 mg/kg i.p.) is lower

than those used in other studies examining ERK activity following cocaine (15 and 20 mg/kg, i.p.) (Valjent et al. 2004; Mattson et al. 2005; Valjent et al. 2006). Discrepancies in CREB and ERK activity within the NAC following acute cocaine injection may be a consequence of handling conditions and the cocaine challenge dose.

ERK phosphorylation in the VTA, sensitization, and cocaine taking

Microinjection of U0126 into the VTA prior to each defeat blocked the development of behavioral sensitization and also prevented the escalation in cocaine intake during a 24-h unlimited access session. We believe this effect to be due to U0126-mediated inhibition of ERK, based on this drug's potency and selectivity for MKK1 (Davies et al. 2000) and based on our finding that intra-VTA U0126 reduced pERK expression after a single defeat stress. For both cocaine and D-amphetamine, the induction of behavioral sensitization is thought to depend primarily on molecular events that occur in the VTA and promote a long-lasting increase in function of the meso-accumbens dopamine pathway (Pierce and Kalivas 1997). The role of ERK in the VTA is supported by the report that local injection of a selective MEK inhibitor, PD98059, before each cocaine administration blocks the initiation of locomotor sensitization without altering the acute locomotor responses (Pierce et al. 1999). However, regulation of ERK phosphorylation in response to psychostimulants is not as robust in the VTA as in the NAC. Indeed, it has been reported that cocaine activates ERK in the VTA following repeated but not single injections (Berhow et al. 1996; Valjent et al. 2004), which is consistent with our finding that VTA pERK was significantly increased after repeated but not single defeat stress exposures. In contrast, D-amphetamine has been shown to activate ERK in the VTA following single but not repeated injections (Rajadhyaksha et al. 2004)—but only in nondopaminergic neurons. Thus, there is a possibility that the ERK activation in other areas could also contribute to the induction of behavioral sensitization. Both glutamate and brain-derived neurotrophic factor (BDNF) are upstream regulators of ERK and thereby modulate ERK phosphorylation. Repeated social defeat results in elevated BDNF and AMPA receptor GluR1 subunit protein levels in the VTA (Miczek et al. 2011; Covington et al. 2008). Daily BDNF injections into the VTA or chronic BDNF administration via osmotic minipump into the VTA or NAC potentiate the acute locomotor-activating effects of cocaine during the induction of sensitization (Horger et al. 1999; Pierce et al. 1999). A single BDNF infusion into the VTA produces long-lasting enhancement of cocaine seeking for up to 30 days and is prevented by intra-VTA administration of the MEK inhibitor, U0126, indicating the involvement of the MAP kinase pathway in BDNF action (Lu et al. 2004). Therefore, social defeat stress-induced changes in BDNF and glutamate signaling in the VTA may underlie alterations in ERK signaling.

There were two findings in the i.v. self-administration experiment that were somewhat surprising. First, while repeated social defeat stress clearly enhanced cocaine intake during a 24-h session of unlimited access, we found no effect of stress on progressive ratio responding. This differs from our previous results in rats (Covington et al. 2005, 2008; Quadros and Miczek 2009) but is consistent with our more recent findings (Cruz et al. 2011; Boyson et al. 2011; Miczek et al. 2011). The potentiating effect of defeat on cocaine self-administration during a PR schedule of reinforcement previously seen diminishes with higher infusion doses (e.g., 0.75 mg/kg/infusion; (Covington and Miczek 2001)) and is difficult to see in mice using the same 0.3 mg/kg/infusion dose (Yap and Miczek 2007). Therefore, the effects of intermittent defeat on cocaine self-administration on a PR schedule are not consistent or robust.

Second, repeated U0126 microinjections into the VTA tended to increase binge cocaine self-administration in nonstressed controls compared to vehicle-treated controls, although this was not found to be statistically significant (p= 0.149). Rats that received repeated U0126 did not show behavioral sensitization on day 20, as measured by walking behavior, and our previous experiments support the notion that the expression of behavioral sensitization is a predictor of escalated cocaine self-administration during a 24-h binge (Covington and Miczek 2005; Covington et al. 2008). The effects of repeated MEK inhibition in the VTA in stress-naive animals on cocaine intake require further investigation. We did not necessarily differentiate between the anterior and posterior VTA, and cannula placements spanned from −4.52 to −6.04 from bregma (Paxinos and Watson 1997). There are regional differences in the VTA with regard to reward-related behaviors, and activity in the VTA can have opposite behavioral consequences depending on the subregion of the VTA involved (McBride et al. 1999; Ikemoto et al. 1997; Shabat-Simon et al. 2008; Olson et al. 2005). Anterior versus posterior VTA differ in both neuronal population and projection to the NAC, differentially regulating reward (Olson et al. 2005). Differences in cocaine intake within nonstressed animals treated with U0126 may be attributable to anatomical dissociation of anterior versus posterior placements within the VTA.

Our social defeat stress protocol was designed to create similar social experiences (Covington et al. 2005; Covington and Miczek 2001; Tornatzky and Miczek 1993). Our criteria for terminating an aggressive encounter are display of 5 s of supine posture by the intruder or a maximum of ten bites in a maximum of 5 min. Additionally, the intruder rat experiences 10 min of "threat" before the fight and another 10 min of threat after the fight. We rotate the aggressive stimulus animals across the four encounters. All this is done to reduce the variability in the quality of the defeat experience. We found

no difference between treatment groups in the proportion of animals that ended the fight by 5-s supine posture, and we found no significant differences in attack latency or attack bites. However, we did see a significant effect of microinjection treatment (U0126 vs vehicle) in the attack duration. This is not surprising to us. Since we have specific criteria that end the defeat (i.e., the display of 5 s of the supine posture or a maximum of ten bites within a maximum of 5 min, whichever appeared first), these criteria will affect attack duration. For example, if an intruder does not go into supine posture, the criterion of a maximum of ten bites within 5 min must be used, thus extending the duration of the defeat. Conversely, the defeat duration will be short and the number of bites will be low if the intruder goes into supine at the beginning of the encounter. Because of this, we employ the 10 min of threat before and after the fight. Collectively, our defeat protocol is aimed at ensuring the intruder rats experience an equivalent amount of stress.

Conclusions

The current experiments reveal that intermittent social defeat stress and its effects on behavioral sensitization and escalated cocaine intake can be attributed, in part, to increased ERK activity in the VTA. These findings are consistent with a role of VTA BDNF signaling in intermittent defeat stress-induced sensitization of cocaine-mediated behavior (Miczek et al. 2011). Taken together, the data suggest a model in which intermittent defeat stress triggers release of BDNF in the VTA, activation of ERK signal transduction pathways, and subsequent potentiation of drug seeking and taking behavior. Given that BDNF can enhance dopamine release, we predict that VTA ERK activation may be necessary. Future studies manipulating upstream regulators of ERK such as the TrkB receptor and downstream effectors of ERK, such as tyrosine hydroxylase will further delineate the molecular pathways necessary for addictive behavior.

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Conflict of interest The authors declare no conflict of interest.

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