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Control over stress accelerates extinction of drug seeking via prefrontal cortical activation

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ABSTRACT

Extinction is a form of inhibitory learning viewed as an essential process in suppressing conditioned responses to drug cues, yet there is little information concerning experiential variables that modulate its formation. Coping factors play an instrumental role in determining how adverse life events impact the transition from casual drug use to addiction. Here we provide evidence in rat that prior exposure to controllable stress accelerates the extinction of cocaine-seeking behavior relative to uncontrollable or no stress exposure. Subsequent experimentation using high-speed optogenetic tools determined if the infralimbic region (IL) of the ventral medial prefrontal cortex mediates the impact of controllable stress on cocaine-seeking behavior. Photoinhibition of pyramidal neurons in the IL during coping behavior did not interfere with subject's ability to control the stressor, but prevented the later control-induced facilitation of extinction. These results provide strong evidence that the degree of behavioral control over adverse events, rather than adverse events *per se*, potently modulates the extinction of cocaine-seeking behavior, and that controllable stress engages prefrontal circuitry that primes future extinction learning.

1. Introduction

A core feature of addiction is the ability of drug-associated cues and contexts to evoke intense craving and compulsive drug-seeking behavior (Childress et al., 1987). Consequently, many treatment strategies are directed at reducing the expression of conditioned behaviors produced by repetitive drug use. In pre-clinical models of drug administration, conditioned drug responses can be extinguished following repeated exposure to the drug-paired environment in the absence of the drug. Despite the extensive use of extinction procedures, little is known about experiential variables that modulate the speed and strength of extinction, as well as the neural mechanisms that mediate the effects of these variables. Only a minority of individuals that use substances with addictive potential go on to develop an addictive disorder (Anthony et al., 1994), and so an understanding of circumstances that promote or mitigate the expression of drug seeking is of clinical importance (Potenza, 2014).

Accumulating evidence indicates that the infralimbic region (IL) of the ventral medial prefrontal cortex (mPFCv) is critical for the suppression of both conditioned fear and appetitive behaviors (Quirk et al., 2006; Peters et al., 2009). Following cocaine self-administration and extinction training, inactivation of the IL is sufficient to elicit cocaine-seeking in the absence of any other reinstatement trigger (Peters et al., 2008). Moreover, recent findings support involvement of the IL in learning the extinction of conditioned reward-seeking behavior as well as the retrieval of extinction memory after cocaine-seeking is extinguished (Hsu and Packard, 2008; Peters et al., 2008; LaLumiere et al., 2010, 2012; Van den Oever et al., 2013). This is noted here because recent work suggests that prior exposure to controllable (escapable shock, ES), but not physically identical uncontrollable (inescapable shock, IS), stressors potently reduce conditioned fear responses through an IL-dependent mechanism (Baratta et al., 2007, 2008). Additionally, exposure to ES *after* fear conditioning facilitates the reduction of fear responses during extinction and eliminates the spontaneous recovery of fear. If controllable stress alters the IL in such a way as to

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suppress later conditioned fear responding, then perhaps prior control would also reduce the later expression of other conditioned responses regulated by the IL.

Adverse life events are thought to be important for the transition from recreational to compulsive drug use in humans (Kosten et al., 1986; Sinha, 2008), and exposure to stressors can facilitate the acquisition of drug self-administration (Piazza et al., 1990; Goeders and Guerin, 1994; Miczek and Mutschler, 1996) and reinstate extinguished drug-seeking (Shaham and Stewart, 1995; Highfield et al., 2000; Bossert et al., 2013). Along these lines, several studies have investigated the impact of stressor controllability on reactions to drugs of abuse. Exposure to a single session of IS, but not ES, enhances the later development of morphine conditioned place preference (CPP) (Will et al., 1998). Furthermore, a prior experience of ES can block later IS-induced potentiation of morphine CPP, even when the ES-to-IS interval is as long as 8 weeks (Rozeske et al., 2012). This stress-buffering effect of ES depends on IL activity during ES.

Here we evaluate the selective impact of stress on the acquisition of extinction and whether or not the dimension of control can modulate its impact. Specifically, the present experiments determine if stressor controllability modulates the extinction of drug-seeking behavior following cocaine self-administration, and use rapid and reversible optogenetic silencing to investigate the role of the IL in mediating the effects of behavioral control.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (300–325 g at the time of testing, Institute for Behavioral Genetics, Boulder, Colorado) were kept on a 12-h reverse light/dark cycle; all experiments were conducted during the dark phase. Animals were pair-housed until catheter implantation, after which they were housed individually. Standard lab chow and water were available *ad libitum*. Procedures were performed in accordance with standard ethical guidelines (National Institutes of Health *Guide for the Care and Use of Laboratory Animals*) and were approved by the Institutional Animal Care and Use Committee at the University of Colorado Boulder.

2.2. Cocaine self-administration

Rats were anesthetized with a ketamine/xylazine mixture (100 mg/kg and 8 mg/kg, respectively, i.p.) and implanted with intrajugular catheters. All incisions were sutured, stapled, and sealed with Vetbond (3M). After surgery, catheters were flushed daily with a sterile solution of heparin (2.5 units/mL) to maintain catheter patency. Five to seven days following catheterization, animals were trained to nose poke for intravenous cocaine HCl (0.5 mg/kg/infusion; National Institute on Drug Abuse) during daily 3-h sessions (65 infusion max) over 7 days. The 65-infusion limit was chosen to prevent adverse effects of the drug. Prior to the start of each session, rats were placed into self-administration chambers ($20 \times 20 \times 26$ cm) that were equipped with two nose-poke portals located 5 cm above the floor on opposite walls. A protective cap was removed from the catheter and its external end was connected via tubing (0.02 inch inner diameter; Saint-Gobain Performance Plastics) to a single-channel liquid swivel mounted in the ceiling of the self-administration chamber. Nose pokes into one of the ports (designated active) resulted in a cocaine infusion over 5 s on a fixed-ratio 1 (FR1) schedule of reinforcement. Each reinforced response resulted in a 20-s timeout period, during which time the active port was illuminated with a white LED positioned at the back of the port. Nose pokes made in the other port (designated inactive) were recorded but had no programmed consequence. At the end of the 7 days of self-administration training, the criteria for achieving stable responding were a) minimum of 30 reinforcers earned; b) preference for the active port greater than 70%; and c) no more than 20% variation (both number of reinforcers and active port preference) for at least two of the final three self-administration sessions.

2.3. Stressor controllability

For manipulation of controllability, subjects were run in a triad design. One subject of each triad received ES, a second received yoked IS, and third received no tailshock (home cage control, HC). Each rat was placed in a Plexiglas box ($14 \times 11 \times 17$ cm) with a wheel mounted in the front of the box. The tail was secured to a Plexiglas rod extending from the back of the box, and affixed with two copper electrodes and electrode paste (Parker Laboratories). Each tailshock session consisted of 80 trials of tailshock (27×1.0 mA, 27×1.3 mA, 26×1.6 mA) on a variable interval 60-s schedule (range = 45–90 s). As in prior studies (Baratta et al., 2007), the following procedure was used to insure that the ES rat learned the operant response to terminate the tail shock. Initially, the shock was terminated by a one-quarter turn of the wheel. The response requirements were increased by a one-quarter turn when three consecutive trials were completed in less than 5 s. Subsequent latencies under 5 s increased the requirement by 50% up to a maximum of four full turns. The requirement was reduced if the trial was not completed in less than 5 s. If the requirement was not reached in less than 30 s, the shock was terminated and the requirement was reduced to one-quarter turn of the wheel. An additional rationale for this procedure is that it maintains shocks at durations sufficient to produce the usual behavioral effects of IS in the yoked IS subject. Rats in the IS group received an *identical* amount of shock but had no control over its termination.

2.4. Extinction of cocaine-seeking behavior

Extinction was conducted in 3-h daily sessions, during which a nose poke in the active port resulted in a 20 s presentation of the white LED, but no cocaine delivery. Nose pokes made in the inactive port had no programmed consequences. Extinction sessions continued for each subject until the number of previously reinforced responses (active port) in a given session was <20% of extinction day 1 levels.

2.5. Viral vectors

Adeno-associated virus (AAV) vector was used to genetically target third-generation halorhodopsin (NpHR), a light-activated chloride pump, to IL pyramidal neurons. CaMKIIα::NpHR-eYFP and CaMKIIα::eYFP cassettes were packaged in AAV vectors serotyped with AAV5 coat proteins (titers: $3.0 - 6.0 \times 10^{12}$ genome copies/mL) by the Vector Core at the University of North Carolina at Chapel Hill.

2.6. Viral delivery and optical fiber implantation

For optogenetic experimentation, rats were anesthetized using a ketamine/xylazine mixture and a stainless steel needle with beveled tip (31 gauge; Hamilton Company) was directed to the IL (+2.5 mm anterior to bregma, ±0.5 mm lateral to the midline, −3.2 mm ventral to the cortical surface), and virus (1.0 μL/hemisphere) was infused over 10 min (0.1 μL/min) followed by an additional 10 min to allow for diffusion. After 2–3 weeks, animals were anesthetized and implanted with an intravenous catheter (as described above) into the right jugular and optical fibers (200 μm diameter core, 0.39 NA, approximately 1.0 mm center-to-center distance) into the bilateral IL

(+2.5 mm anterior to bregma, −3.5 mm ventral to the cortical surface). Optical fibers were secured to the skull with dental cement and stainless steel screws and surrounded by an inverted protective plastic head cap to prevent fiber damage during the tailshock session. All subjects were allowed 5–7 days recovery before behavioral experimentation. Optical fiber placement and viral expression in the IL were verified using a BX-61 Olympus scope, according to the rat brain atlas (Paxinos and Watson, 2007). Subjects were only included if the optical fiber tips were located within the transduced IL. Evidence suggests that the dorsal peduncular cortex, which is situated immediately ventral to the IL, is also part of the mPFCv that regulates extinction; therefore subjects with optical fiber placements close to the dorsal peduncular/IL border were included in all analyses (Peters et al., 2009; LaLumiere et al., 2010; Van den Oever et al., 2013).

2.7. In vivo recording

Single-unit recordings were conducted in a subset of animals ($n = 3$) under urethane anesthesia (1.5 g/kg, i.p.) 18–21 days post-viral injection. Simultaneous optical silencing and electrical recording of IL pyramidal neurons transduced with AAV-NpHR-eYFP were performed using an optrode consisting of a tungsten electrode (1–1.5 MΩ; MicroProbes) attached to an optical fiber. The optical fiber was coupled to a green diode laser ($\lambda = 532$ nm; Shanghai Laser & Optics Century) and yielded a fiber tip irradiance of approximately 150 mW/mm^2 as determined by an optical power meter (Newport) prior to insertion into brain. A small craniotomy was made over the viral injection site in the IL and the optrode was gradually lowered in 50 μm increments using a hydraulic micromanipulator (Narishige) starting from the dorsal boundary of the IL. Recorded signals were bandpass filtered at 300 Hz (low)/8 kHz (high) for unit activity using an ExAmp-20K low-noise spike amplifier (Kation Scientific).

2.8. Experimental overview

Experiment 1 investigated the impact of stressor controllability on the extinction of drug-seeking behavior. Following cocaine self-administration, rats were randomly assigned to either ES or yoked IS or HC treatment ($n = 9$–12/group). One week following stress treatment animals underwent extinction training.

Experiment 2 determined the role of the IL in mediating the facilitated extinction produced by prior ES. Prior to catheter implantation, AAV-NpHR-eYFP or AAV-eYFP was delivered bilaterally to the IL. Following cocaine self-administration, NpHR-eYFP and eYFP rats were assigned to either ES or HC ($n = 8$–10/group). On the day of stress treatment, optical fiber implants were connected to a patch cable that interfaced with a FC/PC fiber-optic rotary joint (Doric Lenses) which then interfaced with a 532 nm solid-state green laser located outside the wheel-turn box. Continuous light delivery coincided with the onset of tailshock and terminated 2 s following the offset of tailshock. An additional ES group ($n = 5$) received NpHR-mediated IL silencing distributed at random during the intertrial interval (ITI) with no specific relationship to shock onset or offset. This group was included to determine whether the observed effects of IL inhibition on ES extinction behavior required silencing to be "tied" to tailshock (when the controlling response occurs). Animals then underwent extinction one week later as described above.

2.9. Data analysis

Data analysis was performed with OriginPro software (OriginLab). The effect of treatment was analyzed with either repeated-measures (number of infusions across self-administration sessions, wheel-turn escape performance, *in vivo* single-unit recordings, number of active pokes for initial extinction sessions) or one-way (total cocaine intake, duration of light delivery, days to extinction) analysis of variance (ANOVA). Main effects and interactions were considered statistically significant if $p < 0.05$. When appropriate, post hoc analyses and planned comparisons were performed using Bonferroni-Holm correction for multiple comparisons. Statistical outliers, indicated by data two standard deviations beyond the group mean, were excluded from analyses. Values in graphs are represented as mean ± SEM.

3. Results

3.1. Effect of stressor controllability on extinction of cocaine seeking

In order to evaluate the impact of stressor controllability on extinction of cocaine-seeking behavior, stress treatment was given 24 h *after* cocaine self-administration training so that any effects of controllability on extinction could not be attributed to modulation of acquisition (Fig. 1A). Rats were trained to nose poke for cocaine (0.5 mg/kg/infusion) under an FR1 schedule of reinforcement. Analysis of cocaine self-administration behavior prior to stress group assignment showed no differences in the number of cocaine infusions across the daily 3-h sessions (group × session interaction: $F_{12,168} = 0.604$, $p = 0.837$; data not shown) and total cocaine intake (group: $F_{2,28} = 0.786$, $p = 0.466$; data not shown). Stress treatment consisted of 80 trials of ES, IS, or HC.

One week following stress exposure, animals were returned to their self-administration chambers for daily 3-h extinction sessions and continued until the extinction criterion was reached (number of previously reinforced responses in a given session was <20% of extinction day 1 levels). Extinction to a criterion was used, rather than a fixed number of extinction sessions, so that facilitation or interference effects of stress could more readily be detected. Prior exposure to ES significantly reduced the number of sessions necessary for extinction, whereas prior IS increased the number of sessions (days to extinction: ES, 6.8 ± 0.969; HC, 14.5 ± 2.00; IS, 23.1 ± 2.83; group: $F_{2,28} = 13.510$, $p < 0.001$; Fig. 1B,C) relative to HC. All three groups were significantly different from each other, with the ES group exhibiting a fewer number of sessions to reach extinction compared to HC ($p < 0.05$) and IS ($p < 0.001$) groups. Analysis of within-session responding during the first extinction session yielded no group differences (group: $F_{2,28} = 0.075$, $p = 0.787$; group × time interaction: $F_{10,140} = 0.831$, $p = 0.600$; Fig. 1D). Similarly, a repeated measures ANOVA revealed that groups did not differ across the initial three extinction sessions (when all subjects were represented) for total number of nose pokes in the active port (group: $F_{2,28} = 0.325$, $p = 0.573$; group × session interaction: $F_{4,56} = 1.14$, $p = 0.345$; Fig. 1E), further demonstrating that the ES-induced acceleration of extinction was not due to a difference in initial levels of responding.

3.2. Optical silencing of IL pyramidal cell activity in vivo

Next we addressed whether temporally specific silencing of the IL during ES would interfere with the ability of ES to facilitate later extinction. AAV vectors encoding the light-driven inward chloride pump, NpHR, driven by the CaMKIIα promoter, were stereotactically delivered in order to transduce glutamatergic neurons locally in the IL. Single unit recordings from NpHR-transduced IL pyramidal neurons in anesthetized rats demonstrated reliable silencing of spontaneous spiking activity in response to 10 s delivery of green light (time: $F_{2,14} = 25.637$, $p < 0.001$, one-way repeated measures ANOVA; Fig. 2). NpHR-mediated silencing was time-locked to the illumination period (firing rate: baseline, 1.69 ± 0.30 Hz; light

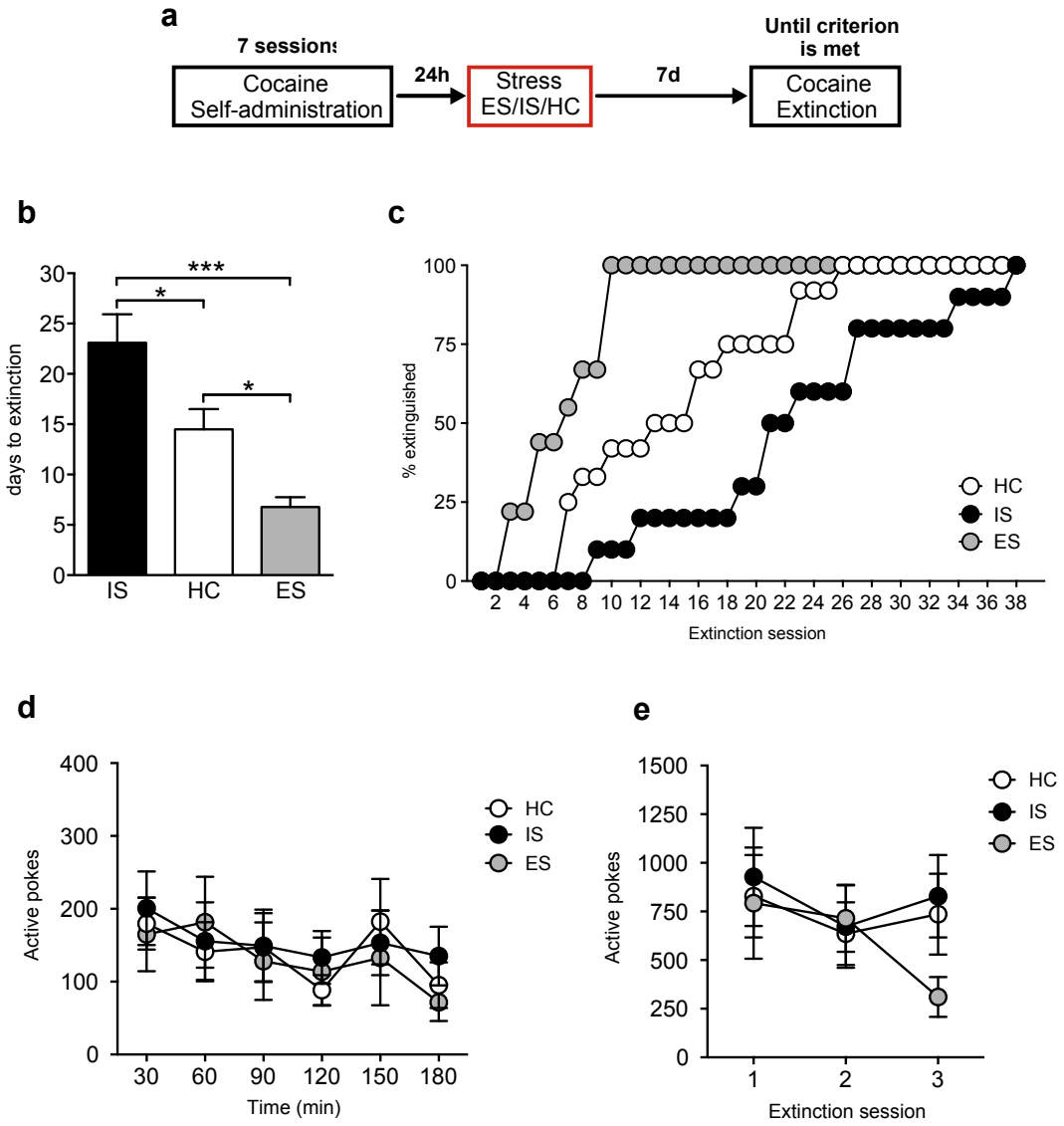

Fig. 1. Prior ES facilitates extinction of drug-seeking behavior. (a) Timeline of the experimental design. (b) Number of days to reach extinction criterion for HC ($n = 12$), IS ($n = 10$), and ES ($n = 9$) groups. (c) Cumulative percentage of HC, IS, and ES subjects extinguished per session. (d) Number of nose pokes made in the active port, in 30-min bins, during the first day of extinction. (e) Number of nose pokes made in the active port during the first three extinction sessions when all subjects were represented. Data are expressed as the mean ± SEM. $^{*}p < 0.05$ compared to HC, $^{***}p < 0.001$ compared to IS.

delivery, 0.04 ± 0.02 Hz; $p < 0.001$, Bonferroni-Holm correction) and the firing rate was restored to levels indistinguishable from baseline following light delivery (post light, 1.85 ± 0.36 Hz; $p = 0.59$, Bonferroni-Holm correction). The average latency of inhibitory responses was 0.23 ± 0.11 s (ranging from 0.1 to 1.0 s).

3.3. *Effect of silencing IL activity during controllable stress on extinction behavior*

Here the question was whether temporally specific silencing of the IL during ES would interfere with the ability of ES to facilitate later extinction of cocaine seeking (Fig. 3). Prior to behavioral experimentation, the IL was bilaterally injected with NpHR-eYFP or eYFP only (control) and subsequently implanted bilaterally with optical fibers (Fig. 3B,C). Analysis of cocaine self-administration prior to stress treatment showed that the number of infusions earned across the daily 3-h sessions (group × session interaction: $F_{18,174} = 0.652$, $p = 0.854$; Supplementary Fig. 1A) and total cocaine intake (group: $F_{3,29} = 0.430$, $p = 0.733$; Supplementary Fig. 1B) were similar between groups.

During ES, the IL was transiently inactivated via bilateral delivery of continuous light during tailshock presentation (when escape responses occur, ES-NpHR). An additional group, ES-NpHR(ITI), received an equal amount of light delivery distributed at random during the ITI with no specific relationship to shock onset or offset. Repeated-measures ANOVA indicated no differences between eYFP- or NpHR-treated ES groups on either response requirement (group: $F_{2,19} = 2.490$, $p = 0.131$; Supplementary Fig. 1C) or time to terminate the shock (group: $F_{2,19} = 4.101$, $p = 0.057$; Supplementary Fig. 1D). Importantly, all ES groups received the same amount of laser light delivery (group: $F_{2,19} = 1.333$, $p = 0.287$; Supplementary Fig. 1E).

One week following stress treatment, animals were returned to their self-administration chambers for daily 3-h extinction sessions. Initial results indicated no differences in extinction rates between HC-eYFP and HC-NpHR subjects (days to extinction:

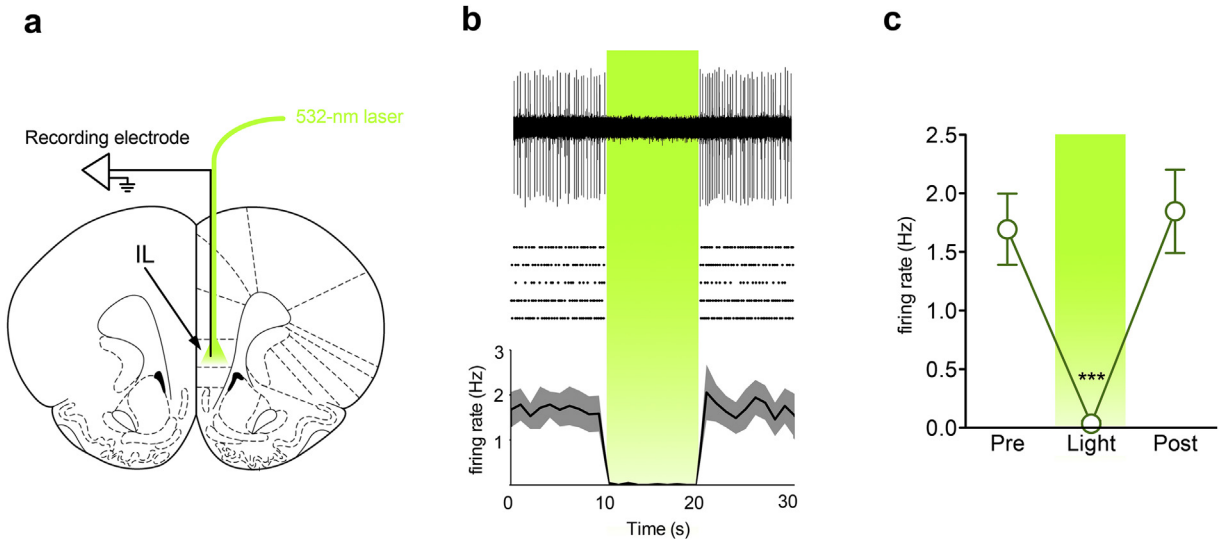

Fig. 2. NpHR rapidly and reversibly silences IL pyramidal cell activity *in vivo*. (a) An optrode (optical fiber attached to a tungsten electrode) was targeted to NpHR-transduced IL. (b) (Top) Representative voltage trace of single unit activity that was inhibited by continuous green (532 nm) light delivery. (Middle) Raster plot showing five repetitions of light-induced silencing in this neuron. Each unit activity is plotted as a dot. (Bottom) Average firing rate (Hz, black line) with SEM (gray shade) calculated from these five repetitions (bin width of 1000 ms). (c) Mean ± SEM of firing rate (Hz) during 10-s of green light illumination for eight different neurons from 3 different subjects. $^{***}p < 0.001$ compared to baseline (Pre).

12.4 ± 2.21 and 13.4 ± 2.69, respectively, n = 5/group; $p = 0.781$, Student's *t*-test), so these groups were pooled to form a single HC group used for statistical analysis. Similar to experiment 1, prior ES-eYFP exhibited accelerated extinction relative to HC subjects (Fig. 3D,E). However, when IL neurons were silenced with NpHR during each of the controllable tailshocks, the number of sessions to extinction was dramatically increased in ES subjects (days to extinction: HC, 12.9 ± 1.65; ES-eYFP, 5.7 ± 1.03; ES-NpHR, 16.75 ± 3.55; ES-NpHR[ITI], 6.4 ± 1.72; group: $F_{3,29} = 5.966$, $p < 0.01$). Planned comparisons using Bonferroni-Holm correction revealed that ES-eYFP required a significantly fewer number of sessions to extinguish compared to HC ($p < 0.05$) and ES-NpHR ($p < 0.001$), but did not differ from subjects that received IL silencing during the ITI ($p = 0.84$). ES-NpHR(ITI) subjects exhibited a trend toward facilitated extinction, although this did not reach statistical significance (vs HC, $p = 0.06$). Additionally, a repeated measures ANOVA revealed that groups did not differ in the mean number of active port nose pokes on the first extinction session when all subjects were represented (group: $F_{3,29} = 1.168$, $p = 0.339$). Together, these data indicate that the IL activity during the controlling response is critical for the later facilitation of extinction learning.

4. Discussion

The present findings indicate that exposure to stressors of differing controllability have bi-directional effects on the later extinction of conditioned drug responses. Exposure to IS profoundly interfered with extinction training that began 7 days later; IS subjects required significantly more sessions to extinguish the cocaine-associated operant response even though responding during the initial extinction sessions was identical to ES and HC. Stress-induced resistance to extinction has also been observed when stress treatment is given in conjunction with extinction training. Kupferschmidt et al. (2009) demonstrated that pretreatment of a pharmacological stressor (yohimbine) prior to daily extinction sessions attenuated the extinction rate of cocaine-seeking behavior.

The IS effect also parallels the large body of literature showing that stressors across a variety of modalities (e.g., footshock, food deprivation, cold swim stress) can reinstate drug-seeking behavior following extinction (Shaham and Stewart, 1995; Highfield et al., 2000; Shalev et al., 2000; Conrad et al., 2010). Similarly, environmental stimuli conditioned to drug delivery or availability can trigger reinstatement when presented alone or in an additive fashion when combined with stress (Liu and Weiss, 2002; Banna et al., 2010). This is particularly relevant to the current findings since active nose pokes during extinction training resulted in contingent presentation of the light cue previously paired with cocaine during training. Typically, IS-induced behavioral effects follow a transient time course (24–72 h) when testing is conducted in a novel environment (Maier and Watkins, 2005). In contrast, the enduring nature of IS extinction resistance observed here occurred in the presence of conditioned stimuli (the drug-paired cue and environment). The prolonged IS-induced resistance is probably not due to an increase in the associative strength acquired by the conditioned stimuli paired with cocaine, given that stress treatment occurred *after* self-administration. Rather, the experience of IS most likely increased the persistence of conditioned responding produced by conditioned stimuli that have a given associative strength.

The main focus of the present experiments was to understand whether the experience of behavioral control over stress alters later extinction of drug seeking. This has not previously been examined. The pattern of extinction behavior that emerged following physically identical ES was the exact opposite to that of IS. ES administered prior to extinction not only prevented the effects on extinction observed with IS, but rather it accelerated the extinction process compared to HC. These results were striking given that ES itself is a highly "stressful" event and produces a hypothalamo-pituitary-adrenal response that is as large as that produced by IS (Maier et al., 1986; Helmreich et al., 1999, 2012). Prior work directed at understanding the mechanisms by which control confers protection from the effects of stressors on conditioned fear responding support the idea that the IL becomes activated during ES, but not IS, and that inactivation of IL activity during ES produces exaggerated

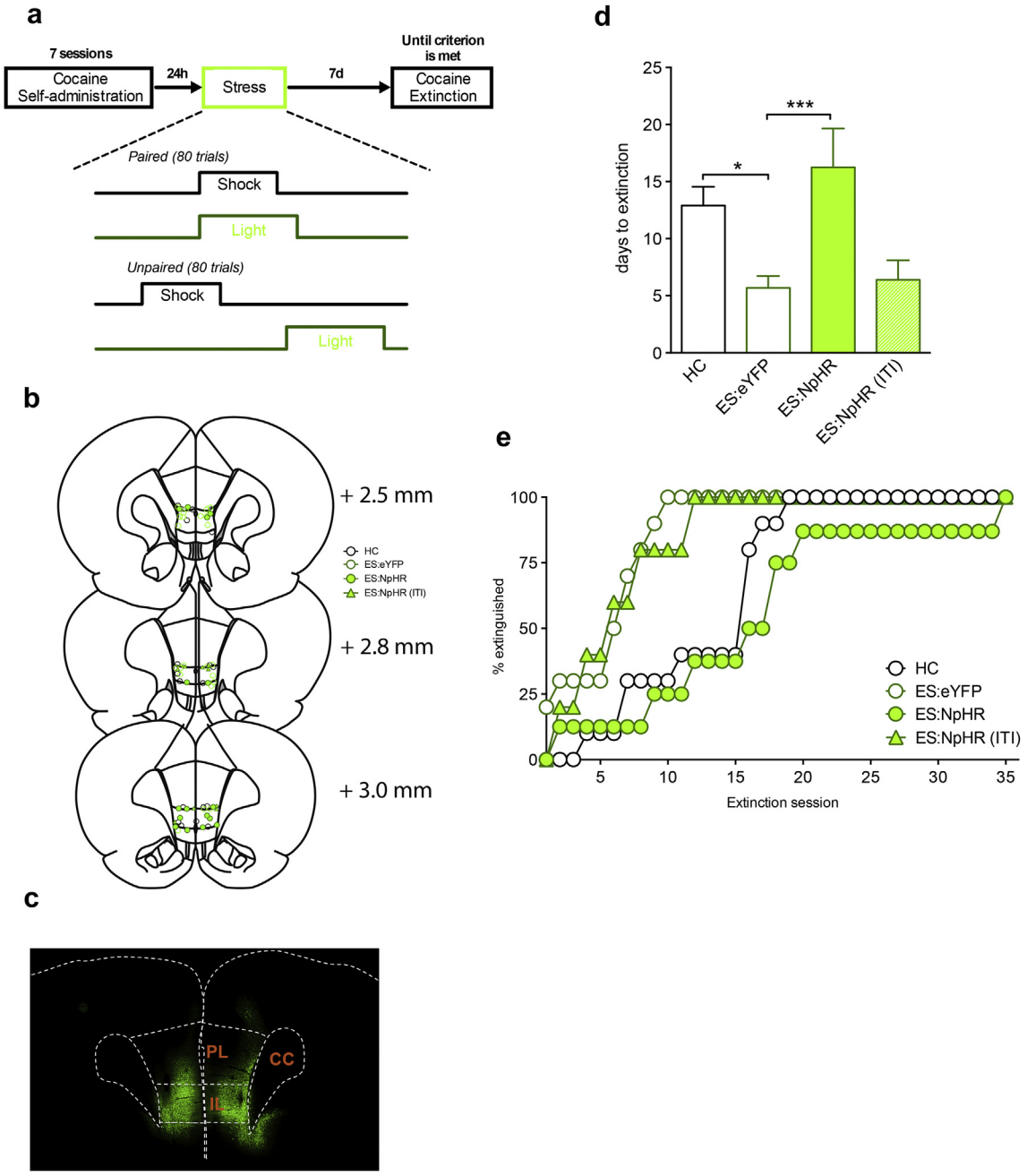

Fig. 3. IL silencing paired with tailshock interferes with accelerated extinction produced by ES. (a) Timeline of the experimental design and schematic illustration of the relationship between light delivery and shock. ES subjects received either continuous light that coincided with the onset of tailshock and terminated 2 s following the offset of tailshock (paired) or continuous light that was distributed in a pseudorandom fashion during the intertrial interval (ITI) with no specific relationship to shock onset or offset (unpaired). (b) Diagram of optical fiber placement within IL. Numbers refer to mm relative to bregma. (c) A representative coronal section of the medial prefrontal cortex with expression of NpHR-eYFP (green) in pyramidal neurons within the IL. (d) Number of days to reach extinction criterion for HC ($n = 10$), ES-eYFP ($n = 10$), ES-NpHR ($n = 8$), and ES-NpHR(ITI) ($n = 5$) groups. (d) Cumulative percentage of HC, ES-eYFP, ES-NpHR, and ES-NpHR(ITI) subjects extinguished per session. Data are expressed as the mean ± SEM. $^{*}p < 0.05$ compared to HC, $^{***}p < 0.001$ compared to ES-NpHR.

fear responding typical of IS exposure (Baratta et al., 2007, 2008). In the present set of experiments, we found that transient bilateral silencing of the IL during tailshock (when the controlling response occurs) dramatically increased the number of sessions required for ES animals to become extinguished. This did not occur when IL activity was transiently silenced outside of the tailshock/controlling response period (ITI), suggesting that the impact of ES on subsequent extinction learning requires the conjoint presence of IL activity with some aspect of the controlling response.

The results of the present experiments are consistent with previous findings indicating that IL activity is necessary for extinction of drug seeking. Peters et al. (2008) showed that only IL inactivation following, but not prior, to extinction learning triggered cocaine-seeking behavior. Additionally, post-training inactivation of the IL following each extinction session impairs the retention of extinction memory of cocaine seeking (LaLumiere et al., 2010). Taken together, these data suggest that the IL is recruited during the extinction process in order to suppress

conditioned responding. It may be the case that ES mimics extinction training by inducing changes in the IL that prime future extinction learning. As noted above, this is similar to data examining the effects of ES on conditioned fear responses. Behavioral control over a stressor facilitates the reduction of fear responses during extinction, a finding that extends from rats to humans (Baratta et al., 2007; Hartley et al., 2014). Intra-IL microinjection of muscimol blocks the ability of prior ES to reduce fear to a conditioned tone and context. Recent evidence points to the IL as a critical site where fear and appetitive extinction circuitries intersect. Direct stimulation of the IL has been shown to inhibit both conditioned fear and drug responses (Milad and Quirk, 2002; Ghazizadeh et al., 2012), and extinction-induced plasticity in the IL has been described following extinction of both types of responses (Burgos-Robles et al., 2007; Ghasemzadeh et al., 2011), although the underlying molecular mechanisms and temporal involvement of the IL may differ between the two extinction forms (Sun and Rebec, 2005; Mueller et al., 2010; Van den Oever et al., 2013; Do-Monte et al., 2015). Recent work has demonstrated that controllable stress elicits a rapid increase in the transcription cascades that follow NMDAR activation in the IL, namely the phosphorylated extracellular signal-regulated kinases 1 and 2 (pERK1/2), relative to uncontrollable or no stress controls (Christianson et al., 2014). Presumably the ability of behavioral control to generalize across appetitive and aversive domains is through the induction of plasticity in IL neurons targeting distinct downstream effectors that regulate the expression of these behaviors (e.g., amygdala and nucleus accumbens shell, respectively), but this has yet to be directly tested.

The current data suggest that the degree of control over adverse events, rather than adverse events *per se*, is a potent modulator of conditioned drug responding, and that control over stress engages the IL to prevent stress-induced resistance to extinction. Many of the psychosocial factors associated with resistance to addiction or relapse involve processes that engage coping strategies (Veenstra et al., 2007; Alim et al., 2012), and current clinical interventions aim to prevent the onset or minimize the extent of drug use by enhancing an individual's coping capacity (Rohsenow et al., 2004). Thus, earlier experiences with control or other types of active coping skills may protect against the development of addiction by priming the prefrontal cortex to respond actively during exposure to drug-related environmental cues and contexts.

Conflicts of interest

The authors report no competing financial interests or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ynstr.2015.03.002.

References

Alim, T.N., Lawson, W.B., Feder, A., Iacoviello, B.M., Saxena, S., Bailey, C.R., Greene, A.M., Neumeister, A., 2012. Resilience to meet the challenge of addiction: psychobiology and clinical considerations. Alcohol Res. 34, 506–515.

Anthony, J.C., Warner, L.A., Kessler, R.C., 1994. Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: basic findings from the National Comorbidity Survey. Exp Clin Psychopharmacol 2, 244–268.

Banna, K.M., Back, S.E., Do, P., See, R.E., 2010. Yohimbine stress potentiates conditioned cue-induced reinstatement of heroin-seeking in rats. Behav. Brain Res. 208, 144–148.

Baratta, M.V., Lucero, T.R., Amat, J., Watkins, L.R., Maier, S.F., 2008. Role of the ventral medial prefrontal cortex in mediating behavioral control-induced reduction of later conditioned fear. Learn Mem. 15, 84–87.

Baratta, M.V., Christianson, J.P., Gomez, D.M., Zarza, C.M., Amat, J., Masini, C.V., Watkins, L.R., Maier, S.F., 2007. Controllable versus uncontrollable stressors bidirectionally modulate conditioned but not innate fear. Neuroscience 146, 1495–1503.

Bossert, J.M., Marchant, N.J., Calu, D.J., Shaham, Y., 2013. The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacol. Berl. 229, 453–476.

Burgos-Robles, A., Vidal-Gonzalez, I., Santini, E., Quirk, G.J., 2007. Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. Neuron 53, 871–880.

Childress, A.R., McLellan, A.T., Ehrman, R.N., O'Brien, C.P., 1987. Extinction of conditioned responses in abstinent cocaine or opioid users. NIDA Res. Monogr. 76, 189–195.

Christianson, J.P., Flyer-Adams, J.G., Drugan, R.C., Amat, J., Daut, R.A., Foilb, A.R., Watkins, L.R., Maier, S.F., 2014. Learned stressor resistance requires extracellular signal-regulated kinase in the prefrontal cortex. Front. Behav. Neurosci. 8, 348.

Conrad, K.L., McCutcheon, J.E., Cotterly, L.M., Ford, K.A., Beales, M., Marinelli, M., 2010. Persistent increases in cocaine-seeking behavior after acute exposure to cold swim stress. Biol. Psychiatry 68, 303–305.

Do-Monte, F.H., Manzano-Nieves, G., Quinones-Laracuente, K., Ramos-Medina, L., Quirk, G.J., 2015. Revisiting the role of infralimbic cortex in fear extinction with optogenetics. J. Neurosci. 35, 3607–3615.

Ghasemzadeh, M.B., Vasudevan, P., Giles, C., Purgianto, A., Seubert, C., Mantsch, J.R., 2011. Glutamatergic plasticity in medial prefrontal cortex and ventral tegmental area following extended-access cocaine self-administration. Brain Res. 1413, 60–71.

Ghazizadeh, A., Ambroggi, F., Odean, N., Fields, H.L., 2012. Prefrontal cortex mediates extinction of responding by two distinct neural mechanisms in accumbens shell. J. Neurosci. 32, 726–737.

Goeders, N.E., Guerin, G.F., 1994. Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. Psychopharmacol. Berl. 114, 63–70.

Hartley, C.A., Gorun, A., Reddan, M.C., Ramirez, F., Phelps, E.A., 2014. Stressor controllability modulates fear extinction in humans. Neurobiol. Learn Mem. 113, 149–156.

Helmreich, D.L., Watkins, L.R., Deak, T., Maier, S.F., Akil, H., Watson, S.J., 1999. The effect of stressor controllability on stress-induced neuropeptide mRNA expression within the paraventricular nucleus of the hypothalamus. J. Neuroendocrinol. 11, 121–128.

Helmreich, D.L., Tylee, D., Christianson, J.P., Kubala, K.H., Govindarajan, S.T., O'Neill, W.E., Becoats, K., Watkins, L., Maier, S.F., 2012. Active behavioral coping alters the behavioral but not the endocrine response to stress. Psychoneuroendocrinology 37, 1941–1948.

Highfield, D., Clements, A., Shalev, U., McDonald, R., Featherstone, R., Stewart, J., Shaham, Y., 2000. Involvement of the medial septum in stress-induced relapse to heroin seeking in rats. Eur. J. Neurosci. 12, 1705–1713.

Hsu, E., Packard, M.G., 2008. Medial prefrontal cortex infusions of bupivacaine or AP-5 block extinction of amphetamine conditioned place preference. Neurobiol. Learn Mem. 89, 504–512.

Kosten, T.R., Rounsaville, B.J., Kleber, H.D., 1986. A 2.5-year follow-up of depression, life crises, and treatment effects on abstinence among opioid addicts. Arch. Gen. Psychiatry 43, 733–738.

Kupferschmidt, D.A., Tribe, E., Erb, S., 2009. Effects of repeated yohimbine on the extinction and reinstatement of cocaine seeking. Pharmacol. Biochem. Behav. 91, 473–480.

LaLumiere, R.T., Niehoff, K.E., Kalivas, P.W., 2010. The infralimbic cortex regulates the consolidation of extinction after cocaine self-administration. Learn Mem. 17, 168–175.

LaLumiere, R.T., Smith, K.C., Kalivas, P.W., 2012. Neural circuit competition in cocaine-seeking: roles of the infralimbic cortex and nucleus accumbens shell. Eur. J. Neurosci. 35, 614–622.

Liu, X., Weiss, F., 2002. Additive effect of stress and drug cues on reinstatement of ethanol seeking: exacerbation by history of dependence and role of concurrent activation of corticotropin-releasing factor and opioid mechanisms. J. Neurosci. 22, 7856–7861.

Maier, S.F., Watkins, L.R., 2005. Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci. Biobehav Rev. 29, 829–841.

Maier, S.F., Ryan, S.M., Barksdale, C.M., Kalin, N.H., 1986. Stressor controllability and the pituitary-adrenal system. Behav. Neurosci. 100, 669–674.
Miczek, K.A., Mutschler, N.H., 1996. Activational effects of social stress on IV cocaine self-administration in rats. Psychopharmacol. Berl. 128, 256–264.
Milad, M.R., Quirk, G.J., 2002. Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420, 70–74.
Mueller, D., Bravo-Rivera, C., Quirk, G.J., 2010. Infralimbic D2 receptors are necessary for fear extinction and extinction-related tone responses. Biol. Psychiatry 68, 1055–1060.
Paxinos, G., Watson, C., 2007. The Rat Brain in Stereotaxic Coordinates, sixth ed. Academic Press, New York.
Peters, J., LaLumiere, R.T., Kalivas, P.W., 2008. Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. J. Neurosci. 28, 6046–6053.
Peters, J., Kalivas, P.W., Quirk, G.J., 2009. Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem. 16, 279–288.
Piazza, P.V., Deminiere, J.M., le Moal, M., Simon, H., 1990. Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Res. 514, 22–26.
Potenza, M.N., 2014. Biased behaviors: towards understanding vulnerability and resilience factors in addictions. Biol. Psychiatry 75, 94–95.
Quirk, G.J., Garcia, R., Gonzalez-Lima, F., 2006. Prefrontal mechanisms in extinction of conditioned fear. Biol. Psychiatry 60, 337–343.
Rohsenow, D.J., Monti, P.M., Martin, R.A., Colby, S.M., Myers, M.G., Gulliver, S.B., Brown, R.A., Mueller, T.I., Gordon, A., Abrams, D.B., 2004. Motivational enhancement and coping skills training for cocaine abusers: effects on substance use outcomes. Addiction 99, 862–874.
Rozeske, R.R., Der-Avakian, A., Watkins, L.R., Maier, S.F., 2012. Activation of the medial prefrontal cortex by escapable stress is necessary for protection against subsequent inescapable stress-induced potentiation of morphine conditioned place preference. Eur. J. Neurosci. 35, 160–165.
Shaham, Y., Stewart, J., 1995. Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacol. Berl. 119, 334–341.
Shalev, U., Highfield, D., Yap, J., Shaham, Y., 2000. Stress and relapse to drug seeking in rats: studies on the generality of the effect. Psychopharmacol. Berl. 150, 337–346.
Sinha, R., 2008. Chronic stress, drug use, and vulnerability to addiction. Ann. N. Y. Acad. Sci. 1141, 105–130.
Sun, W., Rebec, G.V., 2005. The role of prefrontal cortex D1-like and D2-like receptors in cocaine-seeking behavior in rats. Psychopharmacol. Berl. 177, 315–323.
Van den Oever, M.C., Rotaru, D.C., Heinsbroek, J.A., Gouwenberg, Y., Deisseroth, K., Stuber, G.D., Mansvelder, H.D., Smit, A.B., 2013. Ventromedial prefrontal cortex pyramidal cells have a temporal dynamic role in recall and extinction of cocaine-associated memory. J. Neurosci. 33, 18225–18233.
Veenstra, M.Y., Lemmens, P.H., Friesema, I.H., Tan, F.E., Garretsen, H.F., Knottnerus, J.A., Zwietering, P.J., 2007. Coping style mediates impact of stress on alcohol use: a prospective population-based study. Addiction 102, 1890–1898.
Will, M.J., Watkins, L.R., Maier, S.F., 1998. Uncontrollable stress potentiates morphine's rewarding properties. Pharmacol. Biochem. Behav. 60, 655–664.