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Post-Stress Block of Kappa Opioid Receptors Rescues Long-Term Potentiation of Inhibitory Synapses and Prevents Reinstatement of Cocaine Seeking

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Abstract

Background—Dopaminergic neurons in the ventral tegmental area (VTA) of the brain are an important site of convergence of drugs and stress. We previously identified a form of long-term potentiation of GABAergic synapses on these neurons (LTP_{GABA}). Our studies have shown that exposure to acute stress blocks this LTP, and that reversal of the block of LTP_{GABA} is correlated with prevention of stress-induced reinstatement of cocaine-seeking.

Methods—Sprague-Dawley rats were subjected to cold-water swim stress. Midbrain slices were prepared following stress, and whole-cell patch clamp recordings of IPSCs were performed from VTA dopamine neurons. Antagonists of glucocorticoid and kappa opioid receptors were administered at varying time points after stress. Additionally, the ability of a post-stress kappa antagonist to block FSS-induced reinstatement of cocaine self-administration was tested.

Results—We report that an acute stressor blocks LTP_{GABA} for five days after stress through a transient activation of glucocorticoid receptors and more lasting contribution of kappa opioid receptors. Pharmacological block of kappa opioid receptors beginning as late as 4 days after stress has occurred can reverse the block of LTP_{GABA}. Furthermore, post-stress administration of a kappa opioid antagonist prevents reinstatement of cocaine-seeking.

Conclusions—Our results show that a brief stressor can cause days-long changes in the reward circuitry and reveal roles for glucocorticoid and kappa opioid receptors as mediators of the lasting effects of stress on synaptic plasticity. These results indicate that kappa opioid receptor

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antagonists reverse the neuroadaptations underlying stress-induced drug-seeking behavior and may be useful in the treatment of cocaine addiction.

Keywords

Stress; kappa opioid receptor; glucocorticoid receptor; synaptic plasticity; ventral tegmental area; reinstatement

Introduction

Studies utilizing animal models have repeatedly shown that acute or chronic stress escalates intake of addictive drugs (1-5). Moreover, in animals in which cocaine self-administration was extinguished, stress causes reinstatement of drug-seeking behavior (6-11). In particular, acute severe stress increases drug-seeking for several days (11).

These behavioral interactions between stress and drugs of abuse have led to interest in the brain's mesolimbic reward circuitry as a potential locus for stress-induced dysregulation. Within this circuitry, dopamine neurons of the ventral tegmental area (VTA) are important targets of both stress and drugs of abuse (12-17). Although activation of dopamine neurons is classically associated with reward and reinforcement, numerous studies have shown that acute and chronic stressors also enhance dopaminergic function in the mesolimbic system (18-22).

Synapses on dopamine neurons exhibit parallel changes after exposure to stress or to drugs of abuse, suggesting a potential mechanism for the induction of drug-seeking behavior after stress. Many studies have found that excitatory synapses on VTA dopamine neurons are potentiated, as indicated by the ratio of AMPA to NMDA receptors, following exposure to drugs of abuse or by acute stress (23-29). Either in vivo or in vitro administration of the GR agonist dexamethasone increases the AMPA/NMDA ratio, indicating that glucocorticoid receptor activation is sufficient to potentiate these synapses (26).

In addition to these well-documented changes in excitatory synapses, our previous work identified a form of stress-sensitive plasticity of GABAergic synapses on dopamine neurons (LTP_{GABA}) (30, 31). These synapses are potentiated via retrograde signaling of nitric oxide (NO) from the dopamine neuron to a presynaptic GABAergic terminal, leading to a persistent increase GABA release (31, 32). Acute cold water swim stress blocks LTP_{GABA} (25, 30), and we have identified two major pathways that contribute to this block. First, RU486, an antagonist of glucocorticoid receptors (GRs), administered prior to stress prevents the loss of LTP_{GABA} (30). Second, norBNI, an antagonist of κ opioid receptors (κ ORs), also prevents the block of LTP_{GABA} by stress. Importantly, unlike RU486, which normalizes plasticity of both excitatory and inhibitory synapses (23, 30), norBNI prevents the loss of LTP_{GABA} without preventing the stress-induced increase in AMPA/NMDA ratio at excitatory synapses (25). Concurrently, we found that infusion of norBNI directly into the VTA prevents reinstatement of cocaine-seeking after the same cold water swim stress, suggesting that reinstatement can be prevented without restoring AMPA/NMDA ratios to their pre-stress level (25).

In the current study, we expand on our previous work to investigate the time course of the loss of LTP_{GABA} after stress. We find that acute swim stress prevents LTP_{GABA} for at least five days after stress, and that this is dependent on early activation of glucocorticoid receptors in addition to a prolonged contribution of kappa opioid receptors. Surprisingly, we find that treatment with a κ OR antagonist reverses the effects of stress on both GABAergic synaptic plasticity and cocaine-seeking, even at time points considerably after stress has occurred. Together, our results suggest a mechanism by which brief stressors can induce long-lasting changes in synaptic plasticity and in behavior and support a potential target for reversing such neuroadaptations even after the stress has occurred.

Materials and Methods

For detailed methodological information, please see supplementary materials

Animals for slice electrophysiology

All procedures were carried out in accordance with the guidelines of the National Institutes of Health for animal care and use, and were approved by the Brown University Institutional Animal Care and Use Committee.

Acute Forced Swim Stress

Stress was administered by a modified Porsolt forced swim task (FSS) as previously described (23, 30). RU486 (40 mg/kg i.p.) was administered either 60 minutes or 24 hours after FSS. Nor-binaltorphimine (norBNI; 10 mg/kg, i.p.) was administered either two hours, 24 hours, or 4 days after FSS. For corticosterone measurement, serum was isolated from trunk blood and corticosterone levels were determined via EIA.

Self-administration

Self-administration was performed as previously described (25). For self-administration experiments, male Sprague Dawley rats (Taconic) weighing 200-225g at the start of the experiment were individually housed in square plastic cages. All procedures were approved by the University of Pennsylvania Animal Care and Use Committee. Rats were allowed to lever press for cocaine 6 days a week on a FR1 schedule with a time-out of 20 s and a maximum of 30 infusions per session. Once an animal achieved at least 20 infusions of cocaine in a single daily session under the FR1 schedule, the subject was switched to a fixed-ratio 5 (FR5) schedule of reinforcement.

Extinction & Reinstatement

Following 18 days of cocaine self-administration, drug-seeking behavior was extinguished by replacing the cocaine with 0.9% saline. One hour following the final extinction session, animals were exposed to a 3-minute cold-water swim stress. Animals received intraperitoneal (i.p.) injections of either saline or norBNI (10mg/kg) 2 hours following the stress exposure. The reinstatement session, which was identical to the previous extinction sessions, occurred 24 hours after the stress exposure (11).

Preparation of brain slices and electrophysiology

General methods were as previously reported (30). Midbrain slices (250 μ m) were prepared from deeply anesthetized Sprague-Dawley rats (30, 31). In all experiments, the extracellular solution was artificial cerebrospinal fluid containing 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μ M) and strychnine (1 μ M) to block AMPA and glycine receptors. Patch pipettes were filled with a KCl based solution. Dopamine neurons, which comprise about 70% of all VTA neurons, were identified by the presence of a large I_h -current (> 50 pA) during a voltage step from -50 mV to -100 mV. IPSCs were stimulated using a bipolar stimulating electrode in the lateral VTA. LTP_{GABA} was induced by bath application of the NO donor, SNAP (S-nitroso-N-acetylpenicillamine, 400 μ M).

Analysis

Data are presented as means $\pm$ SEM of the percent change of LTP levels. All n's are the number of animals, unless otherwise noted. Significance was determined using a Student's *t*-test or an ANOVA with a significance level of $p < 0.05$, with post-hoc comparisons as noted.

Results

Acute Stress Blocks LTP_{GABA} for at least five days

Our previous studies have shown that acute forced swim stress (FSS) blocks LTP_{GABA} (25, 30), however one outstanding question is how long this effect lasts. To test this, we assayed LTP_{GABA} at varying time points after a single exposure to stress. Dopamine neurons were identified electrophysiologically by the presence of an I_h . In the lateral VTA, where we record, I_h + dopamine neurons are primarily those that project to the nucleus accumbens (33). However, it has been reported that there are also I_h + GABAergic neurons (34). Therefore, using this criterion, a subset of the neurons recorded from and reported here may be non-dopaminergic neurons, and there are likely some subtypes of dopamine neurons that are not included in our data. Bath application of the nitric oxide donor, SNAP, robustly potentiated GABAergic synapses onto VTA dopamine neurons in slices from control animals, but not those from animals that had been stressed one day prior to slicing (Figure 1A-1B) (25, 30-32). This block of LTP_{GABA} persisted for at least five days after stress (Figure 1C). By ten days after the stressor, however, LTP_{GABA} was restored (Figure 1D). Thus, a brief, acute stressor promotes a substantial alteration in GABAergic synaptic plasticity that lasts for at least five days, but is not permanent (Figure 1E-F).

Glucocorticoids are sufficient to block LTP_{GABA}

What signaling molecules are responsible for maintaining the block of LTP_{GABA} for many days after stress? Our previous studies have indicated that both glucocorticoid receptors (GR) and κ opioid receptors (κ OR) contribute to the stress-induced block of LTP_{GABA}. (25, 30), as antagonists of both GRs and κ ORs prevent the block of LTP_{GABA} by stress when administered *in vivo* before stress. We set out to investigate the role of these pathways *after* stress to determine their contribution to the long-term maintenance of the block of LTP_{GABA}.

We first addressed the role of GRs and their endogenous ligand, corticosterone. Previous work has shown that serum corticosterone levels are rapidly increased following swim stress, and return to baseline levels within hours (35). Likewise, we see a robust increase in serum corticosterone concentration one hour following swim stress (Figure 2). As previous studies have shown mixed evidence for whether κ ORs can regulate the levels of corticosterone, we tested whether κ ORs were involved in the induction of serum corticosterone by FSS (Figure 2) (36-38). Pre-treatment with norBNI did not have any effect on the FSS-induced increase in corticosterone (Figure 2), indicating that glucocorticoid signaling does not depend on κ OR activation. These results are consistent with previous studies indicating that the magnitude of corticosterone release induced by repeated FSS is not affected by norBNI administration and does not differ between WT and prodynorphin null mice (38).

To test whether local glucocorticoid receptor activation is sufficient to block LTP_{GABA}, we applied dexamethasone (dex; 10 μ M), a glucocorticoid receptor agonist, or vehicle to midbrain slices from naïve animals for 60 min (Figure 3A). Slices were then washed for at least 60 min in aCSF before recording. As shown in Figure 3B, vehicle-treated slices exhibited characteristic SNAP-induced potentiation. However, GABAergic synapses in slices treated with dexamethasone failed to potentiate (Figure 3B-E). Moreover, dexamethasone itself had no effect on IPSC amplitude in slices from naïve animals (Figure 3F-G). Our data indicate that exposure to stress and subsequent circulation of glucocorticoids could trigger the loss of LTP_{GABA} by acting directly on glucocorticoid receptors within the VTA.

Glucocorticoid receptors play a transient role in the block of LTP_{GABA}

As corticosterone levels are only transiently elevated after FSS, we expected that GRs might have a similarly transient role in maintaining the block of LTP_{GABA} following FSS. To test this idea, we administered the GR receptor antagonist RU486 (40 mg/kg, i.p.) at varying time points after stress, and prepared slices 24 hours after RU486 injection (24-48 hours after FSS) (Figure 4A). As expected, slices from stressed, vehicle-treated animals showed no LTP_{GABA} (Figure 4B). Slices from animals given RU486 one hour after stress exhibited NO-induced LTP_{GABA} (Figure 4C). In slices prepared from animals that were given RU486 24 hours after stress, however, no LTP_{GABA} was elicited (Figure 4D). These results indicate that LTP_{GABA} is rescued when glucocorticoid signaling is blocked shortly after stress, but not 24 hours after stress (Figure 4E-F). These results suggest that glucocorticoid signaling plays an early but transient role in the block of LTP_{GABA} by stress.

κ ORs maintain the block of LTP_{GABA} for multiple days after stress

We next investigated the role of κ ORs in maintaining the block of LTP_{GABA} after stress. Our previous studies found that activation of κ ORs in VTA slices is sufficient to block LTP_{GABA} and that treatment with a κ OR antagonist before FSS prevents both the loss of LTP_{GABA} and reinstatement of cocaine-seeking (25). We hypothesized that these behavioral and physiological changes are due to persistent activation of κ ORs after stress. To test this, rats were subjected to FSS, and given an injection of the κ OR antagonist norBNI (10 mg/kg, i.p.) either two hours, one day, or four days following FSS; slices were

prepared for recording 24 hours after norBNI treatment (Figure 5A). Slices from vehicle-treated stressed animals showed no potentiation in response to SNAP (Figure 5B). Treatment with norBNI two hours after stress reversed the block, as SNAP induced LTP_{GABA} (Figure 5C). Remarkably, LTP_{GABA} was rescued in slices from animals treated with norBNI twenty four hours or four days after stress (Figure 5D-5E). These results demonstrate that treatment with a κ OR antagonist is capable of reversing stress's effects on plasticity, even when administered well after the stressor itself (Figure 5F-G). κ ORs therefore play a critical role in maintaining the block of LTP_{GABA} after stress.

Treatment with a κ OR antagonist after stress prevents reinstatement

Several studies have shown a role for κ ORs at the intersection between stress and drug-seeking behavior, however few studies have addressed ongoing roles for κ ORs in promoting drug-seeking *after* a stressful experience has already occurred. Cold-water forced swim stress is ideal to test this hypothesis, as rats that experience this stress exhibit elevated responding for cocaine for several days after the stressor (11). We recently showed that administration of κ OR antagonist twenty-four hours before stress prevents reinstatement of cocaine-seeking measured one day after cold-water swim stress (25). Given that κ OR antagonist administration is capable of reversing the loss of LTP_{GABA} when administered *after* stress, we next tested if it could also prevent reinstatement of cocaine-seeking when administered after stress. We trained rats to self-administer cocaine and then extinguished this behavior (Figure 6A, 6B). Acquisition of self-administration was similar between the two groups of rats, and no significant difference in lever-pressing was seen between groups during the last extinction session (Figure 6B,C). After extinction criteria were met, rats were subjected to a cold water forced swim. Two hours after swim stress, rats were given a single i.p. injection of norBNI. Twenty-two hours later, rats were returned to the operant chamber for the reinstatement session. Vehicle-treated rats showed a robust increase in lever-pressing compared to the final reinstatement session, but norBNI treated animals did not (Figure 6C). These data show that κ OR receptor antagonists given *after* a stressor were highly effective in preventing reinstatement of drug seeking and provide additional correlative evidence linking LTP_{GABA} and drug seeking after stress.

Discussion

Acute stress has lasting effects on plasticity

In this study we show that an acute stressor triggers neuroadaptations of the mesolimbic reward circuitry that lasts for several days. The block of LTP_{GABA} requires a transient activation of GRs and a more persistent contribution of κ ORs. Although there have been few studies examining long-lasting synaptic consequences of acute stressors in the VTA, other brief experiences can alter plasticity in this region over a similar time scale. For example, a single exposure to cocaine potentiates excitatory synapses on VTA dopamine neurons for seven days (27, 39).

The time course of the block of LTP_{GABA} after forced swim stress closely matches that of reinstatement to cocaine-seeking after the same stressor (11). In this study, rats in which cocaine self-administration was extinguished demonstrated significantly elevated lever-

pressing behavior for three days following the stressor, and a trend towards elevated responding four and five days after stress. Although the rats' responding rate converged with that of control animals by five days, when LTP_{GABA} is still completely blocked, it should be noted that the animals were tested daily in the reinstatement task, and thus were simultaneously re-extinguishing their behavior.

GRs and κ ORs in VTA plasticity and drug seeking

Our results show that the block of LTP_{GABA} is controlled after stress by glucocorticoid and kappa opioid receptors. These results are in line with a growing literature suggesting that κ ORs and GRs are capable of modulating circuit function of the VTA as well as drug seeking behaviors. GRs are expressed in dopaminergic neurons in the VTA (40, 41), and a GR antagonist blocks potentiation of glutamatergic synapses on VTA dopamine neurons by stress (23, 24, 26). Furthermore, GRs appear to play a role in drug taking, as adrenalectomy (which eliminates endogenous corticosterone) decreases self-administration of cocaine (42). Similarly, GRs are necessary for stress-induced reinstatement of cocaine-seeking (43) and injection of a glucocorticoid is sufficient to induce reinstatement (42). However, it is unclear whether glucocorticoid receptors in the VTA itself are important for these effects. Recent work suggests that glucocorticoid receptors in D1-positive neurons in the nucleus accumbens, but not dopamine neurons themselves are required for cocaine-seeking in unstressed animals (44). To our knowledge, the role of GRs specifically within the VTA itself in stress-induced reinstatement has not been tested.

κ ORs have emerged as important mediators of the behavioral consequences of stress (45, 46). They also significantly alter neurotransmission within the VTA. In VTA slices, κ OR agonists transiently decrease EPSC amplitude on dopaminergic and GABAergic neurons (47), IPSCs on BLA-projecting dopaminergic neurons (48), and dopamine-mediated IPSCs (49). Furthermore, stress-induced reinstatement of cocaine self-administration and conditioned place preference are prevented by κ OR antagonist treatment or genetic deletion of κ ORs or their endogenous ligand, prodynorphin (50-52). Activation of κ ORs is sufficient to induce reinstatement of place preference to drugs of abuse in unstressed animals (52, 53). Interestingly, while κ ORs play a significant role in stress-induced reinstatement of both cocaine self-administration and conditioned place preference, these roles may involve distinct circuitry. κ ORs in a circuit encompassing the dorsal raphe and nucleus accumbens, as well as in the locus coeruleus are crucial for stress-induced reinstatement of place preference (54, 55). However, our recently published work shows that a κ OR antagonist administered locally into the VTA prevents reinstatement of cocaine self-administration after cold water swim stress (25), suggesting that κ ORs in the VTA have a critical role in reinstatement of cocaine self-administration. As mentioned above, κ ORs have numerous effects in the VTA in addition to blocking LTP_{GABA} (47-49, 56). These effects are generally transient, persisting only as long as the κ OR agonist is present, and unlike LTP_{GABA}, it is unknown whether they are altered or on what timescale after stress. However, our data do not preclude the possibility of a persistent upregulation of dynorphin or κ OR signaling, which might also modify these other signaling pathways. It is therefore possible that the post-stress effects of norBNI on reinstatement occur independently of the reversal of the blockade of LTP_{GABA}.

Potential mechanisms of long-term alterations in plasticity

By what mechanism is GABAergic plasticity suppressed over several days? Because it remains unknown precisely when LTP_{GABA} begins to be blocked after stress, it is unclear whether administration of RU486 one hour following stress is truly reversing an already established block of LTP_{GABA} or simply preventing a block that has not yet occurred. Nonetheless, by 24 hours after stress, RU486 is not able to reverse the block of LTP_{GABA}, and thus glucocorticoid receptors do not have a significant long-term role in maintaining this block. In contrast, the κ OR antagonist norBNI is effective in restoring LTP_{GABA} when administered twenty-four hours or four days following stress, time points when we are certain that the block of LTP_{GABA} is established (Figure 1; (29)). Several studies have shown that the corticotrophin-releasing factor type two receptors (CRF₂R) induce activation of the dynorphin/ κ OR system after stress, and recruitment of the CRF₂R- κ OR pathway is essential for expression of aversive behavioral responses to stress (46, 57). These effects have been suggested to be independent of glucocorticoid signaling (46). The block of LTP_{GABA} by stress, however, requires both GRs and κ ORs, and the equivalent rise in serum corticosterone following swim stress in both vehicle and norBNI treated animals suggests that activation of GRs occurs upstream of or in parallel with κ OR activation (38). These results do not rule out a role for CRF₂Rs, and future studies determining what role these receptors play in the block of LTP_{GABA} by stress will be intriguing. Alternatively, the block of LTP_{GABA} by stress may result via signaling events distinct from CRF₂R. Glucocorticoids regulate prodynorphin levels in the hippocampus (58), suggesting that a glucocorticoid-mediated upregulation of prodynorphin in VTA-projecting regions such as the BNST or nucleus accumbens could link glucocorticoids and κ OR signaling.

Activation of either GRs or κ ORs in the VTA itself is sufficient to block LTP_{GABA}, as bath application of dexamethasone or the κ OR agonist U69593 (25) *in vitro* prevents SNAP-induced potentiation. The block of LTP_{GABA} could occur through an increase in κ OR activation or number, or in κ OR-triggered signaling molecules, or a combination of both. Alternatively, GR activation could alter the dynamics of dynorphin release from terminals of neurons from other brain regions. A κ OR antagonist delivered into the system would block κ ORs, negating any of these effects. We cannot rule out, however, that bath application of the κ OR or GR agonists block LTP_{GABA} through a distinct mechanism from that occurring after stress in the intact animal, nor that the mechanism of the block immediately after stress differs from the long term maintenance of the block. For example, stress may increase dynorphin levels, as discussed above, or increase the excitability of dynorphin-containing neurons in the nucleus accumbens or BNST that project to the VTA, which would also be functionally prevented by a κ OR antagonist.

Downstream effects of loss of LTP_{GABA}

What consequences for VTA functioning might loss of LTP at GABAergic synapses on dopamine neurons have? GABA_A receptors exert powerful inhibitory control of VTA dopamine neurons (59-62). Dopamine neurons have relatively depolarized membrane potentials, sitting close to action potential threshold (63). Activation of the GABA_A receptor chloride conductance will potentially decrease the spontaneous firing that characterizes these neurons, as opening these channels hyperpolarizes dopamine cells below their firing

threshold. With the field stimulation used in our experiments, we likely are activating a number of inhibitory projections from several regions known to modulate firing and excitability of dopamine neurons (61, 62, 64-66).

We hypothesize that LTP_{GABA} serves as an activity-dependent “brake” on dopamine neurons. These neurons are critical drivers of reward and aversion (67) and the VTA is a crucial hub in reinstatement and other stress-linked behaviors (68-70). The loss of LTP_{GABA} after stress therefore represents removal of a mechanism for control of dopamine neuron firing. Importantly, this loss persists, even after being separated in time and space from the stressor, providing a mechanism by which a single stressor can cause lasting changes in behavior. This is potentially relevant to the situation in patients, where individuals with substance use disorders may encounter opportunities to relapse in times and contexts that are distinct from stressors they may experience.

Recent work has highlighted distinctions between populations of dopamine neurons in the VTA and demonstrated subclasses of neurons with distinct links to behavioral reward and aversion (67). Accumbens projecting neurons, which are found in the lateral VTA, show an increased AMPA/NMDA ratio after a single cocaine injection, and optogenetic activation of these neurons supports place preference (71, 72). In contrast, PFC-projecting neurons in the medial VTA show an increased AMPA/NMDA ratio in response to an aversive stimulus and optogenetic activation of these neurons induces place aversion (71, 72). Although we did not specifically identify neurons by projection target in this study, our selection criteria for neurons (I_h + neurons, primarily in the lateral VTA) suggest that we may primarily be recording from accumbens-projecting “reward” neurons (33). If so, our studies reveal a stress-induced alteration in cells associated with reward. Furthermore, while many studies of κ ORs have focused on specifically aversive processes, our work may indicate a role for κ ORs in the reward-promoting subcircuitry of the VTA.

It is important to note that electrophysiological studies in this work were conducted using young, cocaine-naïve animals. This is an important caveat, as development and exposure to drugs of abuse both cause changes throughout the brain. Although work is still needed to guarantee that the signaling mechanisms are similar in young and adult animals, our previous studies show that LTP_{GABA} can be induced in adult rats following cocaine self-administration and extinction (25). This indicates that the mechanism for induction of LTP_{GABA} is intact in adult animals, that it is not blocked or occluded by long-term exposure to cocaine, and that it is available to be blocked by stress.

Intervention to prevent reinstatement after stress

As discussed above, κ ORs play a significant role in addictive and stress-related disorders (46, 73-76). Human studies have implicated polymorphisms in the dynorphin gene as a risk factor for cocaine dependence (77-79). Furthermore, expression of the prodynorphin gene is higher in individuals with a history of psychostimulant abuse, although it is unclear whether this represents a cause or an effect of exposure to drugs of abuse (80). Our studies suggest that ongoing processes such as stress-induced dysregulation of κ OR signaling and neuroplasticity could contribute to the natural course of addiction.

Most importantly, our studies indicate that stress-induced neuroadaptations can be reversed. Treatment with a κ OR antagonist reversed the stress-induced block of LTP_{GABA}, even when administered four days after stress. Using this information, we also showed that administration of norBNI after stress also prevented reinstatement of drug-seeking. These data strengthen the association between κ ORs and stress-induced drug seeking and raise the possibility that further study of LTP_{GABA} may yield a similarly rich vein of potential pharmacological targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Stress blocks LTP_{GABA} for at least five days

(A) Example experiment showing LTP_{GABA} induced by application of SNAP (black bar) in slices from an unstressed animal. Single experiments demonstrating loss of LTP_{GABA} in slices from animals stressed (B) 24 hours or (C) 5 days prior to slicing. (D) Single experiment showing recovery of LTP_{GABA} measured ten days after stress. (E) Summary graph showing compiled data from all four groups. (F) Comparison of the magnitude of LTP_{GABA} 15-20 minutes after SNAP application. (1-way ANOVA, $F_{3,31}=4.551$, $p=0.009$. IPSC amplitudes, control rats: $142 \pm 10\%$ of baseline values, $n=14$, 24 hours after stress, $90 \pm 17\%$ of baseline values, $n=5$; $p < 0.05$ from control; 5 days post FSS: $101 \pm 13\%$ of baseline, $n=8$, $p < 0.05$ from control; 10 days post FSS: $137 \pm 7\%$ of baseline values, $n=8$, n.s.

from control, Dunnett's Multiple Comparison Test). Insets for this and all figures: IPSCs before (black trace, control) and 15 minutes after drug application (red trace, SNAP, 400 μ M). Scale bars: 20 ms, 100 pA. Insets are averages of ten IPSCs.

Figure 2. FSS elevates glucocorticoids independently of κ OR

Normalized serum corticosterone concentrations from rats pretreated with norBNI or saline before FSS. Serum was collected 1 hour post FSS (saline-control: 1.00 ± 0.37 , $n=5$; saline-FSS: 42.42 ± 3.92 , $n=6$; norBNI-FSS: 45.18 ± 2.62 , $n=7$; 1-way ANOVA, $F_{2,15}=65.42$, $p<0.0001$. * $p<0.05$ in comparison to control, Tukey's multiple comparison test.

Figure 3. Glucocorticoids are sufficient to block LTP_{GABA}

(A) Experimental protocol for bath-applied dexamethasone (dex) experiments. (B) Example experiment from a vehicle-treated slice. (C) Example experiment from a vehicle treated slice. (D) Summary of compiled data for DMSO- and dexamethasone-treated slices. (E) Average magnitude of LTP as measured 15-20 minutes after application of SNAP (IPSC amplitudes, DMSO: $167.0 \pm 24\%$ of baseline, $n=9$ cells/8 animals; Dex: $113.3 \pm 3\%$ of baseline, $n=9$ cells/9 animals. *, $p < 0.05$, Student's t-test) (F) Example experiment showing that IPSC amplitudes are unchanged during dexamethasone application (G) Summary of

compiled data for bath applied dex (30 minutes after dex application: $108.5 \pm 9\%$ of baseline values, $n=9$, no significant difference from baseline, $p=0.36$, Student's t-test).

Figure 4. Transient effect of glucocorticoids in maintaining the block of LTP_{GABA}

(A) Experimental design for post-stress RU486 treatment. (B) Example experiment showing absence of LTP_{GABA} in slices from a vehicle-treated stressed animal. (C) Single experiment showing LTP_{GABA} in a slice from an animal treated with RU486 (40 mg/kg in 5%DMSO/canola oil) 1 hr after stress. (D) Single experiment showing RU486 no longer rescues LTP_{GABA} when administered 24 hours after stress. (E) Summary graph showing compiled data from all three groups. (F) Comparison of the magnitude of LTP_{GABA} 15-20 minutes after SNAP application. (1-way ANOVA, $F_{2,24}=6.003$, $p=0.0077$. IPSC amplitudes, vehicle-

stress: $99 \pm 8\%$ of baseline values, $n=12$, RU486 1 hr post-stress, $141 \pm 11\%$ of baseline values, $n=8$; $p < 0.05$ from control; RU486 24 hours post-stress: $113 \pm 8\%$ of baseline, $n=7$, n.s. from control) Tukey's multiple comparison test.

Figure 5. Long-term role of κ ORs in maintaining the block of LTP_{GABA}

(A) Experimental design for post-stress norBNI treatment. (B) Example experiment showing loss of LTP_{GABA} in slices from a vehicle treated stressed animal. Single experiments showing recovery of LTP_{GABA} in slices from animals treated with norBNI (10 mg/kg i.p.) at 2 hrs (C), 24 hrs (D), or 4 days (E) after stress. (F) Summary graph showing compiled data from all four groups. (G) Comparison of the magnitude of LTP_{GABA} 15-20 minutes after SNAP application. (1-way ANOVA, $F_{3,60}=4.883$, $p=0.0042$. IPSC amplitudes, vehicle-stress: $105\pm4\%$ of baseline values, $n=27$, norBNI 2 hr post-stress, $131\pm7\%$ of baseline

values, n=18; $p<0.05$ from control; norBNI 24 hours post-stress: $138\pm15\%$ of baseline, n=8, $p<0.05$ from control, norBNI 4 days post stress: $130\pm9\%$ of baseline, n=11, $p<0.05$ from control Dunnett's Multiple Comparison Test).

Figure 6. Post-stress norBNI prevents reinstatement of cocaine-seeking

(A) Experimental design for self-administration, extinction and reinstatement behavioral experiment. Animals were subjected to stress and 2 hours later were given either norBNI (10 mg/kg i.p.) or vehicle. (B) Cocaine self-administration training was not significantly different between the two groups prior to stress and norBNI treatment. Two-way repeated measure ANOVA revealed a significant effect of time ($F_{1, 17}=34.23$, $p<0.05$), but no significant effect of group ($F_{1, 17}=0.1671$) or interaction ($F_{1, 17}=0.4725$) (C) Reinstatement of cocaine seeking. Lever presses in vehicle (black) and norBNI (white) treated animals

during the final extinction session (Ext) and reinstatement session (RI). Two-way ANOVA, interaction, $F_{1, 14}=11.29$, $p=.0047$; Sidak's Post-hoc test vehicle vs. norBNI on stress-induced reinstatement, adjusted $p=.0002$. $N= 7$ in norBNI group; 9 in vehicle group.