Report

Neuron

Microglial TNF-α Suppresses Cocaine-Induced Plasticity and Behavioral Sensitization

Highlights

- Cocaine activates microglia in the nucleus accumbens and increases TNF-α production
- TNF-α reduces AMPA/NMDA ratio preferentially on D1-expressing MSNs in the accumbens
- TNF-α antagonizes cocaine-induced synaptic plasticity and reduces sensitization
- Reactivating microglia by TLR4 can suppress cocaine-induced sensitization via TNF-α

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In Brief

Drugs of abuse, such as cocaine, induce changes in reward circuitry, which manifest as long-lasting changes in behavior. Lewitus et al. demonstrate that microglia respond to cocaine by releasing TNF-α, which acts to mitigate cocaine-induced synaptic and behavioral changes.

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Microglial TNF-α Suppresses Cocaine-Induced Plasticity and Behavioral Sensitization

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SUMMARY

Repeated administration of cocaine results in the development of behavioral sensitization, accompanied by a decrease in excitatory synaptic strength in the nucleus accumbens (NAc) through an unknown mechanism. Furthermore, glial cells in the NAc are activated by drugs of abuse, but the contribution of glia to the development of addictive behaviors is unknown. Tumor necrosis factor alpha (TNF-α), an inflammatory cytokine released by activated glia, can drive the internalization of synaptic AMPA receptors on striatal medium spiny neurons. Here we show that repeated administration of cocaine activates striatal microglia and induces TNF-α production, which in turn depresses glutamatergic synaptic strength in the NAc core and limits the development of behavioral sensitization. Critically, following a period of abstinence, a weak TLR4 agonist can reactivate microglia, increase TNF-α production, depress striatal synaptic strength, and suppress cocaine-induced sensitization. Thus, cytokine signaling from microglia can regulate both the induction and expression of drug-induced behaviors.

INTRODUCTION

Changes in striatal processing, particularly in the NAc, are thought to be necessary for the maintenance of addictive behaviors, and repeated exposure to drugs of abuse leads to predictable changes in synaptic strength in the NAc (Lüscher and Malenka, 2011). Drugs of abuse, such as cocaine, elevate dopamine levels in the striatum, and ex vivo treatment of striatal medium spiny neurons (MSNs) with D1 dopamine receptor agonists or with cocaine increases the phosphorylation and insertion of AMPA receptors (Chao et al., 2002; Mangiavacchi and Wolf, 2004; Snyder et al., 2000). However, repeated cocaine treatment in vivo (5 days of noncontingent administration) results in an initial decrease in the AMPA/NMDA ratio on MSNs in the NAc, as measured 24 hr after the last cocaine injection (Kourrich et al., 2007; Mameli et al., 2009). A period of abstinence results in a gradual elevation of AMPA/NMDA ratios and AMPAR surface expression (Boudreau and Wolf, 2005; Schumann and Yaka, 2009), although a challenge dose of cocaine will result in lowered ratios and surface receptor content (Boudreau et al., 2007; Kourrich et al., 2007; Thomas et al., 2001). Self-administration of cocaine also causes similar changes in the NAc, with cocaine exposure causing a loss of AMPA receptors and depressing synaptic strength on MSNs and extended abstinence resulting in synaptic strengthening and accumulation of surface AMPA receptors (Conrad et al., 2008; Ortinski et al., 2012; Schramm-Sapyta et al., 2006). This bidirectional plasticity suggests that other factors, in addition to dopamine, contribute to the synaptic changes induced by drug exposure.

Recently, we have shown that TNF-α drives internalization of AMPARs on MSNs, reducing corticostriatal synaptic strength, and reduces the aberrant changes in striatal circuit function induced by chronic blockade of D2 dopamine receptors (Lewitus et al., 2014). Glia are the main source of TNF-α in the CNS, and both microglia (Sekine et al., 2008) and astrocytes (Bowers and Kalivas, 2003) are activated by psychostimulants. Further, glia have been suggested to regulate drug-induced behavior (Miguel-Hidalgo, 2009). Thus, glia through the release of TNF-α could have a mitigating effect on the circuit changes induced by cocaine. Here we demonstrate that striatal microglia are activated by cocaine, and moderate the synaptic and behavioral changes induced by the repeated administration of cocaine.

RESULTS

To determine the effect of in vivo cocaine exposure on TNF-α levels in the NAc, we measured TNF-α mRNA and protein levels in mice after i.p. injections of saline or cocaine. A single injection of cocaine had no effect on TNF-α levels (measured 24 hr post-injection), but 5 days of daily cocaine treatment (measured 24 hr after the final injection) increased both TNF-α mRNA and protein, compared to saline injected controls (Figures 1A–1D). TNF-α was no longer elevated following 10 days of abstinence from cocaine. To understand the impact of TNF-α on synaptic function in the NAc, we measured AMPA/NMDA ratios on MSNs in the NAc core. Alteration in NAc core AMPA receptors is involved in the expression of behavioral sensitization to psychostimulants (Kalivas, 2009).

We have previously shown that TNF-α drives internalization of AMPARs on MSNs in the dorsal striatum (Lewitus et al., 2014). As repeated cocaine administration primarily affects direct-pathway

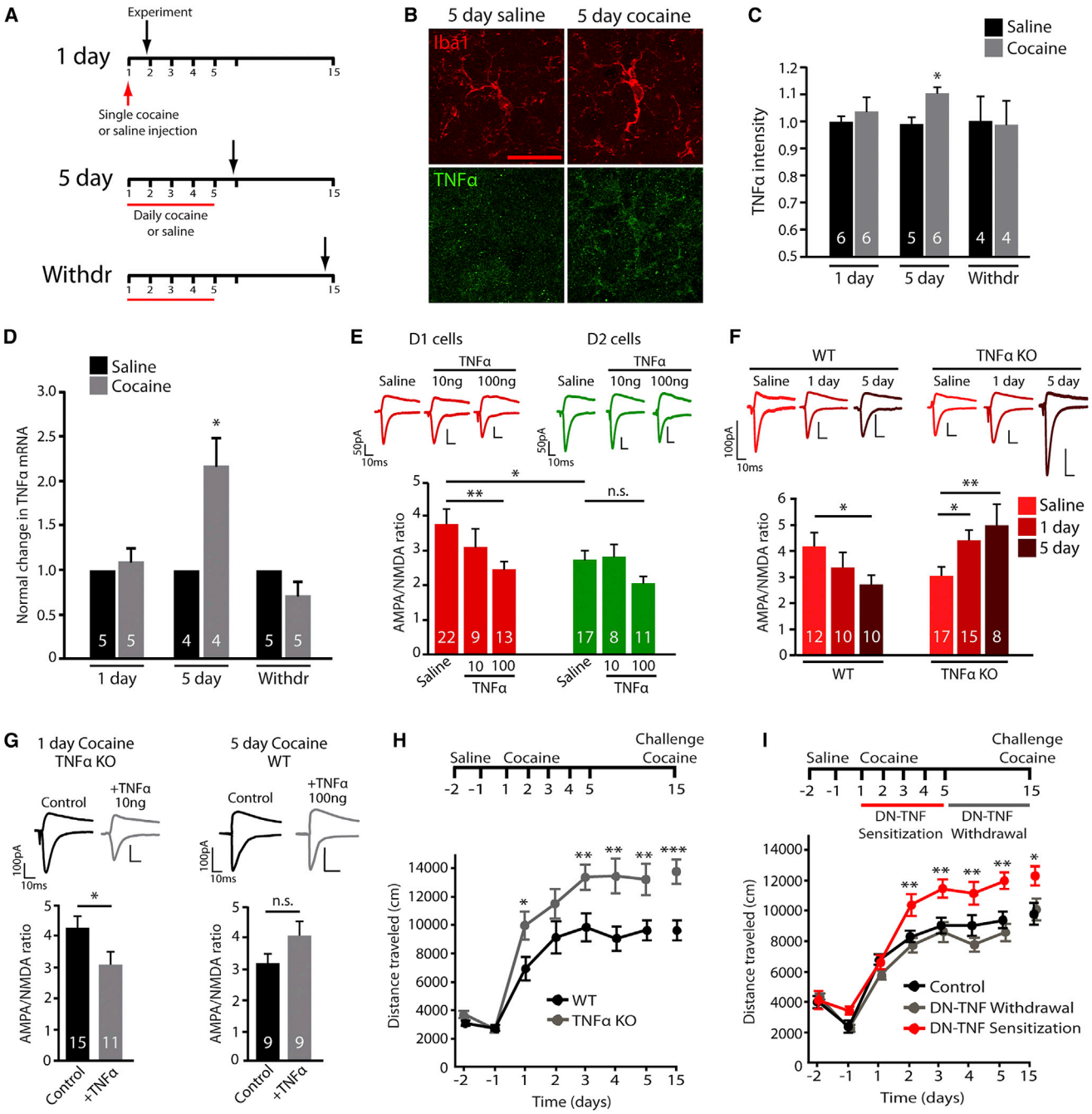

Figure 1. Cocaine Increases TNF-α Levels in the Nucleus Accumbens, which Causes Synaptic Depression on D1-MSNs and Antagonizes Cocaine-Induced Behavioral Sensitization

(A) Diagram of the time points used for experiments: 24 hr after a single injection of saline or cocaine (i.p. 15 mg/kg), 24 hr after five daily injections of saline or cocaine, and 10 days after five daily injections of saline or cocaine.

(B) Representative confocal projection images of NAc immunostained for Iba1 (top) and TNF-α (bottom) from mice injected for 5 days with saline or cocaine (scale bar, 20 μm).

(C) Five daily injections of cocaine increases TNF-α protein in the NAc.

(D) Five daily injections of cocaine increases TNF-α mRNA in the ventral striatum.

(E) Representative recording of EPSCs at −70 mV and +40 mV and mean AMPA/NMDA ratios from control slices and slices treated with 10 or 100 ng/ml TNF-α in D1 (red) and D2 (green) MSNs in the NAc core. AMPA/NMDA ratios were calculated using the peak amplitude at −70 mV for AMPA and the amplitude at +40 mV taken 40 ms after the peak at −70 mV.

(F) Representative traces and mean AMPA/NMDA ratios from D1-MSNs in the NAc core, after 1 and 5 days of cocaine or saline in WT or TNF-α-KO mice. Ratios from mice injected with one or five daily doses of saline were not significantly different, and were combined.

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MSNs, we tested specific subpopulations of MSNs in the NAc core for their response to TNF-α. Acute NAc slices were incubated with TNF-α and whole-cell recording made from Drd1a-td Tomato (D1) positive and negative (D2) MSNs. A low dose of TNF-α (10 ng/ml) had no significant effect on either cell type. However, 100 ng/ml TNF-α significantly reduced the AMPA/NMDA ratio on D1-MSNs, with a nonsignificant reduction in ratios on D2-MSNs (Figure 1E). These results suggest that D1-MSNs are more sensitive to TNF-α than D2-MSNs, although D2-MSNs may respond to a lesser degree.

Repeated noncontingent administration of cocaine results in lower AMPA/NMDA ratios of excitatory inputs onto the NAc specifically on D1-MSNs (Kim et al., 2011; Pascoli et al., 2012). To test whether this decrease in AMPA/NMDA ratios is due to increased TNF-α expression, we evaluated AMPA/NMDA ratios in the NAc core after cocaine or saline administration in WT and *TNF-α*$^{-/-}$ mice. As expected for WT mice, a single injection of cocaine did not significantly reduce AMPA/NMDA ratios on D1-MSNs as measured 1 day later, but five daily cocaine injections did (Figure 1F). Strikingly, in *TNF-α*$^{-/-}$ mice, a single injection of cocaine significantly increased AMPA/NMDA ratios, which remained elevated after 5 days of cocaine treatment (Figure 1F). No significant differences were observed in D2-MSNs for either genotype (see Figure S1A available online). These results suggest that the reduction in AMPA/NMDA ratios in D1-MSNs after repeated cocaine is due to increased TNF-α in the NAc, and that cocaine itself increases synaptic strength. This is consistent with the exocytosis of AMPARs observed with direct stimulation of D1Rs on MSNs (Mangiavacchi and Wolf, 2004). Moreover, this result suggests that, following cocaine treatment, D1-MSNs are more responsive to lower endogenous levels of TNF-α, perhaps because newly inserted AMPARs are more labile, as has been seen at potentiated synapses in the amygdala (Clem and Huganir, 2010). To test the hypothesis that potentiated D1-MSNs are more sensitive to TNF-α, we treated *TNF-α* $^{-/-}$ mice with a single injection of cocaine and evaluated the effect of a low level of TNF-α (10 ng/ml) on AMPA/NMDA ratios. Although this level of TNF-α had no significant effect on MSNs from WT untreated animals (Figure 1E), it significantly reduced AMPA/NMDA ratios on D1-MSNs from cocaine treated knockout animals (Figure 1G). This treatment had no effect on D2-MSNs (Figure S1B). To test whether cocaine-induced TNF-α signaling occludes further synaptic depression by TNF-α, we treated WT animals with cocaine for 5 days and then treated striatal slices ex vivo with TNF-α (100 ng/ml). The AMPA/NMDA ratio on D1-MSNs (already reduced compared to saline treated animals; Figure 1E) was not further reduced by treatment with TNF-α (Figure 1G). This shows that the synaptic depression induced by repeated cocaine injections occludes the TNF-α-mediated reduction in AMPA/NMDA ratio. Overall, these data suggest that repeated cocaine treatment elevates TNF-α, which suppresses the synaptic changes directly induced by cocaine in the NAc core.

Depressing synaptic strength in the NAc can reduce behavioral sensitization to cocaine (Pascoli et al., 2012). Behavioral sensitization is a simple model of drug-induced behavioral change, which measures the progressive increase in locomotor response to psychostimulants. *TNF-α*$^{-/-}$ mice displayed an increased initial locomotor response to cocaine and increased sensitization, compared to WT mice (Figure 1H). This is similar to what has been observed with methamphetamine sensitization in *TNF-α*$^{-/-}$ mice (Nakajima et al., 2004). To exclude compensatory mechanisms resulting from the absence of TNF-α during development, we pharmacologically blocked the soluble form of TNF-α in WT mice using XENP1595 (a dominant-negative variant of TNF-α [DN-TNF]). WT mice were administered DN-TNF either during the 5 days of conditioning (to block TNF-α signaling during acquisition) or during the abstinence period starting immediately after the last cocaine injection (to test the role of TNF-α in the maintenance of the behavior). Blocking TNF-α signaling during acquisition was sufficient to increase sensitization as well as maintain the elevated response on the challenge day, while blocking TNF-α signaling during the 10 day period of abstinence had no effect on the response to the challenge dose (Figure 1I). These results suggest that TNF-α is active during acquisition but not during drug abstinence, consistent with the increased TNF-α expression observed during that period (Figures 1C and 1D). Further, the increased sensitivity observed in *TNF-α*$^{-/-}$ mice on the first day of cocaine is not due to an acute loss of TNF-α signaling and is likely to be unrelated to the increase in sensitization.

In other brain regions, TNF-α that regulates synaptic function is produced by glia (Stellwagen and Malenka, 2006). To assess the source of TNF-α regulating sensitization, we utilized a Cre-loxP system to selectively delete TNF-α from microglia (CX3CR1-Cre; Figure S2A) and astrocytes (GFAP-Cre; Figure S2B). Mice that lack microglial TNF-α showed significantly higher sensitization to cocaine from the second cocaine injection that was maintained through the period of abstinence (Figure 2A). Conversely, mice that lack astrocytic TNF-α did not display a significant change in sensitization compared with littermate controls (Figure 2B). These results suggest that microglia are important for the adaptive TNF-α response to repeated cocaine administration. To verify this, we isolated microglia from the striatum of cocaine and saline treated animals by magnetic bead sorting (Figure S2C) and compared TNF-α mRNA in the

(G) Representative traces and mean AMPA/NMDA ratios from D1-MSNs in the NAc core from control slices or slices treated ex vivo with TNF-α. Treatment with 10 ng/ml TNF-α significantly reduced AMPA/NMDA ratios from *TNF-α*$^{-/-}$ mice treated 24 hr prior with cocaine. Ex vivo treatment with 100 ng/ml TNF-α did not further decrease AMPA/NMDA ratios from WT mice previously exposed to five daily cocaine injections.

(H) Mean locomotor activity in response to cocaine injections in *TNF-α*$^{-/-}$ and WT mice, showing higher sensitization in *TNF-α*$^{-/-}$ mice, that was maintained after abstinence (n = 12 WT, 17 *TNF-α*$^{-/-}$ animals).

(I) Blocking soluble TNF-α signaling only during the sensitization protocol (DN-TNF sensi) with DN-TNF is sufficient to sustain the elevation of the cocaine response to the challenge dose on day 15, while blocking TNF-α signaling during the withdrawal period (DN-TNF withd) had no effect on the response to the challenge dose after withdrawal (n = 16 DN-TNF sensi, 8 DN-TNF withd, 12 Control). Results are expressed as mean ± SEM, n (mice or cells) is given in bars. *p < 0.05, **p < 0.01, ***p < 0.001.

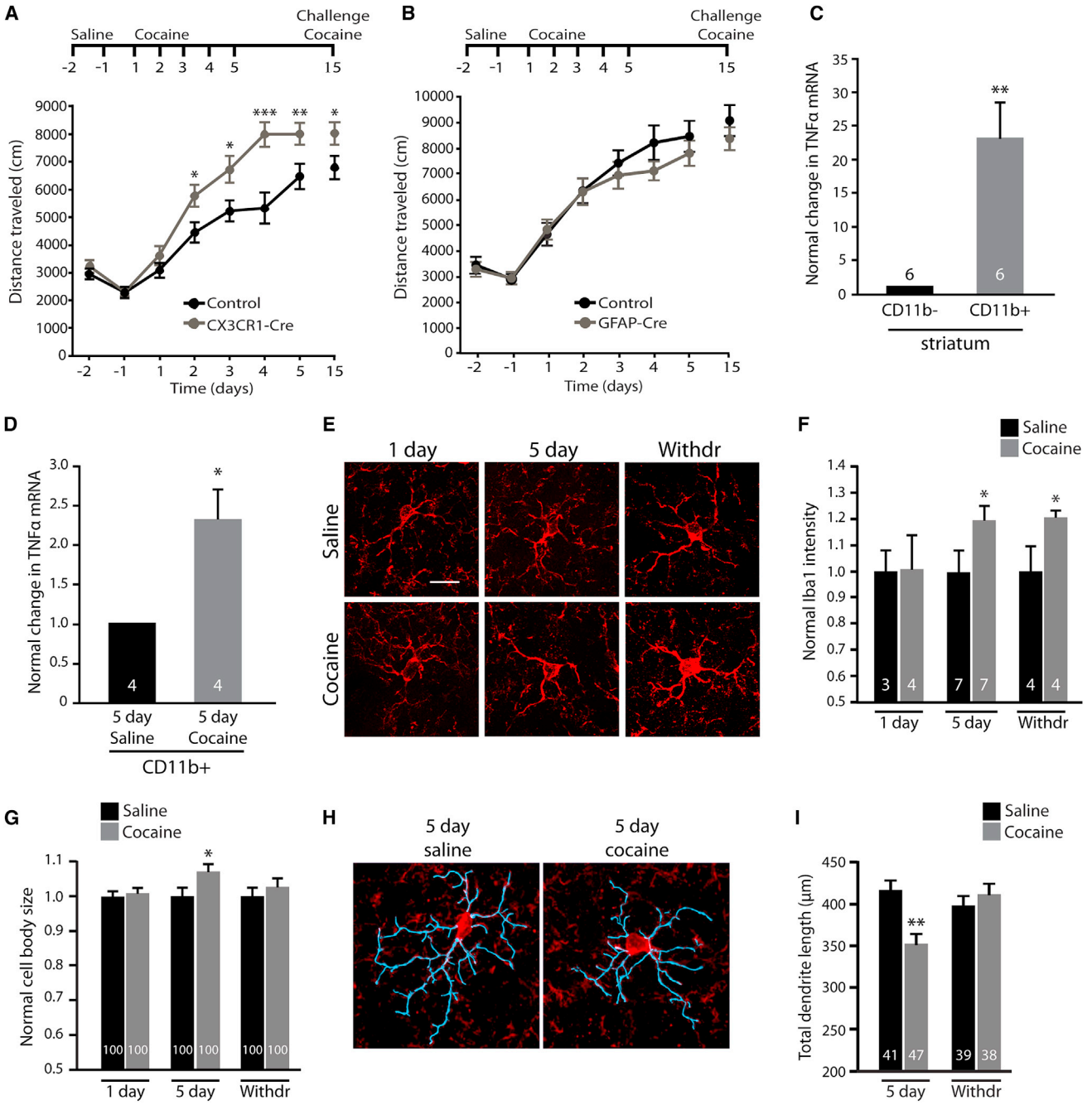

Figure 2. Microglia Are Activated by Cocaine and Release TNF-α to Antagonize Cocaine-Induced Behavioral Sensitization

(A) Mean locomotor activity in response to cocaine in mice that lack microglial TNF-α (CX3CR1-Cre^{+}; TNF-α$^{flox/flox}$) and littermate controls (CX3CR1-cre negative or TNF-α$^{+/flox}$). The elevation was sustained for a final test dose of cocaine (n = 16 control; 12 microglia deletion).

(B) Mean locomotor activity in response to cocaine injections in mice that lack astrocytic TNF-α (GFAP-Cre^{+}; TNF-α$^{flox/flox}$ and littermate controls (GFAP-cre^{-}; TNF-α$^{flox/flox}$). GFAP-Cre mice had normal sensitization and response on the final test day (n = 25 per condition).

(C) Purified microglia (CD11b+ fraction of cells) from whole striatum tissue express significantly more TNF-α mRNA compared to other cell types (CD11b− fraction) (five mice pooled per group in each experiment).

(D) Five daily injections of cocaine increases TNF-α mRNA in striatal microglia (five mice pooled per group per experiment).

(E) Representative confocal projection images of Iba1-labeled microglia in the NAc 24 hr after a single cocaine injection, five daily injections, or 10 days' withdrawal (scale bar, 20μm).

(F) Semiquantitative analysis of Iba1 immunoreactivity in the NAc, normalized to the mean saline intensity for each time point.

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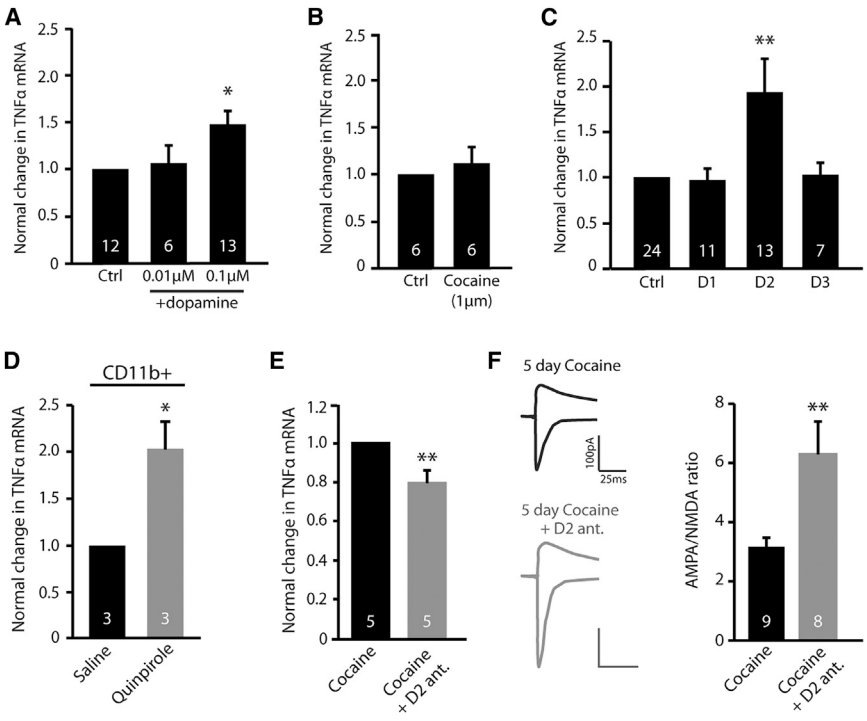

Figure 3. Dopamine Increases TNF-α mRNA in Microglia through D2 Receptors

(A) Primary rat microglia cultures were treated with vehicle or dopamine for 3 hr (n = biological replicates from four independent cultures).

(B) Treatment with cocaine (1 μm, 3 hr) did not alter TNF-α mRNA levels in microglia cultures (n = replicates from three cultures).

(C) Normalized change in TNF-α mRNA in primary rat microglia cultures treated for 3 hr with vehicle (Control), D1-receptor agonist (SKF-38393, 1 μm), D2-receptor agonist (quinpirole, 1 μm), or D3-agonist (pramipexole, 1 μm) (n = replicates from six cultures).

(D) Quinpirole (i.p. 0.5 mg/kg; 24 hr and 1 hr before harvesting) significantly increases TNF-α mRNA in microglia isolated from striatal tissue, compared to saline treatment (n = experiments, four mice pooled per group in each experiment).

(E) Coadministration of the D2-receptor antagonist L741,626 (i.p. 3 mg/kg; 15 min before cocaine) with daily cocaine injections over 5 days significantly decreases TNF-α mRNA in ventral striatum tissue in adult mice.

(F) Coadministration of L741,626 with cocaine results in an increase in AMPA/NMDA ratio on D1-MSNs compared with mice treated with cocaine alone. Results are expressed as mean ± SEM, n (experiments, mice or cells) is given in bars. *p < 0.05, **p < 0.01, ***p < 0.001.

microglial and nonmicroglial fractions. Microglia contained the vast majority of TNF-α mRNA, showing over a 20-fold enrichment compared with the other striatal cell types (Figure 2C). Further, the TNF-α mRNA was increased by cocaine treatment specifically in microglia cells (Figure 2D) and not in other cell types (Figure S2D).

Resting microglia continuously survey the healthy brain and respond to a variety of activation signals by undergoing progressive morphological and functional changes (Kettenmann et al., 2011). Using Iba1, we labeled microglia in adult mice 24 hr after a single cocaine injection, 24 hr after 5 days of daily cocaine injections, or after 10 days of drug abstinence. Although the number of microglia in the NAc did not change at any time point (Figure S2E), Iba1 intensity was increased in microglia by 5 days of cocaine, and after a period of abstinence (Figures 2E and 2F). Microglia cell body size was increased by 5 days of cocaine (Figure 2G), accompanied by a decrease in process length (Figures 2H and 2I). These changes in microglia morphology are consistent with an activated phenotype. In contrast, we did not observe any activation of astrocytes, as judged by the area or intensity of GFAP expression (Figures S2F and S2G). These data strongly suggest that microglia are the source of the cocaine-induced upregulation of TNF-α production in the striatum observed during sensitization.

Cocaine could activate microglia directly by binding the sigma receptor (Navarro et al., 2010) or the Toll-like receptor 4 (TLR4) (Northcutt et al., 2015), or indirectly through the elevation of dopamine. To test this, we treated microglia cultures with dopamine or cocaine for 3 hr. Treatment of microglia with 0.1 μM dopamine, but not 1 μM cocaine, significantly increased TNF-α mRNA (Figures 3A and 3B). This concentration of dopamine is reflective of the concentration found in the NAc in vivo following cocaine administration in rats (Hooks et al., 1992). The same treatment applied to cultured astrocytes had no effect on TNF-α mRNA (Figure S3A). Multiple dopamine receptors are expressed on microglia (Kettenmann et al., 2011). Stimulating microglia with the D2-like agonist quinpirole increased TNF-α mRNA, while the D1 agonist SKF-38393 and specific D3 agonist pramipexole had no effect (Figure 3C). This suggests that dopamine increases TNF-α production in microglia through D2 receptors. Microglia transcriptional profiles change substantially during both development and the culturing process (Butovsky et al., 2014). We therefore cultured microglia from adult animals, in a manner that preserves an in vivo transcriptional profile (Butovsky et al., 2014). We saw a similar response to dopamine and lack of response to cocaine (Figures S3B and S3C). To directly test the response of microglia in vivo, we treated mice with quinpirole (0.5 mg/kg, i.p.; 24 and 1 hr prior to harvest) and isolated

(G) Quantification of microglia cell body size (μm^2) measured by Iba1 immunoreactivity and normalized to the mean saline value for each time point (n = cells from four animals).

(H) Representative examples of microglia processes, after 5 days of cocaine or saline.

(I) Total length of microglia processes is decreased after 5 days' cocaine by 20%, but is not significantly altered after withdrawal (n = microglia from four animals). Results are expressed as mean ± SEM, n (experiments, mice or microglia) is given in bars. *p < 0.05, **p < 0.01.

microglia. Quinpirole treatment increased the TNF-α mRNA in striatal microglia cells (Figure 3D), but not in other striatal cell types (Figure S3D), nor in cortical microglia (Figure S3E), indicating that D2-agonism specifically increases microglial TNF-α in the striatum in vivo. Coadministrating cocaine with the D2 antagonist L741,626 for 5 days reduced the cocaine-induced increase in microglial TNF-α production (Figure 3E). Finally, to verify that D2-like receptor activation was required for the TNF-α-dependent decrease in AMPA/NMDA ratio observed on D1-MSNs, we treated animals for 5 days with cocaine and the D2 antagonist. Blocking D2 receptors (and thus preventing the activation of microglia) resulted in a large cocaine-induced increase in AMPA/NMDA ratio on D1-MSNs, similar to *TNF-α*$^{-/-}$ animals (Figure 3F). Overall, this suggests that cocaine elevates dopamine levels, which act on D1 receptors on direct pathway MSNs to increase synaptic strength; simultaneously activates microglia through D2 receptors; and temporarily increases TNF-α production.

Our data suggest that the activation of microglia limits the cocaine-induced changes to NAc circuitry, but this activation occurs only during a narrow window following cocaine exposure. Because depotentiation of MSNs reduces cocaine-induced behavioral sensitization (Pascoli et al., 2012), we tested if reactivation of microglia to increase TNF-α could depress NAc synapses and suppress sensitization. To do this, we utilized monophosphoryl lipid A (MPLA), a detoxified variant of LPS (Casella and Mitchell, 2008). MPLA is a weak TLR4 agonist that does not induce extensive neuroinflammation or sickness behavior (Michaud et al., 2013). We first verified that MPLA activates microglia in the NAc, by injecting 10 μg MPLA IP after 10 days of abstinence from cocaine. MPLA treatment significantly increased Iba1 intensity within 4 hr compared to saline treated controls (Figure 4A) and was associated with an increase in striatal TNF-α expression, at both 4 and 24 hr after injection (Figure 4B). We next tested if MPLA would depress synaptic strength in the NAc core. After 10 days of abstinence from cocaine, mice were injected with MPLA and evaluated 24 hr later for AMPA/NMDA ratios on D1-MSNs. MPLA treatment significantly reduced AMPA/NMDA ratio in D1-MSNs compared to saline-treated controls (Figure 4C). Because artificially reducing synaptic strength in the NAc can reduce behavioral sensitization (Pascoli et al., 2012), these data suggest that MPLA might suppress drug-induced behaviors.

We first established that MPLA does not alter basal locomotion (Figure S4A). Further, MPLA (10 μg; 24 hr prior to testing) did not decrease the locomotor response to an initial dose of cocaine, as tested in saline-treated animals given an initial dose of cocaine at the challenge time point (Figure 4D). We then tested sensitized mice by injecting 10 μg or 50 μg MPLA or saline, 24 hr prior to the challenge dose of cocaine. Mice treated with MPLA had significantly reduced locomotor response to the cocaine challenge in a dose-dependent manner (Figure 4E). This suggests that MPLA reduces sensitization, rather than the locomotor response to cocaine. The effects of MPLA also did not appear to be due to an increase in sensitivity to cocaine, as we observed no increase in stereotypic behaviors in MPLA treated mice (Figures S4B–S4D). Moreover, MPLA had no effect on sensitization in *TNF-α*$^{-/-}$ mice (Figure 4F), which suggests that MPLA acts through an elevation of TNF-α and not other cytokines. However, this effect is temporary, as MPLA had little impact on sensitization when tested 4 days after injection (Figure S4E). These observations suggest that even after a prolonged period of abstinence from cocaine, increasing TNF-α can be effective in reducing the behavioral response to cocaine, although it does not revert the system to the presensitized state.

DISCUSSION

Chronic cocaine administration produces long-erm neuroadaptions of glutamatergic signaling in the NAc that contribute to addiction-related changes in drug sensitivity and craving. Here we show that microglia in the NAc are transiently activated following cocaine administration, and act to downregulate AMPARs on MSNs through TNF-α signaling. Importantly, this limits the development of behavioral sensitization. Following a period of abstinence, MPLA can reactivate microglia and decrease both synaptic strength in the NAc and locomotor sensitization to cocaine. Our results suggest that microglia have an adaptive role in the response to cocaine and that their modulation could be an effective avenue of treatment. Alterations in the NAc core may be particularly important for the expression of locomotor sensitization (Kalivas, 2009). Infusion of AMPA into the NAc core enhances locomotion in animals that were exposed to cocaine 2–3 weeks earlier (Bell and Kalivas, 1996), while AMPAR antagonists administered into the NAc core prevent the expression of sensitization (Bell et al., 2000; Pierce et al., 1996). The increase in NAc synaptic strength has been hypothesized to correlate with the development of craving (Conrad et al., 2008), and reducing synaptic strength reduces cue-induced self-administration (Wisor et al., 2011). This suggests that increasing TNF-α with mild TLR4 activation may help blunt craving or incentive sensitization.

Further, our results explain a perplexing feature of chronic cocaine administration—that synaptic strength on D1-MSNs decreases initially and then slowly increases during withdrawal. While the formation and subsequent unsilencing of silent synapses likely contribute (Huang et al., 2009) to the changes in AMPA/NMDA ratios, our results suggest an additional mechanism is involved. Our data support the idea that dopamine does, as predicted by in vitro results, increase AMPA/NMDA ratios while simultaneously activating microglia to release TNF-α. This TNF-α release causes the decreased AMPA/NMDA ratios observed following repeated administration of cocaine. This suppression is temporary, as microglia slowly deactivate during abstinence from cocaine, revealing the underlying dopamine-induced potentiation. A challenge dose would reactivate the microglia, increase TNF-α release, and again suppress AMPA/NMDA ratios as observed (Boudreau et al., 2007; Thomas et al., 2001). Taken together, our data suggest that TNF-α has an adaptive role in regulating glutamatergic transmission in the NAc when circuit homeostasis is perturbed, akin to the role of TNF-α in homeostatic synaptic plasticity (Stellwagen and Malenka, 2006).

Moreover, our data suggest that microglia are adaptive regulators of striatal function. This is to suggest not that astrocytes do not regulate striatal function, including the response to drugs,

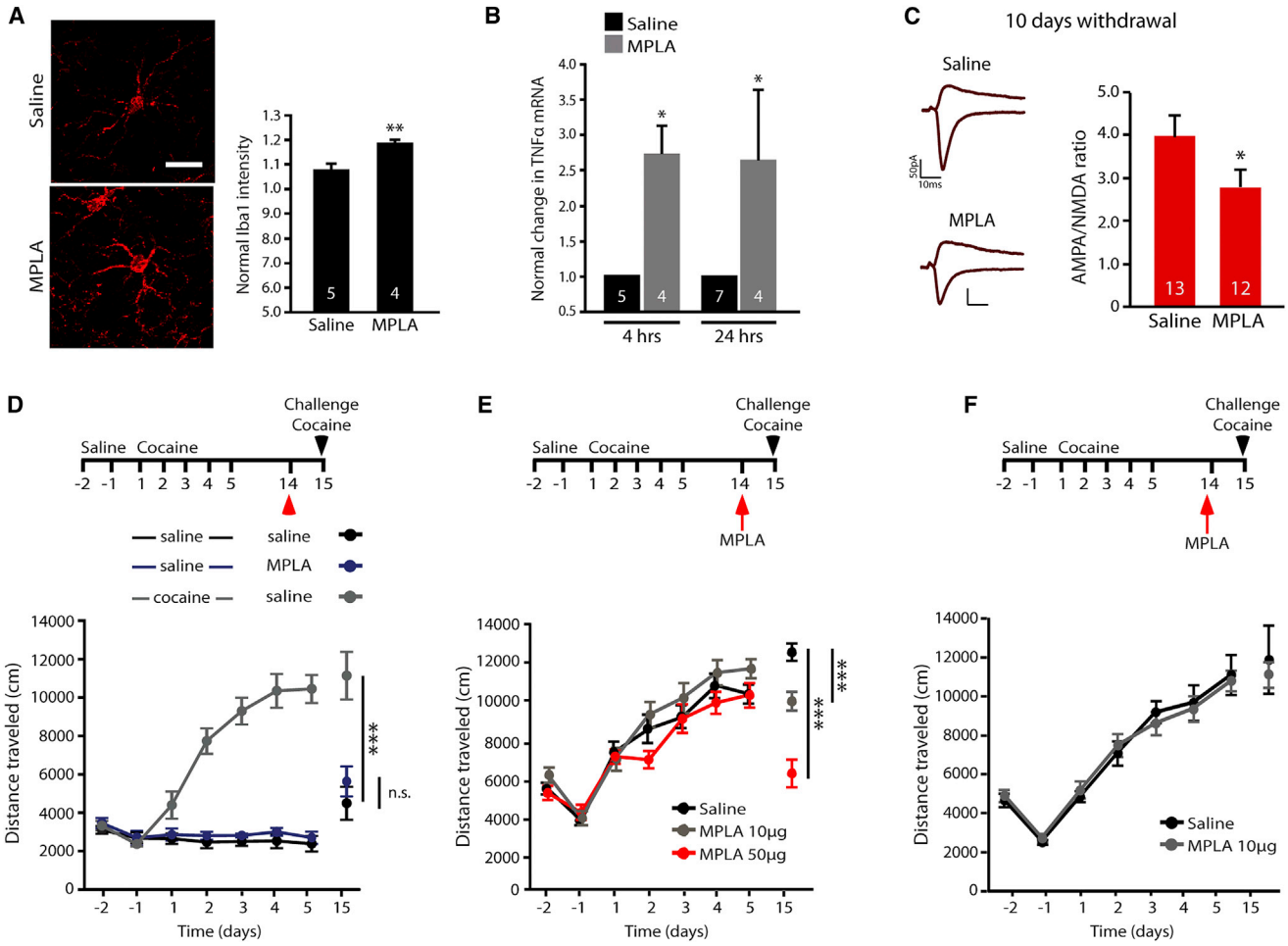

Figure 4. MPLA Activates Microglia in the Nucleus Accumbens and Decreases Behavioral Sensitization to Cocaine via TNF-α

(A) Representative confocal projection images of Iba1 immunostaining in the NAc, after MPLA (10 μg) or saline injection in mice after 10 days' withdrawal. Scale bar, 20 μm. Semiquantification of immunoreactivity reveals that Iba1 intensity was significantly increased 24 hr after a single MPLA injection.

(B) MPLA (10 μg) significantly increases TNF-α mRNA in the ventral striatum at 4 hr and 24 hr.

(C) Representative traces and AMPA/NMDA ratios from D1-MSNs in the NAc core 24 hr after MPLA (10 μg) or saline injection in mice after 10 days' withdrawal.

(D) MPLA does not reduce the initial locomotor response to cocaine. Mice were given seven daily saline injections, then after 9 days of abstinence given an injection of saline or MPLA (10 μg), followed the next day by a challenge dose of cocaine. MPLA did not alter the response to the challenge dose. This response was lower than the sensitized response in control animals given cocaine during training (n = 8 sal/sal, 7 sal/MPLA, 6 coc/sal).

(E) MPLA did reduce the sensitized response to cocaine. After withdrawal, WT mice were injected with MPLA (10 μg or 50 μg) or saline and tested 24 hr later with a challenge dose of cocaine (n = 20 for control, 12 for 10 μg MPLA, and 10 for 50 μg MPLA).

(F) MPLA treatment had no effect on sensitization in *TNF-α*$^{-/-}$ mice, as MPLA (10 μg) did not reduce the response to a challenge dose of cocaine in *TNF-α*$^{-/-}$ mice (n = 10 saline, 11 MPLA). Results are expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

but merely that astrocytes do not supply the TNF-α that opposes the circuit and behavioral changes induced by cocaine. CX3CR1 is expressed in other cell types (including macrophages and a small number of neurons), and a TLR4 agonist like MPLA will act on astrocytes and other cell types. Therefore we cannot exclude the contribution of other cell types to the TNF-α response, but it is difficult to argue that microglia are not the major source of the response. Microglia express a variety of neurotransmitters, neuropeptides, and immune receptors and have the capacity to rapidly respond to physiological changes in the brain (Kettenmann et al., 2011). Our results support the idea that moderate microglia activation has a role in a homeostatic-type response to significant deviations from the basal state (Kierdorf and Prinz, 2013) and has a similar beneficial response in re-establishing homeostasis following stress (Kreisel et al., 2014). Hence, augmenting the microglial response, through TLR4 or other means, might be a useful approach to treat addiction, provided it only moderately activates the microglia. MPLA, a weak TLR4 agonist, has been shown to significantly improve cognitive function in a mouse model of neurodegeneration (Michaud et al., 2013). We found that MPLA can acutely reduce behavioral sensitization after prolonged abstinence from cocaine. If MPLA is found to similarly diminish reinstatement, it would suggest that MPLA could reduce the motivation to acquire drugs and be

used to prevent relapse, a significant problem in the treatment of addiction.

EXPERIMENTAL PROCEDURES

All experiments were approved by the Canadian Council for Animal Care and the Montreal General Hospital Facility Animal Care Committee. Experimental procedures and details of statistics are available in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article at http://dx.doi.org/10.1016/j.neuron.2016.03.030.

AUTHOR CONTRIBUTIONS

All authors did experiments and analyzed data; G.M.L. and D.S. conceived the project; G.M.L., S.C.K., and D.S. wrote the paper.

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