D1/D5 receptors and histone deacetylation mediate the Gateway Effect of LTP in hippocampal dentate gyrus

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The dentate gyrus (DG) of the hippocampus is critical for spatial memory and is also thought to be involved in the formation of drug-related associative memory. Here, we attempt to test an aspect of the Gateway Hypothesis, by studying the effect of consecutive exposure to nicotine and cocaine on long-term synaptic potentiation (LTP) in the DG. We find that a single injection of cocaine does not alter LTP. However, pretreatment with nicotine followed by a single injection of cocaine causes a substantial enhancement of LTP. This priming effect of nicotine is unidirectional: There is no enhancement of LTP if cocaine is administrated prior to nicotine. The facilitation induced by nicotine and cocaine can be blocked by oral administration of the dopamine D1/D5 receptor antagonist (SKF 83566) and enhanced by the D1/D5 agonist (SKF 38393). Application of the histone deacetylation inhibitor suberoylanilide hydroxamic acid (SAHA) simulates the priming effect of nicotine on cocaine. By contrast, the priming effect of nicotine on cocaine is blocked in genetically modified mice that are haploinsufficient for the CREB-binding protein (CBP) and possess only one functional CBP allele and therefore exhibit a reduction in histone acetylation. These results demonstrate that the DG of the hippocampus is an important brain region contributing to the priming effect of nicotine on cocaine. Moreover, both activation of dopamine-D1 receptor/PKA signaling pathway and histone deacetylation/CBP mediated transcription are required for the nicotine priming effect in the DG.

In human populations, there is a well-defined sequence of involvement in drugs of abuse, in which the use of nicotine or alcohol precedes the use of marijuana, which, in turn, precedes the use of cocaine. This developmental sequence of drug involvement has been referred to as the Gateway Effect or Gateway Hypothesis (Kandel 1975, 2002; Yamaguchi 1984). Nicotine is one of the most commonly used drugs of abuse. Prior studies have demonstrated that nicotine not only produces addictive effects by itself, but also sensitizes reward pathways for the addictive effects of other psychostimulants. Thus, nicotine can serve as a "gateway" drug that may lead to a change in the rewarding effect of other drugs, such as cocaine. Behavioral studies in rodents indicated that prior exposure to nicotine produces a greater increase in self-administration, sensitization, and conditioned place preference than the administration of cocaine alone (Klein 2001; Desai and Terry 2003; Collins et al. 2004; McMillen et al. 2005; McQuown et al. 2009; Levine et al. 2011; Mello and Newman 2011). However, the molecular and synaptic mechanisms underlying this gateway effect are not completely understood. Our laboratory has previously documented that the Gateway Effect not only influences behavior but also alters synaptic plasticity in certain brain regions. For instance, prior exposure to nicotine enhances the ability of cocaine to depress LTP in the nucleus accumbens (Levine et al. 2011) and enhances LTP in the amygdala (Huang et al. 2013). This raised the question: Is the priming effect of nicotine on cocaine also evident in the hippocampus, which is critical for spatial memory and other forms of explicit memory concerned with people and objects? Here we focus on the priming effect of nicotine on subsequent cocaine administration in the dentate gyrus (DG) of the hippocampus.

The dentate gyrus of the hippocampus plays a vital role in spatial memory, and is also related to drug associated memory. The administration of cocaine or nicotine modifies spatial memory and synaptic plasticity in the DG (Scerri et al. 2006; Kenney and Gould 2008; Perez et al. 2010; Fole et al. 2011; Iniguez et al. 2012). Lesion of the DG blocks cocaine-induced conditioned place preference (CPP) (Meyers et al. 2006; Hernandez-Rabaza et al. 2008). Moreover, the DG is one of the few regions in the adult brain where neurogenesis continues to take place (Christie and Cameron 2006; Aimons et al. 2010) and neurogenesis is thought to play an important role in the formation of addictive memory (Eisch and Harburg 2006; Canales 2007, 2010; Noona et al. 2010). Finally, the DG is a brain region that is highly sensitive to nicotine and significantly affects synaptic plasticity. Either application of nicotine in brain slices or subcutaneous injection of nicotine, in vivo, enhances LTP in dentate gyrus (Sawada et al. 1994; Curran and Connor 2003; Welsby et al.

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2006, 2009). Acute nicotine treatment prevents sleep deprivation-induced impairment of LTP in the DG (Aleisa et al. 2011) and administration of a high-dose of nicotine causes synaptic potentiation in the DG, without pairing of tetanic stimulation (Matsuyama et al. 2000; Tang and Dani 2009). These studies in the DG have, however, focused on the effects of nicotine alone.

We were interested in knowing, would nicotine also play an important role in priming the DG for the effects of other drugs of abuse, such as cocaine? In our previous studies, we established a mouse model for the sequential use of oral administration of nicotine (10 mg/L) followed by an injection of cocaine (30 mg/kg). We found that the administration of nicotine followed by cocaine altered cocaine-induced changes in synaptic plasticity in the nucleus accumbens and the amygdala (Levine et al. 2011; Huang et al. 2013). We now used this paradigm to examine synaptic plasticity in the DG. We address the following four questions:

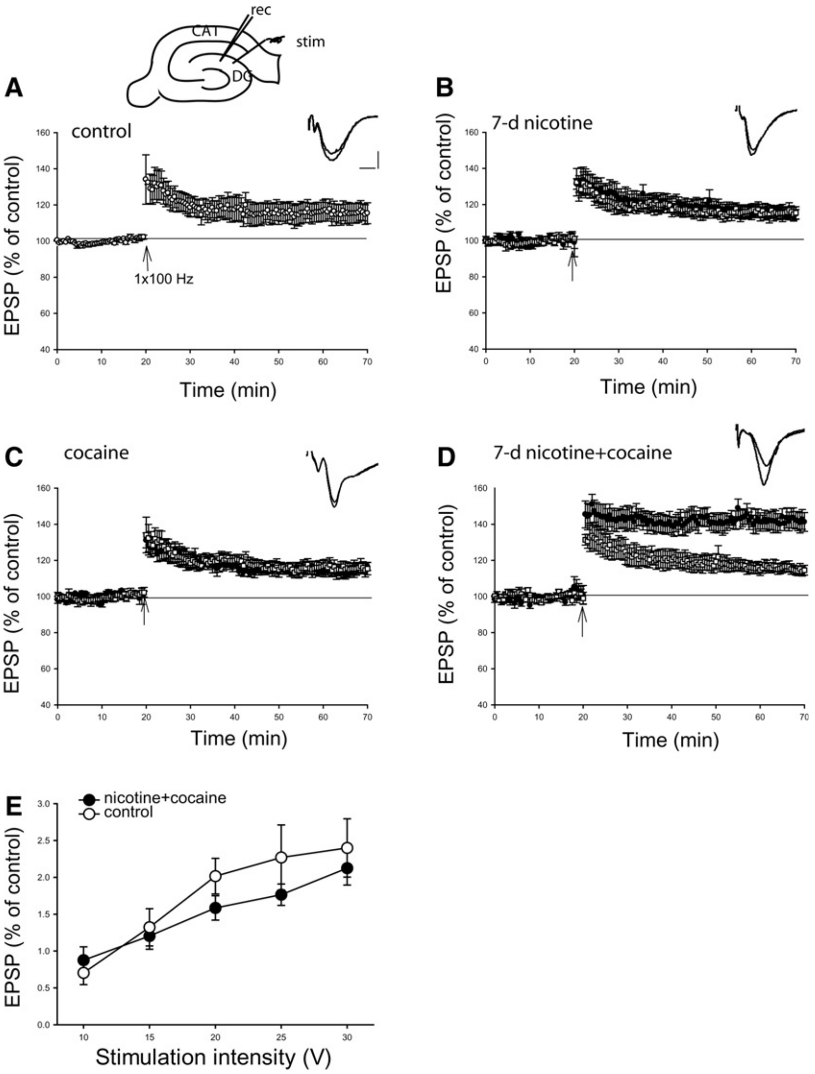

Figure 1. Seven-day nicotine exposure prior to cocaine enhances LTP in the Dentate Gyrus (DG). (*A*) A single tetanus (1 × 100 Hz) induced a weak LTP in DG. Top panel shows the schematic illustration of LTP recording in the DG. (*B*) Oral administration of nicotine for 7 d did not alter LTP. (*C*) Single injection of cocaine did not alter LTP. (*D*) Seven-day nicotine treatment followed by cocaine injection significantly enhanced LTP. (Closed circles) nicotine + cocaine, (open squares) cocaine. (*E*) There is no significant difference in the input–output curves between mice treated with 7-d nicotine/cocaine and control mice. Top panels of each graph show the representative EPSPs before and 50 min after tetanus. Calibration: 10 msec, 1 mV.

1. What are the effects of 7-d oral administration of a low dose of nicotine on LTP in the DG by itself and when the administration of nicotine is followed by an injection of cocaine (priming effect)?
2. Since the central dopaminergic system may play an important role in nicotine-mediated synaptic plasticity (Mansvelder and McGehee 2002; Kauer and Malenka 2007; Tang and Dani 2009), we further ask: Do the dopamine D1/D5 receptors play any role in the priming effect of nicotine on cocaine?
3. Since we have earlier found that nicotine enhances histone acetylation in the striatum (Levine et al. 2011), we examine whether the priming effect of nicotine on cocaine in the DG is simulated by an inhibitor of histone deacetylase (HDAC).
4. Finally, the CREB-binding protein CBP is a histone acetyl transferase (HAT). CBP haploinsufficient mice carry only one functional CBP allele and have a reduction in histone acetylation (Levine et al. 2011). We therefore also ask: How does the sequential treatment paradigm of nicotine on cocaine affect LTP in the DG in CBP haploinsufficient mice?

Results

D1/D5 receptors mediate the priming effect of nicotine in the DG

Since behavioral studies on the priming effect of nicotine on cocaine in humans and in rodents are all performed with the intact GABAergic-system, we studied LTP in DG hippocampal slices without treatment with a GABA-antagonist. With intact GABAergic inhibition, a single tetanus (1 × 100 Hz) induced only a weak LTP in the DG of control animals (115 ± 3%, $n = 10$, 50 min after tetanus) (Fig. 1A).

Does nicotine have any priming effect on cocaine-induced changes of LTP in the DG under those circumstances? To address this question we first examined the effects of nicotine alone and cocaine alone. Oral administration of low doses of nicotine (10 μg/mL) for 7 d did not alter the induction of LTP. In mice treated with nicotine for 7 d, LTP was 116 ± 2% ($n = 8$), which was not significantly different from controls ($P > 0.05$) (Fig. 1B). Similarly, a single injection of cocaine (30 mg/kg) did not significantly alter the induction of LTP (114 ± 3%, $n = 7$) (Fig. 1C). However, when 7-d exposure to nicotine was followed by the injection of cocaine, a significant enhancement of LTP emerged. A single tetanus (1 × 100 Hz) now produced LTP, which was significantly greater than that obtained when mice received a cocaine injection alone (141 ± 6%, $n = 8$, $P < 0.01$) (Fig. 1D). The enhancement of LTP was not associated with any changes in basal synaptic transmission (input–output curve) (Fig. 1E). We next examined the effect of short-term exposure to nicotine. Mice were pretreated with nicotine for 1 d, followed by the injection of cocaine. Although 7-d nicotine exposure followed by cocaine produced a long-lasting enhancement of LTP, 1-d nicotine exposure followed by cocaine only produced a short-lasting enhancement. In this latter group of mice, LTP was enhanced 10 min after tetanus (nicotine+cocaine, 146 ± 5%, $n = 6$; control, 122 ± 5%, $n = 6$, $P < 0.05$) (Fig. 2B). However, LTP decayed 50 min after the tetanus, which was different from the stable enhancement of LTP in mice treated with nicotine for 7 d (Fig. 1D).

Using the protocol of 1-d nicotine exposure, we found that the enhancement of LTP induced by nicotine and cocaine was unidirectional. Reversing the sequence, a single injection of cocaine prior to 1-d nicotine exposure did not produce any significant enhancement of LTP (121 ± 6%, 10 min after tetanus, $n = 6$, $P > 0.05$) (Fig. 2C). Similarly, repeated injection of cocaine for 7 d followed by 1-d nicotine exposure did not produce any enhancement of LTP in DG either 10 min (124 ± 5%) or 50 min (120 ± 6%) after tetanus ($n = 6$, $P > 0.05$) (Fig. 2D).

The central dopaminergic system plays an important role in the effect of nicotine (Mansvelder and McGehee 2002; Kauer and Malenka 2007; Tang and Dani 2009). We therefore asked: Is the priming effect of nicotine on cocaine-induced changes of LTP mediated by the activation of dopamine receptors? Under oral administration, both the D1/D5 agonist SKF 38393 and antagonist SKF 83566 are rapidly absorbed and penetrate the blood–brain barrier. Indeed, the agonist is used clinically for the treatment of Parkinson's disease and nicotine craving (Braun et al. 1987; Nakagome et al. 2011).

We first applied the D1/D5 receptor antagonist (SKF 83566) into the drinking water together with nicotine for 7 d, followed by the injection of cocaine. We found that coadministration of the antagonist SKF 83566 (20 μg/mL) and nicotine (10 μg/mL) significantly blocked the enhancement of LTP induced by pretreatment with nicotine (SKF 83566+nicotine+cocaine, 120 ± 4%, $n = 12$; nicotine+cocaine, 143 ± 5%, $n = 6$, $P < 0.01$) (Fig. 3A). As a control, SKF 83566 alone did not alter LTP (115 ± 6%, $n = 5$) (Fig. 3B). These results indicate that the priming effect of nicotine on cocaine requires the activation of D1/D5 receptors.

We next asked whether the D1/D5 receptor agonist can enhance the priming effect of nicotine. We now added the D1/D5 agonist SKF 38393 (10 μg/mL) into the drinking water together with nicotine, followed by the injection of cocaine. The addition of SKF 38393 to nicotine did not produce further enhancement of LTP by cocaine in mice treated with nicotine for 7 d (SKF 38393+nicotine+cocaine, 137 ± 6%, $n = 7$, $P > 0.05$) (Fig. 3C). However, LTP was enhanced in mice coadministered SKF 38393 and nicotine for 1 d. The coadministration of the D1/D5 receptor agonist SKF 38393 and nicotine for 1 d prior to the injection of cocaine produced a significantly greater LTP at 50 min after HFS (SKF 38393+nicotine+cocaine, 145 ± 7%, $n = 9$), compared to LTP treated with nicotine for 1 d without adding SKF 38393 (nicotine+cocaine, 121 ± 5%, $n = 6$, $P < 0.01$) (Fig. 3D). As a control, oral administration of SKF 38393 alone for 1 d did not produce any significant enhancement of LTP (116 ± 5%, $n = 6$, $P < 0.01$) (Fig. 3E). These results provide evidence that the priming effect of nicotine on cocaine in DG-LTP is at least partly mediated by the activation of the D1/D5 dopamine receptors.

Because the activation of the D1/D5 receptors increases cAMP and activates the protein kinase A (PKA) signaling pathway, we examined the effect of a PKA inhibitor on the priming effect of nicotine on cocaine following short- and long-term exposure to nicotine. We applied the PKA inhibitor KT5720 (2 μM) to the perfusion bath 20 min before tetanus. This did not alter LTP induced by a single tetanus. However, KT5720 completely blocked the enhancement of LTP produced by the treatment of 7-d nicotine and cocaine (KT5720+nicotine+cocaine, 110 ± 4%, $n = 5$; nicotine+cocaine, 143 ± 5%, $n = 6$, $P < 0.01$) (Fig. 3F). These results indicate that the regulation of the D1/D5 receptors on the priming effect of nicotine may be mediated through activation of the PKA signaling pathway.

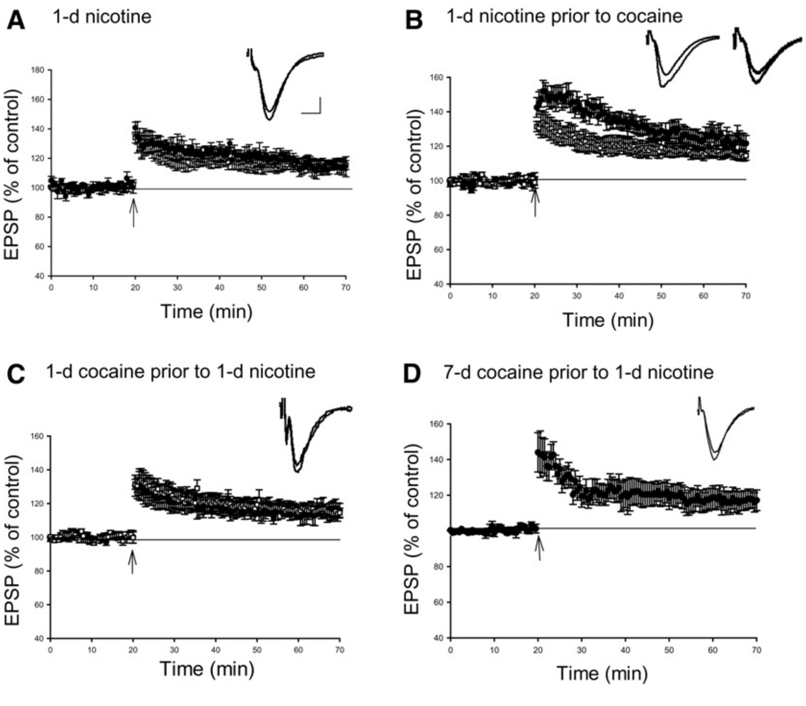

Figure 2. The priming effect of nicotine on cocaine is unidirectional. (*A*) One-day nicotine exposure did not alter LTP. (Open circles) control, (closed circles) nicotine. (*B*) One-day nicotine exposure followed by cocaine injection produces a short-lasting enhancement of LTP. (Open squares) cocaine, (closed circles) nicotine + cocaine. (*C*) Cocaine injection followed by 1-d nicotine exposure (reverse sequence) did not produce any enhancement of LTP. (Open squares) control, (closed circles) nicotine + cocaine. (*D*) Seven-day cocaine followed by 1-d nicotine did not produce significant enhancement of LTP.

Histone acetylation mediates the priming effect in the DG

Histone acetylation plays an important role in learning and memory (Alarcon et al. 2004; Vecsey et al. 2007) as well as in drug addiction (Levine et al. 2005, 2011; Renthal and Nestler 2008; Wang et al. 2010). Our previous work has found that the administration of the HDAC inhibitor SAHA (suberoylanilide hydroxamic acid) can simulate the priming effect of nicotine on cocaine in the expression of LTP in the NAc and amygdala (Levine et al. 2011; Huang et al. 2013). Does histone deacetylation play any role in the priming effect of nicotine on

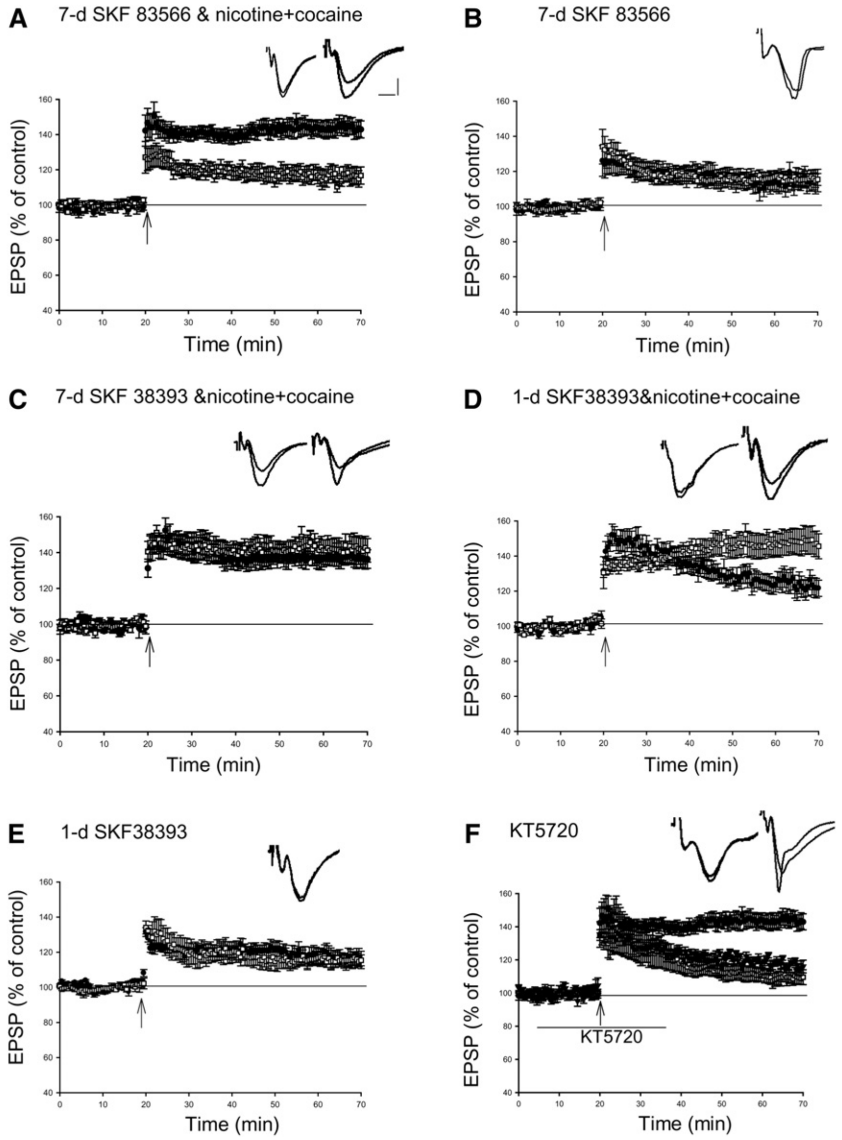

Figure 3. The priming effect on LTP is mediated by D1/D5 receptors. (*A*) Oral administration of the D1 receptor antagonist SKF 83566 together with nicotine for 7 d blocked the enhancement of LTP in the DG. (Open squares) 7-d SKF 83566+nicotine+cocaine, (closed circles) 7-day nicotine+cocaine. (*B*) SKF 83566 alone did not alter LTP. (Closed circles) 7-d SKF 83566, (open squares) control. (*C*) Oral administration of the D1 agonist SKF 38393 together with nicotine prior to cocaine did not further enhance the priming effect on LTP induced by 7-d nicotine/cocaine. (Open squares) 7-d SKF 38393+nicotine+cocaine, (closed circles) 7-d nicotine+cocaine. (*D*) Oral administration of the D1 agonist SKF 38393 significantly enhanced LTP induced by 1-d nicotine exposure and cocaine. (Open squares) SKF 38393+1-d nicotine+cocaine, (closed circles) 1-d nicotine+cocaine. (*E*) Oral administration of the D1 agonist SKF 38393 alone did not alter LTP. (Closed circles) SKF 38393, (open squares) control. (*F*) The protein kinase A (PKA) inhibitor KT5720 blocked the enhancement of LTP induced by 7-d nicotine and cocaine. (Open squares) KT5720+7-d nicotine+cocaine, (closed triangle) KT5720, (closed circles) 7-d nicotine+cocaine.

cocaine in the DG? To address this question we first tested the effect of the HDAC inhibitor SAHA. A single injection of SAHA in vivo (50 mg/kg, intraperitoneal injection [i.p.], 10–20 min before the sacrifice of mice) did not significantly alter DG-LTP induced by a single tetanus (1 × 100 Hz) (SAHA, 115 ± 7%, $n = 6$; control, 114 ± 6%, $n = 6$, $P > 0.05$) (Fig. 4A). However, when the injection of SAHA was followed by an injection of cocaine (SAHA 10 min prior to cocaine), the same tetanus now elicited a greater LTP (142 ± 11%, $n = 10$), which was significantly different from LTP in mice treated with cocaine alone (112 ± 3%, $n = 6$, $P < 0.01$) (Fig. 4B). This result indicates that the HDAC inhibitor SAHA can mimic the priming effect of nicotine on cocaine. Similar to nicotine, the priming effect of SAHA is unidirectional. When SAHA was injected after the injection of cocaine (cocaine 10 min prior to SAHA), no facilitation of LTP was observed (116 ± 5%, $n = 6$, $P > 0.05$) (Fig. 4C). A histogram comparing the effects of SAHA, cocaine by itself, and the combination of SAHA and cocaine is shown in Figure 4D.

Previously, we found that chronic histone treatment (7 d, 10 ng/mL) induced a global hyperacetylation of histone in the striatum (Levine et al. 2011). To further examine the priming effect of nicotine on the DG, we looked at the acetylation levels of histone H3 following 7 d of nicotine and cocaine exposure. We found that, similar to its effect in the striatum, in the DG nicotine increases the acetylation of histone H3 at Lysine 9 (AcH3, 2.37 ± 24%, $P = 0.011$, $n = 5$), while cocaine does not induce a significant increase of histone acetylation (AcH3, 1.10 ± 5%, $P = 0.9$, $n = 5$) (Fig. 4E).

CREB-binding protein CBP is a histone acetyl transferase (HAT). CBP haploinsufficient (CBP+/−) mice carry only one functional CBP allele. In these mice, the basal recruitment of histone (H4) acetylation at the Fos B promoter is significantly decreased compared with wild-type (WT) mice (Alarcon et al. 2004; Levine et al. 2005). To further study the role of HDAC in the priming effect of nicotine, we examined the priming effect in the DG of CBP+/− mice. As in the hippocampal CA1 region (Alarcon et al. 2004), there were no differences in the input–output curves (Fig. 5A) and in LTP induced by single tetanus (1 × 100 Hz) between WT and CBP+/− mice (WT, 115 ± 7%, $n = 6$; KO, 116 ± 8%, $n = 6$, $P > 0.05$) (Fig. 5B). A single injection of cocaine (WT, 120 ± 3%, $n = 5$; KO, 115 ± 5%, $n = 5$, $P > 0.05$) (Fig. 5C), or 7-d exposure to nicotine (WT, 117 ± 3%, $n = 6$; KO, 113 ± 4%, $n = 6$) (Fig. 5D) did not alter LTP either in WT or CBP+/− mice. However, when a cocaine injection was primed by 7-d nicotine exposure, a significant enhancement of LTP was obtained in the WT mice (nicotine+cocaine, 141 ± 3%, $n = 8$, $P < 0.01$), but not in the CBP+/− mice (nicotine+cocaine, 120 ± 3%, $n = 12$, $P < 0.05$) (Fig. 5E). Figure 5F displays a histogram showing the effects of nicotine, cocaine, and nicotine plus cocaine on DG-LTP in WT and CBP+/− mice. These results indicate that the priming effect of nicotine on cocaine is blunted in CBP+/− mice, which are essentially in a chronic hypoacetylated state, and provide genetic evidence that the priming effect of nicotine in the DG is mediated through histone deacetylation.

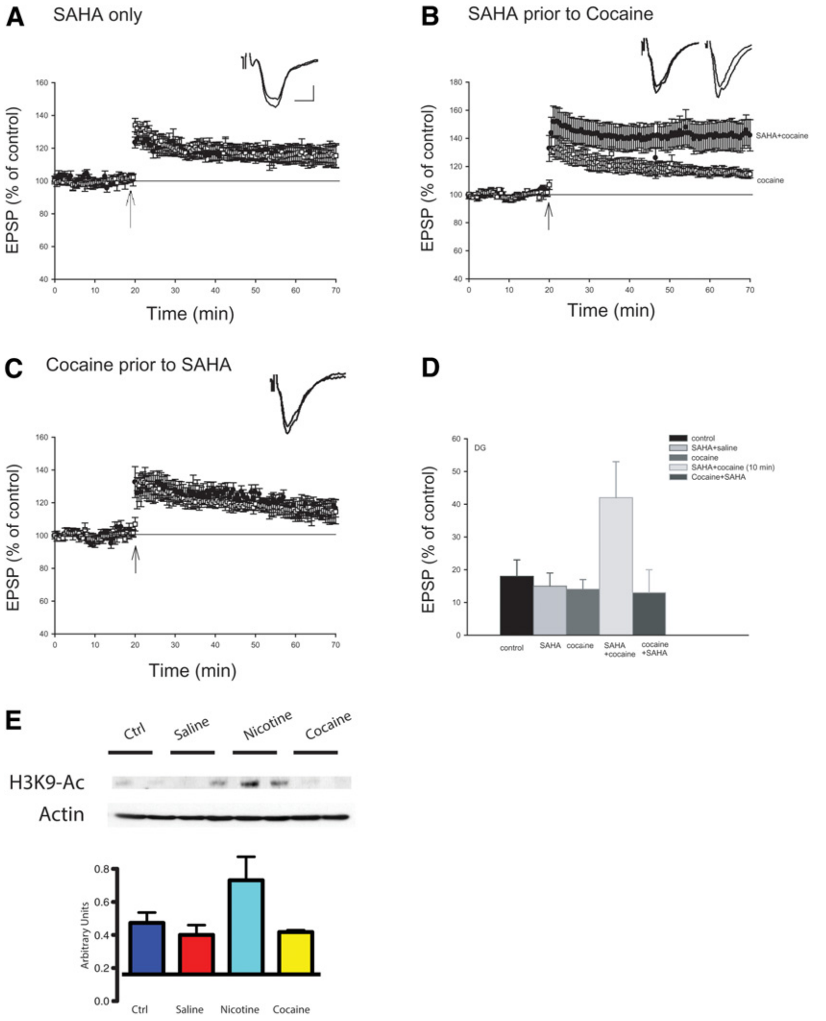

Figure 4. The HDAC inhibitor simulates the priming effect of nicotine on cocaine. (*A*) The injection of SAHA alone did not enhance LTP. (Open squares) control, (closed circles) SAHA. (*B*) SAHA injection prior to (10 min) the injection of cocaine significantly enhances LTP. (Open squares) cocaine, (closed squares) SAHA+cocaine. (*C*) The injection of cocaine prior to (10 min) SAHA injection (reverse) did not alter LTP. (Open squares) cocaine, (closed circles) cocaine+SAHA. (*D*) Histograms show the different changes in LTP in mice treated with SAHA, cocaine, and SAHA+cocaine. (*E*) Seven-day nicotine, but not 7-d cocaine, significantly increases H3 histone acetylation in the DG.

Discussion

The DG in the hippocampus receives dopaminergic innervation from the ventral tegmental area (VTA) and the DG contains dopamine D1/D5 receptors (Fremeau et al. 1991; Meador-Woodruff et al. 1992; Gangarossa et al. 2012). The alteration of the functioning of these receptors may be related to the induction of drug-induced memory (Tanaka et al. 2011). Although many studies have explored the role of the dopaminergic system on the effects of nicotine by itself (Damaj and Martin 1993; Mansvelder and McGehee 2002; Kauer and Malenka 2007; Tang and Dani 2009), the role of the dopaminergic system in the priming effect of nicotine on cocaine has not been previously investigated. Here we have provided evidence showing that dopamine receptors play an important role in the priming effect of nicotine.

We first asked: What are the mechanisms underlying the modulation of D1/D5 receptors in the priming effect? We find that the administration of nicotine activates D1/D5 receptors (O'Neil et al. 1991; Shoaib 1998; Hamada et al. 2004; Hyman et al. 2006; Gozen et al. 2013). Moreover, coadministration of nicotine and D1/D5 receptor agonists seems to produce a higher level of activation of the D1/D5 receptors and may consequently enhance PKA and PKA-mediated phosphorylation of the transcription factor cAMP response element binding protein (CREB). Second, we find that activation of D1/D5 receptors enhances the level of ΔFosB (Doucet et al. 1996; Muller and Unterwald 2005), which is a critical molecular switch for drug addiction (Nestler 2004). Importantly, the administration of nicotine prior to cocaine produces a unidirectional enhancement of ΔFosB. The reverse order has no effect (Levine et al. 2011). ΔFosB controls the unidirectional priming effect of nicotine on cocaine via mediation of histone acetylation (Levine et al. 2005, 2011).When nicotine was coadministered with the D1/D5 receptor agonists, nicotine and the subsequent administration of cocaine produced a higher level of activation of the D1/D5 receptors. This enhanced PKA-mediated phosphorylation of the transcription factor CREB and the expression of ΔFosB and other immediate-early genes, such as Fos and Jun, lead to greater LTP. By contrast, when nicotine was coadministered with the D1/D5 receptor antagonists, the effect of nicotine on the activation of D1/D5 receptors and the increase of ΔFosB was blocked, thereby preventing the priming effect of nicotine on the induction of LTP. Finally, our data suggest that the effect of D1/D5 receptors on the priming effect in the DG may be mediated directly through the regulation of HDAC. Genetic inactivation of dopamine D1 but not D2 receptors inhibits histone acetylation (Darmopil et al. 2009). When a D1 receptor agonist is given together with the HDAC inhibitor, the combination enhances cocaine-induced locomotor response and conditioned place preference (Schroeder et al. 2008). The nicotine-induced activation of D1/D5 receptors may lead to phosphorylation of CREB (Nestler 2004; Hyman et al. 2006), which recruits the CREB-binding protein (CBP), a histone acetyl transferase that acetylates histones and consequently influences the transcription of genes and the modification of synapses (Kandel 2001; Guan et al. 2002; Alarcon et al. 2004; Levine et al. 2005). In addition to the D1/D5 receptors, exposure to nicotine activates dopamine D2 receptors (Hamada et al. 2004; Novak et al. 2010), which may also be involved in the mediation of the priming effect of nicotine on cocaine. However, since D2 receptors are negatively coupled with the cAMP signaling pathway (Beaulieu and Gainetdinov 2011), the involvement of D2 receptors may mediate the nicotine effect through a signaling pathway that is different from that of the D1 receptors and unrelated to HDAC (Hamada et al. 2004; Grieder et al. 2011).

Histone acetylation and deacetylation seem to play a critical role in drug addiction (Levine et al. 2005, 2011; Renthal and

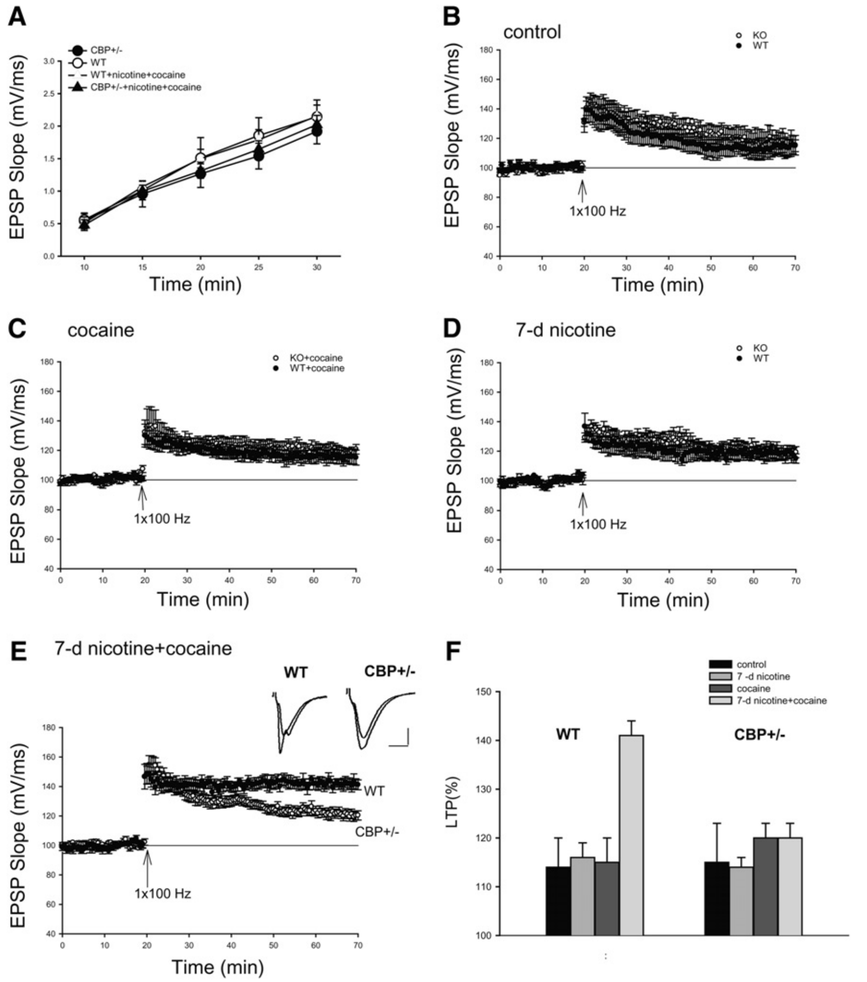

Figure 5. The priming effect is impaired in CBP+/− mice. (*A*) There are no significant differences in the input–output curves between WT and CBP+/− mice. (*B*) LTP induced by a single train of tetanus (1 × 100 Hz) is normal in CBP+/− mice. (*C*) A single injection of cocaine did not alter LTP either in WT or CBP+/− mice. (*D*) Seven days' exposure of nicotine did not alter LTP either in WT or CBP+/− mice. (*E*) The pretreatment with nicotine (7-d) prior to cocaine produced a significant enhancement of LTP in the WT but not the CBP+/− mice. (Open circles) 7-d nicotine+cocaine in CBP+/− mice, (closed circles) 7-d nicotine+cocaine in WT mice. (*F*) A histogram comparing the effect of nicotine, cocaine, and nicotine plus cocaine on LTP in WT and CBP+/− mice. Top panel shows the representative field EPSPs in WT and CBP+/− mice. Calibration: 10 msec, 1 mV.

Nestler 2008). We here provide further pharmacological and genetic evidence showing that the priming of LTP in the DG is also mediated through histone acetylation. The HDAC inhibitor SAHA mimics the priming effect of nicotine. In addition, nicotine priming of cocaine produces an enhancement of DG-LTP induced by a single tetanus in WT mice, but does not do so in CBP+/− mice, which are essentially in a chronic hypoacetylated state. This may be because the association between nicotine and cocaine achieves a sufficient enhancement of histone acetylation in the WT mice that is required for switching a weak form of LTP to a strong form. By contrast, the enhancement of histone acetylation produced by the association of nicotine and cocaine appears to be insufficient in CBP+/− mice. As a result, nicotine and cocaine may be unable to enhance the amplitude of LTP in these mice. This result is consistent with our earlier studies, in which we found that CBP+/− mice have deficits in producing a strong and stable form of LTP in the hippocampal CA1 region (Alarcon et al. 2004).

The present study therefore provides new evidence supporting the idea that, in the dentate gyrus, as in the striatum and in the amygdala, the priming effect of nicotine on cocaine may be mediated, at least partially, by the inhibition of histone deacetylation and the recruitment of CBP-mediated gene transcription.

Materials and Methods

Male C57BL6/J mice (6- to 9-wk-old) (Jackson Laboratories, Bar Harbor, ME) were kept in clear plastic cages (29.2 × 19 × 12.7 cm, N10 cage, Ancare) in groups of five with ad libitum food (Prolab IsoPro RMH3000, PMI Nutrition International LLC) and water (autoclaved tap water). Mice were kept at a 12-h day–night cycle.

The generation of CBP+/− mice was described previously (Tanaka et al. 1997; Alarcon et al. 2004). The WT mice used as control group were litter mates of the CBP+/− mice. Mice were maintained and bred under standard conditions, consistent with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee of Columbia University.

After receiving different drug treatments, mice were quickly decapitated and placed in ice-cold ACSF (artificial cerebrospinal fluid). The hippocampus was removed and transverse slices of brain (400 μM) were cut and transferred to an interface chamber (Harvard Apparatus). Slices were half-submerged and constantly perfused with ACSF at a rate of 2 mL/min and bubbled with 95% O_2 and 5% CO_2. The composition of ACSF was as follows (in mM): 124 NaCl, 1.2 $MgSO_4$, 4 KCl, 1.0 NaH_2PO_4, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 D-glucose. All experiments were performed in the intact GABAergic inhibition (without picrotoxin). The temperature of the slices was maintained at 28°C. Experiments were started 2.5 h after slices were dissected.

Extracellular recordings were made using ACSF-filled glass electrodes (1–3 MΩ). Stimuli were delivered at a rate of every 30 sec (0.033 Hz, 0.05 msec pulse duration) through concentric bipolar stainless-steel electrodes (25 μM wire diameter, CBBRC75, FHC). The stimulating electrodes were placed in the middle of molecular layers of the dentate gyrus (DG), which contained fibers from the perforant pathway. The recording electrodes were placed in the middle molecular layer of the DG (Fig. 1A). Medial pathway responses were confirmed by the depression of fEPSP elicited by paired-pulse stimulation (Nguyen and Kandel 1996). The stimulation intensity was adjusted to evoke the field potential that was ~50% of maximal amplitude. Baseline values were acquired over a period of 20 min before giving the LTP inducing stimulation. LTP was elicited by a single train of tetanus (1 × 100 Hz, 1 sec, and 0.1 msec pulse duration). Changes in synaptic strength were expressed relative to the normalized baseline (mean ± SEM). Statistical comparisons were performed using Student's *t*-test.

Nicotine hydrogen tartrate salt (Sigma) was dissolved in drinking water (10 μg/mL) and administered continuously in the drinking water for 1 d, or 7 d prior to the experiment day. Cocaine hydrochloride (Sigma) was dissolved in sterile saline and administered by intraperitoneal injection (i.p. 30 mg/kg) 10 min before mice were sacrificed. Suberoylanilide hydroxamic acid (SAHA, 50 mg/kg) was dissolved in DMSO and administered by i.p. 10 min prior to the injection of cocaine or, conversely, 10 min after the injection of cocaine (in this experiment cocaine was injected 20 min before the sacrifice of mice). For the oral administration, the D1/D5 antagonist SKF 83566 (20 μg/mL, Tocris) and agonist SKF 38393 (10 μg/mL, Sigma) were dissolved in the drinking water. For the bath application of KT5720 onto brain slices, stock solutions (in DMSO, Sigma) were prepared and were diluted 1000 times when applied into the perfusion solution.

To measure acetylation, mice were treated with saline, nicotine alone, and cocaine alone for 7 d, then the brain was removed and the dentate gyrus of hippocampus was dissected. Striata were dissected in PBS on ice and homogenized in 0.25% Triton X-100, 0.5% Nonidet P-40, 10 mM EDTA, 0.5 mM EGTA, 10 mM Tris·HCl (pH 8.0), and 1 mM PMSF. Thirty micrograms of protein were used for detection of acetylated histone H3 and separated by SDS/15% PAGE. After electrophoresis, and western blotting, the membrane was probed with antibody specific for acetylated Histone H3 at Lysine 9 (Millipore) overnight at 4°C. Blot was incubated with an anti-rabbit IgG-HRP as secondary antibody (1:5000, Sigma) for 1 h at room temperature, and detected by using ECL (Amersham Pharmacia). To verify the accuracy of sample loading, the blot was reprobed with a monoclonal antibody to actin (1:10,000, Sigma). Acetylated H3 bands were measured at 17 kDa. Relative optical density readings for the acetylated Histone H3 and actin bands were determined by comparing H3 and actin densities.

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