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Nicotine primes the effect of cocaine on the induction of LTP in the amygdala

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ABSTRACT

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. The term "Gateway Hypothesis" describes this developmental sequence of drug involvement. In prior work, we have developed a mouse model to study the underlying metaplastic behavioral, cellular and molecular mechanisms by which exposure to one drug, namely nicotine, affects the response to another drug, namely cocaine. We found that nicotine enhances significantly the changes in synaptic plasticity in the striatum induced by cocaine (Levine et al., 2011). Here we ask: does the metaplastic effect of nicotine on cocaine also apply in the amygdala, a brain region that is involved in the orchestration of emotions and in drug addiction? We find that pretreatment with nicotine enhances long-term synaptic potentiation (LTP) in response to cocaine in the amygdala. Both short-term (1 day) and long-term (7 days) pre-exposure to nicotine facilitate the induction of LTP by cocaine. The effect of nicotine on LTP is unidirectional; exposure to nicotine following treatment with cocaine is ineffective. This metaplastic effect of nicotine on cocaine is long lasting but reversible. The facilitation of LTP can be obtained for 24 but not 40 days after cessation of nicotine. As is the case in the striatum, pretreatment with Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, simulates the priming effect of nicotine. These results provide further evidence that the priming effect of nicotine may be achieved, at least partially, by the inhibition of histone acetylation and indicate that the amygdala appears to be an important brain structure for the processing of the metaplastic effect of nicotine on cocaine.

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1. Introduction

In the general population, there is a well-defined sequence of progression to illicit drug use. The vast majority of individuals who use illicit drugs have smoked cigarettes or drank alcohol prior to trying other drugs. This pattern of drug use, labeled the Gateway Hypothesis, derives from the epidemiological finding of a regular sequence of involvement across drug classes, with tobacco and alcohol preceding the use of marijuana, which in turn precedes the use of other illicit drugs (Kandel, 2002; Kandel et al., 2006). The term Gateway Hypothesis has been used to suggest that drugs used earlier in the sequence increase the risk of using other drugs. While this sequence is affected by social, psychological and legal factors, behavioral studies in rodents suggest that biological factors are involved as well. There are also metaplastic effects of an earlier drug on a later drug, such that when a later drug is consumed, the response is different than what it would have been had there not been exposure to the earlier drug (Levine et al., 2011). Multiple studies in rodents document that exposure to drugs used early in the sequence, such as nicotine or alcohol prior to exposure to cocaine (or other drugs of abuse) produces an increase in behavioral phenotypes related to addiction, such as self-administration, sensitization and conditioned place preference, compared with administration of cocaine alone (Klein, 2001; Desai and Terry, 2003; Collins et al., 2004; McMillen et al., 2005; James-Walke et al., 2007; McQuown et al., 2006; Mello and Newman, 2011). Recently, we demonstrated that nicotine acts as a gateway drug by

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increasing histone acetylation in the striatum, thereby enhancing *Fosb* gene expression in response to cocaine (Levine et al., 2011). However, this past work did not examine the priming effect of nicotine on cocaine in other areas of the brain that are known to also play a role in addiction.

The brain circuitry underlying drug addiction is complex and consists of the ventral tegmental area (VTA), the nucleus accumbens (NAc), the hippocampus, cortical areas such as the prefrontal cortex, as well as prelimbic areas, such as the amygdala. The amygdala plays an important role in orchestrating emotion and emotional memory (Blair et al., 2001) and plays a significant role in addiction, as well. For instance, in a discriminative stimulus task in rats, lesions of the amygdala abolish both cue- and cocaine-induced reinstatement (Yun and Fields, 2003; Gardner, 2011). Similarly, lesions of the basolateral amygdala disrupt acquisition of cocaine-conditioned place preference (Fuchs et al., 2002). Conversely, electrical stimulation of the basolateral nucleus of the amygdala reinstates cocaine-seeking behavior in rats (Hayes et al., 2003). Administration of cocaine produces long-lasting effects on fear-potentiated startle in mice and rats, a learned behavior that requires the amygdala (Gordon and Rosen, 1999; Wood et al., 2007). Imaging studies in humans reveal that acute administration of cocaine reduces functional MRI signals in the amygdala, and the volume of the amygdala is reduced in people who suffer from cocaine addiction (Breiter et al., 1997; Makris et al., 2004; Barrós-Loscertales et al., 2011). Thus, the amygdala is thought to play a key role in modulating drug-induced motivational states and drug cravings, which in turn have a powerful effect on maintaining drug behavior (Breiter et al., 1997; Childress et al., 1999; Bonson et al., 2002; Carelli et al., 2003; Carrasquillo and Sweatt, 2005; Crombag et al., 2008).

Both cocaine and nicotine can lead to changes in synaptic plasticity in the amygdala (Goussakov et al., 2006; Huang et al., 2008). Persistent synaptic modifications in the amygdala may, in turn, affect the reward circuitry and may play a role in reward-related behavioral changes (Ambroggi et al., 2008). However, previous studies of long-term synaptic potentiation (LTP) in the amygdala all focused on the effects of nicotine or cocaine alone. The effect of the associated application of these two drugs in a sequential treatment paradigm on LTP in the amygdala is not known. Here we asked: is LTP in the amygdala affected by a sequential treatment paradigm of nicotine and cocaine, as we observed in the striatum (Levine et al., 2011)? Does nicotine prime the effects of cocaine in the amygdala, and if so, what are the mechanisms for this priming effect?

2. Materials and methods

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

Slice preparation: On experimental day, after the designated treatment was administered, mice were removed from their cages and quickly decapitated. The whole brain was placed in ice-cold ACSF (artificial cerebrospinal fluid) and a block of tissue containing the amygdala was removed. Coronal sections of brain (400 μm) were cut and transferred to an interface chamber (Fine Science Tools, Foster City, CA). 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 the 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 2–3 h after slices were dissected.

Extracellular recordings were made using ACSF-filled glass electrodes (1–3 MΩ). Stimuli were delivered at a rate of one per minute (0.017 Hz, 0.05 ms pulse duration) through concentric bipolar stainless steel electrodes (25 μM wire diameter, CBBRC75, FHC, Bowdoinham, ME). The stimulating electrodes were placed in the external capsule (EC), which contained fibers from the cortex to the lateral amygdala (EC–LA). The recording electrodes were placed in the lateral amygdala (Fig. 1A). The stimulation intensity was adjusted to evoke the field potential, which was about 50–60% of maximal amplitude. Baseline values were acquired over a period of 30 min before giving the LTP inducing stimulation. LTP was elicited by a single rain of tetanus (1 × 100 Hz, 1s). The pulse duration of stimulation during tetanus was 0.1 ms. Changes in synaptic strength were expressed relative to the normalized baseline (mean ± SEM). Statistical comparisons were performed using Student's *t*-test.

Drugs: Nicotine hydrogen tartrate salt (Sigma) was dissolved in drinking water (10 μg/ml) and administered continuously in the drinking water for one day, three days or seven days prior to the experimental day. Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in sterile saline and administered by intraperitoneal injection (i.p., 30 mg/kg) 10 min before mice were decapitated. α7 nAChR agonist AR-17779 hydrochloride (Tocris) was dissolved in DMSO and injected intraperitoneally three times (20 mg/kg, 24 h, 16 h and 30 min prior to decapitation). α7 nAChR antagonist methyllycacanitine (MLA, Tocris) and ß2 nAChR antagonist dihydro-ß-erythroidine hydromide (DHßH, Sigma) were dissolved in water and injected intraperioneally 15 min before the injection of cocaine. Suberoylanilide Hydroxamic acid (SAHA, 25 mg/kg, kindly provided by R. Breslow) was dissolved in DMSO and administered by intraperitonealy injection 10 min prior to sacrificing the mice.

3. Results

3.1. The effect of sequential treatment of nicotine and cocaine

We focused our experiments on the cortical-lateral amygdala pathway (Fig. 1A). We first examined the effect of nicotine alone and cocaine alone. Consistent with our previous report, we found that the effect of nicotine on amygdala LTP is stimulation-dependent (Huang et al., 2008). Chronic exposure to nicotine (10 μl/mg, oral administration for 7 days) did not facilitate LTP induced by a single train of tetanus (Nicotine: 106 ± 3%, $n = 6$; Saline: 109 ± 3%, $n = 7$, $P > 0.05$, 1 h after tetanus, Fig. 1B). Similarly, a single injection of cocaine did not lead to facilitation of LTP induced by a single train of tetanus (111 ± 5%, $n = 6$, $P > 0.05$, Fig. 1C). We next examined the effect of the two drugs when given according to a sequential treatment paradigm. We found that in mice treated with nicotine for 7 days followed by a single cocaine injection, LTP was significantly greater than LTP in control mice or in mice treated with cocaine alone (136 ± 5%, $n = 9$, $P < 0.01$, Fig. 1D). A comparison of LTP in mice treated with water, nicotine, cocaine, and nicotine followed by cocaine is shown in Fig. 1E. The facilitation of LTP produced by sequential treatment of nicotine and cocaine was not associated with significant changes of input/output curves (Fig. 1F). These results indicate that nicotine produces a metaplastic effect in the amygdala, so that when cocaine is given after nicotine exposure, it produces a lower threshold for the formation of LTP than when cocaine is given without any prior exposure to nicotine.

In a previous study, we found that different durations of exposure to nicotine produced different types of facilitation of LTP in the amygdala (Huang et al., 2008). We here asked: what is the duration of nicotine exposure that is required for the priming effect on cocaine? Does nicotine prime the amygdala to cocaine when the duration of exposure is shorter than 7 days? We examined LTP in mice pretreated with nicotine for 3 and 1 days followed by a cocaine injection. In mice with 3-day nicotine exposure prior to the cocaine, LTP was increased to 131 ± 7% ($n = 7$), which was significantly different from LTP in control mice (110 ± 2%, $n = 6$, $P < 0.05$, Fig. 2A) and in mice with 1-day nicotine exposure prior to cocaine. LTP similarly was 139 ± 8% ($n = 6$, $P < 0.01$, Fig. 2B). Thus, LTP in mice exposed to nicotine for periods varying from 1 to 7 days followed by cocaine was significantly greater than that in control mice, and there was no statistically significant difference between groups of 1, 3 or 7 days ($P > 0.05$). These results indicate that even short-term exposure to nicotine (1 day) can enhance the effect of cocaine on LTP in the amygdala. Interestingly, the facilitation was unidirectional. Reversing the sequence of nicotine and cocaine (cocaine administration prior to exposure to nicotine) did not produce any facilitation of LTP (116 ± 7%, $P > 0.05$, Fig. 2C). This