Published in final edited form as:

Addict Biol. 2010 July ; 15(3): 277–288. doi:10.1111/j.1369-1600.2010.00222.x.

Effects of fatty acid amide hydrolase inhibition on neuronal responses to nicotine, cocaine and morphine in the nucleus accumbens shell and ventral tegmental area: involvement of PPAR- α nuclear receptors

Antonio Luchicchi¹, Salvatore Lecca¹, Stefano Carta¹, Giuliano Pillolla¹, Anna Lisa Muntoni^{2,3}, Sevil Yasar⁴, Steven R. Goldberg⁵, and Marco Pistis^{1,3,*}

¹B.B.Brodie Department of Neuroscience, University of Cagliari, 09042, Monserrato, Italy

²CNR Neuroscience Institute-Cagliari, University of Cagliari, 09042, Monserrato, Italy

³Center of Excellence for the Neurobiology of Addiction, University of Cagliari, 09042, Monserrato, Italy

⁴Division of Geriatric Medicine and Gerontology, Department of Medicine, John Hopkins University School of Medicine, Baltimore, Maryland 21224

⁵Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch, Intramural Research Program, Department of Health and Human Services, National Institute on Drug Abuse-National Institutes of Health, Baltimore, Maryland 21224

Abstract

The endocannabinoid system regulates neurotransmission in brain regions relevant to neurobiological and behavioral actions of addicting drugs. We recently demonstrated that inhibition by URB597 of fatty acid amide hydrolase (FAAH), the main enzyme which degrades the endogenous cannabinoid N-acylethanolamine (NAE) anandamide and the endogenous non-cannabinoid NAEs oleylethanolamide and palmitoylethanolamide, blocks nicotine-induced excitation of ventral tegmental area (VTA) dopamine (DA) neurons and DA release in the shell of the nucleus accumbens (ShNAc), as well as nicotine-induced drug self-administration, conditioned place preference and relapse in rats. Here, we studied whether effects of FAAH inhibition on nicotine-induced changes in activity of VTA DA neurons were specific for nicotine or extended to two drugs of abuse acting through different mechanisms, cocaine and morphine. We also evaluated whether FAAH inhibition affects nicotine-, cocaine- or morphine-induced actions in the ShNAc. Experiments involved single unit electrophysiological recordings from DA neurons in the VTA and medium spiny neurons in the ShNAc in anaesthetized rats. We found that URB597 blocked effects of nicotine and cocaine in the ShNAc through activation of both surface cannabinoid CB1-receptors and α -type peroxisome proliferator-activated nuclear receptors (PPAR- α). URB597 did not alter the effects of either cocaine or morphine on VTA DA neurons. These results show that the blockade of nicotine-induced excitation of VTA DA neurons, which we previously described, is selective for nicotine and indicate novel mechanisms recruited to regulate the effects of addicting drugs within the ShNAc of the brain reward system.

*Corresponding author: Marco Pistis, M.D., B.B. Brodie Department of Neuroscience, Center of Excellence for the Neurobiology of Addiction, University of Cagliari, Cittadella Universitaria, 09042 Monserrato (CA), Italy, mpistis@unica.it, Phone: +390706754324, Fax: +390706754320.

Keywords

cocaine; dopamine neurons; electrophysiology; nicotine; nucleus accumbens; peroxisome proliferator-activated receptor- α

Introduction

The endocannabinoids are a family of lipid signaling molecules, which play a pivotal role in the modulation of several physiological and pathophysiological conditions within the central nervous system (CNS) and in the periphery. Although there are a number of endogenous compounds with endocannabinoid-like activity, the best characterized are n-arachidonylethanolamide (anandamide) (Devane et al., 1992) and 2-arachidonoyl glycerol (2-AG) (Sugiura et al., 1995). Within the CNS, anandamide and 2-AG are synthesized *on demand* in postsynaptic cell membranes and show affinity for type-1 cannabinoid receptors (CB1), which are mainly located on presynaptic neurons (Kano et al., 2009). Once produced, endocannabinoids inhibit neurotransmitter release and then are moved intracellularly by a putative carrier protein (Hillard and Jarrahian, 2000), where they are then deactivated by two main enzymes, fatty acid amide hydrolase (FAAH), which catabolizes anandamide (Cravatt et al., 1996), and monoacylglycerol lipase (MAG-L), which catabolizes 2-AG (Dinh et al., 2002). Two non-cannabinoid N-acylethanolamines (NAEs), the anorexiant oleoylethanolamide (OEA) and the anti-inflammatory palmitoylethanolamide (PEA), which are structurally similar to anandamide but are endogenous ligands for α -type nuclear peroxisome proliferator-activated receptors (PPAR- α), are also endogenous substrates for FAAH (Cravatt and Lichtman, 2002; Rodriguez de Fonseca et al., 2001). Their centrally-mediated effects have been poorly characterized, although OEA and PEA might be involved in modulation of synaptic signalling as endogenous ligands for an independent endocannabinoid-like system. Evidence is accumulating, which suggests a significant contribution of OEA and PEA and PPAR- α nuclear receptors in effects observed following pharmacological inhibition of FAAH (Mazzola et al., 2009). The endocannabinoid system regulates neurotransmission in brain regions relevant to neurobiological and behavioral actions of addicting drugs or natural rewarding stimuli (Maldonado et al., 2006; Solinas et al., 2008; Solinas et al., 2007). Several lines of evidence indicate that endocannabinoids are released by midbrain dopamine (DA) neurons (Melis et al., 2004; Riegel and Lupica, 2004) to regulate their own afferents. As a consequence, pharmacological manipulation of endocannabinoid signaling fine tunes the effects of different addicting drugs. For example, recent studies have investigated how pharmacological inhibition of FAAH, and the consequent increase in anandamide levels, modulates the effects of nicotine (Forget et al., 2009; Merritt et al., 2008; Scherma et al., 2008). In rats, the FAAH inhibitor cyclohexyl carbamic acid 3'-carbamoyl-3-yl ester (URB597) blocked nicotine-induced conditioned place preference, acquisition of nicotine self-administration behavior, nicotine-induced relapse to drug-seeking behavior and nicotine-induced DA increases in the shell of the nucleus accumbens (ShNAc) (Scherma et al., 2008). We also found that URB597 completely prevents nicotine-induced increases in firing rate and burst firing of ventral tegmental area (VTA) DA neurons of anaesthetized rats (Melis et al., 2008), thus inhibiting one of the primary neuronal responses to nicotine administration in the brain reward system (Maskos et al., 2005).

In this study, we asked whether inhibition of FAAH might prevent not only the effects of nicotine but also the effects of other addicting drugs, such as cocaine and morphine, on VTA DA neurons. In addition, since DA neurons in the VTA directly project to the ShNAc, we also compared the effects of FAAH inhibition on responses to nicotine, cocaine and morphine of GABAergic medium spiny neurons (MSNs) in the ShNAc. Together with the

VTA, the ShNac plays a crucial role in the primary reinforcing properties of addicting drugs and orientates reward-seeking behavior (Carlezon and Thomas, 2009). We found that FAAH inhibition by URB597 specifically modulates neuronal responses to different substances in these two distinct areas through actions on both cannabinoid CB1-receptors and PPAR- α nuclear receptors. This suggests that both endogenous cannabinoid (anandamide) and non-cannabinoid (OEA and PEA) fatty acid ethanolamides, which are all substrates for FAAH, participate in the fine tuning of neurophysiological and behavioral effects of addicting drugs.

Materials and methods

Male Sprague Dawley rats (250–350 g) (Harlan) were used in both the NAc and the VTA experiments. We housed animals in groups of three to six in standard conditions of temperature and humidity under a 12 h light/dark cycle (with lights on at 7:00 A.M.) with food and water available *ad libitum*. We anaesthetized animals with urethane (1300 mg/kg, i.p.), cannulated their femoral vein for intravenous administration of pharmacological agents, and placed in the stereotaxic apparatus (Kopf, Tujunga, CA, USA) with their body temperature maintained at $37\pm 1^\circ\text{C}$ by a heating pad.

We performed the experiments in strict accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2004) and EEC Council Directive of 24 November 1986 (86/609). We made all efforts to minimize pain and suffering and to reduce the number of animals used.

Experiments in the VTA

The scalp was retracted and one burr hole was drilled above the VTA (6.0 mm posterior from bregma, 0.3–0.6 mm lateral from midline) for the placement of a recording electrode. We localized structures according to the stereotaxic atlas of Paxinos and Watson (1997). Single unit activity of neurons located in the VTA (V 7.0–8.0 mm from the cortical surface) was recorded extracellularly with glass micropipettes filled with 2% pontamine sky blue dissolved in 0.5 M sodium acetate (impedance 2–5 M Ω). Single unit activity was filtered (bandpass 500–5000 Hz) and individual spikes were isolated by means of a window discriminator (Neurolog Instruments, Digitimer, UK), displayed on a digital storage oscilloscope (TDS 3012, Tektronics, USA) and digitally recorded. We sampled experiments on line and off line with Spike2 software (Cambridge Electronic Design, Cambridge, UK) by a computer connected to CED 1401 interface (Cambridge Electronic Design, Cambridge, UK). Single units were isolated and identified according to already published criteria (Grace and Bunney, 1983, 1984; Ungless et al., 2004). Since we recorded only one cell per rat, we selected VTA DA neurons when all criteria for identification were fulfilled: firing rate ≤ 10 Hz, duration of action potential ≥ 2.5 ms, inhibitory responses to hindpaw pinching. We defined bursts as the occurrence of two spikes at an interspike interval ≤ 80 ms. Bursts terminated when the interspike interval exceeded 160 ms (Grace and Bunney, 1983). At the end of each recording section, direct current (10 mA for 15 min) was passed through the recording electrode to eject Pontamine sky blue, which allowed the identification of the recorded cells. Brains were removed and fixed in 8% formalin solution. We identified the position of the electrodes microscopically on serial sections (60 μm) stained with cresyl violet.

Experiments in the ShNac

We recorded extracellularly single unit activity of neurons located in the medial part of the NAc (shell) (1.5 mm anterior from bregma, 0.8–1.3 lateral mm from the midline, 6.5–7.0 mm ventral from cortical surface) using the same instruments previously described for the VTA experiments. In addition, since MSNs of the ShNac do not fire spontaneously in

anaesthetized animals, we delivered electrical stimuli in the basolateral amygdala (BLA) to evoke spike firing in the NAc cell. For this reason, we inserted a formvar-coated stimulating stainless steel bipolar electrode with an inclination of 15° anteroposterior on the coronal plane (250 µm tip diameter) in the ipsilateral BLA (3.2 mm posterior from bregma, 5.0 mm lateral from the midline, 7.0 mm ventral from the cortical surface), which is a major excitatory projecting area to the NAc. After the glass electrode had been positioned to the dorsal limit of the NAc, we searched cells that responded to the stimulation of the BLA. Stimuli (~0.5 mA) were delivered to the BLA at 1 s intervals while the microelectrode was lowered incrementally through the NAc. Once we detected a cell, we adjusted the position of the microelectrode in order to maximize the spike amplitude relative to background noise. We identified neurons that responded to BLA stimulation by their robust excitatory response (latency range 10–25 ms). We did not include in this study cells whose latencies were longer than 26 ms following BLA stimulation because they could exhibit a polysynaptic response component (Mulder et al., 1998).

The experimental protocol was essentially that published by Floresco and coll. (2001) with some modifications (Pistis et al., 2002). Once we isolated a cell, we adjusted stimulation currents to approximately half-maximal intensity, such as ~50% of electrical stimuli (1 Hz) in the BLA elicited an action potential in the recorded cell. We calculated evoked spike probability by dividing the number of action potentials observed by the number of stimuli administered in 100 s periods. Once stable levels of evoked spike probability were achieved (<10% changes over 10±15 min), we administered drugs intravenously and assessed spike probability every 100 s. Changes in spike probability were an index of changes induced by the studied compounds over the excitation of NAc cells evoked by BLA stimulation. As well as for the VTA experiments, we recorded only one cell per rat.

Statistical analysis

For VTA experiments, we calculated drug-induced changes in firing rate and pattern by averaging the effects after drug administration (2 min) and normalizing to the predrug baseline.

For ShNAc experiments, we determined pre-drug spike probability baseline as the mean of at least three consecutive assessments (100 s) over 10 min before drug administration. We generated peristimulus time histograms (1 ms bins, 100 cumulative sweeps) by CED Spike2 software (Cambridge Electronic Design, Cambridge, UK). Following drug administration, we calculated spike probability every 100 s and normalized it to the pre-drug baseline.

All the numerical data are given as mean ± SEM. Data were compared and analyzed by using two-way ANOVA for repeated measures (treatment × time), or one-way ANOVA or Student's *t* test for repeated measures, when appropriate. *Post hoc* multiple comparisons were made using the Dunnett's or Bonferroni's tests. We performed statistical analysis by means of the NCSS program. The significance level was established at $p < 0.05$.

Drugs

Nicotine ((-)-nicotine hydrogen tartrate) was purchased from Sigma. Morphine chloridrate and cocaine chloridrate were purchased from S.a.l.a.r.s (Como, Italy) and Akzo Pharmadivision Diosynth (Oss, Netherlands). SCH 23390 was purchased from Sigma/RBI (St. Louis, USA) and L-sulpiride was purchased from Ravizza (Latina, Italy). Rimonabant (SR141716A) was a generous gift of Sanofi-Aventis Recherche (Montpellier, France). URB597 was purchased from Alexis (Lausen, Switzerland). MK886 was purchased from Tocris (Bristol, UK). We diluted nicotine, SCH 23390, L-sulpiride, cocaine and morphine in saline. We adjusted nicotine solution to pH=7 with NaOH. We emulsified rimonabant in 1%

Tween80 and then we diluted in saline and sonicated. We dissolved URB597 in dimethylsulfoxide (DMSO) (100 $\mu\text{g}/\mu\text{l}$) and diluted to the final concentration in saline. The final concentration of DMSO was 0.1%. We emulsified MK886 in 10% of Tween80, dissolved in 20% of DMSO and then diluted to the final concentration in distilled water.

Results

FAAH inhibition does not affect morphine and cocaine effect on VTA DA neurons

We first assessed whether FAAH inhibition modulates responses of VTA DA neurons to cocaine and morphine. We recorded from VTA DA neurons ($n=22$) only when they fulfilled all criteria reported in literature (see methods). A typical waveform of a DA neuron action potential is graphically depicted in Fig. 1A. When we isolated a neuron, we recorded its basal activity for at least 5 minutes before administration of vehicle and, after an additional 4 minutes, we injected morphine or cocaine. The vehicle used for these experiments had no effect on VTA DA neurons. In line with previous studies (Einhorn et al., 1988), cocaine (1.0 mg/kg, i.v.) inhibited firing rate ($61.62 \pm 9.35\%$ of baseline; $F_{(5;30)}=5.996$; $n=6$; $P<0.001$; one-way ANOVA for repeated measures and Dunnett's test) (Fig. 1B,D) and burst firing (-16.42 ± 6.14 of baseline level; $F_{(5;25)}=4.659$; $n=6$; $P<0.01$; one-way ANOVA for repeated measures and Dunnett's test) (Fig. 1E) of VTA DA neurons. On the other hand, as reported in literature (Matthews and German, 1984), morphine (4.0 mg/kg, i.v.) stimulated firing rate (Fig. 2A, C) and burst firing (Fig. 2D) ($139.46 \pm 8.17\%$ of baseline level; $F_{(4;20)}=3.299$; $n=5$; $P<0.05$; one-way ANOVA for repeated measures and Dunnett's test) of VTA DA neurons. In a separate group of rats, we administered the FAAH inhibitor URB597 (0.1 mg/kg, i.v., 1–2 hours before recordings), which persistently ($>6\text{h}$) increases brain levels of anandamide (Kathuria et al., 2003). URB597 pretreatment did not change cocaine effects on firing rate (URB+cocaine: $66.5 \pm 9.98\%$ of baseline level; $F_{(1;60)}=0.0003$; $n=6$, $P>0.05$ vs. vehicle+cocaine) (Fig. 1C, D), and burst firing (URB+cocaine: -18.13 ± 6.68 of baseline level; $F_{(1;60)}=0.15$; $n=6$, $P>0.05$ vs vehicle+cocaine) (Fig. 1E). URB597 pretreatment slightly enhanced the excitatory action of morphine on firing rate but this effect did not reach statistical significance (URB+morphine: 159.63 ± 9.06 of baseline level; $F_{(1;40)}=2.76$; $n=5$, $P=0.13$ vs. vehicle+morphine; two-way ANOVA and Bonferroni's test) (Fig. 2B, C). The effect of morphine on burst firing was similar between controls and URB597 treated animals (URB+morphine: $+15.34 \pm 5.13$ of baseline level; $F_{(1;40)}=0.12$; $n=5$, $P>0.05$ vs. vehicle+morphine; two-way ANOVA and Bonferroni's test) (Fig. 2D).

URB597 had no significant effect on either the frequency or burst firing of DA neurons; the mean baseline frequency of VTA DA neurons recorded was 3.9 ± 1.7 Hz in control animals and 3.8 ± 1.53 Hz in URB597-pretreated animals ($n=11$, $P>0.05$ vs. controls; Student's T test) (Fig. 1F). There was also no significant change in the percent of spikes in bursts after URB597 ($21.5 \pm 3.32\%$ for control rats and $33.9 \pm 10.22\%$ for URB597 pretreated animals, $n=11$, $P>0.05$; Student's T test) (Fig. 1F). Thus, there was no effect of URB597 on cocaine or morphine-induced actions on DA neurons in the VTA, in contrast to our previous findings with nicotine (Melis et al., 2008), demonstrating a selective blockade by FAAH inhibition of nicotine-induced alterations in VTA DA neuron excitability.

FAAH inhibition blocks the effects of nicotine on MSNs of the ShNac

The ShNac plays a pivotal role in the mechanisms underlying the primary reinforcing effects produced by natural stimuli and by drugs of abuse, as well as in reinstatement of drug-seeking behavior in abstinent animals. We next assessed whether FAAH inhibition modulates responses of MSNs in the ShNac to nicotine.

All recorded MSNs ($n=59$) were quiescent, responded to BLA stimulation and were located in the medial part of the ShNAc. BLA stimulation evoked firing in MSNs of the ShNAc with a mean latency of 18.4 ± 0.7 ms (Fig. 3A, B). The average baseline spike probability following BLA stimulation was $46.3 \pm 1.5\%$. We recorded evoked activity of MSNs of the ShNAc for 300 seconds before the administration of nicotine, morphine or cocaine. As previously reported (Hakan et al., 1993), nicotine (0.2 mg/kg, i.v.) depressed the excitability of MSNs in the ShNAc, as measured by their response to BLA stimulation ($64 \pm 12\%$ of baseline level; $F_{(5;40)}=3.44$, $n=6$, $P<0.01$; one-way ANOVA for repeated measures and Dunnett's test) (Fig. 3B, C). This effect required the joint activation of DA receptors by nicotine-induced release of DA, since combined administration of the DA D1 receptor antagonist SCH23390 (1.0 mg/kg, i.v.) and the DA D2 receptor antagonist L-sulpiride (10 mg/kg, i.v.), 4 minutes before nicotine, fully prevented the depression of MSN excitability by nicotine ($122.5 \pm 10.6\%$ of baseline level; $F_{(1;80)}=14.09$; $n=6$; $P<0.001$ vs. control; two-way ANOVA and Bonferroni's test) (Fig. 3D). Neither L-sulpiride nor SCH23390 when administered separately were able to prevent nicotine-induced depression of MSN excitability (SCH23390: $F_{(1;70)}=0.05$, $n=6$, $P>0.05$; L-sulpiride: $F_{(1;70)}=0.02$, $n=6$, $P>0.05$; two-way ANOVA for repeated measures and Dunnett's test) (Fig. 3D).

Pretreatment with URB597 (0.1 mg/kg, i.v., 1–2 hours before recordings) blocked nicotine's depression of MSNs in the ShNAc (Fig. 4A, B) ($126.6 \pm 15.6\%$ of baseline level, $F_{(1;70)}=9.03$, $P<0.01$ vs. control; two-way ANOVA and Bonferroni's test). Consistent with the data obtained in the VTA, URB597 had not significant effect by itself; the mean current administered for spike firing evoked by BLA stimulation in MSNs in the ShNAc was not different between controls and URB597-pretreated animals (1.52 ± 0.16 mA vs. 1.9 ± 0.4 mA, respectively; $n=6$; $P>0.05$; Student's T test) (Fig. 4B, inset). Interestingly, after URB597 treatment, nicotine increased, rather than depressed, firing evoked by BLA stimulation in MSNs ($F_{(8;40)}=3.32$, $n=6$, $P<0.01$; one-way ANOVA for repeated measures and Dunnett's test) (Fig. 4A, B). These results indicate that FAAH inhibition prevents the inhibitory effects of nicotine on MSNs in the ShNAc.

URB597 blocks nicotine's effects in the ShNAc via CB1-receptor- and PPAR- α -dependent mechanisms

Next we explored the mechanism by which URB597 blocks nicotine-induced inhibition of MSNs in the ShNAc. In fact, it is well established that URB597 elevates brain levels of both anandamide, an endogenous CB1-receptor ligand, but also of non-cannabinoid NAEs, such as OEA and PEA, which show no affinity for CB1-receptors but are agonists at peroxisome proliferator-activated nuclear receptors type- α (PPAR- α) (Fegley et al., 2005; Fu et al., 2003). To explore the possible contributions of these two receptor systems to URB597's blockade of nicotine's effects on MSNs in the ShNAc, we treated one group of rats with both URB597 (0.1 mg/kg, i.v.) and the CB1-receptor antagonist rimonabant (SR141716A; 0.5 mg/kg, i.v.) one to two hours before recording and treated another group with URB597 together with the selective PPAR- α antagonist MK886 (Kehrer et al., 2001) (3 mg/kg, i.p., 15 minutes before URB597). Interestingly, both rimonabant and MK886 fully reversed URB597's blockade of nicotine's effects in MSNs of the ShNAc (rimonabant+URB597 vs. URB597: $63.5 \pm 21.8\%$ of baseline level; $F_{(1;63)}=10.3$, $n=5$, $P<0.05$, MK886+URB597 vs. URB597: $56.8 \pm 16\%$ of baseline level; $F_{(1;70)}=5.462$, $n=6$, $P<0.05$, two-way ANOVA and Bonferroni's test) (Fig. 4A; B, C).

FAAH inhibition prevents cocaine's action on MSNs in the ShNAc

We then studied the effects of cocaine and morphine on MSN excitability in the ShNAc, and the consequences of FAAH inhibition by URB597. Cocaine (1.0 mg/kg, i.v.), in agreement with previous studies (White et al., 1993), depressed the excitability of MSNs of the ShNAc

($37.06 \pm 27.7\%$ of baseline level; $F_{(6;48)}=7.28$, $n=7$, $P<0.001$, one-way ANOVA for repeated measures and Dunnett's test) (Fig. 5A, B), as measured by their response to BLA stimulation. When we studied the effect of morphine (4.0 mg/kg, i.v.), but the effects were highly variable (data not shown) and overall did not reach statistical significance, in line with other studies (Hakan and Henriksen, 1987). For this reason, we did not further characterize the effect of URB597 on morphine-induced effects on MSNs. Pretreatment of rats with URB597 (0.1 mg/kg, i.v. 1–2 hs before recordings) prevented cocaine-induced depression of MSNs in the ShNAc ($95.3 \pm 15.1\%$ of baseline level; $F_{(1;77)}=11.97$, $n=6$, $P<0.01$ vs. control, two-way ANOVA and Bonferroni's test) (Fig. 5A, B).

URB597 blocks cocaine's effects in the ShNAc via a PPAR- α dependent mechanism

Finally, we explored the mechanism by which URB597 blocks cocaine-induced inhibition of excitability of MSNs in the ShNAc. When we coadministered URB597 and the CB1-receptor antagonist rimonabant (SR141716A; 0.5 mg/kg, i.v.), we found that URB597's actions were not reversed by CB1-receptor blockade ($98.34 \pm 18.45\%$ of baseline; $F_{(1;70)}=0.04$, $n=6$, $P>0.05$ vs. URB597 pretreated animals, two-way ANOVA and Bonferroni's test) (Fig. 5C), suggesting that CB1-receptors were not involved. However, when we pretreated rats with MK886 (3.0 mg/kg, i.p., 15 minutes before URB597 administration), URB597's blockade of cocaine's inhibition of MSNs was completely prevented and cocaine exerted an inhibitory effect similar to that observed under control conditions ($58.02 \pm 15.59\%$ of baseline level; $F_{(1;70)}=7.028$, $n=6$, $P<0.05$ vs. URB597 pretreated animals, two-way ANOVA and Bonferroni's test) (Fig. 5D). These data suggest that endogenous PPAR- α ligands modulate the effects of cocaine in the ShNAc.

Discussion

In this study, FAAH inhibition by URB597 blocked the acute inhibitory effects of both nicotine and cocaine on firing of MSNs in the ShNAc that was evoked by BLA stimulation in anaesthetized rats. Pharmacological blockade of the target receptors for endogenous lipids that are the primary substrates for FAAH (CB1-receptors and PPAR- α) showed that URB597's blockade of nicotine's inhibition of MSN excitability was due to the combined activation of both surface CB1-receptor and nuclear PPAR- α , while URB597's blockade of cocaine's inhibition of MSN excitability was due only to activation of PPAR- α , and did not involve CB1-receptors.

In the VTA, URB597 did not prevent the decreases in firing rate and burst firing produced by cocaine, or the increases in firing rate and burst firing produced by morphine, in DA neurons of anaesthetized rats. These results extend our previous findings, where FAAH inhibition by URB597 completely abolished nicotine-induced increases in firing rate and burst firing of VTA DA neurons in anaesthetized rats (Melis et al., 2008) and nicotine-induced neurochemical and behavioral effects in rats (Scherma et al., 2008). In that study, URB597 blocked nicotine-induced increases in DA levels in the ShNAc and blocked the development of nicotine self-administration and nicotine-induced conditioned place preferences (Scherma et al., 2008).

The present finding that nicotine-induced depression of MSN excitability requires the combined activation of D1 and D2 DA-receptors suggests that DA release in the ShNAc plays a crucial role in this effect. Moreover, since URB597's blockade of nicotine's inhibition of MSN excitability in the ShNAc was reversed by either the selective CB1-receptor antagonist/inverse agonist rimonabant or by the PPAR- α antagonist MK886, the blockade of nicotine's effects by URB597 appears to involve both surface CB1-receptors and nuclear PPAR- α receptors. Following URB597 administration, we found that nicotine became excitatory on MSNs in the ShNAc. The reason for this reversal of nicotine's effect

is not clear. One possibility is that it might be the result of a combination of factors: (i) the depression of nicotine-induced DA release (Scherma et al., 2008) and (ii) activation of CB1 receptors in the NAc by anandamide and depression of nicotine-induced GABA release. These effects may ultimately unmask the enhancement of glutamate release induced by nicotine (Reid et al., 2000) and the consequent excitation of MSNs.

Although URB597 did not affect the inhibitory actions of cocaine on VTA DA neurons, it completely prevented cocaine-induced inhibition of MSN in the ShNAc. These findings add some complexity to the controversial issue of interactions between the endocannabinoid system and the effects of cocaine, or more generally of psychostimulants (Wiskerke et al., 2008). For example, it has been demonstrated that CB1-receptor knockout mice will self-administrate cocaine (Cossu et al., 2001) and that rimonabant does not modify the development of cocaine-induced conditioned place preference (Martin et al., 2000). Moreover, URB597 does not alter cocaine self-administration by squirrel monkeys under a fixed-ratio schedule (Justinova et al., 2008). In contrast, other studies have shown that rimonabant prevents cocaine-induced increases in DA levels in the NAc (Cheer et al., 2007) and increases the breakpoint for cocaine self-administration under a fixed-ratio schedule in rats with extended access to the drug (Orio et al., 2009). Thus, the present finding that URB597 blocks the inhibitory effects of cocaine on MSN excitability in the ShNAc, although not the inhibitory effects of cocaine on DA neurons in the VTA, was unexpected, particularly since we previously found that URB597 had no effect on cocaine self administration by squirrel monkeys (Justinova et al., 2008). However, besides the difference in experimental subjects (squirrel monkeys versus rats), there was a difference in the protocols of cocaine administration in these two studies. Unlike the self-administration studies in monkeys, where intravenous injections of cocaine were repeatedly self administered during one-hour sessions and the effects of URB597 were examined over three consecutive daily sessions, in the present experiments the electrical activity of neurons was examined in a single brain area following a single acute intravenous injection of cocaine. It is possible that URB597's effect could impair the drug-induced acute responses of a specific neuronal population without significantly affecting behavior induced by chronic administration of the same drug. Thus, this piece of evidence might reveal that, at least for cocaine, FAAH inhibition might prevent the initial acute effects of cocaine administration. In support of this hypothesis, a recent study suggests that FAAH inhibitors do not affect cocaine self-administration but significantly reduced cocaine-induced reinstatement in abstinent animals (Adamczyk et al., 2009).

In the present experiments, URB597 blocked the inhibitory effect of cocaine on MSN excitability in the ShNAc through a non-CB1-receptor-dependent mechanism, since MK886, but not rimonabant, completely reversed URB597's blockade of cocaine's inhibition of MSNs in the ShNAc. This result further supports the conclusion that the effects of FAAH inhibition on the actions of addicting drugs are often due to a combination of different mechanisms, involving both surface CB1-receptors for the endocannabinoid anandamide and PPAR- α nuclear receptors for the non-cannabinoid OEA and PEA. In line with other studies, however, the lack of effect of rimonabant in the present experiments with cocaine, indicates that CB1-receptors are probably not primarily involved in the acute reinforcing effects of psychostimulants (Maldonado et al., 2006; Wiskerke et al., 2008). How PPAR- α modulated acute neuronal responses to cocaine in the present experiments is not known. Among possible explanations, a conservative hypothesis may involve a negative modulation exerted by PPAR- α agonists, such as OEA and PEA, on cholinergic transmission within the ShNAc. In fact, cholinergic interneurons of the NAc were shown to modulate the response of MSNs (de Rover et al., 2002). In that study, the authors hypothesized that this effect occurred through an increase of GABAergic interneuron activity within the ventral striatum. These neurons receive inputs from the cholinergic neurons mediated by nAChRs and their

synapses impinge directly to MSNs. Moreover, other studies have demonstrated an increase in acetylcholine release in the NAc after psychostimulant exposure (Bickerdike and Abercrombie, 1997; Guix et al., 1992; Imperato et al., 1992). In our previous in vitro studies in brain slices, we identified a mechanism by which PPAR- α , activated by endogenous agonists OEA and PEA, specifically modulates nAChRs by inducing their inactivation through phosphorylation (Melis et al., 2008). By analogy, it is likely that PPAR- α activation within the NAc might modulate cocaine's response through inactivation of nAChRs in GABAergic interneurons. This should result in an impairment of GABA transmission to the MSNs that could explain the lack of inhibitory effect of cocaine on MSNs after URB597 pretreatment. Interestingly, interactions between OEA and PEA and acetylcholine transmission might be bidirectional, given that their biosynthesis is increased after stimulation of muscarinic receptors (Stella and Piomelli, 2001), which are present in the terminal regions of GABAergic interneurons (de Rover et al., 2002).

It must be pointed out that, since all drugs were administered systemically, we were unable to determine whether the observed effects of PPAR- α agonists were due to activation of nuclear receptors within the NAc or other brain regions. However, relatively high levels of PPAR- α binding was detected in the rodent striatum (Moreno et al., 2004), coexpressed with tyrosine hydroxylase (Plaza-Zabala et al., 2009), thus a direct action of PPAR- α agonists in this brain region is likely. Endocannabinoids have been involved in the modulation of forms of synaptic plasticity that occur in the NAc or in the VTA early after the administration of addictive substances belonging to different classes (Hoffman et al., 2003; Mato et al., 2004; Pan et al., 2008). It is likely that, beside "classical" endocannabinoids, endocannabinoid-like lipid messengers might also modulate acute effects of addicting substances. Indeed, irrespective of mechanisms involved, pharmacological inhibition of FAAH might represent an opportunity to reveal how homeostatic signals, such as the endocannabinoid anandamide and the non-cannabinoid acetylcannabinoids OEA and PEA, and their respective target receptors, might be recruited to regulate the effects of addicting drugs within brain reward pathways, and might represent a potential new approach to the treatment of drug addiction.

Acknowledgments

This study was supported in part by a grant from Philip Morris USA and Philip Morris International, by the Division of Geriatric Medicine and Gerontology of Johns Hopkins University School of Medicine; and by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services.

MP and SRG were responsible for the study concept and design. AL, SL, SC and GP carried out the experiments and performed data analysis and interpretation of findings. AL and ALM drafted the manuscript. SY, ALM and SRG provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

References

- Adamczyk P, McCreary AC, Przegalinski E, Mierzejewski P, Bienkowski P, Filip M. The effects of fatty acid amide hydrolase inhibitors on maintenance of cocaine and food self-administration and on reinstatement of cocaine-seeking and food-taking behavior in rats. *J Physiol Pharmacol.* 2009; 60:119–125. [PubMed: 19826190]
- Bickerdike MJ, Abercrombie ED. Striatal acetylcholine release correlates with behavioral sensitization in rats withdrawn from chronic amphetamine. *J Pharmacol Exp Ther.* 1997; 282:818–826. [PubMed: 9262346]
- Carlezon WA Jr, Thomas MJ. Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. *Neuropharmacology.* 2009; 56 Suppl 1:122–132. [PubMed: 18675281]

- Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ, Phillips PE, Wightman RM. Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. *J Neurosci.* 2007; 27:791–795. [PubMed: 17251418]
- Cossu G, Ledent C, Fattore L, Imperato A, Bohme GA, Parmentier M, Fratta W. Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behav Brain Res.* 2001; 118:61–65. [PubMed: 11163634]
- Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature.* 1996; 384:83–87. [PubMed: 8900284]
- Cravatt BF, Lichtman AH. The enzymatic inactivation of the fatty acid amide class of signaling lipids. *Chem Phys Lipids.* 2002; 121:135–148. [PubMed: 12505696]
- de Rover M, Lodder JC, Kits KS, Schoffelemeier AN, Brussaard AB. Cholinergic modulation of nucleus accumbens medium spiny neurons. *Eur J Neurosci.* 2002; 16:2279–2290. [PubMed: 12492422]
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science.* 1992; 258:1946–1949. [PubMed: 1470919]
- Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A.* 2002; 99:10819–10824. [PubMed: 12136125]
- Einhorn LC, Johansen PA, White FJ. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J Neurosci.* 1988; 8:100–112. [PubMed: 3339402]
- Fegley D, Gaetani S, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3'-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleylethanolamide deactivation. *J Pharmacol Exp Ther.* 2005; 313:352–358. [PubMed: 15579492]
- Floresco SB, Blaha CD, Yang CR, Phillips AG. Modulation of hippocampal and amygdalar-evoked activity of nucleus accumbens neurons by dopamine: cellular mechanisms of input selection. *J Neurosci.* 2001; 21:2851–2860. [PubMed: 11306637]
- Forget B, Coen KM, Le Foll B. Inhibition of fatty acid amide hydrolase reduces reinstatement of nicotine seeking but not break point for nicotine self-administration--comparison with CB(1) receptor blockade. *Psychopharmacology (Berl).* 2009; 205:613–624. [PubMed: 19484221]
- Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, Rodriguez De Fonseca F, Rosengarth A, Luecke H, Di Giacomo B, Tarzia G, Piomelli D. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. *Nature.* 2003; 425:90–93. [PubMed: 12955147]
- Grace AA, Bunney BS. Intracellular and extracellular electrophysiology of nigral dopaminergic neurons--1. Identification and characterization. *Neuroscience.* 1983; 10:301–315. [PubMed: 6633863]
- Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: burst firing. *J Neurosci.* 1984; 4:2877–2890. [PubMed: 6150071]
- Guix T, Hurd YL, Ungerstedt U. Amphetamine enhances extracellular concentrations of dopamine and acetylcholine in dorsolateral striatum and nucleus accumbens of freely moving rats. *Neurosci Lett.* 1992; 138:137–140. [PubMed: 1407652]
- Hakan RL, Hart C, Eyl C. Specific neurophysiological effects of systemic nicotine on neurons in the nucleus accumbens. *Synapse.* 1993; 15:191–197. [PubMed: 7904087]
- Hakan RL, Henriksen SJ. Systemic opiate administration has heterogeneous effects on activity recorded from nucleus accumbens neurons in vivo. *Neurosci Lett.* 1987; 83:307–312. [PubMed: 3441313]
- Hillard CJ, Jarrahian A. The movement of N-arachidonylethanolamine (anandamide) across cellular membranes. *Chem Phys Lipids.* 2000; 108:123–134. [PubMed: 11106786]
- Hoffman AF, Oz M, Caulder T, Lupica CR. Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. *J Neurosci.* 2003; 23:4815–4820. [PubMed: 12832502]

- Imperato A, Obinu MC, Demontis MV, Gessa GL. Cocaine releases limbic acetylcholine through endogenous dopamine action on D1 receptors. *Eur J Pharmacol.* 1992; 229:265–267. [PubMed: 1362707]
- Justinova Z, Mangieri RA, Bortolato M, Chefer SI, Mukhin AG, Clapper JR, King AR, Redhi GH, Yasar S, Piomelli D, Goldberg SR. Fatty acid amide hydrolase inhibition heightens anandamide signaling without producing reinforcing effects in primates. *Biol Psychiatry.* 2008; 64:930–937. [PubMed: 18814866]
- Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev.* 2009; 89:309–380. [PubMed: 19126760]
- Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med.* 2003; 9:76–81. [PubMed: 12461523]
- Kehrer JP, Biswal SS, La E, Thuillier P, Datta K, Fischer SM, Vanden Heuvel JP. Inhibition of peroxisome-proliferator-activated receptor (PPAR)alpha by MK886. *Biochem J.* 2001; 356:899–906. [PubMed: 11389700]
- Maldonado R, Valverde O, Berrendero F. Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci.* 2006; 29:225–232. [PubMed: 16483675]
- Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, Evrard A, Cazala P, Cormier A, Mameli-Engvall M, Dufour N, Cloez-Tayarani I, Bemelmans AP, Mallet J, Gardier AM, David V, Faure P, Granon S, Changeux JP. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature.* 2005; 436:103–107. [PubMed: 16001069]
- Mato S, Chevalere V, Robbe D, Pazos A, Castillo PE, Manzoni OJ. A single in-vivo exposure to delta 9THC blocks endocannabinoid-mediated synaptic plasticity. *Nat Neurosci.* 2004; 7:585–586. [PubMed: 15146190]
- Matthews RT, German DC. Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. *Neuroscience.* 1984; 11:617–625. [PubMed: 6717805]
- Mazzola C, Medalie J, Scherma M, Panlilio LV, Solinas M, Tanda G, Drago F, Cadet JL, Goldberg SR, Yasar S. Fatty acid amide hydrolase (FAAH) inhibition enhances memory acquisition through activation of PPAR-alpha nuclear receptors. *Learn Mem.* 2009; 16:332–337. [PubMed: 19403796]
- Melis M, Perra S, Muntoni AL, Pillolla G, Lutz B, Marsicano G, Di Marzo V, Gessa GL, Pistis M. Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. *J Neurosci.* 2004; 24:10707–10715. [PubMed: 15564588]
- Melis M, Pillolla G, Luchicchi A, Muntoni AL, Yasar S, Goldberg SR, Pistis M. Endogenous fatty acid ethanolamides suppress nicotine-induced activation of mesolimbic dopamine neurons through nuclear receptors. *J Neurosci.* 2008; 28:13985–13994. [PubMed: 19091987]
- Merritt LL, Martin BR, Walters C, Lichtman AH, Damaj MI. The endogenous cannabinoid system modulates nicotine reward and dependence. *J Pharmacol Exp Ther.* 2008; 326:483–492. [PubMed: 18451315]
- Moreno S, Farioli-Vecchioli S, Ceru MP. Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS. *Neuroscience.* 2004; 123:131–145. [PubMed: 14667448]
- Mulder AB, Hodenprijl MG, Lopes da Silva FH. Electrophysiology of the hippocampal and amygdaloid projections to the nucleus accumbens of the rat: convergence, segregation, and interaction of inputs. *J Neurosci.* 1998; 18:5095–5102. [PubMed: 9634575]
- Orio L, Edwards S, George O, Parsons LH, Koob GF. A role for the endocannabinoid system in the increased motivation for cocaine in extended-access conditions. *J Neurosci.* 2009; 29:4846–4857. [PubMed: 19369553]
- Pan B, Hillard CJ, Liu QS. Endocannabinoid signaling mediates cocaine-induced inhibitory synaptic plasticity in midbrain dopamine neurons. *J Neurosci.* 2008; 28:1385–1397. [PubMed: 18256258]
- Pistis M, Muntoni AL, Pillolla G, Gessa GL. Cannabinoids inhibit excitatory inputs to neurons in the shell of the nucleus accumbens: an in vivo electrophysiological study. *Eur J Neurosci.* 2002; 15:1795–1802. [PubMed: 12081659]
- Plaza-Zabala A, Berrendero F, Suarez J, Bermudez-Silva FJ, Fernandez-Espejo E, Serrano A, Pavon FJ, Parsons LH, De Fonseca FR, Maldonado R, Robledo P. Effects of the endogenous PPAR-alpha

- agonist, oleylethanolamide on MDMA-induced cognitive deficits in mice. *Synapse*. 2009; 64:379–389. [PubMed: 20029832]
- Reid MS, Fox L, Ho LB, Berger SP. Nicotine stimulation of extracellular glutamate levels in the nucleus accumbens: neuropharmacological characterization. *Synapse*. 2000; 35:129–136. [PubMed: 10611638]
- Riegel AC, Lupica CR. Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. *J Neurosci*. 2004; 24:11070–11078. [PubMed: 15590923]
- Rodriguez de Fonseca F, Navarro M, Gomez R, Escuredo L, Nava F, Fu J, Murillo-Rodriguez E, Giuffrida A, LoVerme J, Gaetani S, Kathuria S, Gall C, Piomelli D. An anorexic lipid mediator regulated by feeding. *Nature*. 2001; 414:209–212. [PubMed: 11700558]
- Scherma M, Panlilio LV, Fadda P, Fattore L, Gamaledin I, Le Foll B, Justinova Z, Mikics E, Haller J, Medalie J, Stroik J, Barnes C, Yasar S, Tanda G, Piomelli D, Fratta W, Goldberg SR. Inhibition of anandamide hydrolysis by cyclohexyl carbamic acid 3'-carbamoyl-3-yl ester (URB597) reverses abuse-related behavioral and neurochemical effects of nicotine in rats. *J Pharmacol Exp Ther*. 2008; 327:482–490. [PubMed: 18725543]
- Solinas M, Goldberg SR, Piomelli D. The endocannabinoid system in brain reward processes. *Br J Pharmacol*. 2008; 154:369–383. [PubMed: 18414385]
- Solinas M, Yasar S, Goldberg SR. Endocannabinoid system involvement in brain reward processes related to drug abuse. *Pharmacol Res*. 2007; 56:393–405. [PubMed: 17936009]
- Stella N, Piomelli D. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur J Pharmacol*. 2001; 425:189–196. [PubMed: 11513837]
- Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun*. 1995; 215:89–97. [PubMed: 7575630]
- Ungless MA, Magill PJ, Bolam JP. Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science*. 2004; 303:2040–2042. [PubMed: 15044807]
- Wiskerke J, Pattij T, Schoffelmeer AN, De Vries TJ. The role of CB1 receptors in psychostimulant addiction. *Addict Biol*. 2008; 13:225–238. [PubMed: 18482432]

Figure 1. Effects of URB597 on the responses of VTA DA neurons to cocaine

A) Average trace, acquired from a digital storage oscilloscope, showing the typical broad, notched waveform of an isolated VTA DA neuron recorded from an anaesthetized rat. **B)** Representative firing rate histogram showing the decrease in firing rate of an individual VTA DA neuron produced by intravenous cocaine (COC, 1 mg/kg injected at arrowheads) in control conditions. The injection of vehicle (VEH) is ineffective. **C)** This rate histogram displays that URB597 pretreatment (0.1 mg/kg, i.v.) does not alter cocaine's depression of firing rate of a VTA DA neuron. **D, E)** Graphs illustrating the time course of cocaine's effects on firing rate and burst firing of VTA DA neurons with and without URB597 pretreatment. Pretreatment with URB597 (0.1 mg/kg, i.v.) does not affect the inhibition of VTA DA neurons induced by cocaine (COC, 1 mg/kg, i.v.; arrow) either in firing rate (**D**) or burst firing (**E**). **F)** These histograms show that the pretreatment with URB597 did not affect baseline firing activity (top) or burst firing (bottom) of recorded VTA DA neurons ($P > 0.05$, Student's T test).

Results are means, with vertical bars representing the SEM of firing rate and burst firing, expressed as a percentage of, or difference from, the baseline (BAS). * $P < 0.01$ vs. baseline, one-way ANOVA and Dunnett's test.

Figure 2. Lack of effect of URB597 on morphine-induced increases in firing rate and burst firing of VTA DA neurons

A) Representative firing rate histogram showing that intravenous injection of morphine (MORPH, 4 mg/kg) enhances firing rate of VTA DA neurons in control conditions. **B)** This exemplificative rate histogram displays that the administration of URB597 (0.1 mg/kg, i.v., 2 hours before the recordings) did not affect morphine-induced enhancement of firing rate in a VTA DA neuron.

C, D) Graphical depiction of the time-course of firing rate (**C**) or burst firing (**D**) of VTA DA neurons following intravenous administration of morphine (MORPH, 4 mg/kg).

Pretreatment with URB597 (0.1 mg/kg, i.v.) did not alter the effects of morphine either on firing rate or burst activity of VTA DA neurons.

Results are means, with vertical bars representing the SEM of firing rate and burst firing, expressed as a percentage of, or difference from, the baseline (BAS). * $P < 0.05$ vs. baseline, one-way ANOVA and Dunnett's test.

Figure 3. Nicotine depresses the excitability of MSNs in the ShNAc

A) Superimposed traces acquired from a digital storage oscilloscope showing a relatively constant latency of the orthodromic responses of a representative MSN after BLA stimulation. The arrowhead indicates the artifacts produced by BLA stimulation, the arrow shows evoked action potentials of a MSN. Once a cell was isolated, the current applied to the BLA was adjusted to obtain ~50% of probability to elicit an action potential after a single pulse stimulation. **B)** Representative peristimulus time-histograms displaying the typical inhibitory response of a MSN in the ShNAc after BLA stimulation and injection of nicotine (0.2 mg/kg, i.v.). **C)** Graph showing the time-course of nicotine-induced inhibition of spike firing of MSNs. **D)** Graphical depiction illustrating that nicotine-induced inhibition was prevented by the combined administration (at arrow), but not by the separate injection, of the dopamine D1 receptor antagonist SCH23390 (SCH, 1 mg/kg, i.v.) and the D2 receptor antagonist l-sulpiride (L-Sulp, 10 mg/kg, i.v.).

Results are means with vertical bars representing the SEM of evoked spike firing, expressed as a percentage of the baseline (BAS).

* $P < 0.05$ vs baseline, one-way ANOVA and Dunnett's test; # $P < 0.05$ vs vehicle+nicotine, two-way ANOVA and Bonferroni's test.

Figure 4. URB597 suppresses nicotine's action on MSNs in the ShNAc

A) Exemplificative peristimulus time-histograms showing that nicotine-induced decrease of MSN excitability is reversed by URB597, whereas the CB1-receptor antagonist rimonabant (SR, 0.5 mg/kg) and the PPAR- α antagonist MK886 (3 mg/kg), administered 15 minutes before URB597, prevented the effects of the FAAH inhibitor and restored nicotine-induced inhibition of MSNs responses to BLA stimulation in the ShNAc.

B) and C) Graphical depiction illustrating that URB597 pretreatment prevented nicotine-induced inhibition of MSNs, and that this inhibition by nicotine was reversed by rimonabant (SR, 0.5 mg/kg, i.v.) (**B**) or MK886 (3 mg/kg, i.p.) (**C**). The histogram in the inset displays that the mean current administered in the BLA to evoke spike firing in MSNs was not different between controls (CTRL) and URB597-pretreated animals.

Results are means with vertical bars representing the SEM of evoked spike firing, expressed as difference percentage of the baseline (BAS).

$P < 0.05$ vs vehicle+nicotine, §§ $P < 0.001$ vs. URB+nicotine, two-way ANOVA and Bonferroni's test.

Figure 5. URB597 suppresses cocaine's action on MSNs of the ShNAc

A) Representative peristimulus time-histograms showing the response of recorded ShNAc MSNs neurons after BLA stimulation. The probability of evoking MSN responses after BLA stimulation decreased after cocaine administration. Pretreatment with URB597 reversed cocaine-induced inhibition of MSNs. The PPAR- α antagonist MK886 blocked URB597's effect and restored cocaine-induced inhibition of MSNs. **B,C,D)** Graphical depictions of the time-course of cocaine's effects on MSN excitability in the ShNAc. Cocaine depresses the excitability of MSNs in a long-lasting manner (**B**). This effect was blocked by URB597, which fully prevented cocaine-induced inhibition (**B**). Pretreatment with the CB1-receptor antagonist rimonabant (SR; 0.5 mg/kg, i.v.) did not alter URB597's blockade of cocaine's actions (**C**), whereas MK886 (3 mg/kg, i.p.) (**D**) completely prevented URB597's blockade of cocaine's actions and restored cocaine-induced inhibition of MSNs.

Results are means with vertical bars representing the SEM of evoked spike firing, expressed as a percentage of the baseline (BAS). * $P < 0.05$ vs. baseline, one-way ANOVA and Dunnett's test; # $P < 0.05$ vs. vehicle + cocaine, § $P < 0.05$ vs URB597+cocaine, two-way ANOVA and Bonferroni's test