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ORIGINAL INVESTIGATION

Cortico-limbic circuitry for conditioned nicotine-seeking behavior in rats involves endocannabinoid signaling

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Abstract
Rationale The endocannabinoid system plays an important role in conditioned drug seeking, but the neuronal mechanisms involved in this behavior are unclear.
Objectives Here, we evaluate the role of endogenous cannabinoids in the cortico-limbic circuitry in cue-induced nicotine-seeking behavior in rats.
Methods Animals were first trained to self-administer nicotine (0.03 mg/kg/injection, IV) under conditions in which responding was reinforced jointly by response-contingent nicotine injections and audiovisual stimuli. During subsequent sessions, nicotine was withdrawn and responding was reinforced by contingent presentation of the stimuli only. One month after nicotine removal, the cannabinoid CB1 receptor antagonist, rimonabant, was injected bilaterally into the shell of the nucleus accumbens (ShNAcc, 0.3, 3, or 30 ng/0.5 μl), the basolateral amygdala (BLA, 30 ng/0.5 μl), or the prelimbic cortex (PLCx, 30 ng/0.5 μl).
Results Rimonabant injected into the ShNAcc dose-dependently reduced nicotine-seeking behavior without modifying spontaneous locomotor activity. Similar results were obtained when the drug (30 ng) was injected into the BLA or the PLCx. The anatomical specificity was confirmed in a control experiment using [^{3}H]rimonabant. Fifteen minutes after drug injection, when the behavioral effects of rimonabant were already achieved, radioactivity was detected at the site of injection and had not diffused to adjacent regions.
Conclusions These findings demonstrate that increased endocannabinoid transmission critically triggers conditioned nicotine-seeking behavior in key cortico-limbic regions.

Keywords Cannabinoid · Nicotine self-administration · Cues · Nucleus accumbens · Basolateral amygdala · Prelimbic cortex

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Introduction

The recent findings that the endocannabinoid system is involved in the rewarding and motivational effects of recreational drugs have suggested that the pharmacological modulation of this system may represent a novel approach in the treatment of addictive behaviors (Cohen et al. 2005a; Le Foll and Goldberg 2005; De Vries and Schoffelmeer 2005). Blockade of cannabinoid CB1 receptors with selective antagonists, such as rimonabant or SR147778, reduces oral ethanol intake in a two-bottle choice paradigm and ethanol self-administration using operant procedure (Arnone et al. 1997; Rinaldi-Carmona et al. 2004; Cippitelli et al. 2005; Gessa et al. 2005; Economidou et al. 2006). Rimonabant also decreases nicotine (Cohen et al. 2002, 2005b; Le Foll and Goldberg 2004; Forget et al. 2005) and opiate (Chaperon et al. 1998; De Vries et al. 2003) self-administration, place preference, and cue-induced drug-seeking behavior in laboratory animals. However, little is known about the mechanisms by which CB1 receptor antagonists reduce the addictive properties of drugs. Several findings have suggested that endogenous cannabinoids interact with the mesolimbic dopaminergic system to modulate the addictive properties of nicotine and ethanol (Cohen et al. 2005a). Chronic nicotine and ethanol

treatment increases endocannabinoid levels in the limbic forebrain (Gonzalez et al. 2002) and chronic ethanol down-regulates CB1 receptor function and density in rodents, suggesting an overstimulation of CB1 receptors by endocannabinoids in these regions (Basavarajappa and Hungund 1999). Pharmacological blockade or genetic deletion of the CB1 receptor prevents dopamine release induced by nicotine or ethanol in the shell of the nucleus accumbens (ShNAcc) and in the bed nucleus of the stria terminalis (Cohen et al. 2002; Hungund et al. 2003). Rimonabant reduces firing rate stimulation by ethanol of ventral tegmental area (VTA) dopamine neurons (Perra et al. 2005). Recent data showing that Δ^9-tetrahydrocannabinol is self-administered when injected directly into the VTA and ShNAcc (Zangen et al. 2006) further confirm that CB1 receptors are present (Herkenham et al. 1991; Mailleux and Vanderhaeghen 1992) and are involved in neurotransmitter modulation in these areas (Lupica and Riegel 2005).

The finding that CB1 receptor antagonists prevent nicotine- and ethanol-induced activation of endocannabinoid and dopamine systems in the mesolimbic circuitry underlines their involvement in the blockade of the positive reinforcing effects of drugs of abuse. However, this effect cannot explain why CB1 receptor antagonists reduce drug-seeking behavior induced by conditioned cues in the absence of the primary reinforcer. In a series of previous experiments (Cohen et al. 2005b), we have shown that conditioned stimuli can maintain responding after extensive testing in the absence of nicotine. Conditioned responding was still observed following 60 testing sessions (i.e., 3 months) without nicotine reinforcement. Several control experiments were performed to confirm that responding was maintained by the cue presentation and did not reflect a habit of lever pressing following training. Removal of the cues following 3 months of nicotine withdrawal produced a progressive decrease of responding. In addition, responding could be reinstated by contingent presentation of the cues after 1 month extinction (i.e., testing with no nicotine and no cues reinforcement). In this paradigm, peripheral administration of rimonabant reduced nicotine-seeking behavior maintained by conditioned cues in rats several weeks after nicotine withdrawal (Cohen et al. 2005b). In the present study, using the same paradigm, we investigated the brain regions involved in the effects of rimonabant on nicotine-seeking behavior. Three brain regions expressing CB1 receptors (Herkenham et al. 1991; Mailleux and Vanderhaeghen 1992) and involved in drug-seeking behavior in animals (Neisewander et al. 2000; Weiss et al. 2000; Schroeder et al. 2001; Ciccocioppo et al. 2001; Phillips et al. 2003b; Miller and Marshall 2005) and in humans (Childress et al. 1999; Brody et al. 2002) were chosen: the ShNAcc, basolateral amygdala (BLA), and prelimbic cortex (PLCx). These structures are strongly interconnected and differentially involved in motivational drug effects (Everitt and Robbins 2005; Kalivas and Volkow 2005). The ShNAcc is important in modulating motivational salience (Robinson and Berridge 1993); the BLA is involved in forming associations between motivationally relevant events and sensory stimuli (Everitt et al. 2003); and the PLCx, as part of the prefrontal cortex, mediates the formation of stimulus-reward associations and contributes to executive decision-making processes (Weissenborn et al. 1997). Control studies assessing the effect of rimonabant on locomotor activity were also performed.

Materials and methods

Animals

Male Sprague–Dawley rats (IFFA CREDO, Les Oncins, France) weighing between 200 and 220 g and housed in groups of four upon arrival, were used. After selection, animals were housed in single cages (20×30×18 cm) in a temperature-controlled facility with a 12-h reversed light/dark cycle (lights off at 7:00 a.m.). During the entire procedure, animals were restricted to a daily ration of 15–20 g of standard laboratory food given at the end of each day and over the weekend. Rats were housed and tested in accordance with current European legislation on animal experimentation.

Drugs

Rimonabant was synthesized by Sanofi–Aventis, and (−) nicotine ditartrate was obtained from Sigma, St. Quentin Fallavier, France. All doses are expressed as free bases. Rimonabant was dissolved in saline and 6% dimethylsulfoxide (DMSO) (Scharlau Chemie S.A., Barcelona, Spain) and infused intracerebrally in a volume of 0.5 μl/site at a rate of 0.25 μl/min. [^{3}H]Rimonabant (TRK 1028) was purchased from Amersham Biosciences (Amersham Biosciences Europe GmbH, Orsay, France). [^{3}H]Rimonabant solution was obtained by dilution of an unlabeled solution of rimonabant (30 ng/0.5 μl in saline and 6% DMSO) with 10% of [^{3}H]rimonabant (100 nCi/0.5 μl in ethanol) and infused intracerebrally in a volume of 0.5 μl/site at a rate of 0.25 μl/min. Nicotine (0.03 mg/kg/infusion) was dissolved in a saline solution adjusted to pH=7 by addition of NaOH (0.05 N) and delivered intravenously in a volume of 0.018 ml in 1 s.

Selection of the rats: locomotor screen

Rats were first selected on their locomotor response to a stimulant dose of nicotine, a procedure that avoids the

surgery and training of rats that would not acquire the self-administration. Locomotor activity was assessed in individual activity cages (39×39×16.5-cm high) equipped with two photocells placed perpendicularly and in the middle of the wall. Rats were placed in the activity cages for a habituation period of 60 min. They were then injected with nicotine [0.6 mg/kg, subcutaneous (SC)] and replaced in the activity cages for 30 min. This procedure allows reaching a low level of basal activity before nicotine administration, thus making the test more suitable to display nicotine-induced hyperactivity (Cohen et al. 2003). Rats that reached 100 photocell interrupts during the habituation period and an increase in locomotor activity of at least 80 photocell interrupts after nicotine injection were selected for nicotine self-administration. This criterion led to the selection of about 60–70% of the animals in the present experiments.

Operant responding training

After being selected for their locomotor response to nicotine, the rats were trained to press the left lever in standard two-lever operant responding test chambers (MED-Associates, Georgia, VT, USA) on a fixed-ratio 1 schedule of 45 mg food pellet reinforcement (Noyes, formula P, Lancaster, NH, USA) in 15-min sessions. There was no light in the experimental boxes except for a red house light. Immediately after the rat was put in the chamber, the fan was turned on. A food pellet reinforced each lever press. Tone/light cues were omitted during food reinforcement.

Surgery: catheterization of the jugular vein

After acquiring the operant behavior for food, animals were anesthetized with a mixture of tiletamine 30 mg/kg and zolazepam 30 mg/kg, intraperitoneal (IP) (Zoletil® Virbac, Carros, France) and implanted with a chronic silastic catheter in the right jugular vein extracted from the skin at the level of the back. Catheter patency was maintained by flushing with heparinized saline (30 U/ml; 100 μl/rat) before and after each self-administration session. An antibiotic was also injected the day of the surgery and then every 3 weeks for three consecutive days (enrofloxacin, Baytril® 5%, Virbac, 10 mg/kg, SC). Animals were given a minimum of 5 days to recover from surgery prior to starting self-administration sessions.

Nicotine self-administration

The beginning of the session was signaled by a 1-s cue light above the active lever; a 1-s tone (70 dB); and the fan, which was turned on automatically. Responding on the left lever (active lever) was reinforced with nicotine (0.03 mg/kg/infusion) delivered in a volume of 0.018 ml in 1 s (MED-Associates pump, model PHM100), while responding on the right lever had no consequence. Infusions were coupled to the onset of a 20-s cue light above the active lever and a 1-s tone. During this 20-s time-out period, lever pressing was counted but not reinforced. The infusion pump was located outside the test chamber so that the sound produced by activation of the pump was masked by the ventilator noise and could not serve as a nicotine-paired stimulus. During the first three sessions of nicotine self-administration training, testing was automatically stopped when rats had obtained 20 nicotine infusions (to avoid nicotine overdose, i.e., convulsions) or when 1 h had elapsed. Thereafter, the session duration was set to 1 h and the number of nicotine infusions was not limited. Nicotine self-administration was considered as acquired when performance stabilized with at least 10 nicotine injections per session (12–15 sessions). About 70–75% of the animals previously selected on their locomotor response to nicotine reached this criterion.

Conditioned responding

Following nicotine self-administration training, rats were given daily (five times per week) 1-h sessions during which responding on the active lever produced the contingent presentation of the 1-s tone and 20-s light cue but did not activate the nicotine infusion pump. During this 20-s period, lever pressing was counted but had no consequences. Animals that responding stabilized with at least 12 active lever presses per session (8–12 sessions) underwent surgery for cannulae implantation. About 90% of the animals reached this criterion.

Surgery: implantation of guide cannulae

After acquiring the conditioned responding, animals were anesthetized with Zoletil® (same dosage as above) and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). The skull was exposed and two holes were drilled. Guide cannulae (15-mm length, 26 G, Cooper, London, UK) consisting of stainless-steel tubings with obturators were implanted bilaterally either into the ShNAcc at the coordinates antero-posterior=1.7 mm, lateral=±0.8 mm, dorso-ventral=−6.7 mm; into the BLA at the coordinates antero-posterior=−2.87 mm, lateral=±5.0 mm, dorso-ventral=−7.4 mm; or into the PLCx at the coordinates antero-posterior=+2.9 mm, lateral=±1.0 mm, dorso-ventral=−2.4 mm, tilt 7° angle, according to the atlas of Paxinos and Watson (1998). Guide cannulae were anchored to the skull with a stainless-steel screw and Paladur® dental cement (Heraeus Kulzer, Hanau, Germany).

Effect of rimonabant on conditioned responding

After surgery recovery, rats were given daily testing for operant responding until the number of presses on the active lever varied less than 20% of the average performance of four preceding days. At the beginning of each session, rats were handled and cannulae and cannulae obturators were verified.

Rimonabant was administered after a total of 15–20 sessions of conditioned responding (equivalent to 1 month of nicotine withdrawal). Three minutes prior to testing, rats received bilaterally in the cerebral region of interest and using stainless-steel microinjection cannulae (30 G Cooper) either rimonabant or vehicle (saline+6% DMSO per 0.5 μl for each site). Doses of rimonabant were 0.3 or 3 ng or 30 ng per 0.5 μl for each site in ShNAcc and 30 ng per 0.5 μl for each site in BLA and PLCx. Cannulae were connected by polyethylene tubings (A-M Systems, Carlsborg, WA, USA) to microsyringes (Exmire, Ito, Fuji, Japan) mounted on a motorized pump (CMA, Solna, Sweden). All groups were injected at a rate of 0.25 μl/min. Cannulae were left in place for one additional minute to avoid reflux of the infused fluid inside the guide cannulae. Rats were only used once.

Effect of rimonabant on locomotor activity

Independent series of rats were housed and selected according to the same procedures as those described above (cf. locomotor screen). Guide cannulae were implanted bilaterally in the region of interest (for the coordinates see above, cf. "Surgery: implantation of guide cannulae" section). Three minutes prior to testing, rats received bilaterally either rimonabant at the dose of 30 ng or a saline solution (DMSO 6%). Cannulae were left in place for one additional minute to avoid reflux of the infused fluid inside the guide cannulae. Rats were then placed in the activity cages for 60 min.

Histological control for behavioral studies

Animals were killed just after the behavioral experiment by a pentobarbital bolus (Virbac, 100 mg/kg, IP). The brains were removed and frozen for histological verification of cannulae implantation. Coronal sections (25 μm) of the brain were cut on a cryostat and stained with cresyl violet. The site of injection was microscopically checked and compared to standard stereotaxic plates (Paxinos and Watson 1998). Rats with wrong location of the cannulae were discarded.

Analysis of radioactivity

A control experiment using naïve animals was performed with [^{3}H]rimonabant. Rats were injected in the ShNAcc, BLA, or PLCx with [^{3}H]rimonabant as described above (cf. "Effect of rimonabant on conditioned responding" section). Animals were killed 15 min after radiotracer injection by decapitation. The NAcc, BLA, Cx, and adjacent regions (likely anatomically corresponding to bed nucleus stria terminalis, endopiriform nucleus and/or claustrum and frontoparietal cortex, respectively) were dissected and removed by punches (4 mm in diameter) on slices of 1-mm thickness at the corresponding levels. Tissue samples were assayed for ^{3}H using Beckman scintillation counter. Results were expressed as percent of measured radioactivity on total injected radioactivity per structure (average value on two to three rats).

Statistical analysis

Effect of rimonabant on nicotine-seeking behavior The number of presses on the active and the inactive levers during a 1-h session was analyzed after square root transformation using one-way ANOVA followed by Dunnett's test (ShNAcc) or Student *t* tests (BLA and PLCx). The number of presses on the active lever during the course of the session was analyzed after square root transformation using two-way ANOVA followed by Dunnett's test.

Effect of rimonabant on locomotor activity The number of photocell interrupts after rimonabant or vehicle injections were compared using one-way ANOVA followed by Dunnett's test.

Results

Figure 1 shows the localization of cannulae within the ShNacc, BLA, and PLCx. The final numbers of individuals in each group were, for ShNacc, $n=7$ for each dose of rimonabant and $n=11$ for vehicle; for BLA, $n=7$ per group; and for PLCx, $n=12$ per group. Control experiment with [^{3}H]rimonabant indicates that 15 min after drug injection, radioactivity detected at one site of injection had not diffused to the other structures or to adjacent structures (Table 1).

Nucleus accumbens shell At the end of the nicotine self-administration phase, the average numbers of presses on the active and inactive levers were 33 and 12, respectively, corresponding to 22 nicotine injections per 1-h session. During the session preceding intracerebral drug injection (i.e., conditioned responding), the average numbers of presses on the active and inactive levers were 33 and 6, respectively. Responses were not different between the various experimental groups (data not shown). Bilateral

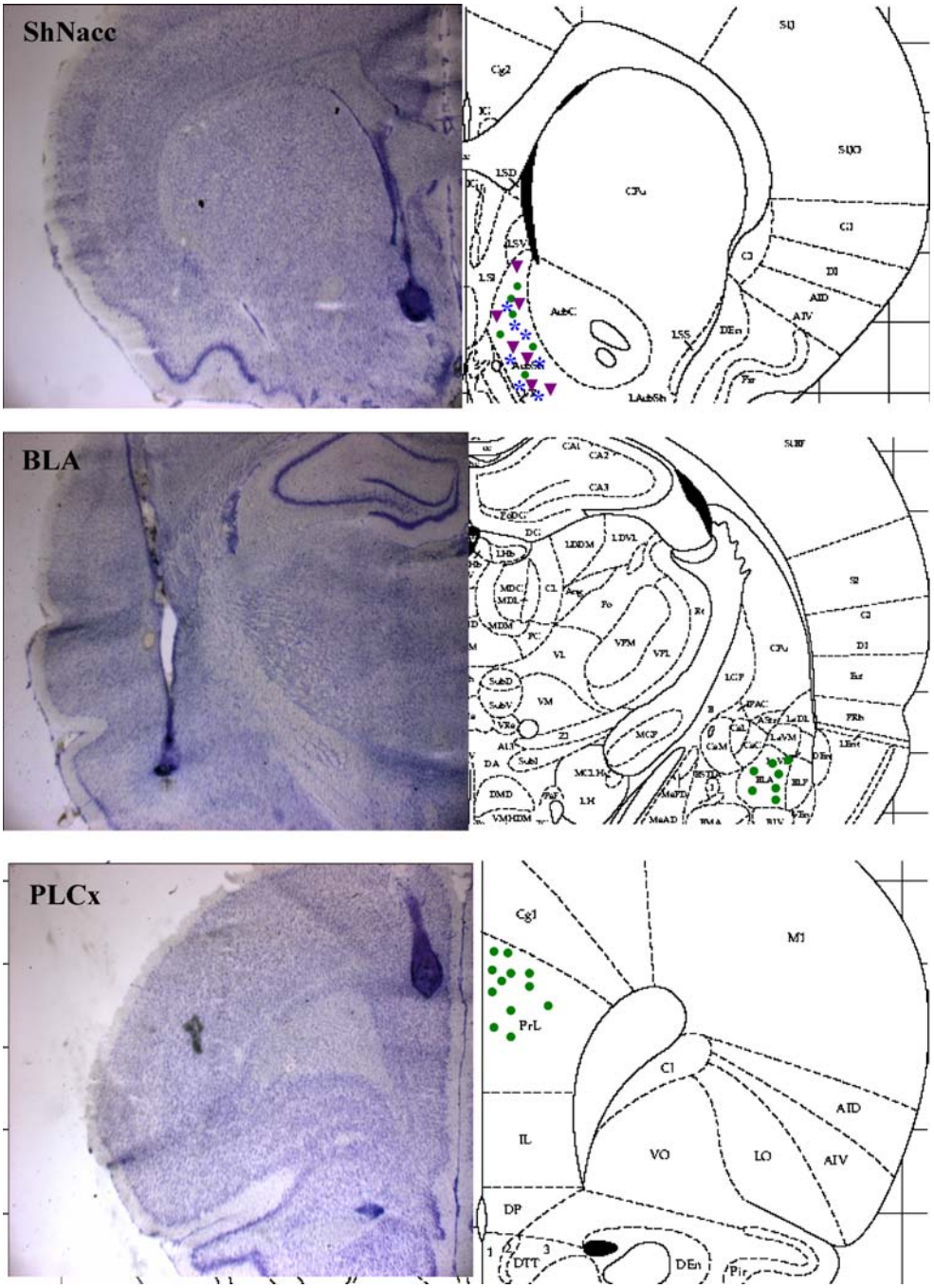

Fig. 1 Verification of cannulae placement on cresyl violet-stained sections and representative photomicrographs of each brain structure of interest: the ShNacc, the BLA, and the PLCx. *Symbols* represent the sites of infusion with the corresponding dose of rimonabant: *asterisks* 0.3 ng, *triangles* 3 ng, *circles* 30 ng

Table 1 Tissue concentrations of ^{3}H

Site of injection of [^{3}H]rimonabant	Tissue radioactivity concentration (% total radioactivity, mean for 2–3 rats)					
	Cx	Adjacent Cx	Nacc	Adjacent NAcc	BLA	Adjacent BLA
Cx	5%	ND	ND	ND	ND	ND
NAcc	ND	ND	8%	ND	ND	ND
BLA	ND	ND	ND	ND	4%	ND

ND not detectable

injections of vehicle (saline + 6% DMSO) did not modify the number of active (36±3 and 34±4 during the preceding control and vehicle days, respectively) or inactive presses (6±2 and 5±2 during the preceding control and vehicle days, respectively). Bilateral injections of rimonabant 3 and 30 ng decreased the number of active lever presses during the 1-h session [$F(3,28)=11.26$, $p<0.01$]. Rimonabant had no significant effects on the number of presses on the inactive lever. The time course of responding was analyzed using two-way ANOVA. There was a significant treatment [$F(3,28)=13.15$, $p<0.01$] and time [$F(3,84)=11.11$, $p<0.01$] effect. As shown in Fig. 2a, 30 ng rimonabant decreased the number of active lever presses from the first 15 min of the session whereas the onset of action of rimonabant at 3 ng and 0.3 ng was delayed.

Basolateral amygdala At the end of the nicotine self-administration phase, the average numbers of presses on the active and inactive levers were 33 and 12, respectively, corresponding to 22 nicotine injections per 1-h session. During the session preceding intracerebral drug injection (i.e., conditioned responding), the average numbers of presses on the active and inactive levers were 30 and 7, respectively. Responses were not different between the various experimental groups (data not shown). Bilateral injections of vehicle (saline + 6% DMSO) did not modify the number of active (30±4 and 34±3 during the preceding control and vehicle day, respectively) or inactive presses (8±2 and 7±2 during the preceding control and vehicle day, respectively). Bilateral injections of rimonabant 30 ng decreased the number of active lever presses during the 1-h session ($t=6.69$, $p<0.01$) and had no significant effects on the number of presses on the inactive lever. Two-way ANOVA indicated significant treatment [$F(1,12)=42.55$, $p<0.01$], time [$F(3,36)=18.86$, $p<0.01$], and treatment × time interaction [$F(3,36)=7.85$, $p<0.01$] effects. As shown in Fig. 2b, rimonabant at 30 ng into the BLA decreased the number of responses on the active lever from the first 15-min period.

Prelimbic cortex At the end of the nicotine self-administration phase, the average numbers of presses on the active and inactive levers were 43 and 11, respectively, corresponding to 28 nicotine injections per 1-h session. During the session preceding intracerebral drug injection (i.e., conditioned responding), the average numbers of presses on the active and inactive levers were 31 and 9, respectively. Responses were not different between the various experimental groups (data not shown). Basal responses during the session preceding drug injection were not different between the experimental groups (data not shown). Bilateral injections of vehicle (saline + 6% DMSO) did not modify the number of active (31±3 and 32±4 during the preceding control and vehicle day, respectively) or inactive presses (11±3 and 9±2 during the preceding control and vehicle day, respectively). Bilateral injections of rimonabant 30 ng decreased the number of active lever presses during the 1-h session ($t=3.14$, $p<0.01$) and had no

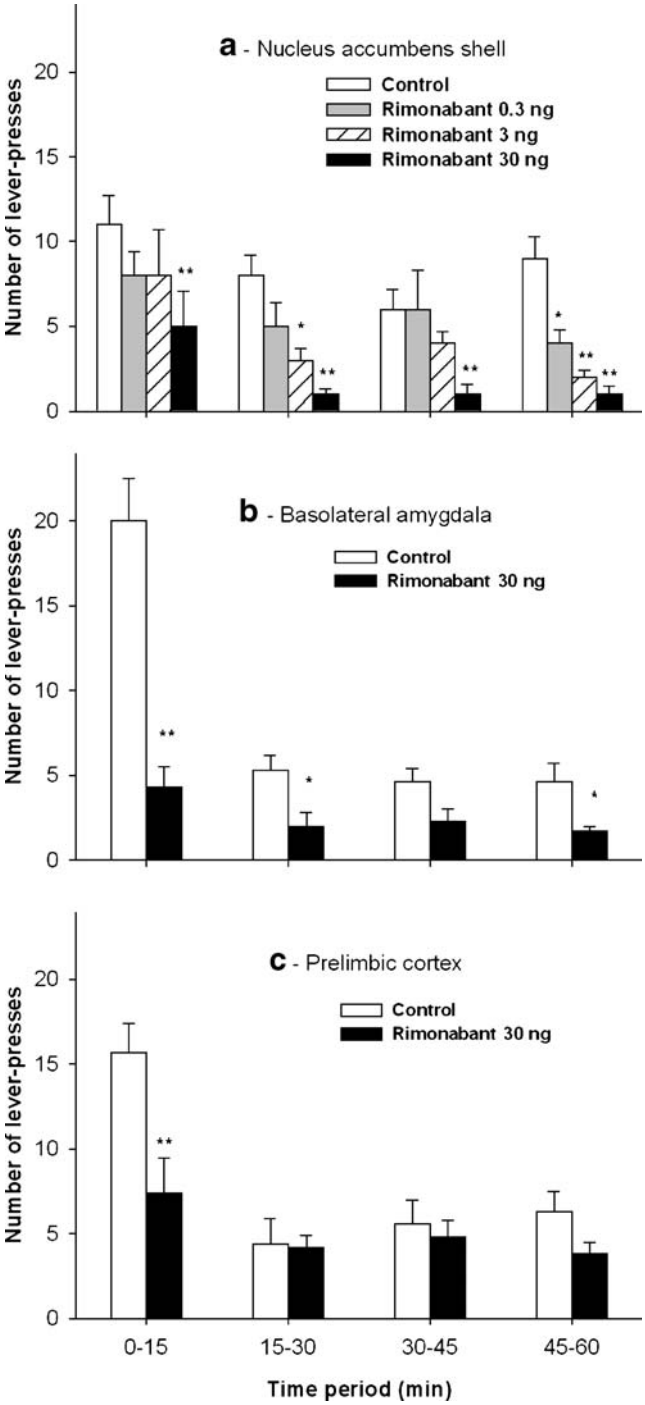

Fig. 2 Effects of rimonabant injected bilaterally into the ShNAcc (**a**), BLA (**b**), and PLCx (**c**) on the number of presses on the active lever (mean + SEM). The volume of injection was 0.5 μl/site. $n=7$–12 rats per group. *Single asterisks* $p<0.05$, *double asterisks* $p<0.01$ vs vehicle

significant effects on the number of presses on the inactive lever. Two-way ANOVA indicated significant treatment [$F(1,23)=4.42$, $p<0.05$], time [$F(3,69)=11.00$, $p<0.01$], and treatment × time interaction [$F(3,69)=5.04$, $p<0.01$] effects. As shown in Fig. 2c, rimonabant 30 ng into the PLCx reduced the number of presses on the active lever during the first 15-min session.

As shown in Fig. 3, bilateral injections of rimonabant 30 ng into the BLA, ShNAcc, or PLCx did not modify spontaneous locomotor activity.

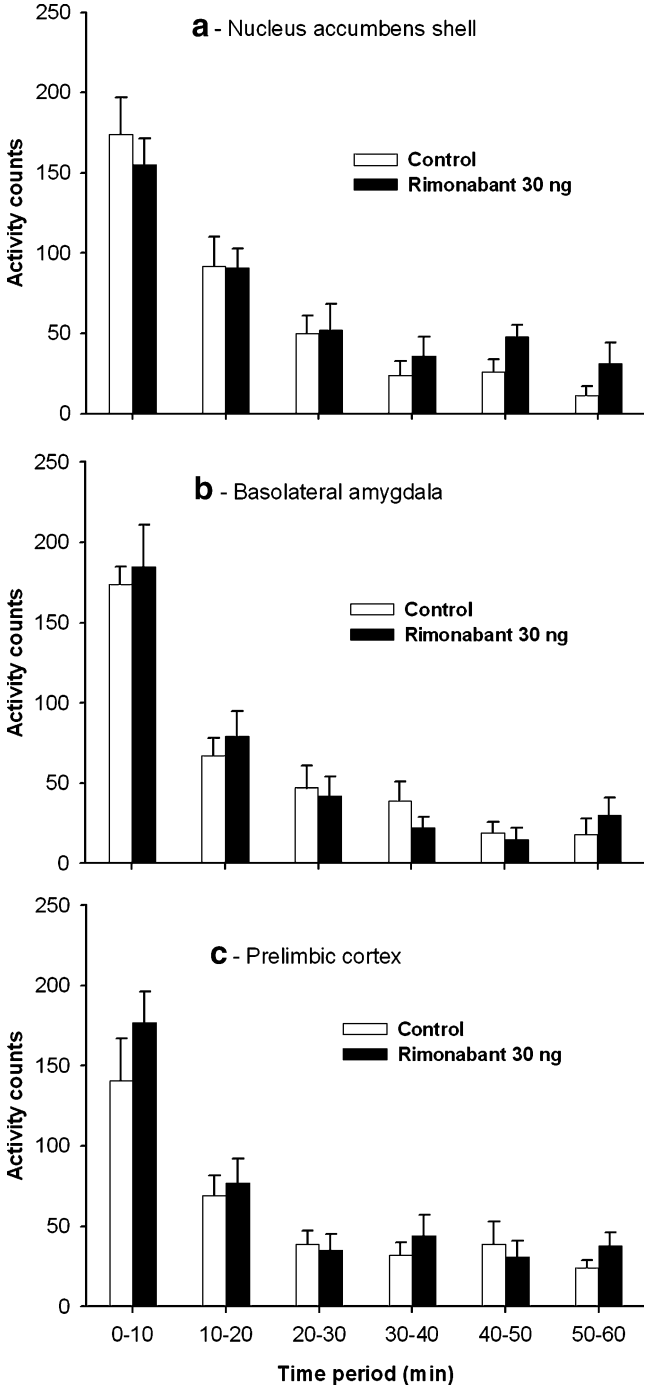

Fig. 3 Effects of rimonabant injected bilaterally into the ShNAcc (**a**), BLA (**b**), and PLCx (**c**) on spontaneous locomotor activity (mean + SEM). The volume of injection was 0.5 μl/site. $n=7–9$ rats per group

Discussion

The present study identifies key cortico-limbic structures, e.g., the ShNAcc, PLCx, and BLA, in mediating the effects of rimonabant on cue-induced nicotine seeking in rats. The effective sites of action appear to be reasonably well delimited, as a control experiment using [^{3}H]rimonabant confirmed that, 15 min after drug injection when the behavioral effects of rimonabant were already achieved, radioactivity was detected at the site of injection and not in the other structures or in adjacent regions.

Bilateral injection of rimonabant into the ShNAcc dose-dependently reduced cue-induced nicotine-seeking behavior without affecting locomotor activity. Previous studies have demonstrated the involvement of the ShNAcc in cue-induced drug seeking behavior under extinction conditions. In particular, exposure to conditioned cocaine cues evoked greater activity of shell neurons than that evoked by neutral stimuli as measured by using single-unit recording (Ghitza et al. 2003) or Fos protein expression as a marker for neuronal activation (Neisewander et al. 2000) in rats. In addition, heroin self-administration produced long-term genomic consequences in the ShNAcc that are profoundly distinct from those caused by the pharmacological effects of passive heroin intake (Jacobs et al. 2002). ShNAcc neuro-adaptations could then subserve the ability of stimuli (drugs, stressors, and drug-associated cues) to elicit relapse to compulsive drug-seeking behavior. That CB1 receptors in the ShNAcc play a role in the motivational effects of drugs fits well with recent findings that the cannabinoid agonist Δ^9-THC is self-administered when injected into the shell but not the core of the NAcc (Zangen et al. 2006). Control experiments with [^{3}H]rimonabant demonstrated that the drug had not diffused to adjacent regions 15 min after injection. Although the volume of administration was small (0.5 μl), we cannot exclude a diffusion of the drug to the core of the Nacc after that time delay. Diffusion to adjacent regions could explain that low doses of rimonabant became effective at the end of the 1-h session. Data on time course of responding should, however, have been interpreted cautiously, as it showed a greater level of variability than the average number of active lever presses during the entire 1-h session. A role for the NAcc core in cue-induced drug seeking behavior has been demonstrated by previous findings that cocaine-associated conditioned stimulus leads to increased dopamine release in the NAcc core and not ShNAcc in rats (Ito et al. 2000; Weiss et al. 2000; Phillips

et al. 2003b). The finding that the involvement of the ShNAcc in drug-seeking behavior is unrelated to the dopamine system in rodents has been recently extended to human drug-addicted subjects when exposed to drug cues (Volkow et al. 2006; Wong et al. 2006). In these studies, cocaine cue presentation in humans produced dopamine release in the dorsal (caudate and putamen) but not in ventral striatum (in which NAcc is located). This contrasts with the described role of dopamine ShNacc in the reinforcing positive effects of drugs (Zangen et al. 2006; Lecca et al. 2006). Previous findings that rimonabant reduced acute nicotine-induced dopamine release in the ShNAcc (Cohen et al. 2002) have suggested that endocannabinoids could modulate dopaminergic systems through a multisynaptic neuronal circuit involving CB1 receptors located in the VTA on inhibitory GABA axon terminals or in the ShNAcc on excitatory glutamate afferents to the long-loop GABA-containing feedback neurons targeting mesolimbic dopamine neurons (Schlicker and Kathmann 2001; Lupica and Riegel 2005; Cohen et al. 2005a). However, because conditioned drug cues do not appear to increase dopamine release in the ShNAcc, an alternative mechanism could be that conditioned cue-induced seeking behavior involved glutamate transmission in the ShNAcc. CB1 receptors have been localized on axonal terminals of the glutamatergic cortical afferents to the NAcc in mice (Robbe et al. 2001). This is consistent with recent data indicating that a presynaptic mGluR2/3 (metabotropic receptor)-dependent mechanism can replace the impaired endocannabinoid system (Mato et al. 2005) and that injection of the mGluR2/3 agonist, LY379268, into the ShNAcc attenuated context-induced reinstatement of heroin seeking (Bossert et al. 2006).

That rimonabant injected into the BLA reduced cue-induced nicotine seeking behavior fits well with recent findings that the BLA plays a critical role in forming associations between specific sensory stimuli and emotional events with motivational valence (Everitt et al. 2003). BLA lesions disrupted cue-elicited cocaine- or heroin-seeking behavior in rats (Parkinson et al. 2001; Kruzich and See 2001; Fuchs and See 2002; McLaughlin and See 2003; Fuchs et al. 2006). On the other hand, BLA electrical stimulation reinstated cocaine-seeking behavior (Hayes et al. 2003). Presentation of conditioned cocaine cues increased BLA expression of Fos protein (Neisewander et al. 2000; Ciccocioppo et al. 2001) and γ protein kinase C, a marker for neuronal plasticity (Thomas and Everitt 2001). Moreover, presentation of conditioned cue tones previously associated with positive stimuli (intracranial self-stimulation) increased neuronal activity in BLA measured by single-unit recording (Muramoto et al. 1993). Recently, it was demonstrated that endocannabinoids, via activation of CB1 receptors, reduced the tonic GABAergic inhibitory control over excitatory glutamatergic neurons in the BLA (Katona et al. 2001; Azad et al. 2004). The blockade of cue-induced nicotine-seeking behavior by intra-BLA injection of rimonabant can thus be attributed to the modulation of glutamatergic projections to the prefrontal cortex and NAcc (Floresco et al. 1998; Phillips et al. 2003a; Miller and Marshall 2005). Although the central nucleus of the amygdala (CeA) has been suggested to be involved in cue-induced drug seeking behavior (Thomas and Everitt 2001; Zarrindast et al. 2003), it is unlikely that the effects of rimonabant, which could have diffused into this nucleus, are mediated by the CeA because no CB1 receptors have been found in this area (Katona et al. 2001).

The present finding that rimonabant injected into the PLCx reduced cue-induced, nicotine-seeking behavior is consistent with previous work showing that exposure to conditioned nicotine, cocaine, or ethanol stimuli increased neuronal activity of prefrontal cortical regions in rats (Ciccocioppo et al. 2001; Schroeder et al. 2001) and humans (Brody et al. 2002; Grusser et al. 2004). Moreover, inactivation of the medial PFCx antagonized cue-elicited reinstatement of lever pressing for cocaine delivery in rats (McLaughlin and See 2003). Differences in frontal cortical activity may be related to individual differences in the vulnerability to drug-taking behavior. Reduced dopaminergic activity in the medial prefrontal cortex has been associated with the facilitated acquisition of psychostimulant self-administration (Piazza et al. 1991; McGregor et al. 1996), and overactive endocannabinoid transmission in PFCx is associated with genetic predisposition to high alcohol preference (Hansson et al. 2006). Rimonabant injected into the prefrontal cortex of alcohol-prefering AA rats suppressed ethanol self-administration (Hansson et al. 2006). Moreover, lesions of the medial prefrontal cortex, and more specifically, the PLCx, produced behavioral disinhibition that may enhance the development of chronic drug taking and alter the response to drug-associated stimuli (Weissenborn et al. 1997; McLaughlin and See 2003). Similarly, dopamine depletion in the medial PFCx altered the ability to withhold responding for a specified period of time to receive food reinforcement (Sokolowski and Salamone 1994). Consistent with a role for PFCx CB1 receptors in behavioral desinhibition, rimonabant has been shown to increase dopamine release in the PFCx (Tzavara et al. 2003) and to increase capacity to wait for a reward (Chaperon and Thiébot 1999).

If the role of rimonabant has been studied separately in the ShNAcc, the BLA, and the PLCx, these regions are strongly interconnected: the BLA and the PFCx, notably the PLCx, have reciprocal glutamatergic projections (Miller and Marshall 2005). The PFCx and the BLA may work in concert to modulate dopaminergic and/or glutamatergic neurotransmissions in the ShNacc (Robbe et al. 2001;

Phillips et al. 2003a). These regions are also interconnected with other structures that are also involved in drug-seeking behavior, in particular the core NAcc and the VTA (Everitt and Robbins 2005). The present demonstration that rimonabant injections into the ShNAcc, BLA, and PLCx are sufficient for preventing cue-induced nicotine seeking behavior in rats further confirms the importance of the endocannabinoid system and its ubiquitous involvement in addictive processes.

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