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$GABA_B$ Receptor Positive Modulation Decreases Selective Molecular and Behavioral Effects of Cocaine

Loic Lhuillier, Ph.D.[†], **Cedric Mombereau, Ph.D.**[†], **John F. Cryan, Ph.D.**[*,#], and **Klemens Kaupmann, Ph.D.**[*]
Neuroscience Research, Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, Switzerland

Abstract

Exposure to cocaine induces selective behavioral and molecular adaptations. In rodents, acute cocaine induces increased locomotor activity whereas prolonged drug exposure results in behavioral locomotor sensitization, which is thought to be a consequence of drug–induced neuroadaptive changes. Recent attention has been given to compounds activating $GABA_B$ receptors as potential anti-addictive therapies. In particular the principle of allosteric positive $GABA_B$ receptor modulators is very promising in this respect, as positive modulators lack the sedative and muscle relaxant properties of full $GABA_B$ receptor agonists such as baclofen. Here we investigated the effects of systemic application of the $GABA_B$ receptor positive modulator GS39783 in animals treated with acute and chronic cocaine administration. Both GS39783 and baclofen dose-dependently attenuated acute cocaine-induced hyperlocomotion. Furthermore, both compounds also efficiently blocked cocaine-induced Fos induction in the striatal complex. In chronic studies GS39783 induced a modest attenuation of cocaine-induced locomotor sensitization. Chronic cocaine induces the accumulation of the transcription factor ΔFosB and up regulates cAMP-response-element-binding-protein (CREB) and dopamine-and-cAMP-regulated-phosphoprotein of 32 kd (DARPP-32). GS39783 blocked the induction/activation of DARPP-32 and CREB in the nucleus accumbens and dorsal striatum and partially inhibited ΔFosB accumulation in the dorsal striatum. In summary our data provide evidence that GS39783 attenuates the acute behavioral effects of cocaine exposure in rodents and in addition prevents the induction of selective long-term adaptive changes in dopaminergic signaling pathways. Further investigation of $GABA_B$ receptor positive modulation as a novel therapeutic strategy for the treatment of cocaine dependence and possibly other drugs of abuse is therefore warranted.

Keywords

addiction; GS39783; abuse; dopamine; delta fosB; CREB

Introduction

In rodents, a behavioral consequence of acute cocaine administration is increased locomotor activity whereas chronic cocaine induces locomotor sensitization, which results in an enduring enhancement of behavioral responses during repeated drug administration (Kalivas and Stewart, 1991). The motor stimulant effects of cocaine are thought to be mediated via an increase in dopaminergic transmission in the mesocorticolimbic system. Cocaine inhibits dopamine (DA), norepinephrine and serotonin reuptake and thereby causes an increase in

***Corresponding authors:** JF Cryan & K Kaupmann, Novartis Institutes for BioMedical Research; Novartis Pharma AG; CH 4002 Basel, Switzerland; please address correspondence to KK (klemens.kaupmann@novartis.com) or JFC (j.cryan@ucc.ie).
[†]LL and CM contributed equally to this work.
[#]Current address: Dept Pharmacology & Therapeutics, School of Pharmacy, University College Cork, Cork, Ireland

synaptic concentrations of these neurotransmitters. In the nucleus accumbens (NAc) elevated DA levels cause a dys-regulation of D1/D2-like DA receptor signaling which in turn leads to the upregulation of several molecular markers (Anderson and Pierce, 2005). Fos is a marker of cell activation and is upregulated in the striatum by acute cocaine (Graybiel et al., 1990). ΔFosB, a truncated form of FosB, slowly accumulates in NAc and dorsal striatum during chronic drug exposure (Hope et al., 1994). Acute and chronic cocaine administration increases the expression of cAMP-response element-binding-protein (CREB) and dopamine-and-cAMP-regulated-phosphoprotein of 32 kD (DARPP-32) in the NAc (Terwilliger et al., 1991; Kano et al., 1995; Nishi et al., 2000, Bibb et al., 2001).

There is accumulating evidence indicating that $GABA_B$ receptor activation could be beneficial in the treatment of cocaine dependence. In rodents the $GABA_B$ receptor agonist baclofen blocks cocaine-induced hyperlocomotion (Kalivas and Stewart, 1991) and cocaine-conditioned hyperlocomotion (Hotsenpiller and Wolf, 2003). Furthermore, baclofen has shown efficacy in human clinical trials in reducing cocaine, opiate and alcohol craving (Addolorato et al., 2002; Brebner et al., 2002; Shoptaw et al., 2003; Cousins et al., 2002). Baclofen's mechanism of action likely involves modulation of dopaminergic neuronal activity in the ventral tegmental area (VTA). Intra VTA application of baclofen attenuates cocaine self administration (Brebner et al., 2000). Furthermore, VTA neurons release DA in the NAc and prefrontal cortex (Kalivas, 1993) and baclofen antagonizes nicotine-, cocaine- and morphine-induced DA release in the NAc (Fadda et al., 2003). Postsynaptic $GABA_B$ receptors reduce cell excitability through activation of potassium channels whereas activation of presynaptic receptors, via Ca^{2+} channel inhibition, reduces the release of various neurotransmitters (Bettler et al., 2004). In the VTA, $GABA_B$ receptors are expressed on dopaminergic cell bodies (Kalivas et al., 1993). Activation of these receptors would be expected to hyperpolarize DA neurons, thereby functionally counteracting the effects of cocaine.

Baclofen however induces unwanted side effects such as muscle relaxation and sedation. Positive $GABA_B$ receptor modulators such as GS39783 are active only in presence of GABA and are devoid of the sedative and muscle relaxant effects of full agonists (Urwyler et al., 2003, Cryan et al., 2004). GS39783 reduces the acute rewarding effects of cocaine self-administration in rodent models (Smith et al., 2004; Slattery et al., 2005). However, the effects of GS39783 on the longterm behavioral and molecular consequences of cocaine exposure are unknown.

The goals of the present study were twofold. The first objective was to assess the efficacy of GS39783 in modulating the locomotor effects of acute and chronic cocaine exposure. Our second objective was to investigate the effects of GS39783 on specific molecular markers of DA signaling affected by cocaine exposure in order to elucidate molecular pathways underlying the anti-addictive properties of $GABA_B$ receptor positive modulation.

Materials and Methods

Animals

Male C57BL/6J mice (18–20g) were obtained from Charles River, France. Housing was at room temperature, in a 12 h light/dark cycle with lights on at 0600. Food pellets and tap water were available ad libitum. All behavioral experiments were conducted during the light cycle. All animals were experimentally naïve unless otherwise noted. Experiments were subject to institutional review and conducted in accordance with the Veterinary Authority of Basel-Stadt, Switzerland.

Acute cocaine-induced hyperactivity

The effects of cocaine, baclofen and GS39783 on locomotor activity were assessed in commercially available test chambers (19 ×31 × 16 cm) (TSE, Bad Homburg, Germany). Activity was recorded using the TSE Moti system which is based on the registration of infrared light beam interruptions along the *x*−, *y*−, and *z*-axes, as caused by an animal's movements (Cryan et al., 2004). Mice were individually placed in test chambers. After 30 min of habituation GS39783 (10, 30, 100 mg/kg p.o.), baclofen (3, 6 mg/kg p.o.) or methylcellulose was applied and the locomotor activity recorded. 30 min later mice were injected with cocaine (10 mg/kg i.p.) or saline and locomotor activity was recorded for additional 60 min. Doses of GS39783 were selected based on previous studies showing activity in anxiety models at this dose range (Mombereau et al., 2004). The doses of baclofen were selected based on them being maximal doses before behavioral inhibition occurs (Cryan et al., 2004; Jacobson and Cryan, 2005). The dose of cocaine was selected as it produced a robust hyperactivity in previous studies (Cryan et al., unpublished).

Chronic cocaine-induced locomotor sensitization

The mice were habituated to the test environment for three days and basal locomotor activity was measured. After an intraperitoneal injection of saline the mice were placed in the test cages (as above) for 30 min and locomotor activity was recorded. From days 4–10, mice were injected with cocaine (20 mg/kg i.p.) or saline and locomotor activity was recorded. To assess the effects of $GABA_B$ receptor positive modulation on the acquisition of behavioral sensitization to cocaine, GS39783 (30 mg/kg p.o.) or vehicle (0.5% methylcellulose) was applied 30 minutes before each cocaine injection. This period of acquisition of sensitization was followed by 14 days without drug treatment. In order to investigate the effect of GS39783 on the expression of cocaine sensitization, we designed a challenge trial (see e.g. Kalivas and Stewart, 1991). On day 23 (challenge day), mice were administrated a dose of 10 mg/kg i.p. cocaine. To assess the effect of $GABA_B$ receptor positive modulator on the expression of sensitization, GS39783 (30 mg/kg p.o.) or methylcellulose was applied 30 min prior to the cocaine injection.

Tissue Preparation

In studies addressing the effects of acute cocaine administration, mice were sacrificed 2 hours after drug application. Chronically treated animals were killed 24 hours after the last cocaine administration. Brains were quickly removed, chilled in ice-cold phosphate-buffered saline (PBS) and cut in 1-mm thick slices using a mouse brain matrix (RBM 2000C, Asi Instruments). NAc and dorsal striatum were dissected on an ice-chilled glass plate and flash-frozen in dry ice.

Immunoblotting

Individual tissue samples for western blot analysis were homogenized with a glass-glass homogenizer in ice-cold buffer containing (in mM) Hepes (pH 7.9) 20, NaCl 400, $MgCl_2$ 5, EDTA 0.5, EGTA 0.1, including complete protease (Roche) and phosphatase inhibitors (Sigma). Homogenates were kept 20 min on ice and centrifuged 15 min at 20000 g, 4°C. Pellets containing particulate fractions enriched in organelles and nuclei were mixed with 2X Laemmli sample buffer (125 mM Tris pH 6.8, 4% (w/v) sodium dodecyl sulfate (SDS), 0.005% (w/v) bromophenol blue, 200 mM dithiothreitol, 20% glycerol), heated to 90°C for 5 min and loaded on 10% SDS acrylamide gels (Bio-Rad). After electrophoretic transfer (Bio-Rad blotting device), nitrocellulose membranes were incubated 1 hour at room temperature in PBS containing 0.1% Tween 20 and 5% fat-free powdered milk (PBST/milk). After three washes in PBST the membranes were incubated overnight at 4°C with primary antibodies in PBST/milk, and washed again three times in PBST. Incubation with horseradish-peroxydase-conjugated secondary antibody was for 1–2 hours at room temperature. Peroxydase activity was detected using Supersignal West Pico substrate (Pierce) and Kodak MR-1 X-ray films (Amersham Biosciences). Afterwards, bound antibodies were removed (restore buffer, Pierce) and the membranes incubated with an anti-actin antibody to check for loading. X-ray films were scanned and analyzed with NIH ImageJ software (http://rsb.info.nih.gov/ij/, v1.31) according to manufacturer's indications. Intensity values are presented as the ratio of the optical density of the band of interest and of its actin control. Individual samples were not pooled and care was taken not to overexpose X-ray films in order to ensure linearity of signals. All experiments were performed in duplicate or triplicate.

Statistical analysis

In behavioral experiments, time courses of motor activity were analyzed using an appropriate two-way or three-way ANOVA with one repeated measure (time) followed by, where appropriate, Fisher's post hoc tests. In molecular studies, significance was assessed by a one-way ANOVA, followed by *post hoc* Newmann-Keuls test.

Reagents

Cocaine was obtained from Sigma (St Louis, MO). L-baclofen and GS39783 (*N*,*N′*-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4, 6-diamine) were synthesized in-house. All drug solutions were made up fresh prior to use. Primary antibodies used were rabbit anti-Fos (sc-253, Santa Cruz Biotechnology, 1:1000), rabbit anti-FosB (sc-48, Santa Cruz Biotechnologies, 1:500), mouse anti-phosphoCREB (clone 1B6, Cell Signaling, 1:1000), rabbit anti-DARPP-32 (2302, Cell Signaling, 1:2000), rabbit anti-CREB (sc-58, Santa Cruz Biotechnology, 1:1000), rabbit anti-actin (A2066, Sigma, 1:5000). Secondary antibodies were HRP-linked goat anti-mouse IgG (1:2000, Bio-Rad) or goat anti-rabbit IgG (7074, Cell Signaling, 1:1000 to 1:5000). All other reagents were from Sigma.

Results

$GABA_B$ receptor activation attenuates cocaine-induced hyperlocomotion

In rodents, a behavioral consequence of acute cocaine administration is increased locomotor activity. We used locomotor activity as readout to assess the behavioral effects of baclofen and GS39783 on cocaine exposure. A single injection of cocaine (10 mg/kg i.p.) resulted in a marked increase in ambulatory activity, compared with administration of saline (Fig. 1). A two-way repeated measures ANOVA revealed a significant effect of cocaine ($F_{1,131} = 57.744$; $p < 0.001$), a significant effect of treatment with the $GABA_B$ receptor agonist baclofen or with the positive modulator GS39783 ($F_{5,131} = 8.807$; $p < 0.001$) and a cocaine x treatment interaction ($F_{5,131} = 6.009$; $p < 0.001$). *Post hoc* analysis revealed that baclofen at 6 mg/kg lowered spontaneous locomotor activity at 0 to 10 minutes timepoint in animals injected with saline ($p < 0.05$; Fig. 1A). Unexpectedly, animals that had been administered baclofen (3 mg/kg) 30 minutes prior to saline injection had a brief, exaggerated increase in locomotor activity immediately after saline injection which normalized 10 minutes later (Fig. 1A). Inspection of the locomotor response in individual animals revealed that only three out of twelve animals showed this increased locomotor activity after application of 3 mg/kg baclofen. We did not observe this phenomenon after treatment with higher doses of baclofen and thus the physiological relevance of this observation remains unclear. In contrast to baclofen, GS39783 (at 10, 30, 100 mg/kg) did not affect spontaneous locomotor activity (Fig. 1C).

The effects of baclofen and GS39783 on cocaine-induced locomotor activity are shown in Fig. 1B and 1D. *Post hoc* analysis revealed that both GS39783 and baclofen blunted the stimulatory effect of cocaine. Although baclofen significantly attenuated cocaine-induced hyperactivity at all doses investigated (Fig. 1B), interpretation of the effect obtained with the higher dose (6 mg/kg) is confounded by baclofen's sedative properties as evidenced by reduced basal activity at this dose (Fig. 1A, B). The $GABA_B$ receptor positive modulator GS39783 significantly attenuated hyperlocomotion between 10 and 60 minutes after cocaine administration (Fig. 1D). However, in contrast to baclofen, GS39783 did not affect basal locomotor activity. No signs of stereotypic behavior after baclofen or GS39783 application were observed. Taken together, these data suggested that activation of $GABA_B$ receptors with the agonist baclofen or with the positive modulator GS39783 can attenuate the locomotor-stimulation induced by a single administration of cocaine. Further, we confirmed previous observations showing that GS39783 is devoid of sedative properties of the $GABA_B$ receptor agonist baclofen (Cryan et al., 2004).

Striatal Fos upregulation by acute cocaine is inhibited by $GABA_B$ receptor activation

To date, in the context of drug addiction only few studies have focused on the investigation of the molecular mechanisms affected by potential therapeutic strategies, including those focused on $GABA_B$ receptors. One of the most robust responses to acute cocaine is the activation of immediate early gene expression, most notably Fos (Curran et al., 1996; Graybiel et al., 1990). To investigate a possible effect of $GABA_B$ receptor activation on cocaine-induced Fos upregulation we conducted an experiment with a separate group of animals. The mice were treated with baclofen, GS39783 or saline 30 min prior to cocaine or saline injection. Fos expression was detected by immunoblots on dorsal striatum and NAc samples and normalized to actin controls (Fig. 2). Treatment with cocaine triggered a robust upregulation of Fos expression in both dorsal striatum and NAc ($p < 0.001$ versus saline; Fig. 2C-F). Baclofen dose-dependently attenuated cocaine-induced Fos expression in both structures ($p < 0.001$, 6 mg/kg baclofen) but did not affect basal Fos levels ($p > 0.1$; Fig. 2C, D). Similarly to baclofen, GS39783 dose-dependently inhibited Fos induction in both dorsal striatum and NAc ($p < 0.001$; 30, 100 mg/kg GS39783; Fig. 2E, F) without affecting basal Fos expression at any dose used ($p > 0.1$). Taken together these data show that $GABA_B$ receptor activation by baclofen and GS39783 attenuated acute cocaine-induced Fos expression.

The effects of the $GABA_B$ receptor positive modulator GS39783 on the acquisition of cocaine sensitization

Chronic cocaine induces locomotor sensitization, which results in an enduring enhancement of behavioral responses during repeated drug administration (Kalivas, 2003). In behavioral sensitization studies at least two different phases are recognized, acquisition and expression (Pierce and Kalivas, 1997). Briefly, acquisition is the phase in which behavioral and physiological changes develop due to repeated, intermittent exposure to psychostimulants. The expression phase defines the long-term behavioral changes that are the result of drug-induced neuroadaptations.

We measured locomotor activity of mice immediately after daily cocaine injection for 30 min during the 7 days of acquisition phase (Fig. 3). Two-way repeated measures ANOVA demonstrated an effect of time ($F_{6,408} = 23.966$; $p < 0.001$) and interaction of cocaine x time ($F_{6,408} = 19.626$; $p < 0.001$) suggesting that behavioral sensitization to cocaine occurred. There was a significant effect of GS39783 ($F_{1,68} = 8.632$; $p = 0.005$), of cocaine ($F_{1,68} = 378.082$; $p < 0.001$) and a significant interaction GS39783 x cocaine ($F_{1,68} = 4.446$; $p = 0.039$). As in the experiments shown in Fig. 1D, GS39783 attenuated the hyperlocomotion induced by a single administration of cocaine (Fig. 3, day 4; $p < 0.01$). In addition, *post hoc* analysis revealed that mice treated daily with cocaine and GS39783 exhibited less hyperactivity than mice treated with cocaine alone (days 7 to 10; Fig. 3). We observed persistent significant effects only after three days of GS39783 treatment thus these data may be interpreted as a delayed response after drug application. However, cocaine-induced sensitization increases with time and the marked increase in locomotor activity between treatment days 6 and 7 could be the reason why the effects of GS39783 are more pronounced and significant only at this protracted stage. Repeated treatments of GS39783 did not affect locomotor activity compared with the vehicle-treated group, confirming the absence of sedative properties of GS39783. It is noteworthy that GS39783 only modestly attenuated sensitization as mice treated with chronic cocaine in combination with GS39783 still appeared sensitized compared to saline treated controls. Further, it is difficult to disentangle GS39783 effects on sensitization from its ability to suppress cocaine-induced hyperactivity acutely.

In order to further examine the effect of GS39783 on locomotor sensitization we designed a cocaine challenge trial, 14 days after the last cocaine injection (Kalivas and Stewart, 1991). During this challenge, all mice received 10 mg/kg of cocaine (Fig. 4). Three-way ANOVA revealed significant effects of repeated cocaine treatment between days 4–10 ($F_{1,63} = 113.408$; $p < 0.001$). These data confirmed the presence of behavioral cocaine sensitization. There was an effect of GS39783 when administered during the acquisition period ($F_{1,63} = 4.708$; $p = 0.034$) and prior to the challenge ($F_{1,63} = 6.795$; $p = 0.005$), and an interaction between repeated cocaine treatment and GS39783 administrated during the acquisition phase ($F_{1,63} = 4.022$; $p = 0.049$). As expected, mice treated repeatedly with cocaine exhibited an enhancement of total distance traveled compared to mice treated only with the challenging dose of cocaine (Fig. 4, groups 3 versus 1; $p < 0.001$). Mice treated concomitantly with GS39783 and cocaine during the acquisition phase exhibited significantly less locomotor activity compared to mice treated only with cocaine (Fig. 4, groups 7 versus 3; $p < 0.05$), suggesting once again that GS39783 moderately attenuates the acquisition of behavioral sensitization to cocaine. Cocaine sensitized mice pretreated with GS39783 before the cocaine challenge did not differ significantly in their locomotor response compared with mice receiving only the challenging dose of cocaine (Fig. 4, groups 4 versus 3) suggesting that GS39783 does not affect the expression of sensitization. However, we note that in this experiment a single application of GS39783 failed to significantly reduce hyperlocomotion in control group 1 (versus group 2). Therefore our data do not allow for definite conclusions on the potential effects of GS39783 on the expression of cocaine sensitization. Dual administration of GS39783 during the acquisition and prior to the challenge reduced cocaine-induced increases in locomotor activity (Fig. 4, groups 8 versus 3; $p < 0.01$). Again it is important to reinforce that it is difficult to discriminate acute effects of GS39783 on cocaine induced hyperactivity from that on the acquisition of sensitization. Further, as in the experiments described under Fig. 3, we observed that animals treated with chronic cocaine in combination with GS39783 were still sensitized at the cocaine challenge as shown by increased locomotor activity compared to saline controls. In summary, GS39783 application had only a modest effect on the acquisition of sensitization.

GS39783 blunts chronic cocaine-associated ΔFosB upregulation in dorsal striatum

In the mesolimbic circuit, chronic cocaine administration triggers the accumulation of ΔFosB, which is thought to play a pivotal role in long-lasting effects of a variety of drugs of abuse including cocaine. Overexpression of ΔFosB increases the sensitivity to cocaine and the motivational aspects of reward (Kelz et al., 1999; Colby et al., 2003). We studied ΔFosB expression after 7-days of daily treatment with saline/cocaine or GS39783/cocaine (Fig. 5). The animals were sacrificed 24 hours after the last administration. A separate group of animals from those used in behavioral studies were used. ΔFosB expression was measured using semi-quantitative Western blot analysis employing selective antibodies (Zhang et al, 2002; Muller and Unterwald, 2005). Cocaine stimulated a robust increase in ΔFosB expression in dorsal striatum (Fig. 4A; $p < 0.001$ versus saline) that was partially blocked by GS39783 ($p < 0.05$). In NAc, ΔFosB levels were also upregulated by chronic cocaine (Fig. 5B; $p < 0.001$) however, in this brain region GS39783 failed to modulate ΔFosB induction by cocaine ($p = 0.92$). Basal levels of ΔFosB expression were not affected in either structure ($p > 0.1$). During protracted withdrawal ΔFosB levels decrease to basal levels, i.e. 10–12 days after cessation of chronic cocaine treatment (Hope et al., 1994; Perrotti et al., 2005). In line with these observations we did not detect ΔFosB 14 days after cessation of cocaine repeated administration (Fig. 5C).

Chronic treatment with GS39783 before withdrawal had no effect on ΔFosB levels. In summary, our data therefore suggest that the mode of action of GS39783 has a modest impact on cocaine-modulated ΔFosB expression in the dorsal striatum but not in NAc.

GS39783 blocks chronic cocaine-induced upregulation and activation of DARPP-32 and CREB

Previous studies have shown that chronic cocaine induces a strong activation of DARPP-32 and CREB through increased DA signaling (Kano et al, 1995; Bibb et al, 2001). We therefore examined whether such changes inDARPP-32 and CREB are modulated by GS39783 (Fig. 6, Fig. 7). An experimental setup identical to the ΔFosB studies as described above was used. In the dorsal striatum a small, non-significant reduction of DARPP-32 expression was observed after treatment with cocaine or GS39783 ($F_{3,50} = 2.44$; $p = 0.07$; Fig. 6). In NAc however, chronic cocaine stimulated DARPP-32 expression. In agreement with previous studies (Lin et al., 2002; Hu et al., 2005) chronic cocaine administration increased DARPP-32 expression in NAc ($p < 0.001$), but not in dorsal striatum. DARPP-32 upregulation was not observed when GS39783 was applied 30 minutes prior to each daily cocaine administration ($p < 0.001$). GS39783 did not affect basal DARPP-32 levels ($p > 0.1$; Fig. 6B).

CREB is activated by phosphorylation and this active form is shuttled to the nucleus where it drives expression of its target genes (Shaywitz and Greenberg, 1999). In addition to total CREB we therefore investigated the levels of phosphorylated CREB (pCREB), and determined the pCREB/CREB ratios. In dorsal striatum, cocaine and GS39783 had a minor effect and the ratio of pCREB/CREB remained unmodified (Fig. 7A; $F_{3,81} = 1.75$; $p = 0.16$). In the NAc chronic cocaine increased CREB activation as evidenced from the pCREB/CREB ratio, confirming previous studies (Fig. 7B; $p < 0.01$, Terwilliger et al., 1991; Kano et al., 1995). Calculation of the pCREB/CREB ratios demonstrated that GS39783 effectively inhibited chronic cocaine-induced CREB stimulation (Fig. 7B; $p < 0.01$).

Discussion

Although molecular adaptations to chronic cocaine have been hypothesized to play a major role in the manifestation of cocaine dependence (Nestler and Aghajanian, 1997) very few studies to date have investigated the ability of potential therapeutic agents to modulate such responses. In the present study we have demonstrated that systemic application of the $GABA_B$ receptor positive allosteric modulator GS39783 attenuates cocaine-induced locomotor activity and is also effective in preventing the induction of several molecular markers of acute or chronic cocaine exposure.

Both the $GABA_B$ receptor agonist baclofen and the positive modulator GS39783 attenuated the hyperlocomotion induced by a single administration of cocaine. Further, we have shown that GS39783 attenuated, albeit modestly the acquisition of cocaine sensitization. GS39783 induced similar effects as a non-sedative dose of baclofen (3 mg/kg) in attenuating acute cocaine-induced hyperlocomotion (Fig. 1), which is relevant as clinical studies suggest efficacy of baclofen in cocaine dependence (Shoptaw et al., 2003). The observations that GS39783, in contrast to baclofen, does not alter baseline locomotor activity is in agreement with the lack of effects in other behavioral tests in both rats and mice which are sensitive to baclofen administration; these include the rotarod motor co-ordination task, cognitive tasks and hypothermia measurements (Cryan et al., 2004;Jacobson and Cryan, 2005). Of note, the effects of $GABA_B$ receptor ligands on cocaine-induced hyperactivity appear not as robust as that reported with D1 selective DA receptor antagonists such as SCH23390, which almost completely block the locomotor stimulant effects of cocaine (O'Neill et al., 1999;Adams et al., 2001). However, a thorough evaluation of $GABA_B$ receptor positive modulators in comparison to other drugs attenuating locomotor stimulant effects of cocaine requires side-by-side experiments and a careful investigation of their side effect profile which may influence locomotor-based behavioral readouts. It is evident that the locomotor effects of stimulant drugs and their reinforcing effects are not homologous. Therefore further investigation of $GABA_B$ receptor modulators in animal models of reward and addiction are necessary.

Locomotor activity is critically dependent on activation of dopaminergic neurotransmission (Kuczenski, 1983). A pivotal role of VTA DA neurons in mediating the hyperlocomotor effects of DA has been described (Kalivas and Stewart, 1991). VTA DA neurons project to numerous limbic loci including the NAc and the prefrontal cortex. Activation of $GABA_B$ receptors on the cell bodies of VTA DA neurons reduce their excitability which in turn leads to a reduction in DA release in the NAc (Olpe et al., 1977; Lacey, 1993; Westerink et al, 1996; Wirtshafter and Sheppard, 2001). In addition, the activity of VTA DA neurons is modulated by excitatory (glutamatergic) and inhibitory (GABAergic) afferents. Activation of $GABA_B$ receptors on glutamatergic afferents would reduce excitatory inputs into the VTA (Johnson and North, 1992; Wu et al., 1999; Hotsenpiller and Wolf, 2003). In support of a key role of $GABA_B$ receptors in the VTA in blocking cocaine-induced hyperlocomotion baclofen pretreatment dose dependently reduced nicotine-, morphine, and cocaine-evoked DA release in the NAc (Fadda et al. 2003). Furthermore, baclofen injection into the VTA blocked an increase in firing of NAc neurons induced by reward-predictive cues (Yun et al., 2004). $GABA_B$ receptor positive modulators such as GS39783 are devoid of intrinsic agonistic activity (Urwyler et al., 2003; Cryan et al., 2004). Their action therefore is dependent on the presence of GABA (i.e. synaptically released GABA). Several lines of evidence suggest that VTA DA neurons are under tonic inhibitory control by $GABA_B$ receptors. Intra-VTA application of the selective $GABA_B$ receptor antagonist CGP55845A increased extracellular DA levels (Giorgetti et al., 2002). Furthermore, systemic application of the $GABA_B$ receptor antagonist CGP35348 increased firing of VTA DA neurons (Erhardt et al., 2002) whereas the $GABA_B$ receptor positive modulator CGP7930 decreased the firing frequency of VTA DA neurons in midbrain slice preparations (Chen et al., 2005). Taken together with the data for baclofen as described above these studies suggest that $GABA_B$ receptor positive modulators such as GS39783 may attenuate effects of cocaine by decreasing VTA DA neuron excitability. However, contributions of other components of the mesocorticolimbic circuitry such as the ventral pallidum or the prefrontal cortex are also possible (Gong et al., 1998; Kalivas and Volkow, 2005). Interestingly, Liu et al. (2005) recently reported that cocaine exposure in vivo facilitates LTP formation in midbrain DA neurons and that drug-induced synaptic plasticity could be prevented by enhanced GABAergic inhibition. The consequence of $GABA_B$ receptor activation on the formation of drug-associated memories however has not yet been investigated.

We have demonstrated that the modulation of several molecular markers by chronic cocaine exposure is attenuated by GS39783. In the NAc GS39783 blocked the induction of both CREB (as evidenced by the ratio of pCREB/CREB) and DARPP-32, without affecting basal levels (Fig. 6, Fig. 7). Cocaine treatment increases synaptic DA concentrations which in turn causes a dys-regulation of DA receptor signaling. In the NAc this leads to an upregulation of the adenylyl cyclase signaling pathway, via increased D1-like DA receptor activity (Anderson and Pierce, 2005). Increased cAMP pathway activation in turn augments the expression and activation by phosphorylation of CREB and DARPP-32 (Terwilliger et al., 1991;Kano et al., 1995;Bibb et al., 2001). Therefore, most likely GS39783 attenuated CREB and DARPP-32 induction through $GABA_B$ receptor mediated reduction of DA neuron excitability, thus preventing selective cocaine-induced changes in DA receptor signaling. Furthermore, $GABA_B$ receptors negatively couple to adenylyl cylase (Bettler et al., 2004). GS39783 mediated inhibition of cAMP formation would be expected to decrease the effect of DA receptor signaling induced upregulation of the adenylyl cyclase pathway, in addition to reducing DA neuron excitability.

Fos expression can be stimulated by a variety of regulators including CREB. After acute treatment Fos is induced in NAc and striatum by several drugs of abuse, including cocaine (Graybiel et al., 1990; Young et al., 1991; Curran et al., 1996; Zhang et al., 2002; Zhang et al., 2004; Nye et al., 1995). Leite-Morris et al. (2002) provided evidence that baclofen treatment in the VTA blocks Fos immunoreactivity in the NAc, by inhibiting the activation of dopaminergic neurons. We have shown that systemic applications of either baclofen or GS39783 effectively blocked cocaine-induced Fos induction. These data are in line with the observed attenuation of acute behavioral effects of cocaine and support the hypothesis that $GABA_B$ receptor activation inhibits the activation of dopaminergic neurons after cocaine treatment. ΔFosB is accumulated in different brain regions in response to various chronic stimuli including cocaine. We observed an increase of ΔFosB levels subsequent to a chronic cocaine treatment regimen in NAc, and to a lesser extent in dorsal striatum (Fig. 5), in agreement with previous studies (Hope et al., 1994; Nye *et* al, 1995; McClung et al., 2004). Interestingly, GS39783 did not significantly affect ΔFosB upregulation in NAc but attenuated the induction in the dorsal striatum. ΔFosB induction is D1-like DA receptor dependent but very little information is available about the downstream signaling cascades/transcription factors responsible for its induction (Nye et al., 1995; McClung and Nestler, 2003). It is conceivable that ΔFosB expression is regulated via several tissue specific signaling pathways, which could explain the differential effects of GS39783 on ΔFosB induction in NAc versus dorsal striatum.

We have shown that GS39783 only modestly attenuated the acquisition of behavioral sensitization to cocaine. However, several molecular adaptations after chronic cocaine exposure such as the induction of DARPP-32 and CREB in NAc were totally suppressed by GS39783 treatment. Thus there is a significant dissociation between the molecular sequelae of chronic cocaine and cocaine-induced locomotor sensitization. Further investigation of the effects of GS39783 in addiction models sensitive to the effects of chronic cocaine are now called for. Such studies will be important to test if the dissociation between the behavioral and molecular consequences of chronic cocaine generalizes to more reward-related aspects of cocaine dependence and its potential treatment.

In summary, our data demonstrate that use-dependent activation of $GABA_B$ receptors, via positive modulation, can decrease both behavioral as well as long-term adaptive molecular changes in DA signaling pathways after cocaine exposure. Possible modes of action include a reduction of excitability of DA neurons but also negative coupling of $GABA_B$ receptors to adenylyl cyclase, which may counteract the increased cAMP pathway activation observed after cocaine exposure (Anderson and Pierce, 2005). It seems evident that several facets to the addiction process need to be addressed in order to develop successful pharmacotherapeutic strategies. Therefore, interventions should not be limited to inhibiting the rewarding effects of a drug, but should also include strategies to enhance the saliency value of natural reinforcers, strengthen inhibitory control, decrease conditioned responses and improve withdrawal-induced deficits in mood and anxiety (Volkow and Li, 2004). The fact that $GABA_B$ receptor positive modulators reduce anxiety in preclinical paradigms (Cryan et al., 2004) and attenuate selective molecular adaptations subsequent to chronic cocaine administration suggests that they may assist in the treatment of addiction beyond simply reducing the primary rewarding effects of the reinforcer.

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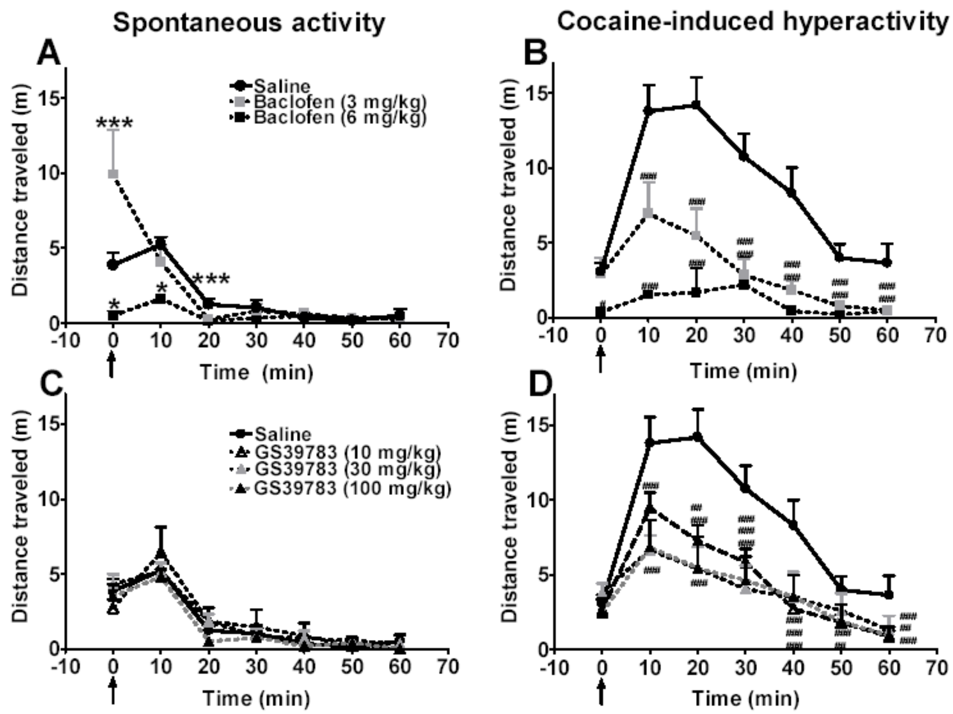

Figure 1. $GABA_B$ receptor activation attenuates cocaine-induced hyperlocomotion

A, C, Effects of baclofen and GS39783 on locomotor activity in mice (12 mice per group). Values are means ± S.E.M. *, ***, groups that differed significantly from vehicle-treated animals ($p < 0.05$ and $p < 0.001$, respectively). B, D, Effects of baclofen and GS39783 on cocaine induced hyperactivity (10 mg/kg i.p.) in mice (n = 12). Values are means ± S.E.M. #, ##, ###, groups that differed significantly from vehicle-treated animals ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively). The arrows indicate the time point of saline or cocaine injection; baclofen or GS39783 were applied 30 min before saline/cocaine.

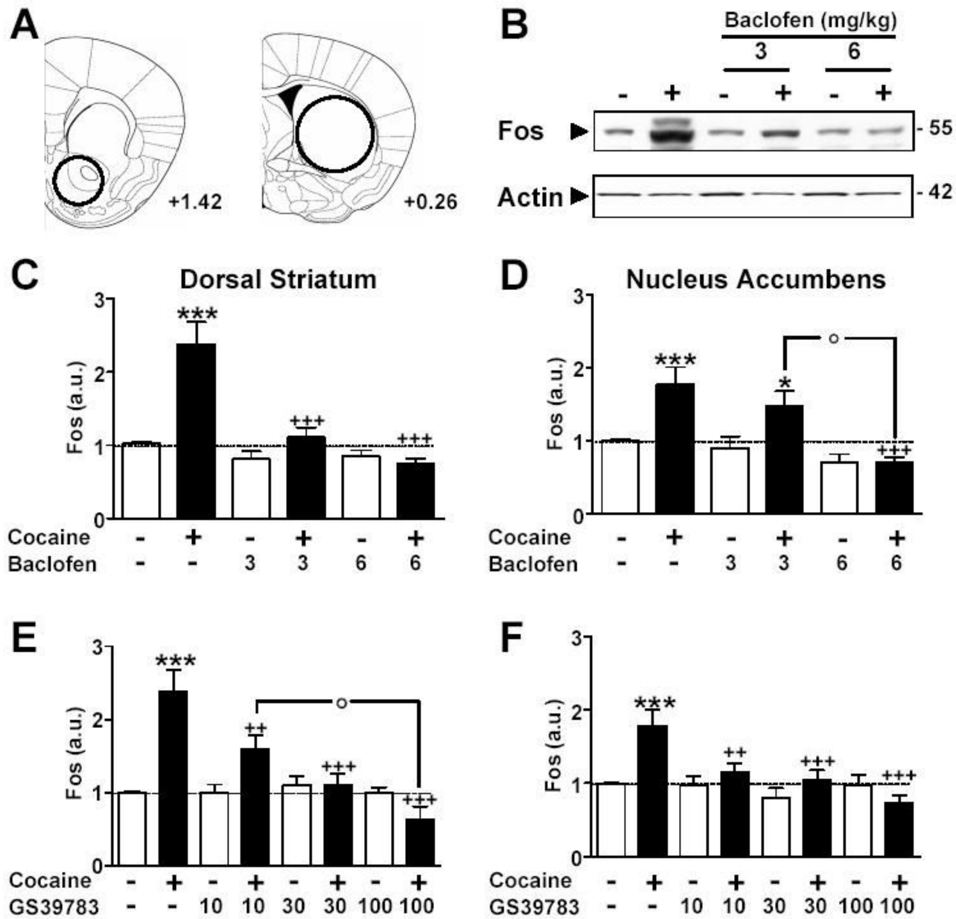

Figure 2. GABA$_B$ receptor activation inhibits cocaine-induced Fos accumulation

Baclofen and GS39783 were applied 30 min prior to cocaine or saline (−) and the mice were sacrificed 2 hours after cocaine/saline injection (20mg/kg i.p; n = 5 for each group). A, circles in schematic drawing after Paxinos and Franklin (2001) indicate dissected brain regions (NAc, left panel; dorsal striatum, right panel; bregma coordinates are given). Fos was detected by immunoblot in dorsal striatum (B, C, E) and NAc (D, E) samples. B, representative Fos immunoblot obtained from dorsal striatum samples, with its corresponding actin control (molecular weights in kilodalton, kda). Mice were either injected with saline (−) or cocaine (+) in absence or presence of baclofen. C, D, Effect of baclofen (3 or 6 mg/kg p.o.) on Fos-upregulation by cocaine. Averaged densitometric values obtained from dorsal striatum (C) and NAc (D) samples are shown. ANOVA and *post hoc* analysis revealed a significant difference between groups and an effect of treatment on Fos expression. E, F, GS39783 (10, 30, 100 mg/kg p.o.) attenuates cocaine-induced Fos upregulation in dorsal striatum (E) and NAc (F) samples. *, + indicate differences to saline controls or cocaine groups, respectively; o indicates differences within treatment groups. *, +, o, $p < 0.05$; **, ++, $p < 0.01$; ***, +++, $p < 0.001$; a.u., arbitrary units.

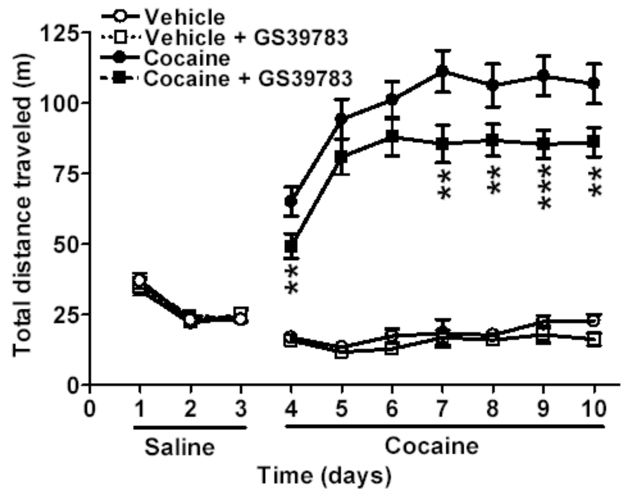

Figure 3. Effects of GS39783 application during the acquisition phase of cocaine sensitization

Mice (n = 12) were habituated to the locomotor activity chambers during three daily sessions (days 1–3) of 30 minutes after receiving intraperitoneal saline injection. GS39783 (30 mg/kg p.o.) or vehicle were administrated 30 minutes before cocaine injection (20 mg/kg i.p.) on 7 consecutives days (days 4–10). Locomotor activity was recorded immediately after cocaine injection for 30 minutes. Values are means (n = 10 per group) ± S.E.M. of the total distance traveled during the total 30 minutes of daily session. Groups that differed significantly from cocaine treated animals are indicated (**, $p < 0.01$; ***, $p < 0.001$).

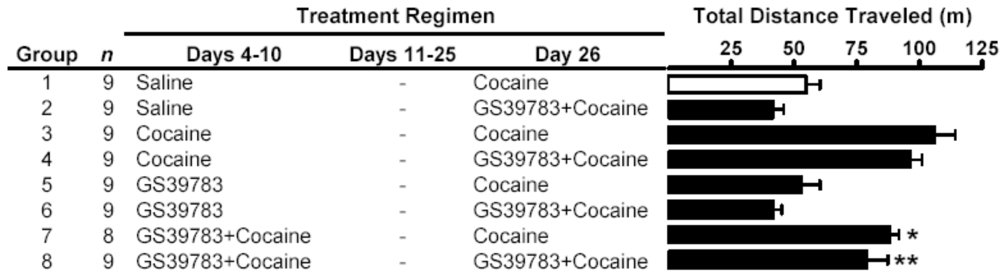

Figure 4. The effects of GS39783 on the acquisition and expression of cocaine sensitization

The effects of different GS39783 regimen (30 mg/kg p.o.) on hyperactivity induced by challenging dose of cocaine (10 mg/kg i.p., day 26) injected after 14 days of drug-free period are shown (n = 8–9 mice per group). Treatment during the acquisition phase (days 4–10) was as described in Fig. 3. Bargraphs show means ± S.E.M. of the total distance traveled during the 30 minutes of challenge session. * and **, groups that differed significantly from cocaine sensitized animals ($p < 0.05$ and $p < 0.01$, respectively).

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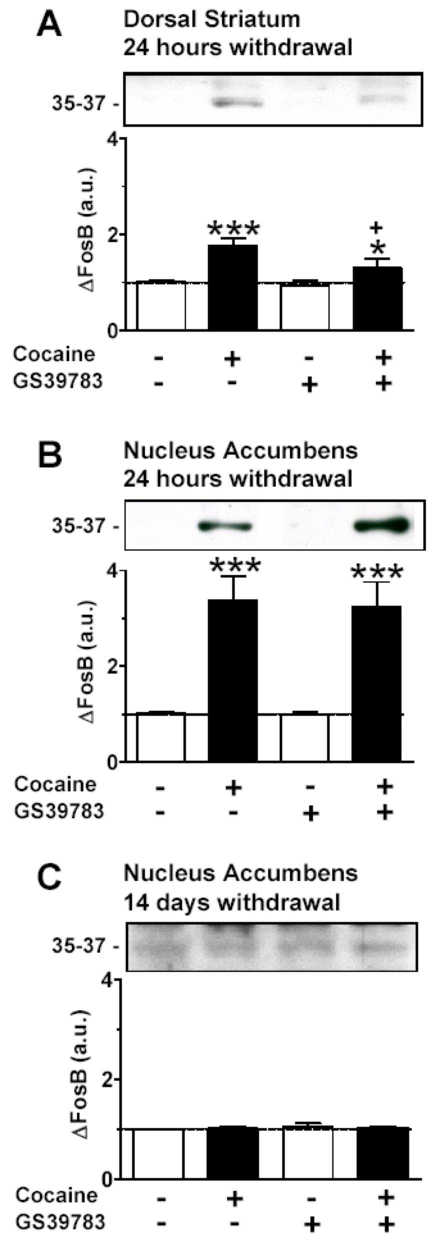

Figure 5. GS39783 has a weak inhibitory effect on ΔFosB induction by chronic cocaine

Mice (n = 5–10 animals/experimental group) were treated daily with cocaine, (20 mg/kg i.p.), GS39783 (30 mg/kg p.o.), saline (−) and respective combinations as indicated on 7 consecutive days. 24 hours after the last treatment the mice were sacrificed and NAc and dorsal striatum dissected and processed for immunoblot analysis. Representative immunoblots (top panels) and averaged densitometry values (bottom panels) are shown. The molecular weight of the ΔFosB isoform analyzed is indicated in kda. A, B, Cocaine induces ΔFosB upregulation in NAc and dorsal striatum. GS39783 partially inhibits ΔFosB induction in dorsal striatum (A) but not in NAc (B). C, Mice (9 animals/group) were administered saline/cocaine for 7 days, after which cocaine exposure was stopped for 14 days. Then the mice received a cocaine challenge (20 mg/kg i.p.) 24 hours after which NAc samples were processed for immunoblotting. ΔFosB protein levels are high immediately after repeated cocaine treatment

(24 hours of withdrawal) whereas expression levels decline to basal levels after 14 days of cessation of chronic cocaine. * and + represent differences to saline or cocaine groups, respectively; *, +, $p < 0.05$; ***, +++, $p < 0.001$.

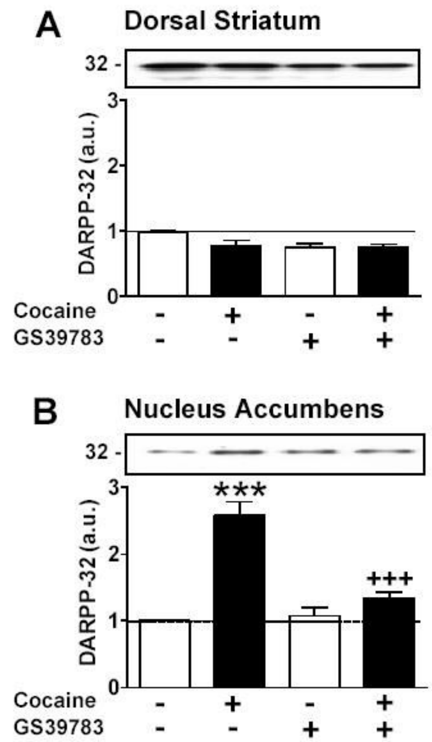

Figure 6. GS39783 inhibits DARPP-32 upregulation by chronic cocaine

Dorsal striatum and NAc samples were prepared 24 hours after cessation of repeated cocaine treatment, as described in Fig. 4. Treatments with cocaine (20 mg/kg i.p.), GS39783 (30 mg/kg p.o.), saline (−) and respective combinations are indicated. Representative immunoblots (top panels) and averaged densitometry values (bottom panels, n = 5–10) are shown (molecular weights in kda). A, GS39783 does not affect DARPP-32 expression in dorsal striatum. B, DARPP-32 upregulation in NAc by repeated cocaine is inhibited by GS39783. * and + indicate differences to saline or cocaine groups, respectively. ***, +++, $p < 0.001$.

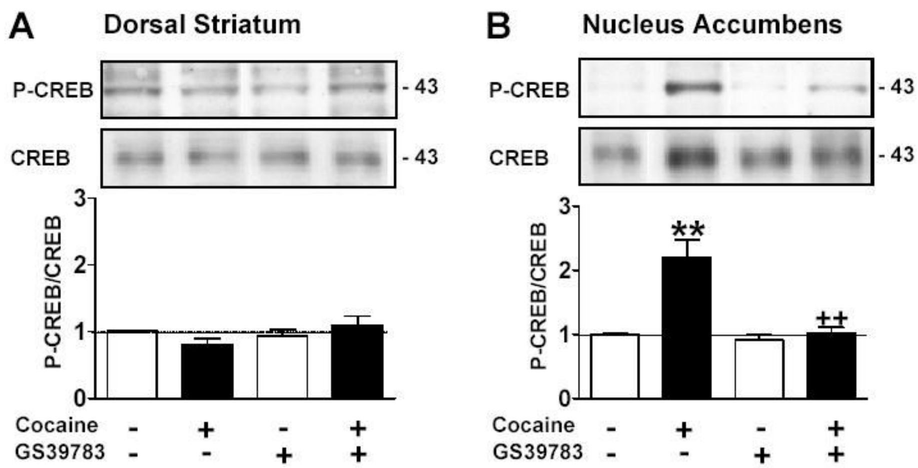

Figure 7. GS39783 inhibits CREB activation by chronic cocaine

Mice were treated for 7 days with daily combinations of cocaine (20 mg/kg i.p.), GS39783 (30 mg/kg p.o.), saline (−) and respective combinations as indicated. dorsal striatum and NAc samples were dissected 24 hours after the end of treatment. P-CREB/CREB ratios were calculated from immunoblots (n = 5–10); representative blots are shown (top panels, molecular weights in kda). GS39783 does not modify CREB expression in dorsal striatum (A) but inhibits cocaine-induced CREB activation in NAc (B).* and + mark differences to saline or cocaine groups, respectively; **, ++, $p < 0.01$