Intracerebral Baclofen Administration Decreases Amphetamine-Induced Behavior and Neuropeptide Gene Expression in the Striatum

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In a previous study, systemic administration of the GABA$_B$ receptor agonist, R-(+)-baclofen (2.5 mg/kg, i.p.) blocked acute amphetamine (2.5 mg/kg, i.p.)-induced rearing and neuropeptide (preprodynorphin (PPD), preprotachykinin (PPT), preproenkephalin (PPE), and secretogranin II (SGII)) mRNA expression in the striatum (Zhou et al, 2004). The purpose of the present study was to investigate the site(s) of action of these baclofen effects in the dorsal and ventral striatal circuitries. Infusion of baclofen (75 ng/side) into the ventral tegmental area (VTA), substantia nigra (SN), nucleus accumbens (NA), caudate-putamen (Cpu), or medial prefrontal cortex (mPFC) had no effect on behavioral activity in saline-treated rats habituated to a photocell apparatus. However, intra-VTA infusion of baclofen (75 ng/side) completely blocked, whereas intra-NA and intra-SN infusion of baclofen attenuated, amphetamine-induced vertical activity without affecting amphetamine-induced total distance traveled. In contrast, intramedial PFC and intra-CPu infusion of baclofen had no effect on behavioral activity in amphetamine-treated rats. Infusion of baclofen into the VTA, NA, or SN decreased amphetamine-induced neuropeptide gene expression in the striatum. These results indicate that GABA$_B$ receptor stimulation within the ventral striatal circuitry is involved in mediating acute amphetamine-induced behaviors and neuropeptide gene expression in the dorsal and ventral striatum. The present study provides information on the potential targets in the brain for baclofen in the initial behavioral and genomic response to amphetamine.
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INTRODUCTION

Acute administration of amphetamine causes direct stimulation of dopamine (DA) release from mesoaccumbal and nigrostriatal terminals (Zetterstrom et al, 1983; Sharp et al, 1987). This increase in striatal synaptic dopamine leads to an increase in locomotion and stereotypic behavior (Robinson and Becker, 1986; Kalivas and Stewart, 1991; White and Wolf, 1991). The increased dopamine release also leads to a cascade of events that involves the activation of neuropeptides in postsynaptic striatal neurons. The induction of striatal neuropeptides, preprodynorphin (PPD), preprotachychinin (PPT), and preproenkephalin (PPE), is well documented after acute administration of amphetamine (Wang and McGinty, 1995a, b; Zhou et al, 2004). After binding to their corresponding receptors, these peptides are able to modulate the release of DA, glutamate, and acetylcholine in the striatum (Heijna et al, 1990; Guzman et al, 1993; Anderson et al, 1994; Gray et al, 1999; Rawls and McGinty, 2000). Recently, we also demonstrated that acute amphetamine increases secretogranin II (SGII) mRNA in the striatum (Gonzalez-Nicolini and McGinty, 2002; Zhou et al, 2004). SGII, a member of the chromogranin family, regulates the packaging, processing, and release of peptides and neurotransmitters (Huttner et al, 1991). Thus, these four neuropeptides are able to modify the changes in striatal neurotransmission caused by psychostimulants.

The GABA system is known to interact with and modulate DA neurotransmission in the nigrostriatal (Engberg et al, 1993) and the mesolimbic dopaminergic pathways (Kalivas et al, 1990). Three types of GABA receptors exist: GABA$_A$, GABA$_B$, and GABA$_C$. GABA$_A$ and GABA$_C$ receptors are GABA-gated chloride channels. GABA$_B$ receptors are G$_i$/G$_o$ protein-coupled receptors that modulate signal transduction pathways, for example, inhibiting adenylate cyclase, stimulating phospholipase A2, activating K$^+$ channels, inhibiting voltage-dependent Ca^{2+} channels, and regulating inositol phospholipids hydrolysis (Bowery, 1993; Misgeld et al, 1995). GABA$_B$ receptors are enriched in areas of the brain that mediate the rewarding and activating effects of

psychostimulants (Chu *et al*, 1990; Lopez-Bendito *et al*, 2002; Boyes and Bolam, 2003). Several investigators have demonstrated that the selective GABA$_B$ receptor agonist, baclofen, attenuates cocaine self-administration across a wide range of conditions, including multiple schedule (Shoaib *et al*, 1998), progressive ratio (Roberts *et al*, 1996), fixed ratio (Campbell *et al*, 1999), concurrent access (Brebner *et al*, 2000), and discrete trial schedules of reinforcement (Roberts and Andrews, 1997). When exposed to a videotape of drug paraphernalia, cocaine addicts taking baclofen reported reduced craving and PET imaging demonstrated reduced limbic activation (Brebner *et al*, 2002). Recently, a clinical trial showed that cocaine addicts receiving baclofen demonstrated significant reductions in cocaine use over those receiving placebo as indicated by urine drug screening results (Shoptaw *et al*, 2003). Furthermore, baclofen decreases cocaine-induced increases in extracellular dopamine (Fadda *et al*, 2003). Thus, the GABA$_B$ receptor agonist, baclofen, is a promising therapeutic candidate in the treatment of cocaine addiction.

Much less is known about the effects of GABA$_B$ receptor stimulation on amphetamine-induced behavioral and neurochemical responses. A previous study from our laboratory demonstrated that GABA$_B$ receptor activation by (+)-baclofen (2.5 mg/kg, i.p.) blocked amphetamine-induced rearing, decreased the peak level of striatal DA release, and blocked mRNA expression of PPD, PPT, PPE, and SGII in the striatum (Zhou *et al*, 2004). In the present study, intracerebral infusion of baclofen was used to investigate the sites of action of baclofen in major nodes of the 'motive circuit' that includes dorsal striatal-related circuitry (including substantia nigra (SN) and caudate putamen (CPu)) and ventral striatal-related circuitry (including ventral tegmental area (VTA), nucleus accumbens (NA), and medial prefrontal cortex (mPFC)). We hypothesized that the GABA$_B$ receptor stimulation within key nodes of the dorsal and ventral striatal circuitries would decrease amphetamine-induced hyperactivity and striatal neuropeptide gene expression.

MATERIALS AND METHODS

Animals

Adult male Sprague–Dawley rats (250–275 g, Charles River, Raleigh, NC) were maintained two per cage on a 12-h light/dark cycle with food and water provided *ad libitum* 3–7 days prior to the experiments. All animal procedures used were in strict accordance with *the NIH Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee. Following surgery, animals were housed individually and allowed at least 1 week to recover before the experiments.

Surgery

After 3–7 days of acclimation, animals were anesthetized with ketamine HCl (87.5 mg/kg Rompum; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (5 mg/kg AnaSed; Shenandoah, Iowa) and implanted with bilateral guide cannulae (26 gauge; Small Parts Inc., Roanoke, VA) aimed at one of five brain regions according to the atlas of Paxinos and Watson (1986) (all coordinates given relative to

bregma): mPFC: +3.0 mm antereoposterior (AP), ±1.0 mm mediolateral (ML), and −3.0 mm dorsoventral (DV); CPu: +0.7 mm AP, ±3.0 mm ML, and −2.7 mm DV; NA: +1.6 mm AP, ±1.4 mm ML, and −2.7 mm DV; SN: −5.2 mm AP, ±2.1 mm ML, −2.7 mm DV; and VTA: −5.2 mm AP, ±1.56 mm ML at an angle of 6°, −7.6 mm DV. Guide cannulae (cut to 10 or 14 mm) were lowered into place and attached to the skull via small stainless steel screws and dental acrylic. The obturators (30 gauge; Small Parts Inc.), cut to extend 0.5 mm shorter than the tip of each cannula, were inserted to prevent obstruction.

Drug Microinjections

Kalivas *et al* (1990) demonstrated that baclofen (75 ng/0.5 µl/side) infusion into the VTA blocked DAMGO, cocaine, and amphetamine-induced hyperactivity. Thus, 75 ng/side baclofen was used in the present study. Before injection, the obturators were removed from the guide cannulae and 30 gauge microinjection cannulae were inserted bilaterally to extend 0.5 mm (mPFC), 2.8 mm (CPu), 3.8 mm (NA), 6.0 mm (SN), or 1.0 mm (VTA) below the end of the guides. Before behavioral testing, rats received a bilateral infusion of either artificial cerebrospinal fluid (ACSF, which consisted of KCl (3.0 mM), NaCl (140 mM), glucose (7.4 mM), CaCl$_2 \cdot$H$_2$O (1.2 mM), MgCl$_2$ (1.0 mM), NaH$_2$PO$_4$ (0.27 mM), Na$_2$HPO$_4$ (1.2 mM), pH 7.4) or 75 ng/side of (+)-baclofen into mPFC, CPu, NAS, SN, or VTA. All infusions were made in a volume of 0.5 µl (with the exception of CPu, in which a volume of 1.0 µl was infused due to the large area) over 5 min. After infusion, 2 min were allowed for adequate diffusion, then the microinjectors were removed, the obturators were replaced, and the rats were given an injection of amphetamine (2.5 mg/kg, i.p.) or saline (1 ml/kg, i.p.). Pilot experiments were performed using pontamine sky blue infused in the volumes and at the coordinates listed above to demonstrate that the extent of diffusion was limited to the region of interest.

Behavior

In each behavioral experiment, rats were randomly assigned to four different groups: ACSF + saline, ACSF + amphetamine, baclofen + saline, or baclofen + amphetamine (*n* = 8/group). The day before the test day, the rats were habituated in Accuscan Photocell Chambers (Accuscan Instruments, Inc., Columbus, OH). Each box contained a series of 16 photocell beams measuring horizontal distance traveled and eight photocell beams measuring vertical activity. Beam breaks were continuously counted and recorded once every 5 min by a PC running VersaMax/Digiscan System Software (Accuscan Instruments, Inc.). On the test day, after 1 h habituation in the chamber, each rat was given an intracranial injection of ACSF or baclofen into one of the structures listed above. After 10 min, each rat received a second injection of saline or amphetamine (2.5 mg/kg, i.p.). Both total distance traveled and vertical activity were recorded for 3 h after the second injection. Immediately after the test, all rats were anesthetized with Equithesin (10 mg/kg, i.p.) and decapitated. The brains were removed and frozen in isopentane at −40°C and stored at −80°C until they were sectioned.

In Situ Hybridization Histochemistry

Quantitative *in situ* hybridization histochemistry to measure the effects of intracranially administered baclofen on amphetamine-induced PPD, PPT, PPE, and SGII mRNA expression in striatal neurons was performed as previously described (Zhou et al, 2004). Briefly, 12 μm coronal sections throughout the striatum of each rat (n = 4–8/group) were cut in a cryostat (four sections per slide) and thaw-mounted onto Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA). Before hybridization, the sections were pretreated in a series of steps that fixed and defatted the tissue and blocked nonspecific hybridization. Synthetic cDNA oligodeoxy-nucleotide probes (48-mers) complementary to rat PPD (bases 862–909), PPT (spanning the first 16 amino acids encoded by exon 3), PPE (bases 388–435), and SGII (bases 862–903) were radiolabeled with ^{35}S-dATP (1250 Ci/mmol; New England Nuclear, Boston, MA) using terminal deoxy-ynucleotide transferase (Roche Diagnostics Corp, Indianapolis, IN). Slides were incubated with 5.0×10^5 cpm/25 μl hybridization buffer/section overnight (16–20 h) at 37°C in a humid environment. After incubation, slides were washed and air dried before being placed into a film cassette, along with ^{14}C standards (American Radiolabeled Chemicals, St Louis, MO), with Kodak Biomax film (Rochester, NY) for 3 days (PPE), 5 days (PPT), 7 days (SGII), or 10 days (PPD).

Quantitation of the PPD, PPT, PPE, and SGII mRNA hybridization signals was performed using NIH image 1.62 (W. Rasband, NIMH) on a Macintosh G3 as previously described (Wang and McGinty, 1995b; Zhou et al, 2004). All measurements were made unilaterally on three adjacent coronal sections ranging from 1.6 to 1.0 mm rostral to Bregma from each animal, matched across animals for their rostral–caudal level. For the experiments in which micro-infusions were made in the CPu or NA, six sections at the center of each injection site were collected for Nissl staining (see below) and adjacent sections (within 75 μm rostral and/or caudal) were used for quantitation of hybridization signals.

In each experiment, the hybridization signals for all four transcripts were measured in each animal using an oval shape: 80×100 pixels in the CPu, 30×70 pixels in the NA shell, and 75×75 pixels in the NA core. The limits of the density slice option were set to eliminate background (minimum) and to avoid saturation (maximum) of the signals in each area. Quantitative changes were expressed as (1) the number of labeled pixels per area (area), (2) mean density of tissue in dpm/mg, and (3) integrated density, which is the product of area times mean density. The integrated density value more accurately depicts the area over which changes in optical density occur because we have found that mean density alone underestimates these changes (Wang and McGinty, 1995b; Zhou et al, 2004). The mean integrated density ± SEM was calculated for each rat by averaging the values of the three adjacent coronal sections.

Histology

Six 12-μm thick sections through the center of the injection site were mounted onto precleaned, charged microscope slides and stained with 0.1% thionin. The placement of each injection cannula was verified according to the rat brain atlas of Paxinos and Watson (1986). Only data from rats with correctly placed probes were analyzed.

Drugs and Chemicals

D-amphetamine sulfate and R-(+)-baclofen hydrochloride were purchased from Sigma Chemical Co. (St Louis, MO) and dissolved in ACSF. All other chemicals used in this study were purchased from either Fisher Scientific or Sigma Chemical Co.

Statistics

Behavioral data were analyzed by calculating the area under the curve (AUC) for the activity counts plotted against time. A one-way analysis of variance (ANOVA) was performed for overall group comparisons. For the gene expression data, a two-way ANOVA was performed on the mean integrated density values. When an ANOVA F-ratio was significant, multiple comparisons were made on behavioral and gene expression data using a least squares means (LSM) test. Results were determined to be significant when $P < 0.05$. All statistical calculations were made using SAS 9.0 (SAS Institute Inc., Cary, NC).

RESULTS

Histology

Figure 1 shows the injection sites of animals used in each experiment. Cannula tips in the mPFC were located in the anterior cingulate cortex (Cg1 and Cg3) from Bregma 3.2 to 2.2 mm AP with the majority at 2.7 mm AP (Figure 1a). Cannula placements in the CPu were in the center of the caudate putamen clustered primarily between Bregma 1.2 and 0.7 mm AP but with a few tips between 1.7 and 1.2 mm AP (Figure 1b). Cannula tips in the NA were located in both the core (the majority) and the shell clustered primarily between Bregma 1.7 and 1.6 mm AP but with some tips extending to 1.0 mm AP (Figure 1b). Cannula placements in the VTA were medial to the medial lemniscus clustered primarily between Bregma −5.6 and −6.3 mm AP (Figure 1c). Cannula tips for the SN were in both the reticulata and the compacta, with a majority of tips clustered primarily between Bregma −4.5 and −5.8 mm AP (Figure 1c).

Intra-VTA, Intra-NA, and Intra-SN Infusion of Baclofen Decreased Amphetamine-Induced Vertical Activity

Intracerebral administration of baclofen did not have a signficant effect on vertical activity as compared to that of ACSF in saline-treated rats habituated to the photocell chamber for 1 h prior to injection (Figure 2). Intra-VTA infusion of baclofen (75 ng/side) completely blocked amphetamine-stimulated vertical activity as compared to that of intra-VTA ACSF in amphetamine-treated rats ($P < 0.001$) (Figure 2a). Both intra-NA (Figure 2b) and intra-SN (Figure 2c) infusion of baclofen attenuated amphetamine-induced vertical activity as compared to that of ACSF in amphetamine-treated rats ($P < 0.01$, for NA and $P < 0.05$ for SN). Neither intra-mPFC or intra-CPu infusion
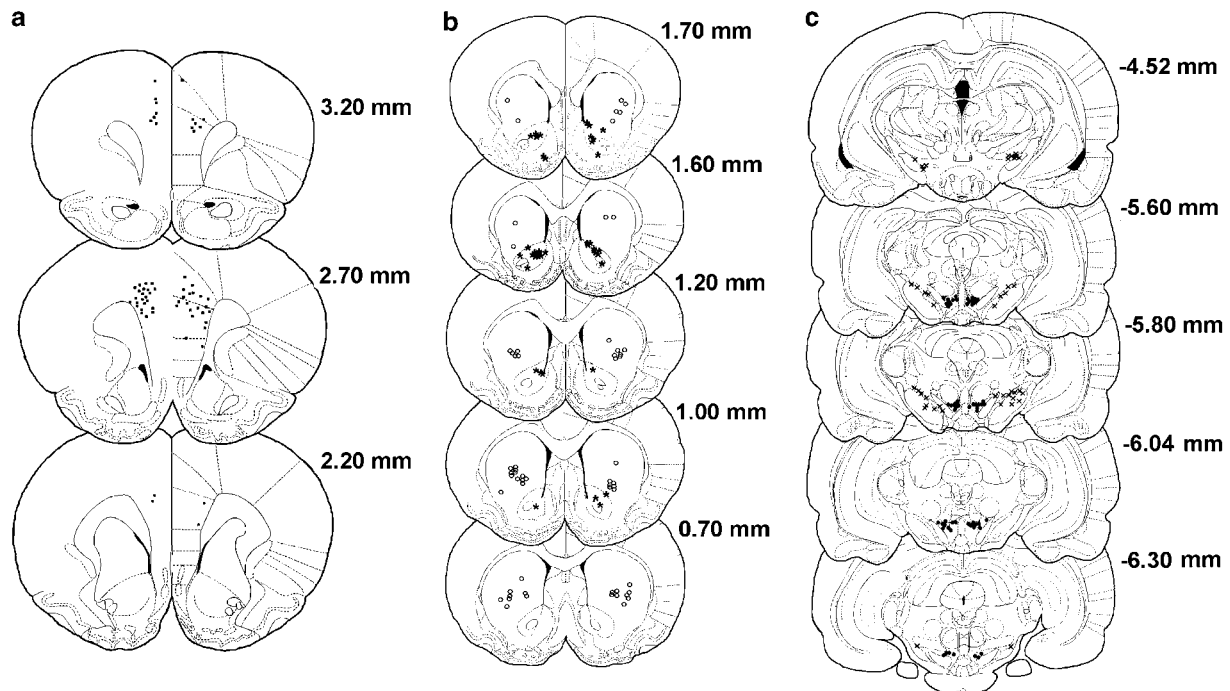
Figure 1 Location of microinjection cannula tips in coronal sections based on the atlas of Paxinos and Watson (1986). Numbers indicate the distance from bregma in the anteroposterior plane. (a) mPFC (■); (b) CPu (○) and NA (*); (c) VTA (●) and SN (x).

of baclofen had any effect on amphetamine-induced vertical activity as compared to that of ACSF in amphetamine-treated rats (data not shown). In contrast, infusion of baclofen into the VTA, NA, SN, mPFC, or CPu did not affect amphetamine-induced total distance traveled as compared to that of ACSF in amphetamine-treated rats (data not shown).

Intra-VTA, Intra-NA, and Intra-SN Infusion of Baclofen Decreased Amphetamine-Induced Neuropeptide Gene Expression in the Striatum

Two-way ANOVA of the effects of intra-VTA infusion of baclofen on amphetamine-induced neuropeptide mRNA expression in the CPu and NA core and shell (Table 1) revealed that treatment 1 (intracerebral infusion) and treatment 2 (intraperitoneal injection) had a significant main effect and the interaction between the two treatments was significant in all cases. LSM comparisons indicated that the intra-VTA infusion of baclofen had no effect on gene expression in saline-treated rats but it decreased amphetamine-induced increases in PPD, PPT, PPE, and SGII mRNA in the CPu. In the NA shell, intra-VTA infusion of baclofen decreased amphetamine-induced PPD and SGII mRNA without altering basal levels of mRNA in saline-treated rats. In the NA core, intra-VTA baclofen decreased amphetamine-induced PPD, PPE, and SGII mRNA levels but had no effect on basal gene expression in saline-treated rats. Figure 3 illustrates representative, digitized micrographs of the effect of baclofen infusion into the VTA on PPD, PPT, PPE, and SGII mRNA expression in the striatum.

Two-way ANOVA of the effects of intra-NA infusion of baclofen on amphetamine-induced neuropeptide mRNA expression in the striatum (Table 2) revealed that treatment 1 (intracerebral infusion) and treatment 2 (intraperitoneal

injection) had a significant main effect and the interaction between the two treatments was significant in all cases. LSM comparisons indicated that the intra-NA infusion of baclofen decreased amphetamine-induced increases in PPD, PPT, PPE, and SGII mRNA in the CPu, NA shell, and NA core without altering basal levels in saline-treated rats. Figure 4 illustrates representative, digitized micrographs of the effect of intra-NA baclofen infusion on PPD, PPT, PPE, and SGII mRNA expression in the striatum. Note that the cannula tracts are either not visible or minimally affect the pattern of gene expression because the sections are adjacent to the center of the injection sites.

Two-way ANOVA of the effects of intra-SN infusion of baclofen on amphetamine-induced neuropeptide mRNA expression in the striatum (Table 3) revealed that treatment 1 (intracerebral infusion) and treatment 2 (intraperitoneal injection) had a significant main effect and the interaction between the two treatments was significant in all cases. The intra-SN infusion of baclofen had no effect on mRNA levels in saline-treated animals. LSM comparisons indicated that intra-SN infusion of baclofen decreased amphetamine-induced increases of all mRNAs in the CPu, NA shell, and NA core. For PPE, baclofen significantly decreased expression in the CPu and NA core but not in the NA shell.

DISCUSSION

The major finding of this study is that $GABA_B$ receptor activation in the VTA, NA, and SN decreased amphetamine-induced vertical activity and striatal gene expression (summarized in Table 4). Further, baclofen had no effect on amphetamine-induced vertical or horizontal activity when infused into the CPu or mPFC nor did it affect total
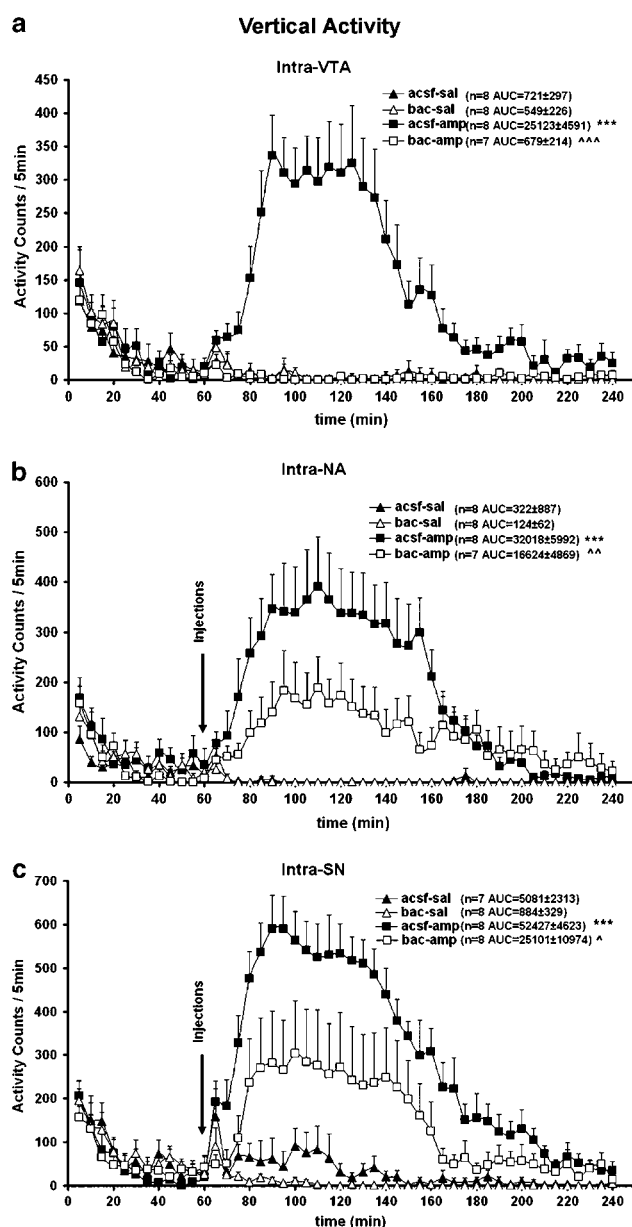
Figure 2 The effects of (a) intra-VTA, (b) intra-NA, and (c) intra-SN infusion of baclofen (bac, 75 ng/side) or ACSF on amphetamine (amp, 2.5 mg/kg, i.p.)-induced vertical activity in rats. The activity and AUC values are expressed as mean ± SEM. ***$P < 0.001$ vs ACSF + saline; ^^^$P < 0.001$ vs ACSF + amphetamine; ^^$P < 0.01$ vs ACSF + amphetamine; ^$P < 0.05$ vs ACSF + amphetamine.

distance traveled after infusion into any of the brain areas investigated. By identifying brain areas that mediate baclofen's effects, these results extend our previous study (Zhou et al, 2004) that demonstrated similar effects of systemically administered baclofen on amphetamine-induced vertical activity and striatal gene expression.

Effects of Intracerebral Baclofen on Amphetamine-Induced Behavior

In addition to mediating amphetamine-induced horizontal activity, the basal ganglia mediate amphetamine-induced vertical activity as part of a rat's exploratory repertoire (Creese and Iversen, 1972; Kelly et al, 1975; Fink and Smith, 1980; Koob et al, 1981; Clarke et al, 1988). However, GABA$_B$ receptor stimulation in the VTA, SN, and NA selectively decreased amphetamine-induced vertical activity, a measure of exploratory rearing, without altering total distance traveled, a measure of horizontal locomotion. There was a difference in the regional sensitivity of baclofen's effects: intra-VTA infusion of baclofen completely blocked, whereas intra-NA or intra-SN infusion of baclofen attenuated, amphetamine-induced rearing. Interestingly, intra-VTA infusion of cholecystokinin (CCK) also significantly blocked amphetamine-induced rearing but not amphetamine-induced forward locomotion in a previous study (Schneider et al, 1983).

It is possible that diffusion from SN to the VTA accounts for the reduced effectiveness of baclofen in the SN. This possibility is made less likely, however, by the fact that baclofen was equally effective when infused into the lateral SN as in the medial SN. Similarly, baclofen's effects in the NA are unlikely to be explained by diffusion to the overlying CPu because intra-CPu baclofen did not affect behavior at all. Even though the intra-CPu infusion coordinates were more lateral and caudal than the NA coordinates, most of the CPu infusion sites were mapped to sections that contained NA sites (Figure 1). Therefore, the difference in the effect of intra-CPu and NA infusions indicates that there was little diffusion from one site to the other. Thus, while these results indicate that GABA$_B$ receptors in the VTA are critical mediators of amphetamine-induced rearing, GABA$_B$ receptors in the SN and NA also appear to contribute, perhaps by altering trans-synaptic activity in the motive circuit.

The mechanisms underlying baclofen's selective effects on amphetamine-induced rearing may include a reduction of amphetamine-evoked extracellular dopamine levels in the striatum that allows locomotion to proceed but more complex exploratory behaviors to be suppressed (Zhou et al, 2004). In support of the regional differences in baclofen's effectiveness, perfusion of baclofen into the SN decreased basal and nomifensine-induced extracellular dopamine levels in the SN and CPu, but infusion into the CPu did not decrease striatal dopamine levels (Westerink et al, 1992; Santiago et al, 1993; Balon et al, 2002). GABA$_B$ receptors are expressed by striatonigral, striatopallidal, and glutamatergic afferents as well as by dopamine and GABA neurons in the SN and VTA (Sugita et al, 1992; Charara et al, 2000; Boyes and Bolam, 2003). However, the amphetamine-suppressing effect of baclofen in the ventral mesencephalon is most consistent with a direct action on dopamine neurons. Baclofen decreases burst firing of dopamine neurons that is associated with phasic dopamine release in the SN and VTA (Engberg et al, 1993; Erhardt et al, 2002). How can baclofen decrease amphetamine-induced dopamine release in the striatum by decreasing burst firing of dopamine neurons? Amphetamine triggers dopamine release by reversing the dopamine transporter. However, we have demonstrated by in vivo microdialysis that approximately 40–50% of the acute amphetamine-induced increase in extracellular dopamine levels in the striatum is calcium and tetrodotoxin-dependent (Gray et al, 1999; Paredes et al, 2001), suggesting that dopamine neuronal firing contributes

Table 1 Intra-VTA Baclofen Infusion Decreases Amphetamine-Induced Striatal Neuropeptide Gene Expression

Region	Group	PPD	PPT	PPE	SGII
CPu	A/S	570±30	3582±144	4605±147	608±35
	B/S	672±80	3921±158	4297±155	570±59
	A/AM	1022±53***	4696±166***	5363±268**	927±47***
	B/AM	658±34^^^	3509±76^^^	4283±138^^^	540±27^^^
NA shell	A/S	525±31	1091±58	746±28	1373±76
	B/S	515±26	1148±50	711±28	1386±77
	A/AM	640±23**	1271±48**	866±36**	1735±57***
	B/AM	513±30^^	1184±46	809±32	1290±44^^^
NA core	A/S	1104±98	1640±79	2693±98	2116±118
	B/S	1084±74	1817±111	2622±80	2024±121
	A/AM	1565±78***	1990±85**	3220±105***	2904±130***
	B/AM	1236±70^^	1863±77	2542±80^^^	1854±92^^^

Integrated density values are expressed as mean±SEM in thousands.
A/S = ACSF+saline ($n = 8$); B/S = baclofen+saline ($n = 8$); A/AM = ACSF+amphetamine ($n = 8$); B/AM = baclofen+amphetamine ($n = 8$).
*$P < 0.05$ vs ACSF+saline; **$P < 0.01$ vs ACSF+saline; ***$P < 0.001$ vs ACSF+saline.
^$P < 0.05$ vs ACSF+amphetamine; ^^$P < 0.05$ vs ACSF+amphetamine; ^^^$P < 0.001$ vs ACSF+amphetamine.

Table 2 Intra-NA Baclofen Infusion Decreases Amphetamine-Induced Striatal Neuropeptide Gene Expression

Region	Group	PPD	PPT	PPE	SGII
CPu	A/S	330±17	1325±161	2803±179	588±28
	B/S	293±13	1481±133	2500±109	567±24
	A/AM	457±11***	2627±175***	3555±184***	1047±23***
	B/AM	266±9^^^	1822±207^^^	2977±146^^^	537±29^^^
NA shell	A/S	320±16	616±37	473±33	468±27
	B/S	306±9	637±25	431±19	392±27
	A/AM	527±18***	806±55**	606±35***	716±29***
	B/AM	328±8^^^	597±26^^	427±18^^^	454±22^^^
NA core	A/S	615±36	1497±99	2201±102	610±36
	B/S	577±24	1559±47	2066±56	588±16
	A/AM	912±46***	1776±80**	2488±107*	852±26***
	B/AM	606±25^^^	1503±74^^	2192±102^	619±25^^^

Integrated density values are expressed in mean±SEM in thousands.
A/S = ACSF+saline ($n = 7$); B/S = baclofen+saline ($n = 8$); A/AM = ACSF+amphetamine ($n = 8$); B/AM = baclofen+amphetamine ($n = 8$).
*$P < 0.05$ vs ACSF+saline; **$P < 0.01$ vs ACSF+saline; ***$P < 0.001$ vs ACSF+saline.
^$P < 0.05$ vs ACSF+amphetamine; ^^$P < 0.01$ vs ACSF+amphetamine; ^^^$P < 0.001$ vs ACSF+amphetamine.

to amphetamine-induced dopamine release *in vivo*. This line of reasoning is consistent with the fact that systemic baclofen decreases cocaine or amphetamine-induced dopamine levels in the striatum by ~ 40–50% (Fadda *et al*, 2003; Zhou *et al*, 2004). Less is known about the cellular distribution of $GABA_B$ receptors and their coupling in the dorsal and ventral striatum of rodents, but Charara *et al* (2000) demonstrated $GABA_B$ immunoreactivity in striatal boutons that make asymmetric contacts with labeled and unlabeled dendritic spines. The presynaptic localization in glutamatergic afferents is consistent with electrophysiological evidence that the predominant effect of baclofen in the striatum is to decrease excitatory synaptic input (Nisenbaum *et al*, 1993). Thus, although the neural substrates that mediate amphetamine-induced rearing are incompletely understood, this study suggests that pre- and postsynaptic $GABA_B$ receptors in the VTA, SN, and NA are differentially involved.
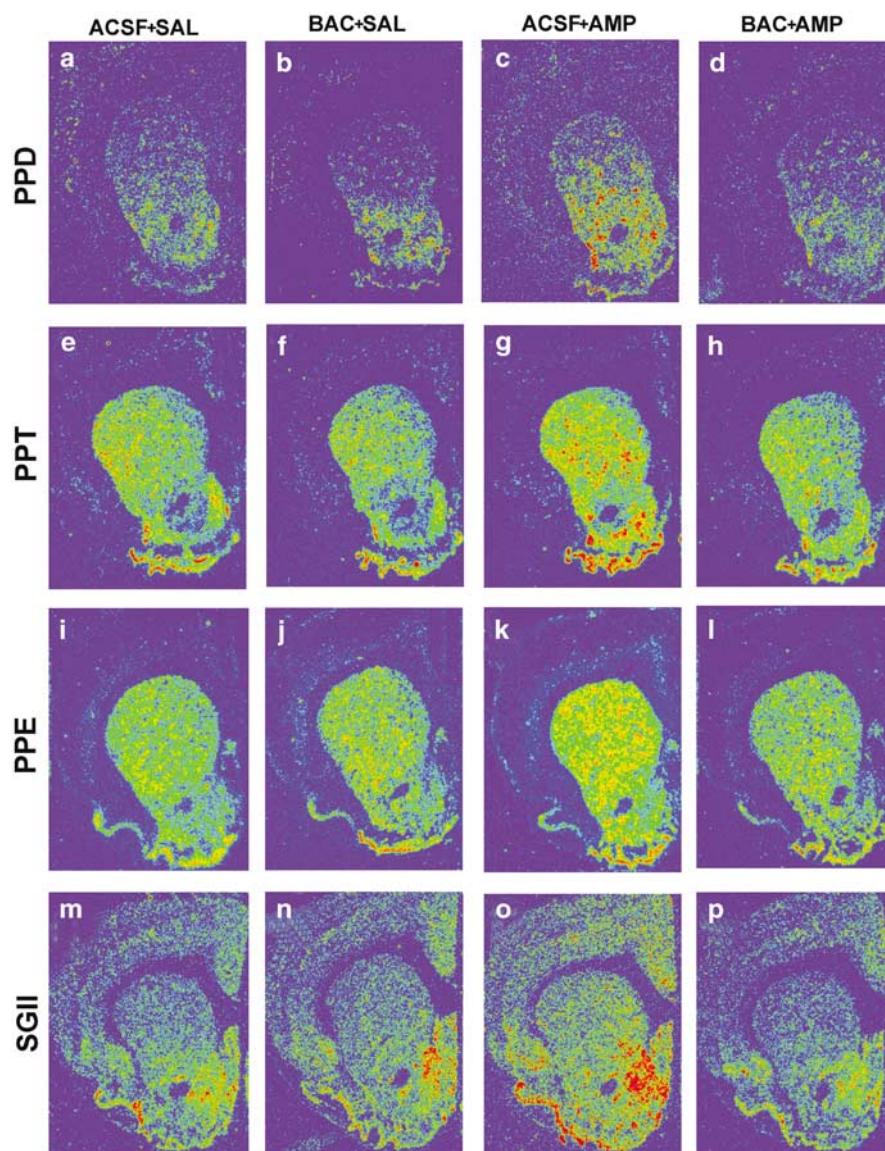
Figure 3 Representative digitized photomicrographs illustrate the effects of intra-VTA infusion of baclofen (bac, 75 ng/side) or ACSF on amphetamine (amp, 2.5 mg/kg, i.p.)-induced (a–d) PPD, (e–h) PPT, (i–l) PPE, and (m–p) SGII mRNA expression in the striatum.

In contrast to the effects of baclofen in VTA, SN, and NA, our results indicate that the stimulatory effect of amphetamine on behavior is not mediated through $GABA_B$ receptors in the mPFC or CPu. Owing to the functional heterogeneity of the striatum, it is possible that even the large volume of baclofen infused into the CPu did not reach the areas that mediate locomotion or rearing. Indeed, Dickson et al (1994) reported that amphetamine infusion into the middle ventromedial region (AP $+2.0$ mm, ML $+2.0$ mm, and DV -7.0 mm) had stimulatory effects on locomotion and rearing but had no effect on oral stereotypy, whereas an injection of amphetamine anterior or posterior in the striatum had no effect on locomotion. However, in contrast to the lack of effect of baclofen, infusions of muscarinic and mGluR ligands into this area of the CPu had profound effects on amphetamine-induced behavioral activity (Wang and McGinty, 1996, 1997). Thus, the lack of effect of intra-

CPu baclofen is more likely because CPu $GABA_B$ receptors are not involved in acute amphetamine-induced behaviors than to the heterogeneity of the striatum. Similarly, $GABA_B$ receptors in the mPFC appear not to be involved in the motor stimulatory effects of acute amphetamine although intra-mPFC baclofen decreases nomifensine-induced extracellular dopamine levels in PFC (Santiago et al, 1993). Similarly, selective quinolinic acid-induced lesions of the prelimbic cortex (Cg3), which only destroy intrinsic neurons, did not affect locomotion and rearing responses induced by amphetamine (Tzschentke and Schmidt, 1998). Once again, however, the selectivity of the neurotransmitter system and the specific circuitry may be key factors because the intra-PFC infusion of the alpha1-adrenergic receptor antagonist, prazocin, blocks amphetamine-induced locomotor activity and mesoaccumbens DA release (Darracq et al, 1998; Ventura et al, 2003).
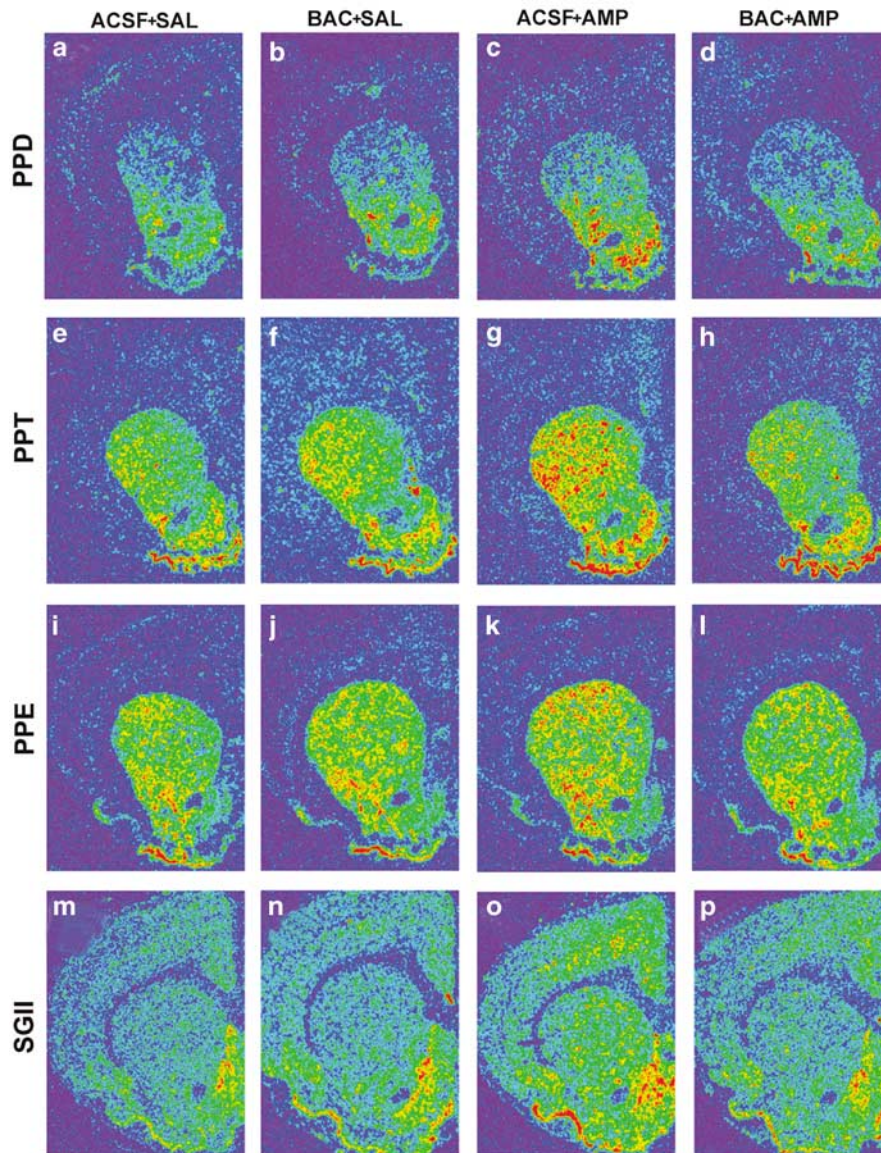
Figure 4 Representative digitized photomicrographs illustrate the effects of intra-NA infusion of baclofen (bac, 75 ng/side) or ACSF on amphetamine (amp, 2.5 mg/kg, i.p.)-induced (a–d) PPD, (e–h) PPT, (i–l) PPE, and (m–p) SGII mRNA expression in the striatum.

Baclofen Effects on Amphetamine-Induced Neuropeptide Gene Expression

With few exceptions, infusion of baclofen into the VTA, NA, or SN decreased amphetamine-induced neuropeptide mRNA expression in the CPu and/or the NA to similar extents. In those cases in which the baclofen effect did not reach significance (PPT in NA shell and core, PPE in NA shell after VTA infusion and PPE in shell after SN infusion), the initial AMPH-induce increase was not robust, possibly due to variability in sampling or section level. Medium spiny neuropeptide genes are not thought to mediate the acute effects of amphetamine, but are triggered as part of the initial cascade of neuroadaptive changes induced by psychostimulants. A strong and/or repeated exposure to drugs is required to alter the mRNA levels of striatal peptides (Wang and McGinty, 1995b; Hanson et al, 2002). Therefore, a decrease in dopamine stimulus strength, as

reflected in reduced vertical activity after SN and NA baclofen infusions, may be as effective in reducing gene expression as complete suppression by VTA infusions. In fact, we have found previously that acute behavioral activity is not 100% correlated to changes in striatal gene expression. For example, ionotropic glutamate receptor antagonists had no effect on acute amphetamine-induced behavioral activity but completely suppressed amphetamine-induced striatal neuropeptide gene expression (Wang et al, 1994a, b). This dissociation is understandable if one considers that glutamate neurotransmission plays a greater role in behavioral sensitization induced by repeated stimulant administration than in behaviors induced by acute stimulant administration (Karler et al, 1990; Vanderschuren and Kalivas, 2000).

The question remains as to why baclofen infusions into the VTA or NA would affect CPu gene expression or infusion into the SN would affect NA gene expression. One

Table 3 Intra-SN Baclofen Infusion Decreases Amphetamine-Induced Striatal Neuropeptide Gene Expression

Region	Group	PPD	PPT	PPE	SGII
CPu	A/S	362±131	2523±111	4008±25	265±17
	B/S	376±16	2724±130	4463±26	310±16
	A/AM	547±22***	3808±180***	5728±30***	480±36**
	B/AM	429±13^^^	2932±121^^^	4001±24^^^	371±16^
NA shell	A/S	393±22	721±34	530±34	384±15
	B/S	405±21	678±53	543±19	329±16
	A/AM	521±19***	887±58***	619±27*	559±22*
	B/AM	384±15^^^	734±25^^^	597±26	398±19^
NA core	A/S	624±25	883±75	2688±166	403±22
	B/S	581±36	838±62	2774±135	438±41
	A/AM	777±51***	1138±50**	3753±190***	600±32***
	B/AM	552±31^^^	878±39^^	2704±104^^^	473±20^^

Integrated density values are expressed in mean±SEM in thousands.
A/S = ACSF+saline ($n = 7$); B/S = baclofen+saline ($n = 8$); A/AM = ACSF+amphetamine ($n = 8$); B/AM = baclofen+amphetamine ($n = 7$).
*$P < 0.05$ vs ACSF+saline; **$P < 0.01$ vs ACSF+saline; ***$P < 0.001$ vs ACSF+saline.
^$P < 0.05$ vs ACSF+amphetamine; ^^$P < 0.01$ vs ACSF+amphetamine; ^^^$P < 0.001$ vs ACSF+amphetamine.

Table 4 Effects of Intracranial Baclofen Infusion on Amphetamine-Induced Behaviors and Striatal Neuropeptide Gene Expression

	VTA	NA	SN	CPu	mPFC
Vertical activity	⇓	↓	↓	↔	↔
Total distance traveled	↔	↔	↔	↔	↔
Neuropeptide gene expression	↓	↓	↓	ND	ND

⇓ baclofen+amphetamine vs ACSF+amphetamine.
↓ baclofen+amphetamine vs ACSF+amphetamine.
↔ No change, ND not done.

possible explanation is that these interactions may be mediated by the often overlooked, direct GABAergic projection from the NA core to the SN and the dopaminergic projection from the VTA to ventromedial CPu (Nauta et al, 1978; Loughlin and Fallon, 1982; Gerfen et al, 1987; Heimer et al, 1991; Zahm and Heimer, 1993). These convergent connections that allow information to flow between dorsal and ventral striatal circuitries of rodents may be similar to those more recently described in the primate as 'spirals' (Haber et al, 2000). By way of this convergence, GABA$_B$ receptors have several presynaptic and postsynaptic targets in VTA, SN, and NA through which they can exert their effects on amphetamine-induced behavior and striatal neuropeptide gene expression.

CONCLUSION

The present study demonstrated that interactions between the VTA, NA, and SN mediate baclofen's ability to decrease amphetamine-induced rearing and neuropeptide gene expression in the striatum. Thus, stimulation of GABA$_B$ receptors in multiple sites in the motive circuit contributes to the initial behavioral and genomic responses to amphetamine.

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