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Alterations in γ-aminobutyric acid$_B$ receptor binding in the rat brain after reinstatement of cocaine-seeking behavior

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Abstract:
We examined neuroadaptive changes in $GABA_B$ receptor binding following reinstatement of cocaine-seeking behavior in rat brain structures using a "yoked" procedure and quantitative autoradiographic analysis. To estimate the distribution of $GABA_B$ receptors in several brain areas, we used [^{3}H]CGP 54626, a $GABA_B$ receptor antagonist. The binding of [^{3}H]CGP 54626 in the nucleus accumbens and the amygdaloid complex was decreased by about 20% in rats that actively administered cocaine. Similar decreases were seen in the animals that were passively administered cocaine; these rats also demonstrated decreased $GABA_B$ receptor binding in the prefrontal and frontal cortices, septum and dorsal striatum. The binding of [^{3}H]CGP 54626 in several rat brain areas was decreased during 10-day withdrawal from self-administered cocaine. The cocaine-priming dose (10 mg/kg, *ip*) induced a significant increase of [^{3}H]CGP 54626 binding in the core of the nucleus accumbens, substantia nigra (reticular part), prefrontal and frontal cortices and septum in rats withdrawn from cocaine self-administration. The presentation of the conditioned stimulus (tone + light) associated with previous cocaine self-administration induced a significant decrease of [^{3}H]CGP 54626 binding in the mediodorsal thalamic nucleus and amygdaloid complex in the rats withdrawn from cocaine self-administration. Increases in $GABA_B$ receptor binding in limbic regions during cocaine-induced reinstatement likely reflect motivational states that were present during active drug self-administration.

Key words:
cocaine-seeking behavior, $GABA_B$ receptors, quantitative autoradiography, yoked procedure

Introduction

Cocaine is one of the most powerful addictive substances in humans; its abusers are at a high risk of relapse. The factors responsible for cocaine relapse are not completely understood; however, research on humans provides evidence that relapse to cocaine use or cocaine craving can be initiated by multiple triggers including self-administered drug or drug-associated environmental cues [4, 5, 17]. In preclinical studies, relapse can be modeled by a reinstatement procedure in which laboratory animals are trained to self-administer drugs and then subjected to extinction training

during which (in an operant version of this procedure) lever presses are not reinforced with drugs. Reinstatement of the extinguished lever response (the operational measure of drug-seeking) is then determined for various conditions: non-contingent priming injections of the drug [6, 17], exposure to cues associated with drug intake [14] or exposure to stress [8]. The reinstatement model has substantial face validity for modeling the activation of craving and arousal by environmentally conditioned stimuli in drug-dependent individuals.

Recent human and rat studies suggest that γ-aminobutyric acid $(GABA)_B$ ligands may be a promising pharmacotherapy for cocaine addiction. In clinical trials, baclofen, a $GABA_B$ receptor agonist, reduced cocaine use in heavy cocaine addicts [19] and decreased limbic activation during cue-induced cocaine craving [2]. In preclinical behavioral studies, acute stimulation of $GABA_B$ receptors by agonists or positive allosteric modulators decreases reinstatement of cocaine-reinforced responding [3, 7, 9]. More recently, we also demonstrated that tonic activation of $GABA_B$ receptors in rats is required for cocaine-seeking behavior since the selective $GABA_B$ receptor antagonist SCH 50911 effectively reduced cue-induced relapse and attenuated cocaine-induced relapse [9]. $GABA_B$ receptors seem to also facilitate neuroadaptations following cocaine exposure. Thus, chronic cocaine administration decreases the functional coupling of $GABA_B$ receptors in the rat ventral tegmental area [13] as well as $GABA_B$ receptor functional responsiveness in the medial prefrontal cortex [12] and in the dorsolateral septal nucleus [18]. We found that $GABA_B$ receptor-selective radioligand binding was decreased in several rat brain areas after 10-day withdrawal from self-administered cocaine, but not from passive cocaine administration [11].

In the present study, we wished to determine if actively self-administered cocaine produces any associated neuroadaptations in brain $GABA_B$ receptors during cocaine-seeking behavior. To investigate this question, we used a quantitative autoradiographic analysis. We employed a "yoked" procedure in which each experimental animal was paired with a rat serving as a "yoked" control. This "yoked" control received an injection of saline (which was not contingent on responding) each time the paired rat self-administered a response-contingent injection of cocaine.

Materials and Methods

Animals

Male Wistar rats (280–300 g) delivered by a licensed breeder (T. Górzkowska, Warszawa, Poland) were housed individually in standard plastic rodent cages in a colony room maintained at 20 ± 1°C and at 40–50% humidity under a 12-h light-dark cycle (lights on at 6.00 a.m.). Animals had free access to food (Labofeed pellets) and water during the 7-day habituation period. Rats were then maintained on limited water during the initial training sessions (see below). All experiments were conducted during the light phase of the light-dark cycle (between 8.00 a.m. – 3.00 p.m.) and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with approval of the Bioethics Commission as compliant with the Polish Law (21 August 1997). The animals were experimentally naive.

Behavioral experiments

Drug

Cocaine hydrochloride (National Institute on Drug Abuse, RTI International, USA) was dissolved in sterile 0.9% NaCl. Cocaine was given either *iv* (0.05 ml/infusion) or *ip* (1 ml/kg).

Surgery

After one week in quarantine, animals were water-deprived for 18 h and then trained for 2 h daily to press a lever for water reinforcement on a fixed ratio (FR) 1 schedule of reinforcement. On the third day of training, the number of responses required to produce reinforcement was increased to a final value of five (a 5-response FR schedule of reinforcement). During this phase of training, the amount of water that each animal received was restricted to that given during daily training sessions and after sessions for 10 min. Two days following lever-press training and free access to water, the rats were anesthetized with ketamine HCl (75 mg/kg, Bioketan; Biowet, Puławy, Poland) and xylazine (5 mg/kg, Sedazin; Biowet, Puławy, Poland) and chronically implanted with a silastic catheter in the external jugular vein, as described previously [10]. For catheter implantation, a guide

cannula (C313G; Plastics One Inc., Wallingford, USA) was attached to microrenathane tubing (MRE-040; Sandown Chemicals Ltd., Hampton, UK) and polypropylene mesh (Bard Mesh; Davol Inc., Cranston, USA) by dental cement; then, it was inserted under the skin between the shoulder blades and exited the skin *via* a dermal biopsy hole (3 mm). The other end of the tubing was threaded under the skin, inserted 3 cm into the right jugular vein, and then sutured securely to the underlying muscles. Catheters were flushed each day with 0.1-ml saline solution containing heparin (70 U/ml) and 0.1-ml cephazolin solution (10 mg/ml; Biochemie GmbH, Kundl, Austria). Catheter patency was tested periodically, or whenever an animal displayed behavior outside baseline parameters, with the ultrashort-acting barbiturate anesthetic methohexital (10 mg/kg, *iv*) for loss of consciousness within 5 s.

Apparatus

Cocaine self-administration experiments were conducted in sixteen standard operant chambers (Med-Associates, St. Albans, USA). Each chamber was equipped with a 24-V house light (located on the ceiling), two retractable levers on one wall, a water-filled dispenser mounted equidistant between levers, a white circular stimulus light illuminated by a 24-V bulb above each lever and a tone generator. Lever pressing on one of the levers (defined as "active") resulted in drug delivery to the animal when scheduled (FR 5) requirements were met, whereas presses on the other lever (defined as "inactive") were recorded but not reinforced. Completion of each FR 5 requirement produced *iv* infusions of cocaine through liquid swivel (Instech, Plymouth Meeting, USA) *via* an infusion pump (Model 3.33 RPM, Med-Associates, St. Albans, USA). The position of the "active" and "inactive" levers remained unchanged throughout the study. A house light was on during the experimental sessions. The operant chambers were enclosed in ventilated, sound-attenuating cubicles (Med-Associates, St. Albans, USA) and controlled by an IBM-compatible computer using the MED Associates MED-PC software package.

Experimental procedures

The experimental design is shown in Table 1. Separate groups of rats were trained to self-administer cocaine ("self administration" group) and to reinstate cocaine-seeking behavior by either cocaine alone or by cue-following cocaine self-administration associated with the conditioned stimulus. A "yoked" procedure was used in which rats were tested simultaneously in groups of two, with one rat actively self-administering cocaine and the second receiving yoked injections of saline.

Maintenance

After a 10-day post-surgery recovery period, all animals were deprived of water for 18 h and trained to press the lever according to a FR 5 schedule of water reinforcement over a 2-h session. Then, the subjects were given access to cocaine during 2-h daily sessions performed 6 days/week (maintenance); from that time, they were given water *ad libitum*. The house light was on throughout each session. Each completion of five presses on the "active" lever complex (FR 5 schedule) resulted in a 5-s infusion of cocaine (0.5 mg/kg per 0.1 ml) and 5-s presentation of a stimulus complex (activation of the white stimulus light directly above the "active" lever together with activation of the tone generator, 2000 Hz; 15 dB above ambient noise levels). Following each injection, there was a 20-s time-out period during which responding was recorded but had no programmed consequences. Response on the "inactive" lever never resulted in cocaine delivery. Acquisition of the operant response lasted a minimum of 10 days until subjects met the following criteria: minimum requirement of 25 reinforcements with an average of 6 days and active lever presses with an average of 6 consecutive days and a standard deviation within those 6 days of < 10%. These criteria were selected based on our prior experi-

Tab. 1. Experimental design

Experimental design			Group number
Maintenance	Extinction	Reinstatement	
Cocaine self-administration (0.5 mg/kg/infusion)	Saline	Cocaine Saline	1 1a
"Yoked" saline	Saline	Cocaine Saline	1b 1c
Cocaine self-administration (0.5 mg/kg/infusion)	Saline	Cue –	2 2a
"Yoked" saline	Saline	Cue –	2b 2c

ments [10]. Once stable rates of responding were established, rats were subjected to extinction/reinstatement sessions. During maintenance, subjects administered an average of 15–18 mg/kg cocaine (*iv*) across the 2 h session.

Extinction and reinstatement

During extinction sessions, subjects had 2-h daily training sessions with no delivery of cocaine and no presentation of the conditioned stimulus. Once they reached the extinction criteria (a minimum of 10 extinction days with responses on the active lever below 10% of the level observed during at least three consecutive maintenance days), the separate groups of rats were tested for response reinstatement induced by a non-contingent presentation of the self-administered reinforcer (cocaine, 10 mg/kg, *ip*) or by a discrete contextual cue (tone + light previously paired with cocaine self-administration). During the reinstatement tests (2-h sessions), active lever presses on the FR 5 schedule resulted only in an *iv* injection of saline.

Quantitative $GABA_B$ receptor autoradiography

Immediately following behavioral experiments, rats were killed by decapitation. The brains were rapidly dissected and frozen by immersion in an *n*-heptane dry-ice bath and stored at –70° C until sectioned. Consecutive coronal sections (12 μm) were cut on a cryostat (Leica CM 1850, Germany) at –22 ± 2°C and were thaw-mounted on gelatin-coated slides. Five coronal sections were mounted on a single slide and stored at –70°C. The slides were kept at –20°C for 30 min and at room temperature for 45 min and then preincubated for 15 min in Krebs-Henseleit buffer (containing 120 mM NaCl, 6 mM glucose, 20 mM Tris, 4.7 mM KCl, 1.8 mM $CaCl_2$ $2H_2O$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, pH 7.4) as described previously [1, 16]. Total binding was performed by incubating [^{3}H]CGP 54626 (American Radiolabeled Chemicals, USA; specific activity: 50 Ci/mmol; 2.4 nM) for 2 h at room temperature in the same buffer with the appropriate tissue sections. To determine the extent of nonspecific binding, parallel sections were incubated in the presence of 10^{-2} M GABA (Tocris Cookson, Bristol, UK), which completely blocked nonspecific binding. Following the incubation period, tissue sections were washed twice in ice-cold (4°C) Krebs-Henseleit buffer for 30 s and twice in distilled water for 30 s and then dried with cool air.

Radiolabeled dried tissue sections were exposed to tritium-sensitive screens (^{3}H-Fujifilm imaging plates; Bas-TR2025, Fuji Photo Film, Tokyo, Japan) for 10 days at room temperature. Quantitative analysis was performed using the MCID image-processing system (Imaging Research, Brock University, Canada). Optical densities of gray values on the film were converted into bound radioactivity with a polynomial regression curve derived from autoradiographic [^{3}H]microscales (RPA 510, Amersham, UK) used as calibration markers. Data (fmol/mg tissue) are expressed as a mean of the percentage of the control level ± SEM shown in Table 2, for 6–8 animals per group. Signal binding was analyzed in several brain areas (Fig. 1, 2), which were identified by comparing autoradiographic images with appropriate figures from the rat brain atlas of Paxinos and Watson [15]. For each brain structure, data were collected from at least four sections per animal, bilaterally.

Statistical analyses

In behavioral experiments, the number of responses on the active and inactive lever were analyzed using paired Student's *t*-test or two-way analysis of variance (ANOVA) for repeated measures. *Post-hoc* Newman-Keuls' test was performed to locate differences between group means. The criterion for statistically significant differences was set at $p < 0.05$.

In autoradiagraphic analyses, optical densities (converted into bound radioactivity) were analyzed using a one- or two-way ANOVA. *Post-hoc* Dunnett's or Newman-Keuls' tests were performed to locate differences between group means. The criterion for statistically significant differences was set at $p < 0.05$.

Results

Behavioral experiments

Animals that self-administered cocaine (see Tab. 1) showed stable responses rates during the last six self-administration sessions, with less than 10% variability in daily cocaine intake. The mean cocaine infusions (± SEM) per day during the last six self-

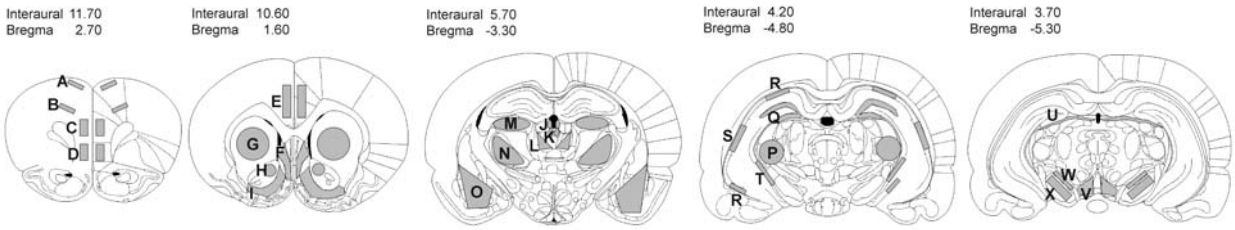

Fig. 1. The brain regions used for quantitative analysis, chosen according to Paxinos and Watson [14]. The grey outlines show the brain areas in which optical densities were quantified. Abbreviations: A – frontal cortex (layers I–III), B – frontal cortex (layers IV–VI), C – prelimbic cortex, D – infralimbic cortex, E – cingular cortex, F – septum, G – dorsal striatum, H – nucleus accumbens (core), I – nucleus accumbens (shell), J – habenula, K – thalamus (paraventricular nucleus), L – thalamus (laterodorsal nucleus), M – thalamus (mediodorsal nucleus), N – thalamus (ventroposterolateral and ventroposteromedial nuclei), O – amygdaloid complex (the basolateral, basomedial, central, lateral nuclei and intra amygdaloid division), P – medial geniculate nucleus, Q – hippocampus (dentate gyrus), R – hippocampus (CA1 layer), S – hippocampus (CA2 layer), T – hippocampus (CA3 layer), U – superior colliculus, V – ventral tegmental area, W – substantia nigra (compact part), X – substantia nigra (reticular part)

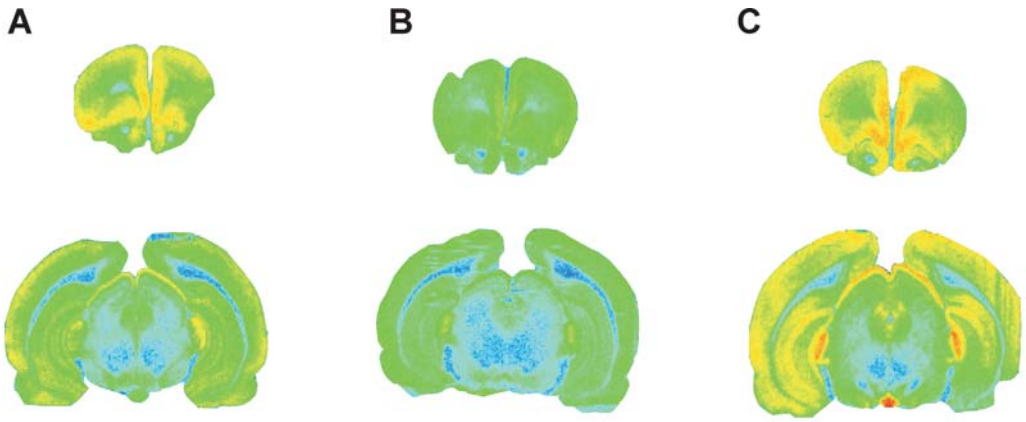

Fig. 2. Representative autoradiograms of [^{3}H]CGP 54626-specific binding sites in the brain of rats following "yoked" injections of saline **(A)**, cocaine-associated cue-induced reinstatement of cocaine-seeking behavior **(B)** and cocaine-primed reinstatement of cocaine-seeking behavior **(C)**

administration sessions in groups 1 and 2 were 35 ± 4.1 and 30 ± 6.4, respectively. During 14 sessions of cocaine self-administration, animals received approximately 171–187 mg/kg throughout the experiment. Rats responded significantly more on the active lever than on the inactive lever ($p < 0.001$) (Fig. 3).

The extinction phase lasted 10 days, during which neither drug nor drug-paired stimuli were available. Extinction resulted in gradually decreasing responses on the active lever. During the last three sessions of extinction, active lever presses did not differ by more than 10% (Fig. 3).

After 10 days of extinction, the rats were tested for response reinstatement induced by cocaine (10 mg/kg, *ip*) (Fig. 3). For group 1, the ANOVA for repeated measures of active and inactive lever presses during the last self-administration session, the last extinction session and the cocaine-induced reinstatement test indicated a significant lever × session interaction [$F(2, 36) = 8.4$, $p < 0.001$], as well as lever [$F(1, 36) = 28.9$, $p < 0.001$] and session [$F(2, 36) = 8.2$, $p < 0.001$] main effects.

After 10 days of extinction, the rats were tested for response reinstatement induced by the presentation of discrete contextual cues (tone + light previously paired with cocaine self-administration) (Fig. 3). For group 2, the ANOVA for repeated measures of active and inactive lever presses during the last self-admi-

Tab. 2. Density of [^{3}H]CGP 54626 binding to $GABA_B$ receptors in various rat brain areas

Brain area	"Yoked" saline/saline (*ip*)	"Yoked" saline/–
Frontal cortex		
• layers I–III	362 ± 10	248 ± 14
• IV–VI layers	178 ± 4	180 ± 8
Prefrontal cortex		
• cingular cortex	294 ± 10	272 ± 10
• infralimbic cortex	308 ± 16	306 ± 12
• prelimbic cortex	302 ± 16	292 ± 14
Septum	267 ± 14	244 ± 16
Dorsal striatum	174 ± 6	176 ± 6
Nucleus accumbens		
• core	178 ± 10	186 ± 8
• shell	186 ± 12	180 ± 8
Habenula	422 ± 64	432 ± 39
Thalamus		
• paraventricular nucleus	498 ± 76	428 ± 94
• laterodorsal nucleus	348 ± 14	364 ± 22
• mediodorsal nucleus	334 ± 20	338 ± 28
• ventroposterolateral and ventroposteromedial nuclei	228 ± 10	254 ± 14
Amygdaloid complex	334 ± 8	330 ± 12
Medial geniculate nucleus	296 ± 6	310 ± 12
Hippocampus		
• dentate gyrus	282 ± 16	258 ± 14
• CA1 layer	230 ± 6	214 ± 12
• CA2 layer	224 ± 8	228 ± 8
• CA3 layer	260 ± 10	214 ± 12
Ventral tegmental area	136 ± 6	130 ± 8
Substantia nigra		
• compact part	144 ± 8	144 ± 8
• reticular part	114 ± 6	134 ± 8
Superior colliculus	324 ± 16	294 ± 6

The number of animals per group was as follows: "yoked" saline/saline group (*ip*) (group 1c; n = 8), "yoked" saline/– group (group 2c; n = 8). Data are presented as the mean specific binding (fmol/mg tissue) (± SEM). For details on the experimental protocol see Table 1

Fig. 3. The figure shows the number of active (*cross bars*) and inactive (*white bars*) lever presses during stabilized self-administration (0.5 mg/kg/infusion) (last three sessions; **A**), extinction (last three sessions; **B**), and reinstatement of cocaine-seeking behavior induced by cocaine priming (10 mg/kg, *ip*) or cocaine-associated cue **(C)** in rats. Each bar represents the mean (± SEM) of 6–8 animals. * $p < 0.001$ *vs.* active lever presses during stabilized self-administration or during reinstatement; # $p < 0.001$ *vs.* active lever presses during stabilized self-administration; + $p < 0.001$ *vs.* extinction of group 1; ^ $p < 0.01$ *vs.* extinction group 2

nistration session, the last extinction session and the cue-induced reinstatement test indicated a significant lever × session interaction [$F(2, 42) = 18.9$, $p < 0.001$] as well as lever [$F(1, 42) = 41.5$, $p < 0.001$] and session [$F(2, 42) = 17.9$, $p < 0.01$] main effects.

Autoradiography

The administration of cocaine (10 mg/kg, *ip*) induced a significant increase of [^{3}H]CGP 54626-specific binding in the core of the nucleus accumbens (~32%), the substantia nigra (reticular part) (~27%), the prefrontal cortex (the prelimbic, infralimbic and cingular cortex) (21–27%), the frontal cortex (~20%) and the septum (18%) in rats withdrawn from cocaine self-administration (Tab. 3).

Challenge with *ip* saline in rats withdrawn from cocaine self-administration evoked a significant decrease (~25%) in [^{3}H]CGP 54626-specific binding in the laterodorsal and mediodorsal thalamic nuclei (Tab. 3).

The challenge dose of cocaine (10 mg/kg, *ip*) in rats withdrawn from passive saline administration ("yoked" saline control) evoked a significant decrease (~25%) in [^{3}H]CGP 54626-specific binding in the frontal cortex (18–25%), the cingular and infralimbic cortices (16–18%), the shell of the nucleus accumbens (19%), thalamic nuclei (22–29%), the amygdaloid complex (34%), the hippocampus (22–35%), the substantia nigra (compact part) (25%), the medial geniculate nucleus (22%) and the superior colliculus (34%) (Tab. 3).

Presentation of the conditioned stimulus (tone + light) associated with previous cocaine self-administration induced a significant decrease of [^{3}H]CGP 54626-specific binding in the mediodorsal thalamic nucleus (~37%) and the amygdaloid complex (~27%) in rats withdrawn from cocaine self-administration (Tab. 4).

No changes in [^{3}H]CGP 54626-specific binding were seen following the presentation of the stimulus (tone + light) in rats withdrawn from passive saline administration ("yoked" saline control) (Tab. 4).

Discussion

Many preclinical studies and some controlled clinical trials indicate the involvement of $GABA_B$ receptors in cocaine addictive behaviors (see Introduction). In the present study, we evaluated the effects of the reinstatement of cocaine seeking-behavior on *ex vivo* $GABA_B$ receptor binding in several rat brain areas by quantitative autoradiography of $GABA_B$ receptors using labeled [^{3}H]CGP 54626.

Our results indicate that the cocaine-induced reinstatement of seeking behavior (confirmed by the behavioral experiment) induced increases of $GABA_B$ receptor binding in numerous rat brain structures. The highest increase (~30%) in [^{3}H]CGP 54626 binding was seen in the core of the nucleus accumbens, while a smaller (~20%) increase was observed in the prefrontal and frontal cortices, the septum, the dorsal striatum and the substantia nigra (reticular part). These changes do not seem to be linked directly with the pharmacological action of cocaine, since the same dose of the drug given to animals withdrawn from passive saline administration ("yoked" saline group) induced either decreases or no change in binding to $GABA_B$ receptors. Furthermore, the increased binding of $GABA_B$ receptors following cocaine *ip* challenge is not a stress-related response to *ip* injection since the animals withdrawn from cocaine self-administration and challenged with *ip* injection of saline showed no alteration in [^{3}H]CGP 54626 binding. The increases in [^{3}H]CGP 54626 binding may be compensatory to a decrease of GABA-ergic transmission. As indicated recently by *in vivo* microdialysis, noncontingent cocaine administration during reinstatement of cocaine-seeking behavior decreased GABA release in the ventral pallidum [20]. Increased $GABA_B$ receptor binding during presentation of a cocaine-priming dose may partly confirm our earlier behavioral results showing that agonists of these receptors, administered peripherally, reduced cocaine-induced reinstatement of cocaine-seeking behavior [10]. Whether this increased binding is a consequence of chronic cocaine exposure or represents a vulnerability to develop cocaine addiction remains to be established.

In direct contrast to the cocaine-induced reinstatement, the present quantitative autoradiographic analyses showed that presentation of the conditioned stimulus (the cocaine-associated cue) induced a significant decrease (16–36%) in $GABA_B$ receptor binding in the mediodorsal thalamic nucleus and the amygdaloid complex. The above changes do not seem to be related to the presentation of the complex stimulus since the same procedure did not evoke any change in [^{3}H]CGP 54626 binding in the "yoked" saline group. However, it should be emphasized that the decreases in radioligand binding were observed during withdrawal from cocaine self-administration [11]; therefore, it is difficult to conclude whether they persist after 10 days of withdrawal from chronic daily exposure to self-administered cocaine or result from the conditioned cue.

Since we used only one concentration of the ligand in this autoradiographic experiment (based on the previous report of Bischoff et al. [1]), it was therefore

Tab. 3. Effects of cocaine (10 mg/kg, *ip*)-induced reinstatement of cocaine-seeking behavior on [^{3}H]CGP 54626 binding to $GABA_B$ receptors in various rat brain areas

Brain area	"Yoked" saline/saline (*ip*)	"Yoked" saline/cocaine (10 mg/kg, *ip*)	Cocaine self-administration/saline (*ip*)	Cocaine self-administration/cocaine (10 mg/kg, *ip*)
Frontal cortex				
• layers I–III	100 ± 3.8	74.5 ± 5.3***	85.3 ± 8.7	122.1 ± 3.5*** ##+++
• layers IV–VI	100 ± 3.2	81.8 ± 5.9*	99.9 ± 5.3	119.2 ± 1.3*** ##+++
Prefrontal cortex				
• cingular cortex	100 ± 3.7	84.1 ± 3.2**	87.3 ± 5.7	120.7 ± 3.2*** ##++
• infralimbic cortex	100 ± 4.5	81.7 ± 5.6*	100.3 ± 8.6	125.6 ± 3*** ##+++
• prelimbic cortex	100 ± 6.5	82.3 ± 6.3	103.1 ± 7.3	127.4 ± 3.7** ##+++
Septum	100 ± 5.9	98.5 ± 2.8	93.9 ± 2.3	118.2 ± 5.1** ##++
Dorsal striatum	100 ± 3.1	89.7 ± 5.8	98.9 ± 4.2	110 ± 3.5* # +
Nucleus accumbens				
• core	100 ± 4.8	91.9 ± 4.3	108.9 ± 7.5	132.1 ± 4*** #+++
• shell	100 ± 5.9	81.3 ± 3.2*	98.5 ± 6.2	115 ± 2.7#+++
Habenula	100 ±17	125.5 ± 5.7	82.6 ± 3.5	98.7 ± 2.8++
Thalamus				
• paraventricular nucleus	100 ±13.1	128.8 ± 7.1	72.6 ± 6.5	81.9 ± 7.8+++
• laterodorsal nucleus	100 ± 3.2	70.7 ± 3.5***	77.5 ± 6.7**	94.2 ± 2.4+++
• mediodorsal nucleus	100 ± 6.3	73.8 ± 6.3**	71.6 ± 5.4**	84.3 ± 6.4
• ventroposterolateral and ventroposteromedial nuclei	100 ± 3.8	78.1 ± 4.7**	94.3 ± 6.9	105.7 ± 2.2++
Amygdaloid complex	100 ± 1.9	65.5 ± 4.4***	93.2 ± 9.5	94.5 ± 1.8+++
Medial geniculate nucleus	100 ± 3	77.5 ± 3.7***	94.5 ± 9.9	105.7 ± 2.5+++
Hippocampus				
• dentate gyrus	100 ± 5.2	65.2 ± 3.3***	82.1 ± 9.3	100.4 ± 3+++
• CA1 layer	100 ± 2.7	71.3 ± 3.9***	91.9 ± 9.1	108.3 ± 2.9+++
• CA2 layer	100 ± 5.1	78.4 ± 4.3**	95 ± 10.9	112.7 ± 5.6+++
• CA3 layer	100 ± 4.9	71 ± 4.4***	92.3 ± 10.5	110.4 ± 3.4+++
Ventral tegmental area	100 ± 3.9	91.2 ± 5.8	95.6 ± 10.1	114.2 ± 5.8+++
Substantia nigra				
• compact part	100 ± 6.5	75.2 ± 2.7***	85.2 ± 13.1	106.5 ± 4.5+++
• reticular part	100 ± 4.9	112.6 ± 4.5	98.1 ± 14.6	127.4 ± 5.9**
Superior colliculus	100 ± 4.7	66.1 ± 4.7***	87.5 ± 11	105.4 ± 3.2+++

The number of animals in each group was as follows: "yoked" saline/saline group (*ip*) (group 1c; n = 8), "yoked" saline/cocaine group (10 mg/kg, *ip*) (group 1b; n = 6), cocaine self-administration/saline group (*ip*) (group 1a; n = 8) and cocaine self-administration/cocaine group (10 mg/kg, *ip*) (group 1; n = 8). Data are presented as the mean percent specific binding (fmol/mg tissue) (± SEM) relative to control ("yoked" saline/saline (*ip*) and analyzed using a two-way ANOVA and *post-hoc* Newman-Keuls' test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* "yoked" saline/saline (*ip*); # $p < 0.05$, ## $p < 0.01$ *vs.* cocaine self-administration/saline (*ip*); + $p < 0.05$, ++ $p < 0.01$; +++ $p < 0.01$ *vs.* "yoked" saline/cocaine (10 mg/kg, *ip*). For details on the experimental protocol, see Table 1

Tab. 4. Effects of the cocaine-associated cue (tone + light)-induced reinstatement of cocaine-seeking behavior on [^{3}H]CGP 54626 binding to $GABA_B$ receptors in various rat brain areas

Brain area	"Yoked" saline/–	"Yoked" saline/cue	Cocaine self-administration/–	Cocaine self-administration/cue
Frontal cortex				
• layers I–III	100 ± 3.5	95 ± 5.5	82.3 ± 9.7	96 ± 5.6
• layers IV–VI	100 ± 2.7	100.5 ± 6.2	89.9 ± 7.3	98.6 ± 6.4
Prefrontal cortex				
• cingular cortex	100 ± 5.5	108.5 ± 4.5	85.3 ± 9.7	97.3 ± 5
• infralimbic cortex	100 ± 5.5	99.8 ± 4.2	100.5 ± 8.9	103.5 ± 5.7
• prelimbic cortex	100 ± 5.5	97.2 ± 4.4	101.1 ± 9.3	103.5 ± 7
Septum	100 ± 7.1	100.5 ± 3.1	83.9 ± 2.5	83.9 ± 9.1
Dorsal striatum	100 ± 3	95.9 ± 4.9	88.7 ± 4.5	108 ± 4.2
Nucleus accumbens				
• core	100 ± 5.6	104.7 ± 4.7	100.1 ± 8.5	115.8 ± 5.5
• shell	100 ± 6.3	97 ± 4.7	96.5 ± 5.3	104 ± 4.4
Habenula	100 ± 6.3	89.5 ± 8.7	85.2 ± 2.5	79.4 ± 13.6
Thalamus				
• paraventricular nucleus	100 ± 15.4	86.8 ± 18.7	73.6 ± 8.2	78.1 ± 16.6
• laterodorsal nucleus	100 ± 3.3	92.1 ± 5.8	79.5 ± 7.5**	84.3 ± 4.2
• mediodorsal nucleus	100 ± 5.3	89.2 ± 6.9	61.1 ± 5.4**	63.4 ± 5.5***^^
• ventroposterolateral and ventroposteromedial nuclei	100 ± 4.4	100.7 ± 5.4	94.5 ± 8.1	92.7 ± 4.8
Amygdaloid complex	100 ± 2.2	98.7 ± 3.3	84.2 ± 8.3	83.6 ± 3.3**^^
Medial geniculate nucleus	100 ± 2.4	105 ± 4.3	85.5 ± 10.9	96.4 ± 3.7
Hippocampus	100 ± 3.9	93.4 ± 4.4		95 ± 2.8
• dentate gyrus	100 ± 5.6	91.5 ± 5.2	72.1 ± 11.3	90.8 ± 3.5
• CA1 layer	100 ± 2.9	93.1 ± 5	81.9 ± 8.1	100.7 ± 2.7
• CA2 layer	100 ± 4.4	91.8 ± 5.7	85 ± 10.9	95.9 ± 4.2
• CA3 layer	100 ± 4.4	99.1 ± 5.3	82.5 ± 10.2	99.3 ± 3.4
Ventral tegmental area	100 ± 4.5	96.2 ± 7.8	85.6 ± 10.2	109.0 ± 9.2
Substantia nigra				
• compact part	100 ± 5.5	99.6 ± 5.3	84.2 ± 13.1	94.5 ± 5.7
• reticular part	100 ± 4.4	115.6 ± 7.4	89.1 ± 14.8	118.0 ± 4.1
Superior colliculus	100 ± 4.9	91.1 ± 2.1	88.5 ± 11.1	91.5 ± 4.8

The number of animals in each group was as follows: "yoked" saline/– group (group 2c; n = 8), "yoked" saline/cue group (group 2b; n = 8), cocaine self-administration (0.5 mg/kg/infusion)/– group (group 2a; n = 8) and cocaine self-administration (0.5 mg/kg/infusion)/cue group (group 2; n = 8). Data are presented as the mean percent specific binding (fmol/mg tissue) (± SEM) relative to control ("yoked" saline). Data were analyzed using a two-way ANOVA and *post-hoc* Newman-Keuls' test. ** $p < 0.01$; *** $p < 0.001$ *vs.* "yoked" saline/–; ^^ $p < 0.01$ *vs.* "yoked" saline/cue. For details on the experimental protocol, see Table 1

not possible to determine whether the changes in [^{3}H]CGP 54626 binding were related to changes in the number of $GABA_B$ receptors, an altered affinity for that ligand or changes in $GABA_B$ receptor downstream signaling. Recently, Xi et al. [21] reported that cocaine self-administration did not alter total protein levels of $GABA_{B1}$ or $GABA_{B2}$ subunits, but it did reduce the functional capacity of $GABA_B$ receptors. To verify whether changes in radioligand binding in our studies were linked with alterations in B_{max} or K_D of $GABA_B$ receptor [^{3}H]CGP 39653 binding, further saturation experiments are required.

In summary, our results suggest that increases in $GABA_B$ receptor binding in limbic regions during cocaine-priming-induced reinstatement likely reflect motivational states originating from cocaine injection dependent on active drug self-administration.

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