Pharmacology Biochemistry and Behavior, Vol. 65, No. 3, pp. 399–406, 2000

0091-3057/00/$–see front matter

PII S0091-3057(99)00211-7

Reinforcing and Neurochemical Effects of Cocaine: Differences Among C57, DBA, and 129 Mice

ALEXANDER KUZMIN AND BJÖRN JOHANSSON

Section of Molecular Neuropharmacology, Department of Physiology and Pharmacology, Karolinska Institutet, SE-171 77 Stockholm, Sweden

Received 22 March 1999; Revised 30 August 1999; Accepted 3 September 1999

KUZMIN, A. AND B. JOHANSSON. *Reinforcing and neurochemical effects of cocaine: Differences Among C57, DBA, and 129 mice.* PHARMACOL BIOCHEM BEHAV **65**(3) 399–406, 2000.—C57BL/6J, DBA/2J, and 129/OlaHsd mice were compared as to their propensity to self-administer cocaine, the ability of cocaine injection to prevent extinction of nose poking in the absence of cocaine infusion, and cocaine's effect on NGFI-A and secretogranin II mRNA. Baseline nose-poke activity tended to be highest in C57 and lowest in DBA mice. C57 and DBA mice self-administered cocaine, as indicated by more frequent nose pokes in mice allowed to infuse cocaine through nose pokes than in mice passively receiving the same amount of cocaine. DBA mice had the larger ratio between active and yoked-control animals, but the response rate in DBA mice was four times lower, and cocaine intake 10 times lower than in C57 mice. The 129 mice showed little response to cocaine. C57 and DBA mice that had been self-administering cocaine showed an extinction of responding when infusions were stopped, preventable by IP cocaine (5 mg kg^{-1}). A single cocaine injection (2 mg kg^{-1}) increased NGFI-A mRNA and decreased secretogranin II mRNA in the caudate putamen in C57 mice. These effects were more widespread in DBA and absent in the 129 mice. The marked differences among mouse strains described here will be important to consider when transgenic mice are to be used in cocaine-related studies. © 2000 Elsevier Science Inc.

Self-administration Extinction Immediate-early gene NGFI-A Secretogranin II Inbred mouse strains

MICE provide an excellent model for studies of the genetic basis of drug intake. The mouse strains C57 and DBA are often compared in such studies, because they are genetically divergent, and are the two progenitor strains for the BXD congenic strains used in the mapping of chromosomal loci that influence drug related phenotypes [reviewed in (7)]. Targeted gene disruption, so-called knockout animals, has normally been made in substrains of the 129 mouse (25) that are then often mated with C57 mice. Because behavioral characteristics differ between the parental strains, the interpretation of behavioral responses in transgenic or knockout animals may be complicated.

There are several reports suggesting differences between strains in sensitivity to cocaine. Carney et al. (6) found that C57 mice readily acquired cocaine self-administration without prior drug experience, whereas DBA mice failed to acquire self-administration within a 5-day access period. Using the same technique, Grahame and Cunningham (11) demonstrated that cocaine was an effective reinforcer in both C57 and DBA strains. Recently, Rocha et al. (26) reported that, when a low unit dose of cocaine was made contingent upon the operant reaction, DBA mice met the cocaine self-administration acquisition criteria in 6 days, whereas even after 14 days none of the C57 mice were able to reach cocaine self-administration criteria. A possible explanation for the divergent results could be that complete dose–effect curves for cocaine reinforcement was not constructed in the studies mentioned. In one study, major differences between the 129 and C57 mice were found in behavioral tests (23).

The aim of the present study was to compare three different inbred mouse strains C57BL/6J (C57), DBA/2J (DBA), and 129OlaHsd (129) as to their sensitivity to reinforcing effects of increasing doses of IV cocaine. The 129/OlaHsd substrain of the 129 mice was chosen, as it is one of the most

Requests for reprints should be addressed to B. Johansson, Section of Molecular Neuropharmacology, Department of Physiology and Pharmacology, Karolinska Institutet, SE-171 77 Stockholm, Sweden.

common background strains of knockouts with behavioral phenotypes (24). In addition, we studied the effect of a single injection of cocaine on the expression of NGFI-A (nerve growth factor-induced clone A, also named zif/268) and secretogranin II, genes regulated by neuronal activity.

METHOD

Animals

Experiments were carried out on male DBA/2J, C57BL/6J (Bomholtgard, Denmark), and 129/OlaHsd (Harlan, UK) mice (18–22 g, age: 4–5 weeks). Animals were kept under standard laboratory conditions with unlimited access to food and water. Animals were housed 12 per cage in a light-controlled room (12 h light/dark cycle, light on at 0600 h) at 21°C and 60% humidity, and used in accordance with the European Community Council Directive. Approval was obtained from the Northern Ethics Board on Animal Experiments of Stockholm.

Drugs and Self-Administration Method

Cocaine (as hydrochloride, Sigma, St. Louis, MO) was dissolved in saline and the pH of the solution was adjusted to 7.2 with 0.01 N NaOH. Doses of cocaine HCl refer to the salt.

Self-administration by naive mice was initiated during a single 30-min session (21). The unit dose ranged from 10 to 320 μg per kg per infusion in a volume of 1.6 μl per infusion, at a fixed ratio = 1, i.e., each nose poke produced one infusion. Mice were tested in pairs in cages (8 × 8 × 8 cm) with a frontal hole (1.5 cm) for nose poking, fitted with infrared sensors (3 mm into the hole from the inner surface of the cage) interfaced to a computer controlling the syringe pump. Mice were partially immobilized by fixing their tails with adhesive tape to the horizontal surface. The tails protruded through a vertical slot in the back wall of the box.

First, the number of nose-poke reactions during a 10-min period was recorded for all experimental animals. On the basis of this 10-min test, pairs of mice were chosen so that animals in each pair exhibited approximately equal nose-poke activity. The matched pairs were thereafter (about 1-h delay from initial testing) placed into the experimental boxes, and cannulas (external diameter 0.4 mm) were introduced into the lateral tail veins of both animals in the pair. To verify the proper placement of the needles, test infusions (3 × 1.6 μl) were made. Improper positioning of the needle resulted in local paling of the tail. In this case the needle was removed and reintroduced. When needles were properly placed the response-contingent injections were activated. Each nose poke of one mouse (called the active mouse) resulted in a response-contingent infusion of 1.6 μl of cocaine solution or saline to both the active mouse and the yoked control. The injection had a constant duration of 1 s. The nose-poke counter was not interrupted during the injection. Nose pokes of the yoked-control animal were counted, but had no programmed consequences. The self-administration session lasted 30 min, after which the animals were labeled and returned to their home cages.

About 15% of active mice failed to display sufficient nose-poke activity to acquire cocaine self-administration These pairs were eliminated from data analysis. The criterion for elimination of a pair was low nose-poke activity in the active animal (nose pokes less than five in 30 min) and/or a blocked or wrongly positioned cannula. Investigation usually showed that the reason for atypically low activity was improper fixation of the animal, which kept it from reaching the infrared sensor. Other reasons for lack of self-administration were extravascular positioning of the cannula or its blockade by coagulated blood. Cannulas were routinely checked before and after each self-administration session.

As a quantitative measure of the reinforcing effect of the drug solution, an *r* (ratio) criterion was used. This was calculated as the $\log_{10}$ of the ratio between the cumulative number of nose pokes by the active and yoked-control mouse in the matched pair during a 30-min self-administration or extinction session. Logarithms were used to normalize the distribution of the data. If an infused solution has a reinforcing effect, the number of operant reactions (nose pokes) in active mice will exceed that in the yoked-control animal independent of any motor stimulant effect of the drug and the $\log_{10}$ of the ratio will become a positive value. Because mice were matched, the initial (predrug) ratio was always close to unity. Because of the matching, the *r* criterion was slightly modified compared to our previous publications (21), as the subtraction of the logarithm of the initial ratio was not necessary.

The numbers of nose pokes for active and yoked-control mice in experimental groups were analyzed using two-way ANOVA; mode (active mice vs. yoked-control mice) and dose (saline and different unit doses of cocaine) were the independent factors. Next, paired comparison (two-tailed Student's *t*-test) was performed to compare 1) active and yoked control activity within unit dose group, and 2) active and yoked control activity in cocaine dose group and saline group. The whole study was designed as a between subjects (independent groups) experiment (i.e., each treatment described was done on a separate set of animals).

Next the ratio criterion was analyzed in a similar way: one-way ANOVA for ratio criteria (with cocaine unit dose as an independent factor) followed by paired comparison of ratio criteria (two-tailed Student's *t*-test) in cocaine unit dose groups with saline group value.

Extinction of Cocaine Reward-Associated Responding

On day 2, 24 h after the mice had been trained with a cocaine dose that produces self-administration behavior, extinction sessions were carried out. Both active and passive (yoked-control) mice were injected with saline or one of the doses of cocaine. All injections were made IP in a volume of 1 ml kg^{-1}. Immediately after, pairs of mice were placed into experimental boxes, but the needles were not introduced and cocaine infusions were not activated. As in the initiation session, the number of nose pokes of each mouse in the pair was counted and these data were analyzed using the *r* criterion.

Sectioning of Brains, In Situ Hybridization

For neurochemistry, mice (not previously used in behavioral experiments) were decapitated under carbon dioxide anesthesia 1 and 4 h after injection of cocaine (2 mg kg^{-1} in approx. 20 μl) into the lateral tail vein, and the brains were removed and rapidly frozen on dry ice. From each brain, coronal sections (14 μm) were cut with a Leitz cryostat and thaw mounted on Polysine slides (Menzel-Gläser, Germany). Sections for quantitative analysis of different brain structures were cut according to the Franklin and Paxinos (10) atlas at the level (IA) 5.5 mm. The slides were stored at −20°C for 2–4 weeks before being used.

The 45-mer NGFI-A probe (Scandinavian Gene Synthesis, Köping, Sweden) was complementary to nucleotides encoding amino acids 2–16 of mouse NGFI-A, and has been checked for specificity (33). The 44-mer secretogranin II (SG-II) probe

TABLE 1
BASAL NOSE-POKE ACTIVITY IN 10 MINUTES

	129 Mice	C57 Mice	DBA Mice
Active group	50.83 ± 5.28	82.63 ± 5.42	23.15 ± 1.83
Yoked control group	52.86 ± 5.22	83.55 ± 5.23	25.65 ± 2.06

Data as mean ± SEM, n = 40–60 mice. All three strains differ significantly from each other ($p < 0.01$; ANOVA followed by unpaired two-tailed Student's t-test).

was complementary to nucleotides 676–719 of mouse secretogranin II mRNA (28). Our secretogranin II probe gives the same labeling pattern in the rat and mouse brain as that described (30) using a different probe for the same target.

Oligodeoxyribonucleotides were labeled using terminal transferase (Amersham) with [^{35}S]-dATP (Amersham) to a specific activity of about 10^9 cpm μg^{-1}. Slides were hybridized for 16 h at 42°C in a cocktail containing 50% deionized formamide (Fluka), 4 × SSC, 1 × Denhardt's solution (Sigma), 1% sarcosyl (Sigma), 0.02 M $NaPO_4$ (pH 7.0), 10% dextran sulfate (Sigma), 0.5 mg ml^{-1} yeast tRNA (Sigma), 0.06 M dithiotreitol (Fluka), 0.1 mg ml^{-1} sheared salmon sperm DNA (Sigma) and labeled oligodeoxyribonucleotide probe (10^7 cpm ml^{-1}). Slides were washed 4 × 15 min in 1 × SSC at 55°C, then dipped briefly in water, 60, 95, and 99.5% ethanol, and air dried.

Finally, sections were apposed to Hyperfilm-Betamax (Amersham) for 4 weeks.

The optical density of the Hyperfilm was measured in areas corresponding to the core and shell parts of nucleus accumbens, the lateral and medial parts of the caudate putamen, prefrontal cortex (the combined pre- and infralimbic cortex was measured), and piriform cortex with a Microcomputer Imaging Device M4 (Imaging Research, Canada). Significant differences between two means were determined using one-way ANOVA followed by Student's t-test.

RESULTS

Initiation of IV Self-Administration

The mouse strains differed in basal nose-poke activity during the 10-min test before cocaine administration (ANOVA: $p < 0.01$, see Table 1). Thus, C57 mice were about four times more active than their DBA counterparts and the 129 mice exhibited an intermediate activity (i.e., C57 > the 129 > DBA). The ratio criterion during self-administration of saline was similar in all strains used and close to zero. During self-administration of saline, the nose-poke ratio was less than during the preceding 10-min test (in all likelihood due to habituation to the environment). When animals were followed for 1 h without drug/saline infusions (data not shown), it was found that about 80% of nose-poke activity occurred in the first 10-min period of testing. In DBA mice, the nose-poke activity tended to be lower than in C57 and the 129 strains during self-administration of saline (again C57 > the 129 > DBA), but the difference did not reach significance (p = 0.068; ANOVA).

Cocaine, when made contingent to the nose-poke responses of active mice, modified the activity in a different manner, depending on the unit dose, mouse strain used, and administration mode (active versus passive; see Table 2).

The unit dose of cocaine had a significant influence on the number of nose pokes in the 129 mice, as revealed by two-way ANOVA, $F(5, 60) = 4.73$, $p < 0.01$, but there were no differences between active and passive mice. Paired comparison revealed that only animals that received cocaine in a unit dose of 320 $\mu g\ kg^{-1}\ inf^{-1}$ showed a significant difference (a reduction) in nose-poke activity ($p < 0.05$) compared with the corresponding saline group. In agreement, we did not observe any significant influence of cocaine dose on the r criterion (Fig. 1A). There was a significant influence of cocaine unit dose on the cumulative intake of cocaine, $F(5, 30) = 8.87$, $p < 0.01$, Fig. 1B).

In C57 mice, there was a significant effect of cocaine dose, $F(5, 68) = 12.46$, $p < 0.01$, and administration mode, $F(1, 68) = 5.38$, $p < 0.05$, on the number of nose-poke responses. Also, the interaction between mode and dose was significant, $F(5, 68) = 2.27$, $p < 0.05$. There was a strong increase in the num-

TABLE 2
COCAINE ASSOCIATED NOSE-POKE ACTIVITY IN 30 MINUTES

Cocaine dose $\mu g\ kg^{-1}\ inf^{-1}$	129 Mice		C57 Mice		DBA Mice	
	Active	Control	Active	Control	Active	Control
0 (sal)	28.7 ± 6.5	31.2 ± 5.0	32.5 ± 6.6	32.5 ± 4.5	19.9 ± 3.4	24.8 ± 4.6
10					35.7 ± 3.6*	36.7 ± 8.9
20					31.8 ± 4.3*	21.3 ± 5.4
40	47.6 ± 7.2	43.8 ± 9.7	97.2 ± 16.5*	85.7 ± 10.9*	57.3 ± 7.3*†	25.2 ± 7.9
60					34.3 ± 5.6†	15.3 ± 4.6
80	56.2 ± 13.2	46.8 ± 5.1	127 ± 16.1*†	99.5 ± 15.0*	26.2 ± 5.8	22.2 ± 3.0
120	41.7 ± 9.0	38.3 ± 14.6	203.6 ± 19.8*†	131.9 ± 21.2*		
160	36.8 ± 6.3	26.6 ± 4.1	157.2 ± 36.6*	88.87 ± 7.1*		
240			94.7 ± 12.0*	121.3 ± 16.2*		
320	16.8 ± 1.6*	17.3 ± 1.8*				

Data as mean ± SEM, n = 8–10 mice.
*$p < 0.05$ (unpaired two-tailed Student's t-test) compared to the corresponding group with saline self-administration.
†$p < 0.05$ (unpaired two-tailed Student's t-test) compared to control mice receiving cocaine passively.

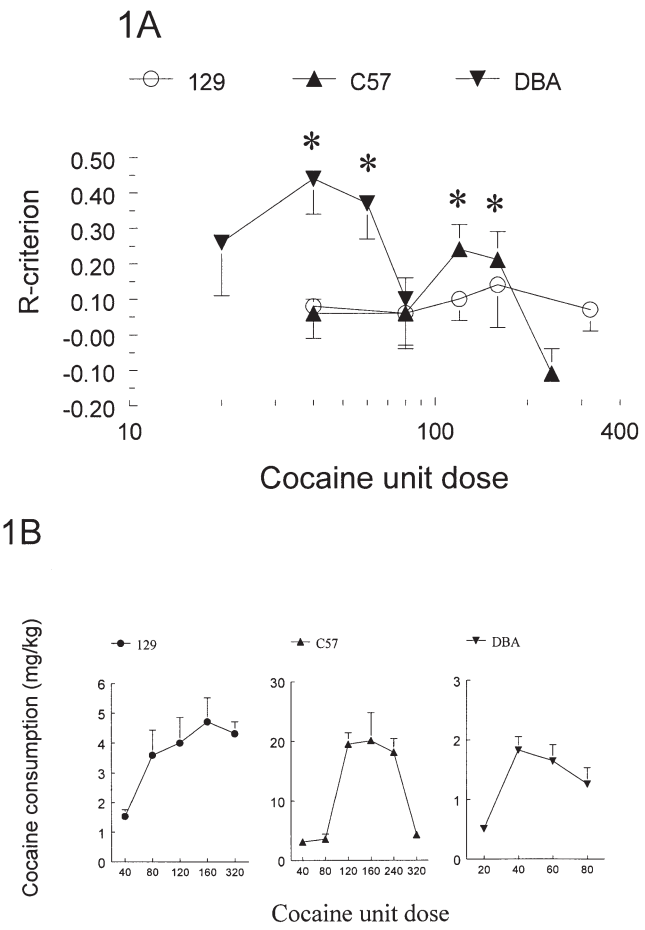

FIG. 1. Effect of increasing doses of cocaine on reinforcement and total cocaine intake in three mouse strains. (A) Influence of cocaine unit dose on the *R*- (ratio-) criterion, i.e., $\log_{10}$ of the ratio of nose pokes in active over passive mice, in experiments with acquisition of cocaine self-administration. Data as mean with SEM (n = 8–10 mice). *indicates significant acquisition of self-administration according to *R*-criterion ($p < 0.05$, Student's t-test, comparison with the group with saline self-administration). (B) Influence of cocaine unit dose on cocaine consumption during the 30 min self-administration session. Mean and SEM (n = 8–10 mice). ○ 129, ▲ C57, ▼ DBA.

ber of nose pokes of the active animals in experiments with cocaine with the strongest effect at a unit dose of 120 μg kg^{-1} inf^{-1} compared with saline self-administration (see Table 2). The control animals also displayed an increase in operant behavior compared to the corresponding saline group (see Table 2). Further increase of the cocaine unit dose (>120 μg kg^{-1} inf^{-1}) produced a decrease in the number of nose pokes, which indicates that C57 mice adjusted their responding to receive cocaine at a certain infusion rate. Accordingly, there was a significant influence of cocaine concentration on the dose consumed, $F(5, 30) = 8.7$, $p < 0.01$. The consumption was not a linear function of unit dose, as there was a plateau phase in the unit dose range of 120–240 μg kg^{-1} inf^{-1} of cocaine (Fig. 1B). In this range of concentrations, mice consumed around 20 mg kg^{-1} of cocaine per session. With respect to the *r* criterion, there was a typical bell-shaped unit dose to response curve [significant differences between doses; $F(5, 36) = 3.53$, $p < 0.01$], with significant acquisition of cocaine self-administration at the dose of 120 and 160 μg kg^{-1} inf^{-1} (Fig. 1A).

Two-way ANOVA revealed significant effect of cocaine dose, $F(5, 68) = 4.0$, $p < 0.01$, and mode of cocaine administration, $F(1, 68) = 9.96$, $p < 0.01$, on nose-poke activity of DBA mice, and a significant dose × mode interaction, $F(5, 68) = 3.38$, $p < 0.01$. There was a significant increase in active nose pokes at the doses of 10, 20, and 40 μg kg^{-1} inf^{-1} ($p < 0.01$, compared with active animals receiving saline). In contrast, none of the cocaine doses produced an increase in nose pokes in control animals. Further increase of the cocaine dose, as in experiments with C57, caused a decrease in nose-poke activity of active animals (but had no influence on the control animals). Consequently the *r* criterion, an index of reinforcement, showed a typical bell-shaped unit dose to response curve, $F(5, 34) = 3.60$, $p = 001$), indicating significant acquisition of cocaine self-administration at the unit doses of 40 and 60 μg kg^{-1} inf^{-1} (Fig. 1A). There was significant influence of cocaine dose on the cocaine intake, $F(5, 30) = 8.7$, $p < 0.01$. The relationship between unit dose and intake was not linear, as there was a plateau at cocaine doses in the range of 40–60 μg kg^{-1} inf^{-1} (Fig. 1B). In this range of doses mice consumed around 2 mg kg^{-1} of cocaine per session. The relationship between unit dose and *r* criterion was strongly shifted to the left along the unit-dose scale in DBA mice in comparison to C57 mice. DBA mice exhibited a larger ratio (compared to C57 mice) between active and yoked-control animals at the unit dose of cocaine that gave maximal reinforcement, but the response rate in DBA mice was about four times lower than in C57 mice, and cocaine intake was 10 times lower than in C57 mice.

Extinction of Cocaine Self-Administration

Because only DBA and C57 mice acquired cocaine self-administration, the extinction experiments were performed only with these strains (trained with 40 μg kg^{-1} inf^{-1} and 120 μg kg^{-1} inf^{-1} on day 1, respectively). On day 2 (24 h after the acquisition session), mice were given noncontingent IP injections of different doses of cocaine or saline and placed into exactly the same experimental boxes as in the initiation experiment. In saline-treated animals the number of nose pokes were not significantly different between active and passive mice (see Fig. 2A). Hence, the *r* criterion did not differ from 0 (Fig. 2B). This was interpreted as extinction, in the absence of response-contingent cocaine, of the behavior acquired on day 1. In experiments with saline injection C57 mice (both active and yoked control) exhibited a significantly higher level of nose-poke activity than DBA mice ($p < 0.01$).

Two-way ANOVA revealed significant influence of cocaine dose, $F(3, 46) = 5.5$, $p < 0.01$, and strain of mice, $F(1, 46) = 57.2$, $p < 0.01$, on the number of nose pokes in active groups of animals (Fig. 2A). The interaction between dose and strain was also significant, $F(3, 46) = 5.99$, $p < 0.01$. In the control groups of mice only strain had a significant influence, $F(1, 46) = 32.41$, $p < 0.01$. In DBA mice (Fig. 2A), IP injection of cocaine at the dose of 5.0 mg kg^{-1} specifically increased the nose-poking response of the active animals that had learned to self-administer cocaine on day 1 ($p < 0.05$ compared with saline-treated group). However, this effect of cocaine at 5.0 mg kg^{-1} was not seen in the active animals trained with saline self-administration on day 1 (data not shown) or in yoked-control animals that received either cocaine or saline infusion on day 1. Similar results were obtained in C57 mice in experiments with administration of co-

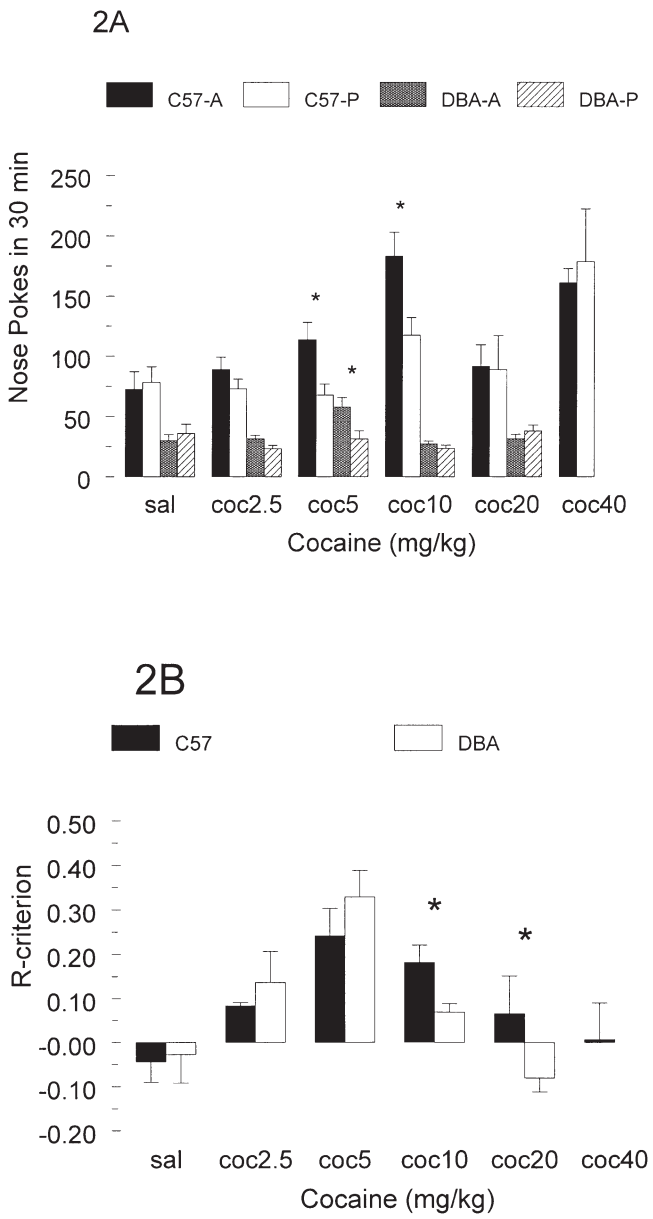

FIG. 2. Extinction of cocaine self-administration. (A) Nose-poke activity during a 30-min session after IP administration of saline or different doses of cocaine. Data as mean with SEM (n = 8–10 mice). *indicates significant difference between active and passive mice ($p < 0.05$, Student's t-test). A, active animals; P, passive animals. (B) Influence of cocaine dose on the R- (ratio-) criterion. Data as mean with SEM (n = 8–10 mice). *indicates significant difference between strains ($p < 0.05$, Student's t-test).

caine at the doses of 5 and 10 mg kg^{-1}. The dose–response relationship was biphasic, so that there was no significant difference in nose-poking activity between the animals in the pair at the higher doses of cocaine (10 and 20 mg kg^{-1} in DBA mice and 20 and 40 mg kg^{-1} in C57 mice).

ANOVA also showed a significant influence of cocaine dose on r criteria in both strains ($p < 0.01$; Fig. 2B). Paired comparison revealed that in the DBA strain the extinction of self-administration-like behavior (according to the r criterion) occurred after saline administration, and cocaine administration at the doses of 10 and 20 mg kg^{-1}. Cocaine at the dose of 2.5 mg kg^{-1} had a tendency to stabilize the self-administration-like behavior, whereas cocaine at the dose of 5.0 mg kg^{-1} prevented the extinction of self-administration-like behavior. In C57, extinction of self-administration was found after saline administration and cocaine administration at the dose of 40 mg kg^{-1}. In this strain, doses of 2.5 and 20 mg kg^{-1} produced a tendency to stabilize the self-administration-like behavior, whereas cocaine at the doses of 5.0 and 10 mg kg^{-1} prevented its extinction. When the two strains were compared with respect to r criterion on day 2, significant differences (Student's t-test, $p < 0.05$) were found at the dose of 10 and 20 mg kg^{-1} (Fig. 2B).

NGFI-A mRNA Expression

Effects of IV cocaine injection (2 mg kg^{-1}) on NGFI-A mRNA expression were found only in C57 and DBA mice (Fig. 3) In C57 mice, significant alteration of NGFI-A expression after a single cocaine injection was found in the caudate putamen ($p < 0.05$, lateral part; and $p < 0.01$, medial part). In the DBA strain cocaine significantly elevated the expression of NGFI-A mRNA in the caudate putamen (both in the medial and lateral part $p < 0.01$), prefrontal cortex ($p < 0.01$, the area measured was the combined pre- and infralimbic cortex), and piriform cortex ($p < 0.05$). The changes occurred 1 h after injection. After 4 h there were no detectable effects of cocaine, and NGFI-A seemed to have returned to a possible basal level (data not shown). Control levels of NGFI-A mRNA (after saline injections) were similar in the three strains in all structures measured. Although not shown in the figure, there was a significant increase in NGFI-A mRNA in the piriform cortex 1 h after cocaine in DBA mice (0.471 ± 0.027 OD units after cocaine and 0.404 ± 0.015 OD units after saline, $p < 0.05$).

Secretogranin II mRNA Expression

Significant effects of cocaine injection on secretogranin II (SG-II) mRNA expression were found only in C57 and DBA mice (Fig. 4). In C57 mice, significant inhibition of SG-II mRNA expression after a single cocaine injection was found in the caudate putamen ($p < 0.05$, lateral part). In the DBA strain, cocaine significantly decreased the expression of SG-II mRNA in the caudate putamen (both in medial and lateral parts, $p < 0.05$) and in the shell part of the n. accumbens ($p < 0.05$). Interstrain comparison revealed significant differences in SG-II mRNA expression in the prefrontal cortex, $F(2, 23) = 5.6$, $p < 0.01$, and in the medial part of the caudate putamen, $F(2, 23) = 4.9$, $p < 0.05$. The post hoc comparison revealed higher ($p < 0.01$) expression of SG-II mRNA in the 129 strain in prefrontal cortex. The rank order of SG-II expression in the mouse strains was 129 >> C57 = DBA. Conversely, the DBA mice exhibit the highest expression of SG-II mRNA in the medial part of the caudate putamen. There the rank order was DBA > C57 >> 129. The basal level of SG-II in the piriform cortex differed between strains (0.275 ± 0.02 in the 129 mice, 0.514 ± 0.047 in C57 mice, and 0.420 ± 0.022 in DBA mice, mean ± SME, OD units, n = 10 mice). Here, there was no effect of cocaine in the 129 mice (0.284 ± 0.0167) but a tendency to a decrease in C57 and DBA (0.426 ± 0.027 and 0.399 ± 0.002 OD units after cocaine treatment, repectively).

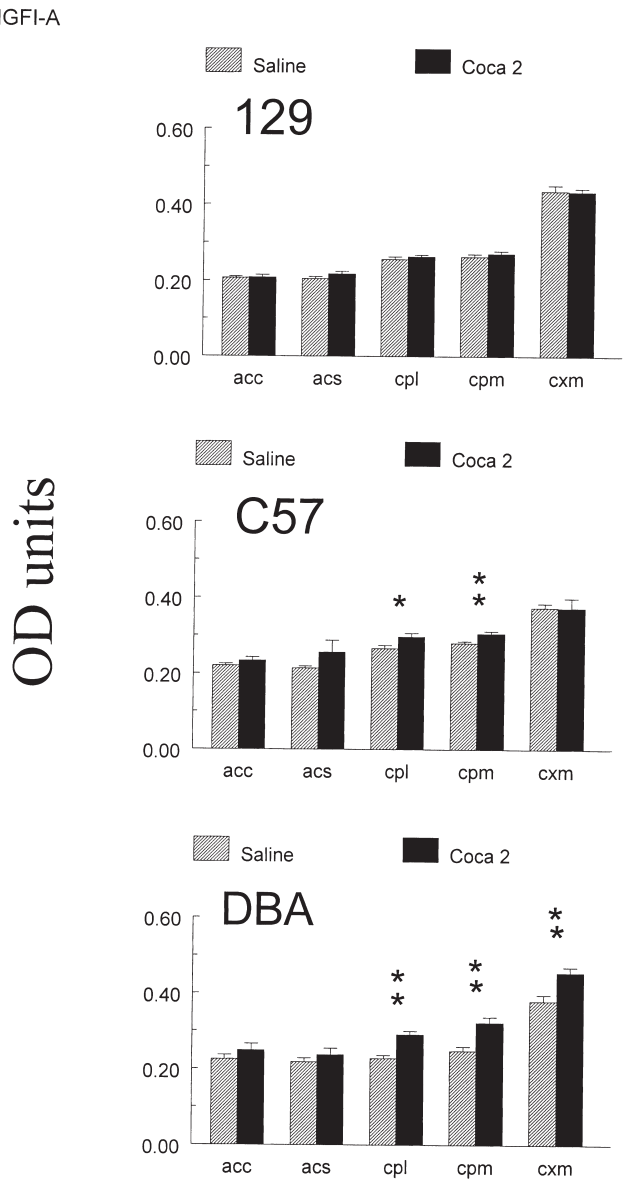

FIG. 3. Influence of acute cocaine injection on NGFI-A mRNA expression in mouse brain. *,**indicate $p < 0.05$ and $p < 0.01$ vs. saline (Student's *t*-test). ACC, n.accumbens core; ACS, n.accumbens shell; CPL, caudate putamen (lateral part); CPM, caudate putamen (medial part); CXM, prefrontal cortex. Data as mean with SEM (n = 8–10 mice).

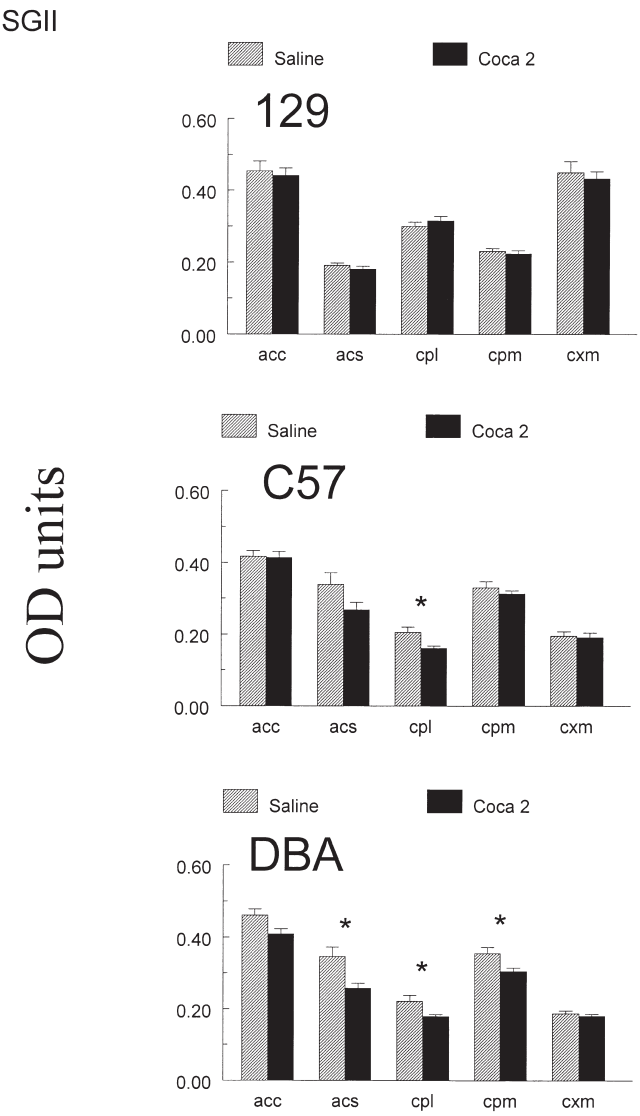

FIG. 4. Influence of acute IV cocaine on secretogranin II mRNA expression in the mouse brain. Data as mean with SEM (n = 8–10 mice). *indicates $p < 0.05$ vs. saline (Student's *t*-test). ACC, n. accumbens core; ACS, n. accumbens shell; CPL, caudate putamen (lateral part); CPM, caudate putamen (medial part); CXM, prefrontal cortex.

DISCUSSION

DBA and C57 mice rapidly acquired cocaine self-administration behavior. The 129 mice failed to acquire self-administration when tested under identical conditions. DBA and C57 mice were responsive to cocaine's stimulatory effects on nose pokes, although this was only true for active mice in experiments with DBA.

Our findings confirm previous reports (11,26) showing that cocaine dose dependently induces self-administration in both DBA and C57 strains, and that the rate of cocaine intake is lower in the former. However, our data contradict the study that showed an inability of DBA mice to acquire cocaine self-administration (6). These researchers reported data from only three DBA mice tested at one unit dose (100 (g kg^{-1} inf^{-1}) and apparently did not check the patency of the cannula after the self-administration session. In the present study, DBA mice also failed to acquire cocaine self-administration in the range of unit doses 80–120 (g kg^{-1} inf^{-1} but exhibited stable self-administration at the dose of 40 μg kg^{-1} inf^{-1}. C57 mice readily acquired self-administration behavior at the doses of 80 and 120 μg kg^{-1} inf^{-1}. Thus, the discrepancy between Carney's study (6) and ours is probably explained by the dose used by those authors, and that the doses at which cocaine is reinforcing are lower in DBA in comparison with C57 mice.

The shift to the right of the unit dose–intake curve in C57 mice [present study; (26)] might be construed as evidence of lower reinforcing potency of cocaine in the C57 strain than in

the DBA strain. It is also possible that C57 mice self-administer larger doses due to reduced sensitivity to the toxic effects of cocaine. That the lowest dose of cocaine that supports acquisition of self-administration was less in DBA mice than in C57 mice (40 $\mu g\ kg^{-1}\ inf^{-1}$ and 120 $\mu g\ kg^{-1}\ inf^{-1}$, respectively) may argue that the sensitivity to cocaine's primary action is greater in this strain. The greater response of NGFI-A and SG-II to single-dose cocaine in DBA mice than in C57 further supports this hypothesis. Similar concentrations of cocaine were measured in the brains of DBA and C57 mice following injection (2,5,9,13,18,22,26,27,32). Furthermore, cocaine has a comparable half-life in these strains (3), suggesting that differences in cocaine self-administration are not due to differential cocaine kinetics among strains.

The absence of an increase in nose pokes or gene expression in response to cocaine in the 129 mice is in line with the results of Miner (23), who showed an absence of place preference after cocaine treatment in the closely related 129/SvJ substrain. Furthermore, Schlussman and co-workers (29) reported a lack of activation of locomotion by cocaine in the 129/J mice. However, Miner (23) reported that the 129/SvJ mice were very sensitive to the locomotor activating effects of cocaine, although locomotor activity was strongly increased also after saline injection from a very low basal level. In the present study, there was no motor activation (revealed by the nose-poke activity) in the 129 mice but there was a significant decrease in nose-poke activity at the highest cocaine unit dose tested. This is in line with the study of Schlussman and coworkers (29), and may indirectly indicate that the 129 mice are sensitive to the side/toxic effects of cocaine. The absence of a response of NGFI-A and SG-II to cocaine in the 129 mice suggests a reduced sensitivity to the primary effects of cocaine in this strain. However, it is possible that the 129 mice have some difficulties in the formation of association between operant reaction and consequent drug infusion, as the 129 substrains perform poorly in some memory tests (8).

NGFI-A, a transcription factor encoded by an immediate early gene, is thought to couple extracellular signals to changes in gene expression, for example, in long-term potentiation. Increased NGFI-A expression in the striatal areas after cocaine has previously been described in rats [reviewed in (12)], and is consistent with current views about mechanisms of the stimulatory effects of cocaine. In addition, there was an increase in NGFI-A mRNA in the piriform cortex in DBA mice. The role of the piriform cortex, if any, in drug effects is not well known. However, it has dopamine autoreceptors similar to those in the mesolimbic dopamine system (1) and responds to cocaine administration (4). The absence of any effect of cocaine on NGFI-A expression in the 129 mice could possibly be attributed to the reduced glutamatergic signalling found at least in the 129/SvEv substrain (20) as NGFI-A is regulated at least partly by glutamate receptors (14).

Cocaine produced only increases in NGFI-A mRNA, but only decreases in SG-II mRNA. Chromogranins, such as secretogranin II, are glycoproteins, found in secretory granules and possibly involved in processing and packaging of peptides (15). As levels of chromogranin mRNA are markedly increased in chronically stimulated neurons, it has been suggested that chromogranin mRNAs may better reflect changes in production and secretion of peptides or transmitters than the more commonly measured immediate early genes (30).

There were interstrain differences in basal (saline treatment) SG-II mRNA in the prefrontal cortex and the caudate putamen. The rank order was 129 >> C57 = DBA for the prefrontal cortex, and DBA > C57 >> 129 for the striatum. Because secretoneurin, derived from SG-II, can stimulate the release of dynorphin (35), and because C57 mice have been found deficient in kappa-receptors in striatum in comparison to DBA mice (17,31) and in endogenous substrates for kappa-receptors (16), differences in the function of endogenous kappa-opioid system might possibly explain the strain differences in cocaine self-administration. Cocaine has stimulatory effects on behavior if administered into the striatum, but inhibitory actions in the frontal cortex (19). The strain difference in basal SG-II mRNA expression could possibly also reflect a shift in the balance in neuronal activity between the striatum and cortex.

Studying strain differences with the procedure for cocaine self-administration used in this article is quick and easy, and may prove valuable for cocaine pharmacogenetics. We demonstrated differences in the sensitivity to cocaine among the C57, DBA, and the 129 mouse strains. The 129/OlaHsd mice should be used with caution in cocaine-related studies, as this background strain apparently lacks some effects of cocaine.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council (Project No. 12587 and 2553), Å. Wiberg, M. Bergvall, OE. and E. Johansson and F. and I. Thuring Foundations, Syskonen Svensson's Fund for Medical Research, the Brain Fund, the Swedish Board of Health and Welfare, the Royal Swedish Academy of Sciences and Karolinska Institutet.

REFERENCES

1. Bannon, M. J.; Wolf, M. E.; Roth, R. H.: Pharmacology of dopamine neurons innervating the prefrontal, cingulate and piriform cortices. Eur. J. Pharmacol. 92:119–125; 1983.
2. Benuck, M.; Lajtha, A.; Reith, M. E.: Pharmacokinetics of systemically administered cocaine and locomotor stimulation in mice. J. Pharmacol. Exp. Ther. 243:144–149; 1987.
3. Boyer, C. S.; Petersen, D. R.: Pharmacokinetic analysis of the metabolism of cocaine to norcocaine and N-hydroxynorcocaine in mice. Drug Metab. Dispos. 20:863–868; 1992.
4. Brown, E. E.; Robertson, G. S.; Fibiger, H. C.: Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: Role of forebrain limbic structures. J. Neurosci. 12:4112–4121; 1992.
5. Caine, B. S.; Lintz, R.; Koob, G. F.: Intravenous drug self-administration techniques in animals. In: Sahgal, A., ed. Behavioral neuroscience: A practical approach. Oxford: Oxford University Press; 1993:117–143.
6. Carney, J. M.; Landrum, R. W.; Cheng, M. S.; Seale, T. W.: Establishment of chronic intravenous drug self-administration in the C57BL/6J mouse. Neuroreport 2:477–480; 1991.
7. Crabbe, J. C.; Belknap, J. K.; Buck, K. J.: Genetic animal models of alcohol and drug abuse. Science 264:1715–1723; 1994.
8. Crawley, J. N.; Belknap, J. K.; Collins, A.; Crabbe, J. C.; Frankel, W.; Henderson, N.; Hitzemann, R. J.; Maxson, S. C.; Miner, L. L.; Silva, A. J.; Wehner, J. M.; Winshaw-Boris, A.; Paylor, R.: Behavioral phenotypes of inbred mouse strains: Implications and recommendations for molecular studies. Psychopharmacology (Berlin) 132:107–124; 1997.
9. De Wit, H.; Stewart, J.: Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berlin) 75:134–143; 1981.

10. Franklin, K. B. J.; Paxinos, G.: The mouse brain in stereotaxic coordinates. San Diego: Academic Press; 1997.
11. Grahame, N. J.; Cunningham, C. L.: Genetic differences in intravenous cocaine self-administration between C57Bl/6J and DBA/2J mice. Psychopharmacology (Berlin) 122:281–291; 1995.
12. Harlan, R. E.; Garcia, M. M.: Drugs of abuse and immediate-early genes in the forebrain. Mol. Neurobiol. 16:221–267; 1998.
13. Heyser, C. J.; McDonald, J. S.; Beauchamp, V.; Koob, G. F.; Gold, L. H.: The effects of cocaine on operant responding for food in several strains of mice. Psychopharmacology (Berlin) 132:202–208; 1997.
14. Hughes, P.; Dragunow, M.: Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. Pharmacol. Rev. 47:133–178; 1995.
15. Huttner, W. B.; Gerdes, H. H.; Rosa, P.: The granin (chromogranin/secretogranin) family. Trends Biochem. Sci. 16:27–30; 1991.
16. Jacob, J.; Michaud, G.; Nicola, M.-A.; Prudhomme, N.: Genetic differences in opioid binding sites and in antinociceptive activities of morphine and ethylketo-cyclazocine. Life Sci. 33:86–92; 1983.
17. Jamensky, N. T.; Gianoulakis, C.: Content of dynorphins and kappa-opioid receptors in distinct brain regions of C57BL/6 and DBA/2 mice. Alcohol. Clin. Exp. Res. 21:1455–1464; 1997.
18. Jones, B. C.; Reed, C. L.; Radcliffe, R. A; Erwin, V.G.: Pharmacogenetics of cocaine: I. Locomotor activity and self-selection. Pharmacogenetics 3:182–188; 1993.
19. Karler, R.; Calder, L. D.; Thai, D. K.; Bedingfield, J. B.: The role of dopamine and GABA in the frontal cortex of mice in modulating a motor-stimulant effect of amphetamine and cocaine. Pharmacol. Biochem. Behav. 60:237–244; 1998.
20. Kolesnikov, Y.; Jain, S.; Wilson, R.; Pasternak, G. W.: Lack of morphine and enkephalin tolerance in 129/SvEv mice: Evidence for a NMDA receptor defect. J. Pharmacol. Exp. Ther. 284:455–459; 1998.
21. Kuzmin, A.; Semenova, S.; Ramsey, N. F.; Zvartau, E. E.; Van Ree, J. M.: Modulation of cocaine intravenous self-administration in drug-naive animals by dihydropyridine Ca^{2+} channel modulators. Eur. J. Pharmacol. 295:19–25; 1996.
22. Mahata, S. K.; Mahata, M.; Fischer-Colbrie, R.; Winkler, H.: In situ hybridization: mRNA levels of secretogranin II, VGF and peptidylglycine alpha-amidating monooxygenase in brain of salt-loaded rats. Histochemistry 99:287–293; 1993.
23. Miner, L. L.: Cocaine reward and locomotor activity in C57Bl/6J and 129/SvJ mice and their F1 cross. Pharmacol. Biochem. Behav. 58:25–30; 1997.
24. Montkowski, A.; Poettig, M.; Mederer, A.; Holsboer, F.: Behavioural performance in three substrains of mouse strain 129. Brain Res. 762:12–18; 1997.
25. Papaioannou, V.; Johnson, R.: Production of chimeras and genetically defined offspring. In: Joyner, A. L., ed. Gene targeting: A practical approach. New York: Oxford University Press; 1993:107.
26. Rocha, B. A.; Odom, L. A.; Barron, B. A.; Ator, R.; Wild, S. A.; Forster, M. J.: Differential responsiveness to cocaine in C57BL/6J and DBA/2J mice. Psychopharmacology (Berlin) 138:82–88; 1998.
27. Ruth, J. A.; Ullman, E. A.; Collins, A. C.: An analysis of cocaine effects on locomotor activities and heart rate in four inbred mouse strains. Pharmacol. Biochem. Behav. 29:157–162; 1988.
28. Schimmel, A.; Braunling, O.; Ruther, U.; Huttner, W.B.; Gerdes, H. H.: The organisation of the mouse secretogranin II gene. FEBS Lett. 314:375–380; 1992.
29. Schlussman, S. D.; Ho, A.; Zhou, Y.; Curtis, A. E.; Kreek, M. J.: Effects of "binge" pattern cocaine on stereotypy and locomotor activity in C57Bl/6J and 129/J mice. Pharmacol. Biochem. Behav. 60:593–599; 1998.
30. Shen, P. J.; Gundlach, A. L.: Chromogranin mRNA levels in the brain as a marker for acute and chronic changes in neuronal activity: Effect of treatments including seizures, osmotic stimulation and axotomy in the rat. Eur. J. Neurosci. 8:988–1000; 1996.
31. Shuster, L.; Webster, G. W.; Eleftheriou; B. E.: A genetic analysis of the running response to morphine in mice. Psychopharmacologia 42:249–254; 1975.
32. Tolliver, B. K.; Carney, J. M.: Sensitization to stereotypy in DBA/2J but not C57BL/6J mice with repeated cocaine. Pharmacol. Biochem. Behav. 48:169–173; 1994.
33. Wisden, W.; Errington, M. L.; Williams, S.; Dunnett, S. B.; Waters, C.; Hitchcock, D.; Evan, G.; Bliss, T. V.; Hunt, S. P.: Differential expression of immediate early genes in the hippocampus and spinal cord. Neuron 4:603–614; 1990.
34. Womer, D. E.; Jones, B. C.; Erwin, V. G.: Characterization of dopamine transporter and locomotor effects of cocaine, GBR 12909, epidepride and SCH 23390 in C57BL and DBA mice. Pharmacol. Biochem. Behav. 48:327–335; 1994.
35. You, Z. B.; Saria, A.; Fischer-Colbrie, R.; Terenius, L.; Goiny, M.; Herrera-Marschitz, M.: Effects of secretogranin II-derived peptides on the release of neurotransmitters monitored in the basal ganglia of the rat with in vivo microdialysis. Naunyn Schmiedebergs Arch. Pharmacol. 354:717–724; 1996.