Pharmacology Biochemistry & Behavior, Vol. 40, pp. 941–948. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Cocaine Actions, Brain Levels and Receptors in Selected Lines of Mice

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Received 15 April 1991

JONES, B. C., A. D. CAMPBELL, R. A. RADCLIFFE AND V. G. ERWIN. *Cocaine actions, brain levels and receptors in selected lines of mice.* PHARMACOL BIOCHEM BEHAV **40**(4) 941–948, 1991.—The effects of cocaine (15 mg/kg IP) versus IP saline on open-field behaviors were evaluated using a crossover design in long-sleep (LS) and short-sleep (SS) mice. Under treatment order 1, mice received saline injection on day 1 followed 24 h later by cocaine (saline-cocaine, S-C). Under treatment order 2, animals received cocaine on day 1 and saline on day 2 (cocaine-saline, C-S). Immediately following injection, animals were placed into an automated open-field apparatus with behavioral samples taken at 5-min intervals for 30 min. The behaviors measured were distance traveled, stereotypy and time spent in proximity to the margins of the test apparatus (thigmotaxis). Cocaine increased locomotor activity in both lines of mice, with S-C producing more pronounced initial activation than C-S in LS mice. Compared to S-C, C-S also increased thigmotaxis, an effect more pronounced in SS mice. In a separate experiment, brain cocaine levels were measured in brains of adapted and nonadapted LS and SS mice 5 min following injection of 15 mg/kg cocaine. Regardless of order, SS mice had significantly higher brain cocaine levels than did LS mice. Mazindol and cocaine binding studies in the forebrain indicated higher B_{max} values for both ligands in LS compared to SS mice. The results of this study indicate that genetically based differences in cocaine receptors as well as treatment order contribute to behavioral actions of cocaine.

Long-sleep and short-sleep mice Cocaine Locomotor activity Mazindol Receptors

IN most species studied, cocaine has been shown to stimulate locomotor activity. This cocaine-induced locomotor activation is asserted to be related to its rewarding effects (34), and there is recent work which shows locomotor activation to be positively correlated with increased concentrations of cocaine in the CNS (1). It is widely held that the rewarding properties of cocaine, as well as its action to increase locomotor activity, are related to its capacity to increase postsynaptic dopamine action in meso-cortico-limbic and nigrostriatal pathways (10, 17, 26, 28, 32, 35). Thus hyperlocomotion in animals may be a useful criterion to identify substances with potential liability for misuse. The studies cited above and numerous others suggest involvement of presynaptic cocaine binding sites (monoamine uptake sites) in psychomotor stimulant effects and self-administration of cocaine. Indeed, recent studies have characterized specific, reversible, stereoselective and saturable cocaine binding sites which function as dopamine reuptake receptors (2,21) in striatum and other brain regions of rat, mouse, monkey and human (4, 20, 23, 27, 30).

One aspect of cocaine's actions which is not well known is the extent to which individuals vary in initial sensitivity to cocaine and how initial sensitivity is related to risk for its compulsive use. It is likely that individual differences in sensitivity to cocaine are attributable, in part, to the genetic makeup of the organism, much as individual differences in vulnerability to ethanol misuse have been shown to have a heritable basis (5, 8, 15). Both human and animal research point to genetic factors as mediating sensitivity to, tolerance to, rewarding properties of and, most importantly, susceptibility to ethanol addiction (11, 12, 16). Because of the less open use of cocaine, as compared to ethanol, individual variability in sensitivity, rewarding properties, tolerance and liability for its abuse is virtually unknown. Consequently, rather little can be asserted concerning genetically based differences among humans for misuse liability concerning cocaine. One study has, however, reported a familial association between ethanol and cocaine addiction (33).

In studies using animals, a limited number of researchers have shown genetic makeup to be an important covariate of cocaine's behavioral (vide infra) and toxic effects (3). A recent study showed differential locomotor sensitivity and propensity for self-administration in two inbred strains of rats (13). Interestingly, the strain which was more sensitive to the locomotor activating effect of cocaine also showed greater tendency for cocaine self-administration in an operant procedure. Among lines and strains of genetically defined mice, a small number of investigators have reported varying direction and magnitude of locomotor response following acute treatment with cocaine. In a recent study, four inbred strains of mice were shown to vary widely in locomotor and other activity measures taken in a Y-maze in response to doses of cocaine ranging from 2.5–15 mg/kg (29). One strain (BALB) was refractory to all doses of cocaine, two strains (C3H and DBA) showed dose-related locomotor stimulation and one strain (C57) showed biphasic response with 2.5 mg/kg inhibiting, and higher doses stimulating, loco-

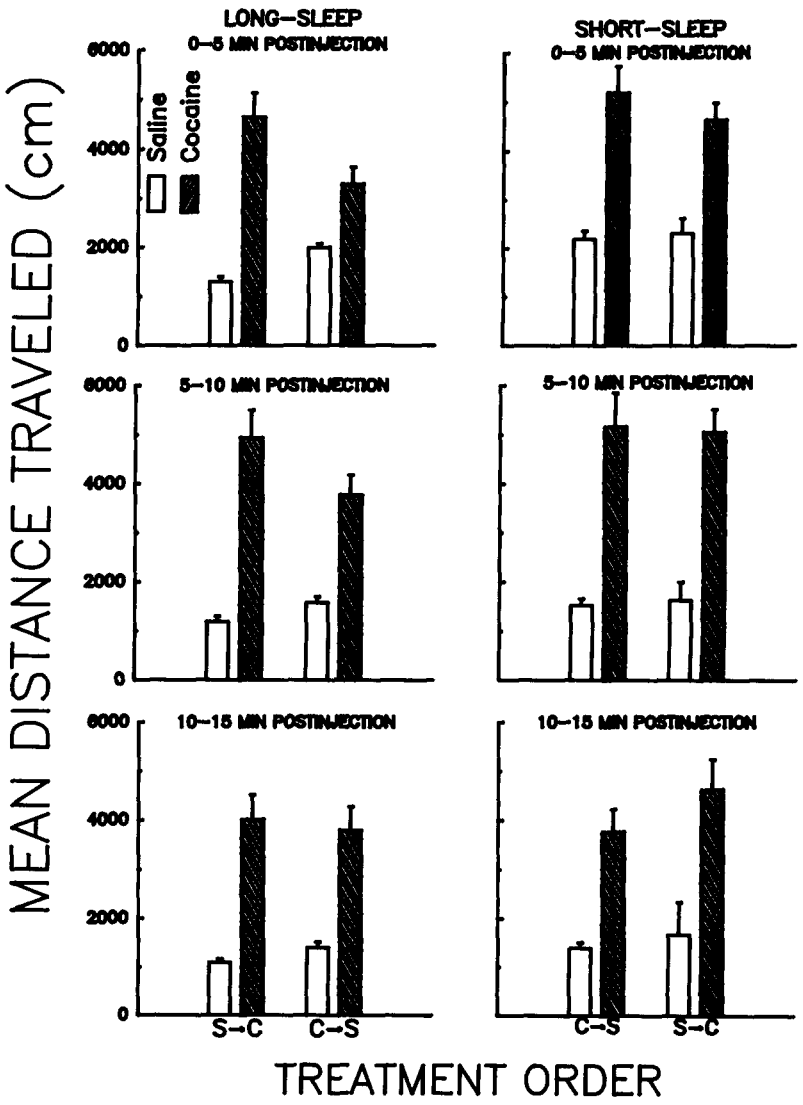

FIG. 1. Effect of cocaine and saline injection on mean (±S.E.M.) distance traveled over three 5-min time blocks, 0–15 min. Male LS (n = 20) and SS (n = 19) mice were tested on 2 consecutive days in an automated open field. Cocaine (15 mg/kg) and saline were administered, IP, under one of two treatment orders, saline day 1–cocaine day 2, or cocaine day 1–saline day 2.

motion. Of interest in this study was the observation of apparent dissociation between strain-related differential activation and brain cocaine levels.

Two studies reported cocaine effects on locomotor activity in long-sleep (LS) and short-sleep (SS) mice, animals selectively bred for differential sensitivity to hypnotic doses of ethanol (25). One study (9) reported dose-related stimulation of Y-maze locomotor activity for both LS and SS mice, with LS showing greater stimulation at 20 mg/kg (doses used were 10, 20 and 40 mg/kg). The other study reported differential effects of cocaine on locomotor activity, with LS not stimulated and SS stimulated at doses ranging from 30 to 75 mg/kg IP. Disparate results using the same, genetically defined animals pose a problem for validity of interpretation of outcomes, and as such, indicate methodological conditions which may be at least partially confounded with the effects of cocaine. For instance, in each of the studies cited above, cocaine was administered acutely to naive animals (i.e., in a novel environment), a condition which has been shown to influence behavioral actions of CNS-active agents (6).

There were three objectives to be met in the conduct of this study. First, we sought to examine the possible influence of situational novelty on cocaine's effect on locomotion in two lines of mice which had been shown previously to differ in this response to cocaine. Second, we sought to examine brain levels of cocaine at the time which showed the earliest effect of cocaine on locomotion (1). Finally, we were interested in possible, genetically determined differences in brain cocaine receptors which might partially explain observed differences in cocaine's locomotor activating effects in genetically defined animals.

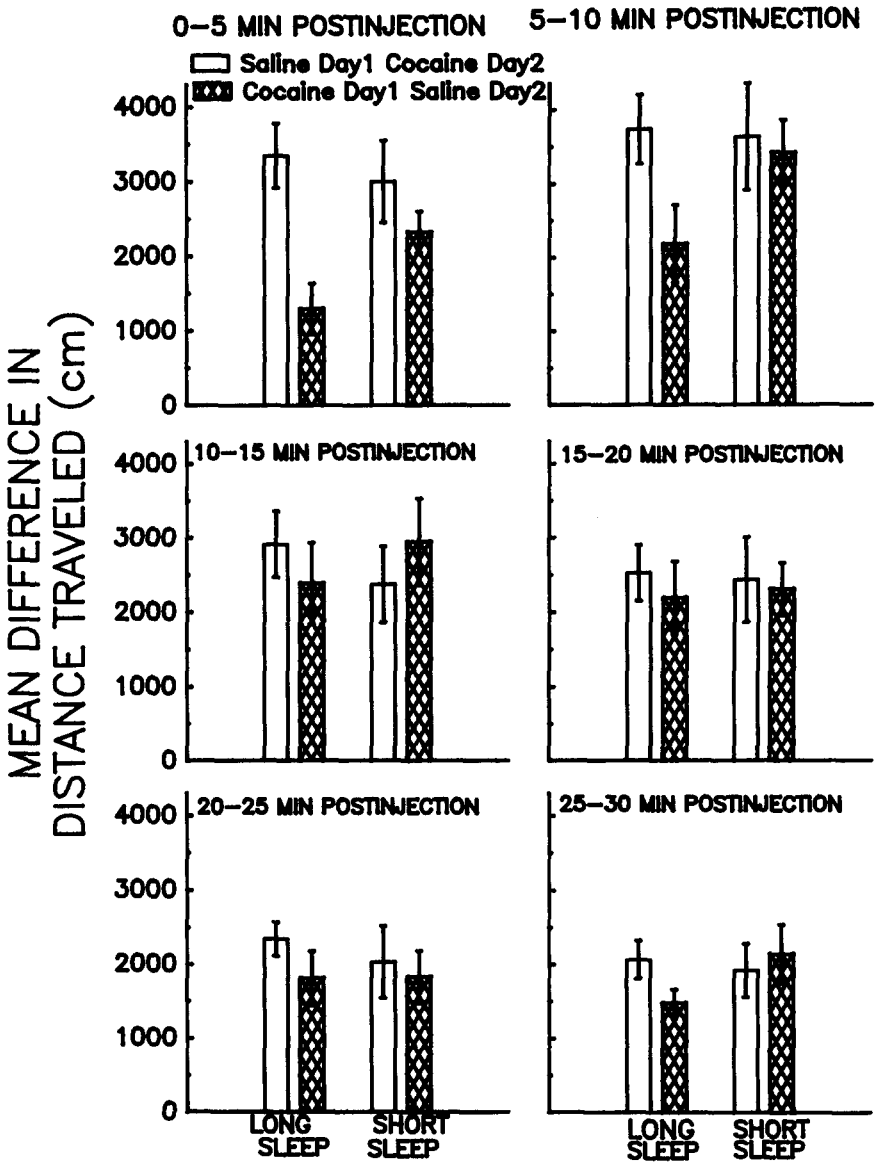

FIG. 2. Locomotor activation by cocaine in male LS (n=20) and SS (n=19) mice. The mice were tested on 2 consecutive days in an automated open field. Cocaine (15 mg/kg) and saline were administered, IP, under one of two treatment orders, saline day 1–cocaine day 2, or cocaine day 1–saline day 2. Data are mean difference (±S.E.M.) in distance traveled, cocaine minus saline.

METHOD

Animals

Male LS and SS mice served as subjects for study. For behavioral experiments, two replicate experiments separated by 4 weeks as well as littermate identification were used to minimize the assignment of littermates to treatments. All animals were housed in the School of Pharmacy colony with constant access to food and water. Temperature and humidity were maintained at 22°C and 20%, respectively, with light cycle, 0700 L:1900 D.

Open-Field Testing

At 60–75 days of age, animals were assigned to one of two treatment orders: saline injection on day 1 and cocaine injection on day 2 (S-C) or cocaine on day 1 and saline on day 2 (C-S). Immediately following injection, all animals were placed into an automated activity monitor (Omnitech, Inc., Columbus, OH) for 30 min, with locomotion, rearing, stereotyped movements and time spent in proximity to the walls (thigmotaxis) sampled during successive 5-min periods. The cocaine as hydrochloride salt was dissolved in sterile saline and injected IP at a volume of 0.01 ml/g body weight to deliver a final dose of cocaine of 15 mg/kg. Saline treatments were isovolumetric.

Brain Cocaine Levels

Brain cocaine concentrations were estimated by the methods of Jatlow and colleagues (18). Male LS and SS mice, adapted

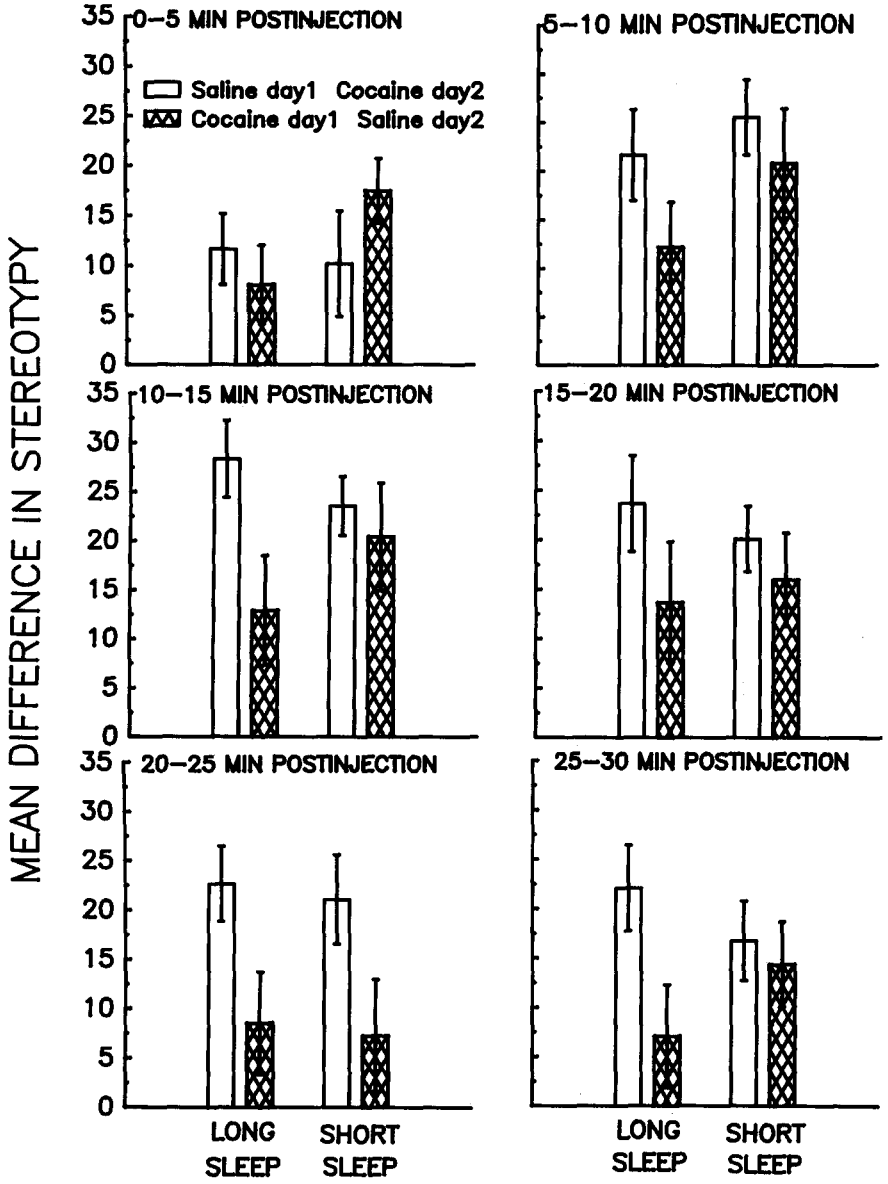

FIG. 3. Effect of cocaine on stereotyped movements in male LS (n = 20) and SS (n = 19) mice. The mice were tested on 2 consecutive days in an automated open field. Cocaine (15 mg/kg) and saline were administered, IP, under one of two treatment orders, saline day 1–cocaine day 2, or cocaine day 1–saline day 2. Data are mean difference (±S.E.M.) in stereotypy, cocaine minus saline.

to the test apparatus as above or nonadapted to the testing apparatus, were injected IP with 15 mg/kg cocaine as described above. At 5 minutes postinjection, all animals were killed by cervical dislocation, the brain removed and placed into 5.0 ml of ice-cold 119 mM NaF. The whole brain was homogenized in a Potter-Elvehjem mortar fitted with a motor-driven teflon pestle (six passes at 1100 revolutions per minute). The homogenate was added to 1 ml of 1.0 N H_2SO_4 containing 5 μg of tropacocaine dissolved in 100 μl of ethanol as internal standard. Ten ml of diethyl ether was added to the homogenate-mix and the tubes were shaken for 10 min. The samples were centrifuged at 180 × g for 10 min. The ether was removed by aspiration and discarded and 300 mg of $Na_2CO_3/NaHCO_3$ (7:8) was added to the aqueous layer to bring the pH to 9.0. Ten ml of a chloroform/ethanol (4:1) mixture was added and the tubes shaken for 10 min. The samples were then centrifuged for 10 min at 180 × g and the organic phase removed and prepared for HPLC assay by evaporation to dryness at 35°C under N_2.

Samples were reconstituted in 100 μl of ethanol, and 50-μl portions were injected onto a Waters μBONDAPAK 3.9 mm × 30 cm C_{18} column. The mobile phase consisted of 50 mM of KH_2PO_4 containing 17.5% CH_3CN with pH adjusted to 2.7. Flow rate was 1.0 ml/min and cocaine peaks were detected by UV absorption at 235 nM by a Gilson Holochrome detector. This procedure has been shown to separate cocaine from its metabolites, viz., norcocaine, N-OH-cocaine and benzoylecognine (18,31) and analysis of standard curves showed the detector response to be linear within the assay range.

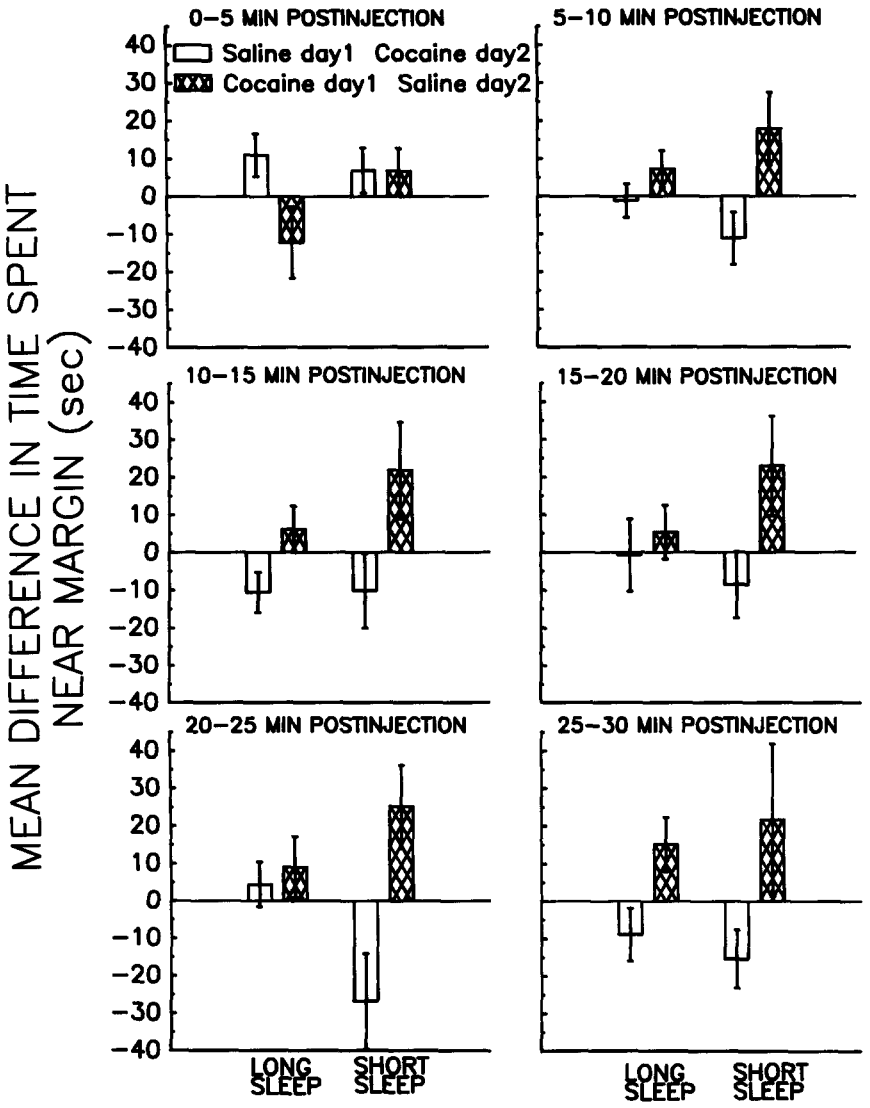

FIG. 4. Effect of cocaine on thigmotaxis in male LS (n=20) and SS (n=19) mice. The mice were tested on 2 consecutive days in an automated open field. Cocaine (15 mg/kg) and saline were administered, IP, under one of two treatment orders, saline day 1–cocaine day 2, or cocaine day 1–saline day 2. Data are mean difference (±S.E.M.) in time spent near the margins of the apparatus, cocaine minus saline.

Estimation of Cocaine Receptor Characteristics in Forebrain

Male LS and SS mice were killed by cervical dislocation, and the brain was rapidly removed and immersed into ice-cold saline for 30 s. The forebrain was dissected by coronal section at the optic chiasm so as to yield frontal cortex as well as dorsal and ventral striatum. Binding assays required the pooling of two forebrains to yield sufficient tissue (ca. 300 mg wet weight). Tissues were prepared by homogenization in 320 mM sucrose by Teflon on glass followed by centrifugation at 1500 × g for 10 min. The supernatant was then centrifuged for 30 min at 20,000 × g and the resulting pellet (P_2) resuspended in ice-cold 25 mM Na_2HPO_4, pH 7.4 and recentrifuged as above. This wash procedure was repeated, and the final membrane pellets were resuspended in ice-cold 25 mM Na_2HPO_4, pH 7.4, to yield a suspension of 100 mg/ml of the original tissue wet weight. All tissue preparative steps were performed at 4°C and the tissue was kept on ice until assay. The protein content of all samples was assessed colorimetrically (22). Binding of (−) [^{3}H]-cocaine (specific activity ca. 35 Ci/mmol) to forebrain membranes was performed by adding 50-μl aliquots containing 250 to 400 μg of protein to a total volume of 100 μl. Saturation binding for cocaine was carried out in 1.5-ml microcentrifuge tubes containing (−) [^{3}H]-cocaine ranging in concentration from 2 nM to 10 μM. Nonspecific binding was estimated in parallel sets of microcentrifuge tubes containing 2 nM to 10 μM (−) [^{3}H]-cocaine plus 50 μM (−) cocaine. Binding was allowed to proceed to equilibrium at 25°C for 1 h, at which time the reaction was stopped by addition of 1.0 ml of ice-cold binding buffer followed by rapid filtration over Whatman GF/B glass fiber filters which were prepared by presoaking them for 60 min in 0.05% polyethylenimine to

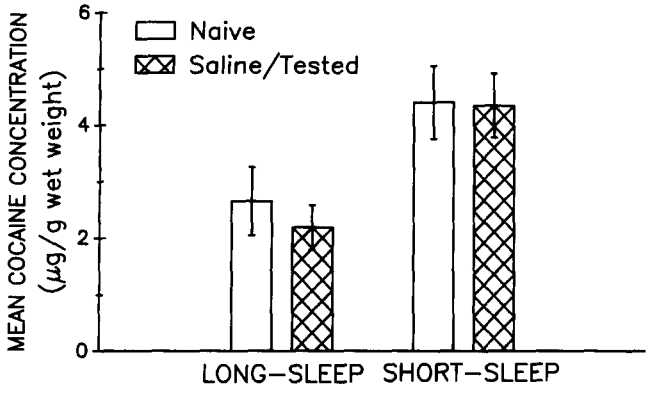

FIG. 5. Mean cocaine concentration in male LS (n=8) and SS (n=7) mice 5 min after IP injection with 15 mg/kg cocaine. The mice were tested on 2 consecutive days in an automated open field. Cocaine (15 mg/kg) and saline were administered, IP, under one of two treatment orders, saline day 1–cocaine day 2, or cocaine day 1–saline day 2. Data are mean (±S.E.M.) cocaine concentrations in whole brain as determined by HPLC-EC detection. Methods are described in the text.

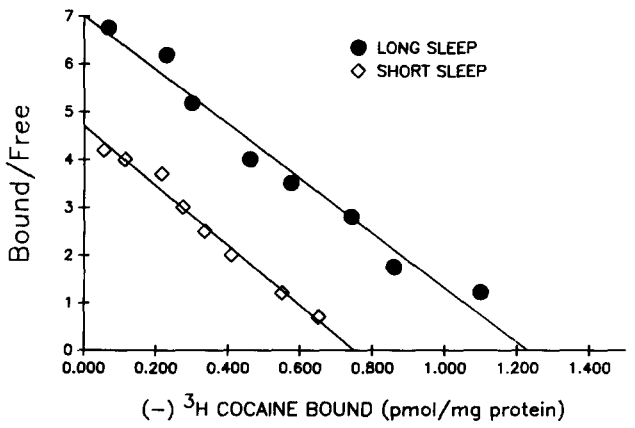

FIG. 6. Scatchard analysis of specific (−)[^{3}H]-cocaine binding to forebrain membranes from LS and SS mice (typical experiment shown). Brains were sectioned at the optic chiasm, and the anterior portion was prepared for receptor binding experiments as described in the text. Data were analyzed by the linear/nonlinear least squares regression analysis program, LIGAND. Membranes were prepared as described in the text.

reduce nonspecific binding to the filters. Each filter was washed twice with 5 ml of ice-cold binding buffer and was placed into a vial containing scintillation cocktail for counting. In a separate series of experiments, we investigated (−) cocaine displacement of [^{3}H]-mazindol, a high-affinity ligand which binds predominantly to dopamine uptake receptors (19). Membranes, prepared as above, were incubated at 25°C for 1 h in 4 nM [^{3}H]-mazindol and 1 μM unlabeled mazindol (for nonspecific binding). Concentrations of (−) cocaine ranging from 1 nM to 10 μM were added to the reaction tubes containing the [^{3}H]-mazindol, and the reactions were allowed to proceed to equilibrium and stopped as described above.

Data Analysis

Statistical analysis of the behavioral data was performed using analysis of variance as appropriate for between-subjects and mixed between- and within-subjects experiments. Scatchard analysis of (−)-[^{3}H]-cocaine binding was conducted using the Ligand software package (22).

RESULTS

Figure 1 presents mean distance traveled in each 5-min interval for the first 15 min postinjection. In the S-C treatment order, cocaine stimulation of locomotion was nearly the same for LS and SS, while in the C-S treatment order LS appeared to show less locomotor stimulation by cocaine than did SS mice. These data were further analyzed as difference scores (cocaine-saline) and are presented for the entire 30 min of testing in Fig. 2. Analysis of variance for a two between-subjects factors (line, treatment order) and one within-subjects factor (time) experiment revealed a significant effect for time, $F(5,165)=15.67$, $p<0.001$ (conservative p estimate at 1,33 *df*), and a significant time by treatment interaction, $F(5,165)=5.25$, $p<0.05$ (conservative p estimate at 1,33 *df*). Further analyses revealed the treatment-order effect to be significant (Tukey HSD method, $p<0.05$) at 0–5 and 5–10 min postinjection and in LS mice, with this line showing less locomotor stimulation by cocaine in the C-S treatment order as compared to the S-C treatment order.

Stereotypy difference scores (Fig. 3) also changed significantly in response to treatment order, $F(1,33)=4.25$, $p<0.05$, with C-S producing less stereotypy overall. Time and time by treatment interaction for stereotypy approached, but failed to reach statistical significance at $p<0.05$.

Margin time difference scores (Fig. 4) responded to treatment order, $F(1,33)=8.624$, $p<0.01$, with C-S resulting in greater margin time differences than S-C. Treatment order by line interaction approached significance, $F(1,33)=3.85$, $p<0.06$, with SS tending to show a greater effect of treatment order than LS.

It was of interest to determine whether LS and SS differences in cocaine-stimulated locomotion in the C-S treatment order might be due to differences in brain cocaine concentration and/or cocaine receptors. Additional studies were conducted, therefore, to measure these characteristics in LS and SS mice.

Brain cocaine concentrations taken 5 min following injection of 15 mg/kg cocaine HCl are shown in Fig. 5. Mean LS brain levels (2.43 μg/g wet weight) were significantly lower than the mean levels observed in SS brains (4.39 μg/g wet weight) $F(1,11)=11.86$, $p<0.01$; treatment order and order by line in-

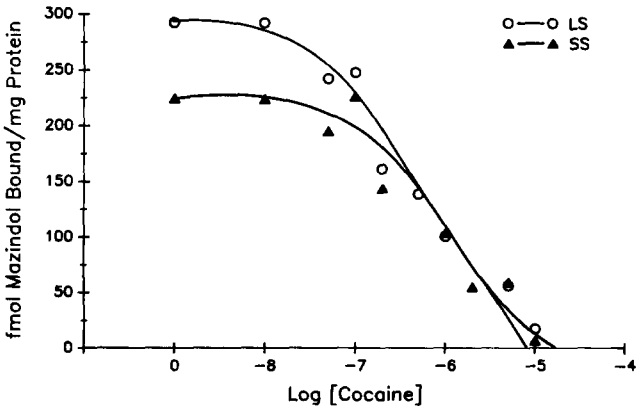

FIG. 7. Cocaine displacement of [^{3}H]-mazindol from forebrain tissues from male LS and SS mice. Each lot represents the mean of three replicate experiments. Brains were sectioned at the optic chiasm, and the anterior portion was prepared for receptor binding experiments as described in the text.

teraction effects were not significant. We also observed SS whole brain weights to be significantly lower than LS brain weights, with mean weights SS = 326 ± 17 mg and LS = 420 ± 4.6 mg, $F(1,11) = 121,87$, $p<0.001$.

Scatchard analysis of (−)-[^{3}H]-cocaine binding (Fig. 6) in forebrain revealed plots consistent with one binding site for both LS and SS with similar affinities but somewhat higher density in LS mice. Mean B_{max} for LS was 1250 ± 40 (S.E.M.) pmol/mg protein, and mean B_{max} for SS was 699.8 ± 73.8 (S.E.M.) pmol/mg protein, $t(4) = 9.29$, $p<0.001$. Alloreceptor competition of (−) cocaine with [^{3}H]-mazindol (Fig. 7) revealed nearly identical IC_{50} values for LS and SS, with maximum binding higher in LS than in SS.

DISCUSSION

The results of this study point to a number of important factors which come into play in the actions of cocaine on animal behavior. First, familiarization with the test apparatus does affect cocaine's actions on locomotion. In LS mice not adapted to the test apparatus, there was considerably less activation by cocaine than in LS previously exposed to the open field. This may be relevant to the previous finding (14) that LS are not as stimulated as SS. In fact, in adapted animals, LS evince as much stimulation of locomotion as SS.

Cocaine levels at 5 min in LS and SS revealed large differences, regardless of whether or not the animals had been exposed to the test apparatus. Furthermore, although LS had significantly lower brain cocaine levels than SS, adapted LS were just as activated as SS. The effect of familiarization and our observation of different cocaine levels point to genetically based variance in reactivity, and target tissue sensitivity, respectively, as probable covariates in cocaine actions. Indeed, others have shown cocaine to be anxiogenic in mice not adapted to the test apparatus (7). The importance of genetically based differences in target tissue sensitivity is also supported by our finding of greater cocaine receptor density in LS forebrain.

We conclude that pharmacokinetic and receptor characteristic measures are necessary, but not sufficient in determining cocaine actions on locomotor activity. The influence of novelty, for example, points to the probable importance of state of activity of a number of limbic and/or cortical circuits in determining cocaine actions. In fact, others have shown that compared to other mesocorticolimbic dopaminergic systems, prefrontal cortical dopaminergic neurons are preferentially activated by novelty (35). In the nonadapted, cocaine-treated mice, therefore, it is likely that novelty-induced dopaminergic activation in the prefrontal cortex was adding to cocaine-induced CNS-wide dopaminergic activation. The line by treatment order interaction may, therefore, reflect inherited differences in mesocorticolimbic function, especially with respect to response to novelty, between LS and SS mice. Finally, as regards the relevance of this work to human drug misuse, R. T. Jones showed in humans, that behavioral effects of CNS-active drugs also vary, depending upon the setting in which they are administered. Thus, in a social setting, tetrahydrocannabinol tends to produce euphoria, while in a socially isolated clinical setting, the same agent may elicit reports of dysphoria (20).

ACKNOWLEDGEMENTS

This research was supported by USPHS grants AA08454, AA00079 and AA07330. The authors wish to thank Dr. C. S. Boyer for sharing his expertise on the brain cocaine assays.

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