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Withdrawal From Continuous or Intermittent Cocaine: Behavioral Responsivity to 5-HT_1 Receptor Agonists

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KING, G. R., C. M. JOYNER AND E. H. ELLINWOOD, JR. *Withdrawal from continuous or intermittent cocaine: Behavioral responsivity to 5-HT_1 receptor agonists.* PHARMACOL BIOCHEM BEHAV **45**(3) 577–587, 1993.—Research on chronic cocaine administration indicates that both the dose and route of administration influences the effects of chronic cocaine. Rats were pretreated with 40 mg/kg/day cocaine for 14 days by either SC injections or osmotic minipumps. Rats were then withdrawn from the pretreatment regimen for 7 days and their behavior rated following injections of 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) or 5-HT_{1B} agonists. In Experiment 1, rats received 0- to 4.0-mg/kg IP injections of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a selective 5-HT_{1A} receptor agonist. In Experiment 2, rats received 0- to 16.0-mg/kg IP injections of 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2*a*]quinoxaline (CGS 12066B), a selective 5-HT_{1B} receptor agonist. The results of Experiment 1 indicated that rats receiving cocaine via osmotic minipumps exhibited marked 5-HT_{1A} receptor subsensitivity. In contrast, rats receiving daily cocaine injections sometimes demonstrated evidence of 5-HT_{1A} supersensitivity and sometimes demonstrated evidence of 5-HT_{1A} normosensitivity. The results of Experiment 2 indicated there were no consistent differences between the pretreatment groups in the behavioral response to CGS 12066B, although there were significant differences at single, isolated time points. Overall, the results indicate that, at least in the present behavioral paradigm, the effects of chronic cocaine administration are mediated by changes in 5-HT_{1A} receptor sensitivity but not by changes in 5-HT_{1B} receptor sensitivity.

Cocaine withdrawal 5-HT_{1A} and 5-HT_{1B} receptors Sensitization Tolerance Rats

RESEARCH on chronic cocaine administration indicates that both the dose and route of administration influence the effects of chronic cocaine [see (32) for a review]. For example, daily IP injections of cocaine produce sensitization (i.e., reverse tolerance) to its locomotor- and stereotypy-inducing properties (35,46,50). Schedule-induced cocaine intake has also been found to produce sensitization; however, oral administration of a single, acute, daily dose of cocaine does not (17,35). Last, daily SC injections also produce sensitization to the locomotor effects of cocaine, while continuous infusion of cocaine via Alza minipumps produces tolerance (33,34,48). These results indicate that the residual behavioral effects of chronic cocaine depend upon the method and temporal pattern of administration.

Although much research has focussed on changes in dopaminergic functioning as a mechanism underlying the effects of chronic cocaine administration [see (32) for a review], several lines of evidence indicate that serotonin [5-hydroxytryptamine (5-HT)] plays a role in stimulant-induced, DA-mediated behaviors [see (34) for a brief review]. Central 5-HT receptors have been divided into four general "families": the 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors. The 5-HT_1 class demonstrates extensive heterogeneity, and has been further divided into four subtypes: the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_{1D} receptors (22,45). The 5-HT autoreceptor seems to belong to the 5-HT_1 family of receptors (22). As a general statement, autoreceptor agonists inhibit firing rates, neurotransmitter release, and biosynthesis.

The 5-HT_{1A} receptor is extensively distributed in the limbic areas such as the hippocampus, lateral septum, the frontal and entorhinal cortex, the central amygdala, and the dorsal and median raphe; it is not, however, extensively found in the hypothalamus, thalamus, and extrapyramidal areas such as the caudate putamen, globus pallidus, and substantia nigra (25,26,55). The restriction of 5-HT_{1A} receptor distribution to limbic areas would indicate that this receptor is probably important in the control of arousal, affect, and motivational processes, possibly via disinhibition dopamine (DA) systems following activation of 5-HT_{1A} receptors. Consistent with this idea is the fact that activation of 5-HT_{1A} receptors results in a behavioral pattern consisting of hyperlocomotion, lateral headweaving, flat body posture, and reciprocal forepaw tread-

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ing, and this behavioral syndrome is partially dependent upon subsequent DA release (2,3,28,47,53).

Consistent with the identification of presynaptic 5-HT_{1A} receptors as the somadendritic autoreceptor, and consistent with the above idea of tonic inhibition of DA systems via 5-HT_{1A} receptors, cocaine has been shown to decrease the firing rates of dorsal raphe neurons (12,13), presumably via activation of 5-HT_{1A} receptors. Second, Broderick (7) found that SC cocaine injections resulted in a dose-dependent decrease in extracellular 5-HT concentrations, and an increase in DA concentrations, in the nucleus accumbens, presumably via activation of 5-HT_{1A} receptors.

In contrast to the 5-HT_{1A} receptor, the 5-HT_{1B} receptor is found primarily in the basal ganglia, with particularly high concentrations in the striatum, substantia nigra, dorsal subiculum, and caudate putamen. This receptor is also found in the dorsal and median raphe nucleus (40). The restriction of 5-HT_{1B} receptor distribution to the basal ganglia and striatal areas would indicate that this receptor is probably important in the control of the expression of motor behavior and stereotypies.

Consistent with the identification of the 5-HT_{1B} receptor as the terminal autoreceptor, administration of TFMPP and 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2*a*]quinoxaline (CGS 12066B), both 5-HT_{1B} agonists, inhibits 5-HT turnover as measured by reduced basal 5-hydroxyindoleacetic acid (5-HIAA) (18), results in a reduced rate of 5-HIAA accumulation following pretreatment with probenecid (18), and results in a decrease in 5-HT biosynthesis (41). Further, Benloucif and Galloway (3) reported that local infusion of RU 24969 into the anterior striatum resulted in a 300% increase in extracellular DA concentrations. Activation of 5-HT_{1B} receptors with either systemic (23) or central (15) administration of the 5-HT_{1B} agonist RU 24969 results in a dose-dependent increase in locomotor activity. The locomotor-stimulating effects of RU 24969 are eliminated with reserpine pretreatment (54). Thus, these results are consistent with a general hypothesis of 5-HT_{1B} receptor modulation of locomotion and stereotypies.

Previous research in this laboratory suggested, but did not clearly indicate, that daily SC cocaine injections resulted in 5-HT_{1A} receptor supersensitivity (34), which is inconsistent with the electrophysiological work of Cunningham and colleagues (11-14) indicating dramatic sensitization of dorsal raphe 5-HT_{1A} receptors following daily cocaine injections. However, the continuous infusion of an equivalent daily cocaine dose clearly resulted in 5-HT_{1A} receptor subsensitivity in our previous study (34), which examined the ability of NAN-190, a putative 5-HT_{1A} receptor antagonist, to inhibit cocaine-induced hyperactivity. Although there is considerable evidence indicating the NAN-190 is a potent and selective postsynaptic 5-HT_{1A} receptor antagonist (21), there is also some evidence that NAN-190 acts as an agonist at presynaptic 5-HT_{1A} autoreceptors (27): Hjorth and Sharp (27) found that NAN-190 dose dependently decreased extracellular 5-HT concentrations, indicating that NAN-190 acts as a partial autoreceptor agonist. However, this ability of NAN-190 to decrease 5-HT concentrations was less than the inhibition produced by the selective 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Thus, it is not clear to what extent the results from our previous study are due to the mixed agonist/antagonist properties of NAN-190. Further, it is unknown to what extent alterations in 5-HT_{1B} receptor functioning contribute to the effects of chronic cocaine administration.

The present experiments examine changes in 5-HT_{1A} and 5-HT_{1B} receptor functioning produced by withdrawal from either the continuous infusion of cocaine or daily SC cocaine injections. Rats were pretreated for 14 days with either continuous or intermittent daily injections of cocaine and then withdrawn from the pretreatment regimen for 7 days. Changes in 5-HT_{1A} receptor functioning were assessed by behavior ratings over several challenge doses of the 5-HT_{1A} receptor agonist 8-OH-DPAT (Experiment 1). Changes in 5-HT_{1B} receptor functioning were assessed in a similar manner by administering the 5-HT_{1B} receptor agonist CGS 12066B (Experiment 2).

METHOD

Animals

Male Sprague-Dawley rats, initially weighing 100–125 g (Charles River Laboratories, Wilmington, DE), were acclimated to the vivarium on a 12 L : 12 D cycle (light between 7:00 a.m.–7:00 p.m.) for 1 week prior to treatment. They were housed in pairs in plastic cages with continuous access to food and water.

Drugs

8-OH-DPAT (RBI Inc., Natick, MA) was dissolved in distilled water. CGS 12066B HBr (RBI) was dissolved in DMSO. All doses are calculated as the base, and injection volume was based upon the body weight.

Minipump Preparation

Alzet Osmotic pumps (Model 2ML2) from Alza Corp. (Palo Alto, CA) were filled with either 2 ml 100 mg/ml cocaine HCL or saline (0.9%); the infusion rate was 5 μl/h, resulting in an overall average dose of 40 mg/kg/day for the cocaine pumps. The pump was primed by warming in a beaker of saline in a waterbath at 37°C for 4 h prior to surgical implantation. The minipumps have been modified by adding a microdialysis fiber to the output portal to increase the surface area over which cocaine is distributed; this modification allows for the continuous infusion of high doses of cocaine without the development of necrotic skin lesions.

Surgery

Animals were shaved and injected locally with (0.2 cc) lidocaine (Abbott, North Chicago, IL) at the dorsal midline incision site. Animals were then anesthetized by inhalation with methoxyflurane (Metofane). A 2-cm vertical incision was made with scissors and a large SC pocket was formed with the scissors. The minipump was inserted into this pocket with the delivery portal toward the head. The opening was closed with metal surgical autoclips. On day 14, the pumps were surgically removed using the same procedure and the residual amount of cocaine measured. The amount was consistently less than 15% of the original volume, indicating that rats approximately received the programmed daily dose.

Pretreatment

Pretreatment was for a 14-day period. On day 1 of treatment, animals were either: a) implanted with 2ML2 Alzet minipumps continuously infusing cocaine at a rate of 40 mg/kg/day (continuous-infusion group); b) injected SC once daily with 40 mg/kg cocaine HCL (injection group); or c) injected SC with 0.9% saline (saline control group) once daily. There was no saline pump control group, as the results of King et al. (33) indicated that there were no differences in the behavioral responses to cocaine challenges in rats that had received either

saline injections or saline pumps. Thus, the surgery itself had no effect on the behavioral response to cocaine.

Behavioral Testing

On day 7 following pretreatment, animals were acclimated to the test room in their home cage for 30 min under normal light conditions. The test cages were standard, clear plastic laboratory animal housing cages, 28 × 18 × 12 cm, with another cage taped, upside down, in place on top. The top cage had five air holes drilled uniformly on either side. Six of these test cages were placed in a row 12 in. apart. A modified version of the Ellinwood and Balster Rating Scale (16) was used (Table 1). A rating was given to each of the animals at 5 min preinjection and at 5-min intervals thereafter for a total of 60 min. The observation period was for 20 s with 10 s between cages.

For the test session in Experiment 1, each rat received one of the following doses of 8-OH-DPAT IP: 0, 0.25, 0.5, 1.0, 2.0, or 4.0 mg/kg immediately prior to the session. For the test session in Experiment 2, each rat received one of the following doses of CGS 12066B IP: 0, 2.0, 4.0, 8.0, or 16.0 mg/kg immediately prior to the session.

For each test session in both experiments, the subject types (i.e., injection, pump, saline) were randomized according to a Latin square design; doses, for each test session, were also randomized by a Latin square design. The significance level was set at $p \leq 0.05$ for all comparisons. There are 10 rats per condition.

RESULTS

Experiment 1

Figure 1 presents the mean behavior rating for each dose of 8-OH-DPAT, separately for each pretreatment group. Panel A presents the behavior ratings of the saline control group. Kruskal-Wallis analyses of variance (ANOVAs) by ranks were performed separately at each time point to determine if there were any differences in the behavior ratings across the doses of 8-OH-DPAT. The results indicated that there was a significant dose effect at 5–30 and 40–55 min. Given the number of time points, and the number of doses used in Experiment 1, it is not feasible to report the results of Mann-Whitney tests for all possible comparisons. Thus, we will only report the results of Mann-Whitney tests for the 5- and 10-min time points, as this is where the differential effects of the pretreatment regimen seem to manifest themselves (see Fig. 2 below). The results of the Mann-Whitney tests indicate that: a) the 0.0-mg/kg dose resulted in significantly lower behavior ratings than all other doses at 5 min and all other doses except the 0.25-mg/kg dose at 10 min; b) the 0.25-mg/kg dose resulted in significantly lower behavior ratings than all other doses at 5 min and all other doses except the 0.5-mg/kg dose at 10 min; c) the 0.5-mg/kg dose resulted in significantly lower behavior ratings than the 2.0- and 4.0-mg/kg doses at both 5 and 10 min; d) the 1.0-mg/kg dose resulted in significantly lower behavior ratings than the 2- and 4-mg/kg doses only at 10 min; and e) the 2.0-mg/kg dose was not significantly different from the 4.0-mg/kg dose at 5 or 10 min.

Panel B presents the behavior ratings of the cocaine injection group. Kruskal-Wallis ANOVAs by ranks were performed separately at each time point to determine if there were any differences in the behavior ratings across the doses of 8-OH-DPAT. The results indicated that there was a significant dose effect at 5–30 and 50–55 min. Similar to panel A, we will only report the results of Mann-Whitney tests for the 5- and 10-min time points. The results of the Mann-Whitney tests indicate that: a) the 0.0-mg/kg dose resulted in significantly lower behavior ratings than all other doses at 5 min and all other doses except the 0.25-mg/kg dose at 10 min; b)

TABLE 1

MODIFIED ELLINWOOD AND BALSTER (1974) RATING SCALE

Score		Definition
1	Asleep	Lying down, eyes closed
2	Inactive	Relaxed muscles, eyes partially shut
3	Abnormal posture	Tense muscles, hunched back (not preseizure behavior)
4	Low active	Lying down, eyes open, infrequent sniffing
5	In place oral behavior	Vacuous oral movements, jaw tremor, yawning
6	Grooming	Grooming of face, body, or groin
7	Normal active movement	Investigation or sniffing of cage, rearing
8	Hyperactive	Running movement characterized by rapid changes in position (jerky)
9	Slow patterned movement	Repetitive exploration of the cage at normal levels of activity
10	Fast-patterned movement	Repetitive exploration of the cage with rapid, intense, stereotyped activities
11	Stereotypy	The types of stereotypies are noted
12	Hyperreactive	The following types of behavior are described and/or counted: jerky hyperactive movements, jumping (popcorn)-like movements, seizures, disjunctive movements, obstinate regression (backing up)

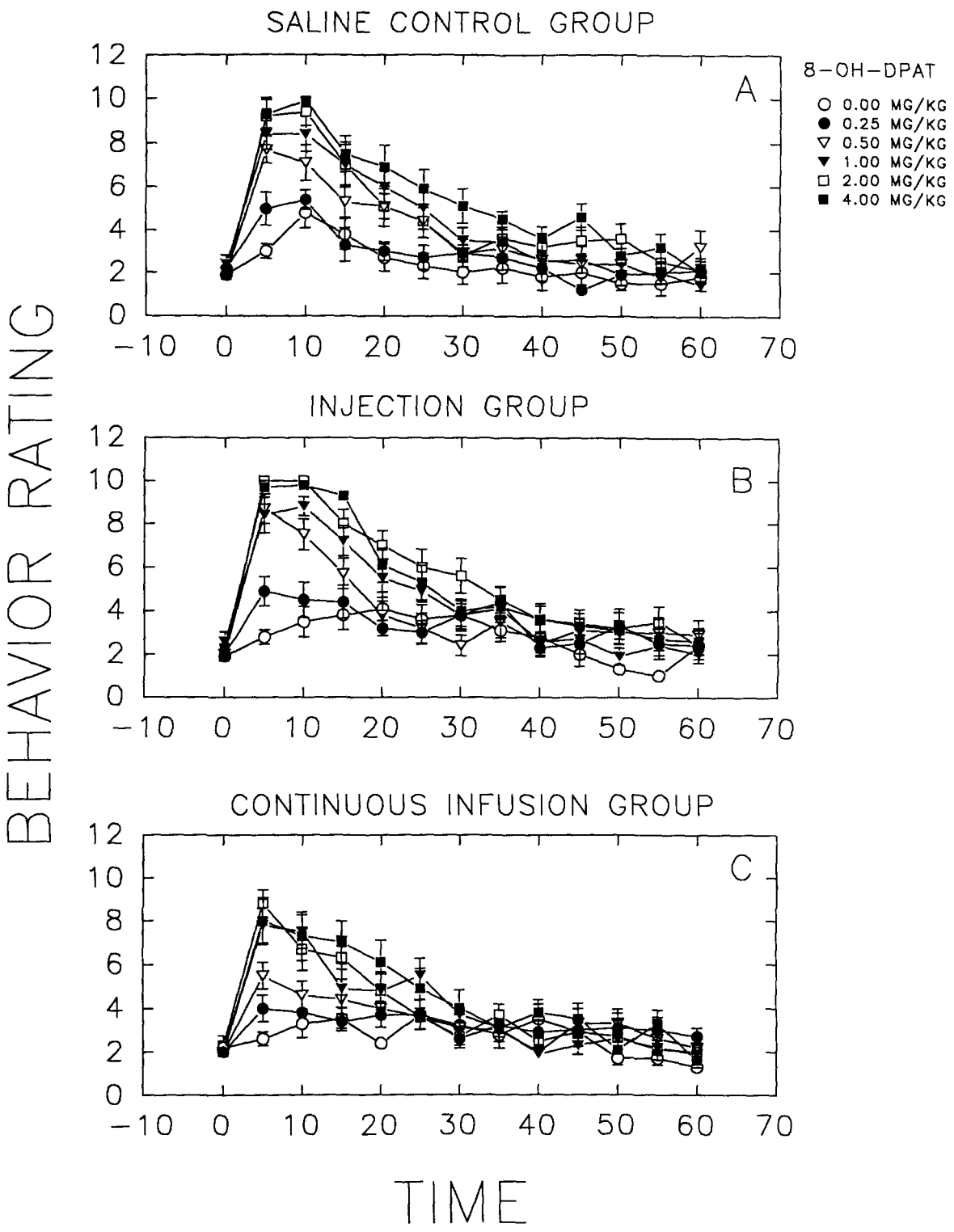

FIG. 1. Mean behavior rating for each dose of 8-OH-DPAT separately for each pretreatment group. The bars represent 1 SE. (○), vehicle (0.0 mg/kg) dose; (●), the 0.25-mg/kg dose; (▽), the 0.5-mg/kg dose; (▼), the 1.0-mg/kg dose; (□), the 2.0-mg/kg dose; (■), the 4.0-mg/kg dose.

the 0.25-mg/kg dose resulted in significantly lower behavior ratings than all other doses at both 5 and 10 min; c) the 0.5-mg/kg dose resulted in significantly lower behavior ratings than the 2.0- and 4.0-mg/kg doses only at 10 min; d) the 1.0-mg/kg dose resulted in significantly lower behavior ratings than the 2.0-mg/kg dose only at 10 min; and e) the 2.0-mg/kg dose was not significantly different from the 4.0-mg/kg dose at 5 or 10 min.

Panel C presents the behavior ratings of the continuous-infusion group. Kruskal–Wallis ANOVAs by ranks were performed separately at each time point to determine if there were any differences in the behavior ratings across the doses of 8-OH-DPAT. The results indicated that there was a significant dose effect at 5–20 and 60 min. Similar to panels A and B, we will only report the results of Mann–Whitney tests for the 5- and 10-min time points. The results of the Mann–Whitney tests indicate that: a) the 0.0-mg/kg dose resulted in significantly lower behavior ratings than all other doses except the 0.25-mg/kg dose at 10 min and the 0.5-mg/kg dose at 10 min; b) the 0.25-mg/kg dose resulted in significantly lower behavior ratings than all other doses except the 0.5-mg/kg dose at 5 and 10 min; c) the 0.5-mg/kg dose resulted in significantly lower behavior ratings than the 1.0-mg/kg dose at both 5 and 10 min and the 2.0-mg/kg dose at 5 min; d) the 1.0-mg/kg dose was not significantly different from the 2.0- and 4.0-mg/kg doses at any time point; and e) the 2.0-mg/kg dose was not significantly different from the 4.0-mg/kg dose at 5 or 10 min.

Figure 2 presents the mean behavior rating for each pretreatment group separately for each dose of 8-OH-DPAT. Panel A presents the behavior ratings of subjects receiving a vehicle injection. The results of Kruskal–Wallis ANOVAs by rank indicated that there were significant differences between the pretreatment groups at 30 and 55 min. Mann–Whitney tests comparing the different pretreatment groups at 30 min indicated that the behavior ratings for the saline control group were significantly higher than the behavior ratings for both the injection group and the continuous-infusion group. Mann–Whitney tests comparing the different pretreatment groups at 55 min indicated that the behavior ratings for the injection group were significantly higher than those for the continuous-infusion group.

Panel B presents the behavior ratings for subjects receiving a 0.25-mg/kg dose of 8-OH-DPAT. The results of Kruskal–Wallis ANOVAs by rank indicated that there were significant differences between the pretreatment groups at 10 and 45 min. Mann–Whitney tests comparing the different pretreatment groups indicated that the behavior ratings for the saline control group were significantly higher than the behavior ratings for the injection group and the continuous-infusion group at both 10 and 45 min.

Panel C presents the behavior ratings for subjects receiving a 0.5-mg/kg dose of 8-OH-DPAT. The results of Kruskal–Wallis ANOVAs by rank indicated that there were significant differences between the pretreatment groups at 5 and 10 min. Mann–Whitney tests comparing the different pretreatment groups at 5 min indicated that the behavior ratings for the saline control group and the cocaine injection group were significantly higher than the behavior ratings for the continuous-infusion group. Mann–Whitney tests comparing the different pretreatment groups at 10 min indicated that the behavior ratings for the saline control group and the cocaine injection group were significantly higher than the behavior ratings for the continuous-infusion group.

Panel D presents the behavior ratings for subjects receiving a 1.0-mg/kg dose of 8-OH-DPAT. The results of Kruskal–Wallis ANOVAs by rank indicated that there were no significant differences between the pretreatment groups at any time point.

Panel E presents the behavior ratings for subjects receiving a 2.0-mg/kg dose of 8-OH-DPAT. The results of Kruskal–Wallis ANOVAs by rank indicated that there were significant differences between the pretreatment groups at 5, 10, 25, and 30 min. Mann–Whitney tests comparing the different pretreatment groups at 5 min indicated that the behavior ratings for the cocaine injection group were significantly higher than the behavior ratings for the continuous-infusion group. Mann–Whitney tests comparing the different pretreatment groups at 10 min indicated that the behavior ratings for the saline control group and the cocaine injection group were significantly higher than the behavior ratings for the continuous-infusion group. Mann–Whitney tests comparing the different pretreatment groups at 25 min indicated that the behavior ratings for the cocaine injection group were significantly higher than the behavior ratings for the continuous-infusion group. Mann–Whitney tests comparing the different pretreatment groups at 30 min indicated that the behavior ratings for the saline control group were significantly higher than the behavior ratings for both the cocaine injection and the continuous-infusion groups.

Panel F presents the behavior ratings for subjects receiving a 4.0-mg/kg dose of 8-OH-DPAT. The results of Kruskal–Wallis ANOVAs by rank indicated that there were significant differences between the pretreatment groups at 35 min. Mann–Whitney tests comparing the different pretreatment groups at 35 min indicated that the behavior ratings for the saline control group were significantly higher than the behavior ratings for the continuous-infusion group.

Experiment 2

Figure 3 presents the mean behavior rating for each dose of CGS 12066B separately for each pretreatment group. Panel A presents the behavior ratings of the saline control group. Kruskal–Wallis ANOVAs by ranks were performed separately at each time point to determine if there were any differences in the behavior ratings across the doses of CGS 12066B. The results indicated that there was a significant dose effect at 10, 15, 30, and 40 min. Mann–Whitney tests comparing the different doses of CGS 12066B at 10 min indicated that the 0.0-, 2.0-, 8.0-, and 16-mg/kg doses resulted in behavior ratings that were significantly smaller than the 4.0-mg/kg dose. Mann–Whitney tests comparing the different doses of CGS 12066B at 15 min indicated that the 2.0- and 8.0-mg/kg doses resulted in behavior ratings that were significantly smaller than the 16.0-mg/kg dose. Mann–Whitney tests comparing the different doses of CGS 12066B at 30 min indicated that the behavior ratings for the 0.0-mg/kg dose are significantly smaller than the behavior ratings for the 4.0-mg/kg dose. Further, the behavior ratings for the 4.0- and 8.0-mg/kg doses resulted in behavior ratings that were significantly smaller than the 16.0-mg/kg dose. Mann–Whitney tests comparing the different doses of CGS 12066B at 40-min 2.0-, 4.0-, and 8.0-mg/kg doses resulted in behavior ratings that were significantly smaller than the 16.0-mg/kg dose.

Similar to panel A, panel B presents the behavior ratings for the cocaine injection group separately for each dose of CGS 12066B. The results of Kruskal–Wallis ANOVAs by ranks did not indicate any significant differences at any time point.

Similar to panels A and B, panel C presents the behavior

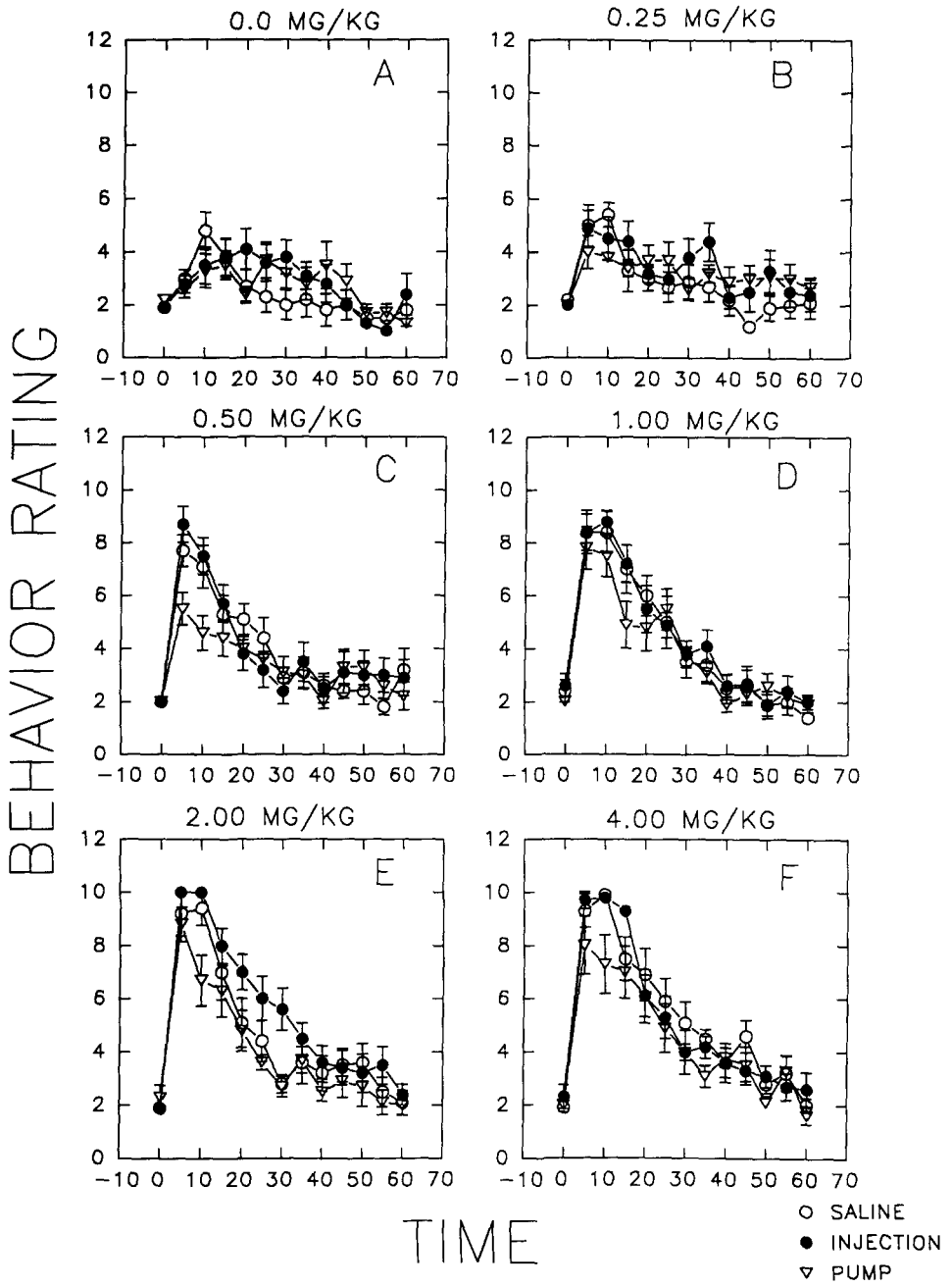

FIG. 2. Mean behavior rating for each group separately for each dose of 8-OH-DPAT. The bars represent 1 SE. (○), saline pretreatment rats; (●), cocaine injection pretreatment rats; (▽), continuous-infusion pretreatment rats.

ratings for the continuous-infusion group separately for each dose of CGS 12066B. The results of Kruskal-Wallis ANOVAs by ranks did not indicate any significant differences at any time point.

Figure 4 presents the mean behavior rating for each pretreatment group separately for each dose of CGS 12066B. Panel A presents the behavior ratings of subjects receiving vehicle injections. The results of Kruskal-Wallis ANOVAs by rank indicated that there were no significant differences between the pretreatment groups at any time point.

Panel B presents the behavior ratings for subjects receiving a 2.0-mg/kg dose of CGS 12066B. The results Kruskal-Wallis ANOVAs by ranks indicated a significant difference at 15 min. Mann-Whitney tests comparing the different pretreatment groups at 15 min indicated that the behavior ratings for the saline control group were significantly less than the behavior ratings for both the injection group and the continuous-infusion group.

Panel C presents the behavior ratings for subjects receiving a 4.0-mg/kg dose of CGS 12066B. The results Kruskal-Wallis

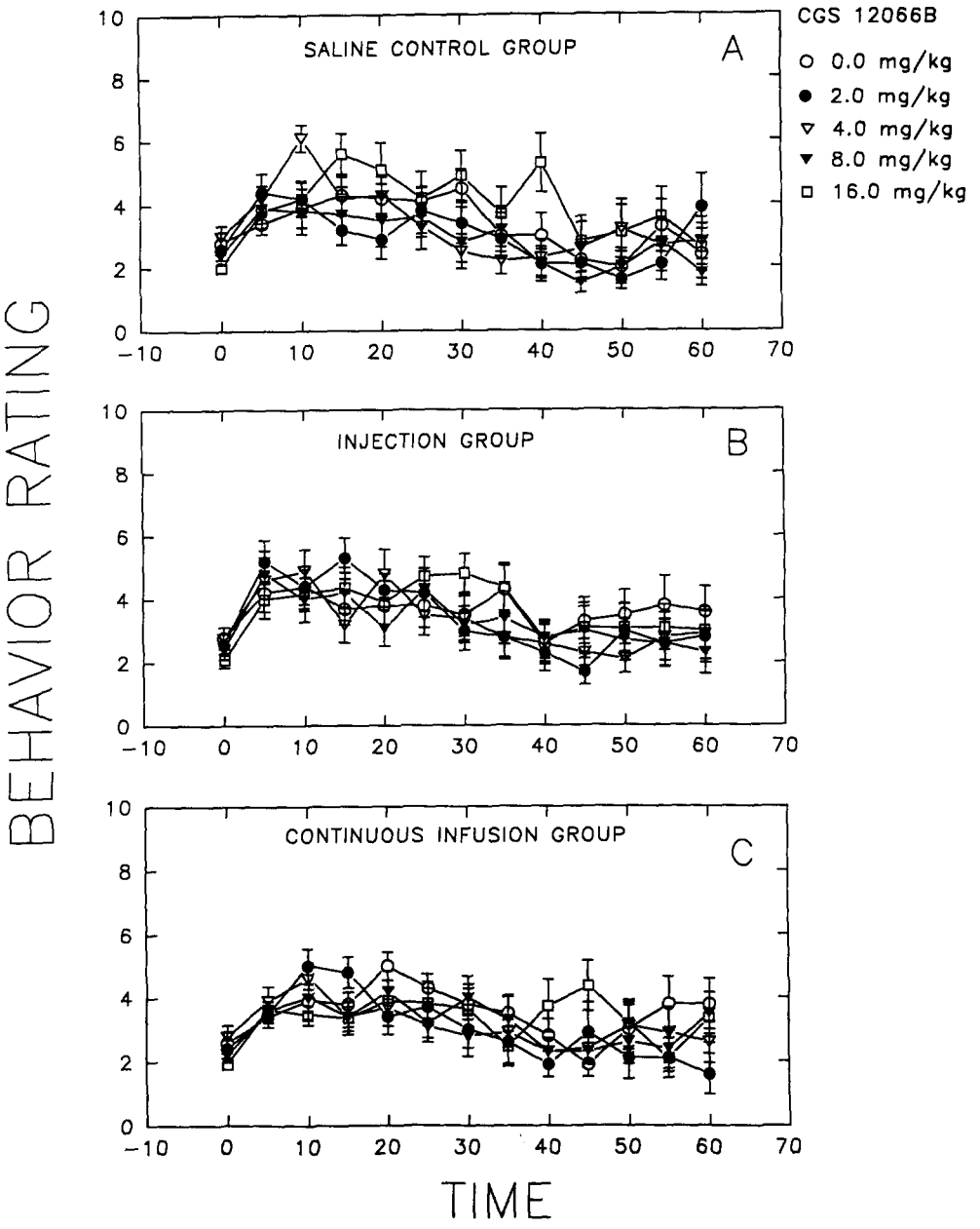

FIG. 3. Man behavior rating for each dose of CGS 12066B separately for each pretreatment group. The bars represent 1 SE. (○), the 0.0-mg/kg dose; (●), the 2.0-mg/kg dose; (▽) the 4.0-mg/kg dose; (▼), the 8.0-mg/kg dose; (□), the 16.0-mg/kg dose.

ANOVAs by ranks indicated a significant difference at 10 min. Mann-Whitney tests comparing the different pretreatment groups at 10 min indicated that the behavior ratings for the saline control group were significantly higher than the behavior ratings for the continuous-infusion group.

Panel D presents the behavior ratings for subjects receiving an 8.0-mg/kg dose of CGS 12066B. The results of Kruskal-Wallis ANOVAs by rank indicated that there were no significant differences between the pretreatment groups at any time point.

Panel E presents the behavior ratings for subjects receiving a 16.0-mg/kg dose of CGS 12066B. The results Kruskal-Wallis ANOVAs by ranks indicated a significant difference at 15 min. Mann-Whitney tests comparing the different pretreatment groups at 10 min indicated that the behavior ratings for the saline control group were significantly higher than the behavior ratings for the continuous-infusion group.

DISCUSSION

The present results support and extend previous findings that indicate that the effects of chronic cocaine depend upon

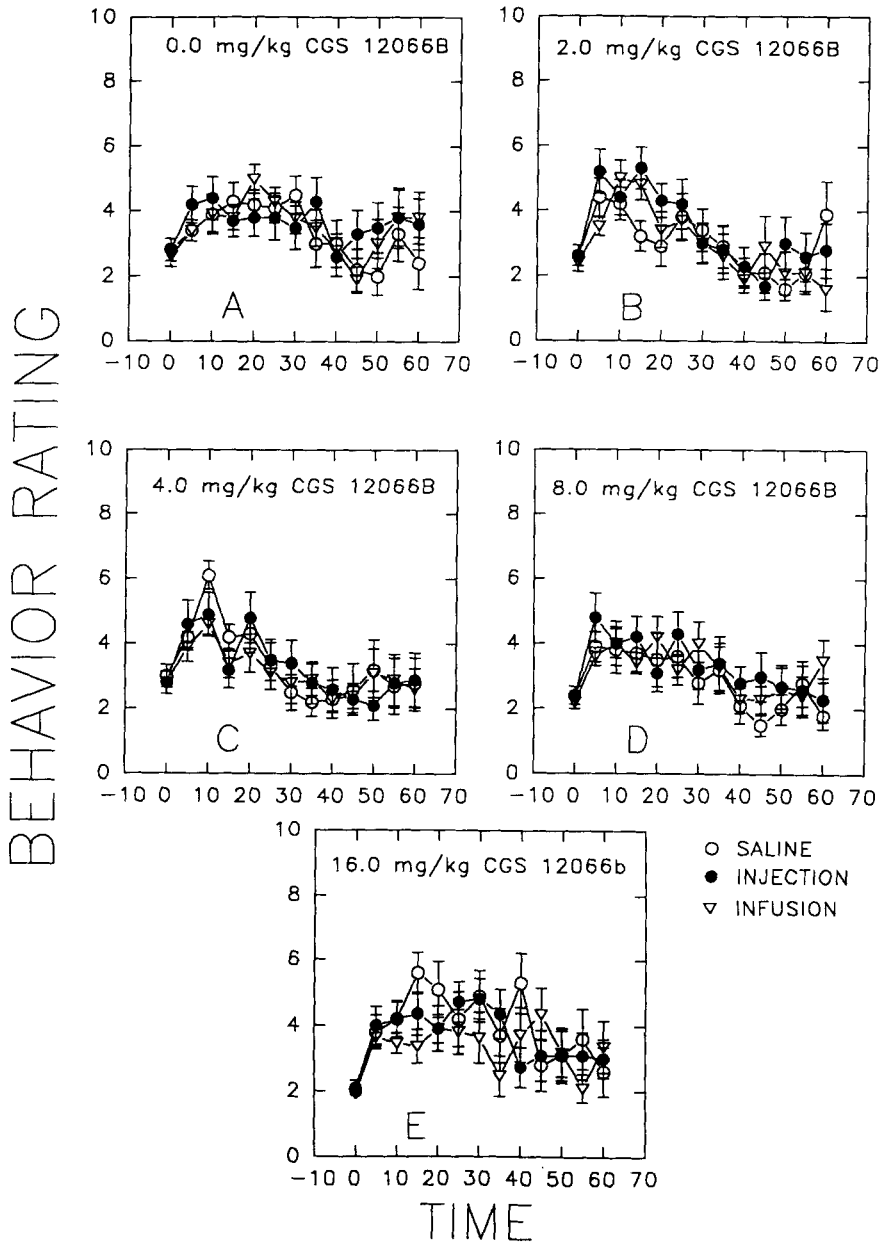

FIG. 4. Mean behavior rating for each pretreatment group separately for each dose of CGS 12066B. The bars represent 1 SE. (○), saline pretreatment rats; (●), cocaine injection pretreatment rats; (▽), continuous-infusion pretreatment rats.

the route and temporal pattern of administration. Chronic, daily SC injections of cocaine may produce an increase in the sensitivity to 5-HT_{1A} receptor agonists, but the results were not consistent. However, there was no evidence that chronic, daily SC injections of cocaine produced any change in sensitivity to 5-HT_{1B} receptor agonists. In contrast to these results, continuous infusion of an overall, equivalent daily dose of cocaine produces a decrease in the sensitivity to 5-HT_{1A} receptor agonists but no change in sensitivity to 5-HT_{1B} receptor agonists.

Previous research indicates that 5-HT has an inhibitory (regulatory) role in stimulant-induced behaviors: a) The midbrain serotonergic raphe (10,31,42) or medial forebrain bundle lesions (24) enhance the locomotor-stimulating effects of stimulants; b) administration of parachlorophenylalanine, 5,6- or 5,7-dihydroxytryptamine enhances the locomotor stimulating properties of the amphetamines and increases the break points of cocaine self-administration on progressive ratio schedules. This increase in the breakpoint indicates an increased reinforcing efficacy of cocaine following elimination of 5-HT neurotransmission (6,37,39,43,51); c) the destruction of cerebral 5-HT neurons increases amphetamine self-admin-

istration (38); and d) pharmacological interventions such as injections of L-tryptophan, which increase 5-HT synthesis, fluoxetine (an uptake inhibitor), or quipazine (a 5-HT agonist) all decrease amphetamine self-administration (36,56).

Given these results, the present series of experiments examined the hypothesis that some of the behavioral effects of chronic cocaine administration may be partially mediated by changes in 5-HT$_1$ receptor sensitivity. For example, behavioral sensitization could be partially mediated by 5-HT$_{1A}$ supersensitivity. This supersensitivity would result in inhibition of 5-HT neuron firing at lower than normal cocaine doses via activation of dorsal or median raphe 5-HT$_{1A}$ autoreceptors. This decreased firing rate would result in a relative disinhibition of the mesolimbic DA system; under these conditions, one would expect an enhanced behavioral response to dopamine-mediated behaviors. Such results have been reported following daily intermittent injections of psychomotor stimulants (33, 46). A similar argument is possible for alterations in 5-HT$_{1B}$ receptor sensitivity as a partial mediator of behavioral sensitization. Serotonergic modulation of the tolerance produced by the continuous infusion of cocaine (33,34,48) is possible by 5-HT$_{1A}$ and/or 5-HT$_{1B}$ receptor subsensitivity.

The 5-HT$_{1A}$ Receptor

The present results are consistent with the hypothesis that continuous infusion of cocaine results in 5-HT$_{1A}$ receptor subsensitivity. The saline control group (i.e., rats receiving chronic saline injections) demonstrated a dose-dependent increase in locomotor activity with 8-OH-DPAT administration. In contrast, although administration of 8-OH-DPAT did increase locomotor activity in continuous-infusion subjects, there was no consistent dose dependency. Further, the behavior ratings produced by different doses of 8-OH-DPAT in continuous-infusion subjects tended to be significantly less than the behavior ratings, produced by the same doses of 8-OH-DPAT, in subjects receiving daily saline or cocaine injections during the chronic pretreatment regimen for virtually all of the doses of 8-OH-DPAT tested. These results are consistent with our previous results (34) using the 5-HT$_{1A}$ receptor antagonist NAN-190 to inhibit cocaine-induced hyperactivity: NAN-190 did not suppress cocaine-induced behaviors in continuous-infusion subjects, which was in stark contrast to the results with saline control and cocaine injection subjects. Hence, both the present and previous (34) results indicate a substantially reduced behavioral responsivity to 5-HT$_{1A}$ receptor ligands during withdrawal from continuous infusion of cocaine.

The results for the daily injection group are mixed regarding the issue of 5-HT$_{1A}$ receptor supersensitivity. First, administration of 8-OH-DPAT resulted in behavioral activation; most of the doses, with the exception of the lowest dose, resulted in near maximal activity levels, implying that the 5-HT$_{1A}$ receptors are supersensitive. Second, the behavior ratings for these subjects were significantly higher than saline control subjects for some of the doses tested. However, these behavioral differences were not consistent across all of the doses tested. This inconsistent pattern of results is consistent with our previous results using NAN-190 (34): Some combinations of NAN-190 plus 15 mg/kg cocaine resulted in a complete elimination of sensitization in these subjects. This is evidence for substantial 5-HT$_{1A}$ receptor supersensitivity. However, other combinations of NAN-190 plus 15 mg/kg cocaine failed to indicate any evidence of 5-HT$_{1A}$ receptor supersensitivity (i.e., there were no differences between cocaine injection and saline control subjects).

The failure to find clear behavioral evidence for an increase in 5-HT$_{1A}$ receptor sensitivity following chronic daily injections of cocaine is somewhat surprising. Cunningham and colleagues (11–14) extensively demonstrated that systemic administration of cocaine inhibits dorsal raphe firing rates and that somadendritic 5-HT$_{1A}$ receptors become supersensitive following daily, intermittent injections of cocaine. Hence, one would expect that functional behavioral effects would also be found. Our failure to find consistent effects may be due to the use of a rating scale that may not be sensitive enough to detect any residual behavioral effects that may be present. Use of a different behavioral paradigm (e.g., differential reinforcement of low rates of behavior) may have detected the presence of alterations in 5-HT$_{1A}$ receptor sensitivity.

The 5-HT$_{1B}$ Receptor

The present results also indicate that the effects of chronic cocaine administration via SC injections or continuous infusion are not mediated by alterations in 5-HT$_{1B}$ receptor sensitivity. This result is also somewhat surprising considering that: a) Benloucif and Galloway (3) recently reported that infusion of RU 24969, a 5-HT$_{1B}$ receptor agonist, into the anterior striatum increased extracellular DA concentrations by 300%. Further, RU24969 also increased extracellular DA concentrations following systemic pretreatment with 8-OH-DPAT; and b) systemic (22) or central (15) administration of the 5-HT$_{1B}$ agonist RU 24969 results in a dose-dependent increase in locomotor activity, and this effect is eliminated with reserpine pretreatment (54).

However, the locomotor-stimulating properties of RU 24969 may not be DA dependent. Oberlander et al. (44) found that the stimulating effects of RU 24969 were not affected by lesions of the globus pallidus, which receives inputs from both the nigrostriatal and mesolimbic DA systems. Further, RU 24969 is not selective for the 5-HT$_{1B}$ receptor; it displays a substantial affinity for the 5-HT$_{1A}$ receptor (40). Thus, the locomotor-stimulating effects of RU 24969 may be partially mediated by the activation of 5-HT$_{1A}$ receptors as opposed to the activation of 5-HT$_{1B}$ receptors. In contrast to RU 24969, CGS 12066B is much more selective for the 1B as opposed to the 1A receptor (40). This difference in selectivity may explain the failure to find any consistent effect of chronic cocaine on the response to CGS 12066B.

SUMMARY

In summary, the present results indicate that daily, intermittent SC injections of cocaine produce marginal 5-HT$_{1A}$ receptor supersensitivity. However, such injections apparently have no effect on 5-HT$_{1B}$ receptor sensitivity. In contrast to these results with SC cocaine injections, the continuous infusion of equivalent daily doses of cocaine clearly results in 5-HT$_{1A}$ receptor subsensitivity. However, the continuous infusion of cocaine apparently has no effect on 5-HT$_{1B}$ receptor sensitivity. Hence, changes in 5-HT$_{1A}$ receptor sensitivity may represent a partial mechanism for the development of sensitization and tolerance. However, the present results do not implicate changes in 5-HT$_{1B}$ receptor sensitivity in the development of sensitization or tolerance.

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