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Involvement of Serotonin in the Modulation of Cocaine-Induced Locomotor Activity in the Rat

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HERGES, S. AND D. A. TAYLOR. *Involvement of serotonin in the modulation of cocaine-induced locomotor activity in the rat*. PHARMACOL BIOCHEM BEHAV **59**(3) 595–611, 1998.—The influence of serotonin (5-HT) antagonists and a selective serotonin reuptake inhibitor (SSRI) on cocaine-induced locomotor activity, rears, and head bobs was investigated in female Glaxo Wistar rats. The SSRI, fluoxetine (10 mg/kg), and the nonselective 5-HT agent, methysergide, at the dose range of 5 and 15 mg/kg enhanced the behaviors produced by cocaine (15 mg/kg) to a similar extent. Moreover, the potentiation of cocaine-induced locomotor activity, rears, and head bobs was even greater after the combined administration of methysergide (15 mg/kg) and fluoxetine (10 mg/kg). In order to investigate a possible involvement of 5-HT_{1A} receptors in the observed potentiation by methysergide and fluoxetine, the potent and selective 5-HT_{1A} antagonist, WAY 100635, was used. WAY 100635 (0.1 and 1.5 mg/kg) markedly reduced the behaviors induced by cocaine preceded by fluoxetine (10 mg/kg) and methysergide (5 and 15 mg/kg) pretreatment, respectively, suggesting an involvement of 5-HT_{1A} receptors in the action of fluoxetine and methysergide on cocaine-induced behaviors. An attenuation of the fluoxetine-enhanced cocaine-induced behaviors was also observed after pretreatment with the 5-HT_{2A} antagonist ketanserin (0.1 and 1.0 mg/kg). Coadministration of ketanserin (1.0 mg/kg) and WAY 100635 (1.5 mg/kg) resulted in the greatest blockade of the fluoxetine-enhanced cocaine-induced behaviors. The antagonists and the SSRI, fluoxetine, did not alter the behaviors in comparison to that of saline-treated animals. These results provide evidence for an involvement of 5-HT_{1A} receptors in the enhancing effect of fluoxetine and methysergide on cocaine-induced locomotor activity, rears, and head bobs, and suggest a stimulatory action of methysergide at the 5-HT_{1A} receptor. In addition, some of the actions may also be mediated by activation of the 5-HT_{2A} receptor and/or inhibition of the 5-HT_{2C} receptor. © 1998 Elsevier Science Inc.

Cocaine Locomotor activity Behavior Fluoxetine Methysergide WAY 100635 Ketanserin 5-HT_{1A} 5-$HT_{2A/C}$

COCAINE, a psychomotor stimulant, elicits an increase in locomotor activity and stereotypic behaviors in rodents [(66), see review (77)]. Previous studies investigating cocaine's mechanism of action have shown that cocaine binds with high affinity to dopamine (DA), noradrenaline (NA), and serotonin (5-HT) transporter sites (37,55–57,61), thereby inhibiting the presynaptic reuptake of these neurotransmitters (39,63,64). The stereotypic behaviors and locomotor activity induced by psychostimulants are thought to be associated with activation of the nigrostriatal and mesolimbic DA systems, respectively (14,35). It has been shown that, selective lesions of DA fibers in the nucleus accumbens (NAC) reduced the locomotor activity induced by psychostimulants (35). Microinjection of cocaine into the NAC induced locomotor activity in the rat, which was blocked by peripheral administration of the DA antagonist, cis-flupenthixol (18). Furthermore, in vivo voltammetry and in vivo microdialysis indicate a significant increase of the extracellular levels of DA and 5-HT in the NAC and the striatum after peripheral cocaine administration (8,9,21). In addition, it has been reported that there is a positive correlation between the increase in extracellular DA and 5-HT levels in these forebrain regions and the observed behavioral effects (8,9). These results support the hypothesis that cocaine-induced behaviors in rodents are associated with an activation of the dopaminergic system. Moreover, the observation that cocaine increased the extracellular 5-HT levels in the NAC and the striatum implicates a contributory role of 5-HT in the behavioral effects of cocaine (8,9).

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Several behavioral studies have investigated the interactive relationship between 5-HT and cocaine's stimulant effects on behaviors. Depletion of 5-HT induced by peripheral pretreatment with *p*-chlorophenylalanine (PCPA), an inhibitor of the 5-HT biosynthesis (38), potentiated the cocaine-induced locomotor activity in rats (66,70). This potentiation of cocaine-induced locomotor activity following 5-HT depletion suggests an inhibitory role of 5-HT on cocaine-induced behaviors [see review (69)], which is also supported by the observation that the 5-HT precursor 5-hydroxytryptophan (5-HTP) attenuated cocaine-induced locomotor activity in rats (54). On the other hand, the selective serotonin reuptake inhibitors (SSRI), fluoxetine and fluvoxamine, have been reported to potentiate the locomotor activity induced by acute cocaine administration in rodents (59,74), which is contrary to an inhibitory role of 5-HT on cocaine-induced locomotor activity. In contrast, fluoxetine and sertraline, another SSRI, failed to alter the locomotor activity induced by cocaine in C57BL/6ByJ mice (59).

Following behavioral studies investigating the serotonergic influence on cocaine's reinforcing effects, it has been shown that fluoxetine and the systemic and dietary administration of L-tryptophan reduced cocaine self-administration in rats (10, 11,44,49,60), whereas depletion of 5-HT following forebrain lesions induced by 5,7-dihydroxytryptamine (5,7-DHT) resulted in an increase of cocaine self-administration (41,62). Since 5-HT decreased cocaine self-administration, it was suggested that 5-HT antagonists may increase the rate of cocaine intake in rats (40). The nonselective 5-HT agent methysergide, the 5-HT_2 antagonist ketanserin, as well as the 5-HT_3 antagonists MDL 72222 and ondansetron failed to alter the rate of cocaine self-administration (40,48).

Recent studies in rodents investigating the 5-HT receptor subtype that might be involved in the modulatory serotonergic influence on cocaine-induced behaviors, reported an attenuation of cocaine-induced locomotor activity by the selective 5-HT_3 antagonists zacopride, ICS 205-930, and MDL 72222 (58,70), whereas studies carried out in this laboratory failed to show any effect of ondansetron (74). In addition, methysergide did not alter the locomotor activity induced by acute cocaine administration in mice (58).

Although it appears from the results of some behavioral studies that 5-HT_3 receptors mediate some of the serotonergic influence on cocaine-induced locomotor activity, but not the cocaine self-administration, the involvement of another receptor subtype cannot be excluded. The present study investigated the effect of peripheral pretreatment with the nonselective 5-HT agent methysergide with affinity at 5-HT_1 and 5-HT_2 receptors, the selective 5-HT_{1A} antagonist WAY 100635 and the selective 5-HT_{2A} antagonist ketanserin on cocaine's stimulant effect on behaviors in rats. Following the observation that SSRIs either potentiate (59,74) or fail to alter cocaine-induced locomotor activity in rodents (59), it was also of interest to assess the effect of the SSRI fluoxetine and the combined administration of the antagonists and fluoxetine on cocaine-induced behaviors.

METHOD

Animals

Female Glaxo Wistar rats, weighing 200–280 g, were obtained from the animal house of the Victorian College of Pharmacy. The animals were housed in group cages of eight rats in a 12 L:12 D cycle in a temperature- and moisture-regulated room. Food and water were available ad libitum. Due to the large number of animals required for the experimental design, each group of eight rats was used at least twice, but no more than four times, with a 10–12 day interval between each use, and no group received the same treatment more than once. A naive group of eight animals was used for each serotonergic pretreatment followed by cocaine administration. Some animals, when reused, may have been exposed to cocaine previously. These animals did not receive the same pretreatment, and the time interval between cocaine injections was at least 4 weeks. No animal received cocaine more than twice. Since the data obtained following the same treatments did not differ from either naive groups or reused groups, a sensitisation to cocaine can be excluded.

Locomotor Activity and Behavioral Testing

All experiments were performed between 0830 and 1630 h. Locomotor activity was quantified using a circular photobeam activity meter. The activity meter was located in an adjacent room isolated from the laboratory where the animals were kept for treatment and the time between the behavioral testing. The activity meter (diameter 67 cm, height 79 cm) was equipped with six horizontal infrared photocell beams located 3 cm above the wire grid floor and spaced approximately 13 cm apart. The activity meter was evenly illuminated by the use of two 40-watt bulbs attached to a metal frame surrounding the activity meter. All photobeam interruptions were automatically recorded by two counters (one for each bank of three beams) and totalled for each behavioral session of 5-min duration.

For each drug treatment 15–16 rats were used. In dose–response experiments 5–30 mg/kg cocaine IP was administered. Antagonists or saline (IP) were given 60 min prior to cocaine (15 mg/kg, 0.1 ml/100 g IP) or saline (IP) administration. Methysergide (5–15 mg/kg) and ketanserin (0.1 and 1.0 mg/kg) were injected IP in a volume of 0.2 ml/100 g. WAY 100635 was administered SC at doses of 0.1 and 1.5 mg/kg in a volume of 0.1 ml/100 g. The first pretreatment was followed 30 min later by fluoxetine (10 mg/kg, 0.2 ml/100 g IP) or saline (IP) administration. The only exception from this injection regimen was the treatment with WAY 100635 (1.5 mg/kg SC)–methysergide (15 mg/kg IP)–saline (IP)–cocaine (15 mg/kg IP). WAY 100635 was injected 90 min prior to cocaine. The pretreatment with WAY 100635 was followed by methysergide at 60 min before cocaine and by saline 30 min later (−30 min). The control groups' behaviors were determined in rats treated with saline using the above described injection regimen.

At the commencement of the behavioral session the home cage lid was removed. The cage lid remained off for the duration of the experiment (maximum 150 min). The basal locomotor activity was quantified during each 5-min observation period 30 min (data not presented) and 10 min before cocaine administration, which allowed the animals to habituate to the new environment and 10, 30, and 60 min after cocaine administration. The animals were exposed to the apparatus on the days before the first behavioral testing. This familiarization resulted in more consistent baseline activities than that in animals not previously exposed to the apparatus. To quantify the behaviors, the animal was placed in the center of the wire grid floor of the activity meter. After 5 min testing period, the rat was replaced by the next one and the counters were rezeroed after noting the scores. The animal was immediately returned to its home cage located in the adjacent laboratory. During the 5-min behavioral sessions, 10, 30, and 60 min after cocaine administration, each rat was videotyped using a Panasonic WV-BL 200 video camera centred 1.6 m above the wire grid floor, connected to a Panasonic Super-VHS FS 90 video cas-

sette recorder located in the adjacent laboratory. The video was stored for the later scoring of the following behaviors: rears, head shakes, and head bobs. The vertical movement rear was defined as when the rat raised both forepaws, either in the open field or against the wall of the activity meter. Head twitches were counted as head shakes excluding whole body shakes. Head bobs were quantified when the animals displayed lateral head movements. Additionally, the upward movements of the head were also counted as head bobs. Due to the location of the video camera, the quantification of oral hyperkinesia (licking and chewing) was not possible.

Statistical Analysis

The locomotor activity and behavioral data were analyzed by one-way analysis of variance (ANOVA). For pairwise multiple comparison between the treatment groups, the Newman–Keuls test was used.

Drugs

The following drugs were used: cocaine HCl (Glaxo Wellcome, Australia), fluoxetine HCl (Eli Lilly, Indianapolis, IN), ketanserin tartrate (Janssen, Beerse, Belgium), methysergide hydrogenmaleinate (Sandoz, Basel, Switzerland), WAY 100635 (Wyeth-Ayerst, Maidenhead, UK). All drugs were freshly prepared and used immediately. The weights used refer to the appropriate salts. Cocaine HCl, fluoxetine HCl, ketanserin tartrate, and methysergide hydrogenmaleinate were dissolved in saline 0.9% w/v. The pH of the methysergide solution was adjusted to 5.5. WAY 100635 was prepared in water for injection.

RESULTS

Effect of Cocaine on Baseline Behaviors

Administration of cocaine (5–30 mg/kg IP) increased activity, the number of rears, and head bobs in a dose-dependent manner [locomotor activity: $F(2, 46) = 46.5$, $p < 0.0001$; rears: $F(2, 46) = 4.35$, $p < 0.05$; head bobs: $F(2, 46) = 45.5$, $p < 0.0001$] (Fig. 1a–c). A significant increase of locomotor activity and the number of head bobs was observed following treatment with cocaine at the high dose of 30 mg/kg compared to animals treated with cocaine at the dose of 15 mg/kg (Fig. 1a and c). At the dose of 5 mg/kg, the locomotor activity and the number of rears induced by cocaine were significantly lower than in animals treated with cocaine at the dose of 15 mg/kg (Fig. 1a and b). To enable either an increase or a decrease in the cocaine-induced behaviors to be observed following pretreatments, a dose of 15 mg/kg cocaine was used. This dose of cocaine resulted in an increase in activity approximately in the middle of the dose–response curve.

Cocaine increased the locomotor activity, rears, and head bobs, which lasted for the duration of the experiment [locomotor activity: $F_{10,30,60\text{min}}(3, 63) = 78.1, 45.4, 16.9$, $p_{10,30,60\text{min}} < 0.0001$; rears: $F_{10,30,60\text{min}}(3, 63) = 18.0, 9.95, 7.24$, $p_{10,30,60\text{min}} < 0.0001, 0.0001, 0.001$; head bobs: $F_{10,30,60\text{min}}(3, 63) = 66.8, 73.6, 34.7$, $p_{10,30,60\text{min}} < 0.0001$] (Fig. 2a–c).

The characteristics of the "5-HT syndrome" such as forepaw treading, flat body posture, and tremors were not observed in any treatment group. ANOVA did not yield significant group effects for the head shakes throughout the experiments, and consequently, the number of head shakes counted have been omitted for clarity.

Effect of Fluoxetine on Cocaine-Induced Behaviors

Fluoxetine did not alter the activity (Fig. 2a), rears (Fig. 2b), and head bobs (Fig. 2c) of saline-treated rats. The stimulant effect of cocaine was enhanced by fluoxetine [locomotor activity: $F_{10,30,60\text{min}}(3, 63) = 78.1, 45.4, 16.9$, $p_{10,30,60\text{min}} < 0.0001$; rears: $F_{10,30,60\text{min}}(3, 63) = 18.0, 9.95, 7.24$, $p_{10,30,60\text{min}} < 0.0001, 0.0001, 0.001$; head bobs: $F_{10,30,60\text{min}}(3, 63) = 66.8, 73.6, 34.7$, $p_{10,30,60\text{min}} < 0.0001$] (Fig. 2a–c). Pairwise comparison between the saline-saline-cocaine (S-S-C) and saline-fluoxetine-cocaine (S-F-C)-treated animals revealed a significant potentiation of the locomotor activity and head bobs at 10, 30, and 60 min (Fig. 2a and c). Rears were potentiated at 10 min (Fig. 2b). The behavioral scores were greatest in both treatment groups in the first 5-min period after cocaine injection. The behavioral scores gradually declined over the following 50 min.

Effect of Methysergide on Cocaine-Induced Behaviors

Methysergide (5 and 15 mg/kg) did not alter the locomotor activity (Fig. 3a), or the number of rears (Fig. 3b) and head bobs (Fig. 3c) of saline-treated rats. At doses of 5 and 15 mg/kg, methysergide potentiated the cocaine-induced locomotor activity [$F_{10,30,60\text{min}}(6, 110) = 41.8, 33.4, 9.0$, $p_{10,30,60\text{min}} < 0.0001$] (Fig. 3a). Pairwise comparison between the S-S-C group and the methysergide pretreated groups yielded statistically significant differences at 10 and 30 min, whereas the S-F-C and methysergide (5 or 15 mg/kg)–saline–cocaine (M5-S-C, M15-S-C) groups showed no significant differences in locomotor activity for the duration of the experiment (Fig. 3a). The number of head bobs was also significantly increased by methysergide pretreatment at 10, 30, and 60 min after cocaine treatment [$F_{10,30,60\text{min}}(6, 110) = 39.8, 36.9, 30.5$, $p_{10,30,60\text{min}} < 0.0001$] (Fig. 3c). Methysergide did not significantly alter the number of rears compared to the S-S-C–treated group [$F_{10,30,60\text{min}}(6, 110) = 11.0, 5.55, 3.52$, $p_{10,30,60\text{min}} < 0.0001, 0.0001, 0.01$] (Fig. 3b). The highest scores for the behaviors were observed 10 min after cocaine administration, followed by a gradual decline.

Effect of WAY 100635 on Cocaine-Induced Behaviors

Analysis of variance yielded a significant group effect (S-S-S, W0.1-S-S, W1.5-S-S, S-S-C, S-F-C, W0.1-S-C, W1.5-S-C) for the locomotor activity [$F_{10,30,60\text{min}}(6, 110) = 46.7, 25.8, 6.16$, $p_{10,30,60\text{min}} < 0.0001$], rears [$F_{10,30,60\text{min}}(6, 110) = 12.2, 6.92, 4.32$, $p_{10,30,60\text{min}} < 0.0001, 0.0001$], 0.001, and head bobs [$F_{10,30,60\text{min}}(6, 110) = 61.8, 55.2, 21.9$, $p_{10,30,60\text{min}} < 0.0001$], which lasted for the duration of the experiment. WAY 100635 (0.1 and 1.5 mg/kg) did not alter the locomotor activity (Fig. 4a) or the number of rears (Fig. 4b) and head bobs (Fig. 4c) in comparison to that of saline-treated animals. Pretreatment with WAY 100635 at both doses did not affect the locomotor activity and number of rears induced by cocaine (Fig. 4a and b). A significant enhancement of the cocaine-induced head bobs were observed after pretreatment with WAY 100635 at a dose of 0.1 mg/kg at 10 min (Fig. 4c). The enhancement of the number of head bobs lasted for the duration of the experiment following pretreatment with WAY 100635 at the dose of 1.5 mg/kg (Fig. 4c).

Effect of Ketanserin on Cocaine-Induced Behaviors

Ketanserin did not alter the locomotor activity (Fig. 5a), rears (Fig. 5b), and head bobs (Fig. 5c) in comparison to that of saline-treated animals. The effects of ketanserin on the co-

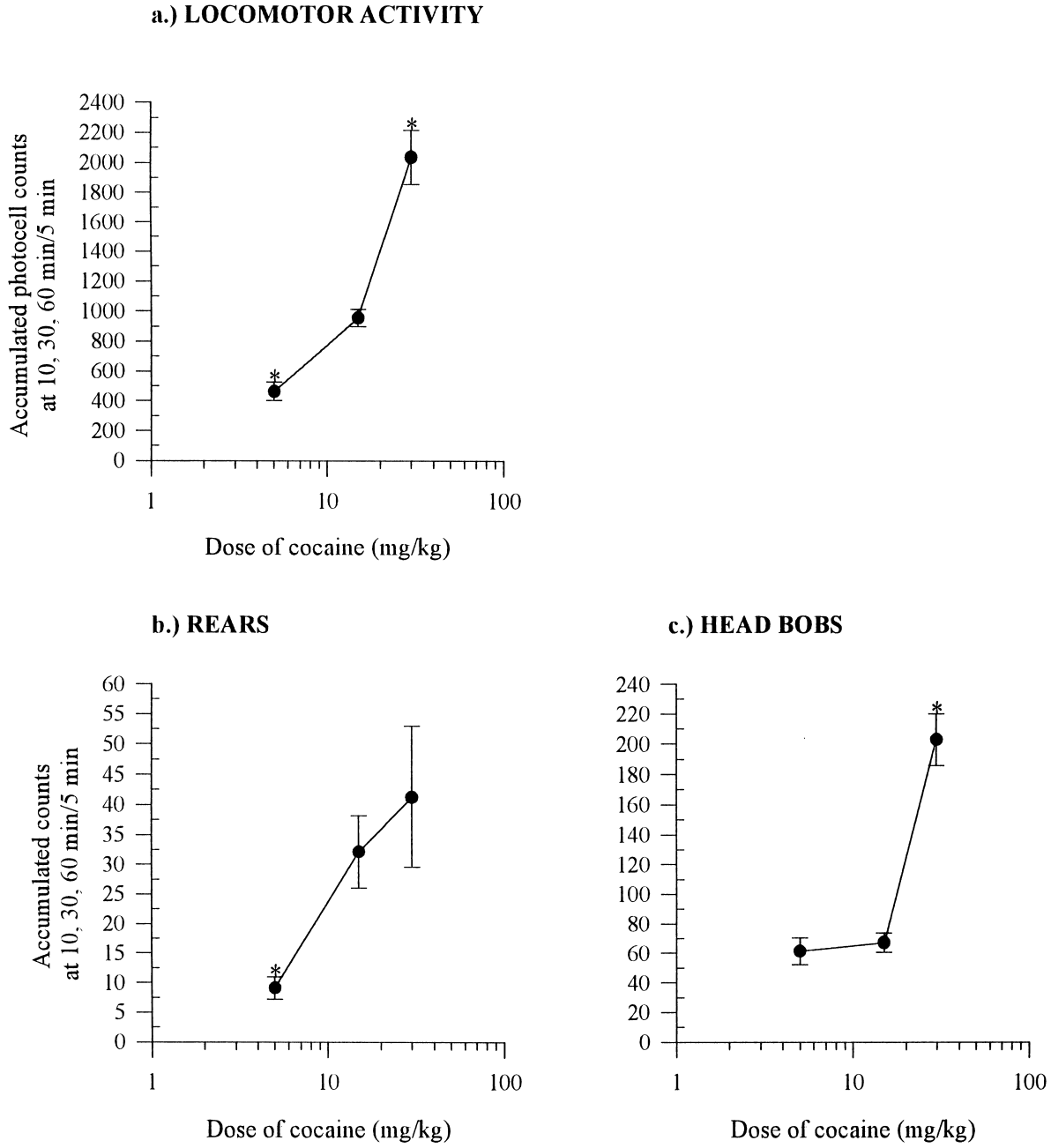

FIG. 1. The locomotor activity (a), rears (b), and head bobs (c) determined in rats treated with cocaine at doses of 5 mg/kg (C5), 15 mg/kg (C15), and 30 mg/kg (C30). All rats were pretreated 30 and 60 min earlier with saline (S). The values represent mean scores ± SEM of 15–16 rats. *$p < 0.05$ (Newman–Keuls test), S-S-C5 and S-S-C30 compared to the S-S-C15–treated group.

caine-induced behaviors were dependent on the dose of ketanserin. A significant reduction of the cocaine-induced locomotor activity at 10 and 30 min was observed following 1 mg/kg ketanserin (K1) pretreatment, whereas 0.1 mg/kg ketanserin (K0.1) significantly increased the cocaine-induced locomotor activity at 10 and 30 min [$F_{10,30,60min}(6, 111) = 48.6, 34.9, 9.14$, $p_{10,30,60min} < 0.0001$] (Fig. 5a). ANOVA yielded a significant group effect (S-S-S, K0.1-S-S, K1.0-S-S, S-S-C, S-F-C, K0.1-S-C, K1-S-C) for the number of rears [$F_{10,30,60min}(6, 111) = 14.4, 9.44, 4.06$, $p_{10,30,60min} < 0.0001, 0.0001, 0.01$] and head bobs, [$F_{10,30,60min}(6, 111) = 48.9, 53.8, 26.2$, $p_{10,30,60min} < 0.0001$] (Fig. 5b and c). The high dose of ketanserin did not alter the number of rears and head bobs compared to the S-S-C group, whereas a significant increase of these behaviors was observed by pretreatment with 0.1 mg/kg ketanserin at 10 min (Fig. 5b and c).

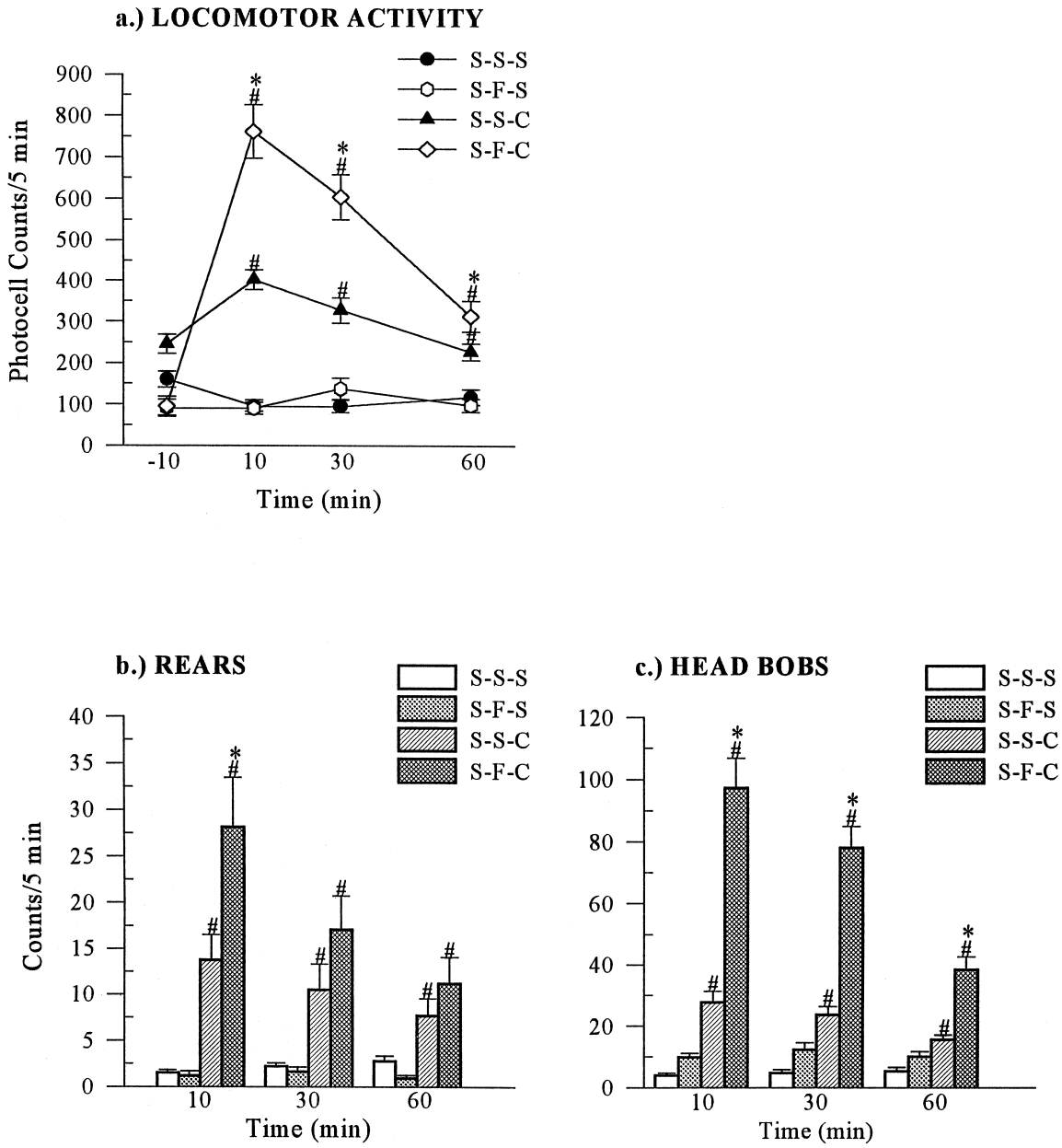

FIG. 2. The locomotor activity (a), rears (b), and head bobs (c) determined in rats treated with saline (S) or cocaine (C) following pretreatment 30 min earlier with saline (S) or fluoxetine (F). All rats were pretreated 30 min earlier than the second pretreatment with saline (S). The values represent mean scores ± SEM of 16 rats. #$p < 0.05$ (Newman–Keuls test), S-S-C and S-F-C compared to saline control group (S-S-S). *$p < 0.05$, S-F-C compared to the S-S-C–treated group.

Effect of WAY 100635 and Methysergide on Cocaine-Induced Behaviors

The combined administration of 1.5 mg/kg WAY 100635 and 15 mg/kg methysergide followed by saline and cocaine resulted in a significant reduction of locomotor activity at 30 min in comparison to the M15-S-C–treated group [$F_{10,30,60min}(3, 62) = 30.0, 25.4, 3.74$, $p_{10,30,60min} < 0.0001, 0.0001, 0.05$] (Fig. 6a). Pretreatment with WAY 100635 and methysergide did not significantly alter the number of rears and head bobs (data not presented).

The coadministration of 1.5 mg/kg WAY 100635 and 5 mg/kg methysergide also resulted in a significant reduction of the locomotor activity at 30 and 60 min compared to the M5-S-C–treated group [$F_{10,30,60min}(3, 63) = 32.6, 20.5, 9.18$, $p_{10,30,60min} < 0.0001$] (Fig. 6b). ANOVA yielded a significant group effect for the number of rears [$F_{10,30,60min}(3, 63) = 6.73, 4.08, 5.21$, $p_{10,30,60min} < 0.001, 0.05, 0.01$] and head bobs [$F_{10,30,60min}(3, 63) = 32.8, 19.2, 22.4$, $p_{10,30,60min} < 0.0001$], which lasted for the duration of the experiment (data not presented as figures). A significant attenuation of the behaviors in com-

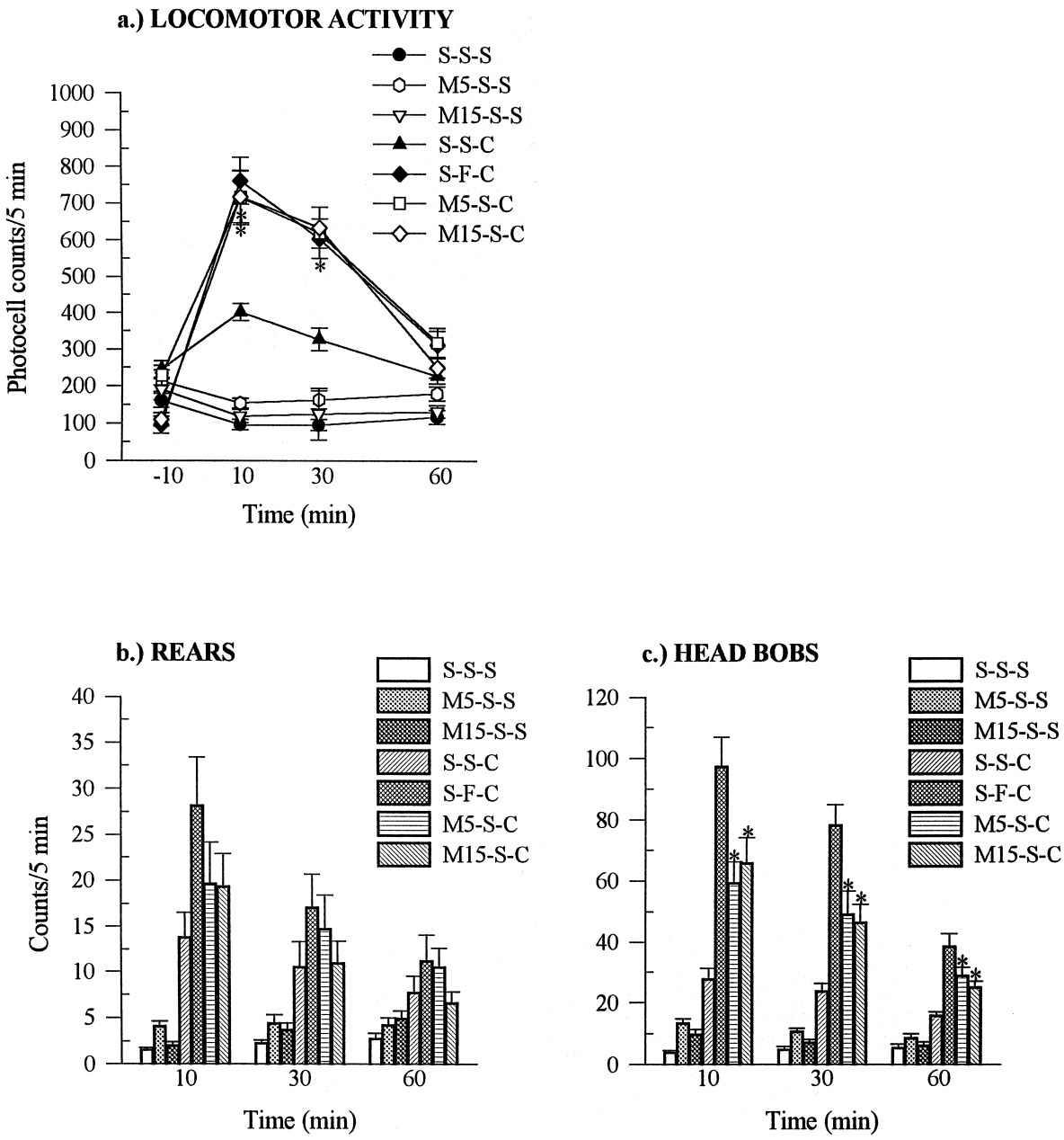

FIG. 3. The locomotor activity (a), rears (b), and head bobs (c) of rats treated with saline (S) or cocaine (C) following pretreatment 30 min earlier with saline (S) or fluoxetine (F). The second pretreatment was preceded 30 min earlier by saline (S), 5 mg/kg methysergide (M5), or 15 mg/kg methysergide (M15) administration. The values represent mean scores ± SEM of 15–16 rats. *$p < 0.05$ (Newman–Keuls test), M5-S-C and M15-S-C compared to the S-S-C–treated group.

parison to the M5-S-C treatment was observed 60 min after cocaine administration.

Effect of Ketanserin and WAY 100635 on Cocaine-Induced Behaviors

The behaviors induced by the coadministration of ketanserin (1 mg/kg) and WAY 100635 (1.5 mg/kg) followed by saline and cocaine showed no significant differences in comparison to the K1-S-C–treated group (data not presented).

Effect of Methysergide on Fluoxetine-Enhanced Cocaine-Induced Behaviors

The effect of methysergide on the fluoxetine enhancement of cocaine-induced behaviors was dependent on dose (Fig. 7a–c). Analysis of variance yielded a significant group effect (S-S-S, S-S-C, S-F-C, M5-F-C, M10-F-C, M15-F-C) for the locomotor activity, which lasted for the duration of the experiment [$F_{10,30,60min}(5, 95) = 27.0, 34.6, 15.5$, $p_{10,30,60min} < 0.0001$] (Fig. 7a). Methysergide, at the dose of 5 mg/kg, reduced the

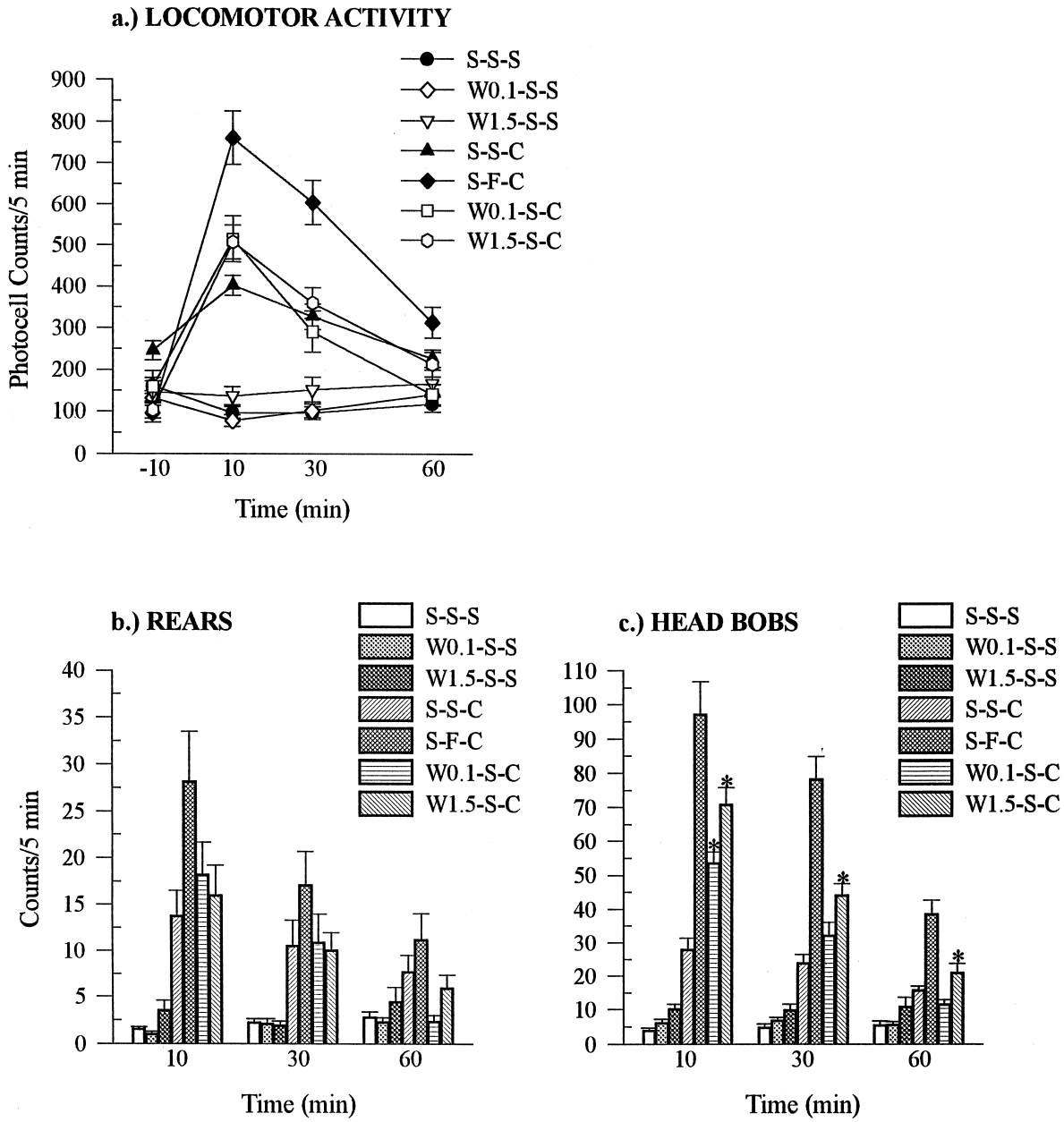

FIG. 4. The locomotor activity (a), rears (b), and head bobs (c) of rats treated with saline (S) or cocaine (C) following pretreatment 30 min earlier with saline (S) or fluoxetine (F). The second pretreatment was preceded 30 min earlier by saline (S), 0.1 mg/kg WAY 100635 (W0.1) or 1.5 mg/kg WAY 100635 (W1.5) administration. The values represent mean scores ± SEM of 15–16 rats. *$p < 0.05$ (Newman–Keuls test), W0.1-S-C and W1.5-S-C in comparison to the S-S-C–treated group.

locomotor activity observed following fluoxetine and cocaine administration in comparison to the activity observed in the S-F-C group (Fig. 7a). Methysergide, at the dose of 10 mg/kg, did not alter the fluoxetine-enhanced cocaine-induced locomotor activity (Fig. 7a). Pretreatment with 15 mg/kg methysergide increased the locomotor activity compared to the S-F-C treatment, which reached statistical significance 30 and 60 min after cocaine administration (Fig. 7a). A similar pattern was observed for the number of rears and head bobs. Analysis of variance yielded a significant group effect (S-S-S, S-S-C, S-F-C, M5-F-C, M10-F-C, M15-F-C) for the number of rears [$F_{10,30,60min}(5, 94) = 5.16, 4.52, 2.97$, $p_{10,30,60min} < 0.001, 0.01, 0.05$] and head bobs [$F_{10,30,60min}(5, 94) = 24.1, 24.2, 17.8$, $p_{10,30,60min} < 0.0001$], which lasted for the duration of the experiment (Fig. 7b and c). The pretreatment with 5 mg/kg methysergide resulted in a significant reduction in the number of head bobs at 10, 30, and 60 min after cocaine administration, compared to the S-F-C–treated group (Fig. 7c). There was a

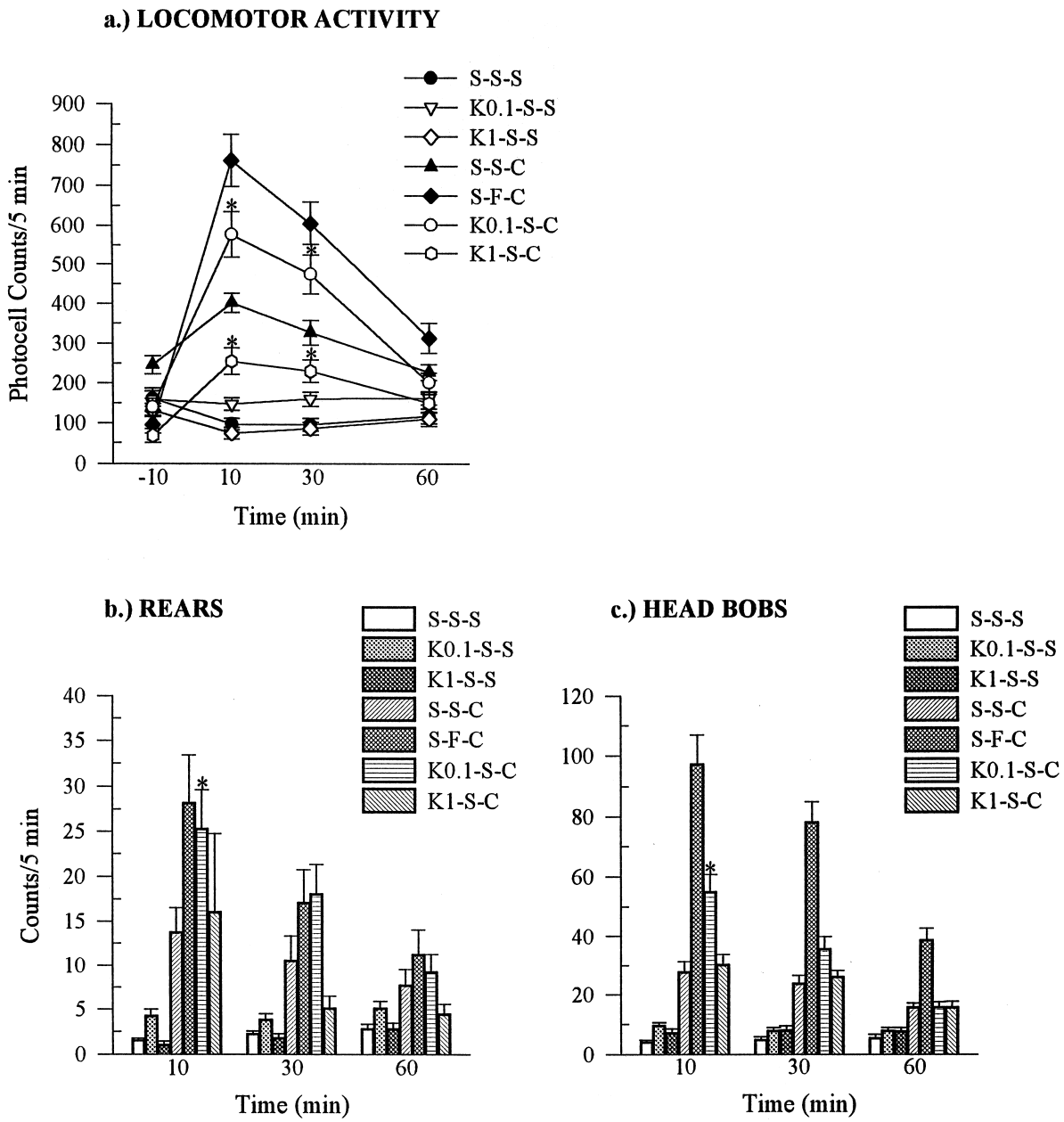

FIG. 5. The locomotor activity (a), rears (b), and head bobs (c) observed in rats treated with saline (S) or cocaine (C) following pretreatment 30 min earlier with saline (S) or fluoxetine (F). The second pretreatment was preceded 30 min earlier by saline (S), 0.1 mg/kg ketanserin (K0.1), or 1.0 mg/kg ketanserin (K1.0) administration. The values represent mean scores ± SEM of 16 rats. *$p < 0.05$ (Newman–Keuls test), K0.1-S-C and K1-S-C compared to S-S-C.

tendency for a reduction of the number of rears following 5 mg/kg methysergide pretreatment, although this did not reach statistical significance compared to the S-F-C treatment (Fig. 7b). Methysergide (10 mg/kg) did not alter the number of rears (Fig. 7b), whereas a significant reduction of the number of head bobs was observed 10 and 30 min after cocaine administration compared to the S-F-C–treated animals (Fig. 7c). The number of rears was increased by 15 mg/kg methysergide pretreatment in comparison to the S-F-C–treated animals (Fig. 7b). The observed potentiation reached statistical significance at 30 min. Methysergide (15 mg/kg) pretreatment did not significantly alter the number of head bobs compared to the S-F-C–treated group (Fig. 7c).

Effect of WAY 100635 on the Fluoxetine-Enhanced Cocaine-Induced Behaviors

An attenuation of the behaviors in comparison to the S-F-C–treated group was obtained after pretreatment with WAY 100635 (0.1 and 1.5 mg/kg) in a dose-dependent manner (Fig.

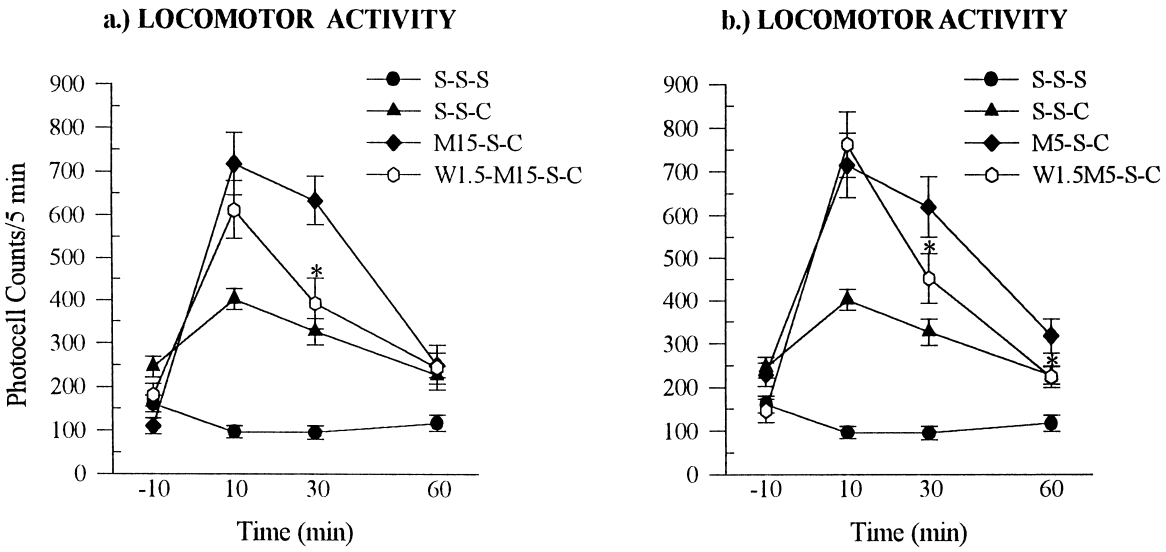

FIG. 6. The locomotor activity (a, b) of rats treated with saline (S) or cocaine (C) following pretreatment 30 min earlier with saline (S). The saline pretreatment was preceded 30 min by saline (S), 5 mg/kg methysergide (M5), 15 mg/kg methysergide (M15), or 1.5 mg/kg WAY 100635 and 5 mg/kg methysergide (W1.5M5) administration. The W1.5-M15-S-C group was pretreated with 1.5 mg/kg WAY 100635 (W1.5) 30 min before 15 mg/kg methysergide (M15) administration. The values represent mean scores ± SEM of 15–16 rats. *$p < 0.05$ (Newman–Keuls test), W1.5-M15-S-C and W1.5M5-S-C compared to M15-S-C and M5-S-C, respectively. For the sack of clarity, the effects of the W1.5M5-S-C treatment on the number of rears and head bobs are described in the text. The effects of the W1.5-M15-S-C treatment on the number of rears and head bobs are not presented.

8a–c). A significant decrease in locomotor activity was observed at 10 and 30 min following pretreatment with WAY 100635 at the dose of 1.5 mg/kg [$F_{10,30,60min}(4, 79) = 24.4, 20.9, 7.06$, $p_{10,30,60min} < 0.0001$] (Fig. 8a). The number of rears and head bobs were significantly decreased at 10 min and 10 and 30 min, respectively, following pretreatment with WAY 100635 at the dose of 0.1 mg/kg [rears: $F_{10,30,60min}(4, 79) = 7.64, 3.83, 3.32$, $p_{10,30,60min} < 0.0001, 0.01, 0.05$; head bobs: $F_{10,30,60min}(4, 79) = 23.7, 27.6, 15.8$, $p_{10,30,60min} < 0.0001$] (Fig. 8b and c). At the dose of 1.5 mg/kg, WAY 100635 pretreatment significantly attenuated the number of rears and head bobs compared to the S-F-C–treated animals at 10 min and 10, 30, and 60 min, respectively (Fig. 8b and c). There was no significant difference in locomotor activity of the 1.5 mg/kg WAY 100635-, fluoxetine-, and cocaine (W1.5-F-C)-treated rats compared to those of the S-S-C group (Fig. 8a).

Effect of Ketanserin on Fluoxetine-Enhanced Cocaine-Induced Behaviors

The locomotor activity, rears, and head bobs following fluoxetine and cocaine treatment were significantly reduced in a dose-dependent manner by ketanserin [locomotor activity: $F_{10,30,60min}(4, 78) = 29.8, 21.9, 6.36$, $p_{10,30,60min} < 0.0001, 0.0001, 0.001$; rears: $F_{10,30,60min}(4, 78) = 4.74, 4.18, 3.44$, $p_{10,30,60min} < 0.01, 0.01, 0.05$; head bobs: $F_{10,30,60min}(4, 78) = 35.5, 34.6, 23.8$, $p_{10,30,60min} < 0.0001$] (Fig. 9a–c). Pairwise comparison between the K0.1-F-C and S-F-C groups revealed a significant reduction for the locomotor activity and head bobs at 10 min and 10, 30, and 60 min, respectively (Fig. 9a and c). The attenuation in locomotor activity and number of head bobs in the K1.0-F-C–treated rats compared to the S-F-C treatment was significant at 10 and 30 min (Fig. 9a and c). The number of rears was significantly reduced at 30 and 60 min (Fig. 9b).

Effect of Ketanserin and WAY 100635 on Fluoxetine-Enhanced Cocaine-Induced Behaviors

The coadministration of ketanserin and WAY 100635 did not alter the locomotor activity (Fig. 10a), rears (Fig. 10b), and head bobs (Fig. 10c) compared to that of saline-treated animals. A significant reduction of the behaviors was observed in the ketanserin (1.0 mg/kg)- and WAY 100635 (1.5 mg/kg)-treated animals followed by fluoxetine and cocaine administration [locomotor activity: $F_{10,30,60min}(4, 79) = 45.3, 23.8, 7.16$, $p_{10,30,60min} < 0.0001$; rears: $F_{10,30,60min}(4, 79) = 3.71, 3.16, 2.98$, $p_{10,30,60min} < 0.01, 0.05, 0.05$; head bobs: $F_{10,30,60min}(4, 79) = 40.4, 31.8, 26.1$, $p_{10,30,60min} < 0.0001$] (Fig. 10a–c). The reduction of the locomotor activity was significant for the duration of the experiment in comparison to the K1-F-C and S-S-C groups (Fig. 10a). Pairwise comparison between the ketanserin and WAY 100635 pretreated group and the K1-F-C group revealed significant differences for the rears and head bobs at 10 min and 10, 30, and 60 min, respectively (Fig. 10b and c).

DISCUSSION

The results described in the present study indicate that the behavioral effects of cocaine can be modified by drugs affecting the serotonergic system. The stimulant effect of cocaine on behavior is thought to be mediated by the dopaminergic neurotransmitter systems (see the introductory paragraphs). From the observation that fluoxetine, presumably by increasing synaptic levels of 5-HT (31,50), enhances the cocaine-induced

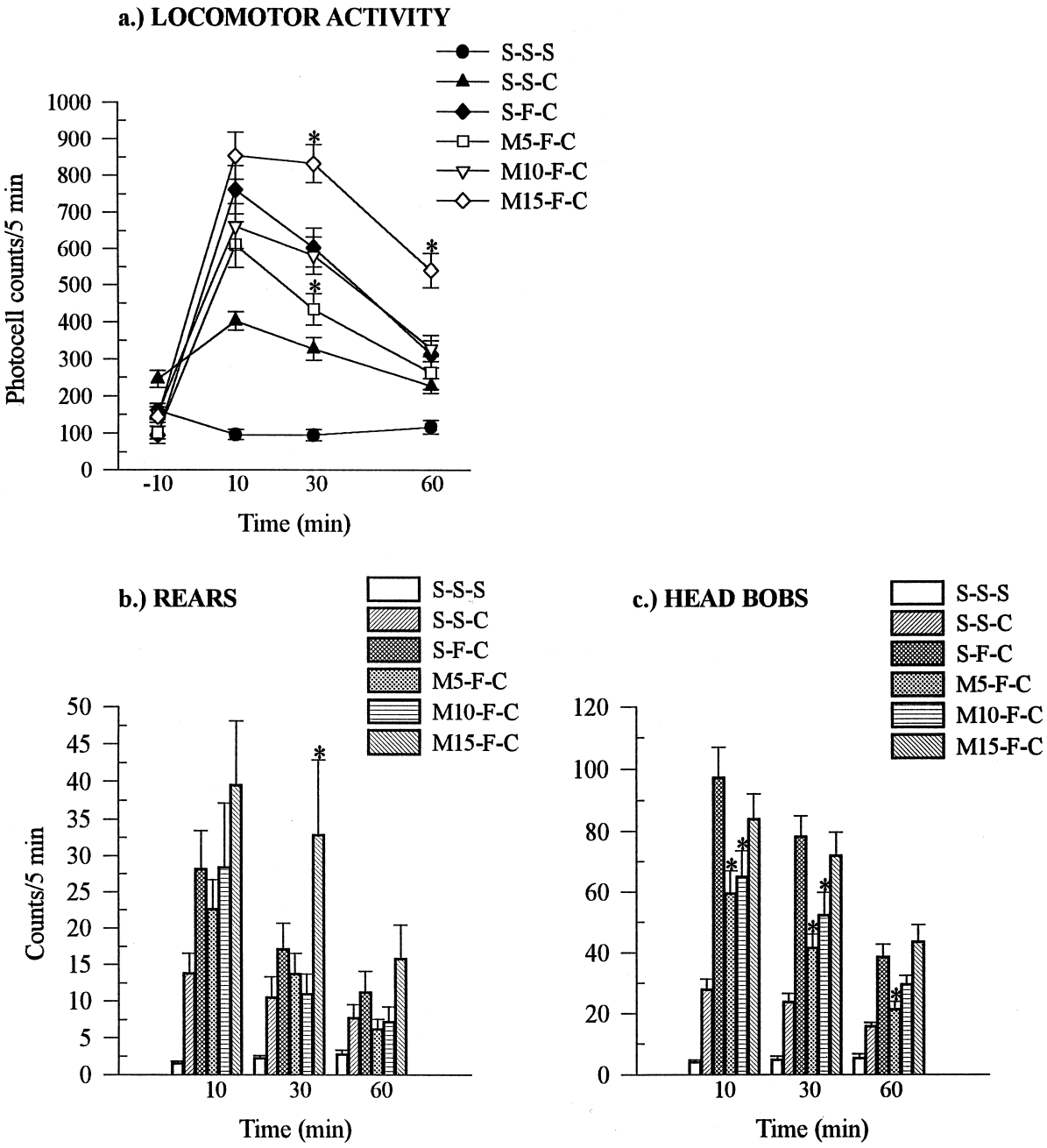

FIG. 7. The locomotor activity (a), rears (b), and head bobs (c) of rats treated with saline (S) or cocaine (C) following pretreatment 30 min earlier with saline (S) or fluoxetine (F). The second pretreatment was preceded 30 min earlier by saline (S), 5 mg/kg methysergide (M5), 10 mg/kg methysergide (M10), or 15 mg/kg methysergide (M15) administration. The values represent mean scores ± SEM of 15–16 rats. *p <0.05 (Newman–Keuls test), M5-F-C, M10-F-C, and M15-F-C compared to the S-F-C–treated group.

locomotor activity, it may be suggested that 5-HT facilitates the stimulatory effect of cocaine. It is possible that the increased activity results from an increase in 5-HT–mediated behaviors being additive to the cocaine-induced behaviors. However, in view of absence of the classical 5-HT–mediated behaviors (5-HT syndrome), including head shakes, at the same time as the cocaine stimulatory effect is enhanced, it is suggested that the increased behavioral scores result from a modulatory influence of 5-HT. In this case, the 5-HT receptor subtypes and their location, involved in modulating the behavioral effects, are complex and remain unclear. This complexity may partially explain why the present results do not agree with previous investigations. For instance, fluoxetine pretreatment (40 mg/kg) did not potentiate or attenuate the locomotor activity

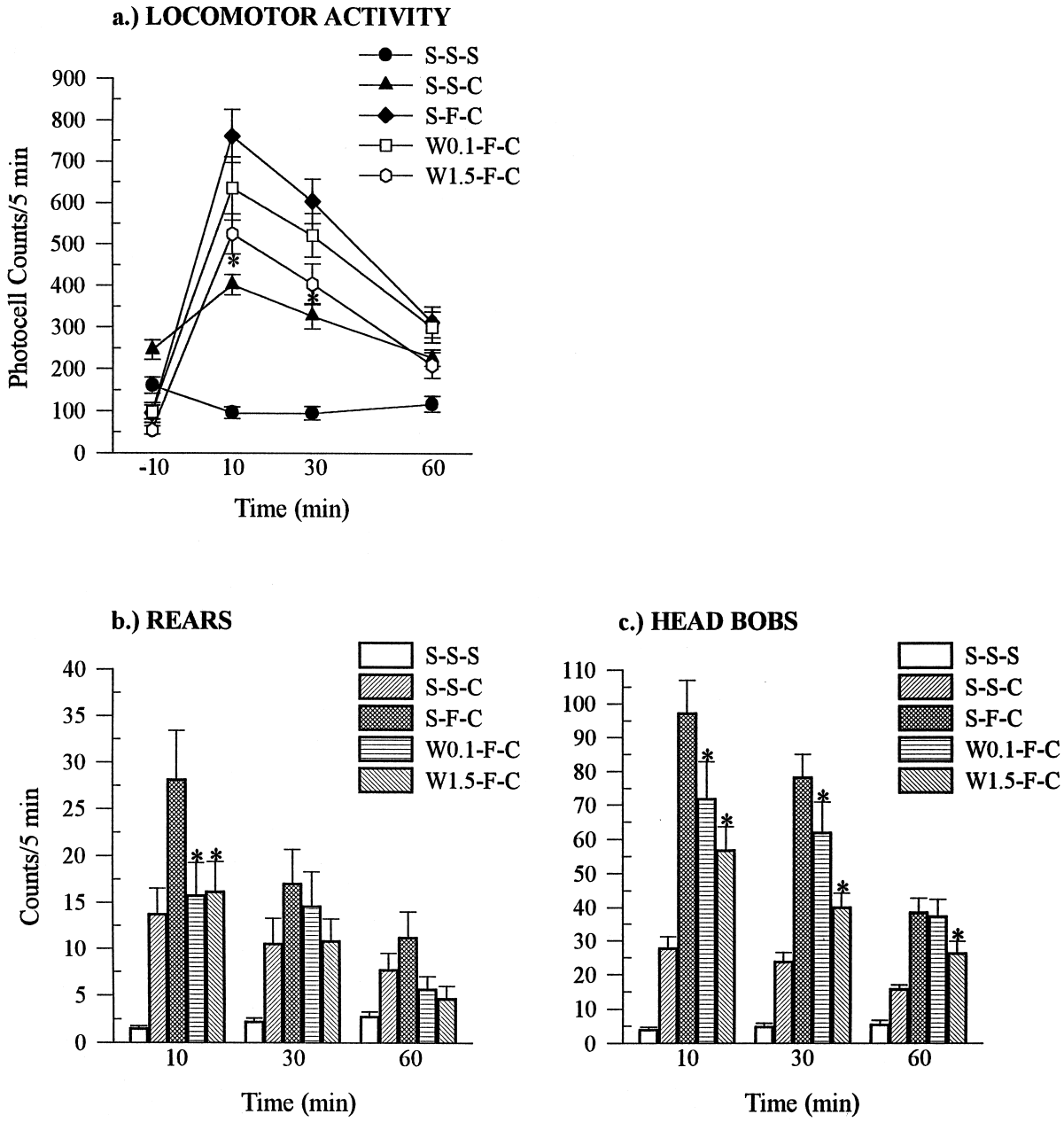

FIG. 8. The locomotor activity (a), rears (b), and head bobs (c) of rats treated with saline (S) or cocaine (C) following pretreatment 30 min earlier with saline (S) or fluoxetine (F). The second pretreatment was preceded 30 min earlier by saline (S), 0.1 mg/kg WAY 100635 (W0.1), or 1.5 mg/kg WAY 100635 (W1.5) administration. The values represent mean scores ± SEM of 16 rats. $*p < 0.05$ (Newman–Keuls test), W0.1-F-C and W1.5-F-C in comparison to the S-F-C–treated group.

produced by cocaine in male and female C57BL/6ByJ mice, whereas another SSRI, fluvoxamine, significantly increased the cocaine-induced locomotor activity (59). It was suggested that the lack of effect of fluoxetine might be due to a stimulation of 5-HT autoreceptors, thereby inhibiting the 5-HT release and suppressing the activity of serotonergic neurones (59). It should also be noted that the dose in the present study corresponds to the dose of selective 5-HT reuptake inhibition (24,78), whereas fluoxetine, at the high dose of 40 mg/kg, can also inhibit the NA reuptake (43), which may yield a stimulation of NA receptors and affect the cocaine-induced locomotor activity. The different doses of the SSRIs, as well as the use of different species and sex, may contribute to the inconsistent results. The present study was conducted in female rats, whereas most behavioral studies cited used male animals. Previous studies have shown that 5-HT–mediated responses are gender dependent. Female rats exhibit the 5-HT–mediated syndrome at lower doses of the 5-HT precursor L-tryptophan

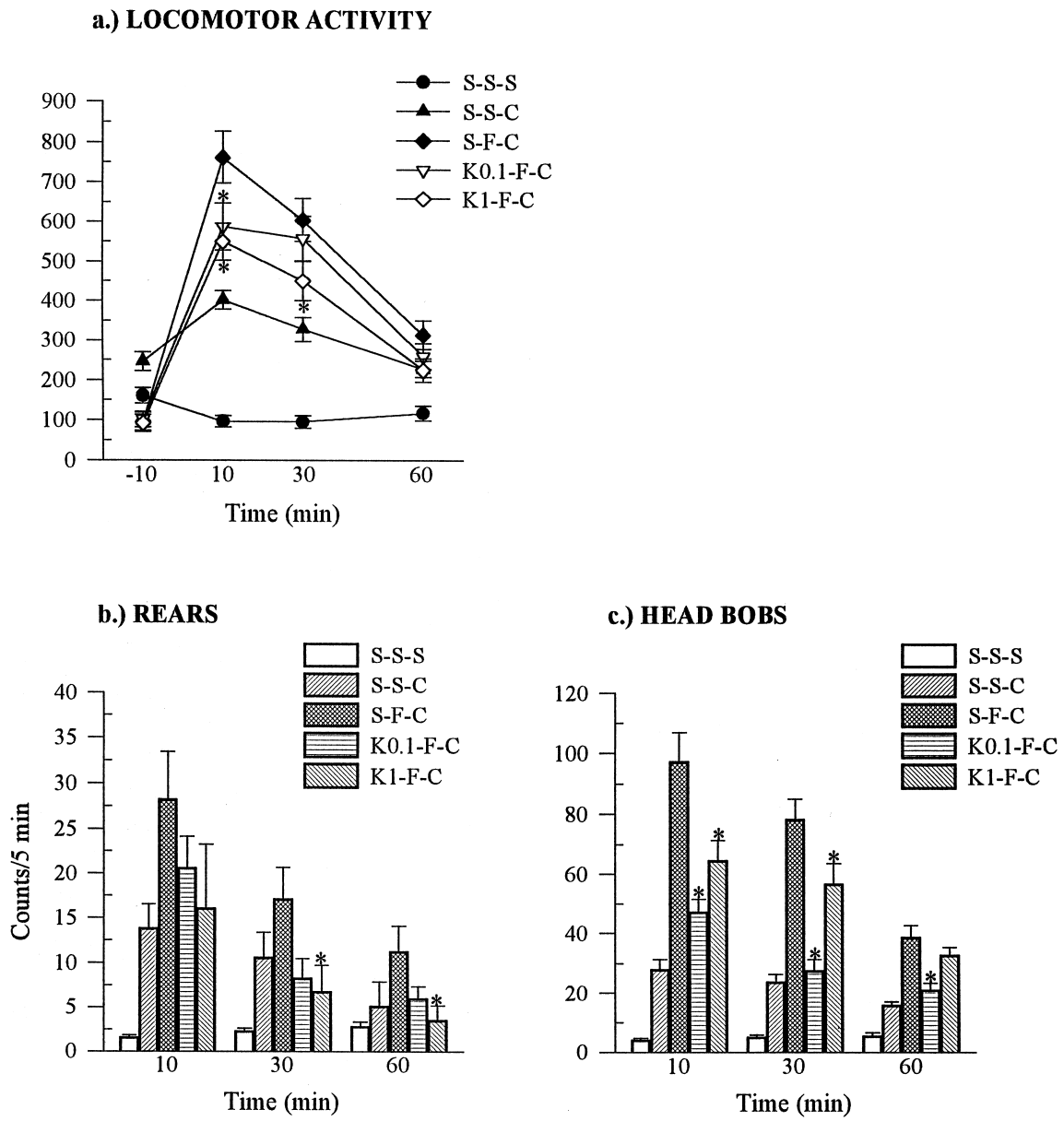

FIG. 9. The locomotor activity (a), rears (b), and head bobs (c) observed in rats treated with saline (S) or cocaine (C) following pretreatment 30 min earlier with saline (S) or fluoxetine (F). The second pretreatment was preceded 30 min earlier by saline (S), 0.1 mg/kg ketanserin (K0.1) or 1.0 mg/kg ketanserin (K1.0) administration. The values represent mean scores ± SEM of 15–16 rats. *$p < 0.05$ (Newman–Keuls test), K0.1-F-C and K1-F-C compared to S-F-C.

than males (6,20,22). Neither the stage of the estrous cycle nor the administration of sex steroids affected the behavioral response of female rats (6,20,22). However, following treatment with the monoamine oxidase inhibitor pargyline and L-tryptophan the increase of brain 5-HT was greater in female rats than in males, which may account for the behavioral supersensitivity of female rats (20). Whilst the influence of sex-dependent differences in brain 5-HT levels on the serotonergic modulation of cocaine-induced behaviors in the present study cannot be ruled out, it is reasonable to assume that the tendency of enhancement or reduction induced by the serotonergic agents would also occur in males.

It has been reported that 5-HT exerts a facilitatory role on striatal DA-dependent behaviors, whereas its action on DA-mediated behaviors associated with the NAC (e.g., locomotor activity, see the introductory paragraphs) is assumed to be inhibitory, although several reports contradict this hypothesis [see review (69)]. The results of the present study suggest a facilitatory role of 5-HT. If this occurred in the striatum, it would agree with the previously suggested facilitatory role of 5-HT, but it is contrary to the inhibitory role of 5-HT in the NAC. Fluoxetine has been shown to increase extracellular 5-HT levels in the striatum (50), which may yield a stimulatory effect on DA-mediated behaviors in the striatum. In support of

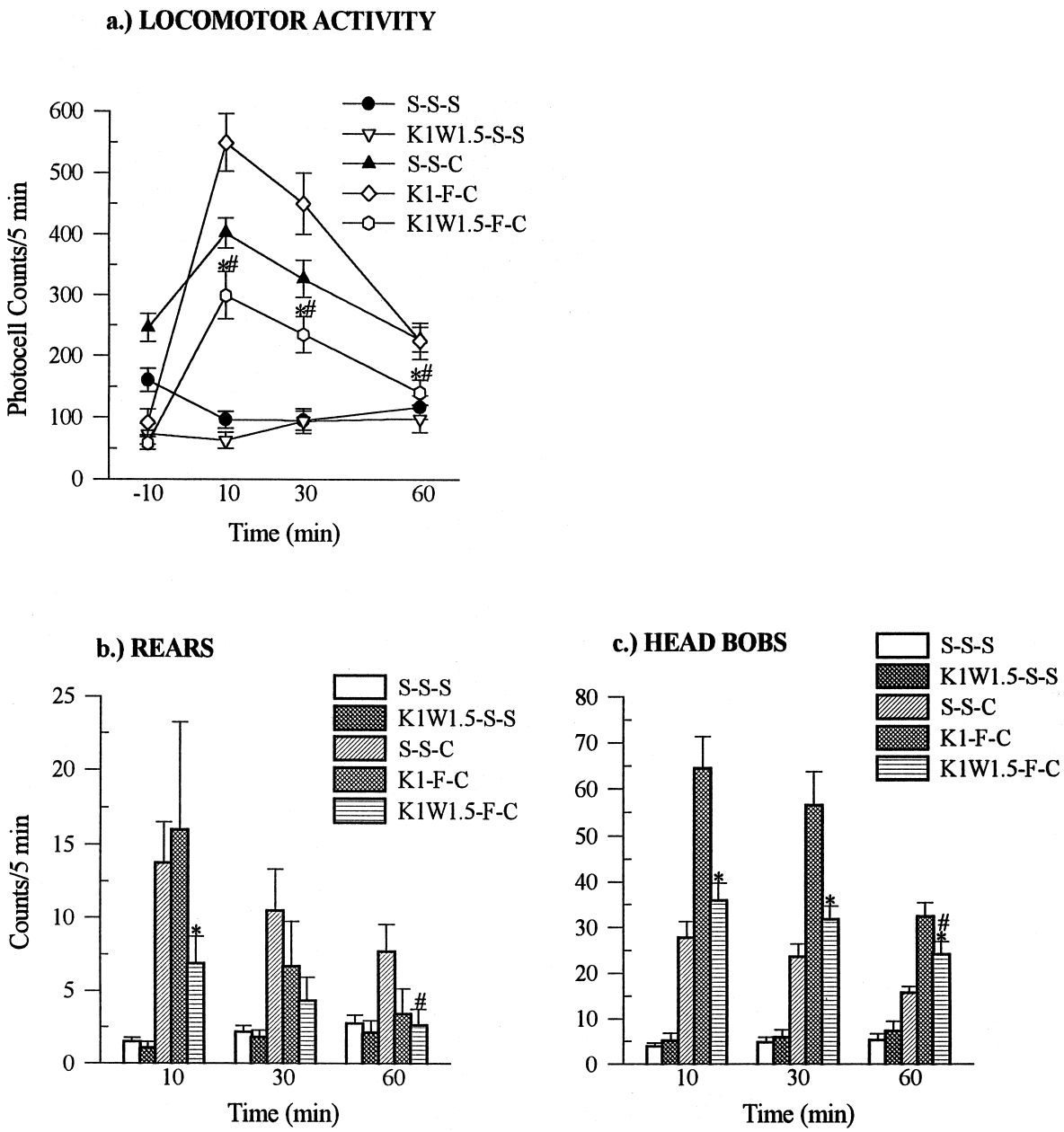

FIG. 10. The locomotor activity (a), rears (b), and head bobs (c) determined in rats treated with saline (S) or cocaine (C) following pretreatment 30 min earlier with saline (S) or fluoxetine (F). The second pretreatment was preceded 30 min earlier by saline (S), 1.0 mg/kg ketanserin (K1), or 1.0 mg/kg ketanserin and 1.5 mg/kg WAY 100635 (K1W1.5) administration. The values represent mean scores ± SEM of 16 rats. *$p < 0.05$ (Newman–Keuls test), K1W1.5-F-C compared to K1-F-C. #$p < 0.05$, K1W1.5-F-C compared to S-S-C.

a facilitatory role of 5-HT in the NAC is the observation that infusion of 5-HT in the VTA and NAC increased the extracellular DA and DA metabolites levels in the NAC (32,46) and the increase in locomotor activity observed in the present study.

5-HT has been shown to alter cocaine's reinforcing properties. Fluoxetine reduced the intravenous self-administration of cocaine in rats (10,49,60). One possible explanation suggested was that facilitating 5-HT with fluoxetine may enhance the reinforcing properties of cocaine, which is reflected in a decreased self-administration of cocaine (44,60). Furthermore, fluoxetine-enhanced the discriminative stimulus properties of cocaine in rats (17), suggesting that 5-HT may facilitate cocaine's stimulus effects. On the other hand, cocaine-induced locomotor activity in rats was attenuated by pretreatment with the 5-HT precursor 5-HTP and enhanced by 5-HT depletion (54,66), which is in support of an inhibitory role of

5-HT. Since 5-HT depletion potentiated cocaine-induced locomotor activity, one may argue that cocaine's activation of the serotonergic system via 5-HT reuptake inhibition normally has a negative influence on its stimulant effects on behaviors. This has been suggested for the increased breaking points on a progressive ratio schedule for intravenous cocaine reinforcement following depletion of forebrain 5-HT (41).

The question then arises which 5-HT receptor subtype(s) might be involved in the modulation of cocaine-induced behaviors. The serotonergic innervation from the DRN to the nigrostriatal and mesolimbic systems (7,71,76) and the high density of 5-HT_{1A} receptors in the DRN, as shown by quantitative autoradiographic mapping of 5-HT_1 receptors in the rat brain (47) may imply an involvement of 5-HT_{1A} receptors in the modulatory effect of 5-HT on cocaine-induced behaviors. Indeed, in the present study the potent and selective 5-HT_{1A} antagonist WAY 100635 (23) markedly reduced the fluoxetine-enhanced cocaine-induced behaviors in a dose-dependent manner. The dual action of WAY 100635 at somatodentritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors (23) and the interaction between WAY 100635 and fluoxetine at the former receptor may contribute to the different extent of reduction by WAY 100635 at the two doses, in addition to the usually observed dose–response effect. Recently, it has been reported that the inhibitory effect of paroxetine (another SSRI) on DRN serotonergic neurons was blocked by WAY 100635, and simultaneously, the effect of paroxetine on extracellular 5-HT at the nerve terminals in the frontal cortex was potentiated (25). Similar electrophysiological and neurochemical alterations may occur by combined administration of WAY 100635 and fluoxetine. However, an assumed increase of 5-HT at the nerve terminal might yield stimulation of vacant postsynaptic receptors, competing with the blockade of postsynaptic 5-HT_{1A} receptors by WAY 100635 and resulting in the observed lower attenuation by WAY 100635 at the dose of 0.1 mg/kg.

Beside an involvement of 5-HT_{1A} receptors in the enhancing effect of fluoxetine, it should also be considered that this receptor subtype may comediate the behavioral effects of cocaine, since cocaine has been reported to inhibit the spontaneous firing of DRN 5-HT neurons (15,16,52,53) which was blocked by the 5-HT_{1A} antagonist spiperone (16). It has been shown that, the selective 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) reduced the reinforcing response of cocaine in rats, indicating an involvement of 5-HT_{1A} receptors in the serotonergic modulation on cocaine self-administration (49). As demonstrated by results from the present study, the 5-HT_{1A} antagonist WAY 100635 failed to alter the locomotor activity induced by cocaine but increased the number of head bobs. A possible explanation for the lack of effect of WAY 100635 on the cocaine-induced locomotor activity could be the peripheral administration of the 5-HT_{1A} antagonist resulting in a blockade of both the somatodentritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors.

The results with methysergide, showing a potentiation of the cocaine-induced behaviors, are inconsistent with 5-HT_{1A} antagonistic properties and the observation that methysergide did not affect the cocaine-induced locomotor activity in male mice (58). The findings of the present study that are inconsistent with the earlier study may be due to differences in cocaine dosages (15 mg/kg compared with 25 mg/kg), as well as different species and sex. In accord with our results with cocaine are the findings that methysergide increased the stereotypy induced by methamphetamine (2) and the hyperactivity produced by 3,4-methylene-dioxymet-amphetamine in rats (26). In regard to its reported action on 5-HT_{1A} receptors, methysergide failed to block lower lip retraction induced by 8-OH-DPAT (4). Notably, methysergide produced lower lip retraction, a behavior that is thought to be associated with activation of 5-HT_{1A} receptors (4,5). Moreover, methysergide, like 8-OH-DPAT, diminished forskolin-stimulated adenylate cyclase activity in guinea pig and calf hippocampus membranes, a functional model to determine interactions of agonists and antagonists at 5-HT_{1A} receptors (19,67), and reduced the 5-HT output in the hippocampal dialysate as measured by in vivo microdialysis (68). In light of these published results, it is reasonable to suggest that methysergide exhibits 5-HT_{1A} receptor agonist activity. This stimulatory effect of methysergide at 5-HT_{1A} receptors [see review (33)] is in agreement with the observed reduction of locomotor activity induced by cocaine and methysergide by WAY 100635, although neither methysergide nor fluoxetine elicited the "5-HT behavioral syndrome" in the present study. The classical 5-HT–mediated behaviors, such as reciprocal forepaw treading, and flat body posture are thought to be associated with activation of 5-HT_{1A} receptors (75). Since methysergide and fluoxetine (via 5-HT) are not selective for the 5-HT_{1A} receptor subtype, the doses required for these drugs to induce the 5-HT behavioral syndrome may be greater than the doses used in the present study, or the observed effects are complicated by actions at other 5-HT receptors.

In addition to its action on 5-HT_{1A} receptors, methysergide has also been classified as a 5-HT_{1B} agonist and 5-HT_{2C} antagonist [see review (33)]. One may argue that its stimulatory effects on cocaine-induced locomotor activity might be due to its action on 5-HT_{1B} receptors. The involvement of postsynaptic 5-HT_{1B} receptors in the stimulatory effect of the nonselective 5-HT_1 agonist RU 24969 on locomotor activity was suggested following the observation that the selective 5-HT_{1A} antagonist WAY 100135 (5 mg/kg) failed to alter the RU 24969-induced locomotor activity (13). However, it has recently been shown that WAY 100635 dose-dependently reduced the RU 24969-induced locomotor activity in rats, suggesting an involvement of 5-HT_{1A} receptors (34).

Surprisingly, the combined pretreatment with methysergide (5 mg/kg) and fluoxetine prior to cocaine resulted in a reduction of the locomotor activity, rears, and head bobs. It might be predicted that the proposed 5-HT_{1A} receptor stimulation by methysergide could be partially compensated by the additional administration of fluoxetine. The results of in vivo microdialysis have demonstrated that after peripheral administration of SSRIs the extracellular 5-HT levels in forebrain regions reflect a balance between decreased 5-HT release and inhibited 5-HT reuptake (1,65). Peripheral administration of fluoxetine preceded by the local infusion of the SSRI into the diencephalon produced a decrease of 5-HT in the dialysate, indicating an inhibition of 5-HT release by fluoxetine (65). Although fluoxetine increased the extracellular 5-HT levels in forebrain regions (31,50,51,65), it would be expected that 5-HT in the synaptic cleft reduces 5-HT release via stimulation of presynaptic autoreceptors (3,12,27,28,45). It could be suggested from the present results that by increasing the dose of methysergide (10–15 mg/kg), the concentration of methysergide was sufficient to stimulate the proposed postsynaptic 5-HT_{1A} receptors to a greater extent and consequently result in a higher locomotor activity and potentiated behaviors.

It should also be considered that methysergide is a 5-HT_{2C} antagonist. The 5-HT_2 receptors have previously been described to possess inhibitory effects on DA-mediated locomotor activity and behaviors (29,30). In particular, the activation of 5-HT_{2C} receptors appears to be involved in suppression of

ambulatory behavior in rats, since the 5-HT_{2C} antagonists methysergide and metergoline have been reported to block the reduction of locomotor activity induced by the 5-HT_{1B} and 5-HT_{2C} agonists 1-(m-chlorophenyl)piperazine (m-CPP) and 1-(m-trifluoromethylphenyl)piperazine (TFMPP) (36,42). Thus, the blockade of the 5-HT_{2C} receptors by methysergide in the present study would abolish the inhibitory action of this 5-HT receptor subtype on behaviors induced by cocaine, and result in a higher locomotor activity and potentiated behaviors. If the 5-HT_{2C} receptor subtype is directly involved in cocaine's effects on behaviors, it might be suggested that, besides cocaine's stimulant effects on behavior, it may also have an inhibitory effect mediated by stimulation of 5-HT_{2C} receptors by endogenous 5-HT following inhibition of 5-HT reuptake by cocaine. Moreover, the blockade of this inhibitory serotonergic effect by methysergide at the dose of 15 mg/kg may also account for the observed potentiation of fluoxetine-enhanced cocaine-induced behaviors 30 and 60 min after administration of the psychostimulant.

The involvement of a second receptor site in the action of methysergide may provide an explanation of the partial blockade produced by WAY 100635, at the dose that abolished the enhancing effect of fluoxetine on cocaine-induced behaviors. The involvement of 5-HT_{2C} receptors in the enhancing effect of fluoxetine is improbable, since fluoxetine reportedly has only a low affinity to 5-HT_{2C} receptors in bovine choroid plexus (79). But the involvement of a second receptor in the potentiation of cocaine-induced behaviors by fluoxetine cannot be excluded, since an attenuation of the fluoxetine-enhanced cocaine-induced behaviors was observed after ketanserin pretreatment. Moreover, the coadministration of ketanserin and WAY 100635 followed by fluoxetine pretreatment diminished the cocaine-induced behaviors to a markedly greater extent than the single administration of either of these two antagonists. It has recently been reported that ketanserin (1 mg/kg), attenuated the apomorphine-induced locomotor activity, underlining a possible involvement of 5-HT_{2A} and/or 5-HT_{2C} receptors in the serotonergic modulation of DA-mediated locomotor activity (80). Because of the 1000-fold higher affinity of ketanserin for 5-HT_{2A} compared to 5-HT_{2C} receptors (33), the blockade of the former receptor at doses of 0.1 and 1 mg/kg in the present experiments is more likely. Whilst the attenuation by ketanserin could be due to nonspecific sedation, this is unlikely since it had no effect on the behaviors compared to that of saline-treated animals [(80); present study]. The modulatory effect of ketanserin on cocaine-induced behaviors also provides evidence for an involvement of the 5-HT_{2A} receptor subtype in the stimulant effect of cocaine on behaviors. Interestingly, ketanserin, at the two doses used, produced an opposite effect on the cocaine-induced behaviors. The low dose of ketanserin enhanced the cocaine-induced behaviors, whereas the high dose reduced them. A plausible explanation for this differential effects of ketanserin at different doses remains to be identified.

The observation in the present study that ketanserin and methysergide modulated the cocaine-induced behaviors is in keeping with other reports of serotonergic modulation of cocaine-induced locomotor activity in rodents (58,70). On the other hand, methysergide, ketanserin, and 5-HT_3 antagonists have reportedly not altered the intravenous self-administration or reinforcement of cocaine in rats (40,48). It appears that the 5-HT receptor subtypes modulating the stimulus effect of cocaine on reinforcement are different from those affecting cocaine-induced behaviors in rodents.

The activation of 5-HT_3 receptors is one possible site of action for fluoxetine since fluoxetine has been reported to increase extracellular DA levels in the prefrontal cortex by stimulation of 5-HT_3 receptors (73). However, this was not observed in the NAC (72), although infusion of 5-HT in the VTA and NAC resulted in increased extracellular DA levels in the NAC (32,46). Previous results from our laboratory did not implicate a role of 5-HT_3 receptors, since ondansetron failed to attenuate the effect of fluoxetine on cocaine-induced locomotor activity (74). An involvement of the 5-HT_3 receptor subtype in the action of methysergide in the present study is improbable since it has reportedly no activity at this 5-HT subtype [see review (33)].

The previous reports, together with the results of our experiments, emphasize that a complex mechanism is involved. Further investigations are required to determine the role of the 5-HT receptor subtypes and their location in the modulation of cocaine-induced locomotor activity and behaviors. In summary, the results of the present study indicate that the 5-HT reuptake inhibition produced by fluoxetine and the effects of methysergide on 5-HT receptor subtypes have a facilitatory effect on the stimulant action of cocaine on behaviors. The blockade of this enhancing effect by WAY 100635 suggests a mediation by 5-HT_{1A} receptors. In addition, an activation of 5-HT_{2A} receptors and/or inhibition of 5-HT_{2C} receptors may also be involved in the serotonergic stimulatory effect on cocaine-induced behaviors.

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