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HYPERLOCOMOTIVE AND DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE ARE UNDER THE CONTROL OF SEROTONIN$_{2C}$ (5-HT$_{2C}$) RECEPTORS IN RAT PREFRONTAL CORTEX

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Abbreviations: **5-HT**, 5-hydroxytryptamine (serotonin); **5-HT_{2C}R**, 5-HT_{2C} receptor; **DA**, dopamine; **GABA**, γ-aminobutyric acid; **MK 212,** 6-chloro-2-(1-piperazinyl)pyrazine hydrochloride; **RS 102221,** 8-[5-(2,4-dimethoxy-5-(4-trifluorophenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazo-spiro[4.5]decane-2,4-dione hydrochloride; **VTA**, ventral tegmental area; **NAc**, nucleus accumbens; **PFC**, prefrontal cortex; **FR**, fixed ratio

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ABSTRACT

The serotonin 5-HT_{2C} receptor (5-HT_{2C}R) is found in abundance in dopamine (DA) mesocorticolimbic pathways and is one of the important target proteins that modulates the behavioral effects of cocaine. In the present study, the hypothesis was tested that the 5-HT_{2C}R in the prefrontal cortex (PFC) may control either spontaneous or cocaine-evoked locomotor activity as well as the discriminative stimulus properties of cocaine. In male Sprague-Dawley rats implanted with bilateral cannulae aimed at the PFC, local microinjections of the preferential 5-HT_{2C}R agonist MK 212 (0.05-0.5 μg/side) did not alter spontaneous activity, but dose-dependently decreased horizontal hyperactivity evoked by cocaine (10 mg/kg, i.p.). Given alone, the selective 5-HT_{2C}R antagonist RS 102221 (5 μg/side) increased basal locomotor activity of rats expressed in the vertical plane. Microinjections of RS 102221 (5 μg/side, but not 0.15-1.5 μg/side) significantly enhanced the horizontal activity induced by cocaine (10 mg/kg). In rats trained to discriminate cocaine (10 mg/kg, i.p.) from saline (i.p.) in a two-lever, water-reinforced FR 20 task, intra-PFC microinjections of MK 212 (0.05 and 0.5 μg/side) did not substitute for cocaine, but attenuated the stimulus effects of cocaine. On the other hand, intra-PFC microinjections of RS 102221 (1.5 and 5 μg/side) evoked 13% and 40% cocaine-lever responding when tested alone and enhanced the recognition of cocaine. These data indicate that the PFC is a brain site at which 5-HT_{2C}R exert an inhibitory control over the hyperactive and discriminative stimulus effects of cocaine known to be dependent upon activation of the DA mesoaccumbens circuit.

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Augmented dopamine (DA) neurotransmission and indirect activation of DA D_1- and D_2-like receptors have been established to play a central role in the *in vivo* effects of cocaine. A sizeable body of literature supports the importance of the mesoaccumbens DA pathway which originates in DA cell bodies in the ventral tegmental area (VTA) and terminates in the nucleus accumbens (NAc) in mediating the behavioral effects of cocaine. In addition to the pronounced involvement of DA in its *in vivo* effects, cocaine also inhibits serotonin (5-hydroxytryptamine; 5-HT) reuptake and enhances 5-HT availability for interaction with potentially all sixteen 5-HT receptors found in the brain (Hoyer et al., 2002). One such receptor, the 5-HT_{2C} receptor (5-HT_{2C}R), is densely localized in brain DA circuits (Lopez-Gimenez et al., 2001), and neurochemical studies implicate a tonic inhibitory control of the 5-HT_{2C}R in brain DA pathways (De Deurwaerdere and Spampinato, 1999). In keeping with an inhibitory role for 5-HT_{2C}R, systemic pretreatment with 5-HT_{2C}R agonists (MK 212 or RO 60-0175) has been reported to suppress the hypermotive (Grottick et al., 2000), discriminative stimulus (Callahan and Cunningham, 1995), and reinforcing effects of cocaine as well as reinstatement of cocaine-seeking behavior following extinction (Grottick et al., 2000). On the other hand, brain-penetrant 5-HT_{2C}R antagonists (SB 242084, SDZ SER 082) have been shown to potentiate these behavioral effects of cocaine (McCreary and Cunningham, 1999; Fletcher et al., 2002). These data suggest that the 5-HT_{2C}R may be a functionally important regulator of the neural substrates that control responsiveness to cocaine.

Two recent microinjection studies tested the hypothesis that one site of action for the 5-HT_{2C}R to modulate cocaine-induced behaviors was the NAc. We demonstrated that intra-NAc shell (but not intra-VTA) microinjection of the 5-HT_{2C}R antagonist RS 102221 blocked the hypermotive (McMahon et al., 2001) and discriminative stimulus effects of cocaine (Filip and Cunningham, 2002), whereas intra-NAc shell pretreatment with the preferential 5-HT_{2C}R agonists MK 212 or RO

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60-0175 enhanced the behavioral actions of cocaine (Filip and Cunningham, 2002). However, based upon systemic studies with 5-HT_{2C}R agonists (above), we would have predicted diametrically opposite outcomes if the NAc were the common site of action for cocaine and the 5-HT_{2C}R ligands. These data suggest that the influence of systemically-administered 5-HT_{2C}R ligands on cocaine-induced behaviors may represent actions at 5-HT_{2C}Rs differentially localized to multiple brain nuclei.

The mesocortical DA pathway which originates in the VTA and terminates in several cortical areas, including the prefrontal cortex (PFC), has been postulated as a forebrain circuit important in the systemic effects of cocaine. In fact, systemic administration of cocaine has been shown to enhance DA release in PFC (Maisonneuve et al., 1990). Interestingly, intra-PFC microinjection of cocaine does not mimic the hyperactivity or discriminative stimulus effects of systemically-administered cocaine (Wood and Emmett-Oglesby, 1989; Delfs et al., 1990), but does support self-administration (Goeders and Smith, 1983) and the reinstatement of cocaine-seeking behavior (McFarland and Kalivas, 2001). Moreover, recent neuroimaging studies in human cocaine abusers show that injections of cocaine, recent withdrawal from cocaine and exposure to cocaine-associated cues are associated with activation of glucose metabolism or an increase in regional blood flow in the PFC (Volkow et al., 1991; Childress et al., 1999). As an important part of a complex neuroanatomical network implicated in regulation of subcortical DA neurotransmission and the behavioral effects of cocaine, the PFC sends direct excitatory glutamate projections to the NAc and VTA (Sesack and Pickel, 1992). Functional studies of these connections suggest that DA in the PFC inhibits excitatory glutamate pyramidal cells to negatively control DA function in the mesoaccumbens pathway with a net outcome of reduced mesoaccumbens-dependent behavioral output (Taber et al., 1995).

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To better elucidate the neurobiological substrates of action for 5-HT_{2C}R to control basal and cocaine-induced behaviors, we have extended our previous findings (McMahon et al., 2001; Filip and Cunningham, 2002) to analyze the manner in which specific behaviors induced by cocaine are controlled by the moderate levels of 5-HT_{2C}R located in the PFC (Lopez-Gimenez et al., 2001). We analyzed the ability of intra-PFC microinjection of a 5-HT_{2C}R agonist or antagonist to mimic or alter spontaneous motor activity as well as the hyperactive or discriminative stimulus effects of cocaine. This study employed the preferential 5-HT_{2C}R agonist MK 212 (Kennett, 1993; Porter et al., 1999) and the selective 5-HT_{2C}R antagonist RS 102221 (Bonhaus et al., 1997).

The PFC itself is an anatomically heterogeneous region composed of ventral and dorsal divisions. The ventral PFC is a component of a ventral "limbic" circuit with connectivity to the NAc shell, medial ventral pallidum, amygdala and VTA (Zahm and Brog, 1992; McFarland and Kalivas, 2001). The functional influence of the ventral PFC has recently been shown to be important to the behavioral effects of cocaine. For example, selective quinolinic acid lesions of the ventral PFC attenuated cocaine-evoked rearing and conditioned place preference as well as the development of behavioral sensitization to cocaine (Tzschentke and Schmidt, 1999). Therefore, given the prominent involvement of the limbic forebrain in the behavioral effects of cocaine (Delfs et al., 1990; Callahan et al., 1994; Filip and Siwanowicz, 2001; Rodd-Henricks, 2002), the important linkage between the ventral PFC and the limbic forebrain and the key control of ventral PFC over the behavioral effects of cocaine, we chose to target our microinfusions of 5-HT_{2C}R ligands to the ventral PFC.

MATERIALS AND METHODS

ANIMALS

Male Sprague-Dawley rats (n=204; Collegium Medicum, Jagiellonian University, Krakow, Poland) weighing 250-330 g at the beginning of the experiment were used. The rats were housed 2 or 3/cage in standard plastic rodent cages in a colony room maintained at 21 $\pm$ 2^{o}C and at 40-50% humidity under a 12 hr light-dark cycle (lights on at 0700 hrs). Rats surgically fitted with indwelling bilateral guide cannulae were housed individually. Rats assigned to locomotor activity assays (n=180) were provided with continuous access to tap water and rodent chow except during experimental sessions. In drug discrimination assays (n=24), rodent chow was available *ad libitum*; the amount of water each animal received was restricted to that given during daily training sessions, after test sessions (10-15 min) and on weekends (36 hrs). All experiments were conducted during the light phase of the light-dark cycle (between 0900-1400 hrs) and were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approval from the Institutional Animal Care and Use Committee.

SURGICAL IMPLANTATION OF CANNULAE AND MICROINJECTION PROTOCOLS

Rats underwent surgical implantation of 26-gauge stainless steel bilateral guide cannulae (Small Parts Inc., Miami Lakes, FL, USA). Each rat was anesthetized using an intramuscular (i.m.) injection of 65 mg/kg of ketamine and 15 mg/kg of xylazine in 0.9% NaCl. With the upper incisor bar of a stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA) positioned at -3.8 mm below the interaural line and using the intersection of the bregma and longitudinal sutures as the origin, the ventral surfaces of the bilateral guide cannulae were positioned 2 mm above the PFC (AP = + 2.7 mm, ML = $\pm$ 0.75 mm, and DV = – 3 mm; Paxinos and Watson, 1998). The guide cannulae were fastened to the skull with stainless steel screws (Small Parts) and cranioplastic

cement (Plastics One, Inc., Roanoke, VA, USA) and were fitted with 28-gauge stainless steel bilateral obturators (Small Parts). Rats received two injections of penicillin (10,000 units/kg, i.m.) after surgery, and were allowed a 1-week recovery period during which rats were handled and weighed daily. Following the initial 1-week recovery period, each rat was habituated to the brief confinement associated with intracranial microinjections by removing the 28-gauge internal obturators, gently restraining the rat for approximately 3 min, and replacing the obturators. For bilateral intra-PFC microinjections, the obturators were removed and two internal cannulae (Plastics One) were positioned so as to extend 1 mm below the tips of the bilateral guide cannulae. The bilateral internal cannulae were attached to two 5 µl syringes (Hamilton Co., Reno, NV, USA) via PE-50 tubing (Clay-Adams, Parsippany, NJ, USA). A microsyringe drive (Baby Bee, Bioanalytical Systems, West Lafayette, IN, USA) driven by a programmable controller (Bee Hive Controller, Bioanalytical Systems) delivered a volume of 0.2 µl/side at a rate of 0.1 µl/min. After completion of microinjections, the injection cannulae remained in place for an additional 1 min to allow for diffusion away from cannulae tips; the obturators were then replaced.

DRUGS

All drugs were dissolved in sterile saline (0.9% NaCl). Cocaine HCl (Merck, Damstadt, Germany) was injected intraperitoneally (i.p.) in a volume of 1 ml/kg. MK 212 (6-chloro-2-(1-piperazinyl)pyrazine HCl; Tocris, Bristol, UK) and RS 102221 (8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione HCl; Tocris, Bristol, UK) were injected intracranially in a volume of 0.2 µl/side. All solutions injected centrally were adjusted to pH 7.2, except the solution of RS 102221 which was adjusted to pH 6-7; control vehicle at pH 6-7 did not alter basal or cocaine-stimulated locomotor activity or the stimulus effects of cocaine (10 mg/kg; data not shown).

MEASUREMENT OF LOCOMOTOR ACTIVITY

Apparatus. Motor activity was monitored and quantified in clear plexiglass chambers (43 cm x 43 cm x 25 cm) housed inside Optovarimex® activity monitors surrounded with a 15 x 15 array of photocell beams located 3 cm from the floor surface (Columbus Instruments, Columbus, OH, USA). Interruptions of these photobeams resulted in horizontal activity defined as distance traveled (expressed in cm). A second set of 15 photocell beams was located 14 cm above the floor surface and interruptions of these photobeams provided for monitoring of rearing (expressed as activity counts). Separate records of horizontal activity and rearing were made by the control software (Columbus Instruments) for subsequent statistical evaluation.

Locomotor activity and microinfusion protocols. Surgically-implanted rats were habituated to the test environment for 2 hrs/day on each of the 2 days before the start of the experiment, and on the test day for 1 hr before the administration of drugs. Rats were assigned to two individual groups according to 5-HT_{2C}R ligand treatment (MK 212 or RS 102221) and each rat underwent only one test session. One group of rats (n=80, divided into 8 subgroups, 10 animals/subgroup) received bilateral intra-PFC microinjections of either sterile saline (0.2 µl/side) or a different dose of MK 212 (0.05, 0.15 or 0.5 µg/0.2 µl/side); bilateral microinjections were followed immediately by an i.p. injection of either saline (1 ml/kg) or cocaine (10 mg/kg). Another group of animals (n=100, divided into 10 subgroups; 10 animals/subgroup) received bilateral intra-PFC microinjections of either saline (0.2 µl/side) or a different dose of RS 102221 (0.15, 0.5, 1.5 or 5 µg/0.2 µl/side); microinjections were immediately followed by an i.p. injection of either saline (1 ml/kg) or cocaine (10 mg/kg). Measurements of locomotor activity began immediately after the systemic injection and lasted 60 min.

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DRUG DISCRIMINATION EXPERIMENTS

Apparatus. The procedures were conducted in commercially available, two-lever operant chambers (MedAssociates, St. Albans, VT, USA). Each chamber was equipped with a water-filled dispenser mounted equidistant between two response levers on one wall and housed in a light- and sound-attenuating cubicle (MedAssociates, St. Albans, VT, USA). A 28-V house light provided illumination; a blower supplied ventilation and masking noise. An interface (MedAssociates) connected the chambers to a computer which controlled and recorded all experimental events using MedState software.

Discrimination training and test protocols. Standard two-lever, water-reinforced drug discrimination procedures were utilized (Callahan and Cunningham, 1995; Filip and Cunningham, 2002). Rats were injected intraperitoneally (i.p.) with cocaine (10 mg/kg) or saline (1 ml/kg) 15 min prior to daily (Monday – Friday) 30-min sessions (n=24). During the initial "errorless training" phase, only the stimulus-appropriate (drug or saline) lever was present. Training began under a fixed ratio 1 (FR 1) schedule of water reinforcement and the FR requirement was incremented until all animals were responding reliably under an FR 20 schedule for each experimental condition. For half of the rats, left-lever responses were reinforced after cocaine administration, whereas right-lever responses were reinforced after saline administration; conditions were reversed for the remaining rats. During this phase of training, cocaine and saline were administered irregularly with the restriction that neither condition prevailed for more than three consecutive sessions.

After responding stabilized, both levers were presented simultaneously during 15-min sessions. The rats were required to respond on the stimulus-appropriate (correct) lever in order to obtain water reinforcement, and there were no programmed consequences for responding on the

incorrect lever. This phase of training continued until the performance of all rats attained criterion (defined as mean accuracies of at least 80% correct for ten consecutive sessions).

Pharmacological test and microinfusion protocols. When rats achieved the criterion for accuracy, test sessions were initiated and training sessions were run during the intervening days to maintain discrimination accuracy. Rats were required to maintain accuracies of at least 80% correct for the saline and cocaine maintenance sessions which immediately preceded a test. During a test session, the rat was placed in the chamber and, upon completion of 20 responses on either lever, a single reinforcer was delivered and the house light was turned off. The rat was removed from the chamber, returned to the colony and allowed free access to water for 10 min beginning 15-30 min after the end of each test. A test session was terminated after 15 min if the rat did not complete 20 responses on either lever. After achieving the criterion for accuracy, the group of trained rats, was separated into two subgroups: one group was tested with intra-PFC infusions of MK 212 (n=12), the other with intra-PFC infusions of RS 102221 (n=12).

Several pharmacological manipulations were performed during test sessions. A systemic dose-response curve for cocaine was established before surgical implantation of cannulae; rats were tested 15 min after an injection of cocaine (1.25-10 mg/kg, i.p.). Following recovery from surgery, discrimination training was reinstated. After several weeks, the systemic dose-response curve for cocaine was reestablished and did not differ from that established prior to surgery (data not shown); the post-surgical dose-response curve served as control in the present experiment.

In intracranial *substitution* tests, lever selection was assessed 10 min after bilateral intracranial infusions of sterile saline (0.9% NaCl; 0.2 µl/side), MK 212 (0.05 or 0.5 µg/side) or RS 102221 (1.5 or 5 µg/side) paired with a systemic injection of saline (1 ml/kg, i.p.). Control tests were also conducted in which rats were assessed for lever selection 10 min following

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administration of either saline or cocaine (10 mg/kg, i.p.) which had been immediately preceded by bilateral intracranial injections of saline (0.2 μl/side). In *combination* tests, bilateral microinjections of MK 212 (0.05 or 0.5 μg/side) or RS 102221 (1.5 or 5 μg/side) immediately preceded an injection of cocaine (1.25-10 mg/kg; i.p.); rats were tested for lever selection 10 min later. The order of drug tests in the group of rats treated with intra-PFC infusions of MK 212 was as follows: saline (0.2 μl/side) + saline (i.p.), cocaine (2.5 mg/kg, i.p.), saline (0.2 μl/side) + cocaine (10 mg/kg, i.p.), cocaine (1.25 mg/kg, i.p.), MK 212 (0.05 μg/side) + cocaine (2.5, 5 and 10 mg/kg, i.p.), cocaine (5 mg/kg, i.p.), MK 212 (0.5 μg/side) + cocaine (2.5, 5 and 10 mg/kg, i.p.), cocaine (10 mg/kg, i.p.), MK 212 (0.05 μg/side) + saline (i.p.) and MK 212 (0.5 μg/side) + saline (i.p.). The order of drug tests in the group of rats treated with intra-PFC infusions of RS 102221 was as follows: saline (0.2 μl/side) + saline (i.p.), cocaine (5 mg/kg, i.p.), saline (0.2 μl/side) + cocaine (10 mg/kg, i.p.), cocaine (1.25 mg/kg, i.p.), RS 102221 (1.5 μg/side) + cocaine (2.5, 5 and 10 mg/kg, i.p.), cocaine (10 mg/kg, i.p.), RS 102221 (5 μg/side) + cocaine (2.5, 5 and 10 mg/kg, i.p.), cocaine (2.5 mg/kg, i.p.), RS 102221 (1.5 μg/side) + saline (i.p.) and RS 102221 (5 μg/side) + saline (i.p.). Test sessions in which intracranial microinjections were performed were spaced 5-7 days apart and the timeframe during which microinjection analyses were conducted was ~ two months. Each rat received a total of 10 bilateral microinfusions.

HISTOLOGY

At the completion of the study, rats were overdosed with chloral hydrate (800 mg/kg, i.p.), the brains were removed and stored in a 20% sucrose/10% formalin solution for at least 3 days before the sectioning. Brain sections (50 μm) were mounted onto gelatin-coated glass slides. The brain sections were defatted, stained with cresyl violet, cleared with xylene and cover-slipped. The cannulae placements were verified using a light microscope. Only those animals whose cannulae

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were within the ventral PFC were included for statistical analysis. No significant tissue damage was evident upon histological examination of sections.

STATISTICAL ANALYSES

For motor activity assays, the dependent measures were horizontal activity (mean total distance traveled in cm) (± S.E.M.) and rearing (mean total counts of photobeam breaks) (± S.E.M.) observed during the 60-min test session. Each experiment was subjected to a one-way ANOVA with levels of the treatment factor corresponding to the drug combinations administered to that group. Planned, pairwise comparisons of the treatment means were made with least significant difference test (SAS for Windows, Version 8.1), which were conducted with an experimentwise error rate of α=0.05.

During drug discrimination training sessions, accuracy was defined as the percentage of correct responses to total responses before the delivery of the first reinforcer; during test sessions, performance was expressed as the percentage of drug-appropriate responses to total responses before the delivery of the first reinforcer. Response rates (responses per second) were also evaluated during training and test sessions as a measure of behavioral disruption. For training sessions, the response rate was calculated as the total number of responses emitted on either lever before completion of the first FR 20 divided by the number of seconds taken to complete that FR 20. During test sessions, the response rate was calculated as the total number of responses before the completion of 20 responses on either lever divided by the number of seconds taken to complete that FR 20. Only data (% drug-lever responding and response rates) from animals that completed the FR 20 during test sessions were used in analyses.

Student's *t*-test for repeated measures was used to compare the percentage of drug-lever responding and response rate during test sessions with the corresponding values for either the

previous drug session (substitution tests), or the training dose tested alone (combination tests). All comparisons were made with an experimentwise type I error rate (alpha) set at 0.05.

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RESULTS

HISTOLOGY

For each animal included in the analyses below, the injection cannulae projected bilaterally past the outer guide cannulae into the ventral PFC. Examples of bilateral placements identified in motor activity (Fig. 1, left) and drug discrimination studies (Fig. 1, right) are illustrated. Inspection of brain tissue revealed slight evidence of gliosis at the site of injection although surrounding tissue was generally intact.

LOCOMOTOR ACTIVITY ASSAYS

Intra-PFC microinjection of saline. Microinjections of saline into the ventral PFC followed by a systemic injection of saline resulted in levels of activity (Figs. 2-3) similar to that reported following a systemic injection of saline tested alone (McCreary and Cunningham, 1999) or following intra-NAc microinfusions of saline using equivalent test protocols (McMahon et al., 2001; Filip and Cunningham, 2002). Microinjections of saline into the PFC followed by a systemic injection of cocaine (10 mg/kg, i.p.) resulted in significant increases in horizontal activity (654-812%) and rearing (323-600%) as compared with saline-saline control values (Figs. 2-3).

Intra-PFC microinjection of MK 212. Eighty rats received bilateral microinjections of saline or the 5-HT_{2C}R agonist MK 212 (0.05, 0.15 or 0.5 μg/side) followed by a systemic injection of saline or cocaine (10 mg/kg). Of these, 69 rats exhibited cannulae placements bilaterally positioned in the ventral PFC at +2.2 to + 3.2 mm posterior to bregma. A main effect of treatment was observed for horizontal activity ($F_{7,55} = 10.64$, $p < 0.001$) and rearing ($F_{7,55} = 2.74$, $p < 0.05$). Intra-PFC pretreatment with MK 212 (0.05-0.5 μg/side) prior to a systemic injection of saline did not alter basal motor activity ($p > 0.05$; Fig. 2). However, intra-PFC microinfusions of MK 212

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significantly decreased cocaine-evoked horizontal activity (0.15 and 0.5 μg/side; $p < 0.05$; Fig. 2, top) in the absence of a significant effect on rearing ($p > 0.05$; Fig. 2, bottom).

***Intra-PFC microinjection of RS 102221*.** Of the 100 rats originally cannulated and tested, 76 rats exhibited cannulae placements bilaterally positioned in the ventral PFC at +2.2 to +3.2 posterior to bregma. For these 76 rats, a main effect of treatment was observed for horizontal activity ($F_{9,65} = 9.39$, $p < 0.001$) and rearing ($F_{9,65} = 3.68$, $p < 0.05$). Intra-PFC microinfusions of RS 102221 (0.15-5 μg/side) prior to a systemic injection of saline did not alter horizontal activity ($p > 0.05$; Fig. 3, top), but did significantly increase rearing at a dose of 5 μg/side ($p < 0.05$; Fig. 3, bottom). Intra-PFC microinfusions of RS 102221 at 5 μg/side also significantly increased cocaine-evoked horizontal activity ($p < 0.05$; Fig. 3, top), but not cocaine-evoked rearing ($p < 0.05$; Fig. 3, bottom).

Drug Discrimination Experiments

Cocaine-saline discrimination and dose-response relationship for cocaine. Acquisition of the cocaine (10 mg/kg) *vs.* saline discrimination was met in an average of 33 sessions (range: 24-41). After recovery from surgery, the performance criterion was reestablished in 15 sessions (range: 13-18). Systemic administration of cocaine (1.25-10 mg/kg) produced a dose-dependent increase in cocaine-appropriate responding prior to (data not shown) and after surgical implantation of cannulae in both subgroups of trained rats (Figs. 4-5); no differences were observed between the pre- and post-surgical dose-response curves for cocaine in either subgroup of trained rats (data not shown). Drug-lever responding after 1.25 and 2.5 mg/kg of cocaine in both subgroups of rats was significantly different from the previous cocaine training session ($p < 0.05$); response rates for all test doses of cocaine did not differ from that observed during the immediately previous cocaine maintenance session for either subgroup of trained rats ($p > 0.05$).

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Control tests were also conducted to assure that the microinjection procedure did not interfere with the discrimination between cocaine and saline. Systemic administration of saline engendered <10% drug-lever responding (data not shown), as has been observed in our numerous drug discrimination studies in rats implanted with intracranial cannulae (e.g., McMahon et al., 2001; Filip and Cunningham, 2002). Intra-PFC microinjections of saline did not alter the low levels of drug-lever responses seen after a systemic injection of saline (far left panels; Figs. 4-5); response rates did not vary between the control tests and the previous maintenance saline sessions. Intra-PFC microinjections of saline did not alter cocaine-lever responding (97.9 ± 0.5%) or response rates (0.67 ± 0.08 responses/sec) seen after systemic injection of cocaine (10 mg/kg) for the group of rats that were tested with MK 212. Likewise, in the group of rats tested with RS 102221, intra-PFC microinjections of saline did not alter cocaine-lever responding (95.4 ± 4.2%) or response rates (0.59 ± 0.07 responses/sec) seen after systemic injection of cocaine (10 mg/kg). These data indicate that the microinjection protocols themselves did not alter the ability of rats to appropriately recognize either a systemic injection of saline or cocaine (10 mg/kg).

***Intra-PFC microinjection of MK 212*.** Of the 12 rats originally cannulated and tested, 8 rats exhibited cannulae placements bilaterally positioned in the ventral PFC at +2.2 to +3.2 posterior to bregma (see Fig. 1, right, for examples of placements). In substitution tests in these animals, intra-PFC microinfusions 0.05 μg/side or 0.5 μg/side of MK 212 evoked 0% and 13% drug-lever responding, respectively; these values were significantly different ($p < 0.05$) from the previous cocaine training session; response rates were unaltered (Fig. 4, left).

A statistically significant suppression of drug-lever responding was observed for the combination of MK 212 (0.05 μg/side or 0.5 μg/side) plus either 5 or 10 mg/kg of cocaine ($p < 0.05$; Fig. 4, top right) as compared to the same dose of cocaine tested alone. Intra-PFC

microinjections of MK 212 (0.05 μg/side) plus cocaine (5 mg/kg) resulted in a significantly higher response rate relative to the same dose of cocaine tested alone ($p < 0.05$; Fig. 4, bottom right).

Intra-PFC microinjection of RS 102221. Of the 12 rats originally cannulated and tested, 7 rats exhibited cannulae placements bilaterally positioned in the ventral PFC at +2.2 to +3.2 posterior to bregma (see Fig. 1, right, for examples of placements). In substitution tests in these rats, 1.5 and 5 μg/side of RS 102221 evoked 11 and 40% drug-lever responding, respectively; these values were significantly different from the previous cocaine training sessions ($p < 0.05$). Response rates were unaltered (Fig. 5, left). Pretreatment with intra-PFC microinfusions of RS 102221 (1.5 and 5 μg/side) dose-dependently increased drug-lever responding observed at submaximal doses of cocaine (1.25 and 2.5 mg/kg) which alone elicited 12% and 34% drug-lever responding, respectively ($p < 0.05$; Fig. 5, top right). Pretreatment with RS 102221 did not affect response rates seen at any tested dose of cocaine (Fig. 5, bottom right).

DISCUSSION

Our findings strongly indicate that the 5-HT_{2C}R within the ventral PFC is important in the regulation of expression of the behavioral effects of cocaine. We found that intra-PFC microinjections of the 5-HT_{2C}R agonist MK 212 into the ventral PFC decreased, and the 5-HT_{2C}R antagonist RS 102221 increased, the hyperactivity induced by cocaine. We also found that this population of PFC 5-HT_{2C}R controlled expression of the discriminative stimulus effects of cocaine, which are thought to model the subjective effects of cocaine in humans (Schuster and Johanson, 1988). The perfectly oppositional effects of these two ligands and the extensive control studies support the concept that the observed outcomes are a consequence of the respective ability of MK 212 and RS 102221 to stimulate and block the 5-HT_{2C}R, respectively, and are not an outcome of lesions induced following implantation of the cannulae or infusions of the drugs.

The choice of drugs in the present study was guided by their selectivity and affinity for the 5-HT_{2C}R and the efficacy of MK 212 in neuropharmacological analyses. Neurochemical studies of MK 212 have demonstrated that this piperazine analog exhibits its highest affinity (Ki ≈ 32-490 nM) for 5-HT_{2C}R and displays full efficacy to stimulate 5-HT_{2C}R (Kennett, 1993; Porter et al., 1999; Cussac et al., 2002). *In vivo* analyses indicate that systemic administration of MK 212 evokes hyperthermia and hypophagia (Clineschmidt, 1979), oral dyskinesias (Eberle-Wang et al., 1996), penile erections (Berendsen et al., 1990), hypomotility (Lucki et al., 1989), and discriminative stimulus effects (Cunningham et al., 1986), all of which are blocked preferentially by 5-HT_{2C}R antagonists. In contrast, MK 212 has a much lower affinity for 5-HT_{2A}R (Ki ≈ 17,400 nM; Kennett, 1993; Porter et al., 1999) and does not evoke behavioral effects consistent with efficacy to stimulate 5-HT_{2A}R (e.g., Lucki et al., 1989). Although MK 212 does exhibit affinity and partial

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agonist actions at the 5-HT_{2B}R (Porter et al., 1999; Cussac et al., 2002), the 5-HT_{2B}R is unlikely to transduce the effects observed here due to its absence in the PFC (Duxon et al., 1997).

MK 212 binds to the 5-HT_3R (Glennon et al., 1989), although its action as either an agonist or antagonist is not well defined. If MK 212 has efficacy as a 5-HT_3R agonist, intra-PFC microinfusion would be expected to increase extracellular DA levels in PFC and suppress the firing of PFC neurons similar to that seen following intra-PFC microinfusion of selective 5-HT_3R agonists (Ashby et al., 1989; Chen et al., 1992; Gobbi and Janiri, 1999). Such an agonist action at 5-HT_3R might culminate in a functional suppression of stimulant-induced behaviors similar to that seen following intra-PFC microinfusion of DA agonists (Sokolowski and Salamone, 1994; Karler et al., 1998). However, although MK 212 is reported to block the actions of the 5-HT_3R agonist 2-methyl-5-HT in dorsal root ganglion cells (Todorovic and Anderson, 1990), we have been unable to locate evidence to suggest that MK 212 acts as a 5-HT_3R agonist *in vivo*. In drug discrimination studies, MK 212 substitutes for preferential 5-HT_{2C}R agonist *m*-chlorophenylpiperazine (mCPP; Callahan and Cunningham, 1994) and the selective 5-HT_{2C}R agonist RO 60-0175 (Dekeyne et al., 1999). Neither 5-HT_3R agonists nor antagonists mimicked these 5-HT_{2C}R-dependent cues and 5-HT_3R antagonists did not block the mCPP- or RO 60-0175-mediated cues (Callahan and Cunningham, 1994; Dekeyne et al., 1999). Furthermore, 5-HT_3R antagonists were not effective in blocking the ability of MK 212 to enhance adrenocorticotropin hormone release in male rats (Jorgensen et al., 2002). Although indirect, these studies offer evidence to exclude an agonist action of MK 212 to act at 5-HT_3R *in vivo*.

The 5-HT_{2C}R antagonist utilized in the present study was RS 102221 which displays high affinity (3.8-7.4 nM) for 5-HT_{2C}R and 100-fold selectivity compared to 5-HT_{2A}R and 5-HT_{2B}R; the affinity of RS 102221 for all other assayed receptors is low (pKi < 6.5; Bonhaus et al., 1997).

Consistent with its ability to function as a full antagonist *in vitro* (Bonhaus et al., 1997), RS 102221 lacks efficacy at h5-HT_{2C}R expressed in CHO cells (Cussac et al., 2002). Although brain distribution is limited, systemic administration of RS 102221 does increase food intake and weight gain and block a well-characterized effect of 5-HT_{2C}R stimulation to induce hypophagia (Bonhaus et al., 1997). After local application, RS 102221 has been shown to block the inhibitory effects of MK 212 on neurons in the rat nucleus tractus solitarius (Sevoz-Couche et al., 2000). These data suggest that the ability of intra-PFC infusion of RS 102221 to block the behavioral effects of cocaine is most likely related to antagonism of 5-HT_{2C}R.

Serotonin neurons of the dorsal raphe nuclei densely innervate the ventral PFC (Lidov et al., 1980) and 5-HT_{2C}R mRNA and protein have been localized in this region (Clemett et al., 2000; Lopez-Gimenez et al., 2001). Furthermore, the ventral PFC (over the dorsal PFC) provides significant afferent input to raphe and regulation of raphe 5-HT neurons (Hajos et al., 1998; Varga et al., 2001). In the ventral PFC, afferent 5-HT terminals contact primarily interneurons (Smiley and Goldman-Rakic, 1996) which are likely to be γ-aminobutyric acid (GABA) interneurons (Lopez-Gimenez et al., 2001), although a possible serotonergic innervation of glutamate pyramidal neurons has not been ruled out (Smiley and Goldman-Rakic, 1996; Lopez-Gimenez et al., 2001). The ventral PFC is also a primary cortical target of the DA pathway originating in the VTA; afferent DA terminals synapse on both glutamate pyramidal neurons and GABA interneurons (Sesack et al., 1995). A DA-dependent suppression of activity of PFC pyramidal neurons and the subsequent reduction of excitatory glutamate output appears to have a net depressive effect on DA function in the mesoaccumbens pathway and on expression of behaviors evoked by psychostimulants (Sokolowski and Salamone, 1994; Karler et al., 1998). Stimulation of raphe nuclei (Mantz et al., 1990) or iontophoretic application of 5-HT ligands (Bergqvist et al., 1999)

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suppresses spontaneous and/or glutamate-activated firing of PFC neurons in a 5-HT_{2C}R-dependent manner (Bergqvist et al., 1999) suggesting that the 5-HT_{2C}R limits the excitability of cortical pyramidal neurons (Carr et al., 2002). Given the parallel between the behavioral profile observed upon intra-PFC microinjections of the 5-HT_{2C}R ligands with that seen following DA ligands, 5-HT_{2C}R in ventral PFC may influence the output of cocaine-evoked behaviors via modification of PFC output to the DA mesoaccumbens circuit.

Intra-PFC application of 5-HT_{2C}R ligands might act at receptor protein localized in several different cellular populations in the PFC, either at the level of the 5-HT [see (1); Fig. 6] or DA axon terminals [see (2); Fig. 6], on excitatory glutamate neurons [see (3); Fig. 6] or on GABA interneurons [see (4); Fig. 6]. If 5-HT_{2C}R localize to *5-HT terminals* in PFC, these receptors could presynaptically control 5-HT release in this region [see (1), Fig. 6]. The PFC receives a dense, possibly exclusive, innervation from dorsal raphe 5-HT neurons (O'Hearn and Molliver, 1984). However, the 5-HT_{2C}R appears to be localized somatodendritically in PFC (Lopez-Gimenez et al., 2001). Furthermore, although the influence of *direct* application of 5-HT_{2C}R ligands on PFC 5-HT efflux has not yet been established, neither the selective 5-HT_{2C}R agonist RO 60-0175 nor the selective antagonist SB 242084 affected 5-HT release in the frontal cortex upon systemic administration (Millan et al., 1998). The 5-HT_{2C}R might also localize to *DA terminals* in PFC, and 5-HT_{2C}R activation could presynaptically control DA function in this region after local infusion of 5-HT_{2C}R ligands [see (2), Fig. 6]. However, although the 5-HT_{2C}R transcript has been localized to regions containing DA cell bodies, expression of 5-HT_{2C}R mRNA does not appear to co-localize with tyrosine hydroxylase in VTA neurons (Eberle-Wang et al., 1997) suggesting that membranes of DA terminals in the PFC are unlikely to possess 5-HT_{2C}R. Thus, these data suggest that the

modulatory influence of intra-PFC 5-HT_{2C}R ligands on the behavioral effects of cocaine are not likely to be related to the actions of the 5-HT_{2C}R localized to either 5-HT or DA terminals in PFC.

The majority of PFC pyramidal neurons express 5-HT_{2C}R mRNA as shown by single cell PCR (Carr et al., 2002) and a direct effect of locally infused ligands at somatodendritic 5-HT_{2C}R (Lopez-Gimenez et al., 2001) may account for observed effects upon behavior. In the present experiments, inhibition of reuptake by cocaine would result in increased DA and 5-HT efflux, resulting in enhanced, indirect stimulation of DA D_2R (Karler et al., 1998) and 5-HT_{2C}R (Carr et al., 2002), respectively, and a subsequent dampening of excitability of PFC pyramidal neurons [see (3), Fig. 6]. Co-stimulation of 5-HT_{2C}R with MK 212 might be expected to further depress excitability of this pathway. In contrast, RS 102221 may block the actions of 5-HT at 5-HT_{2C}R, perhaps enhancing excitability to some degree and further potentiating the behavioral effects of cocaine.

Interneurons in the PFC appear to be a primary target of 5-HT terminals, many of which are GABA interneurons (Smiley and Goldman-Rakic, 1996). Stimulation of 5-HT_{2C}R found within GABA interneurons [see (4), Fig. 6] would be expected to control PFC projection neurons, and could provide a mechanism through which locally-administered 5-HT_{2C}R ligands could affect behavioral responses to cocaine. In keeping with this hypothesis, the 5-$HT_{2A/2C}$R agonist DOI has been shown to increase extracelullar GABA in rat brain (Abi-Saab et al., 1999) and electrophysiological studies indicate that stimulation of 5-HT_{2C}R excites the activity of GABA neurons in the VTA (e.g., Di Giovanni et al., 2001). If this is the case for GABA interneurons in PFC, GABA released consequent to 5-HT_{2C}R stimulation would be expected to reduce excitatory output to medium spiny neurons in the NAc and/or the DA cell bodies in the VTA (Sheldon and Aghajanian, 1990; Kalivas et al., 1993) and decrease stimulant-evoked behaviors. In fact, the

$GABA_AR$ agonist gaboxadol injected intracortically has been shown to decrease cocaine-induced stereotypy (Karler et al., 1998). However, the preferential 5-$HT_{2C}R$ agonist mCPP *inhibits* the activity of pyramidal neurons, an effect not blocked by the $GABA_AR$ antagonist bicuculline (Bergqvist et al., 1999). These findings cast doubt on a mechanism of action for our observed effects based on a 5-$HT_{2C}R$ control of GABA function in the PFC.

Our present findings help clarify the discrepancy between the influence of systemic and intracranial application of 5-$HT_{2C}R$ ligands on cocaine-evoked behaviors (see Introduction). The modulation of cocaine-evoked behaviors by systemic administration of the 5-$HT_{2C}R$ ligands is directionally identical with the influence of the 5-$HT_{2C}R$ ligands after local application to the ventral PFC. Extending our previous observations (McMahon et al., 2001; Filip and Cunningham, 2002), the present findings suggest that separate populations of 5-HT_2R within the PFC, NAc and VTA differentially control the mesoaccumbens DA pathway and that the ventral PFC is a specific brain site at which the 5-$HT_{2C}R$ exerts an inhibitory control over behavioral responses to cocaine.

The localization of an inhibitory influence of the 5-$HT_{2C}R$ to the ventral PFC is important to our understanding of the acute behavioral effects of cocaine and the processes involved in the development of cocaine use disorders. When taken in the context of studies of systemic administration of the 5-$HT_{2C}R$ ligands in animals and the brain imaging studies of human cocaine abusers (see above), the present findings suggest the therapeutic potential of 5-$HT_{2C}R$ manipulations in the treatment of cocaine dependence, the maintenance of abstinence and/or the reduction of craving. In addition, abnormal post-transcriptional regulation of the 5-$HT_{2C}R$ has been linked to depression and suicide (Niswender et al., 2001; Gurevich et al., 2002) and 5-$HT_{2C}R$ ligands might ultimately be useful for the treatment of such major psychiatric disorders, some of which may be pathological states dependent upon limbic forebrain malfunction.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Histological verification of infusion sites. The schematic diagrams show the representative sites of intra-PFC cannulae placements for animals included in the motor activity (left) and drug discrimination studies (right). Plates are taken from Paxinos and Watson (1998) and the numbers beside each plate correspond to millimeters from bregma. Due to the large number of animals utilized for both motor activity and drug discrimination studies, bilateral placements are shown for only a subset of the experimental pool.

Figure 2. Basal and cocaine-stimulated activity following intra-PFC microinjection of MK 212. The mean horizontal activity (expressed as distance traveled in centimeters, cm ± S.E.M.; top panel) and mean rearing (expressed as counts of photobeam breaks ± S.E.M; bottom panel) summed over the 60-min session after intra-PFC microinjections of saline (SAL) or MK 212 (MK; 0.05, 0.15 or 0.5 μg/side) followed by an i.p. injection of saline (SAL) or cocaine (COC; 10 mg/kg) are shown. Data points represent the mean of data from 6-8 rats. * $p < 0.05$ vs. SAL-SAL; ^ , $p < 0.05$ vs. SAL-COC.

Figure 3. Basal and cocaine-stimulated activity following intra-PFC microinjection of RS 102221. The mean horizontal activity (expressed as distance traveled in centimeters, cm ± S.E.M.; top graph) and mean rearing (expressed as counts of photobeam breaks ± S.E.M; bottom panel) summed over the 60-min session after intra-PFC microinjections of saline (SAL) or RS 102221 (RS; 0.15, 0.5, 1.5 or 5 μg/side) followed by an i.p. injection of saline (SAL) or cocaine (COC; 10 mg/kg) are shown. Data points represent the mean of data from 5-8 rats. * $p < 0.05$ vs. SAL-SAL; ^ , $p < 0.05$ vs. SAL-COC.

Figure 4. Effects of intra-PFC microinjection of MK 212 in rats trained to discriminate cocaine (10 mg/kg) from saline. Symbols on the top panel denote the mean percentage of

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cocaine-lever responses (± S.E.M.); symbols on the bottom panel denote the mean number of responses/sec (± S.E.M.). **Left**. Performance is denoted after intra-PFC injections of saline (SAL; 0.2 μl/side; open circle), MK 212 (MK; 0.05 μg/side; open triangle) or MK 212 (0.5 μg/side; open square) followed by a systemic injection of saline (i.p.). **Right.** Performance is denoted after systemic administration of cocaine (1.25-10 mg/kg) preceded by intra-PFC microinjections of saline (SAL; 0.2 μl/side; closed circles) or a fixed dose of MK 212 (MK; 0.05 μg/side; closed triangles) or MK 212 (0.5 μg/side; closed squares). All data points represent the means of data from 7-8/8 rats [n/N, number of rats (n) completing the FR 20 on either lever out of the number of rats tested (N)]. Asterisks (*) represent performances during test sessions that were significantly different from that observed after the appropriate dose of cocaine ($p < 0.05$).

Figure 5. Effects of intra-PFC microinjection of RS 102221 in rats trained to discriminate cocaine (10 mg/kg) from saline. Symbols on the top panels denote the mean percentage of cocaine-lever responses (± S.E.M.); symbols on the bottom panels denote the mean number of responses/sec (± S.E.M.). **Left**. Performance is denoted after intra-PFC injections of saline (SAL; 0.2 μl/side; open circle), RS 102221 (RS; 1.5 μg/side; open triangle) or RS 102221 (5 μg/side; open square) followed by a systemic injection of saline (i.p.). **Right.** Performance is denoted after systemic administration of cocaine (1.25-10 mg/kg) preceded by intra-PFC microinjections of saline (SAL; 0.2 μl/side; closed circles) or a fixed dose of RS 102221 (RS; 1.5 μg/side; closed triangles) or RS 102221 (5 μg/side; closed squares). All data points represent the means of data from 7-7/7 rats [n/N, number of rats (n) completing the FR 20 on either lever out of the number of rats tested (N)]. Asterisks (*) represent

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performances during test sessions that were significantly different from that observed after the appropriate dose of cocaine ($p < 0.05$).

Figure 6. Schematic representation of the possible sites of action for 5-HT_{2C}R to modulate PFC function and behavioral responses to cocaine. The projection from 5-HT neurons in the raphe nuclei (Raphe) to the prefrontal cortex (PFC) is represented by the solid grey line; 5-HT_{2C}Rs located in the PFC are represented by aqua hexagons. Dopamine projections from the ventral tegmental area (VTA) to innervate medium spiny neurons (MSN) in the nucleus accumbens (NAc) (mesoaccumbens circuit) and neurons in the PFC (mesocortical circuit) are represented by solid dark blue lines. Pyramidal glutamate (GLU) projections from the PFC to the VTA and to the NAc are represented by dotted pink lines. The 5-HT_{2C}R may be localized to (1) 5-HT terminals from the raphe, (2) DA terminals from VTA, (3) glutamate pyramidal neurons or (4) γ-aminobutyric acid (GABA) interneurons. A brief discussion of the probable influence of 5-HT_{2C}R localized to each site is found in the Discussion.

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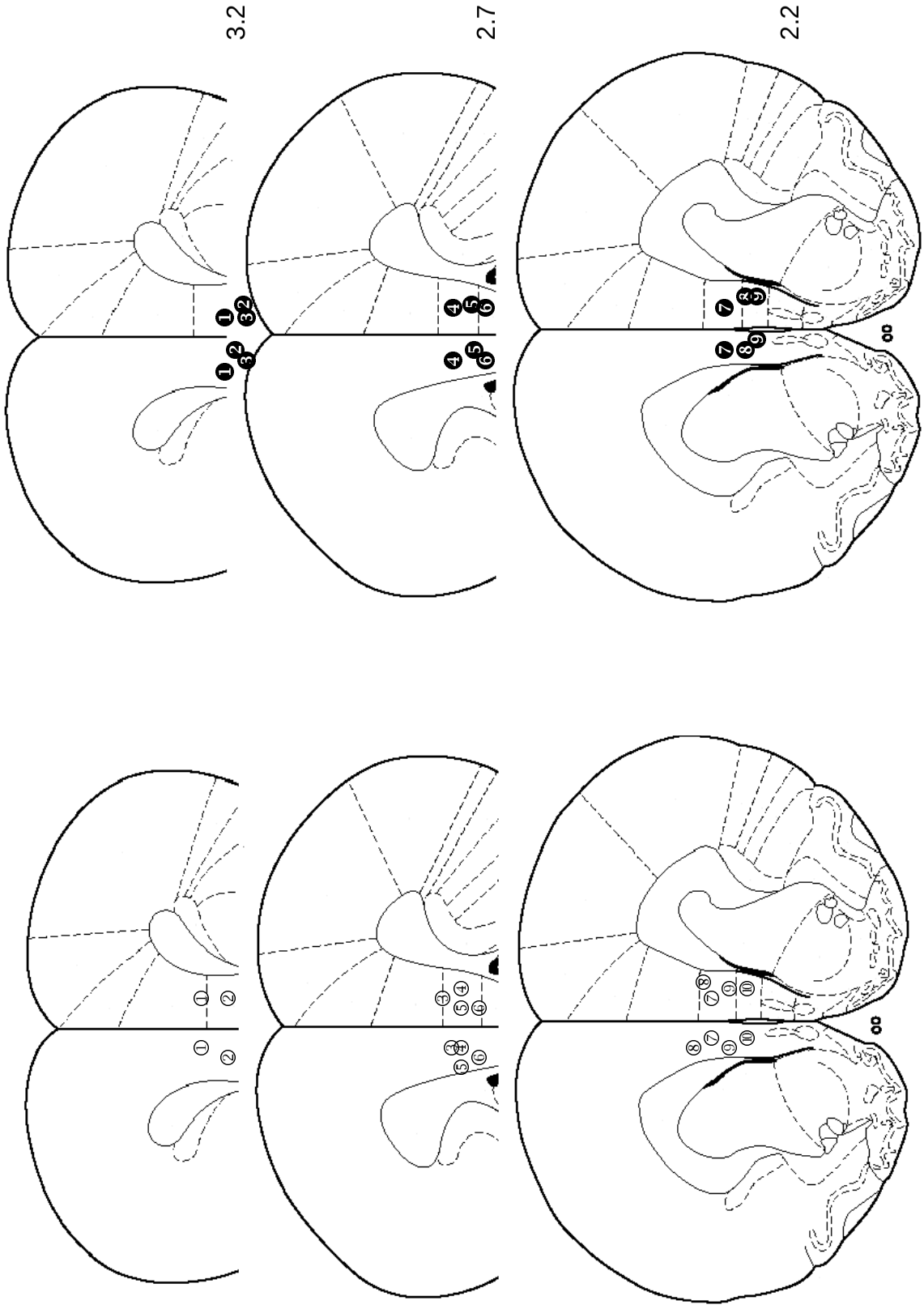

Fig. 1

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Fig. 2

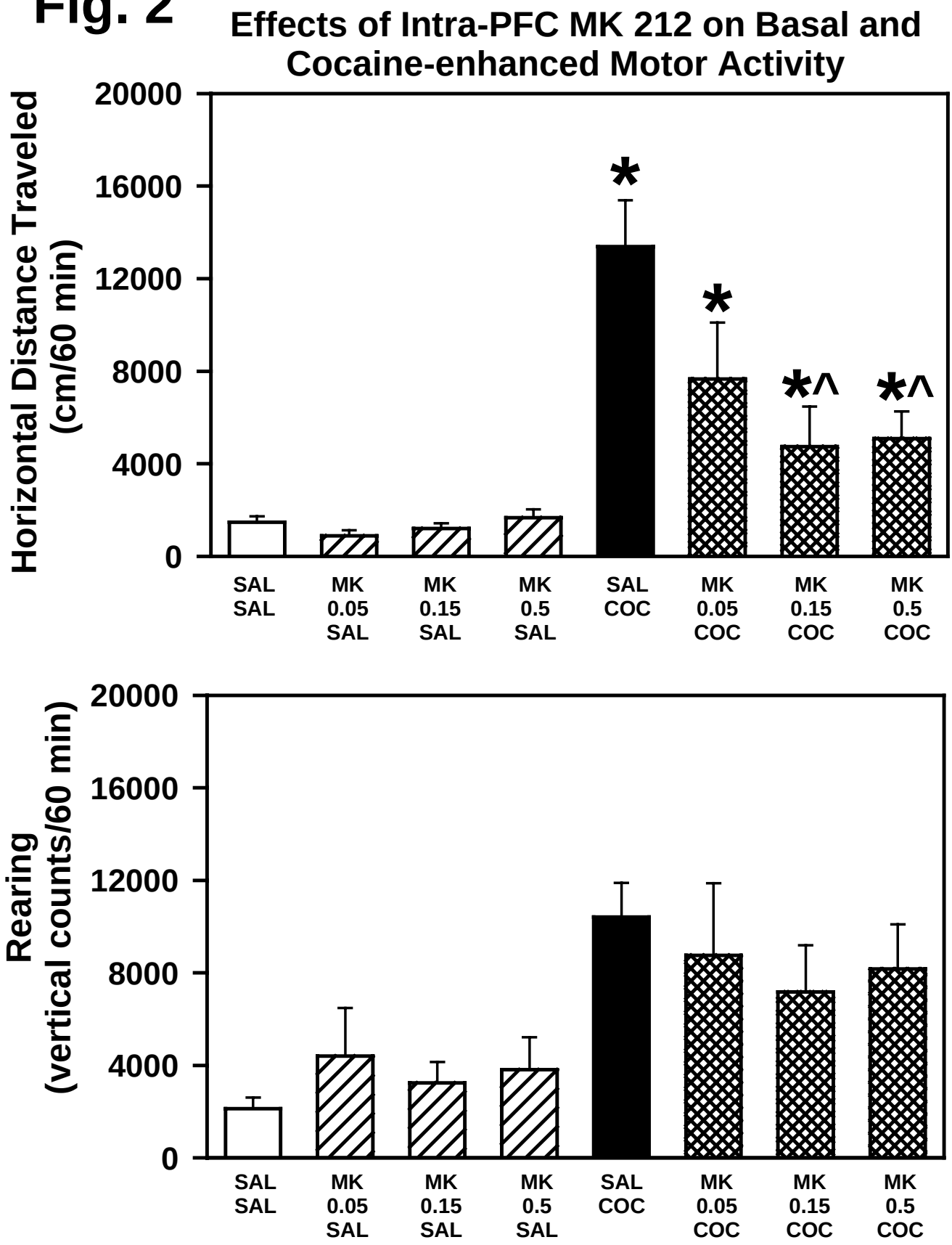

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Fig. 3

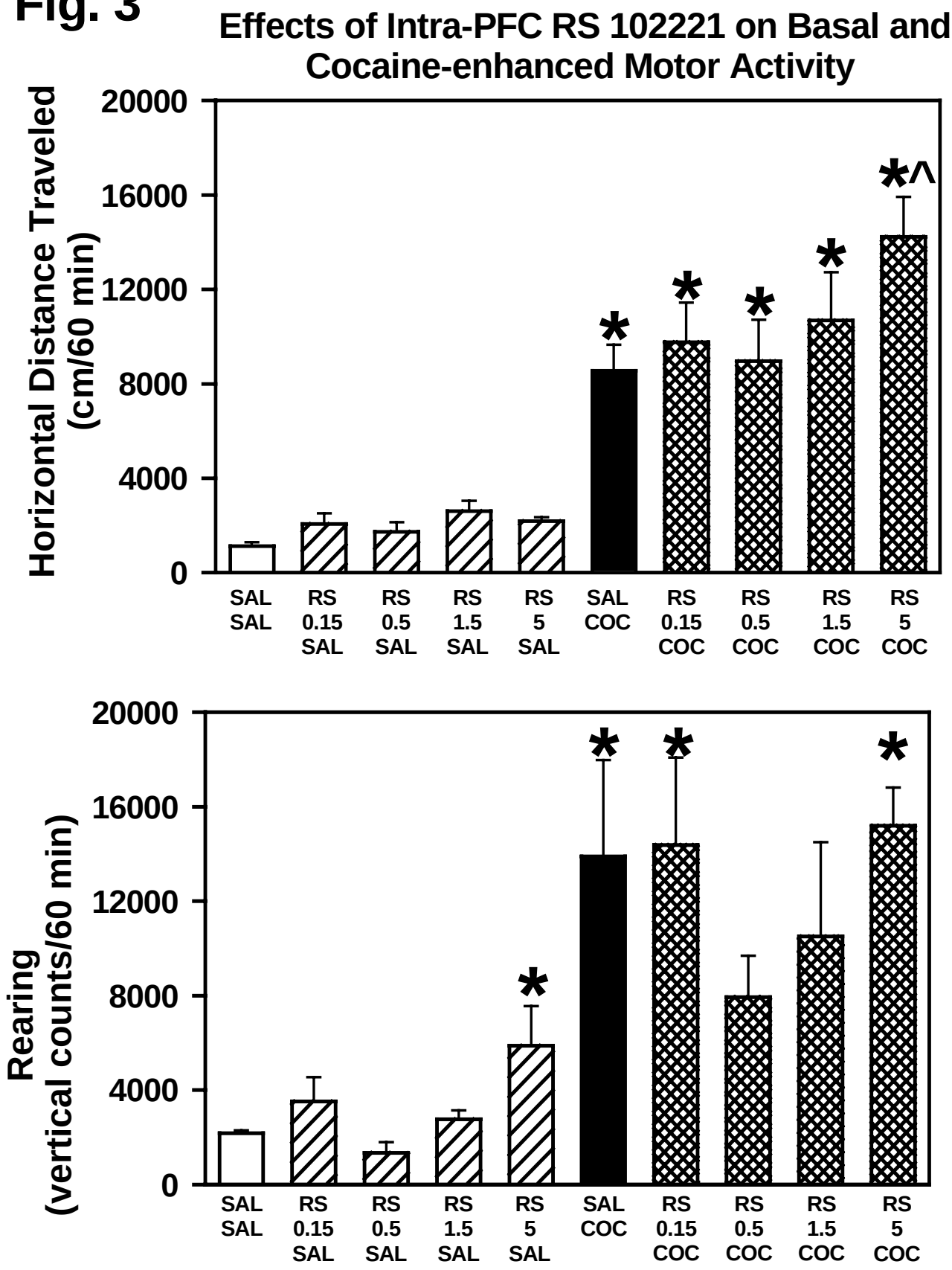

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Fig. 4

Effects of Intra-PFC MK 212 on the Discriminative Stimulus Effects of Cocaine

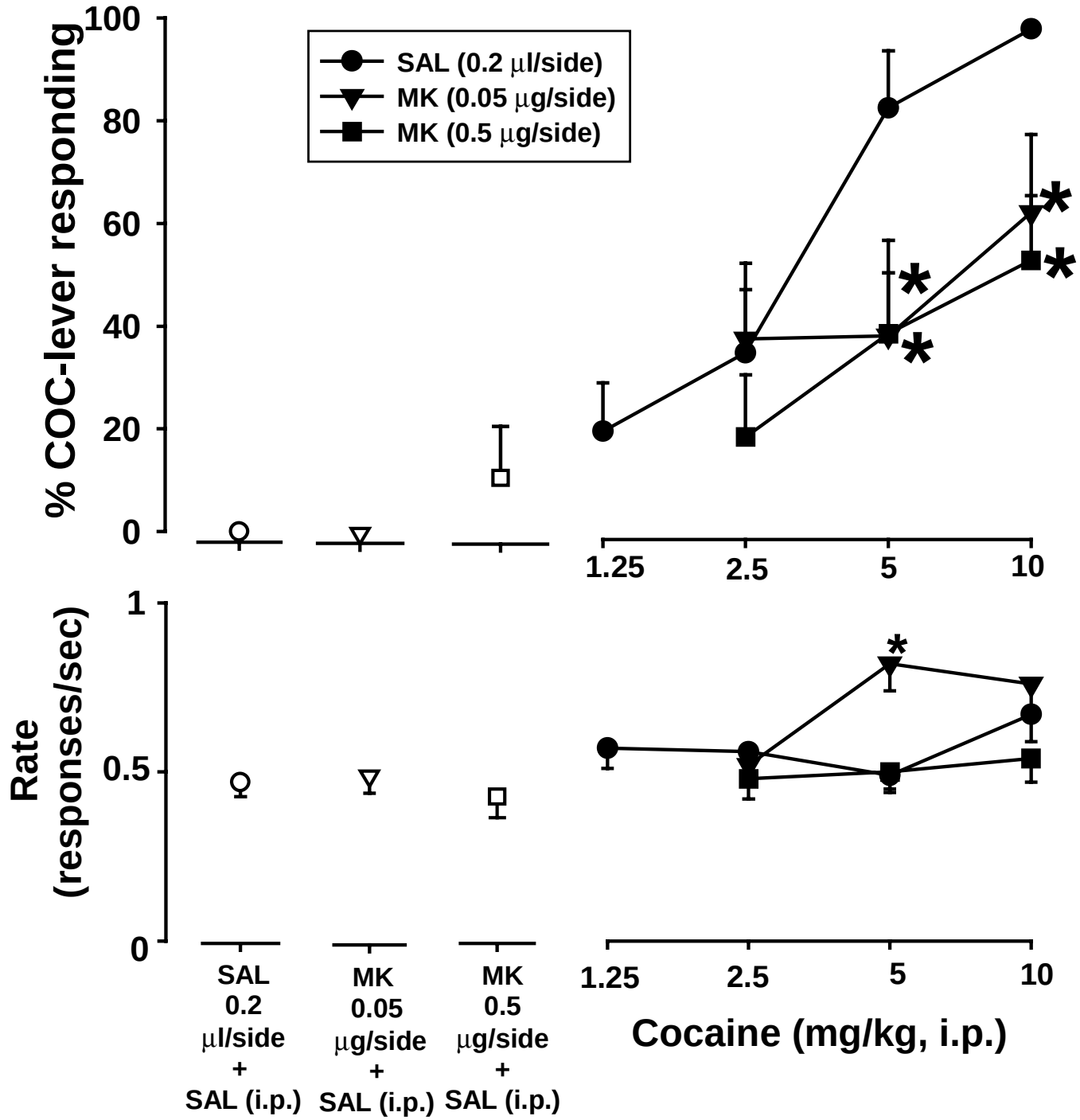

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Fig. 5

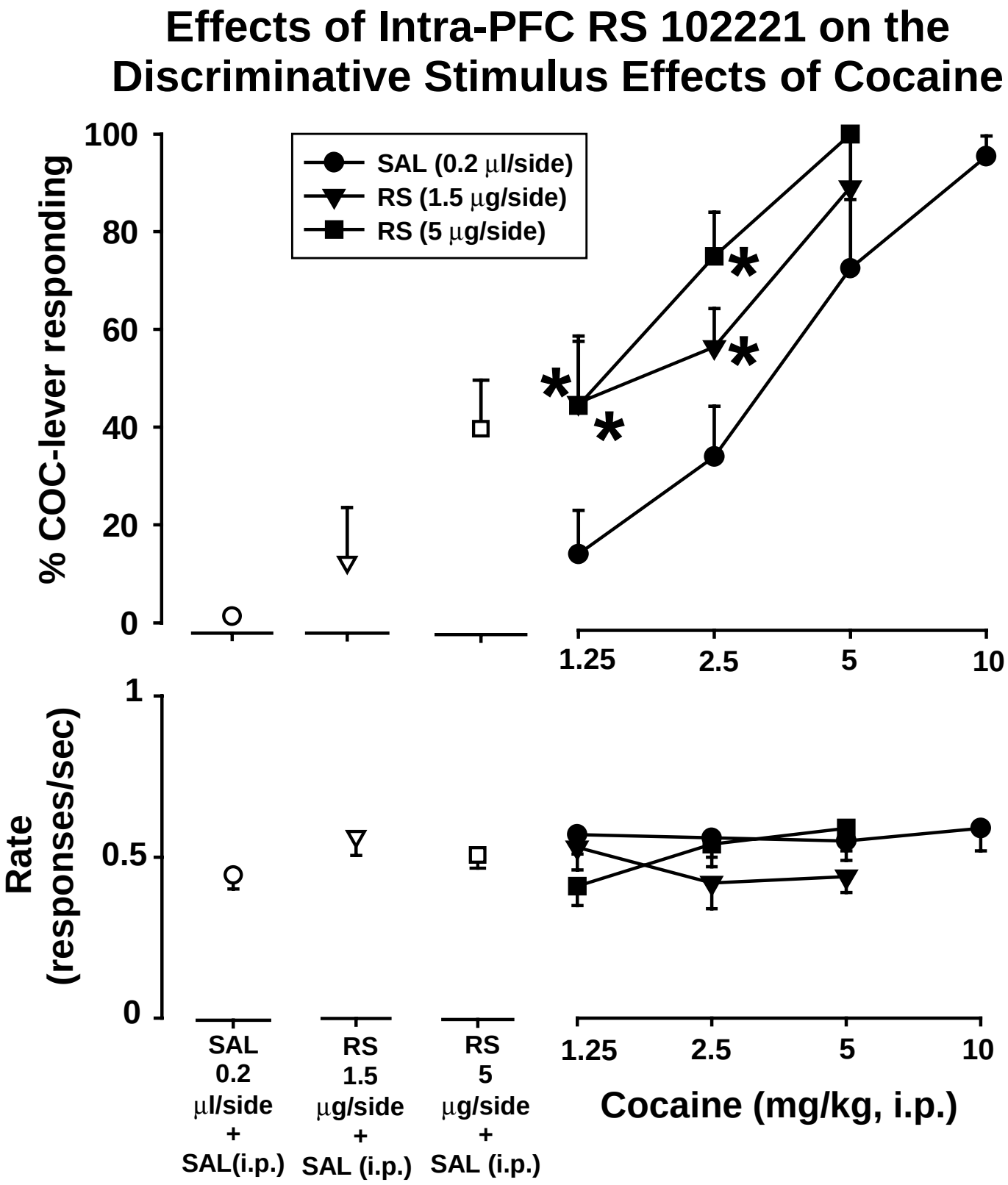

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Fig. 6