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Differential regulation of cocaine-induced locomotor activity in inbred long-sleep and short-sleep mice by dopamine and serotonin systems

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Abstract

Acute injection of cocaine increases locomotor activity of inbred long-sleep (ILS) mice to a greater extent than inbred short-sleep (ISS) mice. Strain differences in dopamine and/or serotonin (5-HT) neurotransmission could underlie these behavioral differences. Here, we found that dopamine D1, 5-HT_{2A} and 5-HT_3 receptor antagonists reduced cocaine-stimulated activity selectively in ILS mice. In contrast, 5-HT transporter (SERT) or 5-HT_{1A} receptor antagonists potentiated cocaine-stimulated activity in ISS, but not in ILS, mice; this potentiation in ISS mice was abolished by dopamine D1 receptor blockade. Thus, in ILS mice, cocaine-induced activation of D1, 5-HT_{2A} or 5-HT_3 receptors is sufficient to produce locomotor stimulation. In contrast, ISS mice require pharmacologically increased 5-HT levels, which appear to result in increased dopamine neurotransmission, for cocaine-induced activation. Our results demonstrate strain differences in dopamine/5-HT receptor subtypes and their interactions that contribute to the differential behavioral responsiveness of ILS and ISS mice to cocaine.

Keywords: Cocaine; Locomotor activity; Dopamine receptor; Serotonin receptor

1. Introduction

Cocaine addiction continues to be a major problem in today's society. The psychomotor stimulant effects of cocaine are most often thought to be mediated through enhanced dopamine neurotransmission in mesolimbic areas of the brain (Kelly and Iversen, 1976; Wise and Bozarth, 1987). This enhancement is primarily due to the ability of cocaine to inhibit the dopamine transporter (DAT; Ritz et al., 1990), an idea confirmed by the findings of Giros et al. (1996), in that cocaine does not induce locomotor activation in DAT knockout mice. However, cocaine also inhibits transporters for serotonin (SERTs) and norepinephrine (NETs; Ritz et al., 1990). In fact, DAT knockout mice show conditioned place preference to cocaine (Sora et al., 2001; Rocha et al., 1998) whereas only the double-mutant mice lacking DAT and SERT fail to show this behavior (Rocha et al., 1998). In addition, SERT can modulate the locomotor and discriminative-stimulant effects of cocaine (Bubar et al., 2003; Cunningham and Callahan, 1991; Herges and Taylor, 1998, but see Reith et al., 1991). Thus, in addition to dopamine, serotonin (5-HT) clearly plays an important role in the behavioral effects of cocaine.

In rodents, acute injection of cocaine increases locomotor activity, as well as dopamine and 5-HT levels in the nucleus accumbens (Andrews and Lucki, 2001; Bubar et al., 2003; Teneud et al., 1996). Subsequent activation of the various dopamine and 5-HT receptor subtypes contributes to the locomotor-stimulant effects of cocaine. For example, dopamine D1 and D2 receptor antagonists attenuate cocaine-

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induced activity in rodents (Chausmer and Katz, 2001; Kita et al., 1999; McCreary and Marsden, 1993; Ushijama et al., 1995). However, the effects of dopamine D2 receptor antagonists are difficult to interpret because most of these drugs depress spontaneous locomotor activity (Chausmer and Katz, 2001; Ushijama et al., 1995). 5-HT on the other hand, can be either stimulatory or inhibitory with respect to cocaine-induced locomotion. For example, 5-HT_{2A} and 5-HT_3 receptors appear to play a stimulatory role in cocaine-induced activity (Fletcher et al., 2002; Herges and Taylor, 2000; McMahon and Cunningham, 2001; Reith, 1990; Svingos and Hitzemann, 1992). However, inhibition of 5-HT_{1A} receptors can either increase or decrease cocaine-induced locomotor activation (Carey et al., 2001; Herges and Taylor, 1998, 1999; Müller et al., 2002). These opposing effects are likely dependent on whether presynaptic or postsynaptic 5-HT_{1A} receptors are blocked.

The complexity of the contribution of 5-HT to cocaine behaviors is further enhanced by interactions between 5-HT and dopamine systems. 5-HT neurons projecting from the dorsal raphe nucleus and innervating both the ventral tegmental area and nucleus accumbens can modulate dopamine neurotransmission (Broderick and Phelix, 1997). For example, 5-HT and 5-HT receptor agonists can increase dopamine release in striatum and nucleus accumbens (Benloucif and Galloway, 1992; Benloucif et al., 1993; Parsons and Justice, 1993). In addition, SERT inhibitors can potentiate the behavioral effects of cocaine, as well as cocaine-mediated dopamine release in the nucleus accumbens (Bubar et al., 2003; but see Clark et al., 1996). Thus, increased 5-HT neurotransmission is correlated with an increase in dopamine release.

Long-sleep (LS) and short-sleep (SS) mice were selectively bred for their differential sensitivity to the sedative effects of higher doses of ethanol (McClearn and Kakihana, 1981). Inbred long-sleep (ILS) and inbred short-sleep (ISS) mice, developed by 20 generations of sibling mating of LS or SS mice, show similar differential sensitivity to ethanol sedation (Markel et al., 1995). When tested with cocaine and other DAT inhibitors, we found that ILS mice show markedly higher locomotor activation compared to ISS mice (Hanania and Zahniser, 2002; Hanania et al., 2004). We also found that ILS mice have ~24% fewer striatal DAT binding sites compared to ISS mice and that the rate of in vivo dopamine clearance is slower in ILS mice under basal conditions and is further slowed upon injection of 10 mg/kg cocaine (Hanania et al., 2004). These small changes in DAT could contribute to, but are unlikely to be solely responsible for, the differential behavioral sensitivity of ILS and ISS mice to cocaine. Because both dopamine and 5-HT clearly play a role in cocaine-induced locomotor activation, we determined the contribution of dopamine and/5-HT receptor subtypes to the locomotor-stimulant effects of cocaine in ILS and ISS mice.

2. Materials and methods

2.1. Animals

Adult (80–90 days old) male ILS and ISS mice were obtained from the Institute for Behavioral Genetics, Boulder, The mice were housed in groups of five and exposed to a 12-h light–dark cycle with food and water available ad libitum. All animal use procedures were in strict accordance with the European Community guidelines for the use of experimental animals and were approved by the Institutional Animal Care and Use Committee, University of Colorado Health Sciences Center.

2.2. Locomotor activity

Drug naïve ILS and ISS mice were transferred to the behavioral testing room and were allowed to habituate for 60 min with the lights turned off in clear acrylic open-field activity chambers (16×16×15 in.) with 8×8 photobeams (San Diego Instruments, San Diego, CA). Following habituation, mice were injected with saline, vehicle or dopamine/5-HT drugs, and their locomotor activity was measured in the dark for 30 min, after which the mice were injected with cocaine (10 mg/kg) and their activity was monitored for an additional 60 min. All injections were given by the i.p. route at 1 ml/100 g body weight. Doses for dopamine/5-HT drugs used in this study were based on our previous studies (Hanania et al., 2002). Horizontal locomotor activity was determined from the number of consecutive photobeam breaks/5 min. Each mouse was tested only once. (−)Cocaine HCl, raclopride, (*R*)-(+)-7chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390), fluoxetine, *N*-(2-(4-(2-methoxyphenyl)-1-piperzinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635), *N*-(1-Azabicyclio[2.2.2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide hydrochloride (Y-25130) and ketanserin were dissolved in saline. [R]-(+)-alpha-(2,3-dimethoxyphenyl)-1-{2-(4phenylrthyl)]-4-piperidine-methanol (M 100907) was dissolved in saline containing 2% dimethyl sulfoxide (DMSO).

The effects of the dopamine/5-HT drugs on (1) spontaneous baseline locomotor activity and (2) cocaine-stimulated activity were determined. The effects on baseline activity were measured by averaging the distance traveled during the 30-min period following either saline, vehicle or drug injection. The effects of the dopamine dopamine/5-HT drugs on cocaine-stimulated activity were measured by averaging the distance that the mice traveled during the 30-min period following cocaine injection.

2.3. Statistical analysis

Locomotor activity data were analyzed using either analyses of variance (ANOVAs) or Student's *t*-tests. In all tests, $P<0.05$ was considered to be statistically significant.

2.4. Materials

Raclopride, eticlopride, SCH 23390, ketanserin and DMSO were purchased from Sigma/RBI (St. Louis, MO, USA). Y-25130 was purchased from Tocris Cookson (Ellisville, MO, USA). WAY 100635 and M 100907 were synthesized at Solvay Pharmaceuticals Research Laboratories (Weesp, the Netherlands). (−)Cocaine HCl was obtained from the National Institute on Drug Abuse (RTI International, Research Triangle Park, NC, USA). Fluoxetine was a gift from Eli Lilly (Indianapolis, IN, USA).

3. Results

3.1. Effect of dopamine receptor antagonists on cocaine-stimulated locomotor activity

To test the involvement of dopamine receptor subtype activation in the locomotor-stimulant effects of cocaine in

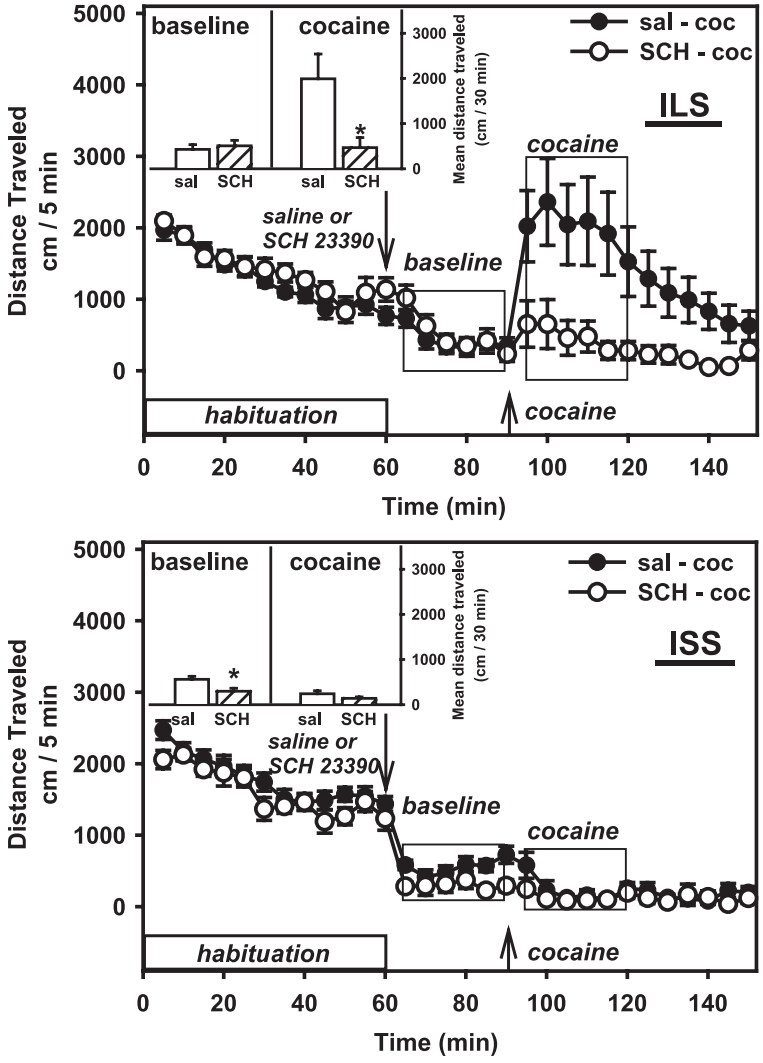

Fig. 1. Time–response for the effects of pretreatment with either saline (sal) or the dopamine D1 receptor antagonist SCH 23390 (SCH; 0.03 mg/kg) on cocaine (coc; 10 mg/kg)-stimulated locomotor activity in ILS (top) and ISS (bottom) mice. Mice were placed in the activity chambers and allowed to habituate with the lights off for 60 min prior to injection. Mice were injected i.p. with saline or SCH 23390 (arrow, n=9–13 mice per group), and activity was measured in the dark for 30 min. Following the first injection, mice were injected i.p. with cocaine (arrow), and activity was measured in the dark for an additional 60 min. Insets: Mean values±S.E.M. for the effects of SCH 23390 on baseline and cocaine-induced locomotor activity in ILS and ISS mice. Data were averaged for the 30 min (min 61–90) after either saline or SCH 23390 injection and for the 30 min (min 91–120) after cocaine injection. *t*-Test analysis showed a significant effect of SCH 23390 on saline-stimulated activity in ISS mice only (*$P<0.05$; bottom). In addition, a significant effect of SCH 23390 was found on cocaine-stimulated activity in ILS (*$P<0.05$; top), but not ISS, mice.

Table 1
The effects of dopamine D2 receptor antagonists on baseline and cocaine-induced locomotor activity in ILS and ISS mice

Drugs	*N*	Baseline locomotor activity (cm/30 min)		Cocaine-induced activity (cm/30 min)	
		ILS	ISS	ILS	ISS
Saline	13–14	429±104	560±68	1994±547	240±67
Raclopride					
(0.3 mg/kg)	8	120±34	286±56*	1507±489	642±384
(1.0 mg/kg)	8–9	52±16*	225±39*	827±386	341±140
Eticlopride					
(0.01 mg/kg)	12	411±116	NT	1091±397	NT
(0.03 mg/kg)	12	617±73	NT	1685±263	NT

Mean values±S.E.M.; NT: not tested. ANOVA analysis found a significant effect of raclopride on baseline locomotor activity in ILS mice at the dose of 1 mg/kg and in ISS mice at the 1- and 3-mg/kg doses.

* $P<0.05$.

ILS and ISS mice, we determined the effects of pretreatment with selective dopamine D1 or D2 receptor antagonists. The time course for the effects of pretreatment with either saline or the dopamine D1 receptor antagonist SCH 23390 (0.03 mg/kg) on basal and cocaine (10 mg/kg)-stimulated locomotor activity is shown for both mouse strains in Fig. 1. SCH 23390 significantly attenuated cocaine-stimulated locomotor activity in ILS mice without altering baseline

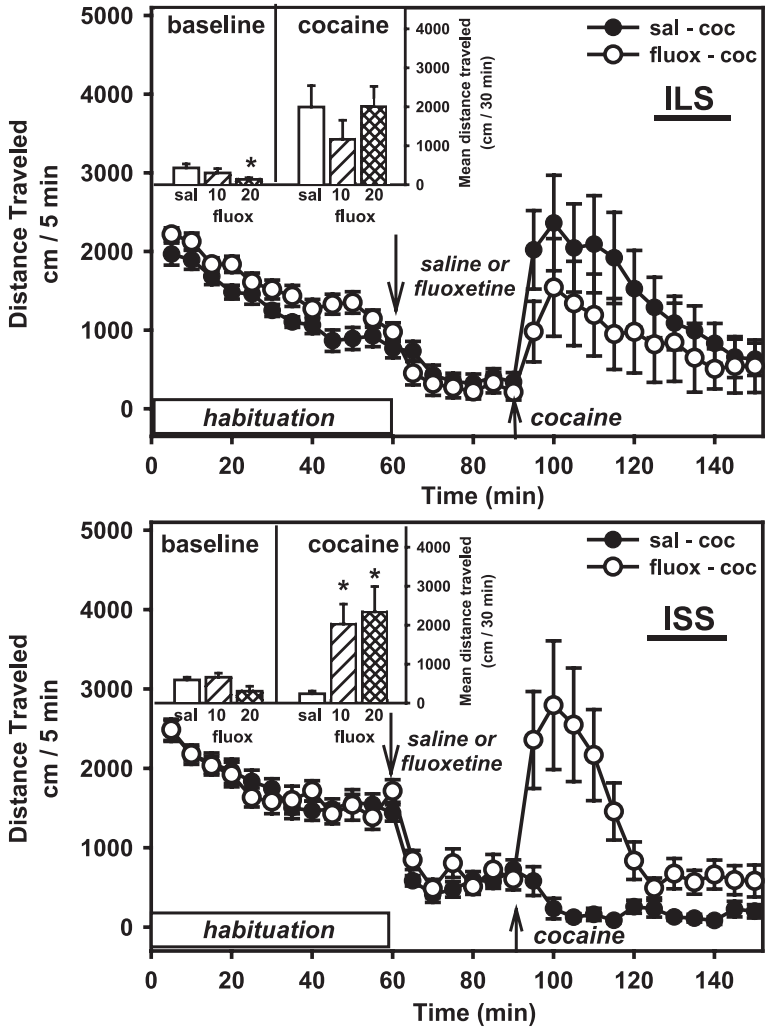

Fig. 2. Time–response for the effects of pretreatment with either saline or the SERT inhibitor fluoxetine (fluox; 10 mg/kg) on cocaine (10 mg/kg)-stimulated locomotor activity in ILS (top) and ISS (bottom) mice (n=10–13 mice per group). See Fig. 1 for methodological details. Insets: Mean values±S.E.M. for the effects of fluoxetine (10 or 20 mg/kg) on baseline and cocaine-induced locomotor activity of ILS and ISS mice (see Fig. 1 for details). ANOVA analysis found a significant effect of fluoxetine on baseline activity only in ILS mice and only at 20 mg/kg (*$P<0.05$; top). Both doses of fluoxetine significantly potentiated cocaine-induced locomotor activity in ISS, but not ILS, mice (*$P<0.05$; bottom).

locomotor activity (Fig. 1, inset). In contrast, SCH 23390 significantly attenuated baseline, but not cocaine-stimulated, locomotor activity in ISS mice (Fig. 1, inset).

We next tested the effects of pretreatment with the dopamine D2 receptor antagonist raclopride (0.3 and 1 mg/kg) on the locomotor-stimulant effects of cocaine in ILS and ISS mice. The results are summarized in Table 1. The lower dose of raclopride had no effect on baseline activity of ILS mice but significantly attenuated that of ISS mice. The higher dose of raclopride significantly attenuated baseline activity of both mouse strains (Table 1). Compared to the saline-pretreated group, pretreatment with both doses of raclopride showed a nonsignificant trend to decrease cocaine-induced locomotor activity in ILS mice (Table 1). Because the trend for raclopride to decrease cocaine-induced activity in ILS mice could be the result of its effect on baseline activity, we tested pretreatment with another selective dopamine D2 receptor antagonist, eticlopride. Eticlorpide (0.01 and 0.03 mg/kg) had no significant effect on either baseline, or cocaine-induced, locomotor activity in ILS mice (Table 1). Similar to ILS mice, raclopride did not alter locomotor activity of cocaine-treated ISS mice (Table 1).

3.2. Effect of a SERT inhibitor on cocaine-stimulated locomotor activity

Fluoxetine is a SERT inhibitor that has been shown to enhance cocaine-induced activity (Bubar et al., 2003). Here, we tested whether pretreatment with fluoxetine (10 or 20 mg/kg) would potentiate cocaine-stimulated activity in ILS and ISS mice. The time course for the effects of 10 mg/kg fluoxetine is shown in Fig. 2. By itself, fluoxetine markedly depressed baseline locomotor activity in ILS mice only at the higher dose tested (Fig. 2, inset). Despite its effects on baseline activity, fluoxetine did not alter cocaine-induced

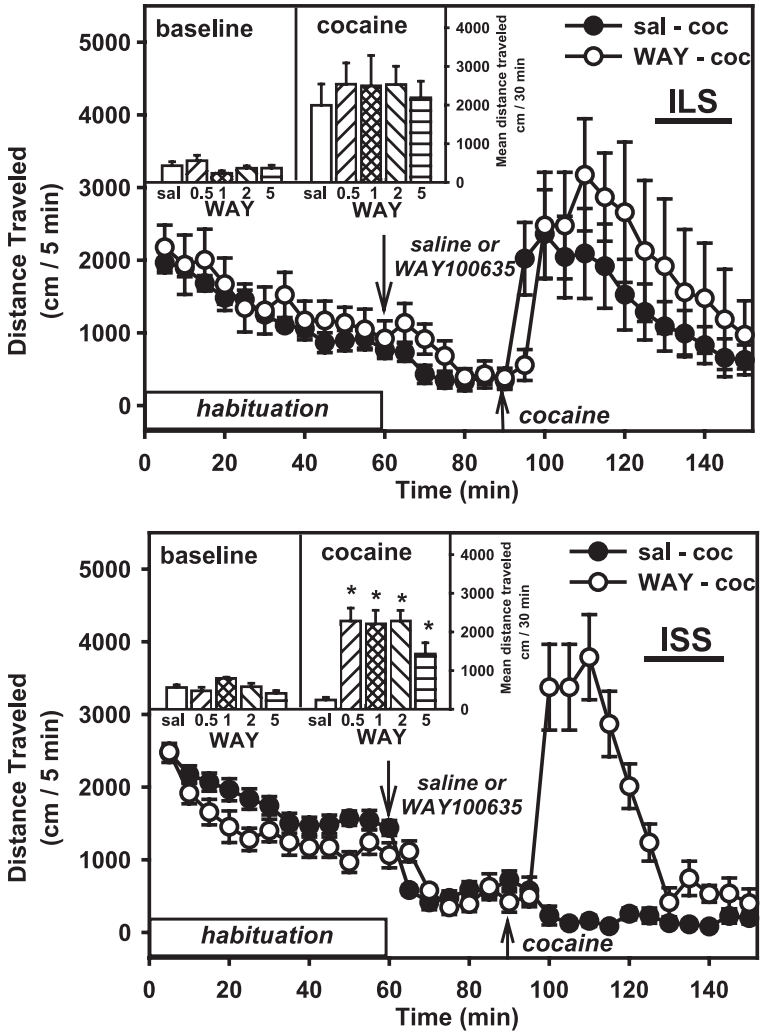

Fig. 3. Time–response for the effects of pretreatment with either saline or the 5-HT_{1A} receptor antagonist WAY 100635 (WAY; 0.5 mg/kg) on cocaine (10 mg/kg)-stimulated locomotor activity in ILS (top) and ISS (bottom) mice (n=8–14 mice per group). See Fig. 1 for methodological details. Insets: Mean values±S.E.M. for the effects of WAY 100635 (0.5, 1, 2 or 5 mg/kg) on baseline and cocaine-induced locomotor activity of ILS and ISS mice (see Fig. 1 for details). ANOVA analysis found that all doses of WAY 100635 significantly potentiated cocaine-induced locomotor activity in ISS mice (*P<0.05; bottom).

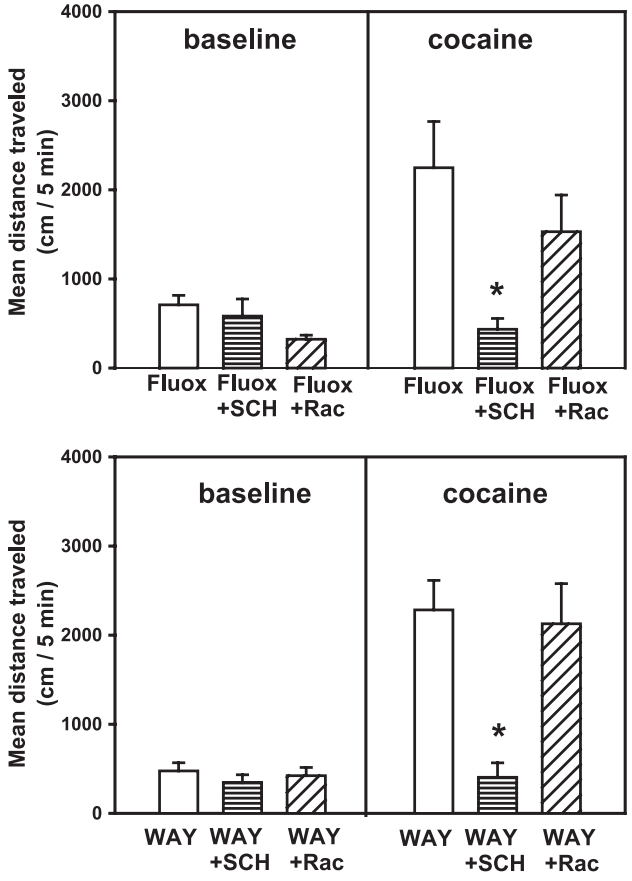

Fig. 4. Summary of the effects of co-pretreatment with fluoxetine (10 mg/kg)+SCH 23390 (0.03 mg/kg; top), fluoxetine (10 mg/kg)+raclopride (0.3 mg/kg; top), WAY 100635 (0.5 mg/kg)+SCH 23390 (0.03 mg/kg; bottom) or WAY 100635+raclopride (0.3 mg/kg; bottom) on baseline and cocaine-stimulated locomotor activities in ISS mice (n=8–9 mice per group). ANOVA analysis showed that ISS mice pretreated with fluoxetine+SCH 23390 (top) or WAY 100635+SCH 23390 (bottom) exhibited significantly depressed cocaine-induced locomotor activity compared to their fluoxetine- or WAY-100635-pretreated counterparts (*P<0.05).

activity in ILS mice (Fig. 2, inset). Interestingly, neither dose of fluoxetine had a significant effect of baseline activity in ISS mice (Fig. 2, inset). Furthermore, cocaine-stimulated locomotor activity in ISS mice pretreated with either dose of fluoxetine was significantly greater than in the saline-pretreated group (Fig. 2, inset).

3.3. Effect of a 5-HT$_{1A}$ receptor antagonist on cocaine-stimulated locomotor activity

The time course for the effects of the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 (0.5 mg/kg) is shown in Fig. 3. By itself, doses of WAY 100635 from 0.5 to 5 mg/kg did not alter baseline locomotor activity in either mouse strain (Fig. 3, insets). Pretreatment with WAY 100635 failed to alter cocaine-induced locomotor activity in ILS mice (Fig. 3, inset). However, all tested doses of WAY 100635 significantly increased cocaine-stimulated locomotor activity in ISS mice (Fig. 3, inset).

3.4. Dopamine involvement in SERT and 5-HT$_{1A}$ receptor regulation of cocaine-stimulated locomotor activity

We next tested whether dopamine was involved in the potentiating effects of fluoxetine and WAY 100635 on cocaine-induced activity in ISS mice. ISS mice were pretreated with either fluoxetine (10 mg/kg) or a combination of fluoxetine+SCH 23390 (0.03 mg/kg), or with fluoxetine or a combination of fluoxetine+raclopride (0.3 mg/kg), prior to injection with cocaine (10 mg/kg). In similar experiments, we pretreated a different group of mice with WAY 100635 (0.5 mg/kg), or combinations of WAY 100635+SCH 23390 or WAY 100635+raclopride, prior to cocaine injection. As shown in Fig. 4, none of the combinations of fluoxetine or WAY 100635 altered baseline locomotor activity, compared to either fluoxetine or WAY 100635, respectively. Pretreatment with either fluoxetine+SCH 23390 (Fig. 4, top) or WAY 100635+SCH 23390 (Fig. 4, bottom) significantly reduced cocaine-induced locomotor activity of ISS mice compared to their respective control groups whereas the combinations with raclopride did not (Fig. 4).

3.5. Effect of 5-HT$_2$ receptor antagonists on cocaine-stimulated locomotor activity

The 5-HT$_2$ receptor antagonist ketanserin (1 mg/kg) had no effect on baseline activity in ILS mice, but significantly decreased that of ISS mice (Table 2). Compared with their saline-pretreated counterparts, ketanserin-pretreated ILS

Table 2
The effects of the 5-HT$_2$ receptor antagonist ketanserin and the 5-HT$_{2A}$ receptor antagonist M 100907 on baseline and cocaine-induced locomotor activity in ILS and ISS mice

Drugs	N	Baseline locomotor activity (cm/30 min)		Cocaine-induced activity (cm/30 min)	
		ILS	ISS	ILS	ISS
Saline	13–14	429±104	560±68	1994±547	240±67
Ketanserin (1 mg/kg)	8–12	374±109	239±92*	586±219*	525±184
Vehicle	8	336±107	329±87	1268±565	202±80
M 100907 (0.3 mg/kg)	7–8	234±54	413±111	92±26*	215±112

Mean values±S.E.M. Student's t-test found a significant effect of ketanserin on baseline locomotor activity of ISS mice. Compared to the saline- or vehicle-treated group, ketanserin and M 100907 had a significant effect on cocaine-induced activity in ILS mice only.

* P<0.05.

mice exhibited significantly reduced cocaine-stimulated locomotor activity. No effect of ketanserin was seen on cocaine-stimulated locomotor activity in ISS mice (Table 2). Moreover, we tested M 100907, a selective 5-HT_{2A} receptor antagonist. M 100907 (0.3 mg/kg) alone did not alter baseline locomotor activity of either mouse strain (Table 2). However, similar to ketanserin, M 100907 significantly reduced cocaine-stimulated locomotor activity selectively in ILS mice (Table 2).

3.6. Effect of a 5-HT_3 receptor antagonist on cocaine-stimulated locomotor activity

Fig. 5 shows the time course for the effects of the selective 5-HT_3 receptor antagonist Y-25130 (3 mg/kg) on cocaine-induced activity in ILS and ISS mice. Doses of Y-25130 from 1 to 5 mg/kg had no effect on baseline locomotor activity on ILS mice (Fig. 5, inset). However, pretreatment with the highest dose of Y-25130 (5 mg/kg) reduced cocaine-induced locomotor activity of these mice (Fig. 5, inset). Compared to the saline-treated group, Y-25130 significantly attenuated baseline activity of ISS mice at the doses of 1 and 3 mg/kg only (Fig. 5, inset). However, none of the doses tested had a significant effect on cocaine-induced locomotor activity of these mice (Fig. 5, inset).

4. Discussion

In this study, we found that cocaine-induced locomotor activity of ILS and ISS mice is differentially regulated by antagonism of dopamine receptors, 5-HT receptors and SERT. This differential regulation in the two mouse strains is most likely due to differences in extracellular levels of

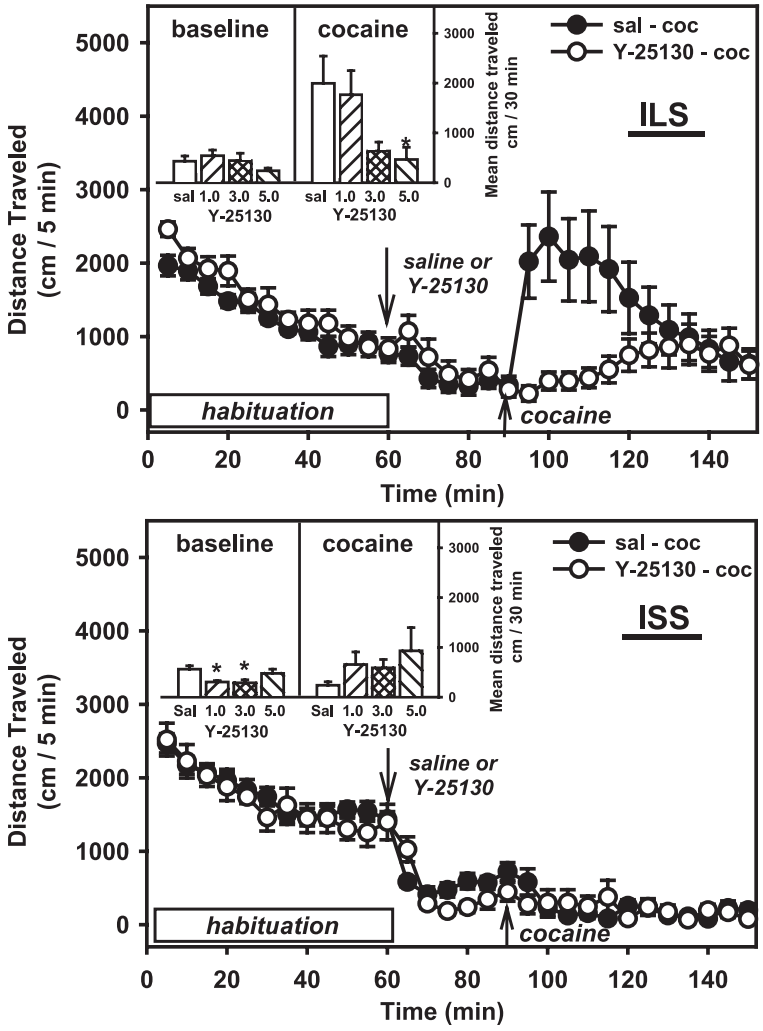

Fig. 5. Time–response for the effects of pretreatment with either saline or the 5-HT_3 receptor antagonist Y-25130 (3 mg/kg) on cocaine (10 mg/kg)-stimulated locomotor activity in ILS (top) and ISS (bottom) mice (n=8–14 mice per group). See Fig. 1 for methodological details. Insets: Mean values±S.E.M. for the effects of Y-25130 (1, 3 or 5 mg/kg) on baseline and cocaine-induced locomotor activity of ILS and ISS mice (see Fig. 1 for details). ANOVA analysis found a significant effect of Y-25130 on baseline locomotor activity only in ISS mice at the 1- and 3-mg/kg doses (*P<0.05; bottom). The higher doses of Y-25130 (3 or 5 mg/kg) attenuated cocaine-induced locomotor activity in ILS mice, but this effect reached significance only at the highest dose tested (5 mg/kg; *P<0.05; top).

dopamine and/or 5-HT following cocaine administration. Thus, ILS mice are highly activated by cocaine, and this activation is blocked by dopamine D1, 5-HT_{2A} or 5-HT_3 receptor antagonists. In contrast, ISS mice show cocaine-induced activation only after antagonism of SERT or 5-HT_{1A} receptors, both of which should increase extracellular 5-HT levels; and this activation is reduced by dopamine D1 receptor blockade.

Cocaine is an indirect acting dopamine receptor agonist (McCreary and Marsden, 1993), and stimulation of both dopamine D1 and D2 receptors is required to increase locomotor activity of rodents (Braun and Chase, 1986; Dreher and Jackson, 1989). However, antagonist studies show that the dopamine D1 receptor clearly plays a pivotal role (Cabib et al., 1991; Kita et al., 1999; McCreary and Marsden, 1993; Ushijama et al., 1995). Furthermore, Xu et al. (1994) found that cocaine failed to stimulate locomotor activity in mice lacking dopamine D1 receptors. In agreement with these studies, we found that the dopamine D1 receptor antagonist SCH 23390 blocked cocaine-induced locomotor activity of ILS mice. ISS mice, on the other hand, exhibited significant cocaine-induced activation only after inhibition of SERT or 5-HT_{1A} receptors; but again, this activation was reduced by SCH 23390. These data suggest a major role for dopamine D1 receptor activation in cocaine-induced activity in these mice.

A concern is that SCH 23390 also binds to 5-HT receptors. The binding affinity of SCH 23390 to dopamine D1 receptors in vitro is ~0.2 nM, whereas 10-fold higher concentrations of SCH 23390 are required for binding to 5-HT_{2A} and 5-HT_{2C} receptors (Bourne, 2001). SCH 23390 demonstrated agonist activity at 5-HT_{2C} receptors with an EC_{50} of 2.6 nM (Millan et al., 2001); however, in vivo studies using doses that block dopamine D1 receptors failed to show any 5-HT_{2C} agonist-like effects (Dr. M. Millan, personal communication to Dr. A.C. McCreary). In addition, a low dose of SCH 23390 inhibited amphetamine-induced locomotor activity and cocaine-induced sensitization, behaviors involving increased extracellular dopamine concentrations (McCreary and Marsden, 1993). Taken together, these data suggest that the effects of the low dose of SCH 23390 used in the present study are most likely mediated via inhibition of dopamine D1 receptors, as opposed to actions at serotonergic receptors.

Our data with both raclopride and eticlopride showing no dopamine D2 receptor regulation of cocaine-induced activity in ILS mice agree with the findings of Cabib et al. (1991). However, they contradict other reports showing inhibition of cocaine-induced locomotion by dopamine D2 receptor antagonists (Chausmer and Katz, 2001; Ushijama et al., 1995). It is important to note that in these later studies, most of the dopamine D2 receptor antagonists that were administered systemically also inhibited spontaneous locomotor activity, as did raclopride in our studies (Table 1). Thus, the effects of these antagonists on cocaine-induced locomotion could be confounded by the attenuated baseline activity. However, our studies show that eticlopride had no effect on baseline activity and no effect on cocaine-stimulated locomotor activity.

The lack of effect of either dopamine D1 or D2 receptor antagonists on cocaine-induced locomotion in ISS mice is similar to our previous findings with the NMDA receptor antagonist MK-801. We found that neither SCH 23390 nor raclopride attenuated MK-801-induced locomotor activity in ISS mice (Hanania et al., 2002). It is possible that 10 mg/kg cocaine does not potentiate dopamine levels sufficiently to activate either dopamine D1 or D2 receptors in these mice. This could also be attributed to the fact that ISS mice have higher number of DATs and thus, this low dose of cocaine may not sufficiently occupy all the transporters in order to increase synaptic dopamine levels and hence locomotor activity in these mice (Hanania et al., 2004). Therefore, it would be difficult to see any measurable modulation by dopamine D1 or D2 receptor antagonists on such a small response.

In contrast to cocaine by itself, pretreatment of ISS mice with either the SERT inhibitor fluoxetine or with the 5-HT_{1A} receptor antagonist WAY 100635 enhanced their cocaine-induced locomotion to a similar extent to that of cocaine-treated ILS mice. Neither drug further increased cocaine-stimulated activity of ILS mice, suggesting that their 5-HT levels are already maximally elevated following cocaine injection, so that neither drug is able to further enhance 5-HT levels and locomotor activity. The strain differences in the effects of fluoxetine are not likely due to differences in number or function of SERT (Hanania et al., 2002).

The potentiating effects of fluoxetine in ISS mice suggest that cocaine alone did not increase 5-HT levels sufficiently in ISS mice to induce locomotor activation. However, pharmacologically increasing 5-HT levels potentiates the effects of cocaine. The enhanced 5-HT levels could, in turn, increase dopamine levels in the nucleus accumbens (Bubar et al., 2003; Parsons and Justice, 1993) and thereby elevate locomotor activity. The more adequate dopamine transmission in ILS mice permits these mice to be more responsive to cocaine-stimulated locomotion. The reduced dopamine tone in ISS mice can be increased by increased 5-HT neurotransmission. With increased serotonergic tone, ISS mice respond to cocaine more like ILS mice. Alternatively, fluoxetine could bind to SERT, allowing higher concentrations of cocaine to bind to DAT and thereby increasing both dopamine levels and locomotor activity. This notion is supported by our previous findings showing that a higher dose of cocaine (20 mg/kg) increased locomotor activity of ISS mice (Hanania et al., 2004). It is important to point out that increasing 5-HT tone does not always play a stimulatory role in cocaine-mediated behaviors. For example, although fluoxetine has been shown to substitute for the discriminative stimulus properties of cocaine (Callahan and Cunningham, 1997), other reports found that fluoxetine attenuates cocaine self-administration in rats (Glatz et al., 2002; Richardson and Roberts, 1991).

Nonetheless, based on our results, 5-HT appears to play a stimulatory role in cocaine-induced locomotor activation in ISS mice.

Pharmacokinetic mechanisms could also be involved in the potentiating effects of fluoxetine on cocaine-induced activity in ISS mice. Fluoxetine potentiates cocaine- and amphetamine-induced activity in rats depleted of brain 5-HT by decreasing the metabolism of both psychomotor drugs and increasing their availability in the brain (Fletcher et al., 2004; Sills et al., 1999). The fact that SCH 23390 inhibited the fluoxetine-induced potentiation of cocaine-induced activity in ISS mice suggests a dopamine-mediated mechanism for the effects of fluoxetine. Thus, it is unlikely that a pharmacokinetic change by itself underlies the actions of fluoxetine.

WAY 100635 also potentiated cocaine-induced locomotor activity selectively in ISS mice. Similar to the current findings, we previously reported that WAY 100635 potentiated locomotor activity mediated by ethanol in ISS mice, suggesting a stimulatory role for 5-HT in both cocaine- and ethanol-mediated locomotor activity in these mice (Hanania et al., 2002). It is possible that, in these mice, cocaine inhibits SERT and increases extracellular levels of 5-HT, which activate 5-HT_{1A} autoreceptors and decrease firing of the dorsal raphe nucleus neurons (Cunningham and Lakoski, 1990; Evrard et al., 1999). This would lead to a decrease in dopamine and 5-HT efflux in the nucleus accumbens (Yoshimoto and McBride, 1992) and a subsequent reduction in locomotor activation. In the presence of WAY 100635, the cocaine-induced feedback inhibition mediated by 5-HT_{1A} autoreceptors in the dorsal raphe nucleus would be blocked, causing an increase in firing of the dorsal raphe nucleus neurons (Fornal et al., 1996) and a resultant increase in dopamine release in nucleus accumbens and enhanced locomotor activation of ISS mice. The fact that the potentiating effects of WAY 100635 were blocked by SCH 23390 supports the above notion. However, if this were the case, fluoxetine should have similar effects as cocaine on dorsal raphe nucleus and yet it potentiates cocaine-induced locomotor activity. It is likely that the effects of fluoxetine on cocaine-induced activity in ISS mice are mediated through different dopamine and 5-HT receptor systems than those involved in the actions of WAY 100635.

It should be noted that other labs have found that a low dose of WAY 100635 (0.4 mg/kg), which did not alter dopamine levels in nucleus accumbens, decreased cocaine-induced locomotor activity in rats (Carey et al., 2001; Müller et al., 2002), an effect opposite to what we, and others, have found (Herges and Taylor, 1998, 1999). In addition to the obvious species difference, the possibility exists that higher doses of WAY 100635 could have effects on dopamine D2 receptors (Müller et al., 2002). However, since the dopamine D2 receptor antagonist raclopride did not alter the potentiated cocaine-induced locomotor activation caused by 0.5 mg/kg WAY 100635 in ISS mice, it is unlikely that this explains our observations.

Inhibition of 5-HT_{2A} and 5-HT_3 receptors attenuates cocaine-induced locomotion in rodents (Fletcher et al., 2002; Herges and Taylor, 2000; Kankaanpää et al., 2002; McMahon and Cunningham, 2001; McMahon et al., 2001; Reith, 1990; Svingos and Hitzemann, 1992). In this study, ketanserin, M 100907 and Y-25130 inhibited locomotor activity of ILS mice without altering basal activity. McMahon et al. (2001) found that 5-HT_{2A} receptors on ventral tegmental area dopamine neurons regulate cocaine-induced locomotor activity. Microdialysis studies have shown that stimulating 5-HT_{2A} receptors under conditions where dopamine levels are elevated, will further increase dopamine release in nucleus accumbens (De Deurwaerdère and Spampinato, 1999). Furthermore, the effects of Y-25130 could be mediated through inhibition of 5-HT_3 receptors in nucleus accumbens (Herges and Taylor, 2000) and decreasing accumbal dopamine efflux (Kankaanpää et al., 1996, 2002; McNeish et al., 1993). An inhibitory role for the 5-HT_3 receptor antagonist has also been reported in cocaine-induced behavioral sensitization and tolerance (King et al., 1999, 2000). Thus, in ILS mice, the locomotor-stimulating effects of cocaine could be due to increased dopamine and 5-HT levels and subsequent activation of ventral tegmental area 5-HT_{2A} and accumbal 5-HT_3 receptors resulting in a further increase in dopamine release in the nucleus accumbens. Since ISS mice showed no activation with cocaine, which is suggestive of insufficient levels of dopamine and 5-HT, it is not surprising that we saw no 5-HT_{2A} or 5-HT_3 receptor modulation of their behavior. These findings support our previous study showing no 5-HT_2 receptor regulation of either MK-801- or ethanol-induced locomotor activity in ISS mice (Hanania et al., 2002). The inhibitory effects of ketanserin, but not M 100907, on basal locomotor activity of ISS mice might be due to the ability of that drug to also inhibit α_1-adrenergic receptors (Marwood, 1994; Stone et al., 2001). It is unclear why the two lower doses of Y-25130 decreased baseline activity in these mice because 5-HT_3 antagonists have not been shown to affect basal dopamine levels in the nucleus accumbens (Palfreyman et al., 1993) or the firing of dopamine neurons in the ventral tegmental area (Sorensen et al., 1989).

Currently, we would summarize our understanding of the locomotor-stimulant effects of cocaine in ILS and ISS mice as follows: In ILS mice, cocaine inhibits DAT and SERT and increases dopamine and 5-HT neurotransmission, which in turn activates dopamine D1, 5-HT_2 and 5-HT_3 receptors, resulting in enhanced locomotor activity. In ISS mice, cocaine also inhibits DAT and SERT. However, these mice have higher number of DATs that clear extracellular dopamine more quickly (Hanania et al., 2004). Thus, synaptic levels of dopamine and/or 5-HT following cocaine administration may be insufficient to activate the receptors necessary to stimulate locomotor activity. However, pharmacologically enhancing the levels of 5-HT by adminis-

tration of either fluoxetine or WAY 100635 potentiates cocaine-induced locomotor activity, perhaps by increasing dopamine neurotransmission.

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