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Effects of dopamine D₁-like and D₂-like antagonists on cocaine discrimination in muscarinic receptor knockout mice

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Abstract

Muscarinic and dopamine brain systems interact intimately, and muscarinic receptor ligands, like dopamine ligands, can modulate the reinforcing and discriminative stimulus (S^D) effects of cocaine. To enlighten the dopamine/muscarinic interactions as they pertain to the S^D effects of cocaine, we evaluated whether muscarinic M₁, M₂ or M₄ receptors are necessary for dopamine D₁ and/or D₂ antagonist mediated modulation of the S^D effects of cocaine. Knockout mice lacking M₁, M₂, or M₄ receptors, as well as control wild-type mice and outbred Swiss-Webster mice, were trained to discriminate 10 mg/kg cocaine from saline in a food-reinforced drug discrimination procedure. Effects of pretreatments with the dopamine D₁ antagonist SCH 23390 and the dopamine D₂ antagonist eticlopride were evaluated. In intact mice, both SCH 23390 and eticlopride attenuated the cocaine discriminative stimulus effect, as expected. SCH 23390 similarly attenuated the cocaine discriminative stimulus effect in M₁ knockout mice, but not in mice lacking M₂ or M₄ receptors. The effects of eticlopride were comparable in each knockout strain. These findings demonstrate differences in the way that D₁ and D₂ antagonists modulate the S^D effects of cocaine, D₁ modulation being at least partially dependent upon activity at the inhibitory M₂/M₄ muscarinic subtypes, while D₂ modulation appeared independent of these systems.

Keywords

Muscarinic receptors; dopamine receptors; knockout mice; drug discrimination; M₁; M₂; M₄; D₁; D₂

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1. Introduction

Selective stimulation of M₁ or M₄ receptors attenuates cocaine's discriminative stimulus (S^D) effects and reinforcing effects in rats and mice (Thomsen et al. 2010a, 2012, 2014; Dencker et al. 2012). Tests using the M₁/M₄-preferring agonist xanomeline in knockout mice lacking M₁ receptors (M₁^{-/-}), M₄ receptors (M₄^{-/-}), or both receptors, indicated that both subtypes are involved in blocking the S^D effects of cocaine, while other receptors, likely M₃, appear to mediate side effects observed with non-selective ligands (Thomsen et al. 2010a, 2012). The mechanisms and neural pathways mediating these effects are still poorly understood, but likely involve striatal muscarinic receptors, based on brain region-specific injections using non-selective ligands or cholinergic neuron toxins (Hikida et al. 2001; Smith et al. 2004; Mark et al. 2006). Striatal muscarinic and dopamine systems are intimately connected, and M₁ and M₄ receptor stimulation can produce functional dopamine antagonism at the cellular and behavioral levels (reviewed in Di Chiara et al. 1994; Pisani et al. 2007; Exley and Cragg 2008; Oldenburg and Ding 2011). However, muscarinic modulation of cocaine effects differs in several ways from the effects of dopamine antagonists, suggesting distinct mechanisms (e.g., selective M₁ agonists did not affect rates of responding maintained by food, and shifted the cocaine self-administration dose-effect function down rather than to the right; Thomsen et al. 2010a, 2012). To uncover similarities and differences between dopamine antagonist and muscarinic agonist modulation of the S^D effects of cocaine at the behavioral level, we evaluated whether effects of D₁ and D₂ antagonists would be affected by the availability of muscarinic receptors.

Studies using intracranial infusions suggested the nucleus accumbens plays the principal role in the S^D effect of cocaine (Wood and Emmet-Oglesby 1989; Callahan et al. 1994). M₁ and M₄ receptors are abundant in ventral and dorsal striatum, with lower levels of M₂ receptors. All or almost all medium spiny neurons (MSN) express M₁ receptors, M₄ receptors are abundant on the D₁-expressing MSN, but are expressed on less than half of the D₂-expressing MSN, and/or are expressed at much lower levels (Weiner et al. 1990; Bernard et al. 1992; Hersch et al. 1994; Hersch and Levey 1995; Ince et al. 1997; Yan et al. 2001; Narushima et al. 2007). M₁ receptors colocalize with D₁ and D₂ receptors on MSN, M₄ receptors co-localize with D₁ receptors and exert opposing effects on cAMP formation (Ince et al., 1997; Onali and Olanas 2002; Jeon et al. 2010). Acetylcholine output by striatal cholinergic interneurons is modulated through the M₂/M₄ autoreceptors, and by dopamine through D₂ and D₅ receptors (Yan et al. 1997; Rivera et al. 2002; Centonze et al. 2003). Thus, M₁, M₂, and M₄ muscarinic subtypes are all candidates to modulate the S^D effect of cocaine and its attenuation by dopamine receptor antagonists.

Here we tested the D₁ antagonist SCH 23390 and the D₂ antagonist eticlopride in M₁^{-/-} mice, M₂^{-/-} mice and M₄^{-/-} mice trained to discriminate 10 mg/kg cocaine from saline. For comparison, outbred Swiss-Webster mice were also tested with SCH 23390, and were tested previously with eticlopride (Thomsen et al. 2010a).

2. Materials and methods

2.1. Animals

Male Swiss-Webster mice were purchased from Taconic Farms (Germantown, NY); male $M_1^{-/-}$, $M_2^{-/-}$ and $M_4^{-/-}$ knockout mice were provided by J. Wess, and were generated as described previously (Gomez et al. 1999a,b; Miyakawa et al. 2001), backcrossed 11 generations to C57BL/6NTac females, then maintained at Taconic Farms. Age- and sex-matched C57BL/6NTac mice from Taconic Farms were used as wild-type controls. Mice were acquired at 4–8 weeks of age, and were acclimated to the housing facilities for at least 7 days before training began, at no earlier than 6 weeks of age. Mice were group housed up to four per cage under standard laboratory conditions, and testing was conducted during the light phase of the circadian cycle. Water was accessible ad libitum and food (rodent diet 5001; PMI Feeds, Inc., St. Louis, MO) was provided daily after training/testing sessions, 4 g/mouse/day. Various flavored rodent treats, nesting material, and exercise/nesting devices were provided for enrichment. All procedures were approved by the McLean Hospital Institutional Animal Care and Use Committee.

2.2. Training and evaluation in cocaine discrimination

Operant-conditioning chambers and experimental procedures were as previously described (Thomsen et al. 2010a). In brief, each chamber contained two nose-poke holes each equipped with a photocell and a cue light. Mice were trained to discriminate 10 mg/kg cocaine from saline (i.p.), reinforced with Vanilla-flavored Ensure nutrition drink. 30 reinforcers were available per 20-min session. Mice were trained first under a fixed ratio (FR) 1 schedule of reinforcement, then, the FR was gradually increased to FR 10, with increasing pretreatment time spent in the chamber rather than the home cage. Eventually sessions were preceded by the entire 10-min pretreatment period in the chamber, during which all lights were off and responding had no scheduled consequences. Cocaine and saline were presented in pseudorandom order across daily training sessions, typically five days/week, and mice were counterbalanced with cocaine trained on the left or right nose-poke. Stable discrimination was defined as at least 7 of 8 consecutive sessions satisfying the following criteria: 1) 10 reinforcers earned per session, 2) 80% correct responses for the first reinforcer, and 3) 90% correct total responses.

Once criteria were met, mice were tested with saline and 0.32, 1.0, 3.2, 10 and 18 mg/kg cocaine to generate dose-effect functions. Then, eticlopride (0.01–0.56 mg/kg) or SCH 23390 (0.01–0.056 mg/kg) was tested as a pretreatment to cocaine, administered s.c., 10 and 5 min. before cocaine, respectively. Antagonists, their initial dose range, vehicles, route of administration, and pretreatment times were based on previous studies: we have shown that eticlopride and SCH 23390 each can shift the cocaine self-administration dose-effect function to the right in C57BL/6J mice (Caine et al. 2002, 2007), and both drugs decreased cocaine-induced hyperlocomotion or gnawing in mice (Tirelli and Witkin 1995; Chausmer and Katz 2001; Prinssen et al. 2004). Further, benzamide antagonists such as eticlopride were found to more reliably attenuate the S^D effect of cocaine relative to butyrophenone antagonist (e.g., haloperidol, spiperone) in monkeys (Soto and Katz 2013). Cocaine and antagonist doses were tested within subjects, in an order counterbalanced between subjects

(modified Latin square). At least one training session was interspersed between each test session, and tests were only performed when mice satisfied discrimination criteria. In the $M_2^{-/-}$ mice tested, several tests were initially performed in duplicate because the lack of effect of SCH 23390 was unexpected. Although re-tests were consistent, we chose to keep all determinations and average the duplicate data points. In cases when responding was suppressed to fewer than 10 responses, the quantity of behavior was considered insufficient to evaluate the percentage of cocaine-appropriate responses, and the response selection was not included in the data presentation or analysis.

2.3. Drugs

Cocaine hydrochloride was supplied by the National Institute on Drug Abuse (National Institutes of Health, Bethesda, MD). (S)-eticlopride hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO), SCH 23390 hydrochloride, from Tocris (Ellisville, MO). Cocaine and SCH 23390 were dissolved in 0.9% saline. Eticlopride was dissolved in ethanol and diluted to 1% ethanol in sterile water.

2.4. Data analysis

Sessions to discrimination criteria were compared between genotypes using the Logrank test. The percentage of drug-appropriate responding (DAR) for the whole session and total rates of responding (i.e., in both holes) are presented. One- or two-way repeated measures ANOVA were performed with dose of pretreatment drug and/or cocaine as factors, on %DAR and response rate, within each genotype separately. In addition, A_{50} values in cocaine dose-effect functions were also calculated in each genotype, i.e., the dose of cocaine estimated to produce 50% DAR. Doses of antagonist estimated to produce 50% decrease in DAR, and 50% decrease in response rates, were each calculated. A_{50} values were obtained in each mouse by interpolation of the dose-effect curves, and group means and 95% confidence intervals (95%CI) were calculated from log-transformed values. Within each genotype, A_{50} values with vs. without pretreatment were compared by two-tailed paired-sample t-test. Significance level was set at $P<0.05$; statistical software was Stata/SE for Mac.

3. Results

3.1. Dopamine antagonist pretreatments in outbred Swiss-Webster mice

We previously showed that the D_2 antagonist eticlopride can decrease cocaine-appropriate responding in Swiss-Webster mice and shift the cocaine dose-effect function about 4-fold to the right (see Fig. 1, Tables 2 and 3), while also decreasing rates of responding (Thomsen et al. 2010a). Here we tested the D_1 antagonist SCH 23390, 0.01 – 0.1 mg/kg, as a pretreatment to 10 mg/kg cocaine in Swiss-Webster mice (Fig. 1). Unlike eticlopride, SCH 23390 produced a U-shaped curve, with a peak effect at 0.032 mg/kg. Repeated-measures ANOVA on all doses up to 0.056 mg/kg did not show a significant effect, however when only doses in the descending portion of the curve were included, a significant effect of SCH 23390 was revealed [$F(3,18)=2.64$, $P<0.05$]. Rates of responding were also significantly suppressed as a function of SCH 23390 dose [$F(4,24)=10.2$, $P<0.0001$]. Pretreatment with 0.032 mg/kg SCH 23390 produced a rightward shift in the cocaine S^D dose-effect curve

(Fig. 2), with a significant effect of the pretreatment [$F(1,39)=21.7$, $P<0.0001$] and a pretreatment by cocaine interaction [$F(4,39)=2.71$, $P<0.05$]. The calculated average potency of cocaine was increased 7-fold by the SCH 23390 pretreatment (see Table 3). Response rates were decreased by the pretreatment [$F(1,49)=36.9$, $P<0.0001$] regardless of cocaine dose.

3.2 Dopamine D₁ antagonist pretreatment in muscarinic knockout mice

Fig. 3 shows cocaine S^D dose-effect functions for all the wild-type mice and knockout M₁^{-/-} mice, M₂^{-/-} mice and M₄^{-/-} mice included in the present data sets. Table 1 shows the calculated potencies (A₅₀ values) for cocaine in each strain, as well as the number of training sessions before discrimination criteria were met. Logrank analysis showed a significant effect of genotype on sessions to criteria [$\chi^2=15.1$, $P<0.01$]. Consistent with a previous report using a set of subjects partially overlapping with those included here (Thomsen et al. 2012), the M₄^{-/-} mice ($\chi^2=9.30$, $P<0.01$), but not the M₁^{-/-} mice, required more sessions to meet discrimination criteria relative to the wild-type mice. A similar trend was observed for the M₂^{-/-} mice, but this did not meet statistical significance ($P=0.08$). Once criteria were met, cocaine dose-effect functions and calculated potencies did not differ significantly between the wild-type mice and the any of the knockout mice (see Fig. 3 and Table 1).

In the muscarinic receptor wild-type mice and in M₁^{-/-} mice, M₂^{-/-} mice and M₄^{-/-} mice, 0.01 – 0.056 mg/kg SCH 23390 was tested as a pretreatment to 10 mg/kg cocaine (see Fig. 4). The 0.056 mg/kg dose suppressed responding to the point that response selection was only obtained from a few mice; those data points are shown as grey shades, with group sizes, and were not included in statistical analyses. Although SCH 23390 appeared to produce moderate reductions in percent cocaine-appropriate responding across strains, the effect reached statistical significance only in the M₁^{-/-} mice [$F(3,15)=6.40$, $P<0.01$]. The effects on response rates were significant in all the strains ($P<0.05$ to $P<0.01$). Table 2 shows the number of mice in which percent cocaine-appropriate responding was reduced to 50% or lower for each strain; this was the majority of mice only in the M₁^{-/-} mice strain, in agreement with the ANOVA analysis.

Based on the above data, a dose of 0.018 mg/kg SCH 23390 was selected to test the hypothesis that the D₁ antagonist would produce rightward shifts in the cocaine S^D dose-effect function in each strain (see Fig. 5). At this dose, SCH 23390 produced a rightward shift in the cocaine S^D dose-effect curve in the wild-type mice (effect of pretreatment [$F(1,52)=13.2$, $P<0.001$], pretreatment by cocaine interaction [$F(4,52)=3.57$, $P<0.05$]) and in the M₁^{-/-} mice (pretreatment [$F(1,45)=12.6$, $P<0.001$], interaction [$F(4,45)=2.78$, $P<0.05$]). In the M₂^{-/-} mice and in the M₄^{-/-} mice, 0.018 mg/kg SCH 23390 had no significant effect on cocaine discrimination. Shifts in the calculated potency of cocaine as an S^D similarly confirmed a significant effect of SCH 23390 in the wild-type mice and M₁^{-/-} mice (each at least a 3-fold shift), but not in the M₂^{-/-} mice or M₄^{-/-} mice (see Table 3).

Effects on rates of responding did not necessarily parallel the effects (or lack of effects) on cocaine discrimination, although the M₁^{-/-} mice again showed patterns comparable to the wild-type mice, while both M₂^{-/-} mice and M₄^{-/-} mice differed. In the wild-type mice and

the $M_1^{-/-}$ mice, SCH 23390 pretreatment decreased rates of responding when combined with saline or low cocaine doses, but not with the highest cocaine dose(s); ANOVA showed a cocaine by pretreatment interaction in the $M_1^{-/-}$ mice [$F(4,45)=3.64$, $P<0.05$] and trend in the wild-type mice ($P=0.07$), and a significant effect of pretreatment in the wild-type mice [$F(1,54)=12.2$, $P<0.001$]. This same pattern was true for the $M_4^{-/-}$ mice (pretreatment [$F(1,54)=15.4$, $P<0.001$], cocaine by pretreatment interaction [$F(4,54)=4.68$, $P<0.01$]), but reductions in rates were more pronounced than in wild-type mice – contrary to the lack of effect on cocaine discrimination. In the $M_2^{-/-}$ mice, rates of responding were related to cocaine dose only [$F(4,26)=5.86$, $P<0.01$], similar to the lack of effect of SCH 23390 on cocaine discrimination.

3.3 Dopamine D_2 antagonist pretreatment in muscarinic knockout mice

In the muscarinic receptor wild-type mice, $M_1^{-/-}$ mice and $M_2^{-/-}$ mice, 0.01 – 0.32 mg/kg eticlopride was tested as a pretreatment to 10 mg/kg cocaine, and up to 0.56 mg/kg in the $M_4^{-/-}$ mice (see Fig. 6). Eticlopride appeared to decrease both percent cocaine-appropriate responding and response rates in all genotypes, although with considerable between-subjects variation. ANOVA revealed a significant effect of eticlopride dose on percent cocaine-appropriate responding in the wild-type mice [$F(4,19)=4.89$, $P<0.01$] and in the $M_1^{-/-}$ mice [$F(3,15)=8.78$, $P<0.01$]. The effect did not reach significance in the $M_2^{-/-}$ mice, of which only $N=3$ completed testing, or in the $M_4^{-/-}$ mice ($P<0.09$). The effects on rates of responding were significant in all the strains ($P<0.01$ to $P<0.001$). Table 2 shows the dose of eticlopride estimated to produce 50% decrease in cocaine-appropriate responding and in rate, respectively, for each strain.

A dose of 0.032 mg/kg eticlopride was selected to test the hypothesis that the D_2 antagonist would produce a rightward shift in the cocaine S^D dose-effect curve in each strain (see Fig. 7). 2-way ANOVA confirmed this effect in all the strains, with a significant effect of the pretreatment in the wild-type mice [$F(1,62)=8.59$, $P<0.01$], the $M_1^{-/-}$ mice [$F(1,42)=12.7$, $P<0.001$], the $M_2^{-/-}$ mice [$F(1,27)=6.12$, $P<0.05$] and the $M_4^{-/-}$ mice [$F(1,44)=12.2$, $P<0.01$], and a significant pretreatment by cocaine interaction in the wild-type mice [$F(4,62)=3.56$, $P<0.05$], the $M_1^{-/-}$ mice, [$F(4,42)=8.12$, $P=0.0001$], and the $M_2^{-/-}$ mice [$F(4,27)=3.00$, $P<0.05$]. Again, significant shifts in the calculated potency of cocaine as an S^D confirmed the effect of eticlopride in each genotype, about 3-fold in the wild-type mice and $M_2^{-/-}$ mice, and about 5-fold in the $M_1^{-/-}$ mice and $M_4^{-/-}$ mice (see Table 3). Although response rates were related to cocaine dose in most strains (wild-type, [$F(4,63)=8.99$, $P<0.0001$]; $M_2^{-/-}$, [$F(4,27)=5.74$, $P<0.01$]; $M_4^{-/-}$, [$F(4,45)=3.60$, $P<0.05$]), the D_2 pretreatment only produced significant decreases in rates in the wild-type mice [$F(1,63)=5.24$, $P<0.05$].

4. Discussion

We tested the effects of a D_1 receptor antagonist, SCH 23390, and of a D_2 receptor antagonist, eticlopride, on the S^D effect of cocaine in knockout mice lacking M_1 , M_2 or M_4 receptors, relative to controls. All strains acquired cocaine discrimination to criteria, although the $M_4^{-/-}$ mice required longer training to do so relative to wild-type mice, as reported previously (Thomsen et al. 2012). Once acquired, cocaine dose-effect functions

were comparable across the genotypes. The D₂ antagonist attenuated the cocaine S^D effect similarly in each genotype, but the effects of the D₁ antagonist were markedly reduced or absent in the M₂^{-/-} mice and in the M₄^{-/-} mice.

Attenuation of the S^D effects of cocaine by D₁ and D₂ antagonists, including SCH 23390 and eticlopride, has been observed fairly reliably, in non-human primates (Kleven et al. 1988, 1990; Spealman et al. 1991; Vanover et al. 1991; Katz et al. 1999), pigeons (Johanson and Barrett 1993), rats (Barrett and Appel 1989; Witkin et al. 1991; Baker et al. 1993), and mice (Beardsley et al. 2001; Elliott et al. 2003; Katz et al. 2003). Effects of D₂ antagonists were varied, but this may be explained by the different ligands used: the butyrophenone antagonist haloperidol often failed to attenuate the cocaine S^D, while eticlopride and other benzamide antagonists appear to reliably antagonize cocaine's S^D effect (see Soto and Katz 2013 for a direct comparison). In the present investigation, both antagonists produced moderate effects on the S^D effect of the training dose, 10 mg/kg cocaine, up to antagonist doses that virtually eliminated responding. When tested against a range of cocaine doses, both antagonists produced at least a 3-fold shift to the right in the cocaine dose-effect curve in the wild-type mice, and a 3 to 7-fold shift in Swiss-Webster mice. While previously reported effects varied, our effect sizes in the intact mouse strains are consistent with the literature.

When tested against 10 mg/kg cocaine, eticlopride attenuated the cocaine S^D effect and decreased rates of responding in the same dose range in the wild-type mice, partially obscuring the S^D effect. This is consistent with our previous reports in Swiss-Webster mice and in C57BL/6 wild-type mice (Thomsen et al. 2010a, 2012). Eticlopride also produced a significant rightward shift in the cocaine S^D dose-effect curve in each genotype. Thus, D₂ receptor antagonist-mediated modulation of the S^D effect of cocaine is not dependent upon M₁, M₂, or M₄ receptors – if anything, effects appeared larger in mice lacking M₁ or M₄ receptors. Though there are few reports on the effects of D₂ antagonists in muscarinic receptor knockout mice, effects generally appear to be preserved. Haloperidol decreased locomotor activity in M₁^{-/-} mice and wild-type mice with comparable dose effect functions (Gerber et al. 2001), and we previously reported that haloperidol increased prepulse inhibition of the startle reflex in mice lacking both M₁ and M₄ receptors (Thomsen et al. 2010b). However, cataleptogenic effects of haloperidol and other typical antipsychotics were attenuated in M₄^{-/-} mice and in D₁-neuron-specific M₄^{-/-} mice (Jeon et al. 2010; Fink-Jensen et al. 2011; but see Karasawa et al. 2003).

SCH 23390 also decreased percent cocaine-appropriate responding and rates of responding in the same dose range in intact mice, when tested against 10 mg/kg cocaine, though effects on cocaine discrimination were smaller than for eticlopride. Unlike eticlopride, which produced monophasic dose-effect curves, SCH 23390 produced U-shaped curves, at least in the subjects that tolerated high doses. Similar dose-response relationships were apparent with SCH 23390 in rats (Barrett and Appel 1989; Witkin et al. 1991; Baker et al. 1993). This biphasic curve, with peak effects occurring at different doses across subjects, accounted largely for the smaller apparent effect size of SCH 23390, because a comparable proportion of Swiss-Webster and wild-type mice showed an effect of the pretreatment with both antagonists, and group means of individual peak effects were similar for eticlopride and

SCH 23390: $19 \pm 9\%$ vs. $24 \pm 9\%$ cocaine-appropriate responding in the Swiss-Webster mice, and $57 \pm 11\%$ vs. $59 \pm 11\%$ in the wild-type mice, respectively. Intriguingly, this biphasic profile of the D_1 antagonist effect is similar to the attenuation of cocaine S^D effect we have typically observed with muscarinic M_1 or M_4 receptor stimulation (Thomsen et al. 2010a, 2012, and manuscript in preparation). A different effect profile was apparent in both the $M_2^{-/-}$ mice and the $M_4^{-/-}$ mice, in which a minority of subjects showed a decreased S^D effect of cocaine, but always at the highest dose of SCH 23390 that did not eliminate responding. SCH 23390 produced a significant rightward shift in the cocaine S^D dose-effect curve in the Swiss-Webster mice, the wild-type mice, and the $M_1^{-/-}$ mice, but had no effect in the $M_2^{-/-}$ mice or the $M_4^{-/-}$ mice. This lack of effect at lower cocaine doses in the $M_2^{-/-}$ mice or $M_4^{-/-}$ mice, together with the fact that effects were observed only at doses of SCH 23390 that strongly affected rates of responding, may suggest a masking effect rather than antagonism of the cocaine S^D effect in the $M_2^{-/-}$ and $M_4^{-/-}$ Mice. In any case, effects of SCH 23390 were, if not completely absent, then very diminished in both $M_2^{-/-}$ mice and $M_4^{-/-}$ mice, indicating an interaction between D_1 - and M_2/M_4 circuits involved in the S^D effects of cocaine.

This lack of D_1 antagonist modulation of cocaine's S^D effects in the $M_4^{-/-}$ mice does not necessarily extend across behavioral endpoints. Here, the $M_4^{-/-}$ mice showed a greater effect of SCH 23390 on rates of responding relative to wild-type mice, and SCH 23390 was previously shown to decrease spontaneous locomotor activity in $M_4^{-/-}$ mice (Gomez et al. 1999a). Such effects on general activity may be centrally or peripherally mediated. In contrast, our findings are in agreement with other endpoints that likely involve striatal functions showing a D_1 /muscarinic (but not D_2 /muscarinic) connection. For instance, a D_1 antagonist but not a D_2 antagonist attenuated muscarinic antagonist-induced stereotypies in rats (Fritts et al. 1998). The muscarinic antagonist scopolamine potentiated behavioral effects of systemic or intrastratial D_1 , but not D_2 agonist administration in rats (Bordi and Meller 1989; Morelli et al. 1993). In mouse striatal slices, the non-selective muscarinic agonist oxotremorine increased DARP-32 phosphorylation in D_1 -expressing striatonigral but not in D_2 -expressing striatopallidal medium spiny neurons (Kuroiwa et al. 2012). As opposed to the D_1 antagonist effects, D_1 agonist effects on locomotor activity were exaggerated in $M_4^{-/-}$ mice relative to wild-type (Gomez et al. 1999a). In medium spiny neurons, postsynaptic M_4 receptors are relatively restricted to the striatonigral population, in which they inhibit excitability, and D_1 and M_4 receptor activation produce opposing effects at the cellular level, (Onali and Olanas 2002; Jeon et al. 2010; Oldenburg & Ding 2011, but see Hernandez- Flores et al. 2015 for a more complex picture). Further, stimulation of pre-synaptic striatal M_4 receptors decreases acetylcholine release by cholinergic interneurons, thereby modulating nicotinic receptor-mediated stimulation of dopamine release (Threlfell et al. 2010, 2012). Finally, M_4 receptors in midbrain and in tegmental nuclei are also thought to regulate striatal dopamine release (Tzavara et al. 2004). Conceivably, $M_4^{-/-}$ mice, without the balancing inhibition by tonic endogenous M_4 receptor stimulation, have a hyperactive striatonigral pathway. This would be consistent with the mild hyperactivity, supersensitivity to D_1 agonist effects, and resistance to D_1 antagonist effects. Indeed, both body-wide and D_1 cell-specific $M_4^{-/-}$ mice have shown elevated levels of extracellular striatal dopamine under some conditions, exaggerated increases in extracellular striatal

dopamine after administration of cocaine or amphetamine, and increased behavioral responses to cocaine (Tzavara et al. 2004; Jeon et al. 2010; Schmidt et al. 2011).

The $M_2^{-/-}$ mice showed no effect of SCH 23390 on either S^D or rates of responding. Because both M_2 and M_4 subtypes function as inhibitory presynaptic receptors, including autoreceptors, it is tempting to draw a parallel between this function and the observed phenotype. M_2 receptors serve as autoreceptors throughout the brain, while M_4 receptors appear to serve this function only in the striatum, which might account for the more general lack of effects (including on rates of responding) in the $M_2^{-/-}$ mice compared to the $M_4^{-/-}$ mice (Hersch and Levey 1995; Zhang et al. 2002; Bonsi et al. 2008). Those interpretations are speculative, the $M_2^{-/-}$ mice having been studied very little with respect to dopamine pathways, compared to $M_4^{-/-}$ mice, and M_2 /dopamine interactions more generally are not well documented. M_2 -receptors are expressed on striatal cholinergic interneurons, which also express D_1 -family receptors, but not on medium spiny neurons (Weiner et al. 1990; Bernard et al. 1998; Smiley et al. 1999). Muscarinic agonist-induced tremors, a preclinical Parkinson's disease model that is responsive to L-DOPA pretreatment, is also absent in $M_2^{-/-}$ mice, further in agreement with an M_2 /dopamine systems interaction (Gomez et al. 1999b).

The use of constitutive knockout mice carries the caveat that compensatory changes may mask effects of the targeted gene deletion, or produce unforeseen additional effects. Compensatory changes in expression levels of the other muscarinic receptor subtypes were not detected in $M_1^{-/-}$, $M_2^{-/-}$, or $M_4^{-/-}$ mice (Gomez et al. 1999a,b; Miyakawa et al. 2001). D_1 (SCH 23390) and D_2 (spiperone or raclopride) binding were not significantly altered in striatal tissues, cortex, olfactory tubercle or ventral tegmental area/substantia nigra from $M_4^{-/-}$ mice (Gomez et al. 1999a; Schmidt et al. 2011), but possible compensatory mechanisms in $M_2^{-/-}$ mice are uncertain.

5. Conclusion

The present findings demonstrate differences in the way that D_1 and D_2 antagonists modulate the S^D effects of cocaine, with only the former appearing dependent upon intact inhibitory M_2 and M_4 muscarinic receptors. Further, while we have shown that M_1 selective agonists can attenuate the cocaine S^D effect in mice (an effect which was absent in $M_1^{-/-}$ mice), we found no attenuation of either D_1 or D_2 receptor antagonist effects in the $M_1^{-/-}$ mice in the present investigation (Thomsen et al. 2010a, 2012). This suggests different mechanisms of action are involved between the D_1 -, D_2 -, M_1 -, and M_4 -mediated modulation of cocaine's S^D effects. Because dopamine antagonist approaches have been unsuccessful in treating psychostimulant abuse (Rothman and Glowa 1995; Haney et al. 2001, 2011), muscarinic agonist approaches are more likely to be useful if they do not act simply as functional dopamine antagonists.

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Fig. 1.

Effects of SCH 23390 and eticlopride pretreatment on the cocaine S^D effect in SW mice 10 mg/kg cocaine was tested with a range of SCH 23390 doses (N=7); effects of eticlopride are included for comparison (N=9, reprinted permission pending). Abscissae: antagonist dose in mg/kg; ordinates: percentage cocaine-appropriate responses (top panels), response rates in responses / s (bottom panels). "V" indicates vehicle. Data are groups means $\pm$ one S.E.M. Numbers next to data points indicates group size where it differs from the overall (due to suppression of behavior).

Fig. 2.

Effect of 0.032 mg/kg SCH 23390 pretreatment on the cocaine S^D effect in SW mice SCH 23390 produced a rightward shift in the cocaine dose-effect function ($N=6$). Abscissae: cocaine dose in mg/kg; other details as in Fig. 1.

Fig. 3.

Cocaine discrimination in muscarinic receptor knockout mice Cocaine dose-effect functions in wild-type mice (N=12), M1^{-/-} mice (N=10), M2^{-/-} mice (N=4), and M4^{-/-} mice (N=10) trained to discriminate 10 mg/kg cocaine from saline. Data represent the group means of all mice included in the investigation. Abscissae: cocaine dose in mg/kg, other details as in Fig. 1.

Fig. 4.

Dose-effect functions of SCH 23390 Effect of SCH 23390 pretreatment on the cocaine S^D effect in wild-type mice (N=8), $M_1^{-/-}$ mice (N=6), $M_2^{-/-}$ mice (N=4), and $M_4^{-/-}$ mice (N=7); doses of SCH 23390 were given as pretreatment to 10 mg/kg cocaine. Abscissae: SCH 23390 dose in mg/kg, other details as in Fig. 1.

Fig. 5. SCH 23390-induced shifts in the cocaine dose-effect function Pretreatment with 0.018 mg/kg SCH 23390 produced a rightward shift in the cocaine dose-effect function in wild-type mice (N=7) and $M_1^{-/-}$ mice (N=6), but not in $M_2^{-/-}$ mice (N=4) or $M_4^{-/-}$ mice (N=7). Abscissae: cocaine dose in mg/kg, other details as in Fig. 1.

Fig. 6. Dose-effect functions of eticlopride Effects of eticlopride pretreatment on the cocaine S^D effect in wild-type mice (N=7), $M1^{-/-}$ mice (N=6), $M2^{-/-}$ mice (N=3), and $M4^{-/-}$ mice (N=6); doses of eticlopride were given as pretreatment to 10 mg/kg cocaine. Abscissae: eticlopride dose in mg/kg, other details as in Fig. 1.

Fig. 7.

Eticlopride-induced shifts in the cocaine dose-effect function Pretreatment with 0.032 mg/kg eticlopride produced a rightward shift in the cocaine dose-effect function in all the genotypes. Group sizes: wild-type, N=8; $M_1^{-/-}$ and $M_4^{-/-}$, N=6; $M_2^{-/-}$, N=4. Abscissae: cocaine dose in mg/kg, other details as in Fig. 1.

Table 1

Cocaine discrimination in muscarinic knockout mice Baseline cocaine discriminative stimulus dose-effect functions in muscarinic receptor wild-type C57BL/6 mice, $M_1^{-/-}$ mice, $M_2^{-/-}$ mice and $M_4^{-/-}$ mice, and sessions needed to meet criteria.

	A ₅₀ DAR	Sessions to criteria	N
Wild-type	1.74 [1.21 – 2.51]	81.8 ± 5.4	12
$M_1^{-/-}$	1.64 [0.98 – 2.75]	84.2 ± 6.0	10
$M_2^{-/-}$	2.88 [1.72 – 4.48]	103.8 ± 7.4	4
$M_4^{-/-}$	1.50 [0.83 – 2.72]	123.6 ± 13.8 ^a	10

The dose of cocaine estimated to produce 50% DAR did not differ significantly in any of the knockout strains relative to wild-type. Reported as group mean [95% confidence interval] of values calculated in each mouse. The $M_4^{-/-}$ mice required longer training to meet discrimination criteria.

^a $P < 0.01$ relative to wild-type, Logrank test. N indicates group size, all mice included in this study.

Table 2

Dopamine antagonist-induced decreases in %cocaine-appropriate responding and in response rates

	A ₅₀ DAR reduction	N/N (DAR)	A ₅₀ rate reduction	N/N (rate)
SCH23390				
Swiss-Webster	0.028 [0.016 – 0.048]	6/7	0.030 [0.025 – 0.036]	7/7
Wild-type	0.014 [0.008 – 0.025]	3/8	0.018 [0.010 – 0.031]	8/8
M ₁ ^{-/-}	0.018 [0.013 – 0.024]	4/6	0.028 [0.024 – 0.033]	6/6
M ₂ ^{-/-}	0.024	1/4	0.021 [0.017 – 0.026]	4/4
M ₄ ^{-/-}	0.014 [0.008 – 0.025]	3/7	0.025 [0.018 – 0.034]	7/7
Eticlopride				
Swiss-Webster ^a	0.06 [0.03 – 0.15]	7/9	0.11 [0.07 – 0.18]	9/9
Wild-type	0.08 [0.02 – 0.38]	3/7	0.03 [0.01 – 0.08]	7/7
M ₁ ^{-/-}	0.10 [0.06 – 0.16]	3/6	0.08 [0.04 – 0.16]	6/6
M ₂ ^{-/-}	0.05 [0.04 – 0.06]	2/3	0.09 [0.05 – 0.19]	3/3
M ₄ ^{-/-}	0.05 [0.02 – 0.11]	5/6	0.11 [0.07 – 0.18]	6/6

The dose of each antagonist estimated to produce 50% reduction in DAR, and the dose estimated to produce 50% reduction in response rate (relative to vehicle) when tested against 10 mg/kg cocaine. Reported as group mean [95% confidence interval] of values calculated in each mouse. N/N indicates the number of mice showing 50% reduction in DAR and 50% reduction in response rates, respectively, over number of mice tested (the first number thus represents the number of mice from which A₅₀ values were calculated).

^aData included in Thomsen et al. 2010a, re-analyzed with current criteria (e.g., using conservative estimates to allow the inclusion of mice for which A₅₀ doses fell outside the range of cocaine doses tested).

Table 3

Dopamine antagonist-induced shifts in cocaine dose-effect functions

	A ₅₀ cocaine alone	A ₅₀ combination	N
SCH23390			
Swiss-Webster	1.29 [0.59 – 2.84]	9.61 [5.93 – 15.6] ^b	6
Wild-type	2.26 [1.68 – 3.03]	8.17 [5.57 – 12.0] ^d	7
M ₁ ^{-/-}	1.96 [0.84 – 4.59]	6.27 [4.67 – 8.42] ^b	6
M ₂ ^{-/-}	2.24 [1.24 – 4.05]	2.39 [1.40 – 4.08]	4
M ₄ ^{-/-}	1.97 [0.83 – 4.70]	2.61 [1.22 – 5.58]	6
Eticlopride			
Swiss-Webster ^a	1.00 [0.60 – 1.66]	3.85 [1.75 – 8.45] ^b	9
Wild-type	1.56 [0.93 – 2.60]	4.71 [2.53 – 8.77] ^c	8
M ₁ ^{-/-}	1.09 [0.55 – 2.16]	5.72 [5.60 – 5.84] ^b	5
M ₂ ^{-/-}	2.24 [1.24 – 4.05]	7.33 [4.69 – 11.5] ^b	3
M ₄ ^{-/-}	1.39 [0.58 – 3.30]	6.86 [3.29 – 14.3] ^b	6

^aData included in Thomsen et al. 2010a, re-analyzed with current criteria (e.g., using conservative estimates to allow the inclusion of mice for which A₅₀ doses fell outside the range of cocaine doses tested).

^b*P* < 0.05,

^c*P* < 0.01,

^d*P* < 0.001 paired-sample two-tailed test vs. cocaine alone A₅₀, calculated on log values.