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ORIGINAL INVESTIGATION

Amphetamine and cocaine suppress social play behavior in rats through distinct mechanisms

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Abstract
Rationale Social play behavior is a characteristic form of social behavior displayed by juvenile and adolescent mammals. This social play behavior is highly rewarding and of major importance for social and cognitive development. Social play is known to be modulated by neurotransmitter systems involved in reward and motivation. Interestingly, psychostimulant drugs, such as amphetamine and cocaine, profoundly suppress social play, but the neural mechanisms underlying these effects remain to be elucidated.
Objective In this study, we investigated the pharmacological underpinnings of amphetamine- and cocaine-induced suppression of social play behavior in rats.
Results The play-suppressant effects of amphetamine were antagonized by the alpha-2 adrenoreceptor antagonist RX821002 but not by the dopamine receptor antagonist alpha-flupenthixol. Remarkably, the effects of cocaine on social play were not antagonized by alpha-2 noradrenergic, dopaminergic, or serotonergic receptor antagonists, administered either alone or in combination. The effects of a subeffective dose of cocaine were enhanced by a combination of subeffective doses of the serotonin reuptake inhibitor fluoxetine, the dopamine reuptake inhibitor GBR12909, and the noradrenaline reuptake inhibitor atomoxetine.
Conclusions Amphetamine, like methylphenidate, exerts its play-suppressant effect through alpha-2 noradrenergic receptors. On the other hand, cocaine reduces social play by simultaneous increases in dopamine, noradrenaline, and serotonin neurotransmission. In conclusion, psychostimulant drugs with different pharmacological profiles suppress social play behavior through distinct mechanisms. These data contribute to our understanding of the neural mechanisms of social behavior during an important developmental period, and of the deleterious effects of psychostimulant exposure thereon.

Keywords Social play · Adolescence · Amphetamine · Cocaine · Dopamine · Serotonin · Noradrenaline · Alpha-2 adrenoceptor

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Introduction

The young of many mammalian species, including humans, display a characteristic form of social interaction known as social play behavior or rough-and-tumble play (Panksepp et al. 1984; Vanderschuren et al. 1997; Pellis and Pellis 2009). Social play behavior is of major importance for social and cognitive development (Potegal and Einon 1989; Van den Berg et al. 1999; Baarendse et al. 2013). Furthermore, social

play is highly rewarding. It is an incentive for maze learning, operant conditioning, and place conditioning in rats and primates (for reviews, see Vanderschuren 2010; Trezza et al. 2011), and it is modulated through neurotransmitter systems implicated in the positive subjective and motivational effects of food, sex, and drugs of abuse (Trezza et al. 2010; Siviy and Panksepp 2011). However, the underlying neurobiological mechanisms of social play behavior are still incompletely understood.

The abundance of social play behavior is an expression of the marked changes in social behavior that take place during post-weaning development (Spear 2000; Nelson et al. 2005). Interestingly, the increased importance of interactions with peers during this phase of life (i.e., the juvenile and adolescent stages in rodents, roughly equivalent to childhood and adolescence in humans) coincides with other changes in behavior, such as increased risk-taking and experimenting with drugs of abuse (Casey and Jones 2010; Blakemore and Robbins 2012). Especially in the early stages of use, drugs are often experienced in a social setting (Boys et al. 2001; Newcomb and Bentler 1989) because of their presumed ability to facilitate interaction with peers, peer acceptance, and group cohesion. However, drug use can have negative consequences for social behavior (for review, see Young et al. 2011). Therefore, investigating the effects of drugs of abuse on social play behavior serves two purposes. First, it increases our knowledge of the neural substrates of social play behavior. Second, it provides important information about how drugs of abuse affect the quality of social interactions during an important period of social development.

In rodent and primate studies, the psychostimulant drugs amphetamine, methylphenidate, and cocaine have been shown to interfere with various social behaviors (Schiørring 1979; Mizcek and Yoshimura 1982; Beatty et al. 1982, 1984; Thor and Holloway 1983; Sutton and Raskin 1986; Ferguson et al. 2000; Vanderschuren et al. 2008; Liu et al. 2010). In particular, these psychostimulants profoundly decrease social play behavior in adolescent rats, without affecting general social interest (Beatty et al. 1982, 1984; Thor and Holloway 1983; Sutton and Raskin 1986; Ferguson et al. 2000; Vanderschuren et al. 2008). We have previously found that the play-suppressant effects of methylphenidate are mediated by stimulation of alpha-2 adrenoceptors, but that they are independent of dopaminergic neurotransmission (Vanderschuren et al. 2008). However, the mechanisms by which amphetamine and cocaine inhibit social play behavior are unknown (Beatty et al. 1984).

It is well established that amphetamine and cocaine increase the synaptic concentrations of dopamine, noradrenaline, and serotonin (5-HT), by stimulating their release and inhibiting their reuptake, respectively (Heikkila et al. 1975; Ritz and Kuhar 1989; Rothman et al. 2001). In addition, there is recent evidence to suggest that amphetamine and cocaine also facilitate exocytotic dopamine release (Venton et al. 2006; Aragona et al. 2008; Daberkow et al. 2013). The relative effectiveness of amphetamine and cocaine on monoamine neurotransmission differs, however. Whereas amphetamine preferentially enhances noradrenaline and dopamine neurotransmission, cocaine most profoundly inhibits the reuptake of 5-HT and dopamine (Ritz and Kuhar 1989; Rothman et al. 2001). Therefore, we investigated the pharmacological mechanisms through which amphetamine and cocaine reduce social play behavior in rats. On the basis of our previous findings (Vanderschuren et al. 2008), and the pharmacological profiles of amphetamine and cocaine, we hypothesized that amphetamine suppresses social play through stimulation of alpha-2 adrenoceptors, but that the effect of cocaine on social play relies on dopamine and/or 5-HT mechanisms.

Materials and methods

Animals

Male Wistar rats (Charles River, Sulzfeld, Germany) arrived in our animal facility at 21 days of age and were housed in groups of four in 40×26×20 cm ($l \times w \times h$) Macrolon cages under controlled conditions (temperature 20–21 °C, 55±15 % relative humidity, and 12/12-h light cycle with lights on at 0700 hours). Food and water were available ad libitum. All animals were experimentally naive and were used only once (i.e., different groups of rats were used for each experiment). All experiments were approved by the Animal Ethics Committee of Utrecht University and were conducted in agreement with Dutch laws (Wet op de Dierproeven 1996) and European regulations (Guideline 86/609/EEC).

Drugs

(+)-Amphetamine sulfate (0.05–0.5 mg/kg, s.c.) was obtained from O.P.G. (Utrecht, The Netherlands). Cocaine hydrochloride (0.5–7.5 mg/kg, s.c.), the dopamine receptor antagonist alpha-flupenthixol dihydrochloride (0.125 mg/kg, i.p.), the 5-HT1A receptor antagonist WAY100635 maleate (0.1 mg/kg, s.c.), and the 5-HT2A receptor antagonist M100907 (0.2 mg/kg, s.c.) were obtained from Sigma-Aldrich (Schnelldorf, Germany). The alpha-2 adrenoreceptor antagonist RX821002 hydrochloride (0.2 mg/kg i.p.), the 5-HT2 receptor antagonist amperozide hydrochloride (0.5 mg/kg, i.p.), the 5-HT1/2 receptor antagonist methysergide maleate (2.0 mg/kg, s.c.), the 5-HT3 receptor antagonist ondansetron hydrochloride (2.0 mg/kg, i.p.), the 5-HT reuptake inhibitor fluoxetine hydrochloride (1–3 mg/kg, s.c.), the dopamine reuptake inhibitor GBR12909 dihydrochloride (3 mg/kg, s.c.), and the noradrenaline reuptake inhibitor atomoxetine hydrochloride (0.1–0.3 mg/kg, i.p.) were obtained from Tocris Bioscience (Avonmouth, UK). All drugs were dissolved in saline, except for GBR12909 which was dissolved in sterile water and

M100907 which was dissolved in saline containing 10 % Tween 80 (Sigma-Aldrich, Schnelldorf, Germany). Amphetamine and cocaine were injected 30 min before the test. The antagonists were administered 30 min before amphetamine or cocaine except for RX821002, which was administered 15 min before amphetamine and cocaine. The reuptake inhibitors were injected 30 min before the test.

We used doses of dopamine-, 5-HT-, and noradrenaline receptor antagonists and reuptake inhibitors that had no effect on social play by themselves (Homberg et al. 2007; Trezza and Vanderschuren 2008b; Vanderschuren et al. 2008). Drug doses and pretreatment intervals were based on our previous work, literature, and pilot experiments. Solutions were freshly prepared on the day of the experiment and administered in a volume of 2 ml/kg. When an experiment involved a combination of antagonists or reuptake inhibitors, the different compounds were dissolved and injected separately to prevent interaction of two or more drugs in the same solution. Because of the importance of the neck area in the expression of social play behavior (Pellis and Pellis 1987; Siviy and Panksepp 1987), subcutaneous injections were administered in the flank.

Procedures

All behavioral procedures were conducted as previously described (Vanderschuren et al. 2008; Trezza et al. 2008a). Briefly, the experiments were performed in a sound-attenuated chamber under dim light conditions. The testing arena consisted of a Plexiglas cage measuring 40×40×60 cm ($l \times w \times h$), with approximately 2 cm of wood shavings covering the floor. At 26–28 days of age, rats were individually habituated to the test cage for 10 min on each of the 2 days before testing. On the test day, the animals were socially isolated for 3.5 h before testing, to enhance their social motivation and thus facilitate the expression of social play behavior during testing. This isolation period has been shown to induce a half-maximal increase in the amount of social play behavior (Niesink and Van Ree 1989; Vanderschuren et al. 1995a, 2008). At the appropriate time before testing, pairs of animals were treated with drugs or vehicle. The test consisted of placing two animals into the test cage for 15 min. The animals in a pair did not differ more than 10 g in body weight. Since dominance status has a profound influence on the intensity and structure of social play (Pellis et al. 1997), and drug effects can be different in dominant versus subordinate animals (e.g., Panksepp et al. 1985; Knutson et al. 1996), animals in a test pair had no previous common social experience (i.e., they were not cage mates), to minimize the influence of dominance/subordination relationships on social play and the effects of drugs thereon. The behavior of the animals was videotaped, and analysis from the video tape recordings was performed afterwards by an observer blind to treatment. Behavior was assessed per pair of animals using Observer 3.0 software (Noldus Information Technology BV, Wageningen, The Netherlands).

In rats, a bout of social play behavior starts with one rat soliciting ("pouncing") another animal, by attempting to nose or rub the nape of its neck. The animal that is pounced upon can respond in different ways: if the animal fully rotates to its dorsal surface, "pinning" is the result, i.e., one animal lying with its dorsal surface on the floor with the other animal standing over it. From this position, the supine animal can initiate another play bout, by trying to gain access to the other animal's neck. Thus, during social play, pouncing is considered an index of play solicitation, while pinning functions as a releaser of a prolonged play bout (Panksepp and Beatty 1980; Pellis and Pellis 1987; Poole and Fish 1975). Pinning and pouncing frequencies can be easily quantified and are considered the most characteristic parameters of social play behavior in rats (Panksepp and Beatty 1980; Trezza et al. 2010). During the social encounter, animals may also display social behaviors not directly associated with play, such as sniffing or grooming the partner's body (Panksepp and Beatty 1980; Vanderschuren et al. 1995a, b). Since social play behavior in rats strongly depends on the playfulness of its partner (Pellis and McKenna 1992; Trezza and Vanderschuren 2008a), both animals in a play pair received the same drug treatment, and a pair of animals was considered as one experimental unit. The following parameters were therefore scored per pair of animals:

- Social behaviors related to play:
  - Frequency of pinning
  - Frequency of pouncing
- Social behaviors unrelated to play:
  - Time spent in social exploration: the total amount of time spent in non-playful forms of social interaction (i.e., one animal sniffing or grooming any part of the partner's body).

Statistical analysis

Data are expressed as mean ± SEM. To assess the effects of single or combined treatments on social play behavior, data were analyzed using one- or two-way ANOVA. ANOVAs were followed by Tukey's post-hoc test, where appropriate.

Results

The play-suppressant effects of amphetamine are mediated by activation of alpha-2 noradrenergic but not dopamine receptors

Amphetamine (amph; 0.2 and 0.5 mg/kg) significantly reduced pinning and pouncing, with no effect on social

exploration [pinning: $F(\text{amph})_{3,28}=16.58$, $p<0.001$; pouncing: $F(\text{amph})_{3,28}=23.12$, $p<0.001$; social exploration: $F(\text{amph})_{3,28}=0.53$, NS, Fig. 1a–c]. We previously found that the reduction in social play behavior induced by treatment with methylphenidate was prevented by pretreatment with the alpha-2 adrenoceptor antagonist RX821002, but not the dopamine receptor antagonist alpha-flupenthixol (Vanderschuren et al. 2008). Therefore, we investigated whether RX821002 and alpha-flupenthixol altered the effect of the lowest effective dose of amphetamine (0.2 mg/kg) on social play. Pretreatment with RX821002 (0.2 mg/kg) blocked the effects of amphetamine on social play behavior (Fig. 1d, e). After saline pretreatment, amphetamine significantly reduced pinning and pouncing frequencies, whereas no significant differences between amphetamine- and vehicle-treated rats were found after pretreatment with RX821002 [pinning: $F(\text{RX})_{1,28}=18.09$, $p<0.001$; $F(\text{amph})_{1,28}=12.72$, $p=0.001$; $F(\text{RX}\times\text{amph})_{1,28}=7.29$, $p=0.01$; pouncing: $F(\text{RX})_{1,28}=5.94$, $p=0.02$; $F(\text{amph})_{1,28}=12.86$, $p=0.001$; $F(\text{RX}\times\text{amph})_{1,28}=23.75$, $p<0.001$]. RX821002 reduced social exploration, but amphetamine did not influence this effect [social exploration: $F(\text{RX})_{1,28}=8.40$, $p=0.01$; $F(\text{amph})_{1,28}=0.36$, NS; $F(\text{RX}\times\text{amph})_{1,28}=0.21$, NS; Fig. 1f]. Pretreatment with alpha-flupenthixol (flup; 0.125 mg/kg) did not affect the reduction in pinning and pouncing induced by amphetamine [pinning: $F(\text{flup})_{1,20}=0.42$, NS; $F(\text{amph})_{1,20}=38.57$, $p<0.001$; $F(\text{flup}\times\text{amph})_{1,20}=0.22$, NS; pouncing: $F(\text{flup})_{1,20}=0.28$, NS; $F(\text{amph})_{1,20}=30.31$, $p<0.001$; $F(\text{flup}\times\text{amph})_{1,20}=0.01$, NS; Fig. 1g–h]. In this experiment, amphetamine-treated rats spent more time in social exploration than vehicle-treated animals [social exploration: $F(\text{flup})_{1,20}=0.30$, NS; $F(\text{amph})_{1,20}=5.71$, $p=0.03$; $F(\text{flup}\times\text{amph})_{1,20}=0.001$, NS; Fig. 1i].

The play-suppressant effects of cocaine are not blocked by administration of dopamine, noradrenaline, or 5-HT receptor antagonists

Cocaine (5.0–7.5 mg/kg) reduced pinning [$F(\text{coc})_{4,35}=8.91$, $p<0.001$] and pouncing [$F(\text{coc})_{4,35}=10.12$, $p<0.001$; Fig. 2a, b], whereas 2.5 mg/kg cocaine increased social exploration [$F(\text{coc})_{4,35}=5.86$, $p=0.001$; Fig. 2c]. Since pretreatment with the RX821002, but not alpha-flupenthixol, blocked the effects of methylphenidate (Vanderschuren et al. 2008) and amphetamine (above) on social play, we next investigated whether these drugs also altered the effect of cocaine on social play. The reduction in social play induced by the lowest effective dose of cocaine (5.0 mg/kg) was not altered by pretreatment with RX821002 [0.2 mg/kg, pinning: $F(\text{RX})_{1,31}=0.90$, NS; $F(\text{coc})_{1,31}=71.00$, $p<0.001$; $F(\text{RX}\times\text{coc})_{1,31}=0.15$, NS; pouncing: $F(\text{RX})_{1,31}=0.90$, NS; $F(\text{coc})_{1,31}=76.78$, $p<0.001$; $F(\text{RX}\times\text{coc})_{1,31}=0.16$, NS; social exploration: $F(\text{RX})_{1,31}=0.99$, NS; $F(\text{coc})_{1,31}=1.45$, NS; $F(\text{RX}\times\text{coc})_{1,31}=0.04$, NS; Fig. 2d–f] or alpha-flupenthixol [0.125 mg/kg, pinning: $F(\text{flup})_{1,20}=0.26$, NS; $F(\text{coc})_{1,20}=42.11$, $p<0.001$; $F(\text{flup}\times\text{coc})_{1,20}=0.37$, NS; pouncing: $F(\text{flup})_{1,20}=0.45$, NS; $F(\text{coc})_{1,20}=37.66$, $p<0.001$; $F(\text{flup}\times\text{coc})_{1,20}=0.32$, NS; social exploration: $F(\text{flup})_{1,20}=0.82$, NS; $F(\text{coc})_{1,20}=3.42$, NS, $F(\text{flup}\times\text{coc})_{1,20}=0.85$, NS; Fig. 2g–i].

Next, we assessed the involvement of 5-HT receptor stimulation in the play-suppressant effect of cocaine. Neither the 5-HT1/2 receptor antagonist methysergide [mts; 2 mg/kg, pinning: $F(\text{mts})_{1,28}=0.30$, NS; $F(\text{coc})_{1,28}=44.00$, $p<0.001$; $F(\text{mts}\times\text{coc})_{1,28}=0.19$, NS; pouncing: $F(\text{mts})_{1,28}=0.20$, NS; $F(\text{coc})_{1,28}=48.64$, $p<0.001$; $F(\text{mts}\times\text{coc})_{1,28}=0.29$, NS; Fig. 3a, b] nor the 5-HT2 receptor antagonist amperozide [apz; 0.5 mg/kg, pinning: $F(\text{apz})_{1,20}=1.50$, NS; $F(\text{coc})_{1,20}=49.55$, $p<0.001$; $F(\text{apz}\times\text{coc})_{1,20}=0.57$, NS; pouncing: $F(\text{apz})_{1,20}=0.40$, NS; $F(\text{coc})_{1,20}=58.62$, $p<0.001$; $F(\text{apz}\times\text{coc})_{1,20}=0.03$, NS; Fig. 3c, d], the 5-HT3 receptor antagonist ondansetron [ond; 1.0 mg/kg, pinning: $F(\text{ond})_{1,28}=2.04$, NS; $F(\text{coc})_{1,28}=55.59$, $p<0.001$; $F(\text{ond}\times\text{coc})_{1,28}=1.22$, NS; pouncing: $F(\text{ond})_{1,28}=1.27$, NS; $F(\text{coc})_{1,28}=62.68$, $p<0.001$; $F(\text{ond}\times\text{coc})_{1,28}=0.42$, NS; Fig. 3e, f], the 5-HT1A receptor antagonist WAY100365 [way; 1 mg/kg, pinning: $F(\text{way})_{1,28}=3.99$, NS; $F(\text{coc})_{1,28}=68.00$, $p<0.001$; $F(\text{way}\times\text{coc})_{1,28}=3.50$, NS; pouncing: $F(\text{way})_{1,28}=2.66$, NS; $F(\text{coc})_{1,28}=96.05$, $p<0.001$; $F(\text{way}\times\text{coc})_{1,28}=3.08$, NS; Fig. 3g, h], or the 5-HT2A receptor antagonist M100907 (m100; 0.2 mg/kg, Fig. 3i, j) altered the effect of cocaine on social play, with no effect on social exploration (Table 1). M100907 itself reduced pinning [$F(\text{m100})_{1,28}=4.77$, $p=0.04$; $F(\text{coc})_{1,28}=17.26$, $p<0.001$; $F(\text{m100}\times\text{coc})_{1,28}=4.77$, $p=0.04$; Fig. 3i], but not pouncing [$F(\text{m100})_{1,28}=1.98$, NS; $F(\text{coc})_{1,31}=37.71$, $p<0.001$; $F(\text{m100}\times\text{coc})_{1,28}=0.81$, NS; Fig. 3j] or social exploration (Table 1), whereas ondansetron altered social exploration (Table 1).

We then hypothesized that the effect of cocaine is mediated by redundant monoaminergic mechanisms. To test this possibility, we investigated the effect of pretreatment with combinations of two or three monoamine receptor antagonists on the play-suppressant effect of cocaine. Pretreatment with a combination of RX821002 (0.2 mg/kg) and methysergide (2 mg/kg, Fig. 4a, b), a combination of alpha-flupenthixol (0.125 mg/kg) and methysergide (2 mg/kg, Fig. 4c, d), or a combination of RX821002 (0.2 mg/kg), alpha-flupenthixol (0.125 mg/kg), and methysergide (2 mg/kg, Fig. 4e, f) did not affect the reduction in pinning [$F(\text{mts+rx})_{1,25}=0.57$, NS; $F(\text{coc})_{1,25}=16.59$, $p<0.001$; $F(\text{mts+rx}\times\text{coc})_{1,25}=0.12$, NS; $F(\text{flup+mts})_{1,28}=1.39$, NS; $F(\text{coc})_{1,28}=50.56$, $p<0.001$; $F(\text{flup+mts}\times\text{coc})_{1,28}=1.39$, NS; $F(\text{rx+flup+mts})_{1,28}=0.15$, NS; $F(\text{coc})_{1,28}=27.47$, $p<0.001$; $F(\text{rx+flup+mts}\times\text{coc})_{1,28}=0.35$, NS] and pouncing [$F(\text{mts+rx})_{1,25}=0.82$, NS; $F(\text{coc})_{1,25}=17.99$, $p<0.001$; $F(\text{mts+rx}\times\text{coc})_{1,25}=0.14$, NS; $F(\text{flup+mts})_{1,28}=1.37$, NS; $F(\text{coc})_{1,20}=51.51$, $p<0.001$; $F(\text{flup+mts}\times\text{coc})_{1,28}=1.37$, NS; $F(\text{rx+flup+mts})_{1,28}=1.47$, NS; $F(\text{coc})_{1,28}=30.57$, $p<0.001$; $F(\text{rx+flup+mts}\times\text{coc})_{1,28}=$

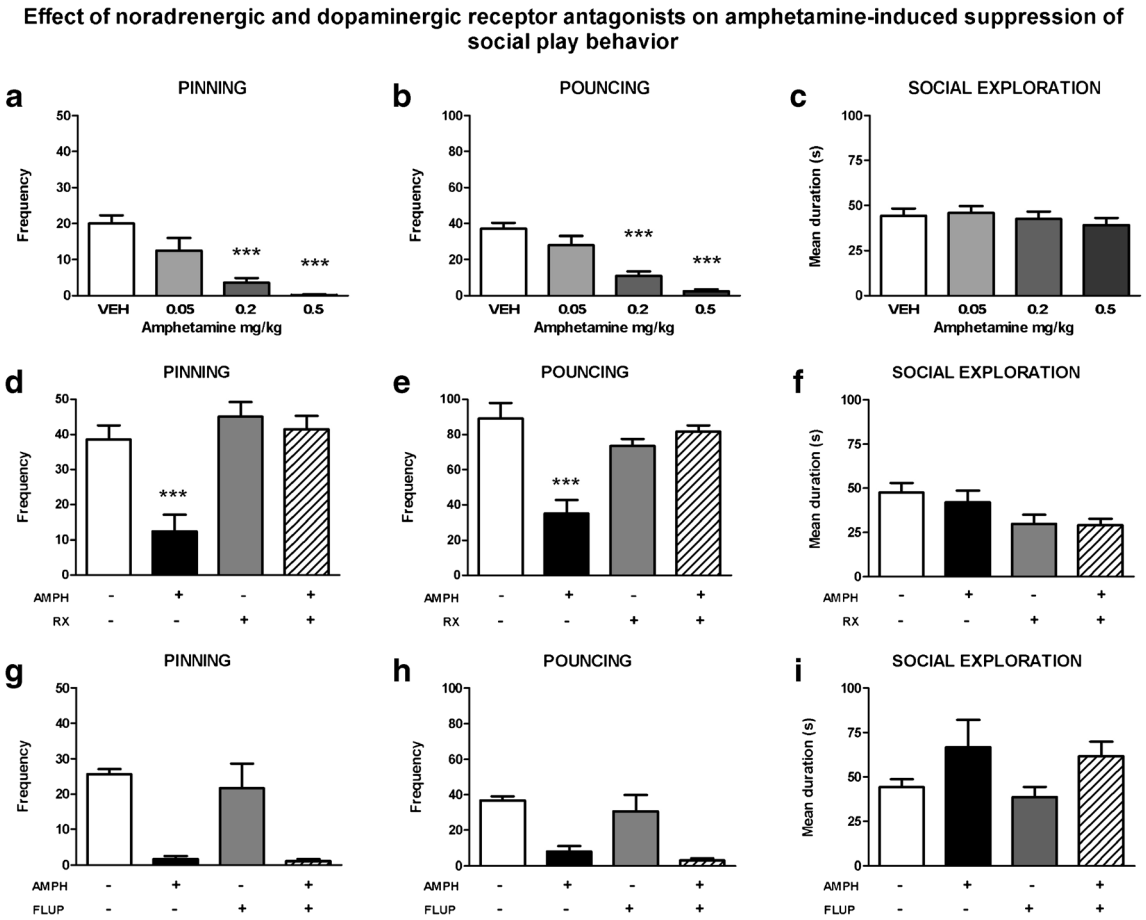


Fig. 1 Effect of noradrenaline and dopamine receptor antagonists on amphetamine-induced suppression of social play behavior. Amphetamine (amph, s.c.) dose-dependently reduced pinning (**a**) and pouncing (**b**) without affecting social exploration (**c**). The effect of amphetamine (0.2 mg/kg) was blocked by the alpha-2 adrenoreceptor antagonist RX821002 (rx, 0.2 mg/kg, i.p.), pinning (**d**), pouncing (**e**), and social exploration (**f**). The effect of amphetamine on pinning (**g**), pouncing (**h**), and social exploration (**i**) was not blocked by the dopamine receptor antagonist alpha-flupenthixol (flup, 0.125 mg/kg, i.p.). *Bars* show the frequency (mean + SEM) of pinning and pouncing and the mean (+ SEM) duration of social exploration (seconds) of the different treatment groups. *Plus sign* indicates couples of animals treated with the test compound; *minus sign* indicates couples treated with the corresponding vehicle. N = 6–8 couples per treatment group. One- or two-way ANOVA with Tukey post-hoc test, ***$p<0.001$, different from vehicle

0.06, NS], induced by cocaine (5.0 mg/kg). These drug combinations did not affect social exploration (Table 1).

The play-suppressant effects of cocaine are mediated by simultaneous blockade of dopamine, noradrenaline, and 5-HT neurotransmission

The data presented in Figs. 2, 3, and 4 did not identify the dopamine, noradrenaline, or 5-HT receptor mechanism through which cocaine exerts its effect on social play. To test whether monoamine reuptake is at all involved in the effect of cocaine, we investigated the effects of combined subeffective doses of cocaine and monoamine reuptake inhibitors on social play. The effect of a subeffective dose of cocaine (0.5 mg/kg) on pinning and pouncing was not changed by treatment with either a subeffective dose of the 5-HT reuptake inhibitor fluoxetine [f3; 3 mg/kg, pinning: $F(\mathrm{f3})_{1,27}=2.44$, NS; $F(\mathrm{coc})_{1,27}=0.04$, NS; $F(\mathrm{f3}\times\mathrm{coc})_{1,28}=0.17$, NS; pouncing: $F(\mathrm{f3})_{1,27}=2.41$, NS; $F(\mathrm{coc})_{1,28}=0.05$, NS; $F(\mathrm{f3}\times\mathrm{coc})_{1,27}=0.11$, NS; Fig. 5a, b] or by a combination of subeffective doses of the 5-HT reuptake inhibitor fluoxetine (3 mg/kg) and the dopamine reuptake inhibitor GBR12909 [g3; 3 mg/kg, pinning: $F(\mathrm{f3+g3})_{1,28}=0.30$, NS; $F(\mathrm{coc})_{1,28}=1.23$, NS; $F(\mathrm{f3+g3}\times\mathrm{coc})_{1,28}=0.09$, NS; pouncing: $F(\mathrm{f3+g3})_{1,28}=0.10$, NS; $F(\mathrm{coc})_{1,28}=0.68$, NS; $F(\mathrm{f3+g3}\times\mathrm{coc})_{1,28}=0.68$, NS; Fig. 5c, d]. Combined administration of fluoxetine, GBR12909, and the noradrenaline reuptake inhibitor atomoxetine (a0.1; 0.1 mg/kg) reduced pinning [$F(\mathrm{f3+g3+a0.1})_{1,26}=20.08$, $p<0.001$; $F(\mathrm{coc})_{1,26}=3.23$, NS] and pouncing [$F(\mathrm{f3+g3+a0.1})_{1,26}=23.72$, $p<0.001$; $F(\mathrm{coc})_{1,26}=2.67$, NS; $F(\mathrm{f3+g3+a0.1}\times\mathrm{coc})_{1,26}=3.51$, NS], and increased social exploration (Table 1). Importantly, a significant interaction between the combination of reuptake inhibitors and a subeffective dose of cocaine was found for pinning [F(f3+

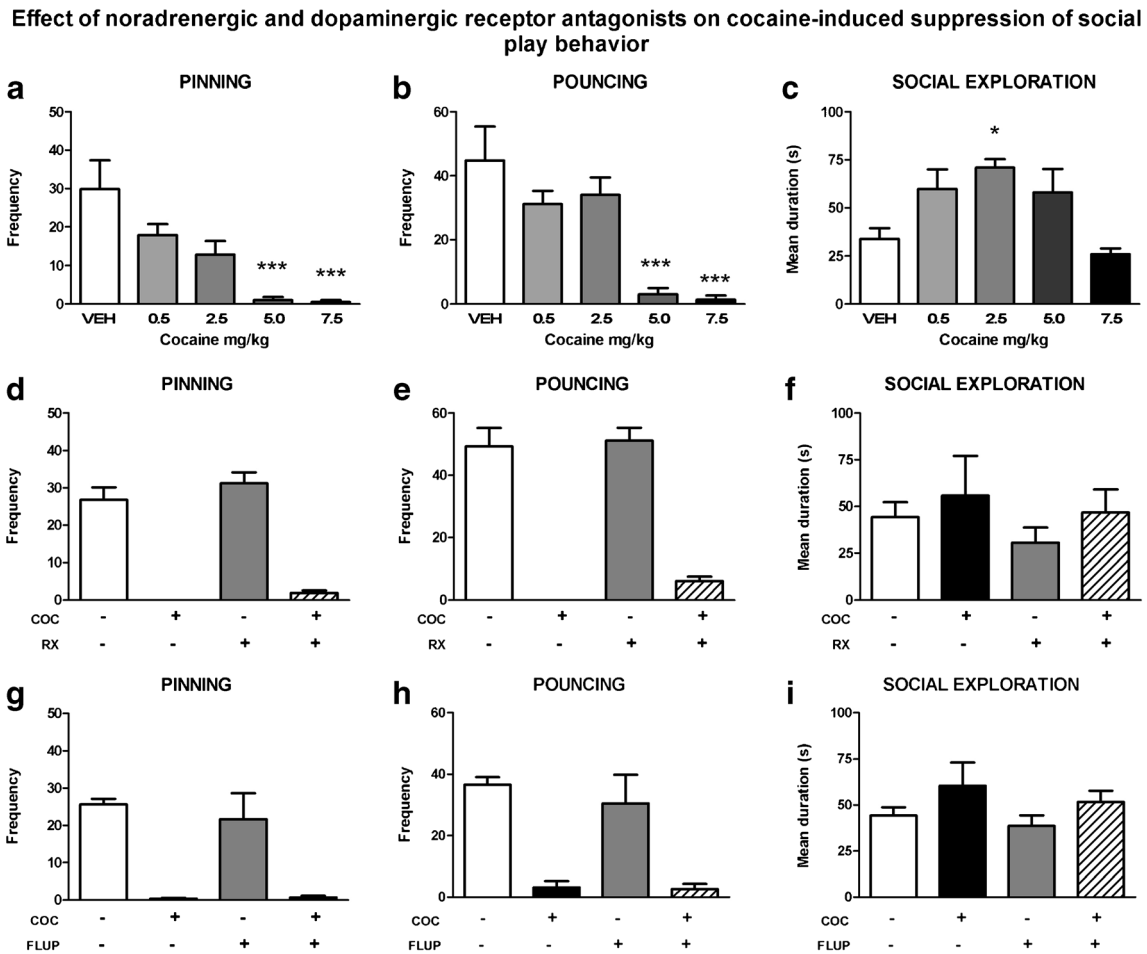


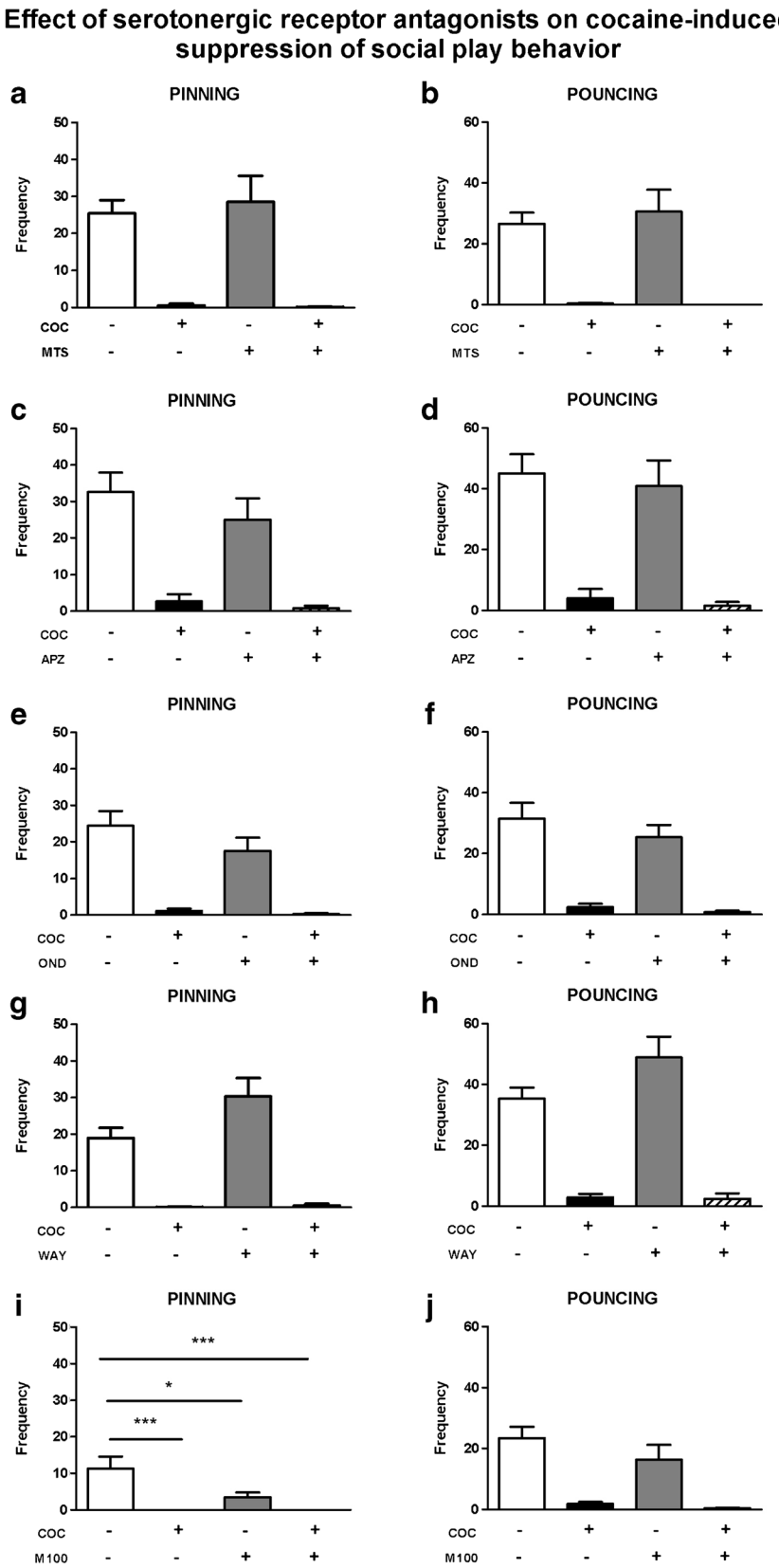
Fig. 2 Effects of noradrenaline and dopamine receptor antagonists on cocaine-induced suppression of social play behavior. Cocaine (coc, s.c.) dose-dependently suppressed pinning (**a**) and pouncing (**b**) and increased social exploration (**c**). The alpha-2 adrenoreceptor antagonist RX821002 (rx, 0.2 mg/kg, i.p.) and the dopamine receptor antagonist alpha-flupenthixol (flup, 0.125 mg/kg, i.p.) did not counteract the effects of cocaine (COC, 5 mg/kg) on pinning (**d**, **g**) and pouncing (**e**, **h**). Social exploration was unaffected by the treatments (**f**, **i**). *Bars* show the frequency of pinning and pouncing and the duration of social exploration (seconds) of the different treatment groups (mean + SEM). *Plus sign* indicates couples of animals treated with the test compound; *minus sign* indicates couples treated with the corresponding vehicle. $N=4$–8 couples per treatment group. One- or two-way ANOVA with Tukey post-hoc test, $^{*}p<0.05$, $^{***}p<0.001$, different from vehicle

g3+a0.1×coc)$_{1,26}$=4.46, $p=0.05$; Fig. 5e, f]. Post-hoc analyses revealed that pinning was reduced in animals treated with the reuptake inhibitors plus a subeffective dose of cocaine compared to the other groups (Fig. 5e). These results suggest that combined blockade of the reuptake of dopamine, noradrenaline, and 5-HT underlies the effect of cocaine on social play behavior in rats.

Discussion

The present study investigated the pharmacological mechanisms underlying the effects of amphetamine and cocaine on social play behavior. We found that low doses of amphetamine and cocaine suppressed social play behavior in adolescent rats. These effects were behaviorally specific, since both psychostimulants did not consistently alter social exploratory behavior. The effects of amphetamine on social play depended on stimulation of alpha-2 noradrenaline but not dopamine receptors. In contrast, the effects of cocaine on social play

Fig. 3 Effects of 5-HT receptor antagonists on cocaine-induced suppression of social play behavior. 5-HT antagonists (methysergide: mts, 5HT1/2 receptor antagonist, 2 mg/kg, s.c.; amperozide: apz, 5HT2 receptor antagonist, 0.5 mg/kg, i.p.; ondansetron: ond, 5HT3 receptor antagonist, 1.0 mg/kg, i.p.; WAY100365: way, 5HT1a receptor antagonist, 0.1 mg/kg, s.c.; M100907: m100, 5HT2a receptor antagonist, 0.2 mg/kg, s.c.) did not counteract the suppression of social play behavior induced by cocaine (coc, 5 mg/kg, s.c.): pinning (**a**, **c**, **e**, **g**, **i**) and pouncing (**b**, **d**, **f**, **h**, **j**). *Bars* show the frequency (mean + SEM) of pinning and pouncing of the different treatment groups. *Plus sign* indicates couples of animals treated with the test compound; *minus sign* indicates couples treated with the corresponding vehicle. $N=5$–8 couples per treatment group. Two-way ANOVA with Tukey post-hoc test, $^{*}p<0.05$, $^{***}p<0.001$


Effect of serotonergic receptor antagonists on cocaine-induced suppression of social play behavior
a
PINNING
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POUNCING
c
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Table 1 Social exploration data and statistics

Drug class	Drug	Mean ± SEM	Statistics
5-HT receptor antagonists	**Methysergide** (mts, 5HT1/2 receptor antagonist, 2 mg/kg, s.c.)	veh-veh: 294.49 ± 35.13 veh-coc: 341.31 ± 22.99 mts-veh: 283.20 ± 28.67 mts-coc: 305.89 ± 39.85	$F(mts)_{1,28}$=0.52, NS $F(coc)_{1,28}$=1.16, NS $F(mts \times coc)_{1,28}$=0.14, NS
	Amperozide (apz, 5HT2 receptor antagonist, 0.5 mg/kg, i.p.)	veh-veh: 44.98 ± 11.88 veh-coc: 44.49 ± 7.75 apz-veh: 38.66 ± 6.23 apz-coc: 40.86 ± 7.32	$F(apz)_{1,20}$=0.30, NS $F(coc)_{1,20}$=0.01, NS $F(apz \times coc)_{1,20}$=0.02, NS
	Ondansetron (ond, 5HT3 receptor antagonist, 1.0 mg/kg, i.p.)	veh-veh: 212.67 ± 12.22 veh-coc: 274.41 ± 17.12 ond-veh: 239.98 ± 16.20 ond-coc: 219.10 ± 23.49	$F(ond)_{1,28}$=5.43, p= 0.03 $F(coc)_{1,28}$=1.33, NS $F(ond \times coc)_{1,28}$=0.62, NS
	WAY100365 (way, 5HT1a receptor antagonist, 0.1 mg/kg, s.c.)	veh-veh: 92.18 ± 10.94 veh-coc: 74.95 ± 8.15 way-veh: 70.17 ± 10.08 way-coc: 56.25 ± 13.29	$F(way)_{1,28}$=3.12, NS $F(coc)_{1,28}$=0.19, NS $F(way \times coc)_{1,28}$=0.02, NS
	M100907 (m100, 5HT2a receptor antagonist, 0.2 mg/kg, s.c.)	veh-veh: 147.70 ± 13.91 veh-coc: 137.75 ± 23.40 m100-veh: 186.13 ± 25.88 m100-coc: 105.48 ± 17.24	$F(m100)_{1,28}$=0.02, NS $F(coc)_{1,28}$=4.81, NS $F(m100 \times coc)_{1,28}$=2.93, NS
Combinations of monoamine receptor antagonists	**RX821002** (rx, α2-adrenoreceptor antagonist, 0.2 mg/kg, i.p.) **methysergide** (mts, 5HT1/2 receptor antagonist, 2 mg/kg, s.c.)	veh-veh: 304.00 ± 25.72 veh-coc: 216.22 ±19.02 rx + mts-veh: 281.77 ± 29.44 rx + mts-coc: 281.28 ± 34.05	$F(rx + mts)_{1,25}$=0.59, NS $F(coc)_{1,25}$=2.51, NS $F(rx + mts \times coc)_{1,28}$=2.46, NS
	α-flupenthixol (flup, dopamine receptor antagonist, 0.125 mg/kg, i.p.) **methysergide** (mts, 5HT1/2 receptor antagonist, 2 mg/kg, s.c.)	veh-veh: 282.61 ± 27.66 veh-coc: 267.18 ± 21.71 flup + mts-veh: 314.69 ± 34.75 flup + mts-coc: 298.90 ± 27.10	$F(flup + mts)_{1,28}$=1.28, NS $F(coc)_{1,28}$=0.31, NS $F(flup + mts \times coc)_{1,28}$=0.00, NS
	RX821002 (rx, α2-adrenoreceptor antagonist, 0.2 mg/kg, i.p.) **α-flupenthixol** (flup, dopamine receptor antagonist, 0.125 mg/kg, i.p.) **methysergide** (mts, 5HT1/2 receptor antagonist, 2 mg/kg, s.c.)	veh-veh: 291.49 ± 22.24 veh-coc: 264.89 ± 22.06 rx + flup + mts-veh: 326.00 ± 41.38 rx + flup + mts-coc: 368.29 ± 43.57	$F(rx + flup + mts)_{1,28}$=4.14, NS $F(coc)_{1,28}$=0.05, NS $F(rx + flup + mts \times coc)_{1,28}$=1.03, NS
Combinations of monoamine reuptake inhibitors	**fluoxetine** (f3, SSRI*, 3 mg/kg, s.c.)	veh-veh: 243.06 ± 27.31 veh-coc: 339.91 ± 20.70 f3-veh: 319.86 ± 39.75 f3-coc: 322.99 ± 31.36	$F(f3)_{1,27}$=3.40, NS $F(coc)_{1,27}$=1.79, NS $F(f3 \times coc)_{1,27}$=0.65, NS
	fluoxetine (f3, SSRI, 3 mg/kg, s.c.) **GBR12909** (g3, DARI# 3 mg/kg, s.c.)	veh-veh: 241.31 ± 19.18 veh-coc: 256.80 ± 20.32 f3 + g3-veh: 270.42 ± 35.49 f3 + g3-coc: 282.83 ± 26.07	$F(f3 + g3)_{1,28}$=1.12, NS $F(coc)_{1,28}$=0.29, NS $F(f3 + g3 \times coc)_{1,28}$=0.00, NS
	fluoxetine (f3, SSRI, 3 mg/kg, s.c.) **GBR12909** (g3, DARI 3 mg/kg) **atomoxetine** (a0.1, NARI$, 0.1 mg/kg, i.p.)	veh-veh: 291.49 ± 20.80 veh-coc: 264.89 ± 20.64 f3 + g3 + a0.1-veh: 326.00 ± 38.70 f3 + g3 + a0.1-coc: 368.29 ± 40.76	$F(f3 + g3 + a0.1)_{1,26}$=9.35, p= 0.01 $F(coc)_{1,26}$= 0.03, NS $F(f3 + g3 + a0.1 \times coc)_{1,26}$=1.85, NS

*SSRI: selective serotonin reuptake inhibitor, # DARI: dopamine reuptake inhibitor, $ NARI: noradrenaline reuptake inhibitor.

depended on simultaneous increases in dopamine, noradrenaline, and 5-HT neurotransmission.

We have previously shown that the reduction in social play induced by the dopamine and noradrenaline reuptake inhibitor methylphenidate was reversed by pretreatment with the alpha-2 adrenoceptor antagonist RX821002, but not the alpha-1 adrenoceptor antagonist prazosine, the beta adrenoceptor antagonist propranolol or the dopamine receptor antagonist alpha-flupenthixol. Furthermore, the play-suppressant effect of methylphenidate was mimicked by the selective noradrenaline reuptake inhibitor atomoxetine but not by the selective dopamine reuptake inhibitor GBR12909 or the dopamine receptor agonist apomorphine (Vanderschuren et al. 2008). In line with these findings, the play-suppressant effects of amphetamine were blocked by RX821002, but not alpha-flupenthixol. These findings are consistent with previous observations that the dopamine receptor antagonist haloperidol, the alpha-1 adrenoreceptor antagonist phenoxybenzamine, the beta-adrenoreceptor antagonist propranolol, and the combined alpha-1 and dopamine D2 receptor antagonist chlorpromazine were ineffective in counteracting the effects of amphetamine on social play behavior (Beatty et al. 1984), and that haloperidol and chlorpromazine did not counteract the disruptive effects of amphetamine and cocaine on social behavior in primates (Miczek and Yoshimura 1982). Together, these data show that the play suppressant effects of amphetamine, like methylphenidate, are mediated by activation of alpha-2 adrenoreceptors, and are independent of dopaminergic neurotransmission.

Cocaine inhibits the reuptake of dopamine, 5-HT and, to a lesser extent, noradrenaline (Heikkila et al. 1975; Ritz and Kuhar 1989; Rothman et al. 2001). We found that

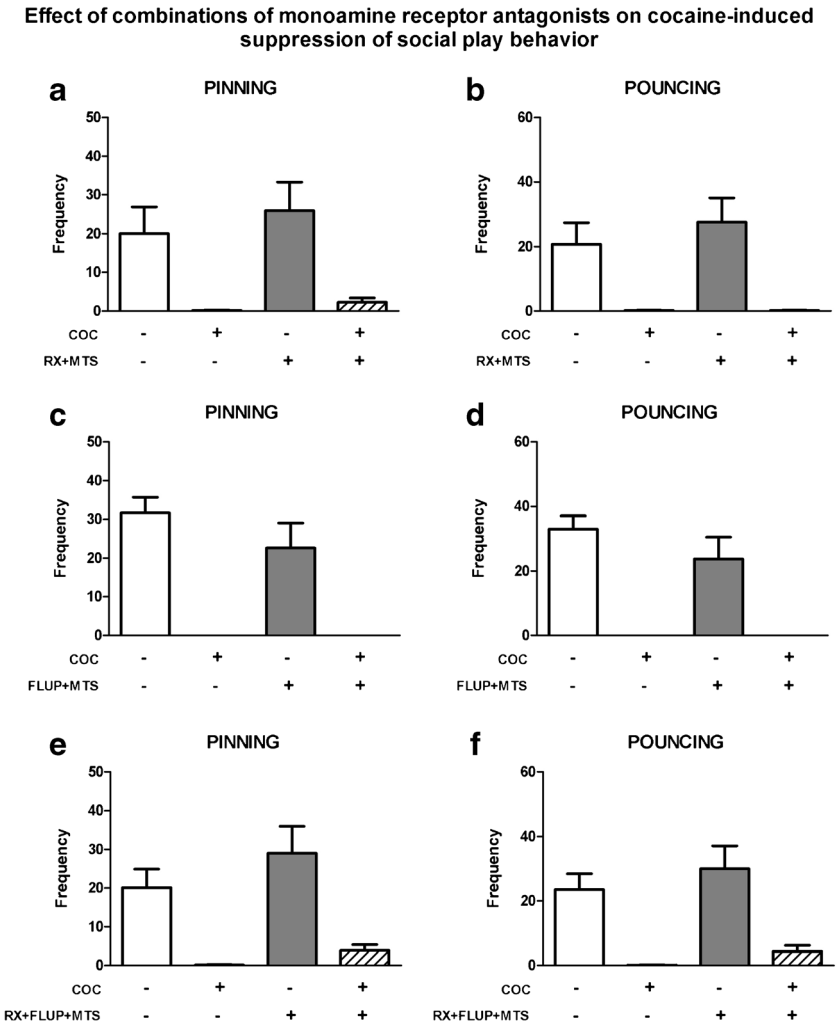

Fig. 4 Effects of combinations of monoamine receptor antagonists on the play suppressant effects of cocaine (coc, 5 mg/kg, s.c.). A combination of RX821002 (rx, α2-adrenoreceptor antagonist, 0.2 mg/kg, i.p.) + methysergide (mts, 5-HT1/2 receptor antagonist, 2 mg/kg, s.c.), a combination of α-flupenthixol (flup, dopamine receptor antagonist, 0.125 mg/kg, i.p.) + methysergide (mts, 5-HT1/2 receptor antagonist, 2 mg/kg, s.c.), and a combination of RX821002 (rx, α2-adrenoreceptor antagonist, 0.2 mg/kg, i.p.)+α-flupenthixol (flup, dopamine receptor antagonist, 0.125 mg/kg, i.p.) + methysergide (mts, 5-HT1/2 receptor antagonist, 2 mg/kg, s.c.) did not antagonize the reduction in pinning (**a**, **c**, **e**) and pouncing (**b**, **d**, **f**) induced by cocaine. *Bars* show the frequency (mean + SEM) of pinning and pouncing of the different treatment groups. *Plus sign* indicates couples of animals treated with the test compounds; *minus sign* indicates couples treated with the corresponding vehicles, $N=7–8$ couples per treatment group

administration of the dopamine receptor antagonist alpha-flupenthixol, the alpha-2 adrenoreceptor antagonist RX821002, or the 5-HT receptor antagonists amperozide (5-HT2), methysergide (5-HT1/2), ondansetron (5-HT3), WAY100365 (5-HT1A), and M100907 (5-HT2A) did not antagonize the reduction in social play behavior induced by cocaine, indicating that it is not likely that one single monoamine receptor mechanism underlies this effect of cocaine. In keeping with these findings, it has previously been found that the reduction in social interaction induced by cocaine in rats was not antagonized by pretreatment with amperozide (Rademacher et al. 2002), and that cocaine-induced social deficits in primates were not altered by pretreatment with chlorpromazine or haloperidol (Miczek and Yoshimura 1982). Interestingly, combinations of methysergide and RX821002, methysergide and alpha-flupenthixol, or a combination of methysergide, RX821002, and alpha-flupenthixol did not counteract the effect of cocaine on social play, which suggests that the play-suppressant effect of cocaine is not exerted through redundant monoamine receptor mechanisms. Since at least 14 subtypes of 5-HT receptors exist (Boess and Martin 1994), the possibility remains that a combination of drugs antagonizing different 5-HT receptors is effective in counteracting the play-suppressant effects of cocaine. To identify which monoamines were involved in the effects of cocaine on social play, we tested the effects of subeffective doses of monoamine reuptake inhibitors administered in combination with a subeffective dose of cocaine. We found that a combination of subeffective doses of the dopamine, noradrenaline, and serotonin reuptake inhibitors GBR12909, atomoxetine, and fluoxetine modestly reduced social play, which was potentiated by a subeffective dose of cocaine. However, fluoxetine alone or a combination of fluoxetine and GBR12909 were ineffective, and co-administration of a subeffective dose of cocaine with either fluoxetine or fluoxetine plus GBR12909 did not reduce social play either. Since cocaine has much lower affinity for the noradrenaline transporter than for the dopamine or 5-HT transporter (Ritz and Kuhar 1989; Rothman et al. 2001), we did not test the effects of atomoxetine combined with fluoxetine, GBR12909, and/or cocaine. We have previously shown that atomoxetine and fluoxetine, at doses higher than those used here, reduced play behavior, while

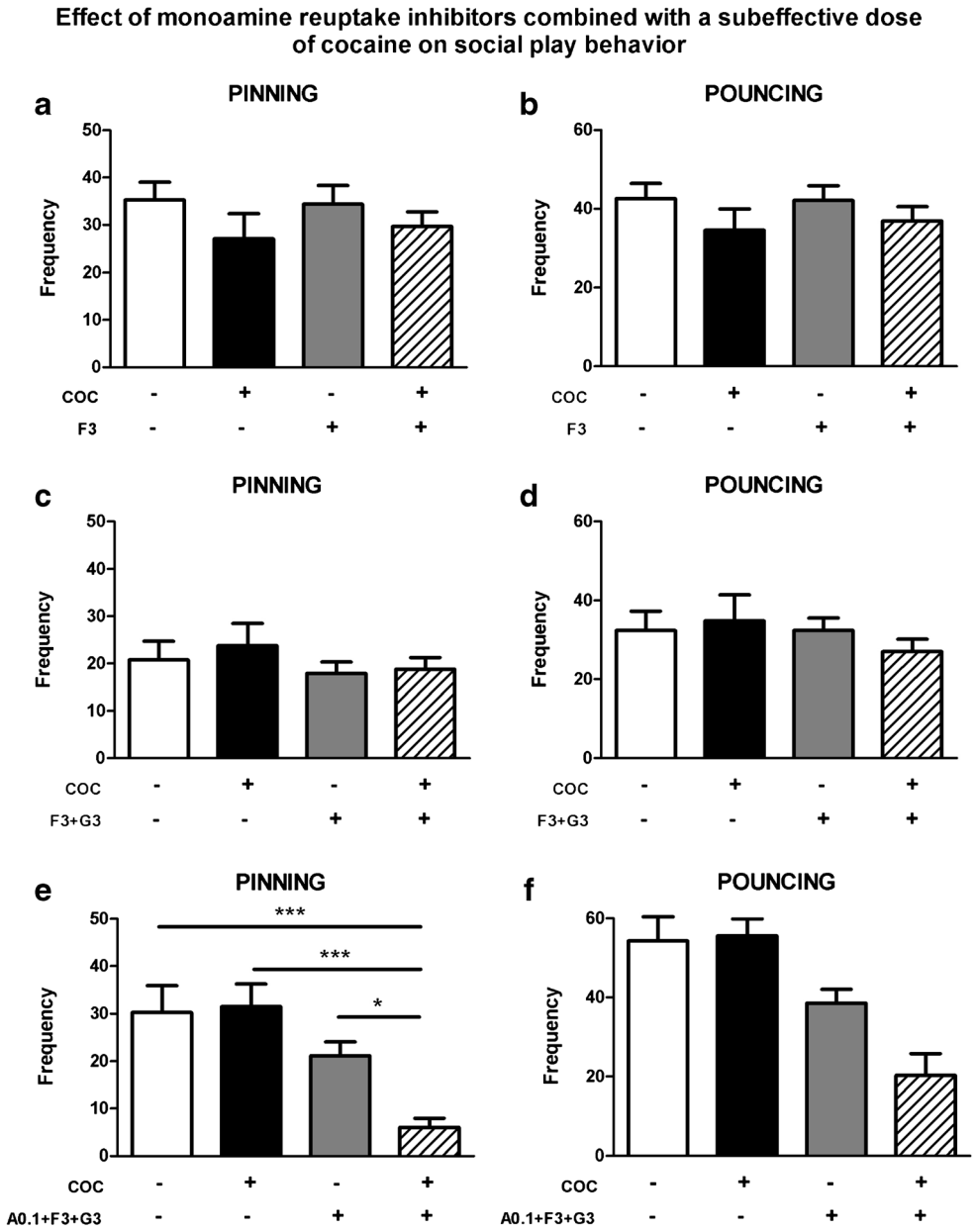

Fig. 5 Effect of (combinations of) subeffective doses of monoamine reuptake inhibitors and a subeffective dose of cocaine on social play. Combined administration of a subeffective dose of fluoxetine (f3, serotonin reuptake inhibitor, 3 mg/kg, s.c.) and a subeffective dose of cocaine (coc, 0.5 mg/kg, s.c.) or combined administration of a subeffective dose of fluoxetine (f3, serotonin reuptake inhibitor, 3 mg/kg, s.c.) and GBR12909 (g3, dopamine reuptake inhibitor, 3 mg/kg, s.c.) together with a subeffective dose of cocaine (coc, 0.5 mg/kg, s.c.) had no effects on pinning (**a**, **c**) and pouncing (**b**, **d**). Combined administration of a subeffective dose of fluoxetine (f3, serotonin reuptake inhibitor, 3 mg/kg, s.c.) + GBR12909 (g3, dopamine reuptake inhibitor, 3 mg/kg) + atomoxetine (a0.1, noradrenaline reuptake inhibitor, 0.1 mg/kg, i.p.) together with a subeffective dose of cocaine (COC, 0.5 mg/kg, s.c.) significantly reduced pinning (**e**) but not pouncing (**f**). *Bars* show the frequency (mean ± SEM) of pinning and pouncing of the different treatment groups. *Plus sign* indicates couples of animals treated with the test compounds; *minus sign* indicates couples treated with the corresponding vehicles. $N=6–8$ couples per treatment group. Two-way ANOVA with Tukey post-hoc test, $*p<0.05$, $***p<0.001$

GBR12909 did not alter social play (Homberg et al. 2007; Vanderschuren et al. 2008). Together, these findings suggest that simultaneous increases in synaptic concentrations of all three monoamines underlie the inhibitory effect of cocaine on social play behavior, although the specific receptors involved remain to be elucidated. Intracranial infusion studies may be helpful in clarifying the mechanism of action by which cocaine inhibits social play behavior.

Social play behavior is a highly vigorous form of social behavior with a strong locomotor component (Panksepp et al. 1984; Vanderschuren et al. 1997; Pellis and Pellis 2009), and amphetamine and cocaine are known to evoke locomotor hyperactivity (Wise and Bozarth 1987). It may therefore be that the psychostimulant-induced suppression of play is the result of behavioral competition, i.e., that the exaggerated hyperactivity induced by amphetamine and cocaine prevents the animals from engaging in a meaningful social interaction. However, we think that this possibility is unlikely, for two reasons. First, the reduction in social play behavior was induced by lower doses of amphetamine and cocaine than those typically used to induced psychomotor hyperactivity (e.g., Sahakian et al. 1975; White et al. 1998), even when taking into account that the sensitivity to psychostimulant drugs may be different for periadolescent vs adult rats (for review, see Schramm-Sapyta et al. 2009). Second, the psychomotor hyperactivity induced by amphetamine and cocaine strongly

depends on dopaminergic neurotransmission (e.g., Kelly et al. 1975; White et al. 1998), whereas their effects on social play behavior are dopamine-independent (Beatty et al. 1984; Vanderschuren et al. 2008; present study). Third, we have previously shown that the effects of methylphenidate on social play and its psychomotor stimulant effects can be dissociated (Vanderschuren et al. 2008).

One may argue that the play-suppressant effects of amphetamine and cocaine reflect an occlusion of social reward. Thus, the positive subjective effects of the psychostimulants could substitute for rewarding effects of social play, so that the animals would no longer need to seek out a social source of positive emotions. Along similar lines, it has been suggested that amphetamine may substitute for the rewarding effects of pair bond formation in prairie voles, and vice versa (Liu et al. 2010, 2011). We do not think that this is the explanation for the present findings, however, for two reasons. First, whereas the rewarding effects of psychostimulant drugs rely on dopaminergic mechanisms (e.g., Veeneman et al. 2011, 2012; for reviews, see Wise 2004; Pierce and Kumaresan 2006), their effects on social play do not. Second, non-psychostimulant drugs of abuse, such as opiates, nicotine, and ethanol, as well as drugs that enhance endocannabinoid signaling, actually enhance social play (for reviews, see Trezza et al. 2010; Siviy and Panksepp 2011). It would then be difficult to conceive why some euphorigenic drugs increase whereas others reduce social play if their positive subjective effects would substitute for those of social play behavior.

An alternative interpretation of the play-suppressant effect of amphetamine and cocaine is that these psychostimulants are anxiogenic (File and Seth 2003). However, amphetamine and cocaine did not affect social exploratory behavior, which is the standard parameter used in the social interaction test of anxiety (File and Seth 2003). Moreover, pharmacological analysis of social play behavior has consistently shown that anxiolytic or anxiogenic drugs do not invariably increase or reduce social play, respectively (Vanderschuren et al. 1997). On the contrary, our recent experiments have shown that doses of nicotine and ethanol, that increase social play in both familiar and unfamiliar environments, did not affect anxiety as tested on the elevated plus maze. Conversely, the standard anxiolytic drug diazepam, which did have an anxiolytic effect on the elevated plus maze, reduced social play, but increased social exploration (Trezza et al. 2009). Thus, it is highly unlikely that the reduction in social play induced by cocaine and amphetamine is secondary to an anxiogenic effect of these drugs.

Several hypotheses can be put forward to explain the effects of psychostimulants on social play behavior. First, on the basis of their effectiveness in attention-deficit/hyperactivity disorder, and the comparable pharmacological profile of the effects of amphetamine and methylphenidate on social play behavior (Vanderschuren et al. 2008; present study) and the stop signal task (Eagle and Baunez 2010), it can be hypothesized that the play suppressant effects of psychostimulants are the result of enhanced or exaggerated behavioral inhibition. That is, through increasing inhibitory control over behavior, psychostimulant drugs may enhance attention for non-social stimuli in the environment, causing the animals to engage less in vigorous playful interactions, that are accompanied by reduced attention for, potentially important, environmental stimuli. Second, and in contrast, psychostimulant-induced increases in tonic noradrenergic neurotransmission may promote disengagement from ongoing (playful) behaviors and facilitate the switching of behaviors (Aston-Jones and Cohen 2005). This may impact social play behavior that requires appropriate behavioral responses from both partners of a dyad. Third, the play-suppressant effect of psychostimulants can be explained by the notion that they increase the intensity of behavior. As not all behaviors can be intensified to the same degree, this causes a narrowing down of the behavioral repertoire with simple behaviors being favored over complex behaviors, such as social play (Lyon and Robbins 1975). One needs to bear in mind though that the present findings add a layer of complexity over the possible behavioral mechanisms of psychostimulant-induced suppression of social play. That is, since amphetamine and methylphenidate reduce social play through a distinct pharmacological mechanism of action than cocaine does, it is well conceivable that the behavioral underpinnings of these effects also differ between different psychostimulant drugs. In this regard, it is worth mentioning that amphetamine and cocaine suppressed pouncing (i.e., play solicitation) and pinning (the most prominent response to pouncing in rats this age, i.e., response to play solicitation) with comparable potency. This is somewhat in contrast with a previous study that showed that amphetamine affects pouncing at lower doses than responding to pouncing by rotating to supine (i.e., pinning; Field and Pellis 1994). It may therefore well be that subcomponents of social play (i.e., pouncing, and the different possible defense strategies) are also differentially affected by amphetamine and cocaine.

In summary, we show here that amphetamine, like methylphenidate, exerts its play-suppressant effect through stimulation of alpha-2 adrenoreceptors. Cocaine, on the other hand, exerts its effect through simultaneous increases in dopamine, noradrenaline, and 5-HT neurotransmission. Positive social interactions in young individuals are essential for emotional well-being and for social and cognitive development. Moreover, the inability to assign a positive subjective value to social stimuli may be a key process in the pathophysiology of childhood and adolescent psychiatric disorders characterized by aberrant social interactions. Our present study advances our understanding of how psychostimulant drugs negatively impact upon social interactions in young individuals. This work has relevance for our understanding of the neural mechanisms of normal social development, as well

as childhood and adolescent psychiatric disorders with a prominent social dimension, such as autism, disruptive behavior disorders, and schizophrenia. Moreover, given the emergence of drug use during early adolescence, increasing our understanding of how psychostimulant drugs affect social behavior has obvious repercussions for the etiology of substance abuse disorders.

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