

Methylphenidate Disrupts Social Play Behavior in Adolescent Rats

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Methylphenidate is the first-choice treatment for attention-deficit/hyperactivity disorder (ADHD), but its mechanism of action is incompletely understood. The cognitive effects of methylphenidate have been extensively studied, but little is known about its effects on spontaneous social behavior. During adolescence, rats display a characteristic, highly vigorous form of social behavior, termed social play behavior, which is of critical importance for social and cognitive development. We investigated the neurobehavioral mechanisms by which methylphenidate affects social play behavior in rats. Methylphenidate (0.3–3.0 mg/kg, s.c. or p.o.) abolished social play behavior, without altering general social interest. This effect of methylphenidate did not depend upon the baseline level of social play and was not secondary to changes in locomotion. Furthermore, the play-suppressant effect of methylphenidate was not subject to tolerance or sensitization. Methylphenidate blocked both the initiation to play and the responsiveness to play initiation. The effect of methylphenidate was mimicked by the noradrenaline reuptake inhibitor atomoxetine, which is also used for the treatment of ADHD, and was blocked by an α -2 adrenoceptor antagonist. In addition, combined administration of subeffective doses of methylphenidate and atomoxetine suppressed social play. However, blockade of α -1 adrenoceptors, β -adrenoceptors, or dopamine receptors did not alter the effect of methylphenidate. These data show that methylphenidate selectively blocks the most vigorous part of the behavioral repertoire of adolescent rats through a noradrenergic mechanism. We suggest that the effect of methylphenidate on social play is a reflection of its therapeutic effect in ADHD, that is, improved behavioral inhibition. However, given the importance of social play for development, these findings may also indicate an adverse side effect of methylphenidate.

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INTRODUCTION

Methylphenidate (Ritalin[®], Concerta[®]) is the first-choice medication for attention-deficit/hyperactivity disorder (ADHD) (Elia *et al*, 1999; Kutcher *et al*, 2004; Fone and Nutt, 2005), a childhood disorder characterized by inattention, hyperactivity, and impulsivity (American Psychiatric Association, 2000; Biederman and Faraone, 2005). Despite the widely recognized efficacy of methylphenidate in the treatment of ADHD, its neurobehavioral mechanism of action is incompletely understood. Methylphenidate blocks the dopamine transporter and the noradrenaline transporter (Ferris and Tang, 1979; Ritz *et al*, 1987), thus increasing the extracellular concentrations of these catecholamines.

Studies in humans have shown that methylphenidate increases impulse control (Tannock *et al*, 1989; Aron *et al*, 2003), attention (Solanto *et al*, 1997; Hawk *et al*, 2003; Overtom *et al*, 2003), and working memory (Mehta *et al*, 2004) in ADHD patients, as well as in healthy subjects (Elliott *et al*, 1997; Mehta *et al*, 2000; Pietras *et al*, 2003; Volkow *et al*, 2004). Comparable findings have been obtained in rodents (Arnsten and Dudley, 2005; Berridge *et al*, 2006; van Gaalen *et al*, 2006; Bizot *et al*, 2007; Eagle *et al*, 2007).

Studies on the effect of methylphenidate on discrete cognitive processes are essential in understanding its mechanism of action. However, the psychopathology of ADHD not only manifests in subjects instructed to engage in a particular task (such as in a classroom setting) but also prominently in spontaneous social interactions, leading to the pattern of unstable relationships, unemployment, and criminal behavior associated with ADHD (American Psychiatric Association, 2000; Biederman and Faraone, 2005). Psychostimulant drugs such as amphetamines are known to disrupt a variety of social behaviors (Schjorring,

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1979; Miczek *et al*, 1989; Moro *et al*, 1997). Studies in rodents have shown that methylphenidate disrupts social behavior in adolescent and young adult rats (Beatty *et al*, 1982; Thor and Holloway, 1983; Arakawa, 1994), but the neurobehavioral background of this effect and its relationship to the therapeutic effects of methylphenidate in ADHD remain elusive. In humans, knowledge on the effects of methylphenidate on social behavior comes from parent and teacher reports, and these studies have for the most part focused on disruptive and aggressive behavior in ADHD (Hinshaw and Lee, 2000; Pelham *et al*, 2001; Schachter *et al*, 2001; Connor *et al*, 2002; Chacko *et al*, 2005).

Between weaning and sexual maturation (postnatal days 21–60, with a peak between postnatal days 25–40), rats display a great deal of a characteristic, highly vigorous form of social behavior, termed social play behavior (Panksepp *et al*, 1984; Vanderschuren *et al*, 1997; Pellis and Pellis, 1998). Social play behavior is thought to subserve social and cognitive development, because social isolation in rats during the 2 weeks in adolescence when social play is most abundant leads to long-lasting behavioral disturbances in the social domain (Hol *et al*, 1999; Van den Berg *et al*, 1999a). Thus, because of (1) the limited understanding of the effects of methylphenidate on social behavior in relationship to its therapeutic effects in ADHD and (2) the importance of social play for behavioral development, we investigated the neurobehavioral mechanisms underlying the effect of methylphenidate on social play behavior in adolescent rats, using doses of the drug that are comparable to those used for the treatment of ADHD (Elia *et al*, 1999; Solanto, 2000; Kuczenski and Segal, 2002; Kutcher *et al*, 2004).

MATERIALS AND METHODS

Animals

Male Wistar rats (Harlan, Horst, The Netherlands, and 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* × *w* × *h*) Macrolon cages under controlled conditions (ambient temperature 20–21°C, 60–65% relative humidity, and 12/12 h light cycle with lights on at 0700 hours). Food and water were available *ad libitum*. Animals were used only once. Group size was *n* = 6–16 in all experiments. All experiments were approved by the Animal Ethics Committees of the VU University Medical Center Amsterdam and the University Medical Center Utrecht and were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Social Play Behavior

All 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* × *w* × *h*) with approximately 2 cm of wood shavings covering the floor.

Social play was assessed as previously described (Vanderschuren *et al*, 1995a). At 26–28 days of age, rats were individually habituated to the test cage for 10 min on 2 days

prior to testing. On the test day, the animals were socially isolated for 3.5 h before 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). In two experiments, animals were isolated for either 0 or 24 h before testing, which induces minimal and maximal levels of social play behavior, respectively (Niesink and Van Ree, 1989; Vanderschuren *et al*, 1995a). At the appropriate time before testing, pairs of animals were treated with drugs or vehicle. In all experiments except for one, both animals of a pair received the same drug treatment. The test consisted of placing two animals into the test cage for 15 min. The animals of each pair did not differ more than 10 g in body weight and had no previous common social experience. The behavior of the animals was videotaped and analyzed afterward. Coding of the drug solutions ensured that both during experimentation and behavior analysis, the experimenter was unaware of the treatment of the animals.

Behavior was assessed per pair of animals, except in one experiment, in which the behavior of both members of a test pair was scored separately, using the 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. 'Pinning,' that is, one animal lying with its dorsal surface on the floor with the other animal standing over it, occurs if the animal that is solicited upon rotates to its dorsal surface. From this position, the supine animal can initiate another bout of play, by trying to gain access to the other animal's neck. Thus, during social play, pinning, which is considered to be the most obvious posture in social play behavior in rats, is not an end point, but rather functions as a releaser of a prolonged play bout (Poole and Fish, 1975; Panksepp *et al*, 1984; Pellis and Pellis, 1987; Pellis, 1988). The animal that is pounced upon can also respond by evading, or by turning around to face the other animal. In the latter situation, a brief period of boxing/wrestling may follow, in which the animals try to push each other away. If the solicited animal evades, the other animal may start to chase it, thus making another attempt to launch a play bout (see Vanderschuren *et al*, 1995b, for a detailed analysis of the temporal structure of social play behavior in rats). The following behaviors were scored per 15 min: frequency of pinning, frequency of pouncing, and time spent in social exploration, that is, sniffing any part of the body of the test partner, including the anogenital area. In the experiment where both members of a test pair were scored separately, pinning was scored when the subject pinned its partner. In one experiment, locomotor activity of the animals was also scored as follows: a grid, dividing the test arena into 25 equally sized compartments, was projected over the social behavior recordings, and the number of line crossings was counted separately for each animal of a pair.

Drugs and Treatment

Methylphenidate-HCl (Bufa and Sigma), GBR-12909-diHCl, apomorphine-HCl, *cis*-(*Z*)-flupenthixol (Sigma), RX821002-HCl, atomoxetine-HCl (Tocris), and propranolol-HCl (ICI) were dissolved in saline, and prazosin-HCl (Pfizer) was dissolved in distilled water. Methylphenidate, GBR-12909,

and apomorphine were administered subcutaneously (s.c.) and atomoxetine was administered i.p. 30 min before testing. In one experiment, methylphenidate was administered by oral gavage 30 min before testing. Prazosin, propranolol, RX821002, and *cis*-(Z)-flupenthixol were administered i.p. 15 min before methylphenidate or atomoxetine. Drug doses and pretreatment intervals were based on the literature and on pilot experiments. Because of the importance of the neck area in the expression of social play behavior (Pellis and Pellis, 1987; Siviy and Panksepp, 1987), s.c. injections were administered in the flank. Injection volume was always 0.2 ml/kg.

To assess the effect of previous exposure on the effectiveness of methylphenidate to suppress social play behavior, animals were pretreated with methylphenidate (1.0 mg/kg, s.c.) or saline for 5 consecutive days (postnatal days 26–30). On day 31, one day after the last pretreatment injection, animals were isolated for 3.5 h. Next, half of both pretreatment groups was treated with methylphenidate (0.1 or 1.0 mg/kg, s.c.), and the other half was treated with saline, 30 min before the test, and tested for social play behavior as described above.

Statistics

Pinning and pouncing frequencies, time spent in social exploration, and line crossings were expressed as mean \pm SEM. To assess the effects of single or combined treatments on social play behavior, data were analyzed using one-way or two-way analysis of variance, respectively, followed by the Student–Newman–Keuls *post hoc* tests where appropriate.

RESULTS

Methylphenidate Specifically Suppresses Social Play Behavior

Treatment with methylphenidate (0.3–3.0 mg/kg, s.c.) dose-dependently suppressed social play behavior. Pinning (Figure 1a) as well as pouncing (Figure 1b) was nearly absent after treatment with the highest dose of methylphenidate. In contrast, social exploration was not affected by methylphenidate treatment (Figure 1c). We next investigated the effect of lower doses of methylphenidate on social play behavior to exclude the possibility that methylphenidate exerts biphasic effects on social play. At doses of 0.01 and 0.1 mg/kg, methylphenidate did not alter pinning, but consistent with the previous experiment, 1.0 mg/kg methylphenidate reduced pinning (Figure 1d) as well as pouncing ($F_{3,30} = 8.76$, $p < 0.0001$; data not shown). To evaluate whether the effect of methylphenidate depended on the baseline level of social play, we assessed its effect in animals that had been socially isolated for 0 or 24 h prior to testing. After 0 h of social isolation, saline-treated rats displayed levels of pinning that were approximately one-third of those isolated for 3.5 h, whereas rats isolated for 24 h showed about twice as much pinning compared to those seen after 3.5 h of isolation. Methylphenidate (0.3–3.0 mg/kg, s.c.) suppressed pinning after both 0 h (Figure 1e) and 24 h of social isolation (Figure 1f). When the effect of methylphenidate was expressed as percentage of saline treatment, its

relative effect after 0, 3.5, and 24 h of social isolation was highly comparable (Figure 1g). After 0 h of social isolation, methylphenidate (at 1.0 and 3.0 mg/kg) also suppressed pouncing ($F_{3,31} = 4.96$, $p < 0.01$) but did not affect social exploration ($F_{3,31} = 0.63$, NS), whereas after 24 h of social isolation, methylphenidate (at 0.3–3.0 mg/kg) decreased pouncing ($F_{3,31} = 71.57$, $p < 0.0001$) and increased social exploration ($F_{3,31} = 16.89$, $p < 0.0001$) (data not shown). Oral administration of methylphenidate also suppressed social play behavior, since both pinning (Figure 1h) and pouncing (Figure 1i), but not social exploration ($F_{3,29} = 1.19$, NS; data not shown), were reduced at doses of 1.0 and 3.0 mg/kg. We also investigated whether the effect of methylphenidate could be secondary to changes in locomotor activity. Methylphenidate enhanced locomotion in animals tested singly (data not shown), but not during a social encounter (no. of crossings: saline-treated: 261.4 ± 10.6 ; methylphenidate (1.0 mg/kg, s.c.)-treated: 275.1 ± 11.3 , $F_{1,31} = 0.83$, NS). To distinguish whether methylphenidate suppressed the initiation to play, the responsivity to play initiation, or both, we next performed an experiment in which none, one, or both members of a test pair were treated with methylphenidate (3.0 mg/kg, s.c.), and behavior of both test partners was scored separately. Consistent with the previous experiments, there was a complete suppression of pinning (Figure 2a) and pouncing (Figure 2b), but no change in social exploration (Figure 2c) when both members of a pair were treated with methylphenidate. When only one animal of a pair was treated, there was also a complete suppression of pinning: the saline-treated rats did not pin the methylphenidate-treated animals, and the methylphenidate-treated rats did not pin the saline-treated animals (Figure 2a). However, whereas the saline-treated animal still solicited play, that is, pounced upon the methylphenidate-treated rat, although less than the saline-treated animals interacting with saline-treated animals, the methylphenidate-treated animal did not pounce upon the saline-treated rat (Figure 2b). Thus, methylphenidate suppressed both the initiation to play as well as the responsivity to play initiation.

The Effect of Methylphenidate on Social Play Behavior Does Not Change after Repeated Treatment

The psychomotor stimulant and positive reinforcing effects of psychostimulant drugs become progressively enhanced after repeated treatment (Stewart and Badiani, 1993; Robinson and Becker, 1986; Vanderschuren and Kalivas, 2000). However, the effects of methylphenidate in the treatment of ADHD are usually immediate and do not change over the course of treatment (Elia *et al.*, 1999; Solanto, 1998). We therefore investigated whether tolerance or sensitization would occur to the effect of methylphenidate on social play behavior after repeated treatment. Animals were treated with methylphenidate (1.0 mg/kg, s.c.) or saline once daily for 5 consecutive days and on the sixth day tested after treatment with an effective (1.0 mg/kg, s.c.) or a subeffective dose (0.1 mg/kg, s.c.) of methylphenidate. Treatment with 1.0 mg/kg methylphenidate suppressed pinning (Figure 3a) and pouncing (Figure 3b) in both saline-pretreated and methylphenidate-pretreated rats, indicating that tolerance to the effect of methylphenidate had

EFFECT OF METHYLPHENIDATE ON SOCIAL PLAY BEHAVIOR

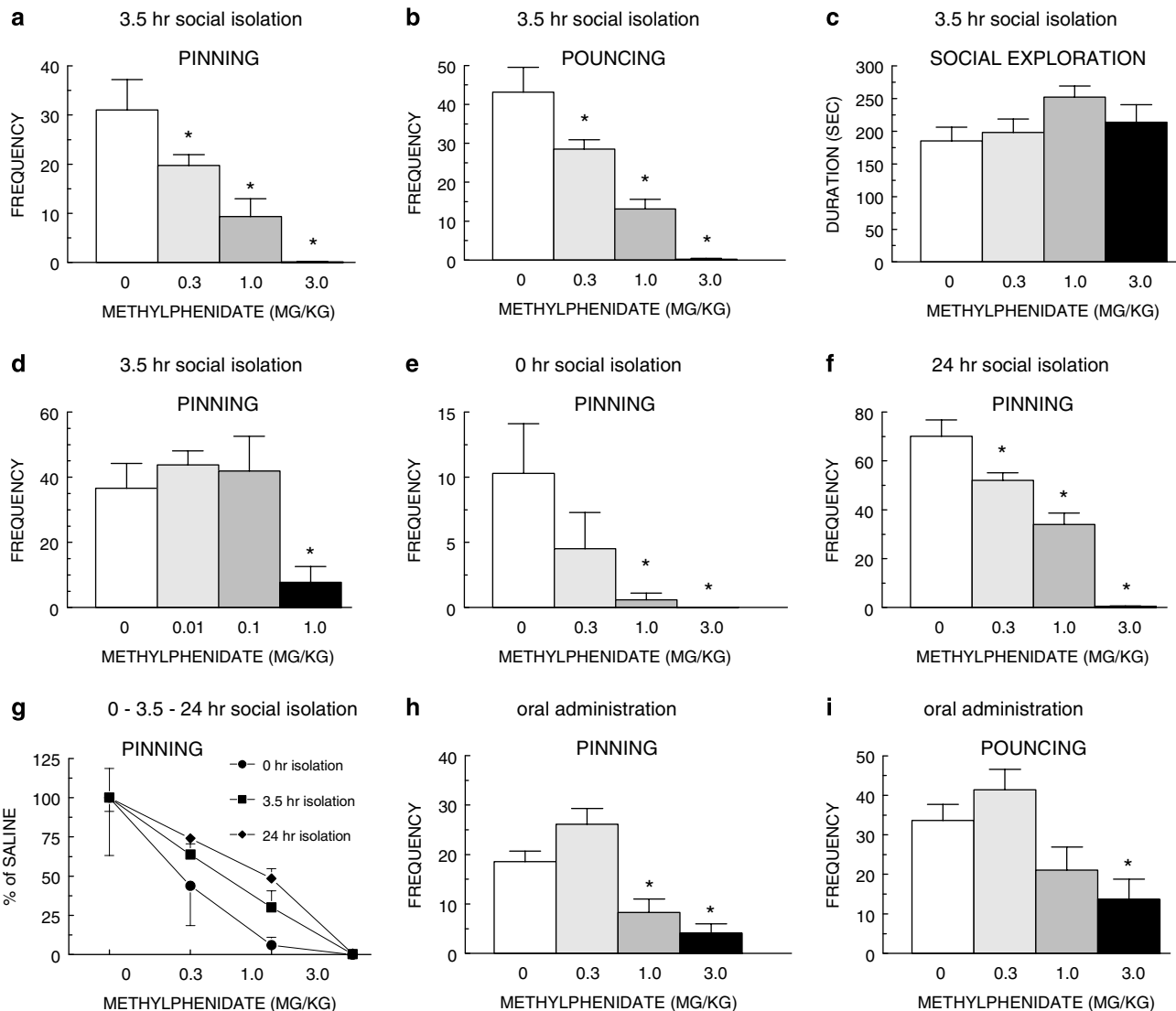


Figure 1 Methylphenidate (0.3–3.0 mg/kg, s.c.) dose-dependently suppressed pinning ((a) $F_{3,29} = 14.96$, $p < 0.0001$) and pouncing ((b) $F_{3,29} = 28.90$, $p < 0.0001$) but not social exploration ((c) $F_{3,29} = 1.72$, NS). Lower doses of methylphenidate (0.01 and 0.1 mg/kg, s.c.) were ineffective in reducing pinning but 1.0 mg/kg methylphenidate, s.c., suppressed pinning ((d) $F_{3,30} = 5.42$, $p < 0.01$). Methylphenidate (0.3–3.0 mg/kg, s.c.) also dose-dependently suppressed pinning in animals showing a minimal induction of social play by 0 h of social isolation ((e) $F_{3,31} = 4.18$, $p < 0.05$), or maximal induction of social play by 24 h of social isolation prior to the experiment ((f) $F_{3,31} = 53.51$, $p < 0.0001$). The potency and efficacy of methylphenidate to suppress pinning were comparable in animals isolated for 0, 3.5, or 24 h (g). Oral administration of methylphenidate also suppressed pinning ((h) $F_{3,29} = 17.50$, $p < 0.0001$) and pouncing ((i) $F_{3,29} = 6.96$, $p = 0.001$) at doses comparable to those effective after s.c. administration of methylphenidate. (a–i) 0 mg/kg methylphenidate = saline vehicle. *Significantly different from saline, $p < 0.05$ (Student–Newman–Keuls test).

not occurred. Social exploration was not affected by methylphenidate pretreatment or treatment ($F(\text{pretreatment})_{1,31} = 0.00$, NS; $F(\text{treatment})_{1,31} = 0.03$, NS; $F(\text{interaction})_{1,31} = 0.77$, NS; data not shown). Treatment with a low dose of methylphenidate that we previously found was subeffective in methylphenidate-naïve rats (see Figure 1d) affected neither pinning (Figure 3c), pouncing (Figure 3d), nor social exploration ($F(\text{pretreatment})_{1,31} = 0.25$, NS; $F(\text{treatment})_{1,31} = 1.08$, NS; $F(\text{interaction})_{1,31} = 0.05$, NS; data not shown), in saline- or methylphenidate-pretreated rats. Thus, sensitization of this effect of methylphenidate did not occur either.

Methylphenidate Suppresses Social Play through α -2 Adrenoceptors

The behavioral and cognitive effects of methylphenidate are usually ascribed to its impact on dopaminergic neurotransmission. However, it also targets noradrenergic neurotransmission, and there is accumulating evidence to show that this property of methylphenidate substantially contributes to its effects in ADHD (Biederman and Spencer, 1999; Bymaster *et al*, 2002; Arnsten and Dudley, 2005; Fone and Nutt, 2005; Berridge *et al*, 2006). We therefore performed a pharmacological characterization of the effects

NONE, ONE OR BOTH ANIMALS TREATED WITH METHYLPHENIDATE

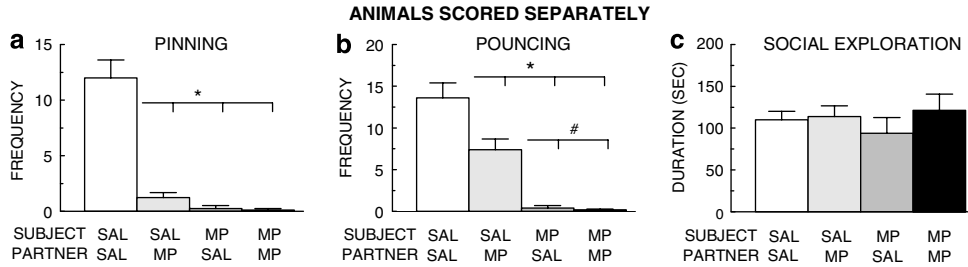
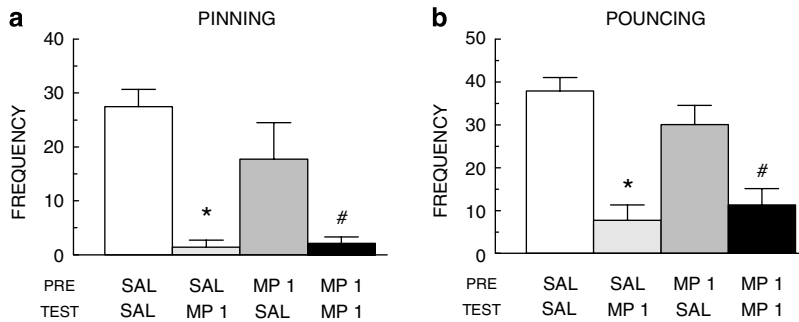


Figure 2 Assessment of social play behavior when animals in a test pair received either methylphenidate (MP; 3.0 mg/kg, s.c.) or saline (SAL). ‘Subject’ represents the treatment of the animal whose behavior was scored; ‘partner’ represents the treatment of its test partner. When either one or both animals in a pair were treated with methylphenidate, pinning was completely suppressed ((a) $F(\text{treatment subject})_{1,47} = 32.06, p < 0.0001$; $F(\text{treatment partner})_{1,47} = 22.87, p < 0.0001$; $F(\text{treatment subject} \times \text{treatment partner})_{1,47} = 21.83, p < 0.0001$), whereas pouncing was attenuated in saline-treated animals interacting with methylphenidate-treated animals, and completely suppressed in methylphenidate-treated animals ((b) $F(\text{treatment subject})_{1,47} = 58.40, p < 0.0001$; $F(\text{treatment partner})_{1,47} = 5.72, p < 0.0001$; $F(\text{treatment subject} \times \text{treatment partner})_{1,47} = 5.06, p < 0.0001$). Social exploration was not affected by methylphenidate ((c) $F(\text{treatment subject})_{1,47} = 0.07, \text{NS}$; $F(\text{treatment partner})_{1,47} = 0.83, \text{NS}$; $F(\text{treatment subject} \times \text{treatment partner})_{1,47} = 0.47, \text{NS}$). *Significantly different from saline-treated rats interacting with saline-treated rats (SAL/SAL), $p < 0.05$ (Student–Newman–Keuls test); #significantly different from saline-treated rats interacting with methylphenidate-treated rats (SAL/MP), $p < 0.05$ (Student–Newman–Keuls test).

METHYLPHENIDATE PRETREATMENT
TEST WITH EFFECTIVE METHYLPHENIDATE DOSE



METHYLPHENIDATE PRETREATMENT
TEST WITH SUBEFFECTIVE METHYLPHENIDATE DOSE

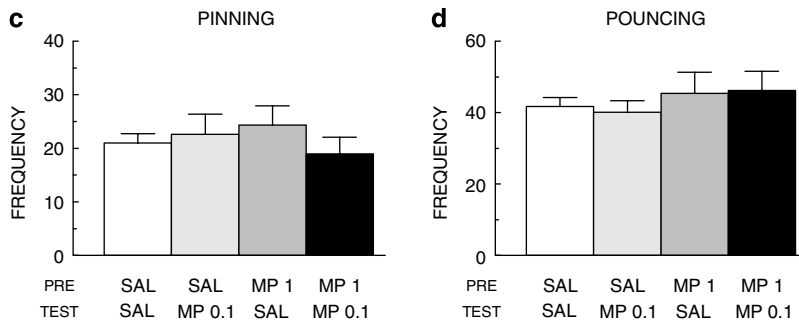


Figure 3 Pretreatment (PRE) with methylphenidate (MP 1; 1.0 mg/kg, s.c., once daily for 5 consecutive days) did not alter the effectiveness of methylphenidate (TEST) to suppress social play. (a) Effect of 1.0 mg/kg methylphenidate, s.c. (MP 1), or saline (SAL) on pinning after 5 days of pretreatment with methylphenidate (1.0 mg/kg, s.c.; MP 1) or saline (SAL) ($F(\text{pretreatment})_{1,31} = 1.57, \text{NS}$; $F(\text{treatment})_{1,31} = 33.73, p < 0.0001$; $F(\text{interaction})_{1,31} = 2.13, \text{NS}$). (b) Effect of 1.0 mg/kg methylphenidate, s.c. (MP 1), or saline (SAL) on pouncing after 5 days of pretreatment with methylphenidate (1.0 mg/kg, s.c.; MP 1) or saline (SAL) ($F(\text{pretreatment})_{1,31} = 0.34, \text{NS}$; $F(\text{treatment})_{1,31} = 48.10, p < 0.0001$; $F(\text{interaction})_{1,31} = 2.61, \text{NS}$). (c) Effect of 0.1 mg/kg methylphenidate, s.c. (MP 0.1), or saline (SAL) on pinning after 5 days of pretreatment with methylphenidate (1.0 mg/kg, s.c.; MP 1) or saline (SAL) ($F(\text{pretreatment})_{1,31} = 0.002, \text{NS}$; $F(\text{treatment})_{1,31} = 0.41, \text{NS}$; $F(\text{interaction})_{1,31} = 1.43, \text{NS}$). (d) Effect of 0.1 mg/kg methylphenidate, s.c. (MP 0.1), or saline (SAL) on pouncing after 5 days of pretreatment with methylphenidate (1.0 mg/kg, s.c.; MP 1) or saline (SAL) ($F(\text{pretreatment})_{1,31} = 1.36, \text{NS}$; $F(\text{treatment})_{1,31} = 0.008, \text{NS}$; $F(\text{interaction})_{1,31} = 0.09, \text{NS}$). *Significantly different from SAL/SAL (pretreated with saline, tested after saline) rats, $p < 0.05$ (Student–Newman–Keuls test); #significantly different from MP 1/SAL (pretreated with methylphenidate, tested after saline) rats, $p < 0.05$ (Student–Newman–Keuls test).

DOPAMINERGIC AND NORADRENERGIC INVOLVEMENT IN SOCIAL PLAY BEHAVIOR

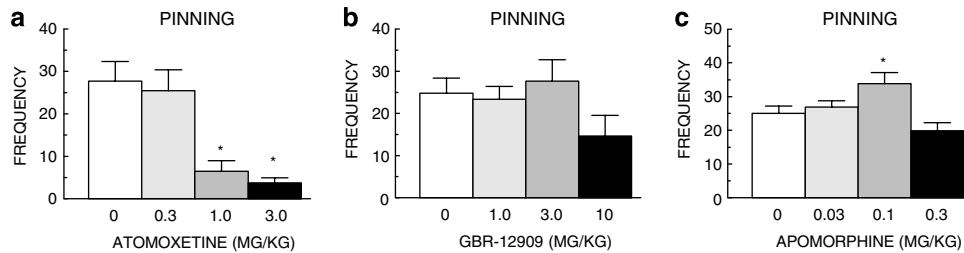


Figure 4 The selective noradrenaline reuptake inhibitor atomoxetine ((a) 0.3–3.0 mg/kg, i.p.; $F_{3,31} = 11.91$, $p < 0.0001$), but not the selective dopamine reuptake inhibitor GBR-12909 ((b) 1.0–10 mg/kg, s.c.; $F_{3,32} = 1.71$, NS), dose-dependently suppressed pinning. The dopamine receptor agonist apomorphine slightly increased pinning ((c) 0.03–0.3 mg/kg, s.c.; $F_{3,31} = 5.44$, $p < 0.01$). (a–c) 0 mg/kg = saline vehicle. *Significantly different from saline, $p < 0.05$ (Student–Newman–Keuls test).

of methylphenidate on social play. The noradrenaline reuptake inhibitor atomoxetine (Strattera[®]), which is also used in the treatment of ADHD (Spencer *et al*, 2002; Michelson *et al*, 2003; Kratochvil *et al*, 2006), suppressed pinning (Figure 4a). However, neither the dopamine reuptake inhibitor GBR-12909 (Figure 4b) nor the dopamine receptor agonist apomorphine (Figure 4c) inhibited social play. In fact, apomorphine stimulated pinning at one dose. We next tried to clarify through which noradrenergic receptor methylphenidate exerts its effects, using the α -2 adrenoceptor antagonist RX821002, the α -1 adrenoceptor antagonist prazosin, the β -adrenoceptor antagonist propranolol, as well as the dopamine receptor antagonist *cis*-(*Z*)-flupenthixol. Since some of these drugs have previously been shown to affect social play themselves (Beatty *et al*, 1984; Siviyy *et al*, 1994), we first performed pilot studies and used doses of these drugs that had no effect on social play by themselves. Pretreatment with the α -2 adrenoceptor antagonist RX821002 prevented the effect of methylphenidate on pinning (Figure 5a). However, neither prazosin (Figure 5b), propranolol (Figure 5c), nor *cis*-(*Z*)-flupenthixol (Figure 5d) influenced the effect of methylphenidate. RX821002 also blocked the suppressant effect on pinning of atomoxetine (Figure 5e). Combined treatment with sub-effective doses of methylphenidate (0.1 mg/kg) and atomoxetine (0.3 mg/kg) also suppressed pinning (Figure 5f). The pattern of effects on pouncing paralleled that seen for pinning, that is, suppression by methylphenidate and atomoxetine, which was blocked by RX821002 but not prazosin, propranolol, or *cis*-(*Z*)-flupenthixol. Moreover, combined treatment with sub-effective doses of methylphenidate and atomoxetine also suppressed pouncing. There were no major drug effects on social exploration (data not shown).

DISCUSSION

The present data show that low doses of methylphenidate block social play behavior in adolescent rats. This effect was behaviorally specific: methylphenidate did not alter social exploratory behavior or locomotor activity during social interaction, demonstrating that changes in general sociability or locomotion did not underlie the effects of methylphenidate on social play. Moreover, the effect of

methylphenidate was not dependent on the baseline level of social play behavior, because it was equally potent in animals isolated for 0, 3.5, and 24 h, which displayed levels of social play that differed approximately sixfold. An experiment in which only one animal in a test pair was treated showed that methylphenidate blocked the initiation to play, as well as the responsiveness to play initiation. The effect of methylphenidate on social play behavior was not subject to tolerance or sensitization. In addition, the play-suppressant effect of methylphenidate was mediated by a noradrenergic mechanism: it was mimicked by the noradrenaline reuptake inhibitor atomoxetine, which is also used for the treatment of ADHD, but not by the dopamine reuptake blocker GBR-12909 or the dopamine receptor agonist apomorphine. The effects of methylphenidate and atomoxetine were blocked by pretreatment with the α -2 adrenoceptor antagonist RX821002, but the effect of methylphenidate was not altered by the dopamine receptor antagonist *cis*-(*Z*)-flupenthixol, the α -1 adrenoceptor antagonist prazosin, or the β -adrenoceptor antagonist propranolol. In addition, combined administration of sub-effective doses of methylphenidate and atomoxetine suppressed social play. Together, these data show that methylphenidate, at doses comparable to those used for the treatment of ADHD, potently suppresses the most vigorous part of the behavioral repertoire of adolescent rats through stimulation of α -2 adrenoceptors.

There are striking parallels between the effect of methylphenidate described here and its therapeutic properties in ADHD. The effect of methylphenidate on social play behavior was immediate and not subject to tolerance or sensitization, which is also the case for its effects in ADHD (Elia *et al*, 1999; Solanto, 2000). In addition, methylphenidate suppressed social play after both subcutaneous and oral administration. This is important, because there is discussion about whether the effects of methylphenidate found in rodents can be translated to the human situation. There is a strong variation in bioavailability of the drug after different routes of administration, and methylphenidate doses that are used in rodents (often 5 mg/kg and higher) have behavioral and neurochemical consequences that can be very different to the low doses of the drug (typically in the range of 0.3–3.0 mg/kg) that are orally effective in humans (Gerasimov *et al*, 2000; Kuczenski and Segal, 2002; Arnsten and Dudley, 2005; Berridge *et al*, 2006).

**EFFECTS OF NORADRENALINE AND DOPAMINE RECEPTOR ANTAGONISTS
ON THE EFFECTS OF METHYLPHENIDATE AND ATOMOXETINE ON SOCIAL PLAY BEHAVIOR**

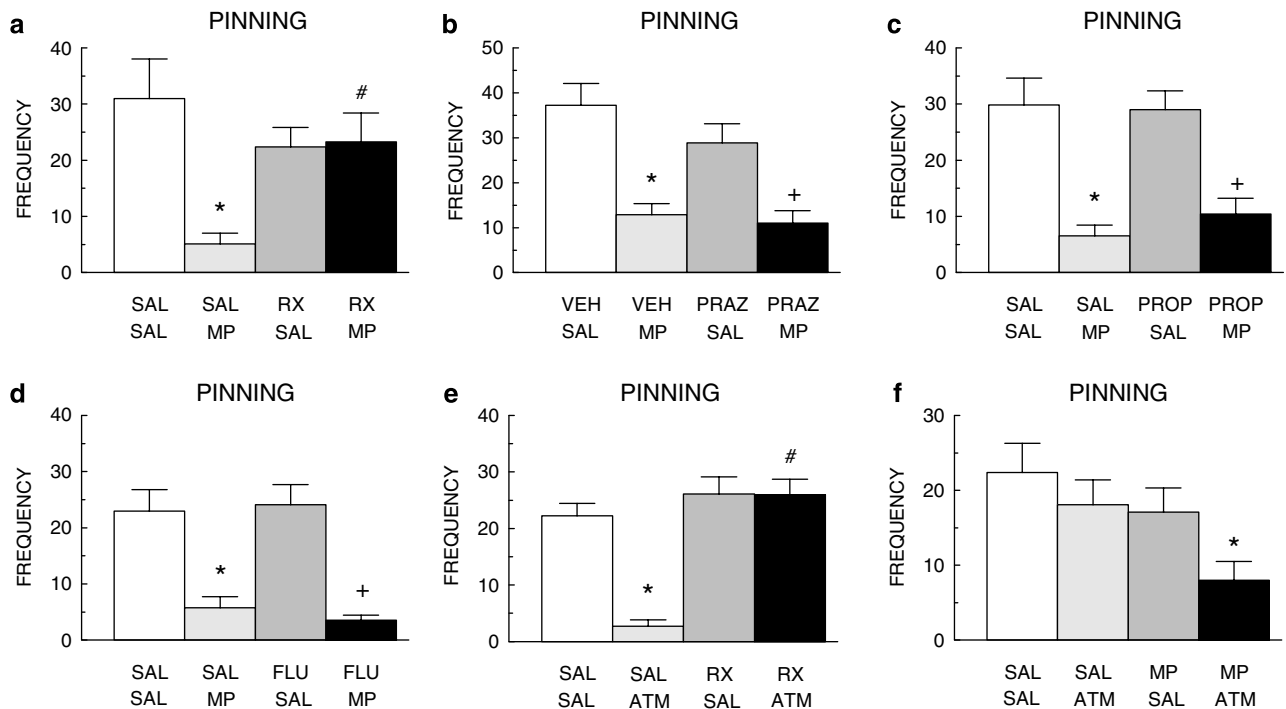


Figure 5 The effect of methylphenidate (MP; 1.0 mg/kg, s.c.) on pinning was blocked by the α -2 adrenoceptor antagonist RX821002 (RX; 0.2 mg/kg, i.p.) ((a) $F(\text{RX821002})_{1,31} = 1.13$, NS; $F(\text{methylphenidate})_{1,31} = 7.83$, $p < 0.001$; $F(\text{interaction})_{1,31} = 8.97$, $p < 0.001$), but not by the α -1 adrenoceptor antagonist prazosin (PRAZ; 0.3 mg/kg, i.p.) ((b) $F(\text{prazosin})_{1,31} = 2.13$, NS; $F(\text{methylphenidate})_{1,31} = 36.12$, $p < 0.0001$; $F(\text{interaction})_{1,31} = 0.86$, NS), the β -adrenoceptor antagonist propranolol (PROP; 3.0 mg/kg, i.p.) ((c) $F(\text{propranolol})_{3,27} = 0.23$, NS; $F(\text{methylphenidate})_{3,27} = 44.97$, $p < 0.0001$; $F(\text{interaction})_{3,27} = 0.57$, NS), or the dopamine receptor antagonist *cis*-(Z)-flupenthixol (FLU; 0.125 mg/kg, i.p.) ((d) $F(\text{cis-(Z)-flupenthixol})_{1,30} = 0.04$, NS; $F(\text{methylphenidate})_{1,30} = 47.58$, $p < 0.0001$; $F(\text{interaction})_{1,30} = 0.36$, NS). RX821002 (RX; 0.2 mg/kg, i.p.) also blocked the effect of atomoxetine (ATM; 1.0 mg/kg, i.p.) on pinning ((e) $F(\text{RX821002})_{1,30} = 35.16$, $p < 0.0001$; $F(\text{atomoxetine})_{1,30} = 18.43$, $p < 0.0001$; $F(\text{interaction})_{1,30} = 17.96$, $p < 0.0001$). Combined treatment with subeffective doses of methylphenidate (MP; 0.1 mg/kg, s.c.) and atomoxetine (ATM; 0.3 mg/kg, i.p.) suppressed pinning ((f) $F(\text{methylphenidate})_{1,63} = 5.94$, $p < 0.05$; $F(\text{atomoxetine})_{1,63} = 4.58$, $p < 0.05$; $F(\text{interaction})_{1,63} = 0.58$, NS). SAL = saline; VEH = vehicle (distilled water). *Significantly different from SAL/SAL (a, c–f) or VEH/SAL (b), $p < 0.05$ (Student–Newman–Keuls test); #significantly different from SAL/MP (a) or SAL/ATM (e), $p < 0.05$ (Student–Newman–Keuls test); + significantly different from PRAZ/SAL (b), PROP/SAL (c), or FLU/SAL (d), $p < 0.05$ (Student–Newman–Keuls test).

In the present study, we chose to administer methylphenidate subcutaneously, which results in a slower rate of absorption and lower peak levels of drug than intraperitoneal administration (Benet *et al*, 1996). We also verified that the effect of methylphenidate after subcutaneous administration was similar to that after oral administration. In fact, the effective oral doses of methylphenidate (1.0–3.0 mg/kg) are within the range used in humans (Elia *et al*, 1999; Kutcher *et al*, 2004).

It is now widely accepted that the effects of psychostimulant drugs in ADHD are not paradoxical or specific to humans. The reduction of hyperactivity induced by psychostimulants is observed in both ADHD patients and normal children (Rapoport *et al*, 1978, 1980), the cognitive effects of methylphenidate in normal subjects and ADHD patients are also similar (Tannock *et al*, 1989; Elliott *et al*, 1997; Solanto *et al*, 1997; Mehta *et al*, 2000, 2004; Aron *et al*, 2003; Hawk *et al*, 2003; Overtom *et al*, 2003; Pietras *et al*, 2003; Volkow *et al*, 2004), and comparable cognitive effects have also been found in rodents (Arnsten and Dudley, 2005; Berridge *et al*, 2006; van Gaalen *et al*, 2006; Bizot *et al*, 2007;

Eagle *et al*, 2007). Together, this indicates that testing the behavioral effects of low doses of psychostimulant drugs in rodents yields information that is relevant for humans, and can help understand their mechanism of action in ADHD.

The effect of methylphenidate on social play behavior is therefore likely related to its therapeutic effects in ADHD, so that the investigation of its neurobehavioral underpinnings may yield information about the mechanism of action of methylphenidate. Of course, our data do not indicate that any drug-induced reduction in social play by itself reflects a therapeutic effect in ADHD. There are many neural and behavioral mechanisms by which drug treatments alter social interaction, and there is a wide variety of drugs that can modulate social play, but not all of them are active in ADHD (Vanderschuren *et al*, 1997; Sivi, 1998). However, on the basis of our behavioral and pharmacological analysis of the effect of methylphenidate on social play behavior, we suggest that this effect is a reflection of part of its therapeutic effect in ADHD.

The effects of psychostimulant drugs on behavior have been suggested to be rate-dependent, that is, psychostimulants

invigorate behavior when activity levels are low, yet have suppressant effects when the intensity of behavior is high (Dews, 1958; Robbins and Sahakian, 1979). However, the finding that methylphenidate was just as potent in suppressing social play in animals showing minimal (after 0 h of social isolation), moderate (after 3.5 h of social isolation), or high levels of social play (after 24 h of social isolation) is inconsistent with this notion. It is also hard to reconcile the present findings with the view that psychostimulants enhance the ability of salient environmental stimuli to direct behavior (Robbins *et al*, 1983; Wyvell and Berridge, 2000). For a socially isolated animal, a conspecific is probably the most salient stimulus, and in this case one would expect that methylphenidate increases, rather than suppresses, social play. Moreover, this behavioral effect of psychostimulant drugs is mediated by dopaminergic neurotransmission (Wolterink *et al*, 1993), whereas the effect on social play behavior is not. Given that the effect of methylphenidate on social play is mediated by a noradrenergic mechanism, this effect could be related to the function of increases in tonic noradrenergic neurotransmission, that is, disengagement of a current task and search for alternative behaviors, which can become apparent as increased distractibility or response switching (Aston-Jones and Cohen, 2005). Quite clearly, however, increased distractibility or response switching would indicate that these low doses of methylphenidate would worsen, rather than ameliorate, ADHD symptoms. Therefore, we think that a more likely explanation for the effect of methylphenidate on social play lies in the observations that methylphenidate improves behavioral inhibition, as measured using a stop-signal reaction time task in rats (Eagle *et al*, 2007) and humans (Tannock *et al*, 1989; Aron *et al*, 2003). This effect on behavioral inhibition may be related to the notion that psychostimulant drugs cut short complex chains of behavior, such as social interactions (Lyon and Robbins, 1975). Interestingly, the pharmacological profile of the effects of psychostimulants in the stop-signal reaction time task is very similar to the effects found here on social play, because the effect of methylphenidate on stop task performance was not blocked by *cis*-(Z)-flupenthixol (Eagle *et al*, 2007) and mimicked by atomoxetine (Chamberlain *et al*, 2006; Robinson *et al*, 2007). Together, these findings suggest that the effects of methylphenidate on social play behavior are the result of increased behavioral inhibition, suppressing vigorous forms of behavior that are likely associated with diminished attention for the environment (Vanderschuren *et al*, 1997; Špinka *et al*, 2001). Increased behavioral inhibition also implies that the effect of methylphenidate would not be remediated by the presence of an untreated rat. Indeed, our observation that both the initiation to play as well as the responsivity to play initiation is reduced by methylphenidate is consistent with this notion.

Pharmacological analysis showed that the effects of methylphenidate and atomoxetine on social play were mediated through α -2 adrenoceptors. At first glance, this may seem counterintuitive, as α -2 adrenoceptors are usually thought to act as presynaptic autoreceptors (Starke *et al*, 1989). Blocking these receptors would then enhance, rather than inhibit, the effects of methylphenidate and atomoxetine. However, α -2 adrenoceptors are also located

postsynaptically, and there is substantial evidence that stimulation of postsynaptic α -2 adrenoceptors can improve prefrontal cognitive functions, such as working memory and behavioral inhibition (Aron *et al*, 2004; Arnsten and Dudley, 2005; Arnsten, 2006). The notion that methylphenidate and atomoxetine suppress social play through a similar mechanism was further supported by the observation that combined treatment with subeffective doses of these drugs reduced play. Our data, showing that the effect of methylphenidate on social play behavior is mediated by a noradrenergic mechanism but is independent of dopaminergic neurotransmission, add to the evidence that methylphenidate has dissociable effects on behavior through both dopaminergic and noradrenergic mechanisms. For example, the effects of methylphenidate on impulsive choice are mediated by both dopaminergic and noradrenergic neurotransmission (Van Gaalen *et al*, 2006; Robinson *et al*, 2007), whereas the effects of this drug on stop-signal reaction time task performance (Chamberlain *et al*, 2006; Eagle *et al*, 2007; Robinson *et al*, 2007) and social play (present study) are mediated by a noradrenergic mechanism.

Social play behavior is a natural reinforcer (Humphreys and Einon, 1981; Normansell and Panksepp, 1990; Calcagnetti and Schechter, 1992; Crowder and Hutto, 1992; Van den Berg *et al*, 1999b), so it may come as a surprise that stimulating dopaminergic neurotransmission with the dopamine receptor agonist apomorphine and the dopamine reuptake blocker GBR-12909 has no major effects on social play (see also Niesink and Van Ree, 1989; Siviyy *et al*, 1996). Dopaminergic neurotransmission plays a critical role in the incentive-motivational, but not the hedonic, pleasurable properties of drugs of abuse and natural rewards (Cardinal *et al*, 2002; Schultz, 2002; Wise, 2004; Berridge, 2007; Salamone *et al*, 2007). Our experimental setup, however, is such that incentive-motivational and pleasurable properties of social play cannot readily be distinguished. Perhaps the latter play a more prominent role in the observed behavior, because the presentation of a gregarious conspecific after a short period of social isolation may be pleasurable in itself, so heightened motivation has no major effects on social play. Our findings are reminiscent of the extensive literature on feeding, which shows that in a free-feeding situation, changes in dopamine neurotransmission do not alter food intake (even though changes in the motivation for food could in theory alter feeding), but in an operant setting, changes in dopaminergic neurotransmission determine whether food is perceived as attractive and how much effort the animal is willing to exert to obtain it (for reviews see Baldo and Kelley, 2007; Barbano and Cador, 2007; Berridge, 2007; Salamone *et al*, 2007).

In summary, our data show that methylphenidate suppresses vigorous social behavior through stimulation of α -2 adrenoceptors, probably by enhancing behavioral inhibition. However, even though we think that this effect of methylphenidate reflects its therapeutic properties in ADHD, the observation that this drug so powerfully suppresses social play also warrants caution. Social play behavior subserves social and cognitive development, and constitutive suppression of this behavior may lead to long-lasting behavioral deficits (Hol *et al*, 1999; Van den Berg *et al*, 1999a; Špinka *et al*, 2001). Even though the few

available studies show that repeated methylphenidate treatment does not cause residual changes in social interaction (Sproson *et al*, 2001; Bolaños *et al*, 2003), there is increasing evidence that repeated treatment with low doses of methylphenidate can have long-lasting effects on behavior (Brandon *et al*, 2001; Andersen *et al*, 2002; Bolaños *et al*, 2003; Carlezon and Konradi, 2004). Thus, further research into the persistent behavioral effects of chronic exposure to low doses of methylphenidate is warranted.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare that, except for income received from their primary employers, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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