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Contrasting Roles of Dopamine and Noradrenaline in the Motivational Properties of Social Play Behavior in Rats

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Social play behavior, abundant in the young of most mammalian species, is thought to be important for social and cognitive development. Social play is highly rewarding, and as such, the expression of social play depends on its pleasurable and motivational properties. Since the motivational properties of social play have only sporadically been investigated, we developed a setup in which rats responded for social play under a progressive ratio schedule of reinforcement. Dopaminergic neurotransmission plays a key role in incentive motivational processes, and both dopamine and noradrenaline have been implicated in the modulation of social play behavior. Therefore, we investigated the role of dopamine and noradrenaline in the motivation for social play. Treatment with the psychostimulant drugs methylphenidate and cocaine increased responding for social play, but suppressed its expression during reinforced play periods. The dopamine reuptake inhibitor GBR-12909 increased responding for social play, but did not affect its expression, whereas the noradrenaline reuptake inhibitor atomoxetine decreased responding for social play as well as its expression. The effects of methylphenidate and cocaine on responding for social play, but not their play-suppressant effects, were blocked by pretreatment with the dopamine receptor antagonist α-flupenthixol. In contrast, pretreatment with the α2-adrenoceptor antagonist RX821002 prevented the play-suppressant effect of methylphenidate, but left its effect on responding for social play unaltered. In sum, the present study introduces a novel method to study the incentive motivational properties of social play behavior in rats. Using this paradigm, we demonstrate dissociable roles for dopamine and noradrenaline in social play behavior: dopamine stimulates the motivation for social play, whereas noradrenaline negatively modulates the motivation for social play behavior and its expression.

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INTRODUCTION

The experience of social interactions during post-weaning development (ie, childhood and adolescence in humans, roughly equivalent to the juvenile and adolescent stages in mammals) is critical for social and cognitive development (Panksepp *et al*, 1984; Vanderschuren *et al*, 1997; Špinka *et al*, 2001; Pellis and Pellis, 2009; Graham and Burghardt, 2010; Baarendse *et al*, 2013a; Vanderschuren and Trezza, 2014). During this developmental period, a characteristic, highly vigorous form of social interaction, ie, social play behavior, is abundantly expressed in most mammalian species (Panksepp *et al*, 1984; Vanderschuren *et al*, 1997; Pellis and Pellis, 2009). Social play behavior is highly rewarding (Vanderschuren, 2010; Trezza *et al*, 2011) and its expression is modulated through neural systems also implicated in other types of reward, such as food, sex, and drugs of abuse (Trezza *et al*, 2010; Siviy and Panksepp, 2011). Reward processes consist of pleasurable, incentive motivational, and learning components, which are mediated through different neural mechanisms (Berridge *et al*, 2009). For example, opioids and endocannabinoids have been implicated in the pleasurable properties of rewards, whereas dopamine is thought to mediate their motivational aspects (Kelley, 2004; Barbano and Cador, 2007; Robbins and Everitt, 2007; Berridge, 2007; Salamone and Correa, 2012; Berridge and Kringelbach, 2015).

The pleasurable properties of social play behavior have previously been studied using place conditioning, in which young rats develop a preference for an environment associated with social play if the play encounter is perceived as pleasurable (Calcagnetti and Schechter, 1992; Crowder and Hutto, 1992; Douglas *et al*, 2004; Thiel *et al*, 2008; Trezza *et al*, 2009a; Peartree *et al*, 2012). However, the incentive motivational properties of social play have only been sporadically investigated in the past (Mason *et al*, 1962; Humphreys

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and Einon, 1981; Normansell and Panksepp, 1990). Therefore, in order to be able to measure the motivational properties of social play behavior, we developed an operant conditioning task, in which rats responded for brief periods of social play under a progressive ratio (PR) schedule of reinforcement (Hodos, 1961; Richardson and Roberts, 1996). In this setup, observation of behavior during reinforced periods also allowed for the assessment of the expression of social play.

Previous studies have shown that social play behavior is modulated by dopaminergic and noradrenergic neurotransmission. For example, treatment with dopamine receptor agonists and antagonists alters the expression of social play behavior (Niesink and Van Ree, 1989; Siviy *et al*, 1996; Vanderschuren *et al*, 2008; Trezza and Vanderschuren, 2009). In addition, the stimulation of social play by endocannabinoids, ethanol, and nicotine depends upon dopamine receptor stimulation (Trezza and Vanderschuren, 2008, 2009; Trezza *et al*, 2009b). Administration of the α2-adrenoreceptor agonist clonidine and the α2-adrenoceptor antagonist RX821002 reduced and enhanced social play, respectively (Normansell and Panksepp, 1985; Siviy *et al*, 1994; Siviy and Baliko, 2000). Furthermore, amphetamine, methylphenidate, and the noradrenaline reuptake inhibitor atomoxetine reduced social play through stimulation of α2-adrenoceptors (Beatty *et al*, 1982; Vanderschuren *et al*, 2008; Achterberg *et al*, 2014). However, it is unknown whether dopamine and noradrenaline are involved in the motivational properties of social play behavior. On the basis of its well-known role in incentive motivational processes (Kelley, 2004; Barbano and Cador, 2007; Robbins and Everitt, 2007; Berridge, 2007; Salamone and Correa, 2012), it is likely that dopamine modulates the motivational properties of social play. Noradrenergic neurotransmission has traditionally been implicated in attention and arousal processes (Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005), rather than motivation or reward. However, emerging work in rodents (Ventura *et al*, 2008) and primates (Bouret and Richmond, 2015) has indicated that noradrenergic neurotransmission may also modulate reward processes. Indeed, our recent work implicates prefrontal, amygdala, and habenula noradrenaline in social play behavior (Achterberg *et al*, 2015), which hints at the possibility that limbic noradrenaline is involved in certain emotional aspects of this behavior.

In the present study, we therefore investigated whether dopamine and noradrenaline are involved in the motivational aspects of social play behavior. To this aim, we tested the effects of the dopamine/noradrenaline reuptake inhibitor methylphenidate, the monoamine reuptake inhibitor cocaine, the dopamine reuptake inhibitor GBR-12909, the noradrenaline reuptake inhibitor atomoxetine, the dopamine receptor antagonist alpha-flupenthixol, and the α2-adrenoceptor antagonist RX821002, alone or in combination, on responding for social play behavior under a PR schedule of reinforcement. We also assessed the expression of social play behavior during reinforced play periods. We hypothesized that dopaminergic neurotransmission is involved in the motivational properties of social play, and that by suppressing social play, increased noradrenaline neurotransmission reduces responding for social play.

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 $40 \times 26 \times 20$ cm ($l \times w \times h$) Macrolon cages under controlled conditions (temperature 20–21 °C, 60–65% relative humidity, and 12/12 h light cycle with lights on at 0700 h). Food and water were available *ad libitum*. All experiments were approved by the Animal Ethics Committee of Utrecht University and were conducted in accordance with Dutch laws (Wet op Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Drugs

Methylphenidate hydrochloride, cocaine hydrochloride (BUFA, Castricum, The Netherlands), atomoxetine hydrochloride, RX821002 hydrochloride (Tocris Bioscience, Bristol, UK), and α-flupenthixol dihydrochloride (Sigma-Aldrich, Schnelldorf, Germany) were dissolved in saline. GBR-12909 dihydrochloride (Sigma-Aldrich) was dissolved in MilliQ water. Methylphenidate, cocaine, and GBR-12909 were administered subcutaneously (s.c.). Atomoxetine, α-flupenthixol and RX821002 were administered intraperitoneally (i.p.). Drug doses and pretreatment intervals were based on previous studies (Baarendse *et al*, 2013a, b; Baarendse and Vanderschuren, 2012; Trezza and Vanderschuren, 2009; Vanderschuren *et al*, 2008; Achterberg *et al*, 2014). Drug doses were calculated as salt. Drugs were administered 30 min before testing, except when methylphenidate or cocaine treatment was combined with α-flupenthixol or RX821002 treatment, in which case α-flupenthixol and RX821002 were administered 30 and 15 min before methylphenidate or cocaine administration, respectively. In view 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.

Apparatus

Behavioral testing was conducted in an operant conditioning chamber (Med Associates, Georgia, VT, USA) divided into two equally sized compartments ($25 \times 30 \times 25$ cm, $l \times w \times h$). The compartments were separated by a Plexiglas wall with 42 small holes (diameter, $\varnothing = 0.5$ cm) and an automated metal door in the middle. Both compartments had a metal grid floor and a Plexiglas lid which contained a house light (2 W). One compartment was equipped with two 4.8-cm-wide retractable levers, located on opposite sides of the compartment. Above each lever was a cue light (2.5 W). One lever was designated as the active lever and the other as the inactive lever; allocation of the left or right lever as active was counterbalanced between animals. Experimental events and data recording were controlled using Med PC software (Med Associates).

Operant Conditioning

All experiments were performed under red light conditions. Animals were randomly paired with a test partner from

another home cage. Animals in a test pair did not differ by more than 10 g in body weight at the start of the experiment. A test pair consisted of one experimental animal and its stimulus partner. At 24 days of age, test pairs were habituated to the test cage for 10 min. During the habituation session, the animals could freely explore the entire apparatus. After the habituation session, animals were isolated for 24 h/day for 5 consecutive days/week, except in the first validation experiment, in which we also included a group of animals isolated for 2 h/day for 5 days/week. Next, the animals received two shaping sessions on two consecutive days. During these shaping sessions, the cue light was presented, the lever retracted and the door opened when the experimental animal approached the active lever. Rats were allowed to interact for 2 min after which the door closed and each rat was placed back into its starting compartment by the experimenter. This procedure was repeated seven times in each shaping session. In addition, if an animal did not perform any active lever presses during acquisition sessions, it received an additional shaping session later that day or on the next day.

On the fourth day, the lever pressing sessions (20 min) commenced under a fixed ratio (FR)-1 schedule of reinforcement. Under this FR-1 schedule of reinforcement, each active lever press resulted in presentation of the cue light, retraction of both levers, and opening of the door, after which animals were allowed to freely interact for 2 min. After 2 min, the door automatically closed and the house light was illuminated during a 25 s inter-trial interval. During this interval, the experimenter placed each rat back into its starting compartment. After acquisition of the task under the FR-1 schedule (ie, when an animal obtained at least six out of eight possible rewards on two consecutive days), a PR schedule of reinforcement was introduced. Under this schedule, the animals had to meet a response requirement on the active lever that progressively increased after every earned reward (1, 2, 4, 6, 9, 12, 15, 25, etc.; Hodos, 1961; Richardson and Roberts, 1996). When rats met the response requirement, the cue light was illuminated, both levers retracted and the door opened for 1 min, during which the animals could freely interact. A PR session continued until an animal failed to obtain a reward within 10 min. Animals received one session per day, for 5 consecutive days/week. During the other 2 days/week animals were socially housed with their original cagemates. After responding had stabilized, defined as obtaining at least six rewards on three consecutive days with a variation of no more than two rewards, drug treatment started according to a Latin square design. Inactive lever presses were recorded, but had no programmed consequences.

During earned social interactions, behavior of the playing rats was assessed online using the Observer 5.1 software (Noldus Information Technology B.V., The Netherlands). In addition to the online analysis, behavior of the animals was recorded using a camera with zoom lens, video tape recorder, and television monitor. Three behavioral elements were scored (Panksepp *et al*, 1984; Vanderschuren *et al*, 1997; Trezza *et al*, 2010). (1) Frequency of pinning: one animal lying with its dorsal surface on the floor with the other animal standing over it. (2) Frequency of pouncing: one animal attempts to nose/rub the nape of the neck of the partner, which is an index of play solicitation (Supplementary Figure 1). Pinning and pouncing frequencies are considered the most characteristic parameters of social play behavior in rats (Panksepp and Beatty, 1980; Vanderschuren *et al*, 1997). (3) Time spent on social exploration: one animal sniffing or grooming any part of the partner's body. This is a measure of general social interest.

Statistical Analysis

Data were analyzed using SPSS software 15.0 for Windows and expressed as mean ± SEM. To correct for differences in earned social interaction time, the frequency of pinning and pouncing and the duration of social exploration during operant conditioning were calculated per min or as a percentage of the interaction time, respectively. Pinning, pouncing, social exploration, rewards obtained and inactive lever presses were analyzed using a paired Student's *t*-test with isolation time as within-subjects factor or using a repeated measures ANOVA with drug/dose as within-subjects factor followed by a paired Student's *t*-test when appropriate. Breakpoints under the PR schedule of reinforcement, ie, the highest number of lever presses made for a single reward in a session, are derived from an escalating curve, which violates the homogeneity of variance. Therefore, breakpoints were analyzed using the non-parametric Friedman test, followed by a *post hoc* Wilcoxon signed ranks test when appropriate, or using a Wilcoxon signed ranks test when only two groups were compared.

RESULTS

Validation of the Operant Conditioning Task

To verify that our operant conditioning task was sensitive to differences in social motivation, we compared rats that were socially isolated for 2 or 24 h, since these isolation periods are known to induce moderate and maximal increases in social play behavior, respectively (Niesink and van Ree, 1989; Vanderschuren *et al*, 1995, 2008). All rats acquired the task, ie, pressed the active lever for the opportunity for a social interaction under the FR-1 schedule of reinforcement. However, only after 24 h of isolation did all tested animals (6/6) reach performance criterion under the FR-1 schedule of reinforcement within 8 days of training, whereas only one-third (2/6) of the animals isolated for 2 h reached criterion (data not shown). Next, a group of rats was trained under the PR schedule of reinforcement, and tested after either 2 or 24 h of social isolation in a within-subjects design. After 24 h isolation, the rats obtained more rewards ($t = 5.15$, $df = 13$, $p < 0.001$) (Figure 1a), reached a higher breakpoint ($Z = -2.97$, $p = 0.003$) (Figure 1b), pinned more ($t = 3.82$, $df = 13$, $p = 0.002$; Figure 1c) than after 2 h of social isolation. Social exploration ($t = -0.19$, $df = 13$, $p = 0.85$; Figure 1d) and inactive lever presses (Supplementary Table 1) were not different after 2 or 24 h of social isolation.

Methylphenidate and Cocaine Enhance Operant Responding, but Reduce Social Play Behavior

Treatment with methylphenidate (1–3 mg/kg) enhanced the number of rewards obtained ($F_{treatment}(2,10) = 19.94$,

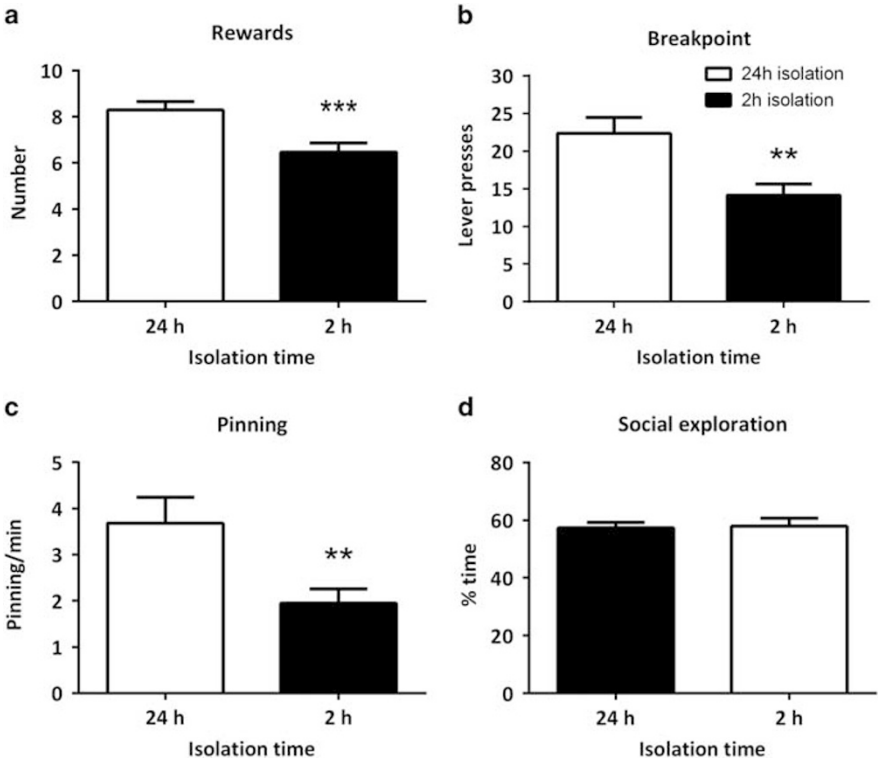

Figure 1 Effect of social isolation duration on responding for social play behavior. After 24 h of social isolation, rats obtained more rewards (a) and reached a higher breakpoint (ie, the largest number of lever presses made for a single reward) (b) than after 2 h of social isolation. Frequency of pinning was higher after 24 h of isolation (c), whereas social exploration did not differ as a result of isolation (d). $n = 14$; all rats were tested after both 2 and 24 h of social isolation. Data are presented as mean+SEM. $^{**}p < 0.01$, $^{***}p < 0.001$.

$p < 0.001$), the breakpoint ($X^2 = 8.27$, df = 2, $p = 0.02$) (Figure 2a and b), but not inactive lever presses (Supplementary Table 1). However, methylphenidate treatment decreased the frequency of pinning ($F_{\text{treatment}}(2,10) = 65.97$, $p < 0.001$) (Figure 2c) and increased the duration of social exploration ($F_{\text{treatment}}(2,10) = 8.73$, $p = 0.01$) (Figure 2d). Treatment with cocaine (5–10 mg/kg) enhanced the number of rewards obtained ($F_{\text{treatment}}(3,12) = 3.64$, $p < 0.05$) (Figure 2e), the breakpoint ($X^2 = 7.89$, df = 3, $p < 0.05$) (Figure 2f), but not inactive lever presses (Supplementary Table 1). Cocaine treatment decreased the frequency of pinning ($F_{\text{treatment}}(3,12) = 4.36$, $p = 0.03$) (Figure 2g), and did not affect the duration of social exploration ($F_{\text{treatment}}(3,12) = 1.02$, $p = 0.42$) (Figure 2h).

Selective Inhibition of Dopamine or Noradrenaline Reuptake Differentially Affects Operant Responding and Social Play

To investigate the role of dopamine and noradrenaline neurotransmission in responding for social play separately, we treated rats with the dopamine reuptake inhibitor GBR-12909 or the noradrenaline reuptake inhibitor atomoxetine. Treatment with GBR-12909 (3–10 mg/kg) increased the number of rewards obtained ($F_{\text{treatment}}(2,20) = 5.49$, $p = 0.01$) (Figure 3a) and the breakpoint ($X^2 = 8.26$, df = 2, $p = 0.02$) (Figure 3b), but not inactive lever presses (Supplementary Table 1). GBR-12909 treatment did not affect pinning ($F_{\text{treatment}}(2,20) = 2.54$, $p = 0.10$) (Figure 3c) or social exploration ($F_{\text{treatment}}(2,20) = 0.95$, $p = 0.41$) (Figure 3d).

Administration of atomoxetine (1–3 mg/kg) reduced the number of rewards obtained ($F_{\text{treatment}}(2,14) = 48.31$, $p < 0.001$) (Figure 3e), the breakpoint ($X^2 = 15.00$, df = 2, $p < 0.001$) (Figure 3f) and inactive lever presses (Supplementary Table 1). Atomoxetine treatment reduced pinning ($F_{\text{treatment}}(2,14) = 9.65$, $p = 0.002$) (Figure 3g) but not social exploration ($F_{\text{treatment}}(2,14) = 2.01$, $p = 0.17$) (Figure 3h).

Doubly Dissociable Roles for Dopamine and Noradrenaline Receptors in the Effects of Methylphenidate on Operant Responding and Social Play Expression

The data presented above, combined with our previous work (Vanderschuren *et al*, 2008; Achterberg *et al*, 2015) suggest that the effects of methylphenidate on the motivation for and the expression of social play are the result of increases in dopamine and noradrenaline neurotransmission, respectively. To investigate this possibility directly, we assessed the effect of methylphenidate on social play motivation and expression after pretreatment with the dopamine receptor antagonist α-flupenthixol and the α2-adrenoceptor antagonist RX821002, respectively. At the doses used, α-flupenthixol and RX821002 had no effect on the parameters measured, albeit that treatment with a higher dose of α-flupenthixol reduced responding for social play, but not pinning or social exploration (Supplementary Figures 2 and 3).

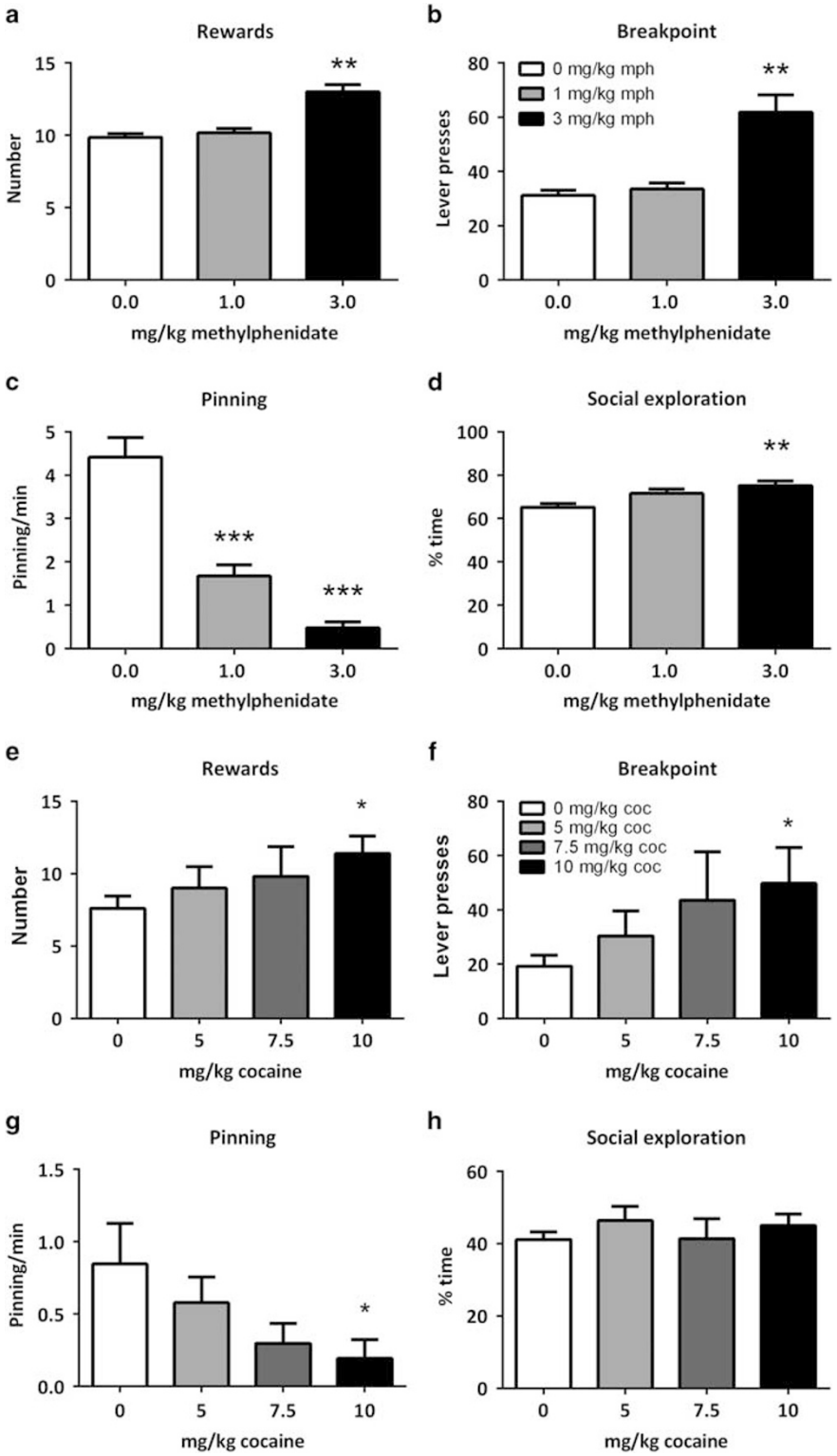

Figure 2 Methylphenidate (mph; $n=6$) and cocaine (coc; $n=5$) enhanced operant responding, but inhibited the expression of social play. Treatment with methylphenidate (1–3 mg/kg, s.c.) and cocaine (5–10 mg/kg, s.c.) enhanced the number of rewards obtained (a, e) and the breakpoint (b, f). Both treatments reduced the frequency of pinning (c, g). Methylphenidate enhanced, while cocaine did not affect, the time spent on social exploratory behavior (d, h). Data are presented as mean+SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$, relative to saline (0 mg/kg mph/coc) treatment.

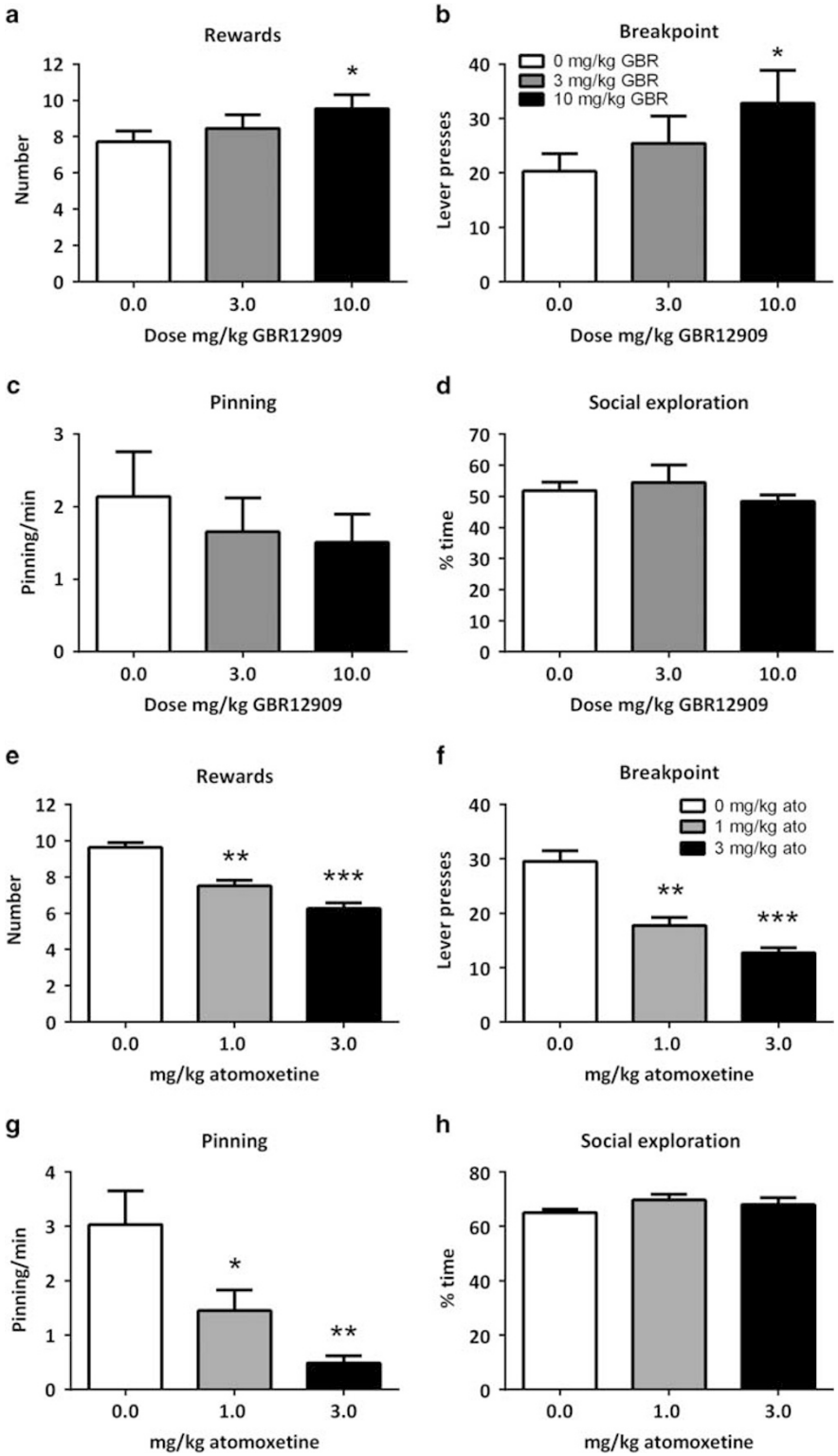

Figure 3 The effect of GBR-12909 (GBR; $n = 11$) and atomoxetine (ato; $n = 8$) on operant responding for social play behavior. Treatment with GBR-12909 (3–10 mg/kg, s.c.) enhanced responding for social play. GBR-12909 increased the number of rewards obtained (a) and the breakpoint (b). Administration of GBR-12909 did not affect the frequency of pinning (c), or the time spent on social exploration (d). Treatment with atomoxetine (1–3 mg/kg, i.p.) reduced operant responding and social play behavior. The number of rewards obtained was reduced (e) and the breakpoint was lower (f). In addition, the frequency of pinning (g) was reduced. The time spent on social exploration was unaffected (h). Data are presented as mean+SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, relative to saline (0 mg/kg GBR/ato) treatment.

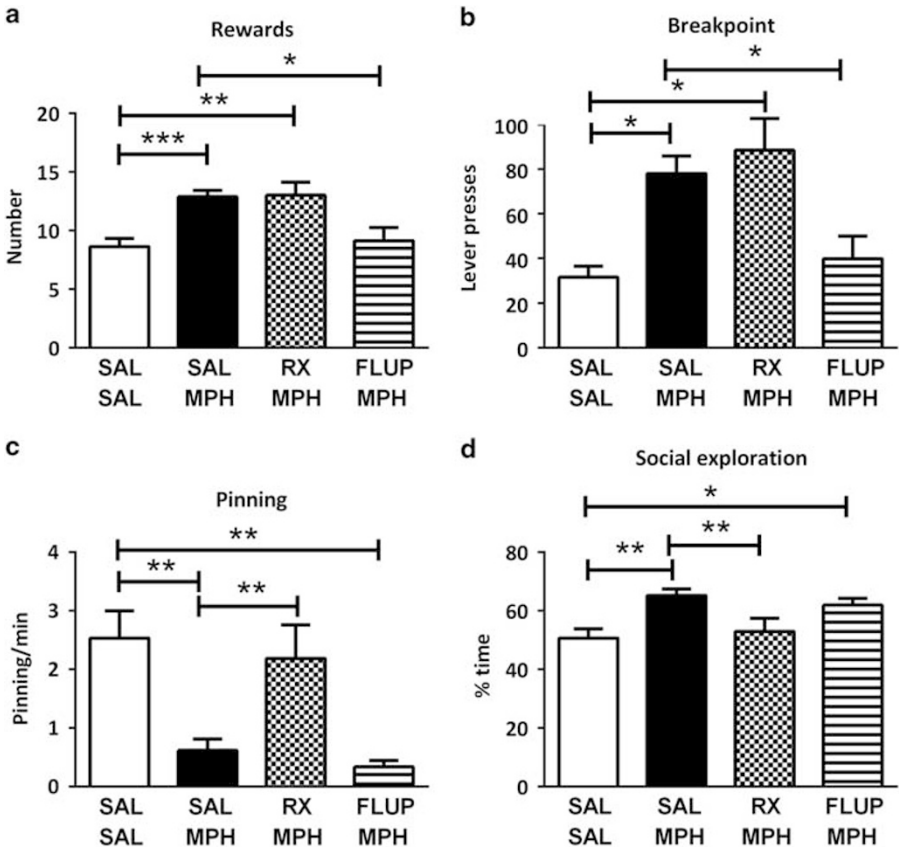

Figure 4 A double dissociation in the effect of methylphenidate on operant responding for social play behavior ($n = 8$). Methylphenidate (MPH; 3 mg/kg, s.c.) increased the number of obtained rewards (a) and the breakpoint (b); this effect was prevented by pretreatment with α-flupenthixol (FLUP; 0.125 mg/kg, i.p.) but not RX821002 (RX; 0.2 mg/kg, i.p.). Methylphenidate reduced the frequency of pinning (c) and increased the time spent on social exploration (d); this effect was prevented by pretreatment with RX821002, but not α-flupenthixol. SAL, saline. Data are presented as mean+SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Treatment with methylphenidate, in combination with α-flupenthixol, RX821002 or vehicle, affected the number of rewards obtained ($F_{\text{treatment}}(3,21) = 10.51$, $p < 0.001$), break point ($X^2 = 13.50$, df = 2, $p = 0.004$), pinning ($F_{\text{treatment}}(3,21) = 10.09$, $p = 0.002$), and social exploration ($F_{\text{treatment}}(3,21) = 5.07$, $p = 0.002$), but not inactive lever presses (Supplementary Table 1). *Post hoc* tests showed that, consistent with the previous experiment, 3 mg/kg methylphenidate increased the number of rewards obtained and breakpoint, decreased pinning and increased social exploratory behavior. Pretreatment with RX821002 (0.2 mg/kg) did not antagonize the increase in rewards obtained and breakpoint induced by methylphenidate, but it counteracted the effects of methylphenidate on pinning and social exploration. In contrast, pretreatment with α-flupenthixol (0.125 mg/kg) antagonized the effects of methylphenidate on rewards obtained and breakpoint, but not the effects of methylphenidate on pinning and social exploration (Figure 4a-d).

Cocaine Enhances Operant Responding via Dopaminergic Neurotransmission, but its Effect on Expression of Social Play is Dopamine-Independent

On the basis of the data presented above, we reasoned that the effect of cocaine on responding for social play are mediated by dopaminergic neurotransmission. In contrast, we have recently shown that the play-suppressant effect of cocaine is not altered by pretreatment with the dopamine receptor antagonist α-flupenthixol (Achterberg *et al*, 2014). We therefore tested whether pretreatment with α-flupenthixol influences the effect of cocaine on operant responding, and the expression of social play during reinforced periods.

Treatment with cocaine, in combination with α-flupenthixol or vehicle, affected the number of rewards obtained ($F_{\text{treatment}}(3,18) = 21.53$, $p < 0.001$), breakpoint ($X^2 = 13.57$, df = 3, $p = 0.004$), pinning ($F_{\text{treatment}}(3,18) = 10.74$, $p = 0.008$) but not social exploration ($F_{\text{treatment}}(3,18) = 0.45$, $p = 0.72$) or inactive lever presses (Supplementary Table 1). *Post hoc* tests revealed that treatment with 10 mg/kg cocaine increased rewards obtained and breakpoint, decreased pinning and did not affect social exploratory behavior. Pretreatment with α-flupenthixol (0.125 mg/kg, i.p.) antagonized the effects of cocaine on rewards obtained and breakpoint, but not the effect of cocaine on pinning (Figure 5a-d).

All drug treatments tested altered pinning and pouncing in the same direction (for pouncing data see Supplementary Figure 1).

DISCUSSION

An Operant Conditioning Task for Social Play

The purpose of this study was to investigate the role of dopamine and noradrenaline in the incentive motivational

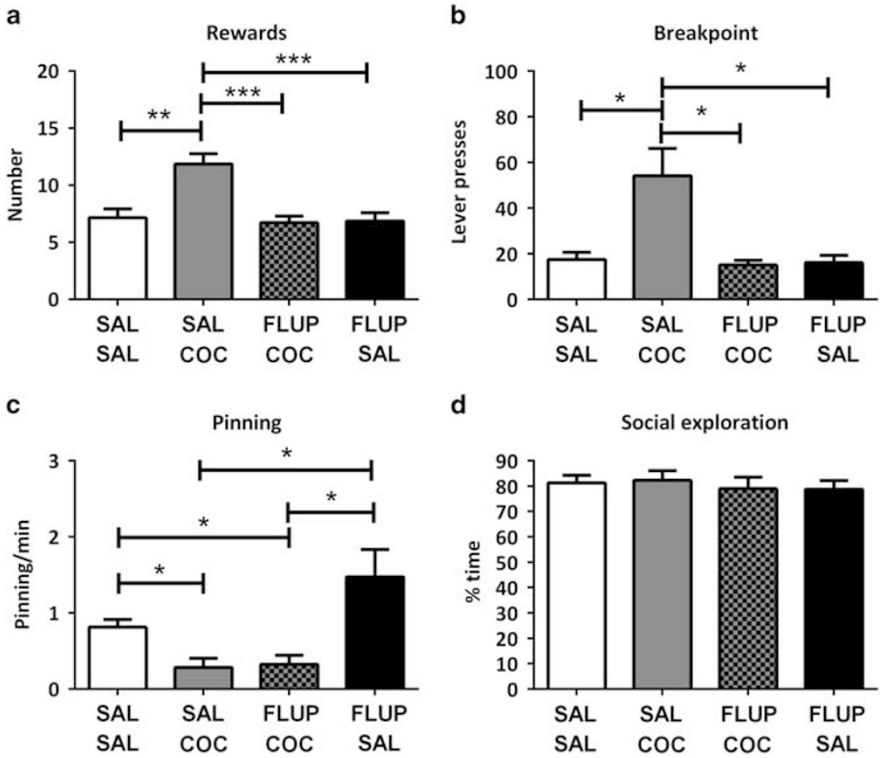

Figure 5 Cocaine enhances responding for social play behavior via dopaminergic neurotransmission ($n=7$). Cocaine (COC; 10 mg/kg, s.c.) increased the number of obtained rewards (a) and the breakpoint (b). This effect was prevented by pretreatment with α-flupenthixol (FLUP; 0.125 mg/kg, i.p.). Cocaine reduced the frequency of pinning (c) but did not affect the time spent on social exploration (d). The effect of cocaine on pinning was not altered by pre/treatment with α-flupenthixol. SAL, saline. Data are presented as mean+SEM. $*p<0.05$, $**p<0.01$, $***p<0.001$.

properties of social play behavior in rats. To that aim, we developed an operant conditioning task, in which rats were trained to lever press under a PR schedule of reinforcement for brief periods of social play. Responding under a PR schedule of reinforcement is a widely used method to measure the motivational properties of rewards (Hodos, 1961; Richardson and Roberts, 1996). In the past, lever pressing for play (with a human experimenter) was demonstrated in chimpanzees (Mason *et al*, 1962), and T-maze tasks have been used to assess motivational aspects of social play behavior in rats (Humphreys and Einon, 1981; Normansell and Panksepp, 1990). To the best of our knowledge, however, the present study is the first to show that rats are willing to lever press for social play reinforcement. This demonstration fits into a larger literature that has described reinforcing properties of a wide variety of social behaviors, including maternal, sexual, and aggressive behavior (Everitt, 1990; Fish *et al*, 2002; Trezza *et al*, 2011).

As a first step, to validate our approach, we investigated whether changing the duration of social isolation before training and testing would alter responding. There is a close relationship between the length of social isolation and the amount of social play behavior expressed during testing (Niesink and Van Ree, 1989; Vanderschuren *et al*, 1995, 2008). Therefore, we assumed that longer social isolation would enhance responding for social play as well as its expression. Indeed, animals isolated for 24 h acquired the operant task faster than animals isolated for 2 h. When the animals were subsequently tested after both isolation periods (ie, all animals were tested after 2 and 24 h of isolation), we found that 24 h of social isolation led to higher breakpoints, and that after 24 h of isolation, the rats earned more social play rewards. Moreover, levels of pinning and pouncing were higher after 24 h of social isolation, consistent with previous work (Niesink and Van Ree, 1989; Vanderschuren *et al*, 1995, 2008). These results show that it is possible to measure differences in social play motivation using an operant conditioning task.

These data support the assumption that social play behavior is the most important factor that drives responding in our operant conditioning task. First, there is a substantial literature to show that playful social interaction in rats is more rewarding in place conditioning and T-maze setups than interaction with a drug-treated partner that does show social investigation, but not social play, or with a physically confined partner (Humphreys and Einon, 1981; Calcagnetti and Schechter, 1992; Trezza *et al*, 2009a, b; Peartree *et al*, 2012). Second, as discussed above, our data show that isolation for 24 h enhances acquisition of the task as well as operant responding and social play behavior (but not social exploratory behavior) during testing, compared with a 2 h isolation period. Third, in an initial pilot experiment we found that rats trained in the task without a social partner (ie, responding on the active lever resulted in opening of the door and presentation of the cue light only) did not acquire responding under on a schedule that was more demanding than an FR1 schedule (ie, under FR2, FR5 or FR10 schedules of reinforcement), excluding the possibility that the animals were merely responding for door opening, cue light presentation, or access to another compartment of the

apparatus (data not shown). This latter observation is consistent with data showing that rats will initially press a lever for cue light presentation, but that this responding quickly extinguishes over days of testing (Deroche-Gamonet *et al*, 2002).

Dissociable Roles of Dopamine and Noradrenaline in Social Play Motivation and Expression

Consistent with the well-known role of dopamine, in particular in the nucleus accumbens, in incentive motivation (Kelley, 2004; Barbano and Cador, 2007; Robbins and Everitt, 2007; Berridge, 2007; Salamone and Correa, 2012), treatment with drugs that increase extracellular dopamine concentrations, ie, methylphenidate, cocaine, and GBR-12909, increased responding. Moreover, the effects of methylphenidate and cocaine on lever pressing were prevented by pretreatment with the dopamine receptor antagonist α-flupenthixol, whereas a higher dose of α-flupenthixol reduced responding for social play by itself. These effects were behaviorally specific, since responding on the inactive lever was not affected by these drug treatments, and the expression of social play during reinforced periods was not affected by GBR-12909 and α-flupenthixol, and reduced by methylphenidate and cocaine, consistent with our previous observations (Vanderschuren *et al*, 2008; Trezza and Vanderschuren, 2009; Achterberg *et al*, 2014). Changes in accumbens dopamine levels are known to affect the motivation for a reward, without markedly changing reward consumption (for reviews see: Kelley, 2004; Barbano and Cador, 2007; Robbins and Everitt, 2007; Berridge, 2007; Salamone and Correa, 2012). For example, administration of amphetamine into the nucleus accumbens enhances operant responding for food (Zhang *et al*, 2003), but not food consumption (Hanlon *et al*, 2004). Our observations are therefore consistent with the view that dopaminergic neurotransmission has a critical role in incentive motivation, that is, in the invigoration of appetitive approach towards a goal (Robbins and Everitt, 2007; Salamone and Correa, 2012), but not in reward consumption.

In keeping with previous work (Beatty *et al*, 1982; Ferguson *et al*, 2000; Vanderschuren *et al*, 2008; Achterberg *et al*, 2014), treatment with the psychostimulant drugs methylphenidate and cocaine reduced the expression of social play behavior during reinforced play periods, despite the fact that they enhanced lever pressing. At first glance, these findings suggest that behaviors other than social play serve as a reinforcer after psychostimulant treatment (Thiel *et al*, 2008). For example, rats treated with MDMA show increases in passive social behavior (Thompson *et al*, 2007), and rewarding properties of the tactile aspects of social interaction have indeed been demonstrated (Kummer *et al*, 2011). Although in our experiments, passive social interaction was hardly ever observed, the possibility that social exploratory behavior contributed to responding after treatment with cocaine or methylphenidate can as yet not be excluded.

Our observations resonate well with the notion that different components of reward behavior, such as pleasure, motivation, consumption, and learning are mediated by dissociable neural mechanisms (Kelley, 2004; Barbano and Cador, 2007; Robbins and Everitt, 2007; Berridge *et al*, 2009; Salamone and Correa, 2012; Berridge and Kringelbach, 2015). Indeed, although the effects of methylphenidate and cocaine on operant responding were mediated by dopaminergic neurotransmission, their effects on the expression of social play were not. In fact, the play-suppressant effect of methylphenidate was prevented by pretreatment with the α2-adrenoceptor antagonist RX821002 (see also Vanderschuren *et al*, 2008), which left its effect on operant responding unaltered. Together, these results demonstrate a double dissociation in the effects of methylphenidate on social play behavior. The increasing effects of methylphenidate on social play motivation are mediated through stimulation of dopamine receptors, whereas its suppressant effects on the expression of social play behavior rely on α2-adrenoceptor stimulation. It is therefore likely that treatment with methylphenidate, by virtue of its effects on nucleus accumbens dopaminergic neurotransmission (Gerasimov *et al*, 2000; Kuczenski and Segal, 2001; Bymaster *et al*, 2002), makes animals more motivated for social play, yet through its effects on prefrontal and subcortical limbic noradrenaline (Achterberg *et al*, 2015) makes animals less capable of actually performing the playful actions.

Treatment with the noradrenaline reuptake inhibitor atomoxetine reduced the expression of social play behavior as well as operant responding for social play. We have previously shown that the reduction in the expression of social play behavior induced by atomoxetine depends upon stimulation of α2-adrenoceptors (Vanderschuren *et al*, 2008). These results indicate that enhanced noradrenaline signaling reduces the motivation for, as well as the expression of social play behavior. Importantly, atomoxetine has been shown to increase extracellular prefrontal noradrenaline, prefrontal dopamine, and subcortical noradrenaline concentrations, but not to alter nucleus accumbens dopamine activity (Bymaster *et al*, 2002; Swanson *et al*, 2006). This likely explains why methylphenidate increases responding for social play, but atomoxetine does not. We have recently shown that atomoxetine, like methylphenidate, reduces social play behavior after infusion into the medial prefrontal cortex, basolateral amygdala, and habenula (Achterberg *et al*, 2015). On the basis of those data, we argued that increased noradrenaline activity in these regions interferes with certain cognitive and emotional aspects of social play. In the present context, this may mean that atomoxetine treatment renders animals less capable of performing social play activities, which may then, in the absence of changes in mesoaccumbens dopamine neurotransmission, lead to a reduction in the motivation to respond for social play. However, a direct effect of atomoxetine on the motivation for social play cannot be ruled out.

Concluding Remarks

The present study adds a new dimension to the analysis of social play behavior in rats, by introducing a method by which the incentive motivational properties of social play can be explicitly assessed. Furthermore, our data show that dopaminergic and noradrenergic signaling affect different aspects of social play behavior. Enhancement of endogenous dopamine levels increases the motivation for social play, but does not alter its expression. Increases in noradrenergic neurotransmission reduce the expression as well as the

motivation for social play. These data provide new insights into the intricate mechanisms by which catecholamines modulate social play behavior in rats. Elucidating the neural underpinnings of social behavior in the young may increase our understanding of normal, adaptive social development, and shed light on the pathophysiology of childhood and adolescent psychiatric disorders characterized by aberrant social behavior.

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