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Opioid modulation of social play reward in juvenile rats

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HIGHLIGHTS

- Morphine enhances the expression of social play behaviour but not the motivation to play.
- Morphine facilitates the development of social play-induced conditioned place preference (CPP).
- Naloxone decreases the motivation for social play behaviour, its expression and the development of social play-induced CPP.
- Opioid neurotransmission has an important role in the pleasurable and motivational properties of social play behaviour.

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ABSTRACT

Social play behaviour is a vigorous form of social interaction abundant during the juvenile and adolescent phases of life in many mammalian species, including rats and humans. Social play is thought to be important for social, emotional and cognitive development. Being a rewarding activity, the expression of social play depends on its pleasurable and motivational properties. Since opioids have been widely implicated in reward processes, in the present study we investigated the role of opioids in the pleasurable and motivational properties of social play behaviour in rats. To assess social play motivation, an operant conditioning setup was used in which rats responded for social play under a progressive ratio schedule of reinforcement. Treatment with the opioid receptor agonist morphine reduced responding for social play at the highest dose tested, likely due to its rate-limiting effects. Morphine treatment increased the expression of social play behaviour during reinforced periods. The acquisition of social play-induced conditioned place preference (CPP) in a subeffective conditioning protocol was enhanced by treatment with morphine. Morphine treatment alone also induced CPP. In contrast, antagonizing opioid receptors with naloxone reduced responding for social play, the expression of social play and blocked the development of social play-induced CPP. These data implicate opioid neurotransmission in both the pleasurable and the motivational aspects of social play behaviour in rats.

This article is part of the Special Issue entitled 'The neuropharmacology of social behavior: from bench to bedside'.

1. Introduction

Social play behaviour is a highly vigorous form of social interaction, containing components of other social behaviours in an altered and/or out-of-context manner (Pellis and Pellis, 2009; Vanderschuren et al., 1997). It is abundantly expressed throughout the juvenile and adolescent periods in most mammalian species, including rats and humans (Panksepp et al., 1984; Pellis and Pellis, 1998; Spear, 2000). Engaging in social play behaviour is thought to be important for emotional, social and cognitive development (Baarendse et al., 2013a; Potegal and Einon, 1989; Van den Berg et al., 1999) as it equips animals and humans with a rich behavioural repertoire, allowing them to flexibly adapt to challenges in the (social) environment (Špinka et al., 2001).

Social play behaviour is highly rewarding, as has been shown using operant and place conditioning setups (Trezza et al., 2011a; Vanderschuren, 2010; Vanderschuren et al., 2016). Indeed, the expression of social play is modulated through neural systems that have been implicated the rewarding properties of food, sex, and drugs of abuse (Trezza et al., 2010; Siviy and Panksepp, 2011; Vanderschuren et al., 2016). With regard to reward processes, it is well accepted that

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these can be dissociated into different components: its pleasurable ('hedonic') properties, incentive motivational properties, and effects on learning (Berridge et al., 2009). These component processes are mediated via different neural systems (Berridge et al., 2009). For example, dopamine is thought to be mainly involved in the motivational aspects of reward, whereas opioids are thought to influence its pleasurable properties, although opioids may be involved in incentive motivational processes as well (Barbano and Cador, 2007; Berridge et al., 2009; Kelly, 2004; Salamone and Correa, 2012).

There is a wealth of evidence to show that social behaviour, including social play, is modulated by opioid neurotransmission (for reviews, see Depue and Morrone-Strupinsky, 2005; Løseth et al., 2014; Paredes, 2014). For example, treatment with low doses of drugs that mimic the effects of endogenous opioids (e.g. morphine) enhances social play (Manduca et al., 2014b; Niesink and Van Ree, 1989; Normansell and Panksepp, 1990; Panksepp et al., 1985; Trezza et al., 2010; Trezza and Vanderschuren, 2008a,b; Vanderschuren et al., 1997; Vanderschuren et al., 1995a; b). Conversely, treatment with opioid receptor antagonists (e.g. naloxone) reduces social play (Beatty and Costello, 1982; Jalowiec et al., 1989; Niesink and Van Ree, 1989; Normansell and Panksepp, 1990; Panksepp et al., 1985; Siegel et al., 1985; Siegel and Jensen, 1986; Trezza and Vanderschuren, 2009). In addition, endogenous opioids in the nucleus accumbens modulate social play behaviour (Trezza et al., 2011b) and regulate context-specific social preferences in adolescent rats (Smith et al., 2018), while antagonizing μ-opioid receptors in the nucleus accumbens prevented the development of social play-induced conditioned place preference (CPP) (Trezza et al., 2011b). This latter observation indicates that opioids are involved in the pleasurable effects of social play, but it is not clear to which extent opioids are involved in the motivation for social play (Normansell and Panksepp, 1990; Vanderschuren et al., 2016).

In the present study, we therefore investigated whether opioids are involved in the motivational and pleasurable properties of social play behaviour. To measure the motivational aspects of social play behaviour, we used a recently developed operant conditioning task, in which rats respond on a lever under a progressive ratio schedule of reinforcement in order to obtain brief periods of access to a playful partner (Achterberg et al., 2016a; −2016b). Furthermore, we quantified the amount of social play the animals expressed during the opportunities to play the animals earned. In order to assess pleasurable aspects of social play, we investigated whether changes in opioid neurotransmission affected the acquisition of social-play induced CPP (Calcagnetti and Schechter, 1992; Trezza et al., 2009). In this setup, rats learn to associate a set of environmental cues with social play behaviour. It is commonly assumed that animals will develop a preference for the play-associated environment if the play encounter is perceived as pleasurable and if they are able to encode the context-reward association (Bardo and Bevins, 2000; Tzschentke, 2007). We hypothesized that opioids modulate both the pleasurable and motivational properties of social play behaviour.

2. Materials and methods

2.1. 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 with wood shavings, shelter and a wooden block. Animals were housed under controlled conditions (ambient temperature 20–21 °C, 60–65% relative humidity, and 12/12 h light cycle with lights on at 7.00 a.m.). Food and water were available *ad libitum*. All animals used were experimentally naïve. Experiments were carried out between 8.00 p.m. and 5.00 a.m. All experiments were approved by the Animal Ethics Committee of Utrecht University and were conducted in accordance with Dutch laws (Wet op de Dierproeven, 1996), European regulations (Guideline 86/609/EEC), and the ARRIVE guidelines.

2.2. Drugs

The opioid receptor agonist morphine (O.P.G. Utrecht, The Netherlands) and the opioid receptor antagonist naloxone (Tocris Cookson, Avonmouth, UK; Sigma-Aldrich, Schnelldorf, Germany) were dissolved in saline. Morphine and naloxone were administered subcutaneously (s.c.), 1 h and 30 min before testing, respectively. Drug doses and pre-treatment intervals were based on previous studies (Trezza and Vanderschuren, 2008a; b; Trezza et al., 2009). In view of the importance of the neck area in the expression of social play behaviour (Pellis and Pellis, 1987; Siviy and Panksepp, 1987), s.c. injections were administered in the flank.

2.3. Operant conditioning paradigm

2.3.1. Apparatus

Behavioural testing was conducted in an operant conditioning chamber (Med Associates, Georgia, VT, USA) divided into two equally sized compartments (25 × 30 × 25 cm, $l \times w \times h$). The compartments were separated by a Plexiglas wall with 42 small holes (Ø 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 (the 'lever pressing 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, Georgia, VT, USA).

2.3.2. Experimental procedure

The experiments were conducted as described in Achterberg et al. (2016a,b). Briefly, 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, consisting of one experimental animal and its stimulus partner, did not differ by more than 10 g in body weight at the start of the experiment. 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. 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 two minutes after which the door closed and each rat was placed back into its starting compartment by the experimenter. This procedure was repeated 7 times in each shaping session. If an animal did not perform any active lever presses during acquisition sessions, it received an additional shaping session in the afternoon.

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 (i.e., when an animal obtained at least six out of eight possible rewards on two consecutive days), a progressive ratio (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. Inactive lever presses were recorded, but had no programmed consequences. 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 3 cage-mates. 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. In all experiments, the stimulus animal received a saline injection unless otherwise specified.

2.3.3. Analysis of social play behaviour

During earned periods of social interaction, behaviour of the rats was assessed on-line using the Observer 5.1 software (Noldus Information Technology B.V., The Netherlands). In addition to the on-line analysis, behaviour of the animals was recorded using a camera with zoom lens, video tape recorder and television monitor. Three behavioural elements were scored (Panksepp et al., 1984; Trezza et al., 2010; Vanderschuren et al., 1997), by an experimenter blind to the treatment conditions.

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. Pinning and pouncing frequencies are considered the most characteristic parameters of social play behaviour in rats (Panksepp and Beatty, 1980).
3. Time spent in social exploration: one animal sniffing or grooming any part of the partner's body. This was used as a measure of general social interest.

2.4. Place conditioning paradigm

2.4.1. Apparatus

The place conditioning setup (TSE System, Bad Homburg, Germany) comprised eight boxes, each consisting of three compartments with removable Plexiglas lids. The two conditioning compartments were equally sized (30 cm × 25 cm x 30 cm; *l x w x h*) and separated by a third, neutral compartment (10 cm × 25 cm x 30 cm; *l x w x h*). The two conditioning compartments had different visual and tactile cues: one had black-and-white striped walls and a floor with wide metal mesh, and the other had black walls and a floor with fine metal mesh. The compartment with black walls had a white light (2 W) mounted on the Plexiglas lid, to achieve a comparable light intensity in both conditioning compartments. The middle compartment had white walls, a smooth floor, and a white light (2 W) on the lid. The position of the animal in the apparatus was monitored by an array of photo-beam sensors located 2.5 cm above the floor. The time spent in each compartment (in msec) was recorded by a computer. All experiments were performed in a dimly lit room, since testing under bright light conditions reduces the expression of social play behaviour (Vanderschuren et al., 1995c).

2.4.2. Experimental procedure

Place conditioning was performed as previously described (Achterberg et al., 2012, 2014; 2016b; Trezza et al., 2009b, 2011b). At 26 days of age (experimental day 1), each rat was placed in the middle compartment of the apparatus and pre-conditioning side preference was determined by allowing the rats to move freely in the three compartments for 15 min. On the basis of their preference scores, rats were assigned to a compartment in which they would be allowed social interaction during conditioning. A counterbalanced place conditioning design was used (Tzschentke, 2007; Veeneman et al., 2011), meaning that the pre-conditioning preference in each experimental group for the to-be social-paired or non-social paired side approximated 50%. Thus, based on their pre-conditioning performance, some of the rats were conditioned with social interaction in their preferred compartment, while some were conditioned in their non-preferred compartment. After the pre-conditioning test, the rats were individually housed to increase their motivation for social interaction and to facilitate the development of social play-induced CPP (Achterberg et al., 2012, 2014; 2016b; Niesink and Van Ree, 1989; Trezza et al., 2009b; Vanderschuren et al., 2008). Place conditioning began on day 2. On days 2, 4, 6, and 8, the rats were placed for 30 min in one compartment with an initially unfamiliar partner (social session) in the morning and were placed alone in the other compartment (non-social session) in the afternoon. On day 3, 5, 7, and 9 the order of the sessions was reversed. Social and non-social sessions were separated by at least three hours. Drugs were administered 1 h (morphine) or 30 min (naloxone) before the start of each social conditioning session. On day 10, the rats were placed in the middle compartment and were allowed to explore the entire apparatus for 15 min.

In the drug-induced place conditioning experiment, animals were subjected to the same conditioning schedule as for the social play-induced paradigm, but animals were alone on both sides of the apparatus. The control animals received a vehicle injection before placement in both compartments, whereas others received a morphine injection before placement on one side and a vehicle injection before placement on the other side of the apparatus (in a counter-balanced design).

In one experiment, a suboptimal place conditioning design was used. The animals were conditioned for four days only, as described above, following treatment with morphine or vehicle. On day 5, i.e., 24 h after the last conditioning session, rats were placed in the middle compartment and were allowed to explore the entire apparatus for 15 min.

The time spent in each compartment during this test was recorded to determine place preference.

2.5. Statistical analysis

Data, expressed as mean + SEM, were analysed using SPSS software 15.0 for Windows. Since the amount of time available for social interaction was dependent on the number of rewards earned, the frequency of pinning and pouncing during operant conditioning was calculated per minute of interaction time, and the duration of social exploration was calculated as a percentage of time. The data were analysed using a repeated measures ANOVA with drug dose as within-subjects factor followed by a paired Student's t-test when appropriate. Operant responding was analysed with lever, treatment and, depending on the experiment, isolation time, as a within-subjects factor. The breakpoints under the PR schedule of reinforcement are derived from an escalating curve, which violates the homogeneity of variance. Therefore, breakpoints were analysed using the non-parametric Friedman test, followed by a post-hoc Wilcoxon signed ranks test when appropriate. When analysing the difference between none or both animals treated in a test pair, Student's t-tests or, when analysing breakpoints, a Wilcoxon signed ranks test was used.

Place conditioning data were expressed as mean time spent in the social/drug paired and non-social/vehicle paired compartment + SEM. Place conditioning data were analysed using a two-way ANOVA, with compartment and treatment as factors, followed by paired Student's t-test when appropriate.

3. Results

3.1. Effects of treatment with morphine on responding for social play

At the highest dose tested, i.e., 3.0 mg/kg, treatment with the opioid receptor agonist morphine reduced responding for social play ($F_{treatment}(3,21) = 23.53$, $p < 0.001$). Although the animals discriminated

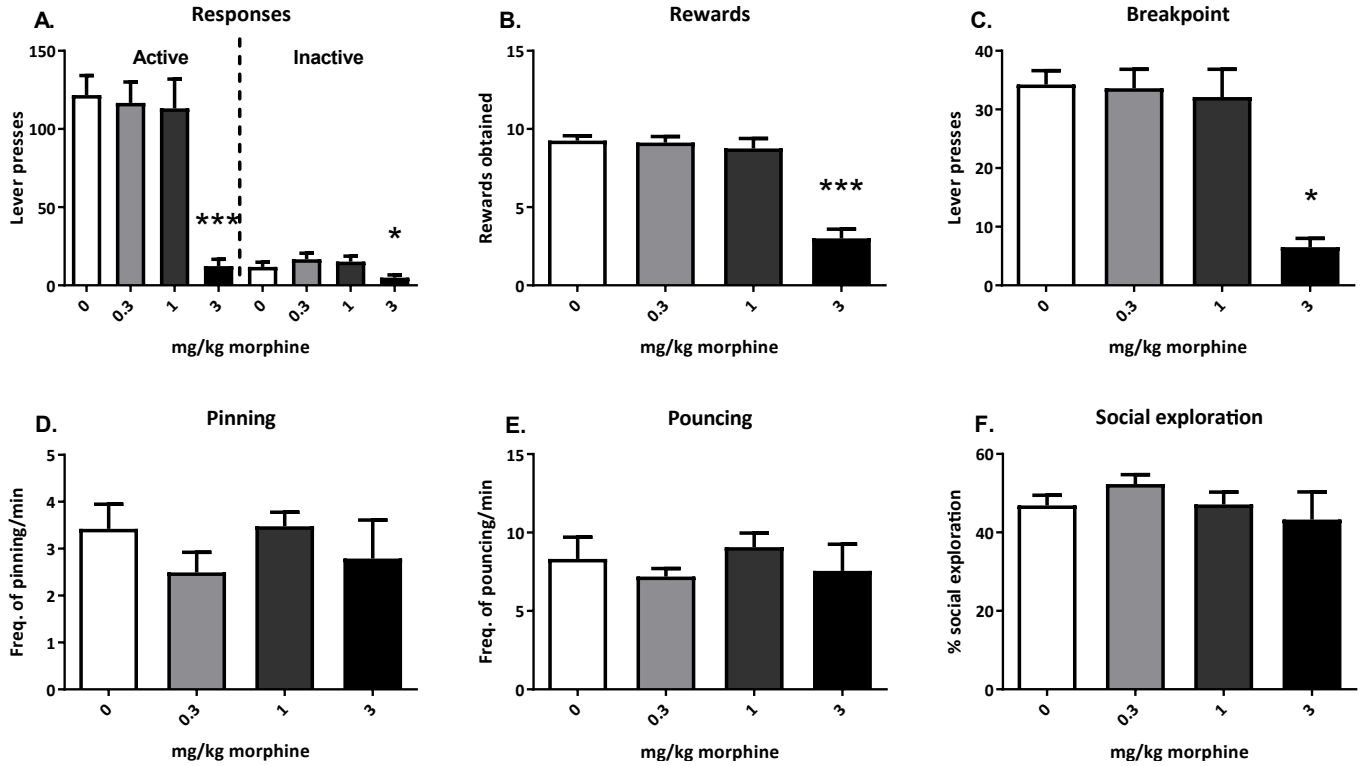

Fig. 1. The effect of the opioid receptor agonist morphine on responding for social play. The highest dose of morphine (0.3-1.0-3.0 mg/kg, n = 8 test pairs) reduced both active and inactive responses (A), rewards (B) and breakpoint (C), without affecting pinning (D), pouncing (E) social exploration (F). Data are presented as mean + SEM. *p < 0.05, ***p < 0.001, relative to vehicle treatment.

between the levers ($F_{lever}(1,7) = 84.33$, $p < 0.001$), the highest dose of morphine reduced the number of both active and inactive responses ($F_{lever*treatment}(3,21) = 14.56$, $p < 0.001$) (Fig. 1A). Furthermore, the number of rewards obtained as well as the breakpoint was reduced after treatment with the highest dose of morphine (rewards: $F_{treatment}(3,21) = 36.12$, $p < 0.001$; breakpoint: $X^2 = 15.64$, df = 3, p = 0.001) (Fig. 1B–C). Morphine treatment did not affect the frequency of pinning ($F_{treatment}(3,21) = 1.09$, p = 0.38), pouncing ($F_{treatment}(3,21) = 0.66$, p = 0.59) or the time spent on social exploration ($F_{treatment}(3,21) = 0.81$, p = 0.50) (Fig. 1D–F).

We next conducted an experiment were we treated none, only the test animal or both animals with 1 mg/kg of morphine. The number of animals treated with morphine (1.0 mg/kg) did not affect responding for social play ($F_{treatment}(2,10) = 1.61$, p = 0.25, n = 6). The animals discriminated between the levers ($F_{lever}(1,10) = 28.87$, p = 0.003), but no interaction effect between responding and the number of animals treated was observed ($F_{lever*treatment}(2,10) = 1.32$, p = 0.31) (Fig. 2A). The number of rewards obtained as well as the breakpoint was unaffected by the amount of animals treated with morphine (rewards: $F_{treatment}(2,10) = 0.91$, p = 0.44; breakpoint: $X^2 = 2.70$, df = 2, p = 0.26) (Fig. 2B–C). The number of animals treated did affect both the frequency of pinning ($F_{treatment}(2,10) = 7.25$, p = 0.01) and pouncing ($F_{treatment}(2,10) = 5.03$, p = 0.03) but not the time spent on social exploration ($F_{treatment}(2,10) = 2.64$, p = 0.12) (Fig. 2D–F). Post hoc analysis revealed that when both animals in the apparatus are treated with morphine, the frequency of pinning and pouncing significantly increased (pinning(0 vs 2 animals treated): $t(5) = -3.90$, p = 0.01; pouncing(0 vs 2 animals treated): $t(5) = -3.16$, p = 0.03). This was not the case when only the test animal was treated (pinning(0 vs 1 animals treated): $t(5) = -2.18$, p = 0.08; pinning(1 vs 2 animals treated): $t(5) = -1.75$, p = 0.14; pouncing(0 vs 1 animals treated): $t(5) = -1.87$, p = 0.12; pouncing(1 vs 2 animals treated): $t(5) = -1.35$, p = 0.23).

3.2. The effect of morphine treatment on the acquisition of social play-induced CPP

Both animals that were vehicle-treated, as well as morphine-treated animals spent significantly more time in the social-paired compartment compared to the non-social paired compartment ($F_{compartment}(1,28) = 29.86$, $p < 0.001$, $n_{morphine} = 8$ $n_{vehicle} = 8$ Fig. 3A). Morphine treatment did not affect the time spent in the social compartment compared to vehicle-treated animals ($F_{treatment}(1,28) = 0.02$, p = 0.97; $F_{compartment*treatment}(1,28) = 2.21$, p = 0.15).

To assess whether treatment with morphine itself was rewarding in juvenile rats, we tested the effect of the opiate on place conditioning (i.e., drug-induced conditioning without the presence of a social partner). The data showed that morphine treatment differentially affected the time spent in the drug-associated compartment ($F_{compartment*treatment}(1,44) = 3.75$, p = 0.02, $F_{compartment}(1,44) = 9.10$, p = 0.004; $F_{treatment}(1,44) = 1.46$, p = 0.70, $n_{morphine} = 8$, $n_{vehicle} = 16$, Fig. 3B). Post-hoc analysis revealed that animals spent significantly more time in the morphine-associated compared to the vehicle-associated compartment ($t_{morphine-veh}(7) = 3.75$, p = 0.007), whereas when animals received vehicle treatment in both compartments, they showed no preference for either compartment ($t_{veh-veh}(15) = 0.30$, p = 0.77).

To test whether morphine could enhance place conditioning with social play behaviour, a subeffective conditioning protocol was used. Rats spent significantly more time in the social-paired compartment ($F_{compartment}(1,40) = 13.10$, p = 0.001, $n_{morphine} = 10$ $n_{vehicle} = 12$, Fig. 3C). In addition, a trend towards a differential effect of morphine on the time spent in each compartment was found ($F_{compartment*treatment}(1,40) = 3.74$, p = 0.06). Post-hoc analyses showed that a subeffective conditioning protocol in combination with morphine-treatment in the play-paired compartment resulted in significantly more time spent in the play-paired

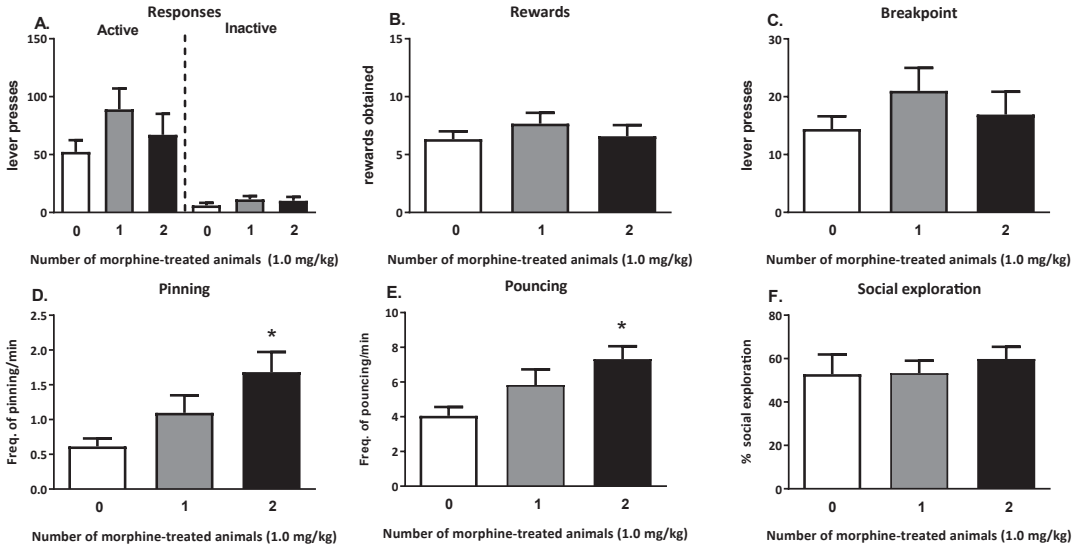

Fig. 2. Treatment of both animals in a test pair with morphine enhances the expression of social play behaviour without affecting motivation. Either 1,2 or none of the animals in a test pair were treated with a dose of morphine (1 mg/kg, n = 6 couples) that is known to enhance social play behaviour. Only when both animals in a test pair were treated animals increased the number of pins (D) and pounces (E). No effect of on motivation was found expressed as active and inactive responses (A), the number of rewards (B) and breakpoint (C). In addition, no effect on social exploration was found (F). Data are presented as mean + SEM. *p < 0.05, relative to none of the animals in a test pair treated with morphine.

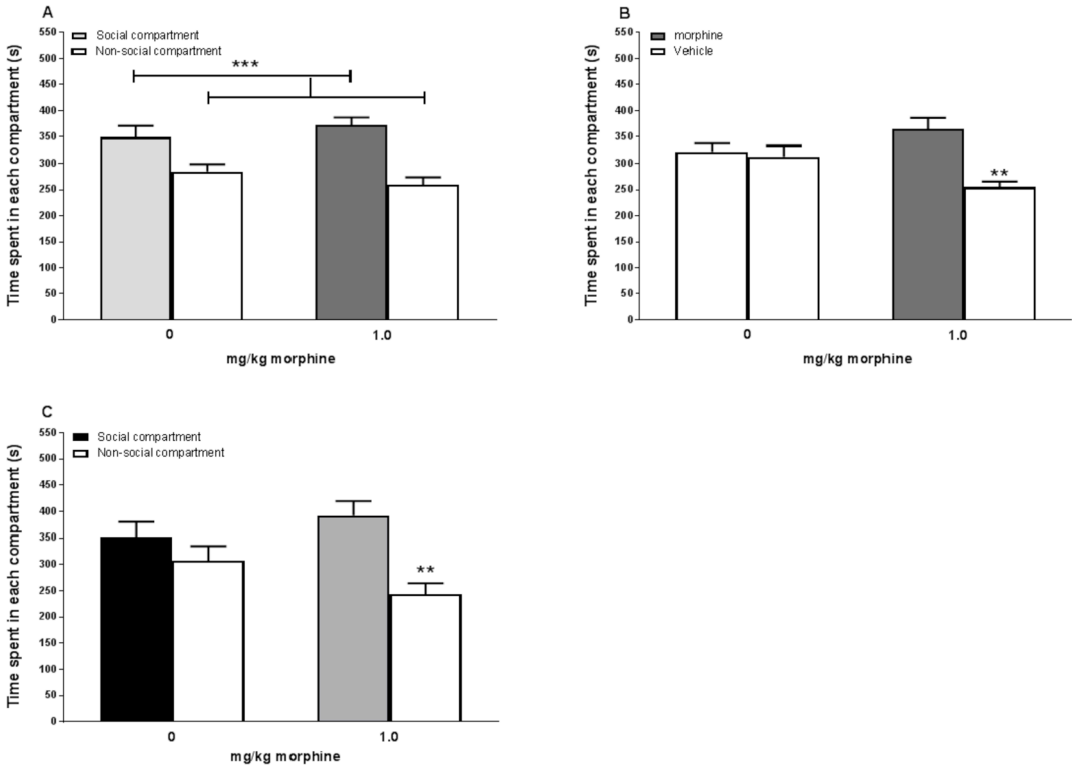

Fig. 3. The effect of morphine on social play-induced conditioned place preference. Morphine (1 mg/kg, n = 8) does not influence social play-induced conditioned place preference compared to vehicle (n = 8) when the standard (8-day) conditioning protocol is used (A), animals spent more time in the social play-associated compartment (grey bars) compared to non-social compartment (white bars). Morphine itself induces CPP (B, n = 8) whereas vehicle-treatment does not (n = 16); animals spent more time in the morphine-associated compartment (grey bar) compared to the vehicle-associated compartment (white bars). Morphine (1 mg/kg) enhances social play-induced place preference in a subeffective (4-day) conditioning protocol (C, $n_{morphine} = 10$ $n_{vehicle} = 12$). Animals spent more time in the social play-associated compartment in combination with morphine (black bar) compared to the non-social compartment (white bar). Also, four days of conditioning is insufficient to induce social play-induced CPP, animals spent an equal amount of time in the compartment associated social play + vehicle treatment (grey bar), compared to the non-social compartment (white bar). Data are presented as mean + SEM. **p < 0.01, ***p < 0.001.

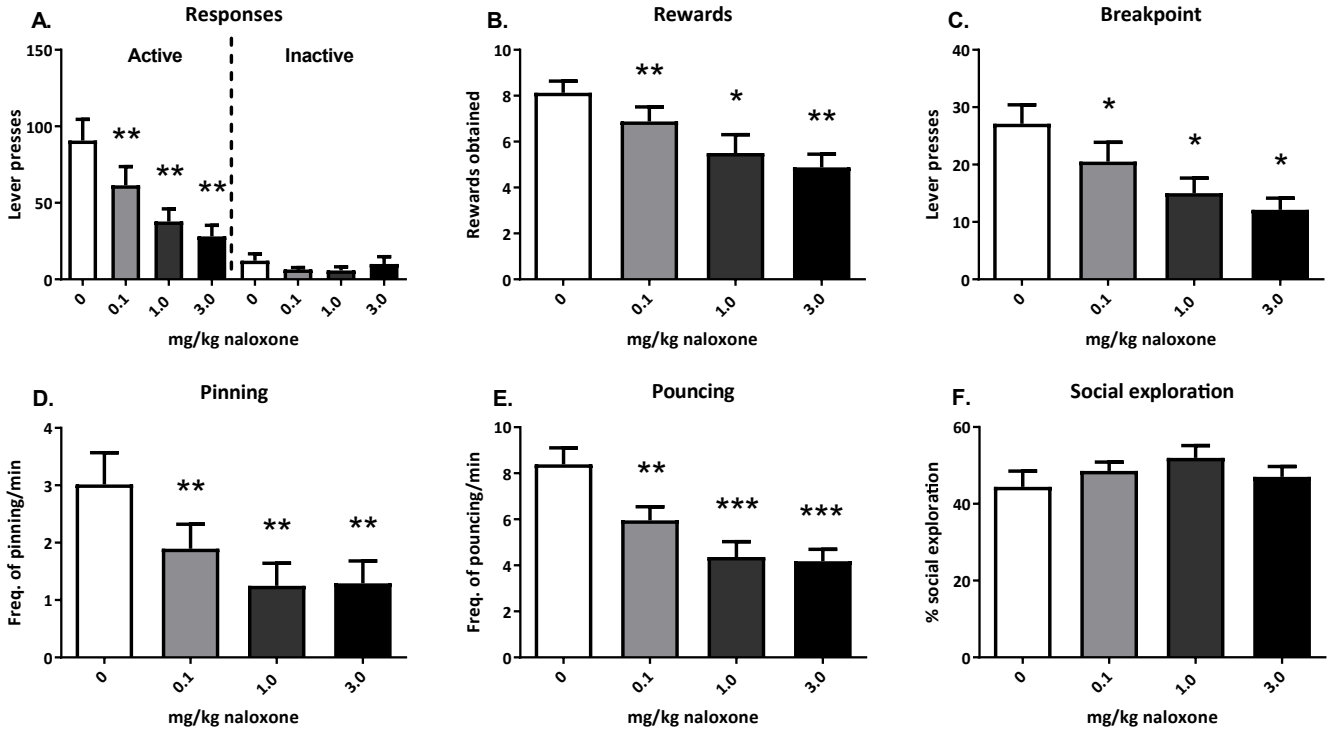

Fig. 4. The effect of the opioid receptor antagonist naloxone on operant responding for social play behaviour. Treatment with naloxone (0.1-1.0-3.0 mg/kg, n = 8 test pairs) reduced the number of active responses without affecting inactive responses (A). Breakpoint (B) and rewards obtained (C) were also reduced. Naloxone-treatment reduced the expression of social play behaviour, i.e. pinning (D) and pouncing (E) without affecting social exploration (F). Data are presented as mean + SEM. *p < 0.05, **p < 0.01, ***p < 0.001, relative to vehicle treatment.

compartment ($t_{morph}(9) = 3.34$, p = 0.009, Fig. 3C), whereas no difference between the compartments in the vehicle-treated rats was found ($t_{vehicle}(11) = 0.82$, p = 0.43).

3.3. Naloxone treatment reduced the motivation for social play

Animals treated with naloxone (0.1-1.0-3.0 mg/kg) showed reduced responding for social play under a PR schedule of reinforcement ($F_{treatment}(3,21) = 10.07$, p < 0.001, n = 8). The rats discriminated between the active and inactive lever ($F_{lever}(1,7) = 40.33$, p < 0.001). After treatment with naloxone, there was a significant, dose-dependent reduction in the number of active responses with no change in responses on the inactive lever ($F_{lever*treatment}(3,21) = 8.94$, p = 0.001) (Fig. 4A). Furthermore, the number of rewards obtained as well as the breakpoint was dose-dependently reduced (rewards: $F_{treatment}(3,21) = 5.94$, p = 0.004; breakpoint: $X^2 = 10.09$, df = 3, p = 0.02) (Fig. 4B–C). In addition to the reduction in operant responding, treatment with naloxone decreased the frequency of pinning ($F_{treatment}(3,21) = 10.48$, p < 0.001) and pouncing ($F_{treatment}(3,21) = 15.58$, p < 0.001) but did not affect the time spent on social exploration ($F_{treatment}(3,21) = 1.14$, p = 0.36) (Fig. 4D–F).

3.4. Naloxone treatment disrupted the acquisition of social play-induced place preference

Naloxone treatment affected the acquisition of social play-induced CPP in a dose-dependent manner ($F_{compartment*treatment}(3,120) = 25.27$, p < 0.001; $F_{compartment}(1,120) = 33.01$, p < 0.001; $F_{treatment}(1,120) = 0.01$, p = 0.99, $n_{vehicle} = 28$, $n_{0.1\,mg/kg} = 10$, $n_{1.0\,mg/kg} = 10$, $n_{3mg/kg} = 16$, Fig. 5). Post-hoc analyses showed that rats treated with vehicle and 0.1 mg/kg naloxone showed a preference for the social play-paired compartment ($t_{veh}(27) = 8.76$, p < 0.001; $t_{0.1}(9) = 4.50$, p = 0.001), whereas rats treated with 1.0 mg/kg did not show any preference ($t_{1.0}(9) = 1.84$, p = 0.10). In addition, 3.0 mg/kg-treated rats showed a trend towards an aversion for the social play-paired compartment ($t_{3.0}(15) = -1.90$, p = 0.08, Fig. 5).

4. Discussion

The aim of the present study was to investigate the role of opioid neurotransmission in the motivational and pleasurable aspects of social play behaviour. The data show that: (1) at the highest dose tested, treatment with the opioid receptor agonist morphine reduced responding for social play but it also reduced the number of inactive responses; (2) the expression of social play during earned periods of social interaction was increased when both animals in a pair were treated with morphine; (3) treatment with morphine induced CPP in juvenile rats, and facilitated the development of social play-induced CPP; (4) treatment with the opioid receptor antagonist naloxone reduced the motivation for social play, the expression of social play, as well as the acquisition of social play-induced CPP.

4.1. Role of opioid modulation in the motivation for social play

Treatment with morphine reduced operant responding for social play behaviour at the highest dose tested (3.0 mg/kg). Although morphine has been found to increase responding for food under a PR schedule (Solinas and Goldberg, 2005), suppressant effects of morphine on operant behaviour have also been documented (Adams and Holtzman, 1990; Leander et al., 1975; Thompson et al., 1970). Indeed, this dose of morphine reduced inactive lever presses as well, which suggests that rate-decreasing effects of morphine underlie this effect of the opiate.

Remarkably, in contrast to previous studies (Niesink and Van Ree, 1989; Panksepp et al., 1985; Trezza and Vanderschuren, 2008a; −2008b; −2009a Vanderschuren et al., 1995a; b), social play behaviour in our first experiment with the operant conditioning task was not altered by treatment with morphine. Previous studies have shown that

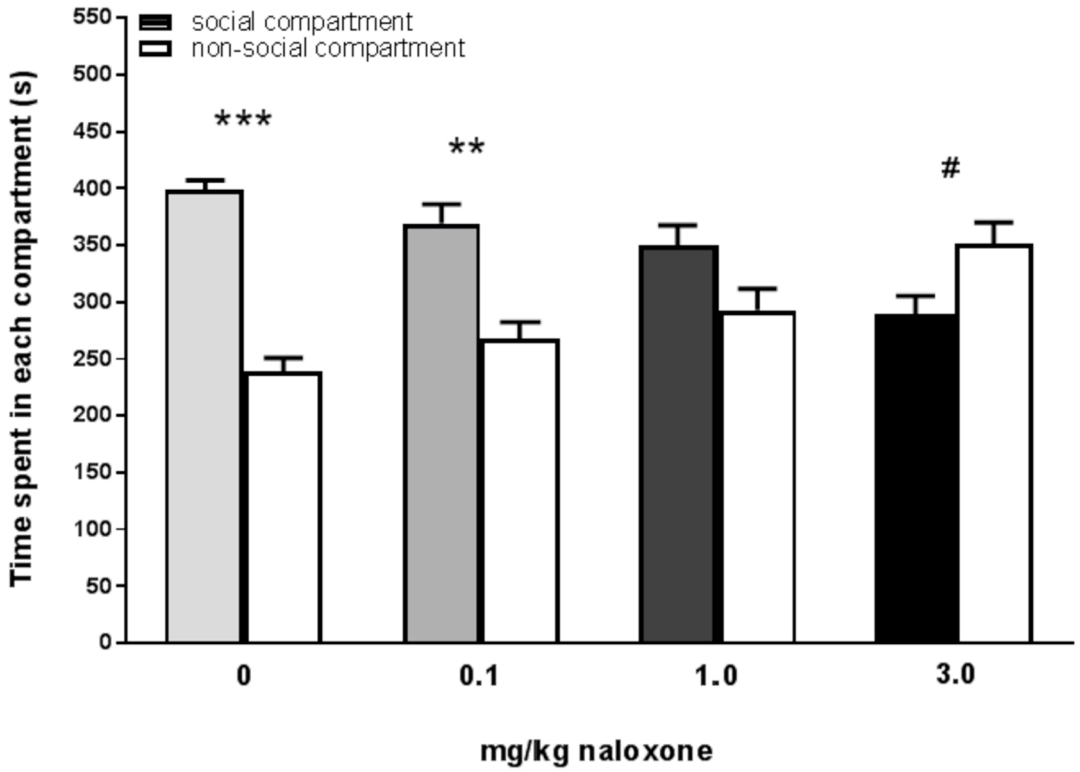

Fig. 5. Naloxone disrupts social play-induced conditioned place preference. Treatment with vehicle or 0.1 mg/kg of naloxone ($n_{vehicle}$ = 28, $n_{0.1\ mg/kg}$ = 10) does not affect social play-induced place preference. Animals fail to differentiate between the social play and the non-social compartment when treated with dose of 1.0 mg/kg naloxone (n = 10). Animals treated with a dose of 3 mg/kg (n = 16) show a trend towards conditioned place aversion when naloxone is coupled with the social play compartment. Data are presented as mean + SEM. **p < 0.01, ***p < 0.001, #p = 0.05–0.07.

morphine enhances social play according to an inverted U-shaped dose-effect curve, whereby 1 mg/kg induced robust increases in both pinning and pouncing (Trezza and Vanderschuren, 2008a; Vanderschuren et al., 1995b-1996). Several methodological factors could explain the discrepancies in findings. First, we socially isolated animals for 24 h, whereas most previous studies used 3.5 h of social isolation. Social isolation for 24 h causes a maximal increase in the amount of social play (Niesink and Van Ree, 1989; Vanderschuren et al., 1995b, 2008), which may obscure the play-enhancing properties of morphine because of a ceiling effect (but see Vanderschuren et al., 1995b).

Second, in the present study, only the experimental animal was treated, and not its stimulus partner. Possibly, drug-treatment of the experimental animal in combination with the 24 h of social isolation may cause a difference in the willingness to play between both animals, so that the play interaction is less rewarding for the stimulus animal, which may reduce the play-enhancing effects of morphine. Trezza and Vanderschuren (2008b) previously showed that treating one animal of a test pair with morphine results in an increase in pouncing (play initiations) but not pinning, when behaviour of a test pair was analysed, whereas the effects of morphine were more pronounced when both animals were treated. In other words, treating only one animal in a test pair may not be sufficient to observe a robust increase (or decrease, for that matter) in social play. We addressed this issue by treating both animals of a play pair with a dose of morphine that is known to enhance social play behaviour (1 mg/kg). Indeed, under these test conditions, treatment with morphine increased social play behaviour, whilst not altering responding for social play. This finding is reminiscent of previous observations that suggest that social interaction is most pleasurable when both animals have a comparable motivation to play (Douglas et al., 2004).

Third, in the operant conditioning setup, animals have only one minute to play per reinforced period, whereas our previous studies on the expression of social play analysed this behaviour for 15 min continuously. It could then be that stimulating effects on social play are less likely to occur because the playful interaction is interrupted after one minute. The present data, together with our previous findings (Achterberg et al., 2016a,b) therefore suggest that social play expression in our operant setup may be more sensitive to manipulations that decrease social play than to those that increase this behaviour. Adjustments to this setup may facilitate the detection of increases in social play expression, as demonstrated with morphine, such as treating both animals in a pair, using a shorter isolation time, or longer interaction time per reinforcement.

Blocking opioid receptors with naloxone reduced responding for social play behaviour as well as its expression. The reduction in responding for social play is in line with studies on the effects of naloxone (Barbano and Cador, 2007; Barbano et al., 2009; Cleary et al., 1996; Solinas and Goldberg, 2005; Schneider et al., 2010), and genetic deletion of μ-opioid receptors (Papaleo et al., 2007) on operant responding for food. Moreover, treatment with naloxone has also been shown to reduce food intake (Carey et al., 1981; Kirkham and Blundell, 1984). Naloxone-treatment also reduced the expression of social play behaviour as demonstrated before with μ-opioid receptor antagonism (Beatty and Costello, 1982; Jalowiec et al., 1989; Niesink and Van Ree, 1989; Normansell and Panksepp, 1990; Siegel et al., 1985; Siegel and Jensen, 1986; Panksepp et al., 1985; Trezza and Vanderschuren, 2009; Trezza et al., 2011b). Together, this implicates opioid receptor modulation in the motivational properties of natural rewards, such as food and social play behaviour.

Interestingly, using a play-rewarded T-maze task, Normansell and Panksepp (1990) found that treatment with naloxone or morphine did not affect the motivational parameter in the task (i.e. latency to enter the goal-box). However, opioid modulation affected extinction in this paradigm, as morphine-treated rats kept making more correct choices during 10 extinction sessions whereas naloxone treatment resulted in a faster decline in correct choices (Normansell and Panksepp, 1990),

suggesting that the motivation to play was affected. This difference in findings, although subtle, may be due to differences in setup (runway vs. operant responding), especially the difference in time and effort necessary to obtain the reward. That is, running down a T-maze requires less time and effort compared to lever pressing under a progressive ratio schedule of reinforcement. Therefore, a runway task may be less sensitive to motivational factors than operant responding.

In summary, a high dose of morphine disrupts operant responding for social play because of its rate-decreasing effects, whereas lower doses do not affect operant responding. The expression of social play in this operant set-up was enhanced by morphine when both animals in a pair were treated. In contrast, treating animals with naloxone reduced both responding for and expression of social play behaviour. Together, these data demonstrate that μ-opioid modulation affects both the motivation for social play as well as its expression.

4.2. Role of opioid modulation in the pleasurable properties of social play

Morphine treatment is known to enhance social play behaviour in adolescent rats (Manduca et al., 2014b; Trezza and Vanderschuren, 2008a; -b; Vanderschuren et al., 1995a; -b; Normansell and Panksepp, 1990; Niesink and Van Ree, 1989; Panksepp et al., 1985). Furthermore, it induces place preference in adult rats (for reviews see, Bardo et al., 1995; Tzschentke, 2007) as well as adolescent rats (present study, Fig. 3b) Here, we show that morphine does not modulate social play-induced CPP in an optimal protocol with 8 conditioning sessions, as we used before (Trezza et al., 2009; Achterberg et al., 2012, 2014), possibly as a result of a ceiling effect. Indeed, when we subsequently used a suboptimal protocol with 4 conditioning sessions, social play behaviour was insufficient to produce CPP in vehicle-treated rats. However, morphine, at a dose known to enhance social play behaviour (1 mg/kg, Trezza and Vanderschuren, 2008a; Vanderschuren et al., 1995b-1996), interacted with social play behaviour to produce CPP in a this sub-effective conditioning paradigm. Consistent with this finding, it has been shown that drugs of abuse like cocaine and nicotine act synergistically with social play to induce CPP (Thiel et al., 2008, 2009, but see Randall et al., 1998). Together these data demonstrate an stimulating effect of morphine on social play-induced place conditioning, suggesting that this drug enhances the rewarding aspects of social play behaviour.

On the other hand, blocking μ-opioid receptors with naloxone disrupted the acquisition of social play-induced CPP suggesting that blockade of opioid receptors reduced the pleasurable properties of social play behaviour in such a way that this behaviour no longer supported learning. The effects of manipulating opioid tone shown are likely due to interference with the pleasurable properties of social play, rather than with conditioning itself, since treatment with opioid receptor agonists, such as morphine, typically disrupts rather than facilitates learning (and vice versa for opioid receptor antagonists, such as naloxone) (Gallagher et al., 1983; Izquierdo, 1979; Izquierdo and Netto, 1990; Martinez, 1983; Tomaz et al., 1990).

It should be noted that the rewarding aspects of social play behaviour that are measured in this study seem to be less sensitive to naloxone treatment compared to operant responding and expression of social play behaviour. That is, in the operant setup we found a reduction in responding for and the expression of social play at the lowest dose of naloxone tested (0.1 mg/kg), while attenuating the acquisition of social play-induced CPP required higher doses of the antagonist (1–3 mg/kg). At face value, these data suggest that the motivational properties of social play behaviour are more sensitive to opioid receptor antagonism that its pleasurable aspects. However, since different opioid receptor subtypes and brain regions may be involved in different components of social play reward (Vanderschuren et al., 2016), we think that more work is required to test the distinction in opioid modulation of social play motivation vs pleasure. Another factor to be pointed out is that both strain and sex differences have been reported in both social play behaviour (e.g. Bredewold et al., 2014; Veenema et al., 2013), the behavioural effects of opioid drugs (Manduca et al., 2014a; b) and μ-opioid receptor binding (Smith et al., 2018). It is possible that the effects we observed may be strain- and sex-dependent; this issue deserves further investigation.

4.3. On μ-opioid receptor modulation and social behaviour

Regarding opioids and social behaviour, Panksepp and colleagues formulated the 'Opioid theory of social attachment' (Panksepp et al., 1978, 1980b). This theory postulated that social contact alleviates social isolation distress and induces a positive affective state through the release of endogenous opioids, whereas social isolation causes opioid withdrawal-like symptoms and a negative affective state. More recently, Løseth et al. (2014) modified and extended this theory. They argued that there is a state dependent μ-opioid modulation of social motivation, whereby social animals will actively seek social contact when in a negative motivational state. Stimulating μ-opioid receptors will then result in less social contact because it alleviates the negative social emotions, whereas blocking μ-opioid receptors makes animals more motivated to seek social contact. In contrast, in a positive motivational state social interaction serves to maintain social bonds, explore social hierarchies and possible sex partners. In this situation, μ-opioid receptor stimulation increases social behaviors and antagonizing these receptors reduces them.

In the case of the expression of social play behaviour and place conditioning, our findings fit well with this theory (Løseth et al., 2014). That is, the pleasurable properties of social play behaviour, its expression as well as its capacity to support place conditioning are enhanced after stimulation, and reduced after blockade of μ-opioid receptors. Moreover, the decrease in responding for social play after naloxone treatment is also in keeping with this notion. Whereas an increase in responding for social play is to be expected after morphine treatment, it is most likely that an enhancing effect on our motivational parameters is obscured by the rate-decreasing effects of morphine. Alternatively, it is possible that there is an optimal brain opioid tone that supports the motivation for social play, so that increasing opioid signaling by morphine does not further enhance responding for social play behaviour, whereas reducing opioid neurotransmission with naloxone will decrease it. Brain region-specific modulation of opioid tone in the paradigms used here may shed more light on these findings.

5. Conclusion

In the present study, we present evidence to support the notion that opioid neurotransmission underlies multiple aspects of social play reward. Blocking opioid receptors reduced the motivation for social play, its rewarding properties as well as the expression of this behaviour. Conversely, stimulating opioid neurotransmission increased the expression of social play behaviour, as well as its rewarding properties. These data therefore increase our understanding of the neural mechanisms of distinct aspects of the positive emotional properties of social play behaviour.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Contributors

EJMA, VT and LJMJV designed the experiments. EJMA, MMHvS, DJH, performed the experiments and analysed the data. EJMA, VT and LJMJV wrote the paper. All authors have approved the final version of the manuscript.

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