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Treatment with low doses of nicotine but not alcohol affects social play reward in rats

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ABSTRACT

Social play behaviour is a vigorous, highly rewarding activity in young animals. It is thought to facilitate social, cognitive and emotional development, but its underlying neural mechanisms are incompletely understood. Previously, we found that low doses of alcohol and nicotine enhanced social play behaviour in young rats. Using place and operant conditioning setups to assess the pleasurable and motivational aspects of social play, we investigated how treatment with nicotine and alcohol affects social play reward. Nicotine-treatment increased the incentive motivational properties of social play as well as the expression of social play itself. Moreover, while nicotine by itself evoked conditioned place preference (CPP), it reduced social play-induced CPP. Alcohol-treatment did not affect the motivation for and expression of social play, nor did it affect social play-induced CPP. The finding that nicotine but not alcohol modulates social play reward increases our understanding of the neural underpinnings of this developmentally important behaviour.

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Social play; nicotine; alcohol; motivation; place conditioning

Introduction

One of the earliest social behaviours to emerge in the life of many mammalian species is social play behaviour. This highly energetic and vigorous social activity has a fundamental role in the development of physical, social as well as emotional and cognitive capacities (Panksepp, 1981; Panksepp, Siviy, & Normansell, 1984; Pellis & Pellis, 2009; Vanderschuren, Niesink, & Van Ree, 1997). That is, by engaging in social play behaviour, animals are thought to acquire a rich and flexible behavioural repertoire that enables them to cope with challenges in the environment (Baarendse, Counotte, O'Donnell, & Vanderschuren, 2013; Pellis & Pellis, 2009; Van den Berg et al., 1999; Vanderschuren & Trezza, 2014). In rats, social play behaviour peaks between post-natal day (PND) 28–40 and its frequency decreases when animals reach sexual maturity. It comprises a combination of altered or exaggerated forms of sexual, aggressive, and predatory behaviour (Panksepp et al., 1984;

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Pellis & Pellis, 2009; Vanderschuren et al., 1997), hence this behaviour is also termed rough-and-tumble play.

Using both operant- and place conditioning paradigms, it has been demonstrated that social play behaviour is a highly rewarding activity (Achterberg, Van Kerkhof, et al., 2016; Achterberg, Van Swieten, Driel, Trezza, & Vanderschuren, 2016; Achterberg, van Swieten, Houwing, Trezza, & Vanderschuren, 2019; Calcagnetti & Schechter, 1992; Trezza, Campolongo, & Vanderschuren, 2011; Trezza, Damsteegt, & Vanderschuren, 2009; Vanderschuren, 2010; Vanderschuren, Achterberg, & Trezza, 2016). In an operant conditioning setup, animals learn to perform certain actions, e.g. pressing a lever, in order to receive rewards (or avoid punishment). In our setup, when an animal makes a lever-press, the cue light goes on and the animal receives its reward, in the form of access to another rat ('play partner'), allowing for a playful social interaction. In this way, the animal learns the contingency between its response (lever-pressing) and the delivery of the reward, thus increasing the likelihood that the animal will press the lever to obtain the reward when placed in the box again (which is termed 'positive reinforcement'). By using a progressive-ratio (PR) schedule, the animals' motivation for rewards can be studied (Hodos, 1961; Richardson & Roberts, 1996). Under this particular schedule, the number of lever presses required to obtain the next reward is increased after every reward, until the animal stops responding. The maximal number of responses performed to obtain one single reward, i.e. the breakpoint, is used as a measure for the animals' motivation for the reward. Place conditioning, on the other hand, is a widely used behavioural paradigm to measure the positive emotional, pleasurable properties of rewards (Bardo & Bevins, 2000; Tzschentke, 2007). A typical place conditioning set-up consists of three linked chambers, i.e. a middle or 'start' compartment and two adjacent chambers with different visual and/or tactile cues. It is based on the principle of association of the primary rewarding properties of, in our case, social play to the distinct environmental cues of a particular compartment. Therefore, when given the choice, a young rat will spent more time in that environment, because these distinct environmental cues acquired secondary rewarding properties and elicit approach behaviour.

Like other rewards, such as food, sex and drugs of abuse, social play behaviour is thought to be regulated via interacting neural systems (Siviy & Panksepp, 2011; Trezza, Baarendse, & Vanderschuren, 2010; Vanderschuren et al., 1997) that mediate its pleasurable, incentive motivational, and learning-related aspects (Berridge, Robinson, & Aldridge, 2009). Within these systems, brain regions like the prefrontal cortex, striatum and amygdala are embedded, and the neurotransmitter systems (e.g. dopamine, opioids, cannabinoids and GABA) that modulate functional activity herein (Barbano & Cador, 2007; Berridge, 2007; Berridge & Kringelbach, 2015; Kelley, 2004; Le Merrer, Becker, Befort, & Kieffer, 2009; Robbins & Everitt, 2007; Salamone & Correa, 2012).

Alcohol and nicotine are typically the first substances used by young people and they are often initially consumed, either alone or in combination, in a social setting (Lipperman-Kreda, Paschall, Robert, & Morrison, 2018; Moss, Chen, & Yi, 2014; Nelson, Van Ryzin, & Dishion, 2015). In fact, these substances are thought to facilitate peer interaction and acceptance. Rodent studies are in concordance with this view, by demonstrating that treatment with low doses of nicotine increases social interaction time in adolescent rats (Cheeta, Irvine, & File, 2001; Cheeta, Irvine, Tucci, Sandhu, & File, 2001) and that alcohol (Trezza, Baarendse, & Vanderschuren, 2009; Varlinskaya & Spear, 2002, 2006, 2009; Varlinskaya, Spear, & Spear, 2001; Willey, Varlinskaya, & Spear, 2009) increases

social behaviour (social play behaviour, social interaction time, social investigation time and social preference) in juvenile, adolescent and adult rats. Importantly, Trezza, Baarendse, et al. (2009) showed that social play behaviour in juvenile rats was increased following treatment with low doses of either alcohol and nicotine but also after treatment with a combination of sub-effective doses of these two substances. This latter finding suggests that alcohol and nicotine modulate social play behaviour through overlapping brain mechanisms. Given that alcohol, nicotine as well as social play behaviour have rewarding effects, it is therefore likely that alcohol and nicotine increase social play behaviour by enhancing its positive emotional properties.

In the present study, we therefore directly investigated whether treatment with nicotine and alcohol affected the motivational and pleasurable properties of social play behaviour. To measure the motivational aspects of social play behaviour, we used an operant conditioning task, in which rats pressed a lever for access to a playful partner under a progressive ratio schedule of reinforcement (Achterberg et al., 2019; Achterberg, Van Kerkhof, et al., 2016; Achterberg, Van Swieten, et al., 2016). Place conditioning was used to gauge whether pleasurable aspects of social play behaviour were affected by treatment with nicotine and alcohol (Achterberg et al., 2019; Achterberg, Van Swieten, et al., 2016; Bardo & Bevins, 2000; Calcagnetti & Schechter, 1992; Douglas, Varlinskaya, & Spear, 2004; Trezza et al., 2009; Tzschentke, 2007; Vanderschuren et al., 2016). Since both alcohol and nicotine enhance the expression of social play behaviour at least partly via dopamine signalling (Trezza, Baarendse, et al., 2009) and dopamine neurotransmission has been implicated in the motivation for social play (Achterberg, Van Kerkhof, et al., 2016), we predicted that alcohol and nicotine increase the motivation to play. Secondly, opioid and cannabinoid neurotransmission, also implicated in the social play-enhancing effects of nicotine and alcohol (Trezza, Baarendse, et al., 2009; Varlinskaya & Spear, 2009), act on the pleasurable but not necessarily the motivational aspects of social play behaviour (Achterberg, Van Swieten, et al., 2016; Achterberg et al., 2019). Therefore we predicted that the pleasurable aspects of social play are also enhanced by treatment with these substances.

Materials and methods

A total of 92 male Wistar rats (Charles River, Sulzfeld, Germany) were used. Animals 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 × w × h) Macrolon cages with wood shavings, shelter and a wooden block until experiments started. Animals were housed under controlled conditions (ambient temperature 20°C–21°C, 60%–65% relative humidity, and 12/12 h light cycle with lights on at 7.00am). Food and water were available ad libitum. All animals used were experimentally naïve. Experiments were carried out between 8.00pm and 5.00am. 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 (Guideline86/609/EEC), and the ARRIVE guidelines.

Drugs

Alcohol (Sigma-Aldrich, Schnelldorf, Germany) was administered intraperitoneally (i.p.) 1 h before the test, as a 12.6% (v/v) solution in physiological saline. Alcohol doses were

varied by changing the volume of the 12.6% alcohol solution. Nicotine (Sigma-Aldrich, Schnelldorf, Germany) was dissolved in saline and administered subcutaneously (s.c.), 10 min before testing. Drug doses and pre-treatment intervals were based on previous studies (Trezza & Vanderschuren, 2008a, 2008b; Trezza, Baarendse, et al., 2009). In view of the importance of the neck area in the expression of social play behaviour (Pellis & Pellis, 1987; Siviy & Panksepp, 1987), s.c. injections were administered in the flank.

Operant conditioning paradigm

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 × w × 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).

Experimental procedure

The experiments were conducted as described in Achterberg, Van Swieten, et al. (2016). Briefly, all experiments were performed under red light conditions. Animals were paired with an unfamiliar test partner from another home cage, so that a test pair consisted of one experimental animal and its stimulus partner. Animals in a test pair did not differ by more than 10 grams in body weight at the start of the experiment. These measures were taken to prevent existing dominance relationships between animals that lead to asymmetries in the expression of playful behaviours (e.g. Pellis, Pellis, & McKenna, 1993), to interfere with behaviour during the experiments. 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 sessions, the cue light was presented, the lever retracted and the door opened whenever 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. In addition, 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 acquisition sessions (20 min) commenced under a fixed ratio (FR)-1 schedule of reinforcement. This schedule was used to train the animals that pressing the lever results in a reward. 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 the 2 min of social interaction time, the door automatically closed and the house-light was illuminated during a 25 sec 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 to assess motivation for social play behaviour. 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 responses, etc; Hodos, 1961; Richardson & 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 cage-mates. After responding had stabilised, 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. The stimulus animal received a saline injection unless otherwise specified. A wash-out day, during which animals received normal training was inserted after every treatment day.

Analysis of social play behaviour

During earned social interactions, behaviour of the playing 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).

1. Frequency of pinning: one animal lying with its dorsal surface on the floor with the other animal is 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 are considered the most characteristic parameters of social play behaviour in rats (Panksepp & 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.

Experiment 1: the effect of alcohol treatment on operant responding for social play

Six test pairs ($n = 12$) were trained to stable responding under the PR schedule of reinforcement after which the experimental animal received vehicle, 0.125 and 0.25 g/kg alcohol in a Latin Square design to obtain a dose–response curve. Prior research showed that 0.25 g/kg alcohol enhances the expression of social play when both

animals in a test pair were treated (Trezza, Baarendse, et al., 2009). Therefore, for comparison to the play expression data by Trezza and colleagues, in this experiment we also included a test in which both animals in a test pair were treated with this dose of alcohol.

Experiment 2: the effect of nicotine treatment on operant responding for social play

Experiment 2a: dose-response curve

Eight test pairs ($n = 16$) were trained to stable responding on a PR schedule after which the experimental animal received vehicle, 0.03 and 0.1 mg/kg nicotine in a Latin Square design to obtain a dose–response curve.

Experiment 2b: the effect of social isolation and treating both animals in a test pair with nicotine on responding for social play

The same eight test pairs that participated in Experiment 2a were used for this experiment. Prior research showed that 0.1 mg/kg nicotine enhances the expression of social play when both animals in a test pair were treated (Trezza, Baarendse, et al., 2009), and in this study, a social isolation time of 3.5 h was used. This isolation period has been shown to induce a half-maximal increase in the amount of social play behaviour (Niesink & Van Ree, 1989; Vanderschuren et al., 2008; Vanderschuren, Niesink, Spruijt, & Van Ree, 1995a). To rule out the possibility that the 24 h social isolation procedure we typically use in the operant conditioning setup generates a ceiling effect, we isolated animals either 24 h or 2 h before the test and treated both animals in the test pair with the dose that was previously found to increase the expression of social play. When animals were isolated for 2 h, they were housed with their test partner for at least 24 h prior to isolation. Animals received at least one wash-out day before the new Latin Square design was introduced, consisting of 1. 24 h isolation-vehicle treatment; 2. 24 h isolation-0.1 mg/kg nicotine treatment; 3. 2 h isolation-vehicle treatment and 4. 2 h isolation-0.1 mg/kg nicotine treatment. A treatment day was followed by a wash out day.

Place conditioning paradigm

Apparatus

The place conditioning setup (TSE Systems, 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 × 30 cm; l × w × h) and separated by a third, neutral compartment (10 cm × 25 cm × 30 cm; l × w × 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, Niesink, Spruijt, & Van Ree, 1995b).

Experiment 3a: the effect of alcohol treatment on acquisition of place conditioning with social play

Place conditioning was performed as previously described (Achterberg, Trezza, & Vanderschuren, 2012, 2014; Achterberg, Van Swieten, et al., 2016; Trezza et al., 2009). At 26 days of age (i.e. 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; Achterberg et al., 2014; Achterberg, Van Swieten, et al., 2016; Niesink & Van Ree, 1989; Trezza et al., 2009; Vanderschuren et al., 1995a; 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 weight-matched partner of the same age (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. Alcohol ($n = 8$) or vehicle ($n = 8$) was administered 1 h before the start of each social conditioning session. The 0.25 g/kg alcohol dose that was used in this experiment was previously found to increase social play behaviour (Trezza, Baarendse, et al., 2009). On day 10, the rats were not treated and placed in the middle compartment and were allowed to explore the entire apparatus for 15 min.

Experiment 3b: acquisition of place conditioning with alcohol

To determine whether alcohol treatment by itself induces CPP, animals were subjected to the same conditioning schedule as for the social play-induced paradigm, but the animals were alone on both sides of the apparatus. The control animals ($n = 8$) received a vehicle injection before placement in both compartments, whereas others ($n = 8$) received an alcohol injection 1 h before placement on one side and a vehicle injection before placement on the other side of the apparatus (in a counter-balanced design; Tzschentke, 2007; Veeneman et al., 2011). On day 10, the rats were not treated and placed in the middle compartment where the time spent in each compartment was recorded to determine place preference.

Experiment 4a: the effect of nicotine treatment on acquisition of place conditioning with social play

Procedures were performed as described under experiment 3a. Nicotine (0.1 mg/kg; $n = 8$) or vehicle ($n = 8$) was administered 10 min prior to conditioning.

Experiment 4b: acquisition of place conditioning with nicotine

Procedures were performed as described under experiment 3b. Nicotine (0.1 mg/kg; $n = 8$) or vehicle ($n = 8$) was administered 10 min prior to conditioning.

Statistical analysis

Data were analysed using SPSS software 24.0 for Windows. The frequency of pinning and pouncing during operant conditioning was calculated per minute of interaction time. The duration of social exploration was calculated as a percentage of interaction 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, one or both animals treated in a test pair, Student's *t*-tests or, when analysing breakpoint, a Wilcoxon signed ranks test was used. 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.

Results

Experiment 1: operant responding for social play: effects of alcohol

Animals treated with alcohol (i.e. the active animal treated with 0.125 or 0.25 g/kg or both animals treated with 0.25 g/kg) did not show significant differences in operant responding for social play ($F_{\text{treatment}}(3,15) = 0.31$, $p = 0.82$, $n = 6$; Figure 1). All animals discriminated between the active and inactive lever ($F_{\text{lever}}(1,5) = 16.41$, $p = 0.01$) but the treatment did not affect responding on the levers ($F_{\text{treatment*lever}}(3,15) = 1.25$, $p = 0.33$). The number of obtained rewards ($F_{\text{treatment}}(3,15) = 0.33$, $p = 0.81$) and breakpoint ($X^2 = 0.60$, df = 3, $p = 0.89$) as well as the expression of play and the time spent on social exploration were unaffected by the treatment (pouncing: $F_{\text{treatment}}(3,15) = 1.82$, $p = 0.19$; pinning: $F_{\text{treatment}}(3,15) = 1.20$, $p = 0.34$; social exploration: $F_{\text{treatment}}(3,15) = 0.57$, $p = 0.64$).

Experiment 2: operant responding for social play: effect of nicotine treatment

Experiment 2a: dose–response curve for nicotine

In the first experiment, only the active (i.e. lever pressing) animals were treated with nicotine. Here, the animals discriminated between the active and inactive lever ($F_{\text{lever}}(1,14) = 45.21$, $p < 0.001$), so animals successfully learned that pressing the active lever leads to play time. Nicotine (0.03 and 0.1 mg/kg) did not affect operant responding for social play ($F_{\text{treatment}}(2,14) = 1.36$, $p = 0.29$; $F_{\text{treatment*lever}}(2,14) = 1.35$, $p = 0.29$; $n = 8$). The number of rewards ($F_{\text{treatment}}(2,14) = 2.44$, $p = 0.12$) and breakpoint ($X^2 = 3.31$, df = 2, $p = 0.19$) were unaffected by nicotine treatment. In addition, nicotine did not affect pinning ($F_{\text{treatment}}(2,14) = 0.45$, $p = 0.65$), pouncing ($F_{\text{treatment}}(2,14) = 2.89$, $p = 0.09$) and the time spent on social exploration ($F_{\text{treatment}}(2,14) = 2.67$, $p = 0.10$) (Figure 2).

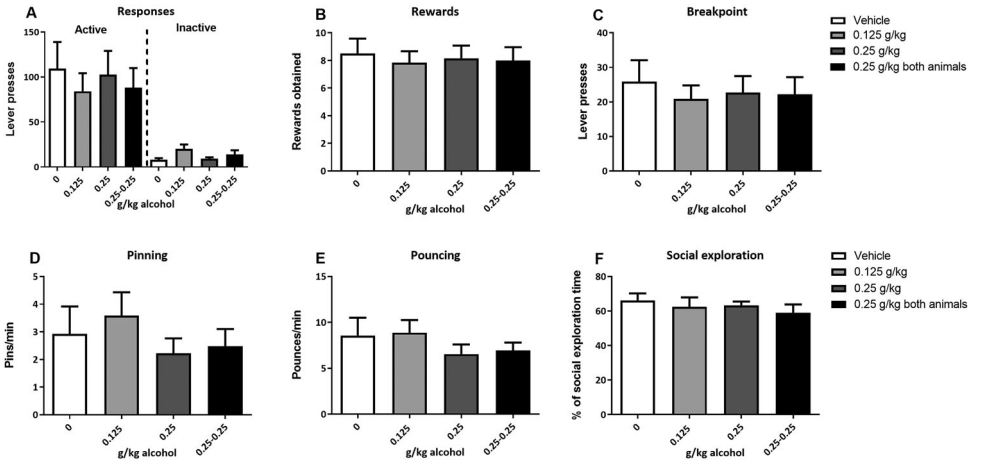

Figure 1. The effect of alcohol treatment on operant responding for social play behaviour. Treatment with alcohol (0.125–0.25 mg/kg, $n = 6$ test pairs) did not affect the number of active or inactive responses (A), rewards obtained (B) and breakpoint (C). Alcohol administration did not modulate the expression of social play behaviour, i.e. pinning (D) and pouncing (E) or social exploration (F). This was also the case when both animals in a test pair were treated with 0.25 g/kg alcohol. Data are presented as mean + SEM.

Experiment 2b: effect of social isolation time and treating both animals in a test pair on the effect of nicotine on the motivation for social play

To test whether treating both animals with nicotine would influence behaviour in the task (Trezza, Baarendse, et al., 2009), in the next experiment both the active lever pressing rat and the test partner were treated with nicotine (0.1 mg/kg). In addition, to test whether

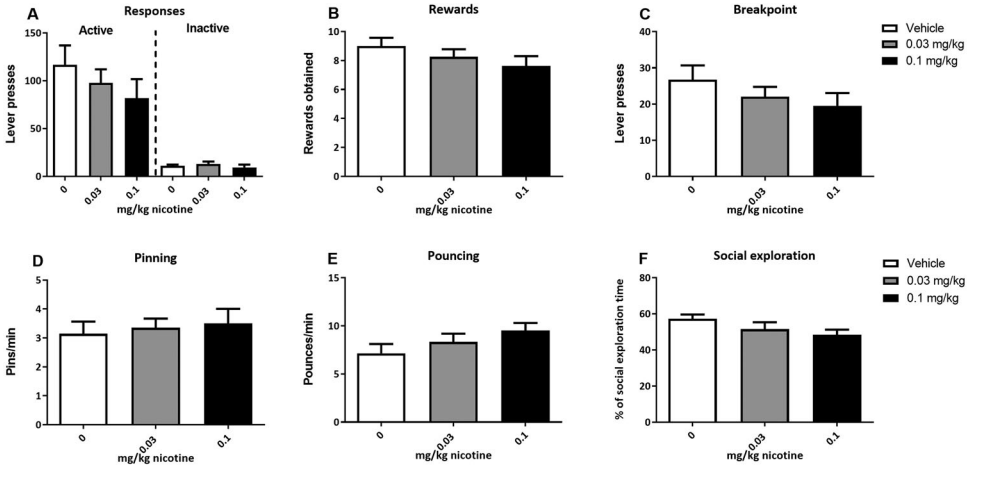

Figure 2. The effect of nicotine treatment on operant responding for social play behaviour. Treatment with nicotine (0.03–0.1 mg/kg, $n = 8$ test pairs) did not affect the number of active or inactive responses (A), rewards obtained (B) and breakpoint (C). Nicotine treatment did not modulate the expression of social play behaviour, i.e. pinning (D) and pouncing (E) or social exploration (F). Data are presented as mean + SEM.

reducing the social motivation would increase the window to observe increases in responding (Achterberg et al., 2016), the duration of social isolation before testing was either 2 or 24 h.

Treating both animals of a test pair with nicotine did not affect operant responding ($F_{treatment}(1,7) = 1.33$, $p = 0.22$; $F_{treatment*lever}(1,7) = 1.05$, $p = 0.34$; $F_{treatment*isolation}(1,7) = 0.86$, $p = 0.38$; $F_{treatment*lever*isolation}(1,7) = 2.98$, $p = 0.13$; $n = 8$; Figure 3(A)), but the animals did discriminate between the levers ($F_{lever}(1,7) = 42.77$, $p < 0.001$). 24 h of isolation time before the test resulted in increased responding on the active lever compared to 2 h of social isolation ($F_{isolation}(1,7) = 6.81$, $p = 0.04$). Both active and inactive lever presses increased due to a longer isolation time ($F_{isolation*lever}(1,7) = 4.71$, $p = 0.07$). Treatment with nicotine increased the number of rewards obtained ($F_{treatment}(1,7) = 7.40$, $p = 0.03$), and isolation time tended to have the same effect ($F_{isolation}(1,7) = 4.84$, $p = 0.06$; $F_{treatment*isolation}(1,7) = 3.\ 62$, $p = 0.10$, Figure 3(B)). Breakpoint was affected by nicotine treatment ($X^2 = 10.64$, df = 3, $p = 0.01$) and isolation time, whereby after 2 h isolation, nicotine-treated animals showed an increased breakpoint ($U_{2h\_veh\text{-}nic} = -2.21$, $p = 0.03$; Figure 3(C)). This was not the case when animals were isolated for 24 h ($U_{24h\_veh\text{-}nic} = -0.52$, $p = 0.62$). A longer isolation period increased the breakpoint in saline- but not nicotine-treated rats ($U_{veh\_2h\text{–}24h} = -2.37$, $p = 0.02$; $U_{nic\_2h\text{–}24h} = -0.70$, $p = 0.48$).

Pinning was affected by nicotine-treatment and isolation time ($F_{treatment*isolation}(1,7) = 5.69$, $p = 0.02$, $F_{treatment}(1,7) = 9.39$, $p = 0.02$; $F_{isolation}(1,7) = 2.43$, $p = 0.16$; Figure 3(D)).

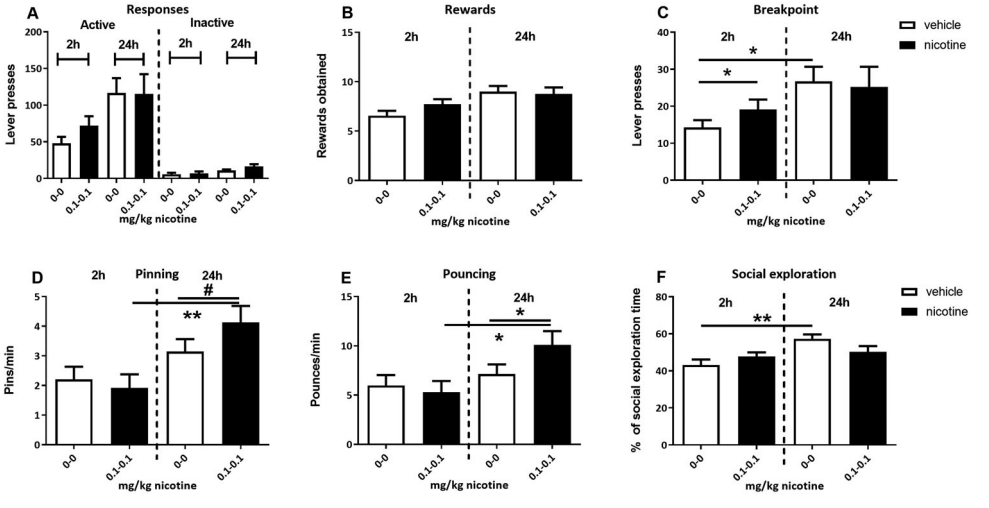

Figure 3. The effect of nicotine treatment and social isolation time on operant responding for social play behaviour. In eight test pairs, twenty-four hours of social isolation resulted in enhanced responding (A), more obtained rewards (B) an increased breakpoint (C) for social play behaviour and increased play (pinning, D; pouncing, E) compared to 2 h of social isolation. Social exploration was not affected by isolation time (F). The isolation-effect was more pronounced in vehicle- compared to nicotine-treated animals (breakpoint, C; time spent on social exploration, F). In addition, nicotine treatment increased breakpoint after 2 h but not 24 h of social isolation (C). After 24 h of social isolation, treating both animals in a test pair with nicotine resulted in more play initiations (pouncing, E) and tended to increase pinning (D). Social exploration after 24hrs of isolation was unaffected by nicotine administration (F). Data are presented as mean + SEM. $^{**}p < 0.01$, $^{*}p < 0.05$, $\#p < 0.075$.

Treating both animals with nicotine after 24 h but not 2 h of social isolation tended to increase the amount of pinning (*post hoc* paired *t*-tests: $t_{24h\_veh\text{-}nic}(7) = -2.28$, $p = 0.06$; $t_{2h\_veh\text{-}nic}(7) = 1.25$, $p = 0.25$). When comparing isolation times, animals treated with nicotine (but not saline) showed higher levels of pinning after 24 h of isolation ($t_{nic\_2h-24h}(7) = -4.16$, $p = 0.004$; $t_{veh\_2h-24h}(7) = -1.52$, $p = 0.17$). Nicotine treatment and isolation time also affected pouncing ($F_{treatment*isolation}(1,7) = 13.14$, $p = 0.008$, $F_{treatment}(1,7) = 4.96$, $p = 0.06$; $F_{isolation}(1,7) = 3.78$, $p = 0.09$; Figure 3(E)). *Post hoc* analysis shows that after 24 h but not 2 h of social isolation, nicotine increased pouncing ($t_{24h\_veh\text{-}nic}(7) = -2.94$, $p = 0.02$; $t_{2h\_veh\text{-}nic}(7) = 1.65$, $p = 0.14$). Like with pinning, nicotine (but not saline) treatment resulted in more pounces after 24 h (compared to 2 h) of social isolation ($t_{nic\_2h-24h}(7) = -3.30$, $p = 0.01$; $t_{veh\_2h-24h}(7) = -0.85$, $p = 0.42$). The percentage of time spent on social exploration was differentially affected by nicotine treatment and isolation time ($F_{treatment*isolation}(1,7) = 11.05$, $p = 0.01$; $F_{treatment}(1,7) = 6.08$, $p = 0.04$; $F_{isolation}(1,7) = 0.26$, $p = 0.62$; Figure 3(F)). At both timepoints, nicotine did not affect social exploration ($t_{2h\_veh\text{-}nic}(7) = -1.85$, $p = 0.11$; $t_{24h\_veh\text{-}nic}(7) = 1.89$, $p = 0.09$), but social exploration was increased in saline-treated (but not nicotine-treated) animals when comparing 24 h to 2 h of social isolation ($t_{veh\_2h-24h}(7) = -3.77$, $p = 0.007$; $t_{nic\_2h-24h}(7) = -0.66$, $p = 0.53$).

Experiment 3a and 3b: place conditioning: effects of alcohol treatment

Alcohol (0.25 g/kg) did not affect acquisition of social play-induced conditioned place preference, as rats showed a preference for the play-paired compartment ($F_{compartment}(1,32) = 4.29$, $p = 0.05$; $F_{treatment}(1,32) = 0.11$, $p = 0.74$; $F_{compartment*treatment}(1,32) = 0.69$, $p = 0.41$, $n = 16$, Figure 4(A)).

In addition, coupling alcohol treatment to a specific compartment did not induce CPP as rats did not show a preference for an alcohol paired compartment over a saline-paired compartment ($F_{compartment}(1,32) = 1.76$, $p = 0.20$; $F_{treatment}(1,32) = 0.34$, $p = 0.56$; $F_{compartment*treatment}(1,32) = 0.09$, $p = 0.76$, $n = 16$, Figure 4(B)).

Experiment 4a and 4b: place conditioning: effects of nicotine treatment

Nicotine treatment (0.1 mg/kg) affected acquisition of social play-induced CPP ($F_{compartment}(1,32) = 21.89$, $p < 0.001$; $F_{compartment*treatment}(1,32) = 4.36$, $p = 0.05$; $F_{treatment}(1,32) = 0.22$, $p = 0.64$; $n = 16$). Post hoc paired *t*-tests demonstrate that saline-treated rats showed a preference for the compartment associated with social play ($t(7)_{vehicle\_soc\text{-}vs\text{-}nonsoc} = 2.90$, $p = 0.02$, Figure 4(C)), whereas this preference was absent in nicotine-treated animals ($t(7)_{nicotine\_soc\text{-}vs\text{-}nsoc} = 1.88$, $p = 0.10$).

In contrast, pairing a compartment solely with nicotine, without the opportunity to play, induced CPP ($F_{compartment*treatment}(1,32) = 6.40$, $p = 0.02$; $F_{compartment}(1,32) = 0.62$, $p = 0.44$; $F_{treatment}(1,32) = 1.48$, $p = 0.23$, post hoc paired *t*-tests: $t(7)_{nicotine} = 3.76$, $p = 0.007$; $t(7)_{vehicle} = -0.57$, $p = 0.59$, $n = 16$, Figure 4(D)).

Discussion

The aim of the present study was to investigate the effect of treatment with alcohol and nicotine that have previously been shown to enhance social play behaviour, on the motivational and pleasurable properties of this behaviour. We found that nicotine increased the motivation to play and its expression, depending on isolation time, when both

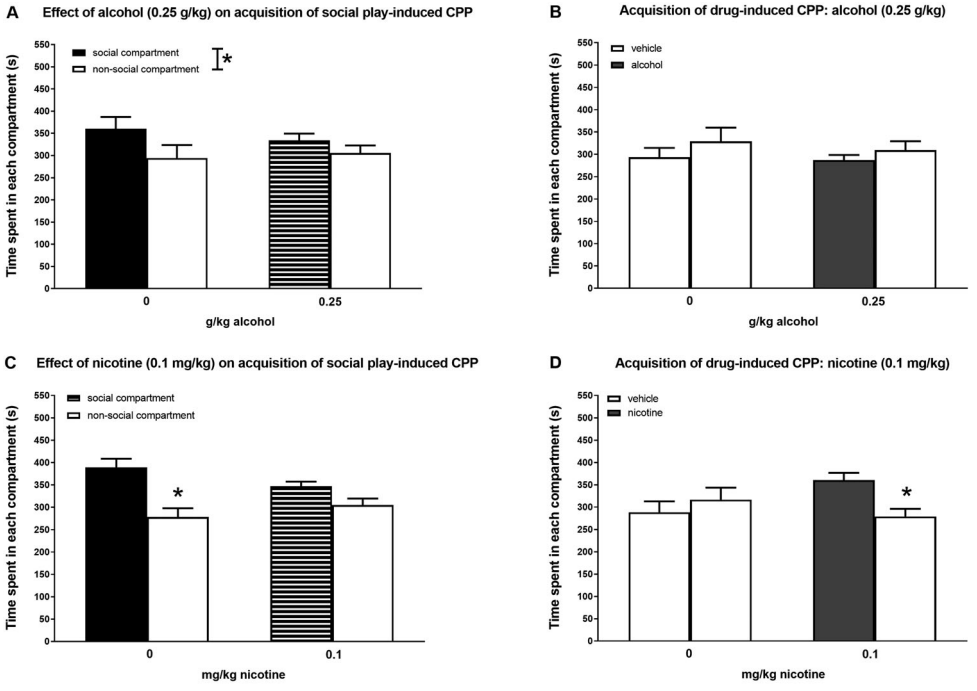

Figure 4. The effect of alcohol- and nicotine treatment on social play-induced conditioned place preference. Alcohol treatment (0.25 g/kg, $n = 8$) did not influence social play-induced conditioned place preference (CPP) compared to vehicle ($n = 8$) (A), animals spent more time in the social play-associated compartment (black/black-striped bars) compared to non-social compartment (white bars). Alcohol itself does not induce CPP (B, $n = 8$); animals spent equal amounts of time in the alcohol-associated compartment (grey bar) compared to the vehicle-associated compartment (white bars). Nicotine (0.1 mg/kg) disrupted the development of social play-induced conditioned place preference (CPP; C, $n = 8$). Animals spent equal amounts of time in the social play-associated compartment in combination with nicotine (black-striped bar) compared to the non-social compartment (white bar). Vehicle-treated animals spent more time in the social play-associated compartment (black bar) compared to the non-social compartment (white bar). Interestingly, nicotine itself did induce CPP (D $n = 8$); animals spent more time in the compartment associated with nicotine treatment (grey bar), compared to vehicle treatment (white bar). Data are presented as mean + SEM. *$p < 0.05$.

animals in a test pair were treated. Furthermore, nicotine disrupted social play-induced CPP but induced CPP by itself. Alcohol, however, did not affect motivation for social play and the expression of social play behaviour under 24 h social isolation conditions. In addition, the pleasurable properties of social play behaviour were unaffected as well.

Lack of effect of alcohol treatment on social play reward

In contrast to our prediction, alcohol, either when one or both animals were treated, did not alter the motivation to engage in social play nor did it affect the expression of social play behaviour itself. Low doses of alcohol have been found to increase social (play) behaviour in juvenile, adolescent and adult rats (Trezza, Baarendse, et al., 2009; Varlinskaya et al., 2001; Varlinskaya & Spear, 2002, 2006, 2009; Willey et al., 2009). A study by Varlinskaya et al. (2001) has suggested an increase in social motivation, as measured by social

contact behaviour, after treatment with low doses of alcohol, comparable to those used in the present study. However, this apparently increased motivation for social play was not observed when pertinently tested under a progressive ratio schedule of reinforcement in the present study. This suggests that social contact behaviour (i.e. social motivation in a situation when social interaction is possible with no boundaries) vs having to exert physical effort for the opportunity to engage in social interaction (i.e. proximal to the actual social interaction) reflect different dimensions of social motivation that rely on different neural substrates. In addition, as mentioned before, the constraints of our operant set-up may have precluded an increase in social play after alcohol treatment to be observed. Also, the increase in the expression of social play behaviour was observed after 3.5 h of social isolation (Trezza, Baarendse, et al., 2009), whereas in the operant conditioning paradigm animals were isolated for 24 h. This could have resulted in a ceiling effect, obscuring an effect of alcohol treatment. Since we did not investigate the effect of alcohol treatment on the motivation to play after a shorter isolation time, the possibility that alcohol does alter social play motivation under these circumstances remains to be investigated.

In the literature, systemic ethanol administration has been reported to decrease responding for food or water under different fixed ratio schedules (FR5, FR10 and FR20). However, this only occurred after treatment with doses higher than 0.9 g/kg (Chuck, McLaughlin, Arizzi-LaFrance, Salamone, & Correa, 2006; Gerak, Hicks, Winsauer, & Varner, 2004; Hiltunen & Jarbe, 1988; McLaughlin, Chuck, Arizzi-LaFrance, Salamone, & Correa, 2008). In addition, to our knowledge, the effects of alcohol treatment on responding for non-social rewards under progressive ratio schedules of reinforcement have not been reported in the literature.

Alcohol did not induce place conditioning by itself and did not affect social play-induced place conditioning. In the literature, both CPP (Bozarth, 1990; Colombo, Kuzmin, Fadda, Pani, & Gessa, 1990; Reid, Hunter, Beaman, & Hubbell, 1985) and conditioned place aversion (Ciccocioppo, Panocka, Froldi, Quitadamo, & Massi, 1999; Gauvin, Briscoe, Goulden, & Holloway, 1994; Philpot, Badanich, & Kirstein, 2003; Schechter Krimmer, 1992) have been found after alcohol exposure. Importantly, in these studies the doses used were often higher than in the present study, and the animals were older at the start of the experiment. In the present study, we used a relatively low dose of alcohol that was previously found to increase social play (Trezza, Baarendse, et al., 2009; Varlinskaya et al., 2001; Willey et al., 2009 but see Varlinskaya & Spear, 2002, 2004, 2006, 2009; Varlinskaya, Truxell, & Spear, 2015) when given acutely. Interestingly, Philpot et al. (2003) have previously found a relation between age of the animals at the start of a four-day place conditioning procedure and whether animals subsequently showed CPP or conditioned place aversion. In general, an increasing aversion to alcohol was observed with increasing doses, but with differences at varying ages in adolescent rats. Animals of PND 25 developed place preference at 0.2 g/kg and developed aversion at 1.0 and 2.0 g/kg. Animals of PND 35 did not develop place preference or aversion at any dose. Our animals were 26 days old at the start of the experiment and were tested at PND 37. Therefore, during training and testing, the subjective effects of alcohol may have changed in such a way that it did not evoke CPP (or aversion, for that matter). In addition, a study by Gauvin et al. (1994) showed that alcohol produced place aversion but this could be attenuated by the opportunity to engage in social interaction with either a sober or intoxicated animal. This latter finding resonates well with the observations (see de Wit & Sayette,

2018, for a review) that the subjective effects of substances of abuse may be different in a social setting.

Effects of nicotine on the motivational and pleasurable properties of social play behaviour

In line with our previous findings (Achterberg, Van Kerkhof, et al., 2016), a longer isolation period (24 h vs 2 h) resulted in higher motivational and play expression parameters. After 24 h of social isolation then, nicotine was found not to affect the motivation for social play, but it increased the expression of social play when both animals in a test pair were treated. Interestingly, after 2 h of social isolation, and when both animals in a test pair were treated, nicotine evoked an increased breakpoint but not the amount of earned rewards or active lever-presses. This indicates that nicotine treatment enhances the motivation to play under moderate isolation conditions (2 h isolation) but that it does not further elevate motivation when the internal motivation to play is already high (i.e. after 24 h isolation). A mechanistic explanation for the increase, albeit small, in breakpoint is that acute nicotine administration, at the dose used in our experiments, results in increased dopamine release in the nucleus accumbens (Fu, Matta, Gao, & Sharp, 2000; Fu, Matta, Gao, Brower, & Sharp, 2000; Nisell, Marcus, Nomikos, & Svensson, 1997; Schilstrom, Svensson, Svensson, & Nomikos, 1998), which has been widely implicated in incentive motivational processes (see Robbins & Everitt, 2007; Salamone & Correa, 2012, for reviews). Indeed, we have previously demonstrated that systemic treatment with dopamine reuptake inhibitors increased the motivation for social play behaviour (Achterberg, Van Kerkhof, et al., 2016). Consistent, studies on operant conditioning with food- (Popke, Mayorga, Fogle, & Paule, 2000 Wing & Shoaib, 2010) and water- (Olausson, Jentsch, & Taylor 2004) reward report an increase in motivation after acute nicotine administration in rats as well. Together, this suggests that nicotine increases the motivation for social play but is less potent compared to amphetamine and cocaine. Whether this effect is mediated via dopaminergic neurotransmission remains to be further explored.

In line with previous research on social interaction in adult (Cheeta, Irvine, & File, 2001; Cheeta, Irvine, Tucci, et al., 2001) and social play behaviour in juvenile rats (Trezza et al., 2009), nicotine was found to enhance the expression of social play behaviour, but only when both animals received nicotine treatment. Consistent, we have previously shown that the widely reported social play-enhancing effect of morphine (e.g. Trezza & Vanderschuren, 2008b; Vanderschuren et al., 1995a) could only be observed in our operant set-up when both animals in a test pair were treated (Achterberg et al., 2019). Furthermore, the effect was solely found after 24 h but not 2 h of social isolation prior to testing. Previously, Trezza, Baarendse, et al. (2009) found a nicotine-induced enhancement in play after 3.5 h of social isolation. Importantly however, the animals were allowed to freely interact for 15 min whereas in the present operant paradigm the reinforced periods to play last only one minute. Therefore, interruption of the playful interaction may have hampered drug treatment-induced increases in social play to be found (discussed in detail in Achterberg et al., 2019). Possible explanations for the effects of nicotine on social play in the present study are therefore that: 1. we used a low dose of nicotine to affect behaviour in the operant set-up; 2. a minimum of play motivation is required for nicotine to enhance play expression; 3. both animals in a test pair need

to be in similar states of motivation for nicotine treatment to enhance social play expression.

Nicotine, when experienced alone, appeared to be rewarding whereas it disrupted the acquisition of social play-induced CPP. The effects of nicotine on place conditioning have been widely investigated before (for reviews, see Cheeta, Irvine, & File, 2001; Cheeta, Irvine, Tucci, et al., 2001; Le Foll & Goldberg, 2005), both thus far, only one study has examined the interaction between social play behaviour and nicotine in place conditioning (Thiel, Sanabria, & Neisewander, 2009). In that study, a sub-effective conditioning protocol (i.e. two 10-minute conditioning sessions per day on two consecutive days) was used to determine whether nicotine and social play had synergistic rewarding effects. Indeed, Thiel et al. reported that while under these conditions, no CPP for either nicotine-treatment or play was found. However combining nicotine-treatment with social play did produce robust CPP. Importantly, Le Foll and Goldberg (2005) reviewed studies that examined the effect of nicotine on the acquisition of CPP. They found that although most studies show either CPP or no effect, a few observed conditioned place aversion after administration of different doses of nicotine. This shows that the effects of nicotine are not exclusively positive or negative, even when tested in the same paradigm. In our study, negative effects of nicotine could have interacted with the rewarding properties of play, thereby diminishing the effects on place preference. Of course, the intriguing question remains why this same dose of nicotine had rewarding effects by itself, but in view of the emerging literature on how the subjective effects of drug depend on whether they are experienced in a social setting or not (de Wit & Sayette, 2018), these findings may not even be that surprising.

Conclusion

Together, these data show that treatment with nicotine and alcohol, that both enhance social play expression, have distinct and complex effects on dissociable aspects of social play behaviour, i.e. motivation and pleasure. The combination of paradigms used to assess these aspects of social play behaviour results in a clearer picture of how these substances of abuse affect the different aspects of social play behaviour.

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References

Achterberg, E. J. M., Trezza, V., & Vanderschuren, L. J. M. J. (2012). β-Adrenoreceptor stimulation mediates reconsolidation of social reward-related memories. *PLoS One*, *7*(6), e39639.

Achterberg, E. J. M., Trezza, V., & Vanderschuren, L. J. M. J. (2014). Glucocorticoid receptor antagonism disrupts the reconsolidation of social reward-related memories in rats. *Behavioural Pharmacology*, *25*(3), 216–225.

Achterberg, E. J. M., Van Kerkhof, L. W. M., Servadio, M., van Swieten, M. M. H., Houwing, D. J., Aalderink, M., … Vanderschuren, L. J. M. J. (2016). Contrasting roles of dopamine and noradrenaline in the motivational properties of social play behavior in rats. *Neuropsychopharmacology*, *41*(3), 858–868.

Achterberg, E. J. M., Van Swieten, M. M. H., Driel, N. V., Trezza, V., & Vanderschuren, L. J. M. J. (2016). Dissociating the role of endocannabinoids in the pleasurable and motivational properties of social play behaviour in rats. *Pharmacological Research*, *110*, 151–158.

Achterberg, E. J. M., van Swieten, M. M. H., Houwing, D. J., Trezza, V., & Vanderschuren, L. J. M. J. (2019). Opioid modulation of social play reward in juvenile rats. *Neuropharmacology*, *159*, 107332.

Baarendse, P. J. J., Counotte, D. S., O'Donnell, P., & Vanderschuren, L. J. M. J. (2013). Early social experience is critical for the development of cognitive control and dopamine modulation of prefrontal cortex function. *Neuropsychopharmacology*, *38*, 1485–1494.

Barbano, M. F., & Cador, M. (2007). Opioids for hedonic experience and dopamine to get ready for it. *Psychopharmacology*, *191*, 497–506.

Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference: What does it add to our preclinical understanding of drug reward? *Psychopharmacology*, *153*, 31–43.

Berridge, K. C. (2007). The debate over dopamine's role in reward: The case for incentive salience. *Psychopharmacology*, *191*(3), 391–431.

Berridge, K. C., & Kringelbach, M. L. (2015). Pleasure systems in the brain. *Neuron*, *86*(3), 646–664. doi:10.1016/j.neuron.2015.02.018

Berridge, K. C., Robinson, T. E., & Aldridge, J. W. (2009). Dissecting components of reward: 'Liking', 'wanting', and learning. *Current Opinion in Pharmacology*, *9*, 65–73.

Bozarth, M. A. (1990). Evidence for the rewarding effects of ethanol using the conditioned place preference method. *Pharmacology Biochemistry and Behavior*, *35*(2), 485–487.

Calcagnetti, D. J., & Schechter, M. D. (1992). Place conditioning reveals the rewarding aspect of social interaction in juvenile rats. *Physiology and Behavior*, *51*, 667–672.

Cheeta, S., Irvine, E., & File, S. E. (2001). Social isolation modifies nicotine's effects in animal tests of anxiety. *British Journal of Pharmacology*, *132*, 1389–1395.

Cheeta, S., Irvine, E. E., Tucci, S., Sandhu, J., & File, S. E. (2001). In adolescence, female rats are more sensitive to the anxiolytic effect of nicotine than are male rats. *Neuropsychopharmacology*, *25*, 601–607.

Chuck, T. L., McLaughlin, P. J., Arizzi-LaFrance, M. N., Salamone, J. D., & Correa, M. (2006). Comparison between multiple behavioral effects of peripheral ethanol administration in rats: Sedation, ataxia, and bradykinesia. *Life Sciences*, *79*, 154–161.

Ciccocioppo, R., Panocka, I., Froldi, R., Quitadamo, E., & Massi, M. (1999). Ethanol induces conditioned place preference in genetically selected alcohol-preferring rats. *Psychopharmacology*, *141*, 235–241.

Colombo, G., Kuzmin, A., Fadda, F., Pani, L., & Gessa, G. L. (1990). Conditioned place preference induced by ethanol in a rat line selected for ethanol preference. *Pharmacological Research*, *22*(3), 48.

Douglas, L. A., Varlinskaya, E. I., & Spear, L. P. (2004). Rewarding properties of social interactions in adolescent and adult male and female rats: Impact of social versus isolate housing of subjects and partners. *Developmental Psychobiology*, *45*(3), 153–162.

Fu, Y., Matta, S. G., Gao, W., Brower, G., & Sharp, B. M. (2000). Systemic nicotine stimulates dopamine release in nucleus accumbens: Re-evaluation of the role of N-methyl-D-aspartate receptors in the ventral tegmental area. *Journal of Pharmacology and Experimental Therapeutics*, *294*, 458–465.

Fu, Y., Matta, S. G., Gao, W., & Sharp, B. M. (2000). Local a-bungarotoxin-sensitive nicotinic receptors in the nucleus accumbens modulate nicotine-stimulated dopamine secretion in vivo. *Neuroscience*, *101*, 369–375.

Gauvin, D. V., Briscoe, R. J., Goulden, K. L., & Holloway, F. A. (1994). Aversive attributes of ethanol can be attenuated by dyadic social interaction in the rat. *Alcohol*, *11*(3), 247–251.

Gerak, L. R., Hicks, A. R., Winsauer, P. J., & Varner, K. J. (2004). Interaction between 1,4-butanediol and ethanol on operant responding and the cardiovascular system. *European Journal of Pharmacology*, *506*, 75–82.

Hiltunen, A. J., & Jarbe, T. U. (1988). Ro 15-4513 does not antagonize the discriminative stimulus- or rate-depressant effects of ethanol in rats. *Alcohol*, *5*(3), 203–207.

Hodos, W. (1961). Progressive ratio as a measure of reward strength. *Science*, *134*, 943–944.

Kelley, A. E. (2004). Ventral striatal control of appetitive motivation: Role in ingestive behaviour and reward-related learning. *Neuroscience and Biobehavioral Reviews*, *27*, 765–776.

Le Foll, B., & Goldberg, S. R. (2005). Nicotine induces conditioned place preferences over a large range of doses in rats. *Psychopharmacology*, *178*, 481–492.

Le Merrer, J., Becker, J. A. J., Befort, K., & Kieffer, B. (2009). Reward processing by the opioid system in the brain. *Physiological Reviews*, *89*, 1379–1412.

Lipperman-Kreda, S., Paschall, M. J., Robert, F. S., & Morrison, C. N. (2018). Places and social contexts associated with simultaneous use of alcohol, tobacco and marijuana among young adults. *Drug and Alcohol Review*, *37*(2), 188–195. doi:10.1111/dar.12537

McLaughlin, P. J., Chuck, T. L., Arizzi-LaFrance, M. N., Salamone, J. D., & Correa, M. (2008). Central vs. peripheral administration of ethanol, acetaldehyde and acetate in rats: Effects on lever pressing and response initiation. *Pharmacology Biochemistry and Behavior*, *89*(3), 304–313.

Moss, H. B., Chen, C. M., & Yi, H. Y. (2014). Early adolescent patterns of alcohol, cigarettes, and marijuana polysubstance use and young adult substance use outcomes in a nationally representative sample. *Drug and Alcohol Dependence*, *136*, 51–62.

Nelson, S. E., Van Ryzin, M. J., & Dishion, T. J. (2015). Alcohol, marijuana, and tobacco use trajectories from age 12 to 24 years: Demographic correlates and young adult substance use problems. *Development and Psychopathology*, *27*, 253–277.

Niesink, R. J. M., & Van Ree, J. M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. *Neuropharmacology*, *28*, 411–418.

Nisell, M., Marcus, M., Nomikos, G. G., & Svensson, T. H. (1997). Differential effects of acute and chronic nicotine on dopamine output in the core and shell of the rat nucleus accumbens. *Journal of Neural Transmission*, *104*(1), 1–10.

Olausson, P., Jentsch, J. D., & Taylor, J. R. (2004). Nicotine enhances responding with conditioned reinforcement. *Psychopharamcology*, *171*, 173–178.

Panksepp, J. (1981). The ontogeny of play in rats. *Developmental Psychobiology*, *14*, 327–332.

Panksepp, J., & Beatty, W. W. (1980). Social deprivation and play in rats. *Behavioral and Neural Biology*, *30*(2), 197–206.

Panksepp, J., Siviy, S. M., & Normansell, L. (1984). The psychobiology of play: Theoretical and methodological perspectives. *Neuroscience and Biobehavioral Reviews*, *8*, 465–492.
Pellis, S. M., & Pellis, V. C. (1987). Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat Rattus norvegicus. *Aggressive Behavior*, *13*, 227–242.
Pellis, S. M., & Pellis, V. C. (2009). *The playful brain*. OneWorld Publications: Oxford.
Pellis, S. M., Pellis, V. C., & McKenna, M. M. (1993). Some subordinates are more equal than others – Play fighting amongst adult subordinate male rats. *Aggressive Behaviour*, *19*(5), 385–393.
Philpot, R. M., Badanich, K. A., & Kirstein, C. L. (2003). Place conditioning: Age-related changes in the rewarding and aversive effects of alcohol. *Alcoholism: Clinical and Experimental Research*, *27* (4), 593–599.
Popke, E. J., Mayorga, A. J., Fogle, C. M., & Paule, M. G. (2000). Effects of acute nicotine on several operant behaviors in rats. *Pharmacology, Biochemistry and Behavior*, *65*, 247–254.
Reid, L. D., Hunter, G. A., Beaman, C. M., & Hubbell, C. L. (1985). Toward understanding ethanol's capacity to be reinforcing: A conditioned place preference following injections of ethanol. *Pharmacology Biochemistry and Behavior*, *22*(3), 483–487.
Richardson, N. R., & Roberts, D. C. (1996). Progressive ratio schedules in drug self-administration studies in rats: A method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods*, *66*, 1–11.
Robbins, T. W., & Everitt, B. J. (2007). A role for mesencephalic dopamine in activation: Commentary on Berridge (2006). *Psychopharmacology*, *191*(3), 433–437.
Salamone, J. D., & Correa, M. (2012). The mysterious motivational functions of mesolimbic dopamine. *Neuron*, *76*, 470–485.
Schechter, M. D., & Krimmer, E. C. (1992). Differences in response to the aversive properties and activity effects of low dose ethanol in LAS and HAS selectively bred rats. *Psychopharmacology*, *107*(4), 564–568.
Schilstrom, B., Svensson, H. M., Svensson, T. H., & Nomikos, G. G. (1998). Nicotine and food induced dopamine release in the nucleus accumbens of the rat: Putative role of a7 nicotinic receptors in the ventral tegmental area. *Neuroscience*, *85*, 1005–1009.
Siviy, S. M., & Panksepp, J. (1987). Juvenile play in the rat: Thalamic and brain stem involvement. *Physiology and Behavior*, *41*, 103–114.
Siviy, S. M., & Panksepp, J. (2011). In search of the neurobiological substrates for social playfulness in mammalian brains. *Neuroscience and Biobehavioral Reviews*, *35*, 1821–1830.
Thiel, K. J., Sanabria, F., & Neisewander, J. L. (2009). Synergistic interaction between nicotine and social rewards in adolescent male rats. *Psychopharmacology*, *204*, 391–402.
Trezza, V., Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2009). Prosocial effects of nicotine and ethanol in adolescent rats through partially dissociable neurobehavioral mechanisms. *Neuropsychopharmacology*, *34*, 2560–2573.
Trezza, V., Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2010). The pleasures of play: Pharmacological insights into social reward mechanisms. *Trends in Pharmacological Sciences*, *31*, 463–469.
Trezza, V., Campolongo, P., & Vanderschuren, L. J. M. J. (2011). Evaluating the rewarding nature of social interactions in laboratory animals. *Developmental Cognitive Neuroscience*, *1*, 444–458.
Trezza, V., Damsteegt, R., & Vanderschuren, L. J. M. J. (2009). Conditioned place preference induced by social play behaviour: Parametrics, extinction, reinstatement and disruption by methylphenidate. *European Neuropsychopharmacology*, *19*, 659–669.
Trezza, V., & Vanderschuren, L. J. M. J. (2008a). Bidirectional cannabinoid modulation of social behavior in adolescent rats. *Psychopharmacology*, *197*, 217–227.
Trezza, V., & Vanderschuren, L. J. M. J. (2008b). Cannabinoid and opioid modulation of social play behavior in adolescent rats: Differential behavioral mechanisms. *European Neuropsychopharmacoly*, *18*, 519–530.
Tzschentke, T. M. (2007). Measuring reward with the conditioned place preference (CPP) paradigm: Update of the last decade. *Addiction Biology*, *12*, 227–462.

Van den Berg, C. L., Hol, T., Van Ree, J. M., Spruijt, B. M., Everts, H., & Koolhaas, J. M. (1999). Play is indispensable for an adequate development of coping with social challenges in the rat. *Developmental Psychobiology*, *34*, 129–138.

Vanderschuren, L. J. M. J. (2010). How the brain makes play fun. *American Journal of Play*, *2*, 315–337.

Vanderschuren, L. J. M. J., Achterberg, E. J. M., & Trezza, V. (2016). The neurobiology of social play and its rewarding value in rats. *Neuroscience and Biobehavioral Reviews*, *70*, 86–105.

Vanderschuren, L. J. M. J., Niesink, R. J., Spruijt, B. M., & Van Ree, J. M. (1995a). Effects of morphine on different aspects of social play in juvenile rats. *Psychopharmacology*, *117*(2), 225–231.

Vanderschuren, L. J. M. J., Niesink, R. J. M., Spruijt, B. M., & Van Ree, J. M. (1995b). Influence of environmental factors on social play behavior of juvenile rats. *Physiology & Behavior*, *58*, 119–123.

Vanderschuren, L. J. M. J., Niesink, R. J. M., & Van Ree, J. M. (1997). The neurobiology of social play behavior in rats. *Neuroscience and Biobehavioral Reviews*, *21*, 309–326.

Vanderschuren, L. J. M. J., & Trezza, V. (2014). What the laboratory rat has taught us about social play behavior: Role in behavioral development and neural mechanisms. *Current Opinion in Behavioral Neuroscience*, *16*, 189–212.

Vanderschuren, L. J. M. J., Trezza, V., Griffioen-Roose, S., Schiepers, O. J. G., Van Leeuwen, N., De Vries, T. J., & Schoffelmeer, A. N. M. (2008). Methylphenidate disrupts social play behavior in adolescent rats. *Neuropsychopharmacology*, *33*, 2946–2956.

Varlinskaya, E. I., & Spear, L. P. (2002). Acute effects of ethanol on social behavior of adolescent and adult rats: Role of familiarity of the test situation. *Alcoholism: Clinical and Experimental Research*, *26*, 1502–1511.

Varlinskaya, E. I., & Spear, L. P. (2004). Changes in sensitivity to ethanol-induced social facilitation and social inhibition from early to late adolescence. *Annals of the New York Academy of Sciences*, *1021*, 459–461.

Varlinskaya, E. I., & Spear, L. P. (2006). Differences in the social consequences of ethanol emerge during the course of adolescence in rats: Social facilitation, social inhibition, and anxiolysis. *Developmental Psychobiology*, *48*, 146–161.

Varlinskaya, E. I., & Spear, L. P. (2009). Ethanol-Induced social facilitation in adolescent rats: Role of endogenous activity at mu opioid receptors. *Alcoholism: Clinical and Experimental Research*, *33*, 991–1000.

Varlinskaya, E. I., Spear, L. P., & Spear, N. E. (2001). Acute effects of ethanol on behavior of adolescent rats: Role of social context. *Alcoholism: Clinical and Experimental Research*, *25*, 377–385.

Varlinskaya, E. I., Truxell, E. M., & Spear, L. P. (2015). Sex differences in sensitivity to the social consequences of acute ethanol and social drinking during adolescence. *Behavioural Brain Research*, *282*, 6–13.

Veeneman, M. M. J., Boleij, H., Broekhoven, M. H., Snoeren, E. M. S., Guitart, M. M., Cousijn, J., … Vanderschuren, L. J. M. J. (2011). Dissociable roles of mGlu5 and dopamine receptors in the rewarding and sensitizing properties of morphine and cocaine. *Psychopharmacology*, *214*, 863–876.

Willey, A. R., Varlinskaya, E. I., & Spear, L. P. (2009). Social interactions and 50 kHz ultrasonic vocalizations in adolescent and adult rats. *Behavioural Brain Research*, *202*(1), 122–129. doi:10.1016/j.bbr.2009.03.025

Wing, V. C., & Shoaib, M. (2010). A second-order schedule of food reinforcement in rats to examine the role of CB1 receptors in the reinforcement-enhancing effects of nicotine. *Addiction Biology*, *15*, 380–392.

de Wit, H., & Sayette, M. (2018). Considering the context: Social factors in responses to drugs in humans. *Psychopharmacology*, *235*(4), 935–945.