ORIGINAL INVESTIGATION

Social play behavior, ultrasonic vocalizations and their modulation by morphine and amphetamine in Wistar and Sprague-Dawley rats

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Abstract

Rationale Social play behavior is the most characteristic social behavior in young mammals. It is highly rewarding and crucial for proper neurobehavioral development. Despite the importance of genetic factors in normal and pathological social behaviors, little information is available about strain influences on social play.

Objective and methods The aim of this study was to investigate differences in social play behavior, 50-kHz ultrasonic vocalizations (USVs) and their modulation by acute morphine and amphetamine administration in two rat strains widely used in behavioral pharmacology studies, i.e., Wistar and Sprague–Dawley rats.

Results Sprague–Dawley rats showed higher levels of social play than Wistar rats. In both strains, no correlation was found between the performance of social behaviors and the emission of 50-kHz USVs. In Wistar and Sprague–Dawley rats, morphine increased and amphetamine decreased social play. The effects of morphine, however, were more pronounced in Wistar than Sprague–Dawley animals. In both strains, morphine did not affect USV emission, while amphetamine increased it during cage exploration. In Sprague–Dawley rats only, amphetamine decreased USVs during social interaction.

Conclusions Wistar and Sprague–Dawley rats differ in their absolute levels of social play behavior and 50-kHz USVs, and quantitative differences exist in their response to pharmacological manipulations of social play. The emission of 50-kHz USVs and the behavioral parameters thought to reflect rewarding social interactions in adolescent rats are dissociable.

Keywords Social behavior · USV · Adolescence · Opioids · Psychostimulants

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Introduction

Social play behavior is the first form of non-parent directed social behavior displayed by most mammalian species (Panksepp et al. 1984; Pellis and Pellis 2009; Vanderschuren et al. 1997). The experience of social play behavior in-between weaning and sexual maturity is crucial for proper social and cognitive development (Baarendse et al. 2013; Panksepp et al. 1984; Pellis and Pellis 2009; Spinka et al. 2001; Van den Berg et al. 1999; Vanderschuren et al. 1997; Von Frijtag et al. 2002), and abnormalities in social play behavior have been observed in developmental psychiatric disorders, such as autism and attention deficit/hyperactivity disorder (Alessandri 1992; Jordan 2003; Manning and Wainwright 2010). In line with its importance for proper neurobehavioral development, social play behavior is a highly rewarding activity (Calcagnetti and Schechter 1992; Crowder and Hutto 1992; Douglas et al. 2004; Falk 1958; Humphreys and Einon 1981; Ikemoto and Panksepp 1992; Mason et al.

1963; Normansell and Panksepp 1990; Thiel et al. 2008; Thiel et al. 2009; Trezza et al. 2009; for reviews see Trezza et al. 2010, 2011a; Vanderschuren 2010).

Genetic factors profoundly affect the development and expression of social behavior from birth to adulthood, and they contribute to normal variation in social behavior between individuals (Ebstein et al. 2010; O'Connell and Hofmann 2010). However, they also play a major role in aberrant social behavior and psychopathology (Meyer-Lindenberg and Tost 2012). Therefore, a better understanding of the extent to which a particular genotype affects social behavior is an important issue in social neuroscience research.

Despite its crucial role in development, to date limited information is available about genetic influences on social play behavior (Ferguson and Cada 2004; Siviy et al. 2003; Reinhart et al. 2006; Siviy et al. 2011). To address this issue, we investigated whether baseline differences in social play behavior exist between adolescent male Wistar and Sprague–Dawley rats. These two outbred strains are widely used in behavioral pharmacology studies (Hedrich 2006; McDermott and Kelly 2008) and are the two preferred rat strains used in developmental neurotoxicity testing in Europe and North America (Clause 1993; Zmarowski et al. 2012). At adulthood, rats from these two strains are known to differ in a wide range of emotional-related behaviors (Rex et al. 2004; Staples and McGregor 2006; Walker et al. 2009), including social interactions (Rex et al. 2004). Therefore, we hypothesized that they also differ in adolescent social play behavior.

During social interactions, rats emit ultrasonic vocalizations (USVs) which are considered a measure of affective states and a means of communication (Knutson et al. 2002; Lahvis et al. 2011; Scattoni et al. 2009; Takahashi et al. 2010; Wohr and Schwarting 2013). In rats, low-frequency (around 22-kHz) USVs have been associated with negative social experiences (e.g., exposure to predator odor, inter-male fighting), while high frequency (around 50-kHz) USVs have been detected in social contexts involving potential reward (e.g., sexual approach, play fighting) (Burgdorf et al. 2011). For this reason, we also investigated whether adolescent Wistar and Sprague–Dawley rats showed a different pattern of USV emission during active social interactions.

We and others have previously shown that opioid and psychostimulant drugs have opposite effects on social play behavior. Thus, systemic administration of opioid receptor agonists such as morphine enhances social play, while psychostimulants such as methylphenidate or amphetamine suppress it (Siviy and Panksepp 2011; Trezza et al. 2010). Opioid neurotransmission is thought to mediate the rewarding and motivational properties of social play behavior (Trezza et al. 2011b), whereas psychostimulants may modulate cognitive aspects of social play behavior (Trezza et al. 2010; Vanderschuren et al. 2008).It has been shown that Wistar

and Sprague–Dawley rats show different sensitivity toward the behavioral effects induced by morphine (Mas et al. 2000; Shoaib et al. 1995) and amphetamine (Kinney et al. 1999; McDermott and Kelly 2008) administration. Therefore, we hypothesized that adolescent Wistar and Sprague–Dawley rats also differ in the effects induced by acute morphine and amphetamine administration on social play behavior and USV emission.

Materials and methods

Animals

Male Wistar and Sprague–Dawley rats (Charles River Laboratories, Calco, Italy) arrived in our animal facility at 21 days of age and were housed in groups of five in $43 \times 26 \times 20$ cm ($l \times w \times h$) Macrolon cages under controlled conditions (i.e., temperature 21 ± 1 °C, 60 ± 10 % relative humidity, and 12/12-h light cycle with lights on at 7:00AM). Food and water were available ad libitum. All animals were experimentally naïve and used only once (i.e., each animal received one injection only, with either drug or vehicle solution). All experiments were approved by the Italian Ministry of Health (Rome, Italy) and performed in agreement with the guidelines released by the Italian Ministry of Health (D.L. 116/92) and the European Community Directive 2010/63/EU of 22 September 2010.

Drugs

The opioid receptor agonist morphine (SALARS, Como, Italy) and the psychostimulant D-amphetamine sulfate (RBI, Research Biochemicals International, MA, USA) were dissolved in saline and given subcutaneously (s.c.) 1 h and 30 min before testing, respectively. For the dose–response experiments shown in Figs. 1 and 4, three drug doses (0.3, 1, and 3 mg/kg of morphine; 0.05, 0.2, and 0.5 mg/kg of amphetamine) were tested. On the basis of these results, for the experiments shown in Figs. 2, 3, 5, and 6, we used the lowest doses of morphine (1 mg/kg) and amphetamine (0.2 mg/kg) that significantly affected social play behavior in both strains. Drug doses and pretreatment intervals were based on our previous experiments and literature data (Beatty et al. 1982; Field and Pellis 1994; Trezza et al. 2010; Trezza and Vanderschuren 2008a; Vanderschuren et al. 1995a, c). We used doses of morphine and amphetamine that are known to have minimal effects on locomotor activity and do not evoke stereotyped behaviors (Bolanos et al. 1998; Fowler et al. 2003; Kuczenski and Segal 1999; Magnus-Ellenbroek and Havemann-Reinecke 1993; Trezza and Vanderschuren 2008a; Vezina and Queen 2000). Solutions were freshly prepared on the day of the experiment and were administered in a
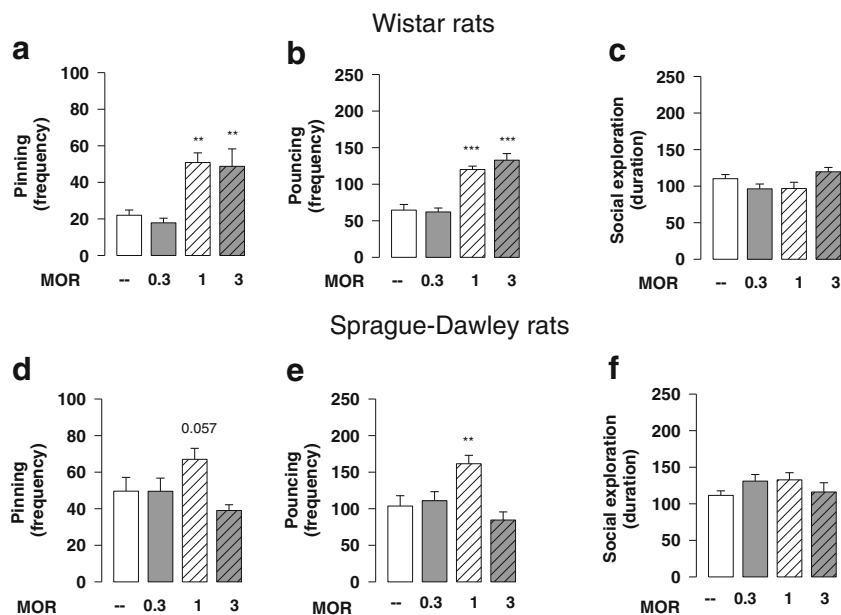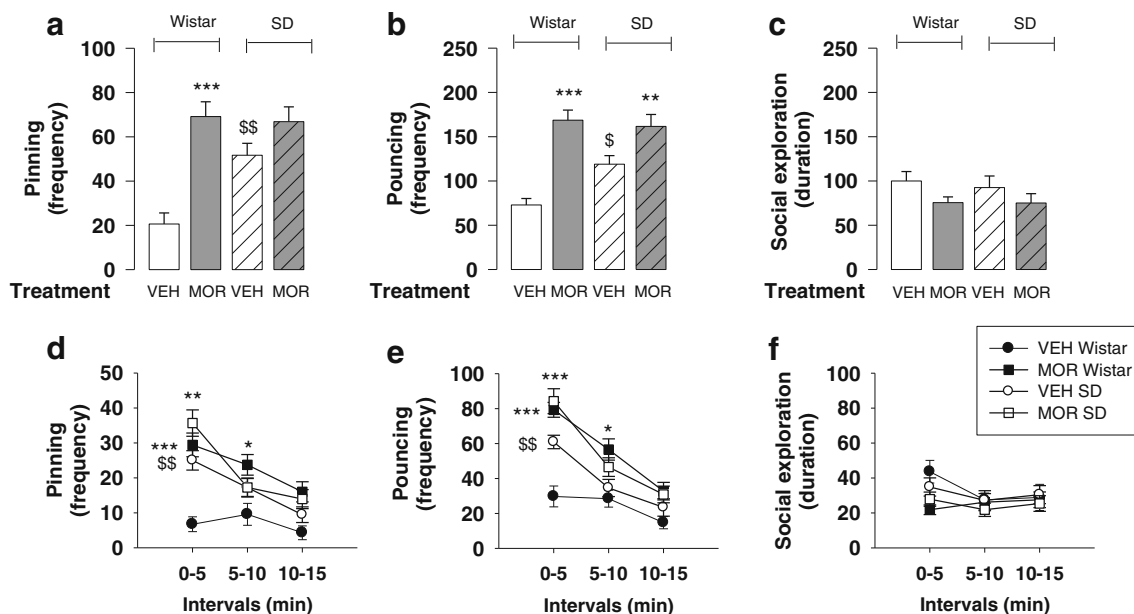
Fig. 1 Effects of graded doses of the opioid receptor agonist morphine (MOR; 0.3, 1, and 3 mg/kg, s.c., 1 h before test) on social play behavior in adolescent Wistar and Sprague–Dawley rats. Morphine, at the doses of 1 and 3 mg/kg, increased pinning (**a**) and pouncing (**b**) frequency in Wistar rats, with no effect on social exploration (**c**). In Sprague–Dawley rats, morphine, at the dose of 1 mg/kg, induced a trend for an increase in pinning (**d**) and significantly increased pouncing (**e**), with no effect on social exploration (**f**). Data represent mean±SEM frequency of pinning and pouncing and mean±SEM duration of social exploration. **$p<0.01$; ***$p<0.001$ vs. vehicle treatment; $N=7$–10 per treatment group

volume of 2 ml/kg. Because of the importance of the neck area in the expression of social play behavior (Pellis and Pellis 1987; Siviy and Panksepp 1987), subcutaneous injections were administered in the flank.

Fig. 2 Effects of the opioid receptor agonist morphine (MOR; 1 mg/kg, s.c., 1 h before test) on social play behavior in adolescent Wistar and Sprague–Dawley rats. Vehicle-treated Sprague–Dawley rats showed higher levels of pinning (**a**) and pouncing (**b**) than vehicle-treated Wistar rats. Morphine administration increased pinning (**a**) and pouncing (**b**) in both strains, although its effects on pinning and pouncing were more pronounced in Wistar than in Sprague–Dawley rats. When behaviors were analyzed per 5-min intervals, vehicle-treated Sprague–Dawley rats played more than vehicle-treated Wistar rats during the first 5-min interval of the test (**d**, **e**); morphine enhanced pinning (**d**) and pouncing (**e**) during the first 5-min interval of the test in both Wistar and Sprague–Dawley rats. In Wistar rats, morphine also increased pinning and pouncing frequencies (**d**, **e**) during the second 5-min periods of the test. No strain or treatment effects were found in the total time spent in general social exploration during social play behavior (**c**, **f**). Data represent mean±SEM frequency of pinning and pouncing and mean±SEM duration of social exploration. *$p<0.05$, **$p<0.01$; ***$p<0.001$ vs. vehicle treatment; $^{\$}p<0.05$, $^{\$\$}p<0.01$ Sprague–Dawley vs. Wistar rats (Student–Newman–Keule post hoc test). $N=7$–12 per treatment group
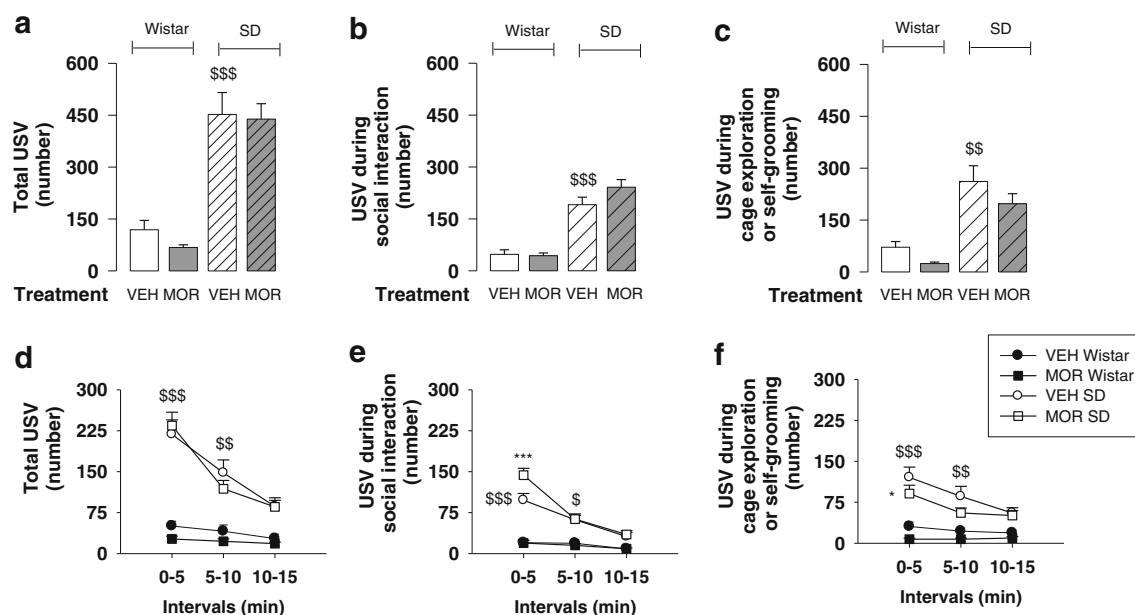
Fig. 3 Effects of morphine (MOR; 1 mg/kg, s.c., 1 h before test) on 50-kHz ultrasonic vocalizations in adolescent Wistar and Sprague–Dawley rats during the social play behavior session. Over the 15-min test session, vehicle-treated Sprague–Dawley rats emitted more USVs than vehicle-treated Wistar rats (**a**, **b**, **c**); no effect of morphine treatment on USV emission was found in both strains (**a**, **b**, **c**). When behaviors were analyzed per 5-min intervals, vehicle-treated Sprague–Dawley rats emitted more USVs, both related and unrelated to the social interaction, than vehicle-treated Wistar rats during the first and the second 5-min interval of the test session (**d**, **e**, **f**). In addition, in the first 5-min interval, morphine increased the number of USVs emitted by Sprague–Dawley rats during social interaction (**e**), whereas it decreased the number of USVs emitted by Sprague–Dawley rats during cage exploration and self-grooming (**f**). Morphine did not affect USV emission at any time point in Wistar rats. Data represent mean±SEM frequency of total USVs (**a**, **d**) and USVs either related (**b**, **e**) and unrelated (**c**, **f**) to the social interaction. *$p < 0.05$, ***$p < 0.001$ vs. vehicle treatment; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$; $^{\$\$\$}p < 0.001$ Sprague–Dawley vs. Wistar rats (Student–Newman–Keule post hoc test). $N = 7$–12 per treatment group

Procedure

Social play behavior

All the experiments were performed in a sound attenuated chamber under dim light conditions. The testing arena consisted of a Plexiglas cage measuring $40 \times 40 \times 60$ cm ($l \times w \times h$), with approximately 2 cm of wood shavings covering the floor. The behavior of the animals was recorded using a video camera with zoom lens, DVD recorder, and LCD monitor.

Social play was assessed as previously described (Trezza et al. 2009; Trezza and Vanderschuren 2008a, b; Vanderschuren et al. 2008). Briefly, at 25–28 days of age, rats were individually habituated to the test cage for 10 min on each of the two days prior to testing. On the test day, the animals were socially isolated for 3.5 h before testing to enhance their social motivation and thus facilitate the expression of social play behavior during testing. This isolation period has been shown to induce a half-maximal increase in the amount of social play behavior (Niesink and Van Ree 1989; Vanderschuren et al. 1995a, 2008). At the appropriate time before testing, pairs of animals were treated with drugs or vehicle. The test consisted of placing two similarly treated animals into the test cage for 15 min. The animals of each pair

did not differ more than 10 g in body weight and had no previous common social experience (i.e., they were not cage mates). Drug treatments were randomized so that cage mates were allocated to different treatment groups.

Behavior was assessed per pair of animals and analyzed by a trained observer who was unaware of treatment condition using the Observer XT software (Noldus, Wageningen, The Netherlands). In rats, a bout of social play behavior starts with one rat soliciting ("pouncing") another animal by attempting to nose or rub the nape of its neck. The animal that is pounced upon can respond in different ways. If the animal that is pounced upon fully rotates to its dorsal surface, "pinning" is the result, i.e., one animal lying with its dorsal surface on the floor with the other animal standing over it. From this position, the supine animal can initiate another play bout by trying to gain access to the other animal's neck. Thus, during social play, pouncing is considered an index of play solicitation, while pinning functions as a releaser of a prolonged play bout (Panksepp and Beatty 1980; Pellis and Pellis 1987; Pellis et al. 1989; Poole and Fish 1975). Pinning and pouncing frequencies can be easily quantified and they are considered to be the most characteristic parameters of social play behavior in rats (Panksepp and Beatty 1980). During the social encounter, animals may also display social behaviors not directly associated with play, such as sniffing or grooming the partner's body

(Panksepp and Beatty 1980; Vanderschuren et al. 1995c). Since social play behavior in rats strongly depends on the playfulness of its partner (Pellis and McKenna 1992; Trezza and Vanderschuren 2008b), in the present study, both animals in a play pair were similarly treated, and a pair of rats was considered as one experimental unit. The following parameters were therefore scored per pair of animals:

Social behaviors directly related to play:

- frequency of pinning
- frequency of pouncing

Social behaviors unrelated to play:

- time spent in social exploration: the total amount of time spent in non playful forms of social interaction (i.e., one animal sniffing or grooming any part of the partner's body).

Measurement of ultrasonic vocalizations during social interaction

USVs were recorded as previously described (Burgdorf et al. 2005; Trezza et al. 2008; Willey et al. 2009). The USVs emitted during the social play behavior session were detected by an ultrasonic microphone (SM2, Ultrasound Advice) fixed at 30 cm above the floor of the test cage in order to record USVs from the whole chamber. The microphone was connected to a Bat Detector (US 30 Ultrasound Advice) tuned to 50 ± 10 kHz and connected to a high-speed tape recorder (Racal Store). The number of USVs was manually and independently recorded by three experimenters blind to the treatment, by listening to the audible output of the tape recorder through headphones (Philips HI-FI stereo SHP9000). The experimenters were listening to the audible output through headphones while watching the behavior of the animals in the LCD monitor, thus being able to discriminate between the USVs emitted during social-related behaviors (i.e., pinning, pouncing, boxing, sniffing, following, social grooming) and USVs emitted during non social-related behaviors (i.e., cage exploration (rearing, wall rearing, digging) and self-grooming). The total number of USVs emitted during the 15-min social play testing session was analyzed per pair of animals. Thus, a pair of rats was treated as a single observation. Total USVs represent the sum of USVs emitted during social-related and social-unrelated behaviors.

Statistical analysis

Pinning and pouncing frequencies and time spent in social exploration were calculated per pair of animals and expressed as mean±SEM. The USVs emitted during social-related and social-unrelated behaviors and the total number of USVs emitted during the 15-min test session were calculated per

pair of animals and expressed as mean±SEM. Data from the dose–response experiments were analyzed using one-way analysis of variance (ANOVA). To assess the effects of the different strains and treatments on social play behavior and USV production, data were analyzed using two-way ANOVA, using treatment (morphine or amphetamine) and strain (Wistar or Sprague–Dawley) as between-subjects factors. One- and two-way ANOVA were followed by Student–Newman–Keuls post hoc test where appropriate. To assess whether the effects of morphine and amphetamine on social play behavior and USV production in Wistar and Sprague–Dawley rats changed over time, the 15-min test session was divided in three blocks of 5 min, which were analyzed using two-way ANOVA for repeated measures, followed by Student–Newmann–Keuls post hoc test where appropriate. P values of less than 0.05 were considered statistically significant.

Results

Effects of morphine on social play behavior in adolescent Wistar and Sprague–Dawley rats

Dose–response experiments performed in Wistar and Sprague–Dawley rats showed that morphine administration increased social play behavior in both strains. In Wistar rats, a one–way ANOVA analysis performed on pinning and pouncing frequencies and on duration of social exploration gave the following results: [pinning: $F_{3,26}=9.822$, $p=0.0002$; pouncing: $F_{3,26}=24.755$, $p<0.0001$; social exploration: $F_{3,26}=2.731$, $p=0.0643$]. Post hoc analysis revealed that morphine (MOR), at the doses of 1 and 3 mg/kg, significantly increased pinning (Fig. 1a; $p<0.01$) and pouncing (Fig. 1b; $p<0.001$), with no effect on social exploration (Fig. 1c; n.s.). In Sprague–Dawley rats, a one-way ANOVA analysis performed on pinning and pouncing frequencies and on duration of social exploration gave the following results: [pinning: $F_{3,30}=3.019$, $p=0.0452$; pouncing: $F_{3,30}=6.630$, $p=0.0014$; social exploration: $F_{3,30}=1.404$, $p=0.2608$]. Post hoc analysis revealed that morphine, at the dose of 1 mg/kg, significantly increased pouncing (Fig. 1e; $p<0.01$), while induced a trend for an increase in pinning (Fig. 1d; $p=0.057$). Morphine did not affect social exploration in Sprague–Dawley rats (Fig. 1f; n.s.).

When we compared the effects of morphine (1 mg/kg) on social play behavior in the two strains, we found that the increase in social play induced by morphine was more pronounced in Wistar than in Sprague–Dawley rats. A two-way ANOVA analysis performed on pinning and pouncing frequencies gave the following results: pinning [$F_{(\text{strain})1,31}=4.929$, $p=0.034$; $F_{(\text{treatment})1,31}=24.164$, $p<0.001$; $F_{(\text{strain x treatment})1,31}=6.637$, $p=0.015$]; pouncing [$F_{(\text{strain})1,31}=2.572$, $p=0.119$; $F_{(\text{treatment})1,31}=32.319$, $p<0.001$; $F_{(\text{strain x treatment})1,31}=4.743$, $p=0.037$]. Post hoc

analysis revealed that baseline levels of social play behavior were higher in Sprague–Dawley than in Wistar rats. Vehicle-treated Sprague–Dawley rats showed higher levels of pinning and pouncing (Fig. 2a, b) than vehicle-treated Wistar rats ($p < 0.01$ for pinning; $p < 0.05$ for pouncing). Morphine administration increased social play behavior in both strains, although its effects on pinning (Fig. 2a) were more pronounced in Wistar ($p < 0.001$) than in Sprague–Dawley rats ($p = 0.064$). As for the time spent in general social exploration, the two-way ANOVA did not show a statistically significant difference between either the two strains or treatment groups: [Fig. 2c: $F_{(strain)1,31} = 0.117$, $p = 0.735$; $F_{(treatment)1,31} = 3.345$, $p = 0.077$; $F_{(strain \times treatment)1,31} = 0.0928$, $p = 0.763$]. When behaviors were analyzed per 5-min intervals, the two-way ANOVA for repeated measures revealed that morphine enhanced pinning (Fig. 2d; [$F_{(interval)2,62} = 25.940$, $p < 0.0001$; $F_{(interval \times strain)2,62} = 6.151$, $p = 0.0037$; $F_{(interval \times treatment)2,62} = 4.080$, $p = 0.0216$; $F_{(interval \times strain \times treatment)2,62} = 0.455$, $p = 0.6364$]) and pouncing (Fig. 2e; [$F_{(interval)2,62} = 65.042$, $p < 0.0001$; $F_{(interval \times strain)2,62} = 4.778$, $p = 0.0118$; $F_{(interval \times treatment)2,62} = 6.581$, $p = 0.0026$; $F_{(interval \times strain \times treatment)2,62} = 0.694$, $p = 0.5035$]) in both Sprague–Dawley and Wistar rats and that there were differences in the baseline levels of social play behavior between the two rat strains. Post hoc analysis revealed that vehicle-treated Sprague–Dawley rats played more than vehicle-treated Wistar rats during the first 5-min interval of the test (Fig. 2d, e; $p < 0.01$ for pinning; $p < 0.01$ for pouncing). In both Wistar and Sprague–Dawley rats, morphine enhanced pinning (Fig. 2d) and pouncing (Fig. 2e) during the first 5-min interval of the test (pinning: $p < 0.001$ for Wistar rats; $p < 0.01$ for Sprague–Dawley rats; pouncing: $p < 0.001$ for both Wistar and Sprague–Dawley rats). In Wistar rats, morphine also increased pinning and pouncing frequencies during the second 5-min period of the test (Fig. 2d, e; $p < 0.05$ for both pinning and pouncing). No differences in the total time spent in general social exploration were observed between either strains or treatment groups (Fig. 2f; [$F_{(interval)2,62} = 2.554$, $p = 0.0859$; $F_{(interval \times strain)2,62} = 0.064$, $p = 0.9381$; $F_{(interval \times treatment)2,62} = 2.533$, $p = 0.0876$; $F_{(interval \times strain \times treatment)2,62} = 1.797$, $p = 0.1743$]).

Effects of morphine on 50-kHz ultrasonic vocalizations in adolescent Wistar and Sprague–Dawley rats

The rate of USVs emitted during the test session differed between Wistar and Sprague–Dawley rats, with the latter emitting more USVs than the former. In both strains, no correlation was found between the performance of social behaviors and the emission of 50-kHz USVs. Morphine administration had no effects in both strains.

A two-way ANOVA analysis performed on the number of total USVs emitted during the 15-min test session (Fig. 3a) and on the USVs emitted exclusively either during active social interaction (Fig. 3b) or during cage exploration or self-grooming (Fig. 3c) gave the following results: total USVs [$F_{(strain)1,31} = 54.902$, $p < 0.001$; $F_{(treatment)1,31} = 0.470$, $p = 0.498$; $F_{(strain \times treatment)1,31} = 0.157$; $p = 0.695$]; USVs during social interaction [$F_{(strain)1,31} = 70.286$, $p < 0.001$; $F_{(treatment)1,31} = 1.302$, $p = 0.263$; $F_{(strain \times treatment)1,31} = 1.789$, $p = 0.191$]; USVs during cage exploration or self-grooming [$F_{(strain)1,31} = 31.186$, $p < 0.001$; $F_{(treatment)1,31} = 2.952$, $p = 0.096$; $F_{(strain \times treatment)1,31} = 0.067$, $p = 0.798$]. Post hoc analysis revealed that, over the 15-min testing session, vehicle-treated Sprague–Dawley rats emitted more USVs ($p < 0.001$), either related ($p < 0.001$) or unrelated ($p < 0.01$) to the social interaction, than vehicle-treated Wistar rats (Fig. 3a–c). Furthermore, no effect of morphine treatment on USV emission was found in both rat strains (Fig. 3a–c).

When behaviors were analyzed per 5-min intervals, two-way ANOVA for repeated measures performed on the total number of USVs revealed that Sprague–Dawley rats emitted more USVs than Wistar rats (Fig. 3d; [$F_{(interval)2,62} = 54.488$, $p < 0.0001$; $F_{(interval \times strain)2,62} = 35.426$, $p < 0.0001$; $F_{(interval \times treatment)2,62} = 1.091$, $p = 0.342$; $F_{(interval \times strain \times treatment)2,62} = 1.425$, $p = 0.248$]). The USVs emitted during social interaction (Fig. 3e; [$F_{(interval)2,62} = 42.524$, $p < 0.0001$; $F_{(interval \times strain)2,62} = 26.874$, $p < 0.0001$; $F_{(interval \times treatment)2,62} = 2.877$, $p = 0.0638$; $F_{(interval \times strain \times treatment)2,62} = 2.551$, $p = 0.0861$]) and during cage exploration or self-grooming (Fig. 3f; [$F_{(interval)2,62} = 17.889$, $p < 0.0001$; $F_{(interval \times strain)2,62} = 12.211$, $p < 0.0001$; $F_{(interval \times treatment)2,62} = 2.215$, $p = 0.117$; $F_{(interval \times strain \times treatment)2,62} = 0.553$, $p = 0.578$]) were higher in Sprague–Dawley than in Wistar rats. Post hoc analysis revealed that vehicle-treated Sprague–Dawley emitted more USVs, either related or unrelated to social interaction, than vehicle-treated Wistar rats during the first (Fig. 3d–f; $p < 0.001$ for total number of USVs and for USVs related and unrelated to social interaction) and the second 5-min interval of the test session (Fig. 3d, f; $p < 0.01$ for total number of USVs and for USVs during cage exploration or self-grooming; Fig. 3e; $p < 0.05$ for USVs related to social interaction). In the first 5-min interval only, morphine increased the number of USVs emitted by Sprague–Dawley rats during social interaction (Fig. 3e; $p < 0.001$), whereas it decreased the number of USVs emitted by Sprague–Dawley rats during cage exploration and self-grooming (Fig. 3f; $p < 0.05$). Morphine did not affect USV emission at any time point in Wistar rats (Fig. 3d–f).

Effects of amphetamine on social play behavior in adolescent Wistar and Sprague–Dawley rats

Separate dose–response experiments performed in Wistar and Sprague–Dawley rats showed that amphetamine administration decreased social play behavior in both strains. A one-way ANOVA analysis performed on pinning and pouncing frequencies and on duration of social exploration in either

Wistar or Sprague–Dawley rats gave the following results: Wistar rats [pinning: $F_{3,29}=14.457$, $p<0.0001$; pouncing: $F_{3,29}=27.509$, $p<0.0001$; social exploration: $F_{3,29}=2.368$, $p=0.0913$; Fig. 4a–c]; Sprague–Dawley rats [pinning: $F_{3,29}=20.249$, $p<0.0001$; pouncing: $F_{3,29}=24.658$, $p<0.0001$; social exploration: $F_{3,29}=2.174$, $p=0.1126$; Fig. 4d–f]. Post hoc analysis revealed that amphetamine (AMPH), at the doses of 0.2 and 0.5 mg/kg, significantly decreased pinning (Fig. 4a; $p<0.001$; Fig. 4d; $p<0.001$) and pouncing (Fig. 4b; $p<0.001$; Fig. 4e; $p<0.001$), with no effect on social exploration (Fig. 4c; n.s.; Fig. 4f; n.s.) in both strains.

When we compared the effects of amphetamine (0.2 mg/kg) on social play behavior in the two strains, we found that the drug induced quantitatively similar effects in Wistar and Sprague–Dawley rats. A two-way ANOVA performed on pinning and pouncing frequencies gave the following results: pinning [$F_{(strain)1,27}=12.496$, $p=0.001$; $F_{(treatment)1,27}=41.976$, $p<0.001$; $F_{(strain\times treatment)1,27}=10.409$, $p=0.003$]; pouncing [$F_{(strain)1,27}=23.805$, $p<0.001$; $F_{(treatment)1,27}=55.153$, $p<0.001$; $F_{(strain\times treatment)1,27}=3.003$, $p=0.095$]. As in the previous experiments, post hoc analysis showed that vehicle-treated Sprague–Dawley rats displayed higher pinning and pouncing frequencies than vehicle-treated Wistar rats (Fig. 5a, b; $p<0.001$ for both pinning and pouncing). Amphetamine reduced the frequency of pinning and pouncing in both Wistar and Sprague–Dawley rats (Fig. 5a, b; Wistar rats: $p<0.05$ for pinning and $p<0.001$ for pouncing; Sprague–Dawley rats: $p<0.001$ for both pinning and pouncing).

No strain or treatment effects were found in the total time spent in general social exploration during social play behavior

([$F_{(strain)1,27}=0.0782$, $p=0.782$; $F_{(treatment)1,27}=2.157$, $p=0.832$; $F_{(strain\times treatment)1,27}=0.431$, $p=0.517$; Fig. 5c].

When behaviors were analyzed per 5-min intervals, two-way ANOVA for repeated measures gave the following results: pinning [$F_{(interval)2,54}=15.931$, $p<0.0001$; $F_{(interval\times strain)2,54}=7.510$, $p=0.0013$; $F_{(interval\times treatment)2,54}=12.167$, $p<0.001$; $F_{(interval\times strain\times treatment)2,54}=5.300$, $p=0.0079$]; pouncing [$F_{(interval)2,54}=49.624$, $p<0.0001$; $F_{(interval\times strain)2,54}=11.012$, $p<0.0001$; $F_{(interval\times treatment)2,54}=4.716$, $p=0.0129$; $F_{(interval\times strain\times treatment)2,54}=0.147$, $p=0.8632$]; social exploration [$F_{(interval)2,54}=11.406$, $p<0.0001$; $F_{(interval\times strain)2,54}=0.642$, $p=0.5301$; $F_{(interval\times treatment)2,54}=1.559$, $p=0.2196$; $F_{(interval\times strain\times treatment)2,54}=1.084$, $p=0.3455$]. Post hoc comparisons revealed that vehicle-treated Sprague–Dawley rats showed higher pinning (Fig. 5d; $p<0.001$) and pouncing (Fig. 5e; $p<0.001$) frequencies than vehicle-treated Wistar rats in the first 5-min interval of the test. In both strains, amphetamine reduced the frequency of pinning and pouncing during the first 5-min interval (Wistar rats: $p<0.01$ for pinning and $p<0.001$ for pouncing; Sprague–Dawley rats: $p<0.001$ for both pinning and pouncing). In Sprague–Dawley rats, amphetamine decreased pinning and pouncing in the second 5-min interval as well (Fig. 5d, e; $p<0.05$ for both pinning and pouncing), with no effects on social exploration in either strain (Fig. 5f).

Effects of amphetamine on 50-kHz ultrasonic vocalizations in adolescent Wistar and Sprague–Dawley rats

As in the previous experiment, Sprague–Dawley rats emitted more USVs than Wistar rats and the performance of social

Fig. 4 Effects of graded doses of amphetamine (AMPH; 0.05, 0.2, and 0.5 mg/kg, s.c., 30 min before test) on social play behavior in adolescent Wistar and Sprague–Dawley rats. Amphetamine, at the doses of 0.2 and 0.5 mg/kg, decreased pinning (**a**, **d**) and pouncing (**b**, **e**) frequency in both Wistar and Sprague–Dawley rats, with no effect on social exploration (**c**, **f**). Data represent mean±SEM frequency of pinning and pouncing and mean±SEM duration of social exploration. ***$p<0.001$ vs. vehicle treatment; $N=7$–10 per treatment group
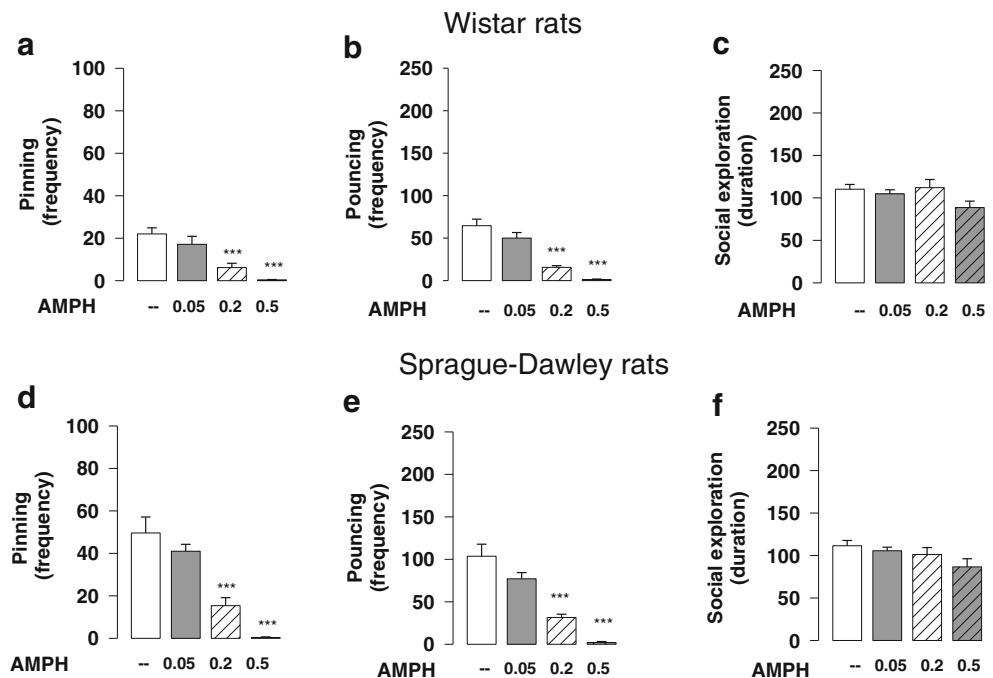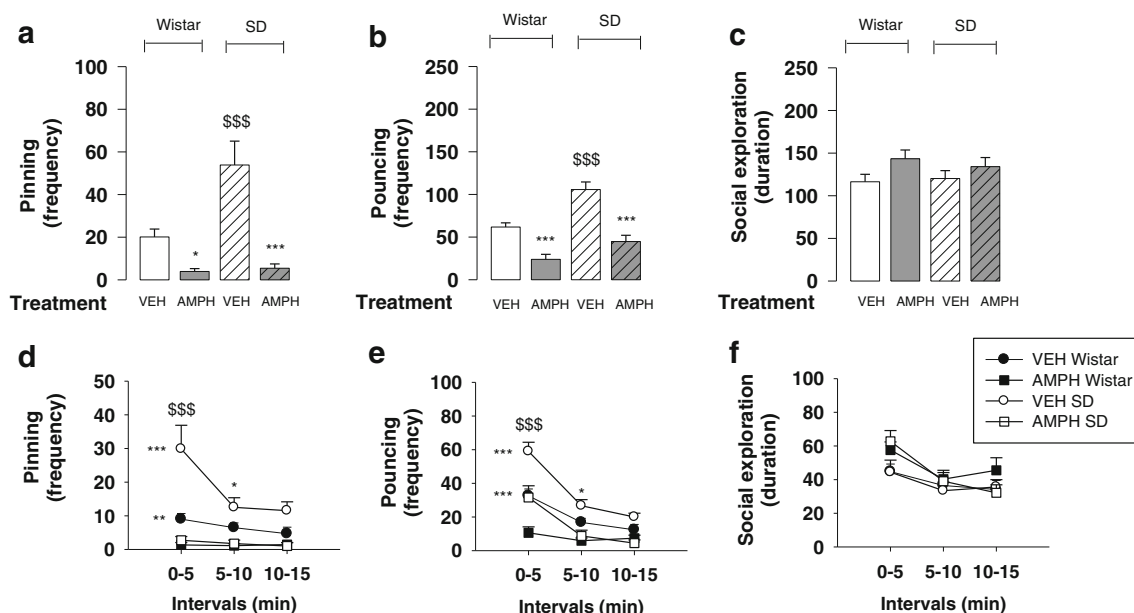
Fig. 5 Effects of amphetamine (AMPH; 0.2 mg/kg, s.c., 30 min before test) on social play behavior in adolescent Wistar and Sprague–Dawley rats. Vehicle-treated Sprague–Dawley rats played more than vehicle-treated Wistar rats (**a**, **b**). Amphetamine decreased the frequency of pinning (**a**) and pouncing (**b**) in both rat strains. When behaviors were analyzed per 5-min interval, vehicle-treated Sprague–Dawley rats showed higher pinning (**d**) and pouncing (**e**) frequencies than vehicle-treated Wistar rats in the first 5-min interval of the test. Amphetamine reduced the frequency of pinning (**d**) and pouncing (**e**) in both Wistar and Sprague–Dawley rats during the first 5-min interval and only in Sprague–Dawley rats in the second 5-min interval. No strain or treatment effects were found in the total time spent in general social exploration (**c**, **f**). Data represent mean±SEM frequency of pinning and pouncing and mean±SEM duration of social exploration. *$p < 0.05$, **$p < 0.01$; ***$p < 0.001$ vs. vehicle treatment; $^{\$\$\$}p < 0.001$ Sprague–Dawley vs. Wistar rats (Student–Newman–Keule post hoc test). $N = 6$–9 per treatment group

behaviors was unrelated to the emission of 50-kHz USVs. Amphetamine administration increased the number of USVs emitted during cage exploration or self-grooming in both strains. In Sprague–Dawley rats only, amphetamine decreased the USVs emitted during social interaction.

A two-way ANOVA analysis performed on the number of total USVs emitted during the 15-min test session and on the USVs emitted exclusively either during social interaction or during cage exploration and self-grooming gave the following results: total USVs emitted: $[F_{(\text{strain})1,27}=22.694, p<0.001;$ $F_{(\text{treatment})1,27}=0.156, p=0.696;$ $F_{(\text{strain}\times\text{treatment})1,27}=0.631,$ $p=0.434]$, Fig. 6a; USVs emitted during active social interaction: $[F_{(\text{strain})1,27}=16.391, p<0.001; F_{(\text{treatment})1,27}=15.824,$ $p<0.001;$ $F_{(\text{strain}\times\text{treatment})1,27}=2.673, p=0.114]$, Fig. 6b; USVs emitted during cage exploration and self-grooming: $[F_{(\text{strain})1,27}=12.613, p=0.001;$ $F_{(\text{treatment})1,27}=11.063,$ $p=0.003;$ $F_{(\text{strain}\times\text{treatment})1,27}=0.007, p=0.935]$, Fig. 6c). Post hoc analysis indicated that vehicle-treated Sprague–Dawley rats emitted more USVs than vehicle-treated Wistar rats during the 15-min testing session (Fig. 6a; $p<0.01$), both during active social interaction (Fig. 6b; $p<0.01$) and during cage exploration or self-grooming (Fig. 6c; $p<0.05$).
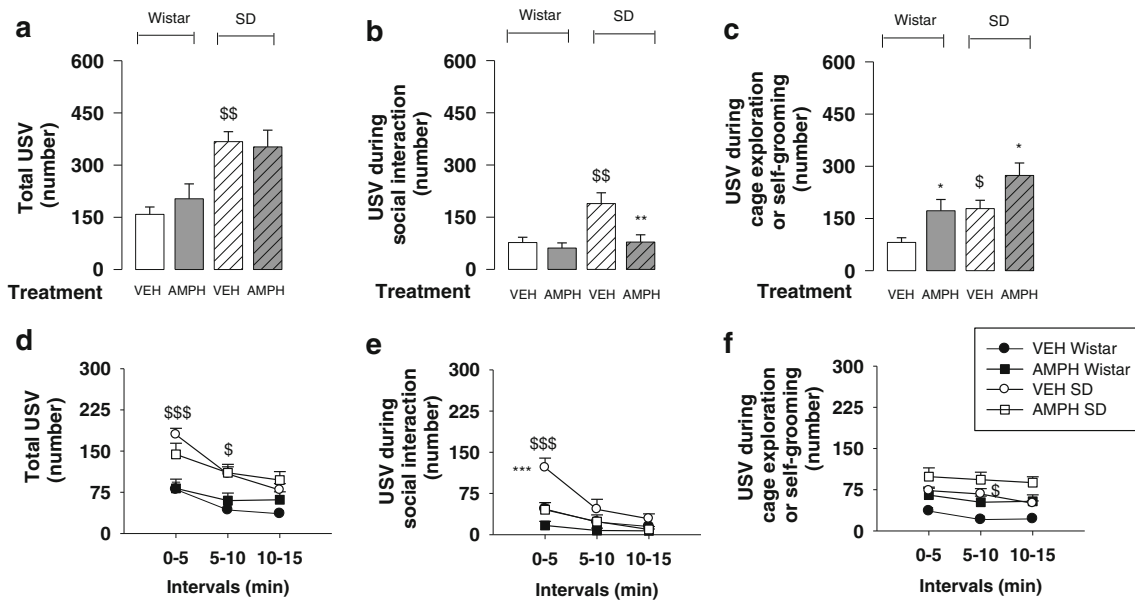
Post hoc analysis showed that Sprague–Dawley rats treated with amphetamine emitted less USVs during active social interaction than vehicle-treated Sprague–Dawley rats (Fig. 6b; $p<0.01$). Conversely, amphetamine-treated Sprague–Dawley and Wistar rats emitted more USVs during

cage exploration and self-grooming (Fig. 6c; $p<0.05$ for both Wistar and Sprague–Dawley rats) than the corresponding vehicle-treated rats.

When ultrasonic vocalizations were analyzed per 5-min intervals, vehicle-treated Sprague–Dawley rats emitted more USVs than vehicle-treated Wistar rats (total USVs emitted: $[F_{(\text{interval})2,54}=53.308, p<0.0001;$ $F_{(\text{interval}\times\text{strain})2,58}=7.367, p=0.0015; F_{(\text{interval}\times\text{treatment})2,54}=$ $6.751, p=0.0024; F_{(\text{interval}\times\text{strain}\times\text{treatment})2,54}=1.084,$ $p=0.3454]$, Fig. 6d; USVs emitted during social interaction: $[F_{(\text{interval})2,54}=35.047, p<0.0001; F_{(\text{interval}\times\text{strain})2,54}=$ $9.068, p<0.0004;$ $F_{(\text{interval}\times\text{treatment})2,54}=8.197,$ $p=0.0008;$ $F_{(\text{interval}\times\text{strain}\times\text{treatment})2,54}=2.096,$ $p=0.1329]$, Fig. 6e; USVs emitted during cage exploration: $[F_{(\text{interval})2,54}=7.014, p=0.002; F_{(\text{interval}\times\text{strain})2,54}=$ $1.253, p=0.2937; F_{(\text{interval}\times\text{treatment})2,54}=0.476, p=0.6241;$ $F_{(\text{interval}\times\text{strain}\times\text{treatment})2,58}=0.255, p=0.776]$, Fig. 6f).

Post hoc analysis revealed that the total number of USVs, both related and unrelated to social behavior, was higher in vehicle-treated Sprague–Dawley than vehicle-treated Wistar rats during the first ($p<0.001$) and the second ($p<0.05$) 5-min intervals of the testing session (Fig. 6d). USVs emitted selectively during either social interaction (Fig. 6e) or cage exploration and self grooming (Fig. 6f) were higher in vehicle-treated Sprague–Dawley than vehicle-treated Wistar rats during the first and second 5-min intervals of the test, respectively (USVs during social interaction in the first 5-min interval:

Fig. 6 Effects of amphetamine (AMPH; 0.2 mg/kg, s.c., 30 min before test) on 50-kHz ultrasonic vocalizations in adolescent Wistar and Sprague–Dawley rats during the social play behavior session. Vehicle-treated Sprague–Dawley rats emitted more USVs than vehicle-treated Wistar rats during the 15-min test session (**a**), both during active social interaction (**b**) and during cage exploration or self-grooming (**c**). Sprague–Dawley rats treated with amphetamine emitted less USVs during active social interaction than vehicle-treated Sprague–Dawley rats (**b**). Conversely, amphetamine-treated Sprague–Dawley and Wistar rats emitted more USVs during cage exploration and self-grooming (**c**) than the corresponding vehicle-treated rats. When ultrasonic vocalizations were analyzed per 5-min interval, the total number of USVs was higher in vehicle-treated Sprague–Dawley than vehicle-treated Wistar rats during the first and the second 5-min intervals of the testing session (**d**). USVs emitted selectively during either social interaction (**e**) or cage exploration and self-grooming (**f**) were higher in vehicle-treated Sprague–Dawley than vehicle-treated Wistar rats during the first and second 5-min intervals of the test, respectively. Amphetamine decreased the number of USVs emitted during social interaction only in Sprague–Dawley rats in the first 5-min interval (**e**). Data represent mean±SEM frequency of total USVs (**a**, **d**) and USVs either related (**b**, **e**) and unrelated (**c**, **f**) to the social interaction. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. vehicle treatment; $^{\$}p<0.05$; $^{\$\$}p<0.01$; $^{\$\$\$}p<0.001$ Sprague–Dawley vs. Wistar rats (Student–Newman–Keule post hoc test). $N=6$–9 per treatment group

$p<0.001$; USVs during cage exploration or self-grooming in the second 5-min: $p<0.05$). Amphetamine decreased the number of USVs emitted during social interaction only in Sprague–Dawley rats in the first 5-min interval (Fig. 6e; $p<0.001$).

Discussion

Comparing different strains of rats or mice is a very useful approach to discover and map genetic influences on behavioral traits (Brand et al. 2012; Yilmazer-Hanke 2008). The aim of this study was to determine whether adolescent male Wistar and Sprague–Dawley rats show different patterns of social play behavior and USV emission during social interaction. Furthermore, we investigated whether adolescent rats from these two strains differ in the effects of acute morphine and amphetamine administration on social play behavior and USV production.

Wistar and Sprague–Dawley rat strains are extensively used in behavioral pharmacology studies. Certain behavioral differences between Wistar and Sprague–Dawley rats have been well characterized (Rex et al. 2004; Staples and

McGregor 2006; Walker et al. 2009; Zmarowski et al. 2012). For instance, Rex et al. (2004) found that adult Sprague–Dawley rats showed higher level of social interaction than adult Wistar rats when tested under high light levels, without any difference in locomotor activity. In addition, adult Sprague–Dawley and Wistar rats also spent more time on the open arms of the elevated plus-maze compared to Wistar rats (Rex et al. 2004). Differences in novelty-seeking behavior (Walker et al. 2009), cognitive performance (Zmarowski et al. 2012), and defensive response to predator odors (Staples and McGregor 2006) between adult Wistar and Sprague–Dawley rats have also been described. However, to our knowledge, differences in social play behavior and high-frequency USVs between adolescents of these strains have never been reported, and these cannot be easily inferred from across-laboratory comparisons.

We found that Sprague–Dawley rats exhibited higher levels of social play than Wistar rats: the frequency of pinning and pouncing, the two most characteristic expressions of social play behavior in rats, was higher in vehicle-treated adolescent Sprague–Dawley than in Wistar rats. These differences were most pronounced in the first 5 min of the test and were specific

for play-related social behaviors, since the two strains did not differ in social behaviors not related to play, such as sniffing or social grooming. In both strains, social play declined over the 15-min test session. This is in line with previous findings showing that, when adolescent rats are tested under dim light in a familiar test cage, where the only novel stimulus is an unfamiliar test partner, levels of social play are already high in the first 5-min of the test period (Trezza and Vanderschuren 2008a; Vanderschuren et al. 1995a, b).

Social play behavior is a natural reinforcer (for reviews, see Trezza et al. 2010, 2011a; Vanderschuren 2010), and neuro-transmitter systems implicated in the motivational, pleasur-able, and cognitive aspects of natural and drug rewards, such as opioids, endocannabinoids, dopamine, and noradrenaline, play an important modulatory role in the performance of social play (Siviy and Panksepp 2011; Trezza et al. 2010; Vanderschuren et al. 1997). For instance, we and others have repeatedly shown that stimulation of μ-opioid receptors by morphine or selective μ-opioid receptor agonists enhances social play behavior, most likely by increasing the hedonic properties of social play (Panksepp et al. 1985; Trezza et al. 2011b; Trezza and Vanderschuren 2008b; Vanderschuren et al. 1995a). In addition, activation of α-2 adrenoceptors by psychostimulants such as amphetamine and methylphenidate suppresses social play, possibly by interfering with cognitive aspects of the performance of social play behavior (Beatty et al. 1982, 1984; Thor and Holloway 1983; Vanderschuren et al. 2008). To evaluate whether adolescent Sprague–Dawley and Wistar rats may be equally useful in neuropharmacology studies of social play behavior, i.e., to assess the effects of drugs that may influence this behavior, we investigated whether rats from these two strains differed in the effects of acute morphine and amphetamine administration on social play behavior. On the basis of dose-response experiments performed separately in the two strains, the doses of morphine 1 mg/kg and amphet-amine 0.2 mg/kg were selected for strain comparison experiments. Morphine increased social play behavior in both strains, although its effects were more pronounced in Wistar than Sprague–Dawley rats. Thus, Wistar rats treated with morphine showed a threefold increase in pinning and pouncing frequencies compared to Wistar rats treated with vehicle; in Sprague–Dawley rats, morphine induced a trend for an increase in pinning and significantly increased pouncing. It has previously been shown that morphine increases social play as a whole, without altering the sequential structure of this behavior (Vanderschuren et al. 1995c). Therefore, in line with the higher levels of social play displayed by vehicle-treated animals at the beginning of the test period, we found that in Wistar rats morphine increased social play in the first and second interval of the testing session, while this happened in Sprague–Dawley rats in the first interval only.

The effects of amphetamine on social play were qualita-tively and quantitatively similar in Wistar and Sprague–Dawley rats, with the drug reducing pinning and pouncing frequencies to the same extent in adolescent rats from both strains. In both strains, morphine and amphetamine did not alter social exploratory behavior, thus confirming previous findings (Beatty et al. 1982; Sutton and Raskin 1986; Trezza and Vanderschuren 2008a; Vanderschuren et al. 2008) show-ing that opioid and psychostimulants effects on social play are behaviorally specific, i.e., not secondary to general changes in locomotion or social interest. Taken together, our results show that morphine increased and amphetamine decreased social play behavior in both Wistar and Sprague–Dawley rats. However, the play-enhancing effects of morphine were more pronounced in Wistar than Sprague–Dawley animals. The higher baseline levels of social play behavior displayed by vehicle-treated Sprague–Dawley compared to vehicle-treated Wistar rats likely account for this finding. These strain differ-ences should be taken into account when testing the effects of drugs that increase social play behavior, in order to avoid possible ceiling effects.

In our experiments, we measured the number of 50-kHz USVs emitted during the testing session. In particular, we discriminated between the 50-kHz USVs emitted during ac-tual social interaction from those emitted during the perfor-mance of non-social behaviors, such as cage exploration or self-grooming. We did not find a positive correlation between the performance of social behaviors and the emission of 50-kHz USVs. In fact, vehicle-treated adolescents from both strains emitted a similar number of 50-kHz USVs while performing non-social behaviors (i.e., cage exploration or self-grooming) or social activities (calculated as the sum of social behaviors both related and unrelated to play). Both categories of 50-kHz USVs were higher in vehicle-treated Sprague–Dawley than Wistar rats. In both strains, morphine administration did not affect the number of USVs emitted during the 15-min testing session, neither while animals were engaged in social activities nor in general exploration. In both Wistar and Sprague–Dawley rats, amphetamine administra-tion increased USV emission during cage exploration, which is in line with previous findings showing increases in 50-kHz USVs in amphetamine-treated Sprague–Dawley and Wistar rats (e.g., Burgdorf et al. 2001; Thompson et al. 2006). In Sprague–Dawley animals only, amphetamine decreased USVs during social interaction.

Controversial data exist in the literature about whether 50-kHz USVs may reflect the rewarding value of adolescent social interactions. Knutson and coworkers were the first to suggest that 50-kHz USVs may index play motivation in rats. They found that adolescent Long-Evans rats emitted more 50-kHz USVs while playing together than while alone (Knutson et al. 1998). Interestingly, rats separated from the play partner through a screen dividing the testing chamber, but given the opportunity to play the day before, vocalized more than rats that played both days. Conversely, separated rat pairs

that had not played before vocalized less than rats that played on both days. On the basis of these data, the authors suggested that one trial of play is sufficient to induce a motivational state that evokes 50-kHz USVs in rats and that general motor activity alone cannot account for the expression of these vocalizations (Knutson et al. 1998, 2002). However, not all data support the use of 50-kHz USVs in adolescent rats as an index of positive affective state during active social interaction or anticipation for social contact. Thus, Willey et al. found that adolescent Sprague–Dawley rats emit fewer 50-kHz USVs than adults in anticipation of a social partner (Willey and Spear 2012) and during social interactions (Willey et al. 2009), although adolescent rats usually engage in higher levels of social behaviors, especially social play behavior (Varlinskaya and Spear 2002; 2008; Willey and Spear 2013; Willey et al. 2009) and show greater conditioned place preference for a social stimulus than adults (Douglas et al. 2004). Together with our data, these findings suggest that the emission of 50-kHz USVs and the behavioral parameters typically thought to measure the rewarding value of social interactions in adolescent rats can be dissociable and may rely on different neurobehavioral mechanisms. In line with this hypothesis, it has been shown that a dissociation exists between the emission of 50-kHz USVs and behavioral measures of affective responses during drug self-administration or anticipation. Thus, while 50-kHz USVs can be elicited by administration of psychostimulant drugs (Barker et al. 2010; Browning et al. 2011; Burgdorf et al. 2000; Burgdorf and Panksepp 2006; Knutson et al. 1998; Maier et al. 2010; Simola et al. 2012; Thompson et al. 2006; Trezza et al. 2010; Wintink and Brudzynski 2001), rewarding doses of morphine, MDMA, and nicotine have been related to no changes or even decreases in 50-kHz USV emission (Hamed et al. 2012; Sadananda et al. 2012; Simola et al. 2012; Wright et al. 2012).

To summarize, two conclusions can be drawn from the present study. First, strain differences in baseline levels of social play behavior exist between adolescent Wistar and Sprague–Dawley rats, with the latter showing higher levels of social play than the former.

Morphine and amphetamine increased and decreased social play behavior, respectively, in the two strains. However, while morphine significantly increased pinning and pouncing frequencies in Wistar rats, it failed to significantly increase pinning in Sprague–Dawley adolescents. Thus, when performing pharmacological manipulations of social play, researchers should be aware that the magnitude of drug-induced changes in social play parameters may differ in these two strains, and this needs to be considered in order to avoid ceiling or floor effects. Second, we show that positive social interactions in adolescent rats do not necessarily correlate with 50-kHz USVs emission and that, under the testing conditions used in the present study, drugs that affected social play did not affect USV production during social interactions. Thus, it

cannot generally be assumed that 50-kHz USVs index positive affective states during social interactions.

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