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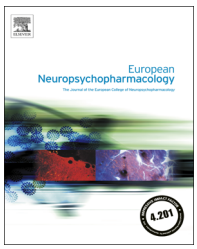

Strain- and context-dependent effects of the anandamide hydrolysis inhibitor URB597 on social behavior in rats

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Abstract
Genetic and environmental factors play an important role in the cannabinoid modulation of motivation and emotion. Therefore, the aim of the present study was to test whether anandamide modulation of social behavior is strain- and context-dependent. We tested the effects of the anandamide hydrolysis inhibitor URB597 on social behavior and 50-kHz ultrasonic vocalizations (USVs) in adolescent and adult Wistar and Sprague–Dawley rats tested in different emotionally arousing conditions (familiarity/unfamiliarity to the test cage, low/high light).
Under all experimental conditions, adolescent and adult Sprague–Dawley rats displayed higher levels of social behavior and emitted more 50-kHz USVs than Wistar rats. URB597 enhanced social play behavior in adolescent Wistar rats under all experimental conditions. However, URB597 only increased social interaction in adult Wistar rats under unfamiliar/high light conditions. URB597 did not affect adolescent social play behavior and adult social interaction in Sprague–Dawley rats under any experimental condition. Moreover, URB597 increased the USVs emitted during social interaction by adolescent Wistar and adult Sprague–Dawley rats tested under familiar/high light and unfamiliar/high light, respectively. These results show that anandamide has distinct roles in adolescent and adult social behaviors. Anandamide modulation

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of adolescent social play behavior is strain- but not context-dependent. Conversely, anandamide modulation of adult social behavior and USV emission depends upon both strain and experimental context. Furthermore, these results confirm that profound behavioral differences exist between Wistar and Sprague–Dawley rats, which may explain the sometimes contradictory effects of cannabinoid drugs on emotionality in different strains of rodents.

1. Introduction

It is known since ancient times that exposure to cannabis-derived drugs produces a wide range of effects on emotionality in humans (Zanettini et al., 2011). Upon discovery and characterization of the endocannabinoid system, it has become clear that these effects are related to the abundance of cannabinoid receptors in brain areas involved in emotional responses, where endocannabinoids influence the activity of neurotransmitter systems involved in emotion and motivation (Haller et al., 2004; Millan, 2003; Trezza et al., 2012a).

In the last 10 years, indirect stimulation of endocannabinoid signaling through inhibition of the enzymes responsible for endocannabinoid degradation has emerged as a useful approach to study the role of endocannabinoid neurotransmission in emotional and motivational states. Thus, drugs that inhibit endocannabinoid deactivation increase endocannabinoid neurotransmission only in active synapses, thus preserving the spatiotemporal specificity of endocannabinoid activity (Di Marzo, 2009). For instance, URB597 is a potent inhibitor of fatty acid amide hydrolase (FAAH, which catalyzes the intracellular hydrolysis of anandamide), but does not bind cannabinoid receptors and does not induce the well known effects of CB1 cannabinoid receptor agonists, such as catalepsy, hypothermia, or hyperphagia (Kathuria et al., 2003). However, it exerts analgesic-, anxiolytic-, and antidepressant-like effects in rodents (Gobbi et al., 2005; Hill et al., 2007; Kathuria et al., 2003; Naidu et al., 2007; Rademacher and Hillard, 2007; Realini et al., 2011). Furthermore, we have previously shown that URB597, administered either systemically (Trezza and Vanderschuren, 2008) or into limbic brain regions such as the nucleus accumbens and amygdala (Trezza et al., 2012b), enhances social play behavior in rats. Social play is a vigorous, characteristic form of social interaction in young mammals, thought to be crucial for proper social and cognitive development (Panksepp et al., 1984; Vanderschuren and Trezza, 2013). Altogether, these studies indicate that anandamide signaling maintains emotional homeostasis and regulates positive aspects of social interactions. However, it has also been shown that the behavioral consequences of pharmacological inhibition of FAAH largely depend on the environmental context (Naidu et al., 2007). For instance, URB597 administration did not affect behavior under non-stressful conditions but protected against the anxiogenic effects of stressful stimuli and circumstances in rats (Haller et al., 2009). The effects of URB597 on social behavior also appear to be context-dependent, since the increase in social play behavior induced by URB597 in adolescent rats was influenced by the level of social activity of the test partner (Trezza and Vanderschuren, 2008).

Strain differences in the effects of cannabinoid compounds on emotional and motivational processes in rodents have also been reported (Arnold et al., 2010; Brand et al., 2012; Cadoni et al., in press; Deiana et al., 2007). The aim of the present study was therefore to investigate whether the effects of URB597 on social behavior in adolescent and adult rats are strain- and context-dependent. To address this aim, we tested the effects of systemic administration of URB597 on social behavior in adolescent and adult Wistar and Sprague–Dawley rats tested under different experimental conditions (i.e., low or high light, familiarity or unfamiliarity to the testing environment). We used Wistar and Sprague–Dawley rats because these outbred strains are widely used in behavioral pharmacology studies (Hedrich, 2006) and 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 adolescent (Manduca et al., 2014) and adult (Rex et al., 2004) social behavior.

In rodents, the emission of ultrasonic vocalizations (USVs) is considered a measure of affective states and a means of communication (Knutson et al., 2002). In rats, low frequency (around 18–30 kHz) USVs have been associated with negative social experiences (e.g., exposure to predator odor or inescapable foot shocks, inter-male fighting), while high frequency (around 50 kHz) USVs have been detected in social contexts involving potential reward (e.g., sexual approach, social play) (Burgdorf et al., 2011). Previous studies have shown that cannabinoid drugs, including URB597, modulate maternal separation-induced USVs in rat pups (Bortolato et al., 2006; Kathuria et al., 2003; McGregor et al., 1996) and adult low frequency USVs (Butler et al., 2012). Since strain differences in the effects of cannabinoid compounds on USV emission in adult rats have been reported (Arnold et al., 2010), we also investigated the effects of URB597 on USV emission during active social interactions in adolescent and adult Wistar and Sprague-Dawley rats tested in different environmental conditions.

2. Experimental procedures

2.1. 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 either 4 (adolescent rats) or 2 (adult rats) in $43 \times 26 \times 20\ cm^3$ ($l \times w \times h$) Macrolon cages under controlled conditions (i.e. temperature $21 \pm 1\ ^\circ C$, $60 \pm 10\%$ relative humidity and 12/12-h light cycle with lights on at 7:00 AM). Food and water were available ad libitum. We used 148 couples of Wistar and 162 couples of Sprague–Dawley rats. All animals were experimentally naïve and were 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 of the Italian Ministry

of Health (D.L.116/92) and the European Community Directive 2010/63/EU of 22 September 2010.

2.2. Drugs

The anandamide hydrolysis inhibitor URB597 (National Institute of Mental Health's Chemical Synthesis and Drug Supply Program, Bethesda, MD) was dissolved in 5% Tween 80/5% polyethylene glycol/saline and administered intraperitoneally (i.p.) 2 h before testing. Drug dose and pre-treatment intervals were based on literature (Hill et al., 2007; Kathuria et al., 2003; Naidu et al., 2007; Trezza et al., 2012b; Trezza and Vanderschuren, 2008) and on pilot experiments. Solutions were freshly prepared on the day of the experiment and were administered in a volume of 2 ml/kg in adolescent rats and 1 ml/kg in adult rats.

2.3. Apparatus

The experiments were performed in a sound attenuated chamber. The testing arena consisted of a Plexiglas cage measuring $45 \times 45 \times 60\ cm^3$ ($l \times w \times h$), with approximately 2 cm of wood shavings covering the floor. The test cage was illuminated by either a 40-W red light bulb (1–2 lx) in the low light conditions, or by a 60-W white light bulb (560 lx) in the high light conditions. Light bulbs were mounted 60 cm above the test cage. The behavior of the animals was recorded using a video camera with zoom lens, DVD recorder and LCD monitor.

2.4. Behavioral procedures

2.4.1. Social behavior in adolescent and adult rats

Social behavior was assessed as previously described (File, 1980; Manduca et al., 2014; Segatto et al., 2014; Trezza et al., 2008; Trezza and Vanderschuren, 2008). Environmental factors are known to influence social behavior in both adolescent (Panksepp et al., 1984; Trezza and Vanderschuren, 2009; Vanderschuren et al., 1995) and adult rats (File, 1980). Therefore, we assessed the effects of URB597 on social behavior in adolescent and adult rats under different experimental conditions. In particular, we manipulated the familiarity of the animals to the test arena and the light level to generate the following different levels of aversiveness (File, 1980):

1. low aversive conditions: low light, familiar arena (LF);
2. moderate aversive conditions: low light, unfamiliar arena or high light, familiar arena (LU or HF, respectively);
3. high aversive conditions: high light, unfamiliar arena (HU).

At either 28–35 (adolescent rats) or 80–90 days of age (adult rats), separate groups of rats were allocated to the different experimental groups. The animals allocated to the LF and HF groups were individually habituated to the test cage for 10 min (adolescent rats) or 5 min (adult rats) on each of the 2 days prior to testing, while the animals allocated to the LU and HU groups were brought into the experimental room but remained in their home cage. Before testing, adolescent animals from all experimental groups were socially isolated for 3.5 h 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 in Wistar rats (Niesink and Van Ree, 1989). In the same way, adult animals from each experimental group were socially isolated for 24 h before testing, to enhance their motivation to interact with a social partner (Niesink and Van Ree, 1982). Two hours before testing, pairs of animals were treated with URB597 (0.1 mg/kg, *i.p.*) or its vehicle. 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 the four different experimental groups (LF, LU, HF and HU). 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).

The test for social behavior consisted of placing two similarly treated animals into the test cage for either 15 min (adolescent rats) or 10 min (adult rats). 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 (Pellis and Pellis, 1987). Pinning and pouncing frequencies can be easily quantified and they are considered to be the most characteristic parameters of social play behavior in adolescent rats. During the social encounter, animals may also display social behaviors not directly associated with play, such as sniffing or grooming the partner's body. Since social behavior in rats strongly depends on the playfulness of its partner (Trezza and Vanderschuren, 2008), in the present study, both animals in a play pair were similarly treated, and a pair of rats was considered as one experimental unit.

In adolescent rats, 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).

In adult rats, total time and total frequency of active social interactions were obtained as the sum of time and frequency of the following behavioral elements scored per 10 min (Segatto et al., 2014):

- Play-related behaviors: pouncing, pinning and boxing;
- Social behaviors unrelated to play: social exploration (sniffing any part of the body of the test partner, including the anogenital area), social grooming (one rat licks and chews the fur of the conspecific, while placing its forepaws on the back or the neck of the other rat), following/chasing (walking or running in the direction of the partner which stays where it is or moves away), crawling under/over (one animal crawls underneath or over the partner's body, crossing it transversely from one side to the other), kicking (the rat kicks backwards at the conspecific with one or both hindlegs).

2.4.2. Measurement of ultrasonic vocalizations (USVs) during social interaction in adolescent and adult rats

USVs were recorded as previously described (Manduca et al., 2014; Trezza et al., 2008). The USVs emitted during the social interaction session in both adolescent and adult rats were detected by an ultrasonic microphone (SM2, Ultrasound Advice) fixed 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 number of USVs emitted during social-related behaviors and during cage exploration and self-grooming was analyzed per pair of animals.

2.5. Statistical analysis

Data are expressed as mean±SEM. To assess the effects of the different strains and treatments on social behavior and USV production, data were analyzed using two-way ANOVA, using treatment (URB597 or vehicle) and strain (Wistar or Sprague-Dawley) as between-subjects factors. Two-way ANOVA was followed by Student-Newman-Keuls post-hoc test where appropriate. *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Effects of the FAAH inhibitor URB597 on social play behavior and USV emission in adolescent Wistar and Sprague-Dawley rats tested under different experimental conditions

3.1.1. Rats familiar to the test cage and tested under low light (LF)

In line with our previous study (Manduca et al., 2014), we found differences in the baseline levels of social play behavior between Wistar and Sprague-Dawley rats. Furthermore, we found that URB597 increased social play behavior only in Wistar rats. A two-way ANOVA of pinning and pouncing frequencies gave the following results: pinning [$F_{(strain)1,30}=8.52$, $p<0.01$; $F_{(treatment)1,30}=2.77$, $p=0.11$; $F_{(strain \times treatment)1,30}=3.13$, $p=0.09$]; pouncing [$F_{(strain)1,30}=5.75$, $p=0.02$; $F_{(treatment)1,30}=10.25$, $p<0.01$; $F_{(strain \times treatment)1,30}=4.19$, $p<0.05$]. Post-hoc analysis revealed that vehicle-treated Sprague-Dawley rats showed higher levels of pinning ($p<0.05$, Figure 1a) and pouncing ($p<0.01$, Figure 1b) than vehicle-treated Wistar rats. URB597 increased social play behavior in Wistar rats (pinning: $p<0.05$, Figure 1a; pouncing: $p<0.01$, Figure 1b), but not in Sprague-Dawley rats. As for the time spent in general social exploration, no significant differences were found between

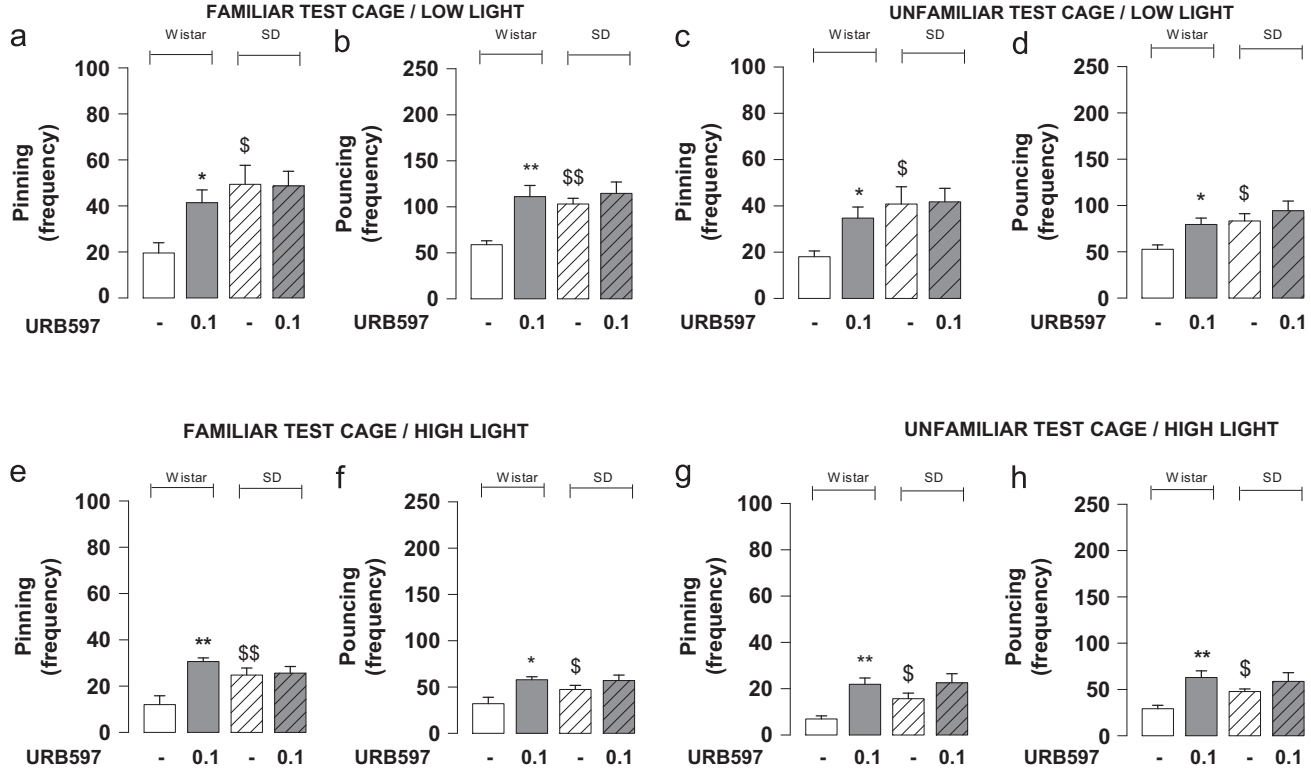

Figure 1 Effects of the FAAH inhibitor URB597 (0.1 mg/kg, *i.p.*, 2 h before test) on social play behavior in adolescent Wistar and Sprague-Dawley rats tested under different experimental conditions. Vehicle-treated Sprague-Dawley rats showed higher levels of pinning (a, c, e, g) and pouncing (b, d, f, h) than vehicle-treated Wistar rats under different experimental conditions (i.e. animals familiar or unfamiliar to the test cage and tested under low or high light). URB597 administration increased pinning (a, c, e, g) and pouncing (b, d, f, h) in Wistar but not in Sprague-Dawley rats tested under all the different experimental conditions. Data represent mean±SEM frequency of pinning (a, c, e, g) and pouncing (b, d, f, h). $^{*}p<0.05$, $^{**}p<0.01$ 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.

Table 1 Effects of the FAAH inhibitor URB597 (0.1 mg/kg, *i.p.*, 2h before test) on the time spent in general social exploration in adolescent Wistar and Sprague-Dawley rats tested under different experimental conditions.

Condition and treatment	Social exploration (s/15 min)	ANOVA *P* value
Familiar test cage/low light		
VEH Wistar	120±9	$F_{(strain)1,30}=0.26$, $p=0.61$
URB597 Wistar	140±14	$F_{(treatment)1,30}=2.41$, $p=0.13$
VEH SD	115±8	$F_{(strainxtreatment)1,30}=0.01$, $p=0.94$
URB597 SD	133±15	
Unfamiliar test cage/low light		
VEH Wistar	101±6	$F_{(strain)1,34}=2.63$, $p=0.11$
URB597 Wistar	107±7	$F_{(treatment)1,34}=1.52$, $p=0.23$
VEH SD	109±6	$F_{(strainxtreatment)1,34}=0.05$, $p=0.83$
URB597 SD	118±5	
Familiar test cage/high light		
VEH Wistar	139±7	$F_{(strain)1,25}=0.07$, $p=0.79$
URB597 Wistar	138±8	$F_{(treatment)1,25}=0.19$, $p=0.66$
VEH SD	139±9	$F_{(strainxtreatment)1,25}=0.08$, $p=0.78$
URB597 SD	134±5	
Unfamiliar test cage/high light		
VEH Wistar	118±11	$F_{(strain)1,27}=2.57$, $p=0.12$
URB597 Wistar	107±11	$F_{(treatment)1,27}=0.03$, $p=0.87$
VEH SD	126±12	$F_{(strainxtreatment)1,27}=0.80$, $p=0.38$
URB597 SD	134±9	

No differences were found in the duration of social exploration between vehicle-treated Sprague-Dawley and Wistar rats tested under different experimental conditions (i.e. animals familiar or unfamiliar to the test cage and tested under low or high light). URB597 administration did not affect social exploration in both rat strains. Data represent mean±SEM duration of social exploration. $N=7$-12 per treatment group.

either the two strains or treatment groups (for statistical values, see Table 1).

When we analyzed the rate of USVs emitted during the test session (Table 2), we found that, over the 15-min testing session, vehicle-treated Sprague-Dawley rats emitted more USVs, either related ($p<0.01$) or unrelated ($p<0.05$) to the social interaction, than vehicle-treated Wistar rats. Furthermore, no effect of URB597 on USV emission was found in both rat strains tested under these experimental conditions (for statistical values, see Table 2).

3.1.2. Rats unfamiliar to the test cage and tested under low light (LU)

As in the previous experiment, vehicle-treated Sprague-Dawley rats played more than vehicle-treated Wistar rats and URB597 increased social play behavior only in Wistar rats. A two-way ANOVA of pinning and pouncing frequencies gave the following results: pinning [$F_{(strain)1,34}=6.70$, $p<0.05$; $F_{(treatment)1,34}=2.45$, $p=0.13$; $F_{(strain\times treatment)1,34}=1.95$, $p=0.17$]; pouncing [$F_{(strain)1,34}=7.49$, $p<0.01$; $F_{(treatment)1,34}=5.18$, $p<0.05$; $F_{(strain\times treatment)1,34}=0.90$, $p=0.35$]. Post-hoc analysis showed that vehicle-treated Sprague-Dawley rats displayed higher pinning and pouncing frequencies than vehicle-treated Wistar rats ($p<0.05$ for both pinning and pouncing, Figure 1c and d). URB597 increased the frequency of pinning and pouncing ($p<0.05$ for both pinning and pouncing, Figure 1c and d) only in Wistar rats. No strain or treatment effects were found in the total time spent in social exploration during social play behavior (Table 1).

As in the previous experiment, vehicle-treated Sprague-Dawley rats emitted more USVs than vehicle-treated Wistar rats both during active social interaction ($p<0.01$) and during cage exploration or self-grooming ($p<0.05$), whereas no effect of URB597 on USV emission was found in both rat strains under LU conditions (Table 2).

3.1.3. Rats familiar to the test cage and tested under high light (HF)

Vehicle-treated Sprague-Dawley rats played more than vehicle-treated Wistar rats and URB597 increased social play behavior only in Wistar rats. A two-way ANOVA of pinning and pouncing frequencies gave the following results: pinning [$F_{(strain)1,25}=1.72$, $p=0.20$; $F_{(treatment)1,25}=10.80$, $p<0.01$; $F_{(strain\times treatment)1,25}=9.05$, $p<0.01$]; pouncing [$F_{(strain)1,25}=1.88$, $p=0.18$; $F_{(treatment)1,25}=11.52$, $p<0.01$; $F_{(strain\times treatment)1,25}=2.36$, $p=0.14$]. Post-hoc analysis revealed higher pinning ($p<0.01$, Figure 1e) and pouncing ($p<0.05$, Figure 1f) frequencies in Sprague-Dawley than Wistar rats. URB597 treatment increased pinning ($p<0.01$, Figure 1e) and pouncing ($p<0.05$, Figure 1f) in Wistar rats only. As for the time spent in general social exploration, no significant difference was found between either the two rat strains or treatment groups (Table 1).

Table 2 Effects of the FAAH inhibitor URB597 (0.1 mg/kg, *i.p.*, 2 h before test) on 50-kHz ultrasonic vocalizations (USVs) emitted during social play behavior in adolescent Wistar and Sprague-Dawley rats tested under different experimental conditions.

Condition and treatment	USVs (num/ 15 min)	ANOVA *P* value	USVs (num/ 15min)	ANOVA P value
Familiar test cage/low light	**USV during social interaction**		**USV during cage-exploration or self-grooming**	
VEH Wistar	49 ± 11	$F_{(strain)1,30}=28.87$,	69 ± 15	$F_{(strain)1,30}=14.34$, $p<0.001$
URB597 Wistar	40 ± 10	$p<0.0001$	77 ± 24	$F_{(treatment)1,30}=0.14$,
VEH SD	167 ± 222;$^{\$\$}$	$F_{(treatment)1,30}=0.06$,	192 ± 35 1;$^{\$}$	$p=0.71$
URB597 SD	188 ± 36	$p=0.81$	210 ± 44	$F_{(strain \times treatment)1,30}=0.02$,
		$F_{(strain \times treatment)1,30}=0.38$,		$p=0.88$
		$p=0.54$		
Unfamiliar test cage/Low Light	**USV during social interaction**		**USV during cage-exploration or self-grooming**	
VEH Wistar	140 ± 22	$F_{(strain)1,34}=24.15$,	154 ± 32	$F_{(strain)1,34}=21.24$,
URB597 Wistar	158 ± 66	$p<0.0001$	238 ± 59	$p<0.0001$
VEH SD	482 ± 682;$^{\$\$}$	$F_{(treatment)1,34}=0.13$,	530 ± 801;$^{\$}$	$F_{(treatment)1,34}=1.12$,
URB597 SD	514 ± 89	$p=0.73$	621 ± 110	$p=0.30$
		$F_{(strain \times treatment)1,34}=0.01$,		$F_{(strain \times treatment)1,34}<0.01$,
		$p=0.93$		$p=0.96$
Familiar test cage/High Light	**USV during social interaction**		**USV during cage-exploration or self-grooming**	
VEH Wistar	64 ± 23	$F_{(strain)1,25}=14.62$,	111 ± 45	$F_{(strain)1,25}=16.08$, $p<0.001$
URB597 Wistar	211 ± 61*	$p<0.001$	105 ± 29	$F_{(treatment)1,25}=0.69$,
VEH SD	291 ± 562;$^{\$\$}$	$F_{(treatment)1,25}=5.07$,	241 ± 45 1;$^{\$}$	$p=0.41$
URB597 SD	370 ± 50	$p<0.05$	319 ± 49	$F_{(strain \times treatment)1,25}=0.95$,
		$F_{(strain \times treatment)1,25}=0.45$,		$p=0.34$
		$p=0.51$		
Unfamiliar test cage/High Light	**USV during social interaction**		**USV during cage-exploration or self-grooming**	
VEH Wistar	148 ± 24	$F_{(strain)1,27}=19.31$,	312 ± 50	$F_{(strain)1,27}=15.34$, $p<0.001$
URB597 Wistar	205 ± 23	$p<0.001$	349 ± 73	$F_{(treatment)1,27}=1.63$,
VEH SD	334 ± 442;$^{\$\$}$	$F_{(treatment)1,27}=0.14$	527 ± 53	$p=0.21$
URB597 SD	301 ± 31	$p=0.71$	660 ± 86	$F_{(strain \times treatment)1,27}=0.52$,
		$F_{(strain \times treatment)1,27}=1.97$,		$p=0.48$
		$p=0.17$		

Over the 15-min test session, vehicle-treated Sprague-Dawley rats emitted more USVs, either related or unrelated to the social interaction, than vehicle-treated Wistar rats. URB597 increased the frequency of USVs related to the social interaction in Wistar rats familiar to the test cage and tested under high light. Data represent mean ± SEM USVs during social interaction and USVs during cage-exploration or self-grooming.

*$p<0.05$ 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.

Vehicle-treated Sprague-Dawley rats emitted more USVs than vehicle-treated Wistar rats both during active social interaction ($p<0.01$) and during cage exploration or self-grooming ($p<0.05$). Furthermore, URB597 increased the USVs emitted by Wistar rats during social interaction ($p<0.05$, Table 2).

3.1.4. Rats unfamiliar to the test cage and tested under high light (HU)

We found differences in the baseline levels of social play behavior between the two strains treated with vehicle. Moreover, we found that URB597 increased social play behavior in Wistar rats only. A two-way ANOVA of pinning and pouncing frequencies gave the following results: pinning [$F_{(strain)1,27}=2.87$, $p=0.10$; $F_{(treatment)1,27}=15.55$, $p<0.001$; $F_{(strain \times treatment)1,27}=2.14$, $p=0.16$]; pouncing [$F_{(strain)1,27}=1.28$, $p=0.27$; $F_{(treatment)1,27}=12.53$, $p<0.01$; $F_{(strain \times treatment)1,27}=3.24$, $p=0.08$]. Post-hoc analysis revealed that Sprague-Dawley rats displayed higher pinning and pouncing frequencies ($p<0.05$ for both pinning and pouncing, Figure 1g and h) than Wistar rats and that URB597 increased social play behavior in Wistar rats ($p<0.01$ for both pinning and pouncing, Figure 1g and h) but not in Sprague-Dawley rats. As for the time spent in general social

exploration, no significant differences were found between either the two strains or treatment groups (Table 1).

Vehicle-treated Sprague–Dawley rats emitted more USVs than vehicle-treated Wistar rats during active social interaction ($p<0.01$), with a trend for higher levels of USVs emitted during cage exploration or self-grooming ($p=0.07$). URB597 did not affect the emission of USVs in both rat strains (Table 2).

3.2. Effects of the FAAH inhibitor URB597 on social interaction and USV emission in adult Wistar and Sprague–Dawley rats tested under different experimental conditions

3.2.1. Rats familiar to the test cage and tested under low light (LF)

Vehicle-treated Sprague–Dawley rats displayed higher levels of social interaction than vehicle-treated Wistar rats, but URB597 did not affect social interaction in both strains. A two-way ANOVA of time and frequency of social interaction gave the following results: time [$F_{(strain)1,40}=16.60$, $p<0.001$; $F_{(treatment)1,40}=0.16$, $p=0.69$; $F_{(strain\times treatment)1,40}=1.04$, $p=0.31$] and frequency of social interaction [$F_{(strain)1,40}=14.91$, $p<0.001$; $F_{(treatment)1,40}=0.40$, $p=0.53$; $F_{(strain\times treatment)1,40}=0.71$, $p=0.41$]. Post-hoc analysis revealed that the duration and frequency of social interaction were higher in vehicle-treated Sprague–Dawley rats than vehicle-treated Wistar rats ($p<0.05$ for both social interaction time and frequency, Figure 2a and b). No effects of URB597 on social interaction were found in either strain (Figure 2a and b).

Vehicle-treated Sprague–Dawley rats emitted more USVs than vehicle-treated Wistar rats during active social interaction ($p<0.05$) and during cage exploration or self-grooming ($p<0.05$). URB597 did not affect USV emission in both strains (Table 3).

3.2.2. Rats unfamiliar to the test cage and tested under low light (LU)

Vehicle-treated Sprague–Dawley rats showed higher levels of social interaction than vehicle-treated Wistar rats and URB597 did not affect social interaction in both rat strains. A two-way ANOVA of time and frequency of social interaction gave the following results: time [$F_{(strain)1,41}=19.91$, $p<0.0001$; $F_{(treatment)1,41}=1.78$, $p=0.19$; $F_{(strain\times treatment)1,41}=0.36$, $p=0.55$] and frequency of social interaction [$F_{(strain)1,41}=25.08$, $p<0.0001$; $F_{(treatment)1,41}=1.09$, $p=0.30$; $F_{(strain\times treatment)1,41}=0.94$, $p=0.34$]. Post-hoc analysis revealed that the duration and frequency of social interaction were higher in vehicle-treated Sprague–Dawley than vehicle-treated Wistar rats ($p<0.05$ for both social interaction time and frequency, Figure 2c and d).

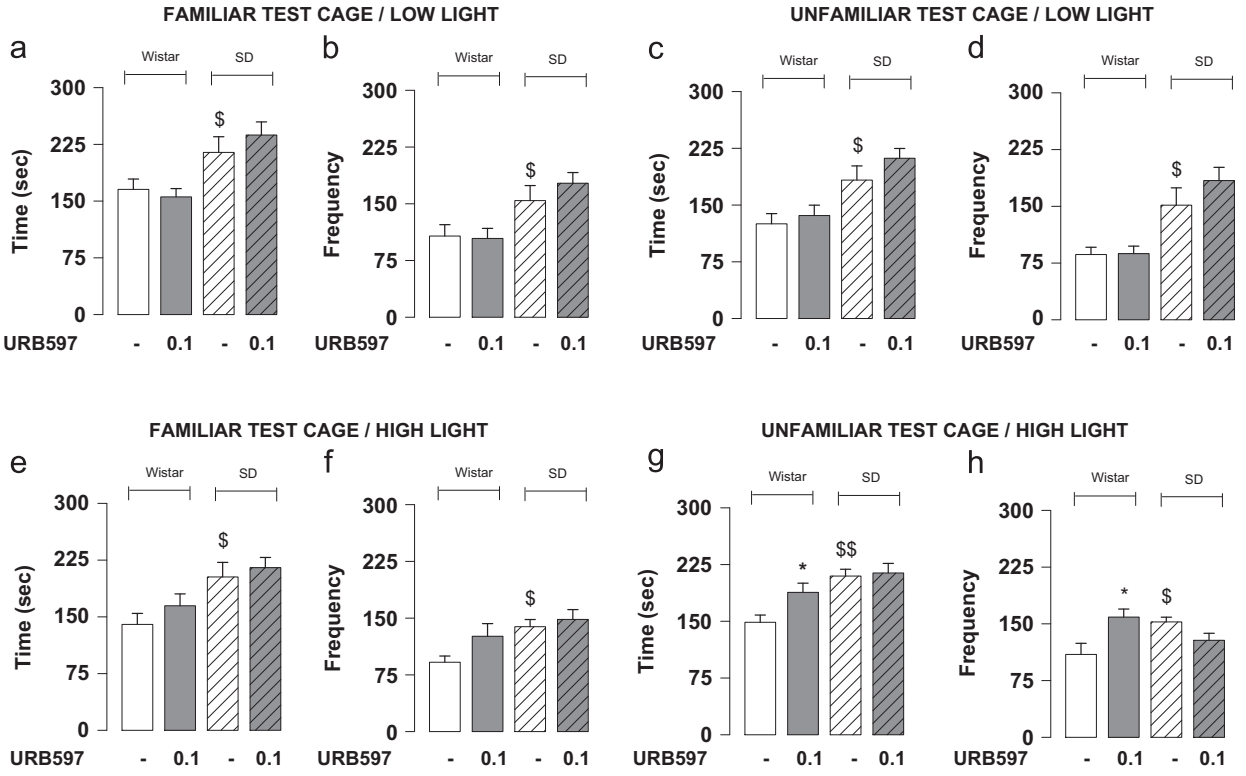

Figure 2 Effects of the FAAH inhibitor URB597 (0.1 mg/kg, *i.p.*, 2 h before test) on social interaction in adult Wistar and Sprague-Dawley rats tested under different experimental conditions. Vehicle-treated adult Sprague-Dawley rats spent more time in social interaction (a, c, e, g) and interacted more frequently with the social partner (b, d, f, h) than vehicle-treated adult Wistar rats. URB597 increased the time (g) and frequency (h) of social interaction in Wistar rats unfamiliar to the test cage and tested under high light. No effect of URB597 on social interaction was found in Sprague-Dawley rats (a-h). Data represent mean ± SEM time (a, c, e, g) and frequency (b, d, f, h) of social interaction during 10-min session. *$p<0.05$ vs. vehicle treatment; $^{\$}p<0.05$, $^{\$\$}p<0.01$ Sprague-Dawley vs. Wistar rats (Student-Newman-Keule post-hoc test). $N=9$–13 per treatment group.

Table 3 Effects of the FAAH inhibitor URB597 (0.1 mg/kg, *i.p.*, 2 h before test) on 50-kHz ultrasonic vocalizations (USVs) emitted during social interaction in adult Wistar and Sprague-Dawley rats tested under different experimental conditions.

Condition and treatment	USVs (num/10min)	ANOVA P value	USVs (num/10min)	ANOVA P value
Familiar test cage/low light	**USV during social interaction**		**USV during cage-exploration or self-grooming**	
VEH Wistar	224±25	$F_{(strain)1,40}=11.97$, $p<0.01$	299±40	$F_{(strain)1,40}=11.67$, $p<0.01$
URB597 Wistar	220±28	$F_{(treatment)1,40}=0.02$,	304±40	$F_{(treatment)1,40}=0.84$,
VEH SD	322±271;$^{\$}$	$p=0.88$	471±531;$^{\$}$	$p=0.36$
URB597 SD	317±31	$F_{(strain\times treatment)1,40}=0.01$, $p=0.99$	396±22	$F_{(strain\times treatment)1,40}=1.05$, $p=0.31$
Unfamiliar test cage/Low Light	**USV during social interaction**		**USV during cage-exploration or self-grooming**	
VEH Wistar	251±37	$F_{(strain)1,41}=12.01$, $p<0.01$	306±47	$F_{(strain)1,41}=34.46$, $p<0.0001$
URB597 Wistar	271±44	$F_{(treatment)1,41}<0.01$,	342±42	$F_{(treatment)1,41}=0.29$,
VEH SD	417±301;$^{\$}$	$p=0.96$	637±362;$^{\$\$\$}$	$p=0.60$
URB597 SD	402±53	$F_{(strain\times treatment)1,41}=0.17$, $p=0.69$	645±45	$F_{(strain\times treatment)1,41}=0.11$, $p=0.74$
Familiar test cage/High Light	**USV during social interaction**		**USV during cage-exploration or self-grooming**	
VEH Wistar	187±47	$F_{(strain)1,38}=8.81$, $p<0.01$	320±28	$F_{(strain)1,38}=11.75$, $p<0.01$
URB597 Wistar	267±35	$F_{(treatment)1,38}=0.60$,	375±22	$F_{(treatment)1,38}=0.50$,
VEH SD	350±271;$^{\$}$	$p=0.44$	466±351;$^{\$}$	$p=0.48$
URB597 SD	327±37	$F_{(strain\times treatment)1,38}=1.90$, $p=0.18$	460±39	$F_{(strain\times treatment)1,38}=0.84$, $p=0.37$
Unfamiliar test cage/high light	**USV during social interaction**		**USV during cage-exploration or self-grooming**	
VEH Wistar	109±24	$F_{(strain)1,43}=38.52$,	325±31	$F_{(strain)1,43}=15.97$, $p<0.001$
URB597 Wistar	92±10	$p<0.0001$	290±32	$F_{(treatment)1,43}=1.23$,
VEH SD	337±212;$^{\$\$\$}$	$F_{(treatment)1,43}=1.17$,	457±371;$^{\$}$	$p=0.27$
URB597 SD	396±20*	$p=0.28$	419±28	$F_{(strain\times treatment)1,43}<0.01$,
		$F_{(strain\times treatment)1,43}=4.17$, $p<0.05$		$p=0.95$

Over the 10-min test session, vehicle-treated Sprague-Dawley rats emitted more USVs, either related or unrelated to the social interaction, than vehicle-treated Wistar rats. In Sprague-Dawley rats, URB597 treatment increased the USVs emitted during social interaction in animals unfamiliar to the test cage and tested under high light. Data represent mean±SEM USVs during social interaction and USVs during cage-exploration or self-grooming.

$^{*}p<0.05$ vs. vehicle treatment.

$^{\$}p<0.05$.

$^{\$\$\$}p<0.001$ Sprague-Dawley vs. Wistar rats (Student-Newman-Keule post-hoc test). $N=9$-13 per treatment group.

URB597 treatment did not alter social interaction in either rat strain (Figure 2c and d).

Vehicle-treated Sprague-Dawley rats emitted more USVs than vehicle-treated Wistar rats during active social interaction ($p<0.05$) and during cage exploration or self-grooming ($p<0.001$). No effect of URB597 on USV emission was found in both rat strains (Table 3).

3.2.3. Rats familiar to the test cage and tested under high light (HF)

Vehicle-treated Sprague-Dawley rats showed higher levels of social interaction than vehicle-treated Wistar rats. URB597 tended to increase the frequency of social interaction in Wistar, but not in Sprague-Dawley rats. A two-way ANOVA of time and frequency of social interaction gave the following results: time [$F_{(strain)1,38}=12.63$, $p<0.001$; $F_{(treatment)1,38}=1.35$, $p=0.25$; $F_{(strain\times treatment)1,38}=0.15$, $p=0.70$] and frequency of social interaction [$F_{(strain)1,38}=7.27$, $p<0.05$; $F_{(treatment)1,38}=2.94$, $p=0.09$; $F_{(strain\times treatment)1,38}=0.94$, $p=0.34$]. Post-hoc analysis revealed that the duration and frequency of social interaction were higher in Sprague-Dawley than in Wistar rats ($p<0.05$ for total time and frequency of social interaction, Figure 2e and f). Furthermore, URB597 induced a trend for an increase in the frequency of social interaction ($p=0.08$, Figure 2f) in Wistar rats.

Vehicle-treated Sprague-Dawley rats emitted more USVs than vehicle-treated Wistar rats during active social

interaction ($p<0.05$) and during cage exploration or self-grooming ($p<0.05$). URB597 did not affect USV emission in both rat strains (Table 3).

3.2.4. Rats unfamiliar to the test cage and tested under high light (HU)

Vehicle-treated Sprague–Dawley rats showed higher levels of social interaction than vehicle-treated Wistar rats. URB597 increased the time and frequency of social interaction in Wistar but not in Sprague–Dawley rats. A two-way ANOVA of time and frequency spent of social interaction gave the following results: time [$F_{(strain)1,43}=13.80$, $p<0.001$; $F_{(treatment)1,43}=3.49$, $p=0.07$; $F_{(strain\times treatment)1,43}=2.30$, $p=0.14$] and frequency of social interaction [$F_{(strain)1,43}=0.35$, $p=0.56$; $F_{(treatment)1,43}=1.48$, $p=0.23$; $F_{(strain\times treatment)1,43}=12.58$, $p=0.001$]. Post-hoc analysis revealed that social interaction time ($p<0.01$, Figure 2g) and frequency ($p<0.05$, Figure 2h) were higher in vehicle-treated Sprague–Dawley than vehicle-treated Wistar rats. Under these experimental conditions, URB597 increased the time ($p<0.05$, Figure 2g) and frequency ($p<0.05$, Figure 2h) of social interaction in Wistar but not in Sprague–Dawley rats.

Vehicle-treated Sprague–Dawley rats emitted more USVs than vehicle-treated Wistar rats expressed as USVs emitted during active social interaction ($p<0.001$) and during cage exploration or self-grooming ($p<0.05$). URB597 increased the USVs emitted during social-related behaviors only in Sprague–Dawley rats ($p<0.05$, Table 3).

4. Discussion

It has repeatedly been shown that drugs that prolong ongoing endocannabinoid activity by inhibiting endocannabinoid deactivation affect emotional reactivity and sociability in rodents, which provides substantial support for a role for endocannabinoids in motivation and emotions (Haller et al., 2004; Patel et al., 2005b; Trezza et al., 2012a). However, it is also thought that the effects of cannabinoid drugs on emotional processes depend on genetic and environmental factors (Arnold et al., 2010; Arnold et al., 2001; Brand et al., 2012; Campolongo et al., 2013; Campolongo et al., 2012; Haller et al., 2009). Therefore, the aim of the present study was to investigate whether anandamide modulation of social behavior is strain- and context-dependent. To address this aim, we tested the effects of the anandamide hydrolysis inhibitor URB597 on social behavior and 50-kHz USVs in adolescent and adult Wistar and Sprague–Dawley rats tested under different environmental conditions (i.e., low or high light, familiarity or unfamiliarity to the testing environment). The results of this study demonstrate that the effects of URB597 on social behavior and USV emission during social interaction in rats are strain- and context-dependent.

The Wistar and Sprague–Dawley rat strains are extensively used in behavioral pharmacology studies and certain behavioral differences between these two rat strains have been well characterized (Rex et al., 2004; Staples and McGregor, 2006; Walker et al., 2009; Zmarowski et al., 2012). In particular, we have recently shown that adolescent Sprague–Dawley rats display higher baseline levels of social play behavior and USV emission than Wistar rats when tested under low aversive conditions (i.e. when the animals were habituated to the test cage and tested under low light) (Manduca et al., 2014). Furthermore, Rex et al. (2004) found that adult Sprague–Dawley rats showed a higher level of social interaction than adult Wistar rats when tested under high light levels. In the present study, we confirm and extend these findings by showing that adolescent and adult Sprague–Dawley rats exhibit higher levels of social behavior than Wistar rats independently of the experimental conditions. Indeed, the frequencies of pinning and pouncing, the two most characteristic postures of social play behavior in rats, were higher in vehicle-treated adolescent Sprague–Dawley than Wistar rats either when animals were tested under low or high light and were familiar or unfamiliar to the test cage. Similarly, at adulthood, we found that vehicle-treated Sprague–Dawley rats spent more time in social interaction than vehicle-treated Wistar rats in all experimental conditions.

The effects of URB597 on adolescent social play behavior were strain- but not context-dependent. Thus, systemic administration of URB597 increased social play behavior in adolescent Wistar but not Sprague–Dawley rats, with no effect on the total time spent in non-playful forms of social behavior, such as social exploration and contact behavior, under all the experimental conditions. Conversely, the effects of URB597 on adult social behavior were both strain- and context-dependent. Thus, URB597 significantly increased social interaction in Wistar but not Sprague–Dawley rats only under highly aversive experimental conditions (i.e. animals unfamiliar to the text cage and tested under high light). Furthermore, URB597 induced a trend for an increase in the frequency of social interaction in Wistar but not Sprague–Dawley rats tested under moderately aversive conditions (i.e. animals familiar to the text cage and tested under high light).

The lack of effects of URB597 in Sprague–Dawley rats may be due to a ceiling effect. Thus, it is possible that in the Sprague–Dawley strain, URB597 administration did not further increase the high social play behavior observed in control animals, while it was effective in Wistar rats that displayed lower baseline levels of social play. In line with this possibility, we have recently demonstrated that the play-enhancing effects of morphine are more pronounced in Wistar than Sprague–Dawley rats (Manduca et al., 2014), indicating that differences exist in the response of these two rat strains to pharmacological manipulations of social play. Similarly, the higher levels of social interaction observed in adult Sprague–Dawley rats compared to Wistar rats were associated with a different response to diazepam, which increased social interaction in Wistar rats and had only sedating effects in Sprague–Dawley rats (Rex et al., 2004).

Different behavioral effects of cannabinoid compounds depending on the rodent strain used have been frequently reported. For instance, compared to other rat strains, Wistar rats were more sensitive to the effects of cannabinoid drugs on catalepsy, body temperature, locomotor activity (Arnold et al., 2001), palatable food intake (Brand et al., 2012) and spatial working memory (Renard et al., 2013). At the neural level, compared to other rat strains, Wistar rats showed higher cannabinoid-induced expression of the transcription factor c-Fos in regions of the brain that subserve stress and anxiety-related behavior (Arnold et al.,

2001), higher expression of the CB1 cannabinoid receptor and FAAH enzyme, as well as higher cannabinoid-induced stimulation of the CB1 cannabinoid receptor in the hippocampus (Brand et al., 2012). Thus, a second possibility is that baseline differences in the endocannabinoid system may be responsible for the different effects of URB597 on social behavior in Wistar and Sprague–Dawley rats.

Together with genetic factor, the ability of cannabinoid drugs to influence emotional behavior depending on the environmental conditions has also been extensively reported (Campolongo et al., 2013, 2012; Haller et al., 2014, 2009; Morena and Campolongo, in press; Naidu et al., 2007; Patel et al., 2005a; Trezza and Vanderschuren, 2008). For instance, it has been shown that the effects of URB597 on anxiety-like behaviors in adult mice and rats tested in the elevated plus-maze test depend largely on the experimental conditions. This conclusion is directly supported by two studies. First, the study performed by Naidu et al. (2007) showed that the anxiolytic effects of URB597 in mice became evident only when the illumination differed in the open and closed arms of the plus-maze. A second study by Haller et al. (2009) showed that the anxiolytic effects of URB597 in the elevated plus-maze test were not detected under non-challenging experimental conditions, for example when rats were tested under low light or were habituated to the testing room. In contrast, robust anxiolytic effects of URB597 were observed when rats were tested under high light without habituation, or when habituated rats were submitted to sudden changes in illumination during testing.

The social interaction test is widely used to assess emotional reactivity in adult rodents (File, 1980). In this test, the dependent measure is the time that pairs of adult rodents spend in social interaction, that is increased by anxiolytic drugs and decreased by anxiogenic compounds. This test can be performed in different experimental conditions, such as familiar or unfamiliar social partner and arena (File, 1980). Our results show that, in line with the results obtained in the elevated plus-maze test (Haller et al., 2009; Naidu et al., 2007), the anxiolytic-like effects of URB597 in adult rats tested in the social interaction test can be detected only when Wistar rats are unfamiliar to the test cage and tested under high light. Conversely, the fact that URB597 increased social play behavior in adolescent Wistar rats in all experimental conditions supports the idea that the increase in social play induced by URB597 is not due to its anxiolytic-like properties. Rather, we suggest that the higher anandamide signaling induced by URB597 increases the motivational and rewarding properties of social play so that, compared to vehicle-treated rats, URB597-treated adolescents prefer to engage in playful interactions with their partners even in aversive environmental conditions. To support this possibility, it has recently been shown that, during social interactions with an unfamiliar partner, anandamide levels are increased in the nucleus accumbens, amygdala and dorsal striatum of adolescent rats (Marco et al., 2011; Trezza et al., 2012b). Interestingly, these increased anandamide signaling may be due to the novelty associated with the social encounter, since higher striatal anandamide levels were only observed after a social interaction session with a non-familiar test partner (Marco et al., 2011). It is also possible that URB597 administration in adolescent Wistar rats not only increased the rewarding value of play, but also altered the attentional processes which are normally recruited in response to a novel and more aversive environment so that, compared to adult animals, adolescent rats treated with URB597 paid more attention to novel social than to novel environmental stimuli.

We also measured the number of 50-kHz USVs emitted during the testing session by both adolescent and adult Wistar and Sprague–Dawley rats. In particular, we discriminated between the 50-kHz USVs emitted during actual social interaction from those emitted during the performance of non-social behaviors, such as cage exploration or self-grooming.

In line with our previous findings (Manduca et al., 2014), we did not find a positive correlation between the performance of social behaviors and the emission of 50-kHz USVs, independently of the experimental conditions. Moreover, as previously reported, we found that vehicle-treated adolescent and adult Sprague–Dawley emitted more 50-kHz USVs, both related and unrelated to the social interaction, than Wistar rats tested under all the different experimental conditions. It has previously been shown that rats emit 22-kHz calls during social defeat, drug withdrawal, as well as in anticipation of aversive events (Burgdorf et al., 2011). In our experiments, we did not observe the emergence of aggressive behaviors between the two rats of each pair, either adolescents or adults. Indeed, in our experimental setting, each animal was subjected to one single social interaction session with an unfamiliar, same age partner, while aggressive behavior usually emerges in rodents following repeated confrontation with an unfamiliar intruder. Therefore, although we did not measure low-frequency USVs and therefore their occurrence cannot be firmly excluded, it is unlikely that in our setting rats emitted 22-kHz calls in the absence of any aggressive or otherwise aversive behavior.

It has previously been shown that cannabinoid drugs affect different kinds of USVs in rodents. That is, Arnold and colleagues found that Wistar rats treated with the cannabinoid receptor agonist CP55,940 and re-exposed to a chamber in which they had previously received a footshock emitted more 18–30-kHz USVs than vehicle-treated rats. Conversely, CP55,940 had no significant effect upon conditioned USVs in Lewis rats (Arnold et al., 2010), highlighting a strain-dependent effect of the cannabinoid agonist on fear-related USV emission. Previous studies have shown that cannabinoid drugs, including URB597, modulate maternal separation-induced USVs in rat pups (Bortolato et al., 2006; Kathuria et al., 2003; McGregor et al., 1996), showing a pivotal role of the endocannabinoid system in the modulation of USVs during early neonatal stages in rodents (for a review, see Manduca et al. (2012)). In line with this possibility, CB1 knock-out mice did not show the characteristic developmental peak in isolation-induced USVs observed between 3 and 6 days of age in wild-type animals, and emitted less USVs in response to stress at adulthood (Fride, 2005). Interestingly, it has been recently demonstrated that URB597 significantly reduced the low frequency USVs (22 kHz) emitted during freezing behavior in the fear conditioning paradigm in male Lister-hooded rats (Butler et al., 2012), thus supporting the possibility that FAAH has

an important role in fear-related behaviors. Our present findings show that URB597 enhanced the frequency of 50-kHz USVs emitted during social interaction by adolescent Wistar and adult Sprague–Dawley rats tested under moderate and high aversive conditions respectively, suggesting that the endocannabinoid system modulates the emission of high frequency USVs (50 kHz) during social behavior in a strain- and context-dependent manner.

In conclusion, this study shows that anandamide modulation of adolescent social play behavior is strain- but not context-dependent. In contrast, anandamide modulation of adult social behavior depends on both strain and experimental context. This supports the idea that anandamide has distinct roles in adolescent social play behavior and adult social interaction. Furthermore, these results confirm that robust behavioral differences exist between vehicle-treated Wistar and Sprague–Dawley rats, which may explain the sometimes contradictory effects of cannabinoid drugs on emotionality in different strains of rodents.

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Contributors

All authors contributed to and have approved the final manuscript. Antonia Manduca and Michela Servadio performed the experiments. Antonia Manduca wrote the first draft of the manuscript. Patrizia Campolongo, Maura Palmery, Luigia Trabace, Louk J.M.J. Vanderschuren and Vincenzo Cuomo analyzed and contributed to the design of the experiments and edited the manuscript. Viviana Trezza supervised the project, designed the experiments and wrote the manuscript.

Conflicts of interest

All authors declare no potential conflicts of interest in relation to the work described.

On behalf of all-co-authors,
Viviana Trezza

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