International Journal of Neuropsychopharmacology (2013), **16**, 661–676. © CINP 2012
doi:10.1017/S1461145712000569

ARTICLE

CINP

Hyperactivity induced by the dopamine D_2/D_3 receptor agonist quinpirole is attenuated by inhibitors of endocannabinoid degradation in mice

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Abstract

The present study was designed to investigate the effect of pharmacological inhibition of endocannabinoid degradation on behavioural actions of the dopamine D_2/D_3 receptor agonist quinpirole in male C57Bl/6J mice. In addition, we studied the effects of endocannabinoid degradation inhibition on both cocaine-induced psychomotor activation and behavioural sensitization. We analysed the effects of inhibition of the two main endocannabinoid degradation enzymes: fatty acid amide hydrolase (FAAH), using inhibitor URB597 (1 mg/kg); monoacylglycerol lipase (MAGL), using inhibitor URB602 (10 mg/kg). Administration of quinpirole (1 mg/kg) caused a temporal biphasic response characterized by a first phase of immobility (0–50 min), followed by enhanced locomotion (next 70 min) that was associated with the introduction of stereotyped behaviours (stereotyped jumping and rearing). Pretreatment with both endocannabinoid degradation inhibitors did not affect the hypoactivity actions of quinpirole. However, this pretreatment resulted in a marked decrease in quinpirole-induced locomotion and stereotyped behaviours. Administration of FAAH or MAGL inhibitors did not attenuate the acute effects of cocaine. Furthermore, these inhibitors did not impair the acquisition of cocaine-induced behavioural sensitization or the expression of cocaine-induced conditioned locomotion. Only MAGL inhibition attenuated the expression of an already acquired cocaine-induced behavioural sensitization. These results suggest that pharmacological inhibition of endocannabinoid degradation might exert a negative feedback on D_2/D_3 receptor-mediated hyperactivity. This finding might be relevant for therapeutic approaches for either psychomotor disorders (dyskinesia, corea) or disorganized behaviours associated with dopamine-mediated hyperactivity.

Received 5 January 2012; Reviewed 10 February 2012; Revised 13 April 2012; Accepted 24 April 2012; First published online 30 May 2012

Key words: Cocaine, dopamine, endocannabinoid system, quinpirole, stereotyped behaviour.

Introduction

The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are retrograde messengers that regulate a variety of brain functions through stimulation of cannabinoid receptors type 1 and 2 (CB_1 and CB_2; Placzek *et al.* 2008; Wang & Ueda, 2008). The CB_1 receptor is highly expressed on axon terminals of glutamatergic and γ-aminobutyric acid (GABA)ergic projecting neurons, through which the endogenous cannabinoid system controls neurotransmitter release and synaptic plasticity (Adermark & Lovinger, 2007; Adermark *et al.* 2009; Gerdeman & Lovinger, 2001; Piomelli, 2003). The endocannabinoid system (ECS) is mainly involved in motor, motivational, emotional and cognitive processes (Giuffrida *et al.* 1999, 2004;

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Luchicchi *et al.* 2010; Rasmussen *et al.* 2009; Rodriguez de Fonseca *et al.* 1998; Solinas *et al.* 2007). In addition, the ECS regulates dopamine-mediated behaviours (Rodriguez de Fonseca *et al.* 2001). This role is related to its anatomical presence in basal ganglia circuits (Martin *et al.* 2008; Rodriguez de Fonseca *et al.* 1998, 2001; Solinas *et al.* 2008).

Due to the presence of the ECS in brain dopaminergic circuits, much work has been performed to clarify its role in brain reward processes (Gardner, 2005). For instance, pharmacological modulation of the ECS appears to regulate dopamine-mediated rewarding effects of alcohol, cannabis, opioids and psychostimulants (Colombo *et al.* 2005; de Vries *et al.* 2001; Ledent *et al.* 1999; Maldonado & Rodriguez de Fonseca, 2002; Scherma *et al.* 2008). However, less work has been performed regarding behavioural consequences of endocannabinoid signalling modulation in motor control. Some evidence suggests that regulation of psychomotor output might depend on the interaction between the endocannabinoid and the dopaminergic systems in both the basal ganglia and the mesolimbic reward system (Giuffrida & Piomelli, 2000; Glass *et al.* 1997).

Analysis of the role of the ECS in dopamine-mediated behaviours suggests that endocannabinoids exert a complex regulatory role in both dopamine-releasing and dopamine receptor-expressing neurons. For instance, activation of the CB_1 receptor induces dopamine release in rodents and humans (Bossong *et al.* 2009; Ng Cheong Ton *et al.* 1988; O'Neill *et al.* 2009). This effect is thought to be mediated by the reinforcing properties of natural cannabinoids (Gardner, 2005). Conversely, activation of dopamine D_2/D_3 receptors stimulates production of the endocannabinoid AEA in the dorsal striatum (Giuffrida *et al.* 1999). In this situation, the ECS would be acting as an inhibitory feedback mechanism that counteracts the dopamine-induced facilitation of motor activity (Beltramo *et al.* 2000). Supporting this hypothesis, previous reports show that the CB_1 receptor agonist WIN 55,212-2 is able to ameliorate dyskinesias (Ferrer *et al.* 2003). Conversely, the CB_1 receptor antagonist SR141716A aggravates the stereotypies induced by pharmacological over-activation of dopamine receptors (Ferrer *et al.* 2007). Moreover, desensitization of CB_1 receptors induced by Δ9-tetrahydrocannabinol administration facilitates dopamine-mediated behaviours (Gorriti *et al.* 1999, 2005).

Although the role of dopamine D_2/D_3 receptors as activators of endocannabinoid release is well known (Giuffrida *et al.* 1999), the effects of psychostimulants on the activation of the ECS are much less understood. Among psychostimulants, cocaine is a monoamine reuptake inhibitor that interferes with the uptake of dopamine, noradrenaline and serotonin (Ritz *et al.* 1990). Cocaine also induces an augmented motor response after repeated administration (sensitization; Blanco *et al.* 2012*a*, *b*). Acute cocaine administration increases AEA levels in the striatum (Arnold, 2005). This effect is mediated by dopaminergic D_2-like receptors (Arnold, 2005). However, as in the case of dopamine D_2/D_3 receptor agonists, there is no clear evidence that the increase of endocannabinoids induced by cocaine also acts as an inhibitory feedback signal for cocaine-induced stimulation. It is important to note that the effects of cocaine, including reinforcing effects, can be mediated by non-dopaminergic neurons through interactions with other neurotransmitter systems, such as the serotoninergic system (Hnasko *et al.* 2007).

From the studies described above, we can hypothesize that endocannabinoids generated by dopamine D_2/D_3 receptor activation serve as counter-regulatory signals that limit behavioural over-activation. To confirm this hypothesis, we performed studies with inhibitors of endocannabinoid degradation. AEA and 2-AG are generated by cells on demand through stimulus-dependent cleavage of membrane phospholipid precursor and undergo rapid biological deactivation after release (di Marzo *et al.* 1994; Stella *et al.* 1997). Both AEA and 2-AG are degraded and eliminated through enzymatic hydrolysis by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively (Cravatt *et al.* 1996, 2001). Blockage of this degradation will result in enhanced availability of endocannabinoids, which may counteract quinpirole-induced locomotion/stereotypy and cocaine-induced sensitization. Thus, the main goals of the present study were as follows: (*a*) to characterize locomotor activity and stereotypic behaviours induced by the dopamine D_2/D_3 receptor agonist quinpirole in a mouse model; (*b*) to evaluate the effects of FAAH and MAGL inhibition on quinpirole-induced behaviours; (*c*) to assess the effects of FAAH and MAGL inhibition on horizontal locomotion, conditioned locomotion and behavioural sensitization induced by cocaine.

Method

Animals

C57B1/6J adult male mice (Charles River, Spain) were maintained in controlled housing conditions (12 h light/dark cycle, lights on 08:00 hours; temperature: 20 ± 2 °C; humidity: 40 ± 5%) with food and water

ad libitum at the University of Malaga's vivarium. The experimental procedures were in accordance with European Communities Council Directives 86/609/EU, 98/81/CEE, 2003/65/EC and Commission Recommendation 2007/526/EC.

Drugs

The dopamine D_2/D_3 receptor agonist quinpirole hydrochloride (QNP; Tocris Cookson Ltd, UK) was dissolved in a solution of DMSO, Tween 80 and sterile saline (1:1:8, Veh) and administered subcutaneously at doses of 0.05, 0.1 and 1 mg/kg. Selective inhibitors of FAAH [URB597 [3′-(aminocarbonyl)[1,1′-biphenyl]-3-yl)-cyclohexylcarbamate)] and MAGL [URB602 ([1,1′-biphenyl]-3-yl-carbamic acid, cyclohexyl ester); Cayman, USA] were dissolved in Veh and injected i.p. at doses of 1 mg/kg (URB597) or 10 mg/kg (URB602). Cocaine hydrochloride (Sigma-Aldrich, Spain) was dissolved in a sterile saline solution and administered i.p. at doses of 10 or 20 mg/kg. All of the drugs were injected in a volume of 1 ml/kg.

Apparatus and general procedures

Animals were handled and habituated to injection procedures once per day for 5 d. All of the experiments were carried out between 08:00 and 20:00 hours. Each day, the animals were acclimatized to the experimental room for 30 min. Performance in the open field (OF) was recorded by a computer-based video tracking system (Smart v2.5®; Panlab, Spain). Stereotyped behaviours were directly observed by placing the animals in transparent glass observational cylinders (OCs). The maximum light intensity in the centre of the OF and inside the OC was 100 lux. All of the apparatuses were cleaned with a 70% ethanol solution.

Open field

Four OFs (50 × 50 × 50 cm; Panlab) with grey backgrounds were used. Animals were placed in the centre of each arena and their behaviour was recorded for 30 or 120 min. Horizontal locomotion was measured as the total distance travelled (cm). The immobility variable was evaluated as the total time of immobility (s) using a detection filter (detection range less than 10 cm/s). Because analysis of locomotion and time spent in the centre of the OF is considered an index of anxiety in cannabinoid studies (Long *et al.* 2010; Thiemann *et al.* 2009), we also registered the distance travelled in periphery and centre area. The centre of the OF was defined as a square of 30 × 30 cm. A mouse was considered to be in the central area when its four paws were in it.

Observational cylinders

Quantification of stereotyped activity was performed by direct observation of the animals after they were placed in the glass transparent OCs (60 cm height × 12 cm diameter). Eight cylinders were attached to the original base of the OF in the perimeter of the arena. Clean bedding material was added after each animal test. Animals were injected with Veh or QNP and/or the inhibitors of endocannabinoid degradation and placed in the cylinders. Evaluation of stereotyped behaviours (such as jumping, rearing and grooming) was performed by trained observers blind to the experimental conditions. Quantification was performed at 10 min time intervals for a total time of 120 min post-injection.

Behavioural procedures

We performed a total of five experiments. In expt 1, we evaluated the effect of QNP administration on locomotion/immobility behaviour in the OF. In expt 2, we analysed stereotyped behaviours in the OC. In expt 3, we assessed the effects of FAAH and MAGL inhibitors on anxiety, habituation, locomotion activity and stereotyped behaviours in the OF and OC. In expt 4, we analysed the effects of FAAH and MAGL inhibitors on QNP-induced locomotion and stereotyped behaviours in the OF and OC. Finally, we evaluated the effects of FAAH and MAGL inhibitors in acute/repeated cocaine administration, conditioned locomotion and behavioural sensitization in the OF (expt 5).

Evaluation of locomotion and stereotyped behaviours after administration of QNP and/or inhibitors of FAAH/MAGL in the OF and OC

Animals were injected with Veh or different single doses of QNP (expt 1 and expt 2), URB597 or URB602 (expt 3) or were co-administered QNP + URB597 or QNP + URB602 (expt 4). When drugs were co-administered, URB597 and URB602 were injected 30 min before QNP.

Evaluation of FAAH/MAGL inhibitors on acute/repeated cocaine administration, conditioned locomotion and behavioural sensitization in the OF

Animals received a single i.p. injection of Veh, cocaine, URB597, URB602, URB597 + cocaine or URB602 + cocaine in the different phases of the experiment (expt 5). Briefly, mice were exposed to acute or repeated cocaine administration (20 mg/kg) for five consecutive days. One half of the animals were treated with cocaine, cocaine + URB597 or cocaine + URB602. The other half of the animals were treated with Veh,

URB597 or URB602. After the 5 d, mice rested in their home cages without drugs for another 5 d. Twenty-four hours later, we evaluated the conditioned locomotion response after administration of Veh, URB597 or URB602. On the last day, we tested the behavioural sensitization by injection of a prime dose of cocaine (10 mg/kg), cocaine (10 mg/kg) + URB597 or cocaine (10 mg/kg) + URB602. Using two other groups of animals, we also evaluated the effects of acute administration of URB597 and URB602 on an already acquired conditioned locomotion and cocaine sensitization response. During all of these phases, the animals were evaluated in the OF immediately after the drug or Veh injections to measure the distance travelled over 30 min. Conditioned locomotion and behavioural sensitization protocols used in this study are based on Pavlovian conditioning. Classical Pavlovian conditioning is a basic process of associative learning that allows an animal to predict and adapt to future events based on previous experience. Conditional learning involves the association of a neutral stimulus with an unconditional stimulus (UCS) that elicits an unconditional response (UCR). After repeated pairing, the neutral stimulus becomes a conditional stimulus (CS) that induces a conditional response (CR) similar to the original UCR. In our study, the psychostimulant cocaine (UCS) produces an increased locomotor response (UCR). Repeated pairing of drug administration (daily cocaine injections as UCS) with a specific context (OF as CS) typically leads to an enhanced locomotor response (conditioned locomotion as CR) when mice are re-exposed without cocaine in the OF. In addition, this conditioned motor-stimulant response is exponentially increased by a single injection of a prime dose of cocaine (behavioural sensitization).

Statistical analysis

Results were expressed as the mean ± S.E.M. Data were analysed by one-, two- or three-way analysis of variance (ANOVA) tests with or without repeated measures, followed by a *post-hoc* Tukey–Kramer test. The Greenhouse–Geisser's correction was employed when appropriate. A probability was considered to be significant at $\leqslant 5\%$. Statistical analyses were performed with SPSS 15.0 (SPSS Inc., USA).

Results

Hyperactivity and immobility induced by acute treatment with quinpirole in C57/Bl6J mice

Mice were injected with QNP (0.1 or 1 mg/kg) or Veh and exposed to the OF for 2 h. Distance travelled was measured in 10 min time intervals. ANOVA showed that the effects of treatment, time interval and the interaction were significant ($F_{2,27}=21.87$, $p<0.001$; $F_{5.28,142.66}=8.40$, $p<0.001$; $F_{10.56,142.66}=19.22$, $p<0.001$). ANOVAs performed on each time interval showed that the effect of treatment was significant in the first eight time intervals ($F_{2,27}=203.94$, $p<0.001$; $F_{2,27}=223.61$, $p<0.001$; $F_{2,27}=86.32$, $p<0.001$; $F_{2,27}=35.93$, $p<0.001$; $F_{2,27}=17.09$, $p<0.001$; $F_{2,27}=6.96$, $p<0.001$; $F_{2,27}=20.31$, $p<0.001$; $F_{2,27}=11.69$, $p<0.001$). *Post-hoc* tests revealed that mice injected with both doses of QNP travelled significantly shorter distances than Veh-injected mice during the first 50 min (Fig. 1*a*). In contrast, mice injected with 1 mg/kg QNP travelled a significantly longer distance compared to other groups at 50–80 min (Fig. 1*a*). When locomotion was expressed as a percentage of the distance travelled by Veh-injected mice, the effects of treatment, time interval and the interaction were significant ($F_{1,18}=5.43$, $p=0.032$; $F_{4.37,78.71}=46.56$, $p<0.001$; $F_{4.37,78.71}=10.34$, $p<0.001$). *Post-hoc* tests revealed that, during the first 30 min, mice injected with 1 mg/kg QNP displayed a significantly lower percentage of distance travelled than mice injected with 0.1 mg/kg QNP. These data show that both groups had lower levels of locomotion in comparison to the Veh group (Fig. 1*b*). In contrast, mice injected with 1 mg/kg QNP showed a significantly higher percentage of distance travelled when compared to mice injected with 0.1 mg/kg QNP or Veh at 50–80 min (Fig. 1*b*). Furthermore, when data were collapsed into 0–50 min and 50–120 min groups, ANOVAs revealed significant differences ($F_{2,27}=270.28$, $p<0.001$; $F_{2,27}=19.37$, $p<0.001$). Furthermore, *post-hoc* tests indicated that during the first 50 min both groups injected with QNP travelled a significantly decreased distance when compared to the Veh group. This effect occurred in a dose-dependent manner (Fig. 1*c*). From 50 to 120 min, the group injected with the highest dose of QNP displayed a significantly longer distance travelled in comparison with the other groups (Fig. 1*d*). Regarding immobility time, ANOVA showed that the effects of treatment, time interval and the interaction were significant ($F_{2,27}=16.54$, $p<0.001$; $F_{3.51,94.80}=6.79$, $p<0.001$; $F_{7.02,94.80}=10.78$, $p<0.001$). ANOVAs showed that the effect of treatment was significant in the first 50 min and from 60 to 80 min (0–10 min: $F_{2,27}=461.37$, $p<0.001$; 10–20 min: $F_{2,27}=377.08$, $p<0.001$; 20–30 min: $F_{2,27}=311.39$, $p<0.001$; 30–40 min: $F_{2,27}=28.94$, $p<0.001$; 40–50 min: $F_{2,27}=21.62$, $p<0.001$; 60–70 min: $F_{2,27}=5.27$, $p=0.012$; 70–80 min: $F_{2,27}=3.79$, $p=0.036$). *Post-hoc* tests indicated that, during the first 50 min, mice injected with both doses of QNP spent a longer time immobilized

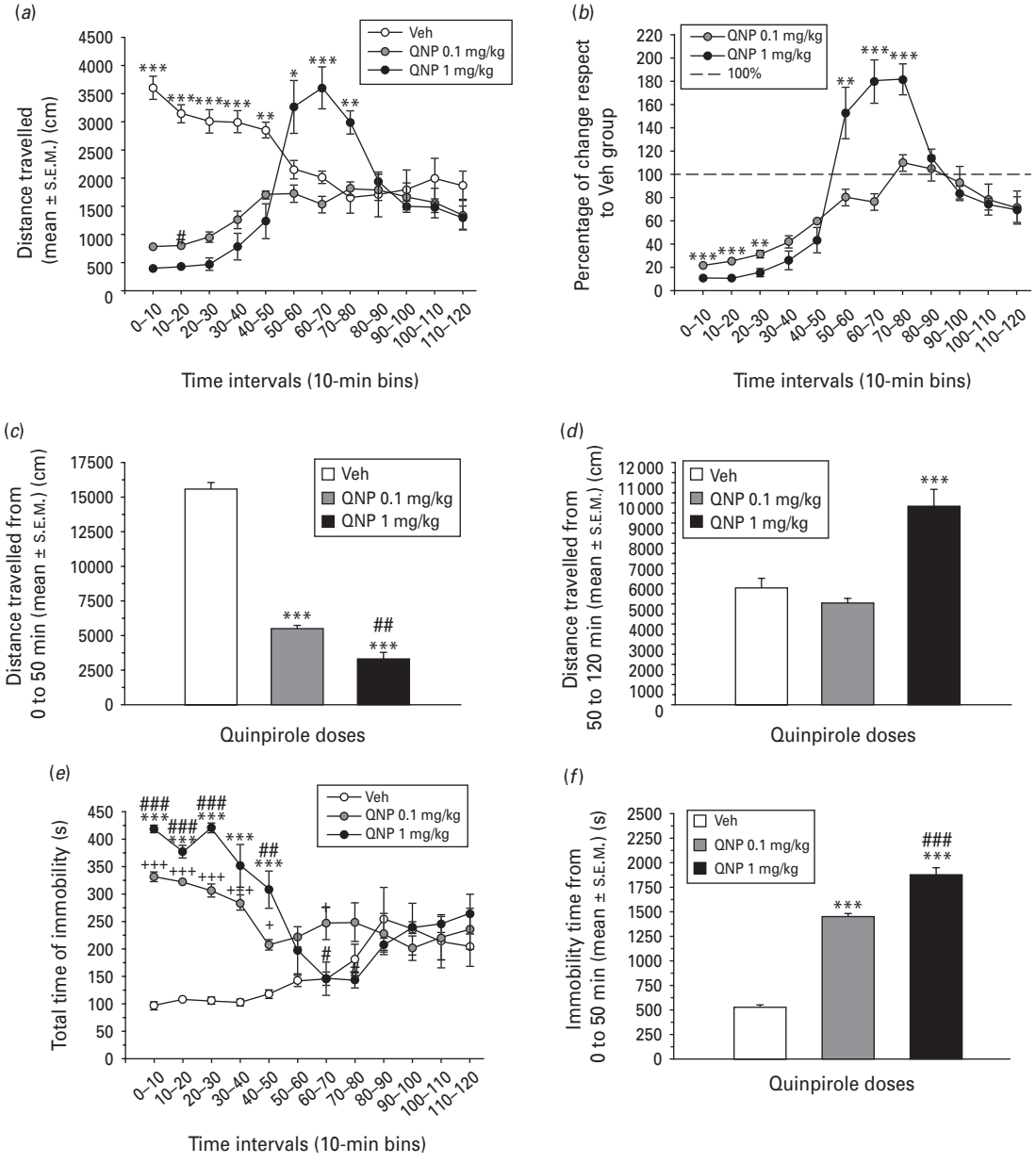

Fig. 1. Effects of acute treatment with the dopamine D_2/D_3 receptor agonist quinpirole hydrochloride (QNP) on locomotion in C57/BI6J mice: characterization of a biphasic response evaluated in the open field (OF). (*a*) Locomotor activity after acute QNP administration (*** $p<0.001$, ** $p<0.01$ and * $p<0.05$ compared to all other groups; $^{\#}$ $p<0.05$ compared to QNP 1 mg/kg); (*b*) percentage change in locomotion after acute QNP administration (*** $p<0.001$ and ** $p<0.01$ compared to the other group); (*c*) effect of QNP-induced hypolocomotion for the first 50 min [*** $p<0.001$ compared to vehicle (Veh) control group; $^{\#\#}$ $p<0.01$ compared to QNP 0.1 mg/kg]; (*d*) the enhanced locomotion for the last 70 min after QNP administration (*** $p<0.001$ compared to QNP 0.1 mg/kg and Veh control groups); (*e*) immobility response after acute QNP administration (*** $p<0.001$, $^{+++}$ $p<0.001$ and $^{+}$ $p<0.05$ compared to Veh control group; $^{\#\#\#}$ $p<0.001$, $^{\#\#}$ $p<0.01$ and $^{\#}$ $p<0.05$ compared to QNP 0.1 mg/kg); (*f*) effect of QNP-induced immobility for the first 60 min (*** $p<0.001$ compared to Veh control group; $^{\#\#\#}$ $p<0.001$ compared to QNP 0.1 mg/kg). Values represent the mean ± S.E.M. ($n=10$ per group).

compared to the Veh group. This effect also occurred in a dose-dependent manner (Fig. 1*e*). From 60 to 80 min, mice injected with the highest dose of QNP had a similar immobility time compared to the Veh group (Fig. 1*e*). ANOVA performed on collapsed data from 0 to 50 min were significant ($F_{2,27}=226.48$,

$p<0.001$) and *post-hoc* tests confirmed that both doses of QNP enhanced immobility (Fig. 1*f*).

Stereotypic behaviours elicited by acute treatment with quinpirole in C57/Bl6J mice

Stereotypic behaviours (jumping, rearing and grooming) were measured during the 2 h after Veh or QNP injections in the OC. Regarding jumping, ANOVA performed between 60 and 110 min indicated that the effects of treatment, time interval and the interaction were significant ($F_{3,36}=15.04$, $p<0.001$; $F_{3.07,110.53}=5.31$, $p<0.001$; $F_{9.21,110.53}=3.25$, $p<0.001$). ANOVAs showed that the effect of treatment was significant in all of the time intervals except the first (60–70 min: $F_{3,36}=2.77$, $p=$n.s.; 70–80 min: $F_{3,36}=7.57$, $p<0.001$; 80–90 min: $F_{3,36}=18.88$, $p<0.001$; 90–100 min: $F_{3,36}=7.45$, $p<0.001$; 100–110 min: $F_{3,36}=3.80$, $p=0.018$). *Post-hoc* tests revealed that from 70 to 110 min mice injected with the highest dose of QNP displayed a significantly higher number of jumps compared to the other groups (Fig. 2*a*). When an ANOVA was conducted on the total number of jumps between 70 and 110 min, the effect of treatment was significant ($F_{3,36}=15.44$, $p<0.001$). *Post-hoc* tests showed that an injection of 1 mg/kg QNP significantly increased the number of jumps (Fig. 2*b*). Concerning rearing behaviour, ANOVA performed between 50 and 90 min indicated that the effect of treatment and the treatment × time interval interaction were significant ($F_{3,36}=4.70$, $p<0.01$; $F_{9,108}=4.83$, $p<0.001$). ANOVAs showed that the effect of treatment was significant in the 60–70 and 70–80 min time intervals ($F_{3,36}=9.90$, $p<0.001$; $F_{3,36}=10.65$, $p<0.001$). *Post-hoc* tests revealed that from 60 to 80 min mice injected with 1 mg/kg QNP displayed a significantly higher number of rearing in comparison to the other groups (Fig. 2*c*). ANOVA performed on collapsed data from 60 to 80 min was significant ($F_{3,36}=15.94$, $p<0.001$). *Post-hoc* tests showed that an injection of 1 mg/kg QNP significantly increased the number of rearing (Fig. 2*d*). Finally, grooming behaviour was not significantly affected by QNP administration (Fig. 2*e*, *f*).

FAAH and MAGL inhibitors did not modify locomotion, anxiety, habituation or stereotypic behaviours in C57/Bl6J mice

To evaluate the effects of FAAH and MAGL inhibitors on horizontal locomotion, anxiety and habituation, mice were injected with URB597 (1 mg/kg), URB602 (10 mg/kg) or Veh and tested in two OF sessions with a 24 h inter-session interval. Locomotion was evaluated during the whole duration of the first OF session. ANOVA indicated that neither the effect of treatment (Fig. 3*b*) nor the interaction was significant ($F_{2,33}<1$, $p=$n.s.; $F_{10.384,171.34}<1$, $p=$n.s.). However, the effect of time interval was significant ($F_{5.192,171.34}=7.88$, $p<0.001$). These results suggest that all of the groups showed the same levels of locomotor activity. Furthermore, locomotor activity in the groups progressively decreased in the same manner over time (Fig. 3*a*). To determine if treatment with FAAH and MAGL inhibitors had any effect on anxiety levels and inter-session habituation, we measured the distance travelled by mice in the periphery and the central zone of the OF during the first 30 min of both sessions. A three-way ANOVA, with treatment (Veh or URB597 or URB602) as between-subject factor and zone and day as within-subject factors, indicated that the effect of treatment was not significant ($F_{2,33}<1$, $p=$n.s.). However, the effects of zone and day were significant ($F_{1,33}=954.70$, $p<0.001$; $F_{1,33}=59.83$, $p<0.001$). *Post-hoc* tests showed that all of the groups displayed significantly higher levels of locomotion in the periphery zone of the OF when compared to the central zone (Fig. 3*c*). Additionally, during the second OF session, all of the groups showed significantly lower levels of locomotion in comparison to the first session (Fig. 3*d*). Finally, to study whether FAAH and MAGL inhibitors had any effect on stereotypic behaviours, another subset of mice was injected with URB597 (1 mg/kg), URB602 (10 mg/kg) or Veh and evaluated in the OC over 2 h. We quantified stereotypic behaviours at the specific time intervals where QNP significantly increased locomotion and stereotypic behaviours (Figs. 1*a–f*, 2*a–f*). ANOVAs showed that neither group differed in the number of jumping, rearing or grooming behaviours ($F_{2,27}<1$, $p=$n.s.; $F_{2,27}=1.64$, $p=$n.s.; $F_{2,27}<1$, $p=$n.s.) (Fig. 3*e–g*). These results lead us to conclude that treatment with FAAH or MAGL inhibitors did not modify locomotor activity, anxiety, habituation or stereotypic behaviours in C57/Bl6J mice.

Co-administration of FAAH or MAGL inhibitors and quinpirole counteracts quinpirole-induced hyperactivity and stereotypic behaviour

Groups of mice were injected with Veh, QNP (1 mg/kg), QNP and URB597 (1 mg/kg) or QNP and URB602 (10 mg/kg) and evaluated in the OF and OC. In the first 50–70 min post-injection, FAAH and MAGL inhibitors did not reverse nor potentiate the motor depressing effects of QNP (distance travelled in the OF in the first 50 min: $F_{3,44}=140.76$, $p<0.001$; rearing in the OC in the first 60 min: $F_{3,44}=30.51$, $p<0.001$)

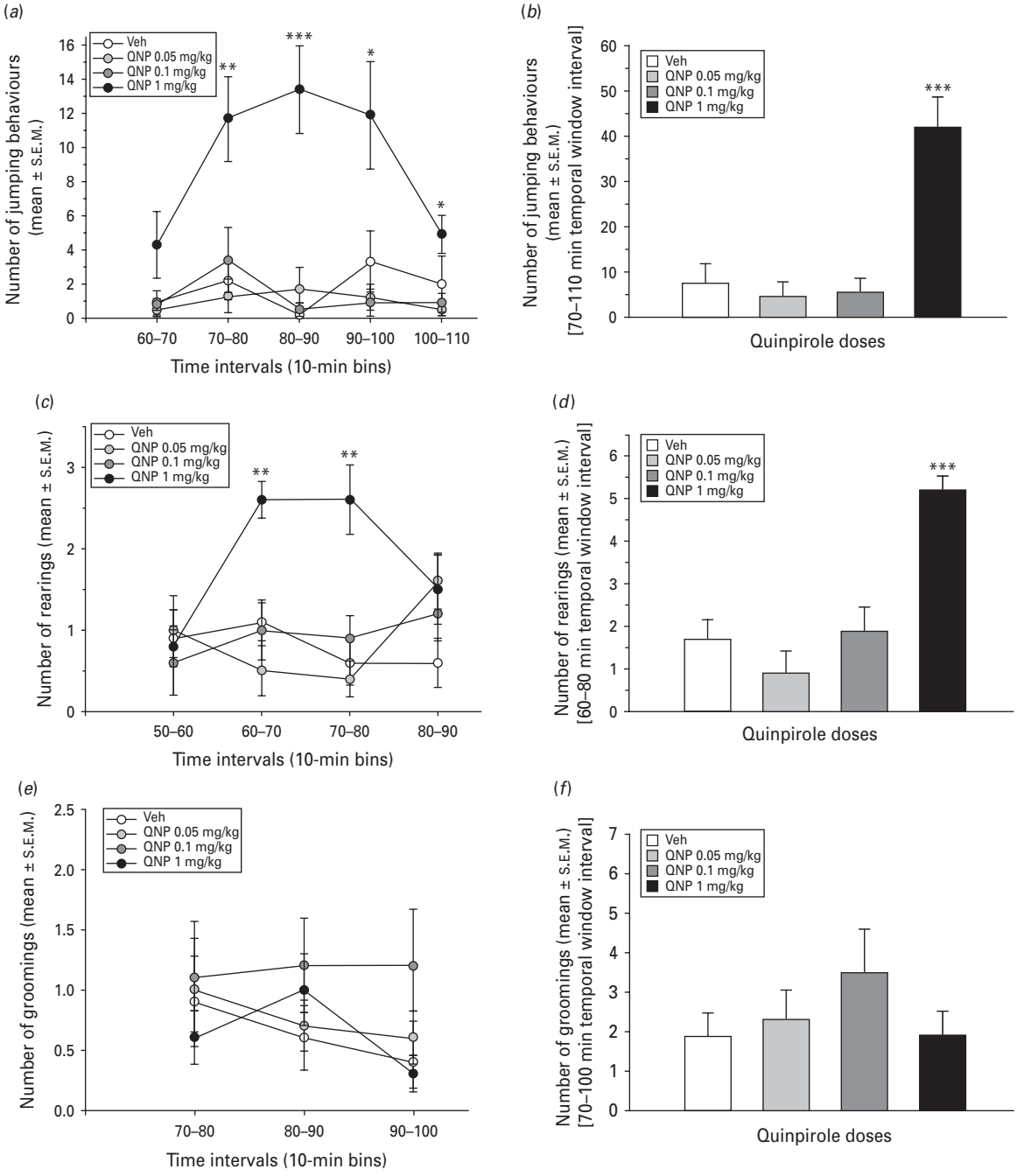

Fig. 2. Effects of acute treatment with quinpirole hydrochloride (QNP) in stereotypic behaviours measured in the observational cylinder. QNP-induced (*a, b*) stereotypic jumping, (*c, d*) rearing and (*e, f*) grooming behaviours (*a*, *c* and *e*, at time-intervals in a specific temporal window; *b*, *d* and *f*, data accumulated). *** $p<0.001$, ** $p<0.01$ and * $p<0.05$ compared to all other groups. Values represent the mean ± S.E.M. ($n=10$ per group).

(Fig. 4*a*, *c*). Number of jumps did not differ at this early phase of QNP action ($F_{3,44}<1$, $p=$ n.s.; Fig. 4*b*). However, inhibition of endocannabinoid degradation did suppress the enhanced locomotion and stereotypic activity that appears in the second phase of QNP action (distance travelled in the OF: $F_{3,44}=15.71$, $p<0.001$; jumps and rearing in the OC: $F_{3,44}=13.98$, $p<0.001$; $F_{3,44}=10.46$, $p<0.001$; Fig. 4*d–f*). *Post-hoc* tests confirmed that mice injected with QNP travelled a longer distance and displayed a higher number of

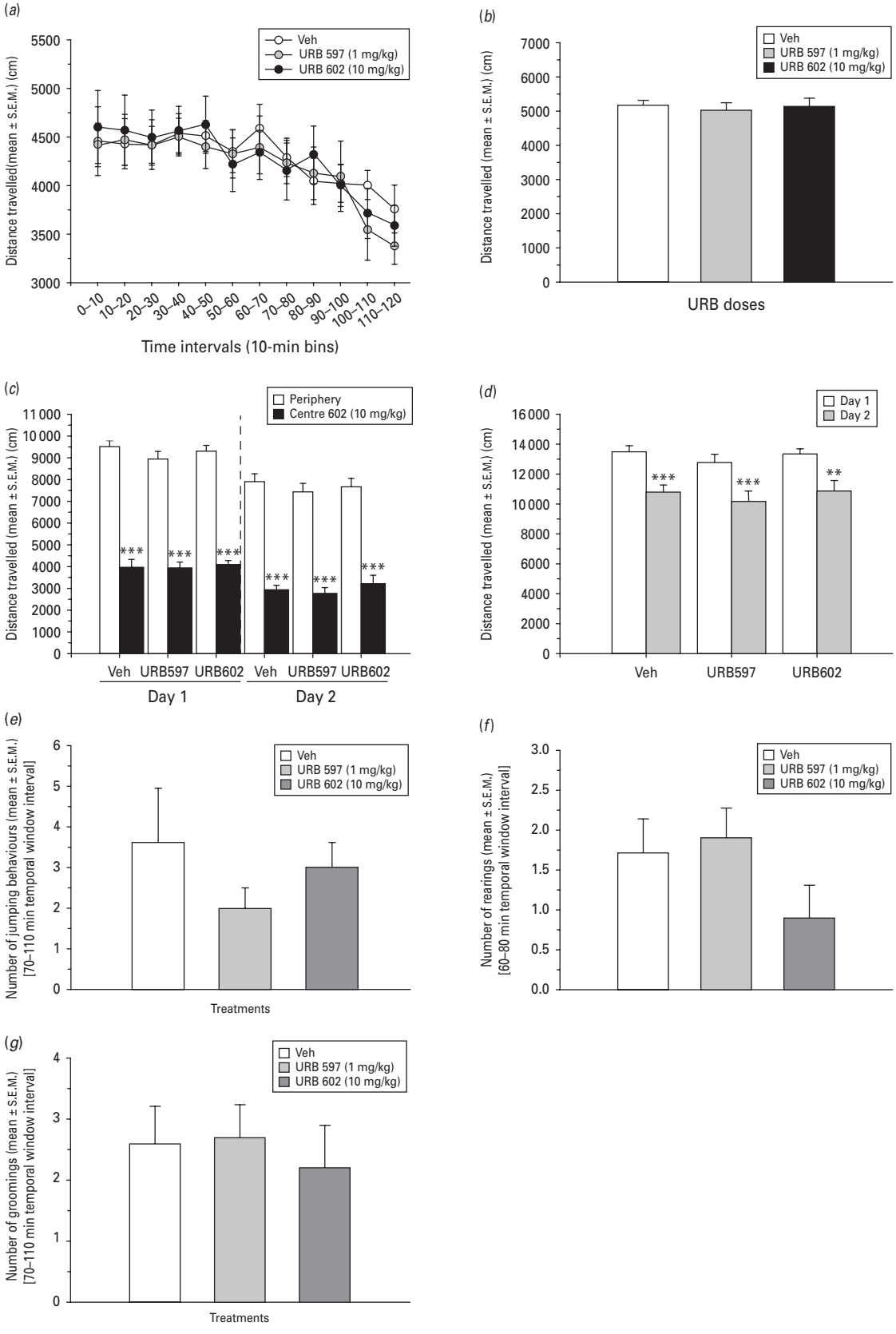

Fig. 3. For legend see opposite page.

jumps and rearing in comparison to the other groups (Fig. 4*d–f*). Furthermore, groups injected with QNP + URB597 or QNP + URB602 did not differ from Veh-injected mice. These results suggest that co-administration of FAAH or MAGL inhibitors with QNP reversed QNP-induced hyperactivity and stereotypic behaviours.

Effects of FAAH and MAGL inhibitors on cocaine-induced locomotion and behavioural sensitization

We conducted a series of experiments to study the influence of FAAH and MAGL inhibitors on: (*a*) the locomotion response elicited by acute cocaine administration; (*b*) the acquisition of cocaine-induced sensitization; (*c*) the expression of conditioned locomotion; (*d*) the expression of cocaine sensitization. In an initial experiment, a group of mice were injected with a single dose of cocaine (20 mg/kg), URB597 (1 mg/kg), URB602 (10 mg/kg), Veh or cocaine with URB597 or URB602. ANOVA indicated that the effect of treatment was significant ($F_{5,42} = 39.10$, $p < 0.001$). *Post-hoc* tests showed that the distance travelled by mice injected with cocaine alone or injected with cocaine and URB597 or URB602 was significantly longer in comparison to the groups injected with Veh, URB597 or URB602 (Fig. 5*a*). This result suggests that FAAH and MAGL inhibitors did not affect the acute cocaine-induced locomotion response.

In a second experiment, another cohort of animals were injected once per day for five consecutive days with cocaine (20 mg/kg), URB597 (1 mg/kg), URB602 (10 mg/kg), Veh or cocaine with URB597 or URB602. ANOVA showed that the effects of treatment, day and the interaction were significant ($F_{5,54} = 63.72$, $p < 0.001$; $F_{3.06,165.30} = 31.36$, $p < 0.001$; $F_{15.30,165.30} = 3.38$, $p < 0.001$). ANOVAs performed on each day were also all significant (day 1: $F_{5,54} = 11.30$, $p < 0.001$; day 2: $F_{5,54} = 56.91$, $p < 0.001$; day 3: $F_{5,54} = 31.88$, $p < 0.001$; day 4: $F_{5,54} = 25.21$, $p < 0.001$; day 5: $F_{5,54} = 41.07$, $p < 0.001$). *Post-hoc* tests revealed that the distance travelled by mice injected with cocaine alone or injected with URB597 or URB602 and cocaine was significantly longer than distance travelled by mice injected with URB597, URB602 or Veh (Fig. 5*b*). This result suggests that the endocannabinoid degradation inhibitors did not produce any effect on cocaine Pavlovian conditioning. Next, mice were left undisturbed for five consecutive days. Twenty-four hours later, groups previously conditioned with cocaine were injected with Veh and exposed to the OF to evaluate the conditioned locomotion response. The next day, mice in the same groups were injected with a prime dose of cocaine (10 mg/kg). Cocaine sensitization was evaluated by comparing the distance travelled by the mice after injection of the prime dose of cocaine to the distance travelled on the previous day (conditioned locomotion). The ANOVA indicated that the effects of pretreatment (cocaine, cocaine + URB597 or cocaine + URB602) and the pretreatment × protocol interaction (conditioned locomotion or cocaine sensitization) were not significant ($F_{2,27} < 1$ in both cases). In contrast, the effect of the protocol was significant ($F_{1,27} = 121{,}02$, $p < 0.001$). *Post-hoc* tests showed that the three groups travelled significantly longer distances during the cocaine sensitization session when compared to the conditioned locomotion session (Fig. 5*c*). These results suggest that administration of FAAH and MAGL inhibitors during cocaine conditioning did not affect the development of conditioned locomotion and cocaine sensitization.

Additionally, we conducted another experiment to study the effects of acute administration of FAAH and MAGL inhibitors on the conditioned locomotion and cocaine sensitization response. Mice were conditioned with cocaine (20 mg/kg) or treated with Veh over five consecutive days (data not shown). Five days after finishing the conditioning protocol, a subset of mice were treated with Veh, URB597 (1 mg/kg) or URB602 (10 mg/kg) and exposed to the OF. ANOVA ($F_{3,36} = 8.07$, $p < 0.001$) followed by *post-hoc* tests indicated that mice conditioned with cocaine travelled a significantly longer distance when compared to mice previously treated with Veh (Fig. 5*d*). Moreover, mice previously conditioned with cocaine and treated with an acute injection of URB597 or URB602 also travelled a significantly longer distance than mice

Fig. 3. Effects of administration of either a fatty acid amide hydrolase (FAAH) inhibitor (URB597) or a monoacylglycerol lipase (MAGL) inhibitor (URB602), on locomotion, anxiety, habituation and stereotypic behaviours in male mice. (*a*, *b*) Locomotion in the open field (OF; A, at time-intervals; B, data accumulated); (*c*) exploration of peripheral *vs.* central zones measured in the OF (*** $p < 0.001$ compared to peripheral zone); (*d*) the environmental novelty (day 1) *vs.* familiarity (day 2) response in the development of habituation to the OF (*** $p < 0.001$, ** $p < 0.01$ compared to day 1); (*e*) stereotypic jumping, (*f*) rearing and (*g*) grooming behaviours measured in the observational cylinder. All of these behaviours were evaluated after acute administration of vehicle (Veh), FAAH or MAGL inhibitors (*a–g*). Values represent the mean ± S.E.M. ($n = 10$–12 per group).

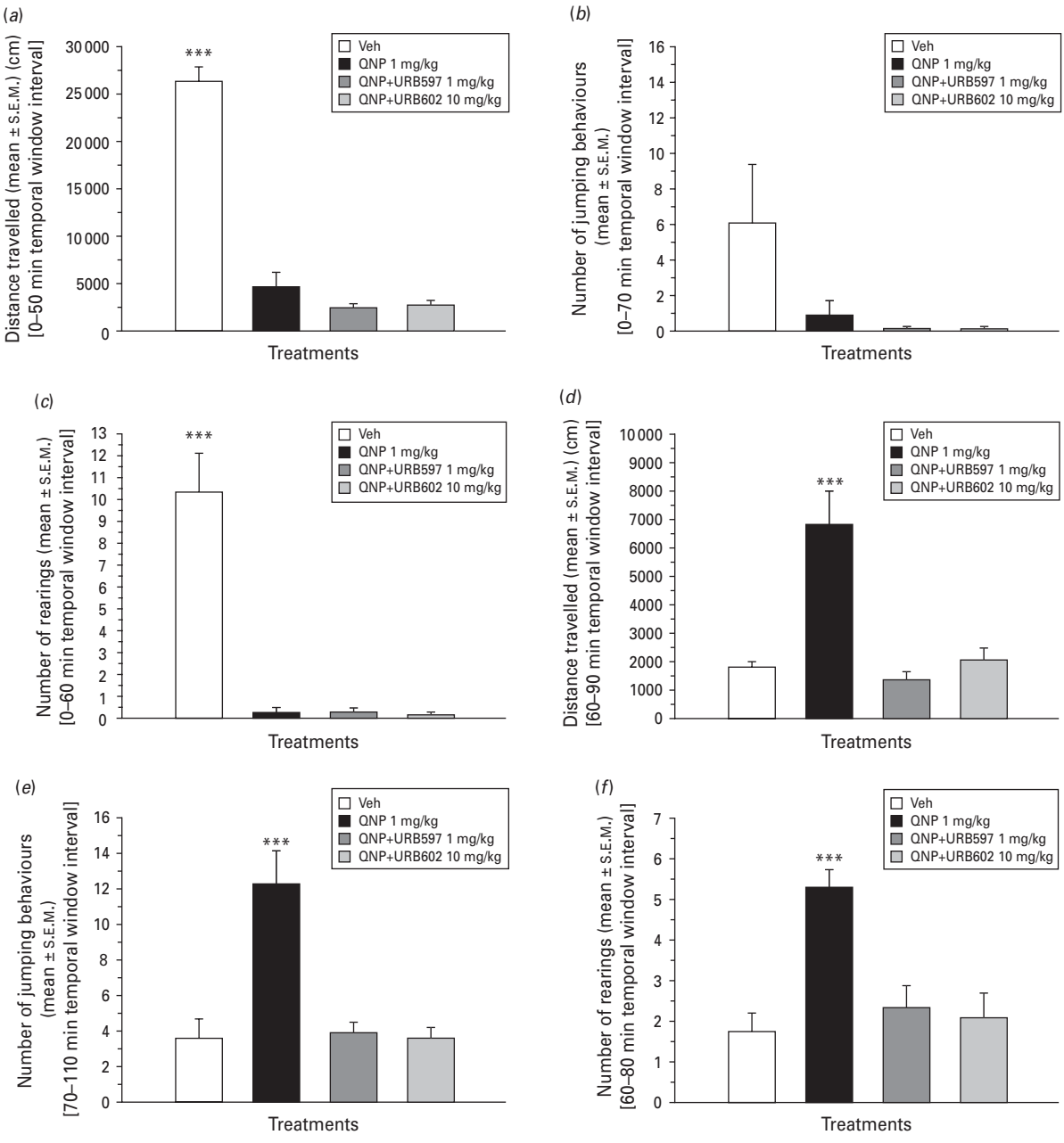

Fig. 4. Effects of pre-treatment with either a fatty acid amide hydrolase inhibitor (URB597) or a monoacylglycerol lipase inhibitor (URB602) on quinpirole hydrochloride (QNP)-induced behaviours in mice. The hypoactivity induced by QNP in the first phase was not potentiated by these inhibitors. This is reflected in the data on (*a*) locomotion (0–50 min), (*b*) jumping (0–70 min) and (*c*) rearing (0–60 min). However, both the hyperactivity and the appearance of stereotyped behaviours induced by QNP in the second phase (60–110 min post-injection) were attenuated by administration of both inhibitors. This is reflected in (*d*) the reduction of locomotion (60–90 min), (*e*) attenuation of jumping (70–110 min) and (*f*) reduction of rearing (60–80 min). *** $p<0.001$ compared to all other groups. Values represent the mean ± S.E.M. ($n=12$ per group).

previously treated with Veh (Fig. 5*d*). This suggests that acute treatment with endocannabinoid degradation inhibitors did not modify the conditioned locomotion response. Finally, another subset of animals previously conditioned with cocaine received a prime injection of cocaine (10 mg/kg) with or without an acute injection of URB597 or URB602. Mice previously treated with Veh received an acute injection of cocaine or Veh. ANOVA ($F_{4,45}=24.93$, $p<0.001$) followed by *post-hoc* tests revealed that mice that

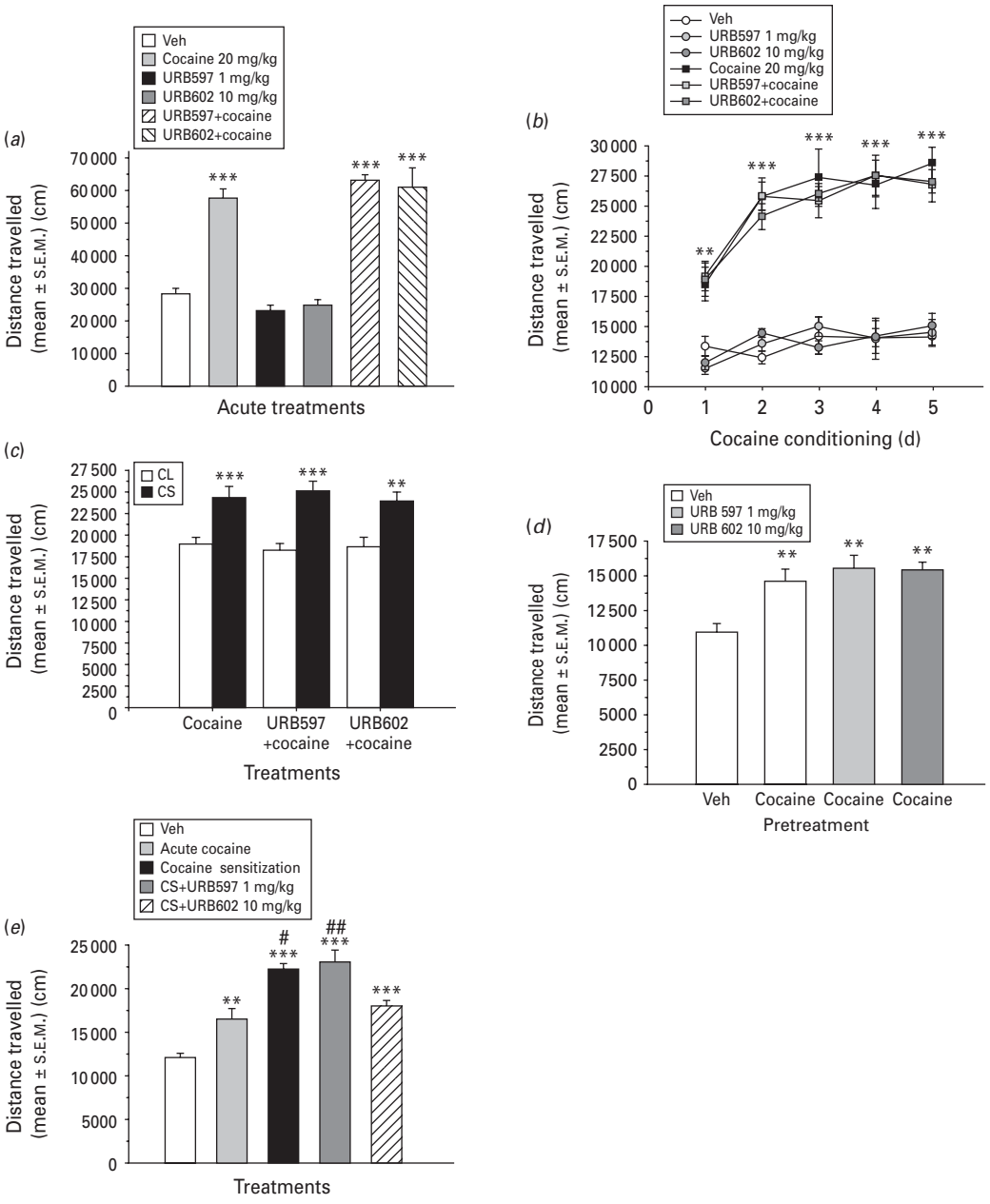

Fig. 5. Effects of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) inhibitors on cocaine-induced activity in the open field. (*a*) The effect of co-administration of FAAH and MAGL inhibitors in acute cocaine-induced locomotion (*** $p<0.001$ compared to groups without acute cocaine injection: vehicle (Veh), URB597 1 mg/kg and URB602 10 mg/kg); (*b*) the effect of co-administration of FAAH and MAGL inhibitors in cocaine-conditioning (*** $p<0.001$ and ** $p<0.01$ compared to control groups without repeated cocaine co-administration); (*c*) the effect of pretreatment with FAAH and MAGL inhibitors during cocaine conditioning phase on cocaine-induced conditioned locomotion (CL) and behavioural sensitization [cocaine sensitization (CS)] (*** $p<0.001$ and ** $p<0.01$ compared to CL groups); (*d, e*) the effect of acute administration of FAAH and MAGL inhibitors in the expression of an already acquired cocaine-induced CL and CS (*** $p<0.001$ and ** $p<0.01$ compared to Veh control group; ## $p<0.01$ and # $p<0.05$ compared to acute cocaine group). Values represent the mean ± S.E.M. ($n=8$–10 per group).

received acute administration of the MAGL inhibitor (URB602) did not develop the sensitization response (Fig. 5*e*).

Discussion

Growing evidence has established that the ECS acts as a modulator of dopamine transmission in the basal ganglia (Giuffrida & Piomelli, 2000; Rodriguez de Fonseca *et al.* 1998). The development of endocannabinoid degradation inhibitors has opened up new alternatives for developing endocannabinoid-based therapeutic strategies in dopamine-related disorders (Fernández-Espejo *et al.* 2009; Piomelli, 2003). To illustrate the effects of these inhibitors on dopamine-mediated behaviours, we characterized the behavioural performance of mice treated with QNP. After QNP treatment, we subsequently studied pharmacological effects of endocannabinoid degradation inhibitors on either QNP- or cocaine-induced behaviours. Results demonstrated the following: (1) similar to rats, QNP produced a biphasic locomotion response in mice (depression of locomotion followed by a marked activation) and a dose-dependent increase in stereotyped behaviours; (2) inhibition of either FAAH or MAGL abolished the increase in locomotion induced by a high dose of QNP and suppressed the induction of stereotyped behaviours; finally, (3) inhibition of both FAAH and MAGL affected neither psychomotor actions of cocaine nor the acquisition of sensitization. However, blockage of 2-AG degradation did reduce the expression of an already acquired cocaine-induced sensitization. These results indicate that inhibition of endocannabinoid degradation exerts a potent suppression of dopamine D_2/D_3 receptor-mediated stimulatory effects on behaviour. However, a very limited suppression of the maximal psychostimulant effects of cocaine was observed.

Activation of the dopamine D_2/D_3 receptor produces a marked decrease in motor activity by inhibiting dopamine release from dopaminergic terminals projecting to the basal ganglia. This effect is mediated through presynaptic dopamine D_2/D_3 receptors (Davis *et al.* 1997). Despite this inhibitory effect, stimulation of post-synaptic dopamine D_2/D_3 receptors produces enhanced locomotion and characteristic stereotyped behaviours (including jumping, climbing and oral movements). Thus, in rats a high dose of QNP produces a typical inhibitory component in behaviour, followed by a temporary activation of locomotion and stereotypies (Eilam *et al.* 1992; Rodriguez de Fonseca *et al.* 1994). We characterized this response in C57Bl/6J mice and found similar pharmacological effects, which have also been recently described (de Haas *et al.* in press; Jung & Shim, 2011). As depicted in Fig. 1, the highest dose of QNP produced stimulation of movement after its initial depressor effect. The activation of behaviour induced by QNP was transient and it was accompanied by characteristic jumping and rearing behaviours, but not grooming (a dopamine D_1-mediated behaviour; Starr & Starr, 1986) as depicted in Fig. 2. On the basis of these results, we selected a dose of 1 mg/kg QNP to analyse the actions of the endocannabinoid degradation inhibitors. These inhibitors were used at doses that did not result in motor depressant effects but are known to fully inhibit enzymatic activity (Fig. 3; Hohmann *et al.* 2005; Kathuria *et al.* 2003; Luchicchi *et al.* 2010). When these inhibitors were injected prior to QNP, its inhibitory effect on behavioural output was not affected. This can be expected because dopamine neuron terminals (which release dopamine) lack cannabinoid CB_1 receptors (Martin *et al.* 2008). However, pharmacological inhibition of either FAAH or MAGL induced an attenuation of behavioural stimulation elicited by QNP. This attenuation indicates that the increase in AEA and 2-AG is sufficient to abolish the stimulatory component derived from dopamine D_2/D_3 receptor activation. Similar findings have been described for AEA transport inhibitor AM404 (Beltramo *et al.* 2000). This finding might have important consequences for therapeutics, particularly in Parkinson's disease and schizophrenia. For example, dyskinesia and stereotyped behaviours associated with repeated stimulation of dopamine D_2 receptors appear after long-term treatment with L-DOPA or dopamine agonists in Parkinson's disease or in the context of psychostimulant abuse (Ferrer *et al.* 2003, 2007; Gorriti *et al.* 1999). In both cases, stimulation of cannabinoid CB_1 receptors reduced their presence in animal models (Ferrer *et al.* 2003, 2007; Gorriti *et al.* 1999). Regarding schizophrenia, positive symptoms (i.e. delusion, hallucination or behavioural disorganization) depend on activation of dopamine D_2 receptors and are inversely correlated with cerebrospinal fluid AEA (Giuffrida *et al.* 2004). Hypothetically, an increase in brain endocannabinoids resulting from pharmacological inhibition of FAAH and/or MAGL might attenuate these symptoms.

Mechanistically, these effects on dopamine D_2/D_3 receptor-mediated responses can be attributed to endocannabinoids released through activation of D_2/D_3 receptors. Either AEA or 2-AG (released after dopamine receptor activation) might act in several places across the basal ganglia circuitry by engaging cannabinoid CB_1 receptors to reduce dopamine-induced

behavioural activation. For instance, endocannabinoids can control pre-synaptic dopamine release (O'Neill *et al.* 2009). This effect most likely occurs through trans-synaptic actions because cannabinoid CB_1 receptors are not present in dopaminergic neurons (Martin *et al.* 2008). Additionally, endocannabinoids can regulate dopamine receptor-mediated transmission. Cannabinoid CB_1 receptors are co-expressed with either dopamine D_1 or D_2/D_3 receptors in medium spiny striatal neurons. Endocannabinoids can regulate signalling at these receptors, most likely through interaction with CB_1 receptor dimers (formed with dopamine D_2/D_3 receptors or adenosine A_{2A} receptors (Navarro *et al.* 2008)) or by regulating dopamine D_2/D_3 receptor availability (Crunelle *et al.* 2011). Finally, endocannabinoids can exert their effects by regulating corticostriatal glutamatergic transmission (similar to dopamine), therefore regulating dopamine-mediated modulation of synaptic plasticity within the basal ganglia (Adermark & Lovinger, 2007; Adermark *et al.* 2009; Gerdeman & Lovinger, 2001).

In the present study, we did not find any effects of endocannabinoid degradation inhibitors on psychostimulant effects of cocaine (Fig. 5). Although the role of endogenous cannabinoids as modulators of dopamine transmission in addiction is clearly established (Colombo *et al.* 2005; Ledent *et al.* 1999; Maldonado & Rodriguez de Fonseca, 2002; Scherma *et al.* 2008; Solinas *et al.* 2007, 2008), there is not a general consensus about the functions of the ECS in cocaine addiction (Arnold, 2005). Some studies indicate that neither pharmacological antagonism nor deletion of the CB_1 receptor alters the acute rewarding effects of cocaine (Adamczyk *et al.* 2012; Lesscher *et al.* 2005; Orio *et al.* 2009). However, there are other studies that show the contrary (Li *et al.* 2009; Soria *et al.* 2005; Xi *et al.* 2008). CB_1 receptors appear to be involved in the association of cocaine reward with environmental cues, reinstatement of cocaine self-administration and acquisition of behavioural sensitization (Adamczyk *et al.* 2012; de Vries *et al.* 2001; Gerdeman *et al.* 2008). Additionally, a new role for cannabinoid CB_2 receptors in cocaine addiction is emerging (Xi *et al.* 2011). In the present study, inhibition of FAAH or MAGL affected neither acute psychomotor actions of cocaine nor the acquisition of behavioural sensitization or the expression of conditioned locomotion. Because cocaine is not a selective blocker of dopamine uptake, we can hypothesize that the psychostimulant profile of cocaine may be independent of endocannabinoid modulation of dopaminergic transmission. In this sense, it is important to note that the effects of cocaine on serotonin transporters are sufficient to sustain the rewarding effects of the psychostimulant in dopamine-deficient mice (Hnasko *et al.* 2007). Thus, it is feasible to hypothesize that an increase in endocannabinoid availability by inhibiting endocannabinoid degradation may not be sufficient to attenuate psychostimulant actions of cocaine.

Finally, we observed a MAGL-induced decrease in the expression of an already acquired cocaine-induced sensitization. Because sensitization involves effects of external associative cues, this pharmacological effect could be associated with those described for other ECS-dependent associative responses in Pavlovian conditioning protocols with cocaine (Adamczyk *et al.* 2012; de Vries *et al*; 2001; Gerdeman *et al.* 2008). However, the specificity of our observation, together with the lack of effects of endocannabinoid degradation inhibitors on conditioned locomotion, makes it very difficult to draw conclusions about the role of this class of drugs in Pavlovian conditioning. Whether this selective attenuation of behavioural sensitization reflects a state-dependent change in dopamine transmission induced by cocaine (Chefer & Shippenberg, 2002), differential adaptations in 2-AG signalling or 2-AG selective actions on striatal plasticity associated with repeated cocaine exposure remains to be determined.

In summary, the present study demonstrates that inhibition of endocannabinoid degradation attenuates dopamine D_2/D_3 receptor-mediated behavioural activation. This finding might be relevant for neuropsychopharmacological therapies for dopamine-related disorders.

Acknowledgments

This work was supported by the following grants: P07-CTS-03324 (CICE, Andalusian Government); RD06/0001/0000 (Red de Trastornos Adictivos RETICS-network, Health Institute Carlos III); 049/2009 (Plan Nacional sobre Drogas 2009/2011), SAS 111224 (Andalusian Health Service) (to F. R. F.) and the Second University International Cooperation for Development Project and Proper Research Program (University of Malaga) (to E. B.). M. J. Luque-Rojas and E. Blanco are recipients of doctoral and post-doctoral (Juan de la Cierva) fellowships, respectively (Ministry of Science and Innovation, Spain). P. Galeano is a fellowship holder from CONICET, Argentina. J. Suárez is recipient of a postdoctoral contract (Sara Borrell, Health Institute Carlos III, Spain). We thank *American Journal of Experts* for English editing and we state that the authors are entirely responsible for the scientific content of the paper.

Statement of Interest

None.

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