Disruptive effects of the prototypical cannabinoid Δ^9-tetrahydrocannabinol and the fatty acid amide inhibitor URB-597 on go/no-go auditory discrimination performance and olfactory reversal learning in rats

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The effects of Δ^9-tetrahydrocannabinol (Δ^9-THC; 0.3, 1, 3 and 10 mg/kg), and the fatty acid amide hydrolysis inhibitor URB-597 (0.1 and 0.3 mg/kg), on auditory and olfactory go/no-go discrimination tasks were examined in rats. The aims were to assess (i) whether simple olfactory and auditory discrimination tasks are sensitive to cannabinoid interference and (ii) whether manipulation of endogenous cannabinoid levels with URB-597 might have adverse effects on perceptual and cognitive functions. Thirsty rats were trained to nose poke at a 'sniff port', where odours were briefly presented. After one odour (S+, lemon), licks made at an adjacent tube were rewarded with water, whereas licks after a second odour (S−, strawberry) were unrewarded. In an analogous auditory task, nose pokes produced an auditory S+ (beep) or S− (white noise). Δ^9-THC and URB-597 impaired performance on the auditory but not the olfactory discrimination task. Auditory performance was still affected on the day after Δ^9-THC (3 and 10 mg/kg) and URB-597 (0.3 mg/kg) exposure. Δ^9-THC and URB-597 markedly impaired olfactory discrimination reversals without disrupting acquisition of the original discrimination. Rimonabant (CB_1 antagonist; 3 mg/kg) reversed all Δ^9-THC and URB-597 effects on auditory discriminations and olfactory discrimination reversals. These results confirm impairment of cognitive flexibility (reversal learning) by cannabinoids and show remarkable sensitivity of auditory discrimination performance to Δ^9-THC and the augmented endocannabinoid signalling produced by URB-597. *Behavioural Pharmacology* 22:191–202 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Cannabis is the most widely used illicit drug in the world and has well-documented sedative, analgesic, appetite-stimulatory and psychedelic effects, thought to be due to its major psychoactive component, Δ^9-tetrahydrocannabinol (Δ^9-THC) (Ashton, 2001; Iversen, 2003, 2005; Howlett *et al.*, 2004; El-Alfy *et al.*, 2010; Robinson *et al.*, 2010). Acute and chronic exposure to Δ^9-THC has been shown to impair cognitive function, with broadly similar effects reported in humans and laboratory subjects (Egerton *et al.*, 2006; Ranganathan and D'Souza, 2006; Solowij and Battisti, 2008; D'Souza *et al.*, 2009; Sofuoglu *et al.*, 2010). Characteristic effects in rodents include impairment of spatial learning, working memory and attentional processes (Mallet and Beninger, 1998; Presburger and Robinson, 1999; Hampson and Deadwyler, 2000; Verrico *et al.*, 2004; Silva de Melo *et al.*, 2005; Robinson *et al.*, 2007; Varvel *et al.*, 2007; Boucher *et al.*, 2009, 2011). Such effects may reflect cannabinoid-mediated disruption of cortical and hippocampal electrical activity, which underlie the encoding of stimulus features and their associative significance (Robbe *et al.*, 2006; Deadwyler *et al.*, 2007; Hajos *et al.*, 2008; Robbe and Buzsaki, 2009).

Many anecdotal reports suggest that cannabis affects auditory perception in humans, with an increased appreciation of auditory, particularly musical, stimuli during acute intoxication (Fachner, 2006). A small number of laboratory studies have explored cannabinoid effects on tasks involving the auditory modality. Cannabinoids have been shown to cause hyper-reactivity in mice to unexpected, loud auditory stimuli (Holtzman *et al.*, 1969; Boggan *et al.*, 1973). In humans, an impairment of auditory signal detection was observed in subjects intoxicated with cannabis (Moskowitz, 1974). Chronic cannabis use is associated with an impaired ability to filter out irrelevant auditory stimuli in an oddball task and the use of different strategies of attention allocation in auditory information processing tasks (Solowij *et al.*, 1995; Kempel *et al.*, 2003). Smoking marijuana also produced changes in brain metabolism or regional cerebral blood flow in auditory cortices during a

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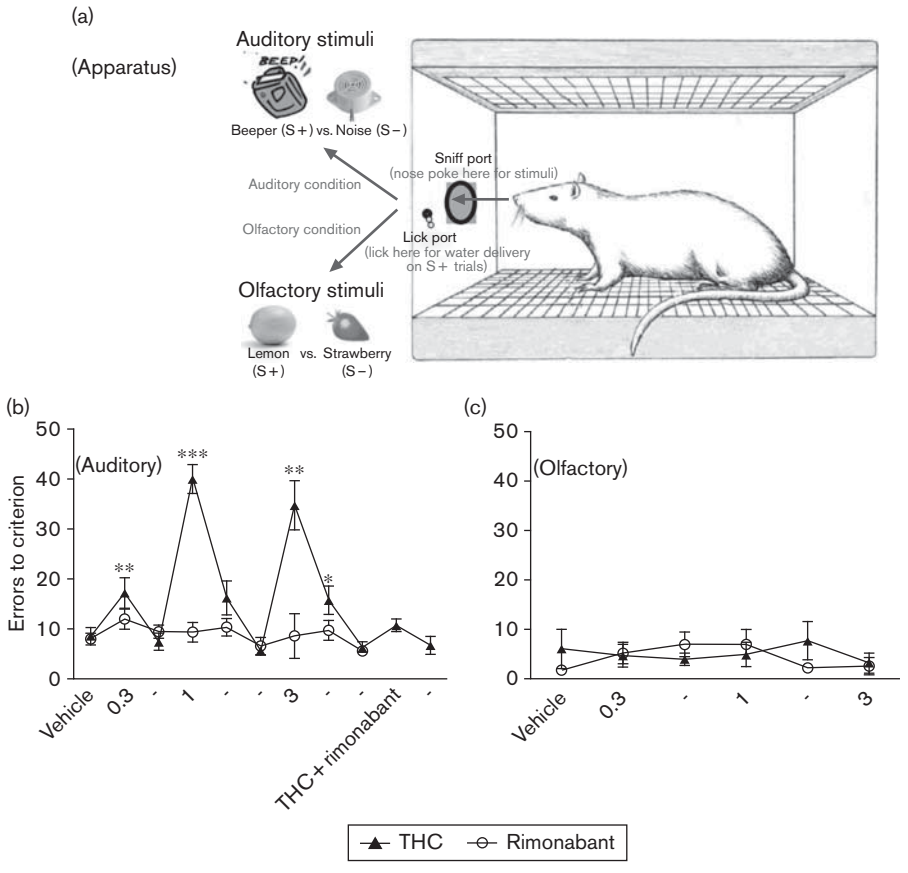
focused attention task (O'Leary *et al.*, 2000, 2002). Cannabinoid disruption of sensorimotor gating to auditory stimuli has also been recently reported in the prepulse inhibition of startle paradigm in rats and mice (Schneider and Koch, 2002; Malone and Taylor, 2006; Boucher *et al.*, 2007, 2011) and human cannabis users (Kedzior and Martin-Iverson, 2006), and this has been linked to cannabinoid-induced changes in auditory stimulus-evoked electrophysiological activity (Hajos *et al.*, 2008).

In contrast, cannabinoid effects on olfactory perception are relatively uncharacterized. The appetite-stimulatory effects of cannabinoid might reflect an increased pleasurable sensory experience of foodstuffs (Yoshida *et al.*, 2010), presumably through an interaction with hedonic processes related to gustation and olfaction (Mahler *et al.*, 2007). With respect to olfactory learning, rats engaged in a foraging task in which odours cued the location of food rewards showed normal performance when tested with Δ^9-THC (THC Pharm GmbH, Frankfurt Main, Germany) but showed an impaired ability when contingencies were reversed (Egerton *et al.*, 2005). This was interpreted as general impairment in cognitive flexibility linked to disruptive effects of Δ^9-THC on prefrontal and orbitofrontal functions (Egerton *et al.*, 2005; Roser *et al.*, 2010).

We have developed a task that allows drug effects on both auditory and olfactory discriminations to be assessed (Sokolic and McGregor, 2007). In this go/no-go discrimination paradigm, rats nose poke to receive either auditory or olfactory stimuli of brief duration that signal either the availability (S+) or nonavailability (S–) of a water reward at an adjacent lick tube (Fig. 1a). Using this task, we found that benzodiazepines selectively impair the acquisition and reversal of olfactory, but not auditory discriminations (Sokolic and McGregor, 2007). As the auditory and olfactory discrimination tasks have identical motor, motivational, and response-inhibition requirements, modality-specific impairment caused by drugs can be readily uncovered using this paradigm.

Fig. 1



(a) The olfactometer apparatus in which olfactory and auditory go/no-go discriminations and discrimination reversals were tested. (b) Δ^9-tetrahydrocannabinol (Δ^9-THC) impairs the performance of a well-learned auditory discrimination task. Impairment was also seen 24 h after administration of Δ^9-THC (3 mg/kg). Asterisks represent a significant within-subjects drug effect relative to previous baseline performance (*$P<0.05$, **$P<0.01$, ***$P<0.001$). Rimonabant had no significant effect on the performance of the auditory group. (c) Effects of Δ^9-THC and rimonabant on the performance of a well-learned olfactory task. There were no significant drug effects on olfactory discrimination performance.

In this study, our primary aim was to assess the effects of the prototypical cannabinoid Δ^9-THC on auditory and olfactory go/no-go discriminations, and to evaluate whether any general performance impairments, or any modality-specific effects, were evident. On the basis of the existing literature, we predicted that cannabinoid might interfere with auditory discrimination ability and with the ability to reverse previously learned discriminations. This study also focused on the fatty acid amide hydrolase (FAAH) inhibitor URB-597. This drug increases brain anandamide levels by preventing the intracellular enzymatic activity of FAAH (Kathuria *et al.*, 2003; Piomelli *et al.*, 2006; Gaetani *et al.*, 2009). The pharmacological properties of URB-597, the most potent member of its class, include anxiolytic, antidepressant-like and antinausea effects in rodents and potentiation of the effects of exogenously administered anandamide (Kathuria *et al.*, 2003; Piomelli *et al.*, 2006; Bortolato *et al.*, 2007; Cross-Mellor *et al.*, 2007; Scherma *et al.*, 2008; Clapper *et al.*, 2009; Gaetani *et al.*, 2009). URB-597 also increases the motivational and rewarding properties of social play, extending the potential therapeutic utility of indirect cannabinoid (CB_1) agonists (Trezza and Vanderschuren, 2008).

URB-597 does not seem to produce classical cannabinoid-like effects such as catalepsy, hyperphagia and hypothermia, and seems to have only subtle effects on cognitive function (Varvel *et al.*, 2006, 2007). A recent study showed that URB-597 enhanced memory acquisition in a passive-avoidance task (Mazzola *et al.*, 2009). However, another study showed that URB-597 caused impairments in working memory in the *T*-maze (Seillier *et al.*, 2010). Here, we aimed to examine the effects of URB-597 and to compare them with Δ^9-THC in auditory and olfactory discrimination paradigms. To ascertain the involvement of CB_1 receptors in any observed effects, this study also evaluated the ability of the CB_1 receptor antagonist rimonabant (Rinaldi-Carmona *et al.*, 1994) to prevent any effects observed with URB-597 or Δ^9-THC.

Methods

Subjects

A total of 32 male Australian Hooded Wistar rats (in-house breeding facility, University of Sydney, Australia) were used. The rats weighed between 116 and 229 g and were approximately 40–50 days old at the start of testing. They were housed in groups of eight in a temperature-controlled colony room (21 2°C) on a reverse light–dark cycle (lights on from 20:00 to 08:00 h). They were maintained on a 10 ml per day water deprivation schedule with free access to water most weekends. All training and test sessions were conducted between 10:00 and 16:00 h. The University of Sydney Animal Ethics Committee approved all experimental procedures used in this study in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Apparatus

All testing occurred in 12-channel air-dilution olfactometers (Fig. 1a) using methods similar to those described by Slotnick (2001) and Slotnick and Schellinck (2001). Two of the four olfactometers were modified so that they could also be used for auditory discrimination tasks (Sokolic and McGregor, 2007). The test chamber for each of the four units was a 17 cm (W) × 24 cm (L) × 25 cm (H) Plexiglas box fitted with a stainless steel grid floor. A brushless ventilation fan was mounted on one side of the box. A 'sniff port' with a diameter of 3 cm was located on the right wall of the chamber 14 cm above the floor. Nose pokes into this port were detected by a photo beam located across the entrance to the port.

To generate olfactory stimuli, an air stream of 200 ml per minute was briefly diverted into one of 12 glass saturator bottles by the operation of two normally closed solenoid valves located on the input and output of the bottles. The saturator bottles contained 5 ml of a specific liquid odorant. The air stream passing through the saturator bottle was mixed with the main system airflow of 1950 ml per minute, which flowed through the sniff port before being pumped to an exhaust tube, which took the air out of the laboratory.

A lick tube, through which water rewards could be delivered, was located 3 cm to the left of the sniff port and at the same height as the port. The outer part of the lick tube was made of glass, whereas the inner part consisted of thin stainless steel tubing. Each time the tongue of the rat made contact with the steel inner of the lick tube, an electrical circuit was completed between the tube and the grid floor of the chambers, which was detected and recorded by the computer. The lick tubes were connected by C flex tubing to a 20-ml syringe filled with water. Operation of a normally closed solenoid pinch valve allowed water to flow under gravitational force from the syringe reservoir down through the tubing and out of the lick tube. Opening time of the water delivery pinch valves was calibrated so that 0.05 ml of water was delivered to the rat on rewarded trials. Cue lamps (Med Associates, St Albans, Vermont, USA, part ENV-221M) mounted 2 cm above the lick tubes were used to signal the intertrial interval (ITI).

Rats initiated each trial by making a nose poke at the sniff port. This nose poke immediately triggered the operation of the 'saturator valves' for a 2-s period so that air flowed over the liquid odourant and was added to the main system airflow to generate the olfactory stimulus. For the first 1 s of the 2-s period, a 'final valve' operated that temporarily diverted all airflow away from the sniff port to an exhaust port. Thus, odour-saturated air only passed through the sniff port in the final second of this 2-s period. The process of temporarily diverting the air stream through a 'final valve' ensures adequate mixing of

odorants with the air stream before presentation of the odour to the rat (Slotnick and Schoonover, 1984). During this 1-s period, as odour flowed through the sniff port, rats were required to nose poke for a cumulative total of at least 300 ms (to ensure adequate stimulus sampling), otherwise the trial was repeated.

The 16-odour stimuli used in the experiments described below were lemon, strawberry, chocolate, aniseed, vanilla, rosewater, cherry brandy, coconut, banana and coffee essence (Queen Fine Foods, Alderley, Queensland, Australia), aromatic oils jasmine, frangipani and honeysuckle (Eco Aroma, Natalie Group, Albion, Queensland, Australia) and butanal (*n*-butyl aldehyde) and heptanal (*n*-heptyl aldehyde; 99% purity; Fluka, Castle Hill, New South Wales, Australia; Table 1). The aldehydes were diluted to 1 : 10 000 with near-odourless 1,2-propandiol (99.5% purity; Sigma-Aldrich, Castle Hill, New South Wales, Australia), whereas all other odourants were used undiluted.

The auditory discrimination tasks were run identically to the olfactory discrimination tasks except that no odours were present in the saturator bottles. Solenoid valves operated in exactly the same way and response requirements were exactly the same as for the olfactory tasks. Instead of the olfactory stimuli, however, each trial involved presentation of a specific auditory stimulus. One stimulus was a beeper (Dick Smith Electronics, Sydney, Australia) and the other a transistor radio tuned to produce a consistent white noise stimulus (Audiosonic, Audiosonic Ltd., Hong Kong, KM360 model). Control of the olfactometers and data acquisition were accomplished by custom programs written in the Strawberry Tree Workbench Mac programming environment (McGregor, 1996).

Basic training

On the first day of training, thirsty rats were rewarded with a 0.05-ml drop of water for licking at the lick tube. After each reward, the cue lights came on to signal a 6-s ITI during which lick responses were not reinforced. On the next 4 days, rats were trained to keep their nose in the sniff port for increasing lengths of time to obtain a water reward. If the nose was kept in the port for the required amount of time, any subsequent lick on the lick tube was reinforced with 0.05 ml of water. Again, an ITI of 6 s was imposed during which the cue lights came on in the test chamber. By the end of this phase, rats had been successfully trained to keep their nose in the sniff port for at least 300 ms of the 1000 ms after the operation of the final valve. After this initial training, a go/no-go successive discrimination task was then introduced. Rats were divided into two groups: olfactory ($n = 16$) and auditory ($n = 16$). The olfactory group was trained on a lemon/S + versus strawberry/S − discrimination, whereas the stimuli used for the auditory group were beeper/S + versus white noise/S −. These rats were used across a total of five experiments that are summarized in Table 1.

In all discrimination sessions, rats initiated each trial with a nose poke. If the poke requirement was met (at least 300-ms poking during the first 1000 ms of odour or auditory stimulus delivery) and an S + stimulus had been presented, then licking at the lick tube was reinforced with a water reward and the trial was scored as correct (a hit). An ITI of 5 s then occurred before the next trial could be initiated. If the rat did not lick the tube during an 8-s period after termination of the S + stimulus, then the trial was scored as an error (a miss). If a rat made a lick response on an S − (strawberry or noise) trial, no water was delivered and the trial was scored as an error (false alarm). A longer ITI of 8 s was given in such incorrect trials. If the rat did not lick the lick tube on an S − trial, the trial was scored as correct (a correct rejection) and an ITI of 5 s ensued. There were an equal number of S + and S − trials in each block of 20 trials with no more than any three consecutive trials being of the same type (S + or S −).

Criterion performance on all auditory and olfactory discrimination tasks was set at 17 correct responses in any given sequence of 20 consecutive trials. When the 17 out of 20 criterion was met, the cue lights in the chamber were illuminated permanently and no further trials could be initiated.

Table 1 Overview of experiments

Experiment	Drug dose (mg/kg)	Olfactory task	Auditory task
1. Discrimination performance	Δ^9-THC (0.3–3)	Lemon vs. strawberry	Beeper vs. noise
	Rimonabant (0.3–3)	Lemon vs. strawberry	Beeper vs. noise
2. Residual effect of Δ^9-THC	Δ^9-THC (10)		Beeper vs. noise
3. Discrimination performance	URB-597 (0.1 and 0.3)		Beeper vs. noise
	URB-597 (0.3) + rimonabant (3)		Beeper vs. noise
4. Acquisition and reversal	Δ^9-THC (3)	Butanal vs. heptanal	
	Δ^9-THC (1)	Chocolate vs. aniseed	
	Δ^9-THC (0.3)	Vanilla vs. rosewater	
	Δ^9-THC (3) + rimonabant (3)	Cherry brandy vs. coconut	
	Rimonabant (3)	Banana vs. ginger	
	URB-597 (0.3)	Coffee vs. jasmine	
5. Acquisition and reversal	URB-597 (0.3) + rimonabant (3)	Frangipani vs. honeysuckle	

Δ^9-THC, Δ^9-tetrahydrocannabinol.

Experiment 1: Δ^9-tetrahydrocannabinol and rimonabant effects on auditory and olfactory discrimination performance

Once rats had achieved this criterion performance on each of seven consecutive daily sessions, drug testing commenced. For initial drug testing, the olfactory and auditory groups were further divided into two treatment subgroups: Δ^9-THC and rimonabant ($n = 8$ per group). Rats in each group were given an ascending sequence of either Δ^9-THC or rimonabant doses across days (0.3, 1 and then 3 mg/kg), 15 min before testing. Washout days were included between the testing days to ensure that rats had regained predrug levels of performance before the next dose was tested. On these washout days, the rats were tested on the discrimination task without any drug treatment. As 1 and 3 mg/kg of Δ^9-THC heavily impaired the performance of the auditory group, two washout days were used between consecutive drug test days in this group. After this testing sequence, a final auditory discrimination task was given to the Δ^9-THC subgroup (after two washout days) in which Δ^9-THC (3 mg/kg) was combined with rimonabant (3 mg/kg) to determine whether the antagonist would block the disruptive effects of Δ^9-THC.

Experiment 2: residual effects of high-dose Δ^9-tetrahydrocannabinol on auditory discrimination performance

This experiment extended the findings from experiment 1 that auditory discrimination performance was impaired 24 h after administration of Δ^9-THC. Here, a high dose of Δ^9-THC (10 mg/kg) was given and performance of the auditory discrimination was tested 24 h later. The auditory group of rats ($n = 16$) used in experiment 1 served as subjects. The rats were divided into two treatment groups counterbalanced for an earlier exposure to Δ^9-THC or rimonabant. The Δ^9-THC group ($n = 8$) was injected once with 10 mg/kg of Δ^9-THC and the controls ($n = 8$) were injected with vehicle. No auditory discrimination testing took place on the drug administration day with rats returned to their home cages immediately after drug or vehicle injection. The performance of rats on the beeper/S+ versus noise/S− task was tested 24 h later (Table 1).

Experiment 3: the effect of URB-597 on auditory discrimination performance

The auditory group used in experiments 1 and 2 were again used as subjects in experiment 3, which was conducted 3 weeks after experiment 2 to minimize the possibility of any long-term residual effects of an earlier Δ^9-THC exposure. The rats were injected with URB-597 15 min before testing the same beeper/S+ versus noise/S− task used in experiments 1 and 2 (Table 1). Testing occurred on two consecutive test days using a within-subjects design. On the first (baseline) day, all rats received vehicle injection 15 min before testing. On the second day, half the rats ($n = 8$) were injected with URB-597 (0.1 mg/kg), whereas the other half ($n = 8$) were given URB-597 (0.3 mg/kg). Two washout days followed in which rats were tested without drug to ensure that performance had returned to the baseline. The process was then repeated in an identical manner except that on the drug treatment day, the groups were reversed so that the original 0.1-mg/kg group was now tested with 0.3 mg/kg of URB-597 and *vice versa*. Two washout days were again given after this test.

One week later, the effect of coadministering rimonabant with URB-597 was evaluated in these same rats. The rats were randomly reallocated to one of two groups that were injected with vehicle ($n = 8$) or rimonabant (3 mg/kg, $n = 8$), 15 min before all received URB-597 (0.3 mg/kg). Rats were then tested on the standard auditory discrimination test.

Experiment 4: effects of Δ^9-tetrahydrocannabinol on acquisition of a novel olfactory discrimination and reversal of a well-learned olfactory discrimination

Experiment 4 determined whether various doses of Δ^9-THC interfere with the acquisition and reversal of a novel two-odour olfactory discrimination task. A go/no-go paradigm identical to that described in experiment 1 was again used, albeit with novel olfactory stimuli. The olfactory group of rats from experiment 1 ($n = 16$) served as subjects. As the auditory group showed major impairment on basic discrimination performance after Δ^9-THC administration (experiments 1 and 2), it was not feasible to test them for specific deficits in the acquisition and reversal of auditory discriminations.

The effects or various doses of Δ^9-THC, Δ^9-THC and rimonabant combined and rimonabant alone were assessed across the acquisition and reversal of five different novel two-odour discriminations (Table 1). The experimental design was between-subjects, whereby for each acquisition and reversal task, rats were allocated to either a drug ($n = 8$) or control ($n = 8$) group. This allowed experimental control for possible differences in the difficulty of each unique odour discrimination task as well as control for possible practice effects over performance of sets of discriminations. The order of tests were Δ^9-THC (3, 1 and 0.3 mg/kg) followed by Δ^9-THC (3 mg/kg) and rimonabant (3 mg/kg) combined, followed by rimonabant (3 mg/kg) alone.

Acquisition of the novel tasks was measured as the number of errors made in reaching a criterion of 17 of 20 consecutively correct responses. Rats usually achieved criterion performance in a single session, but those not reaching criterion within 400 trials in the first session were rerun on the same task the following day until criterion was reached. Once subjects had successfully acquired the discrimination, they were repeatedly tested on this task for 4 further days to allow asymptotic performance to be reached.

The rats were then reallocated to two new groups to test the effects of the same drug treatment used in acquisition on the reversal of the just-acquired discrimination. These subgroups were counterbalanced so that half of the rats had been in the control group for the acquisition of the discrimination and half had been in the drug group. Successful reversal performance was measured using the same criteria as for acquisition.

Experiment 5: the effect of URB-597 on olfactory discrimination acquisition and reversal

The same rats that had been previously used in experiments 1 and 4 were divided into two treatment groups (control or URB-597, $n = 8$ per group). These rats were then tested for their acquisition of a novel coffee/S+ versus jasmine/S− olfactory discrimination (Table 1). The URB-597 group received URB-597 (0.3 mg/kg, intraperitoneally), 15 min before the start of the test session, whereas the control group received vehicle. As in experiment 4, once subjects had reached criterion they were repeatedly tested on this task for a further 4 days until they achieved asymptotic performance.

Rats were then reallocated to two subgroups and were administered with either URB-597 (0.3 mg/kg) or vehicle, 15 min before the test session in which the contingencies of the previous discrimination were reversed. Thus, coffee became the S− stimulus and jasmine became the S+ stimulus (Table 1).

For the second test, the rats were divided into two new subgroups, with groups injected with vehicle ($n = 8$) or rimonabant (3 mg/kg), 15 min before URB-597 (0.3 mg/kg, all rats). They were then tested on the acquisition of a further novel (frangipani/S+ vs. honeysuckle/S−) olfactory discrimination. The same task was then further tested for another 4 days. In the reversal task, rats were again reallocated to drug conditions, and honeysuckle became the rewarded stimulus/S+, whereas frangipani was the unrewarded/S−.

Drugs

Rimonabant (Sequoia Research Products, UK) [*N*-(piperidine-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride] and Δ^9-THC (THCPharm, Germany) were dissolved in Tween 80 and ethanol, and were suspended in saline to make a final mixture of 1 : 1 : 18 Tween 80 : ethanol : saline. URB-597 (Cayman Chemical Company, Ann Arbor, Michigan, USA) was dissolved in 100% dimethyl sulfoxide and suspended in saline to a final concentration of 5% dimethyl sulfoxide. The 0.3 and 1-mg/kg doses are relatively low doses of Δ^9-THC, whereas the 3-mg/kg dose was anticipated to cause some motoric impairment (McGregor *et al.*, 2005).

The doses of URB-597 chosen were based on observations that 0.3 mg/kg maximally blocked FAAH activity in rats but produced no catalepsy, hypothermia, or hyperphagia (Kathuria *et al.*, 2003). All drugs were administered by intraperitoneal injection in a volume of 1 ml/kg. An interval of 15 min was interposed between drug injection and the time of testing, as previous reports indicate strong activity of both URB-597 and Δ^9-THC at this time interval (Kathuria *et al.*, 2003; McGregor *et al.*, 2005; Piomelli *et al.*, 2006).

Statistical analysis

For all experiments, the dependent variable was the number of errors made in reaching the criterion performance of 17 of 20 consecutive correct trials. In experiment 1, errors to criterion with each dose of Δ^9-THC or rimonabant, and those made on the washout days immediately after drug treatment, were compared with those made on the nearest preceding baseline day using within-subjects contrasts. Bonferroni critical values were used to control the family-wise error rate at 0.05. For experiment 2, errors to criterion were compared between the Δ^9-THC and vehicle groups using an independent samples *t*-test. Experiment 3 was divided into three phases. Data from the first and second phases (URB-597 0.1 and 0.3-mg/kg effects) were analysed using within-subjects contrasts to compare performance under URB-597 0.1 and 0.3 mg/kg, and on the washout day after URB-597 administration, with performance on the nearest preceding baseline day. For the third phase of experiment 3, an independent samples *t*-test was used to compare the number of errors to criterion in the two treatment groups (URB-597 0.3 mg/kg vs. URB-597 0.3 mg/kg + rimonabant). For experiment 4, errors to criterion on each of the five separate olfactory discrimination acquisition and discrimination reversal tasks were compared across the vehicle and drug groups using the independent samples *t*-tests. Repeated measures of analyses of variance could not be used because half of the rats that were in the control condition for the acquisition of each task were reallocated to the drug condition for the reversal of the same task. Similarly, in the first part of experiment 5, errors to criterion on the discrimination acquisition and discrimination reversal tasks were compared for the URB-597 and vehicle groups using the independent samples *t*-tests. The URB-597 and URB-597 plus rimonabant groups were similarly compared in the second part of that experiment.

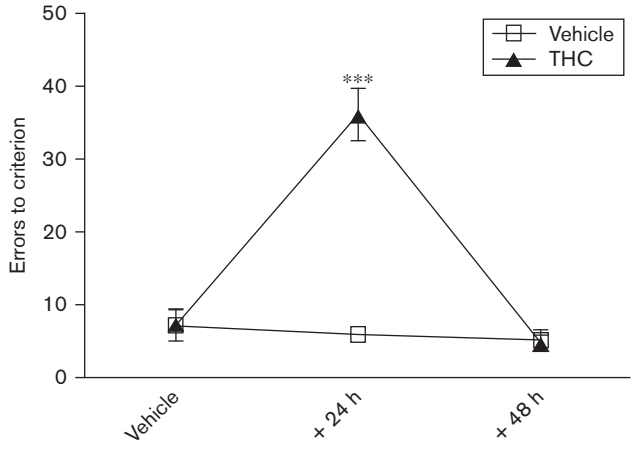
Results

Experiment 1

As shown in Fig. 1b, Δ^9-THC clearly impaired the performance of a well-learned auditory discrimination task. Within-subjects contrasts (Bonferroni $F_{crt} = 8.07$) indicated that the 0.3, 1 and 3-mg/kg doses of Δ^9-THC produced a significant impairment relative to baseline performance: Δ^9-THC [0.3 mg/kg; $F(1,7) = 9.74$; $P < 0.05$], Δ^9-THC [1 mg/kg; $F(1,7) = 147.87$; $P < 0.001$] and Δ^9-THC [3 mg/kg; $F(1,7) = 39.75$; $P < 0.001$]. Performance was also impaired relative to baseline on the day after the

3-mg/kg dose [$F(1,7) = 15.99$; $P < 0.01$]. The disruptive effect of Δ^9-THC (3 mg/kg) on auditory discrimination performance was reduced by coadministration of rimonabant (3 mg/kg), such that performance under the drug combination was far better than under Δ^9-THC (3 mg/kg) alone [$F(1,7) = 27.66$; $P < 0.01$]. Rimonabant given alone had little effect on the performance of the auditory discrimination task (Fig. 1b) with none of the within-subjects contrasts reaching significance: 0.3 [$F(1,7) = 4.27$; $P = 0.077$], 1 and 3 mg/kg (Fs < 1). Performance on the olfactory discrimination task was not affected by rimonabant or Δ^9-THC at any of the doses tested (Fig. 1c). None of the within-subjects contrasts reached statistical significance (Fs < 1).

Fig. 2



Significant impairment of performance on a well-learned auditory discrimination 24 h after administration of Δ^9-tetrahydrocannabinol (Δ^9-THC; 10 mg/kg). ***P<0.001 for Δ^9-THC relative to vehicle treatment.

Experiment 2

Performance on a well-learned go/no-go auditory discrimination task was severely impaired 24 h after the administration of 10 mg/kg of Δ^9-THC [(Fig. 2); $t(14) = 8.03$; $P < 0.001$] but not 48 h later.

Experiment 3

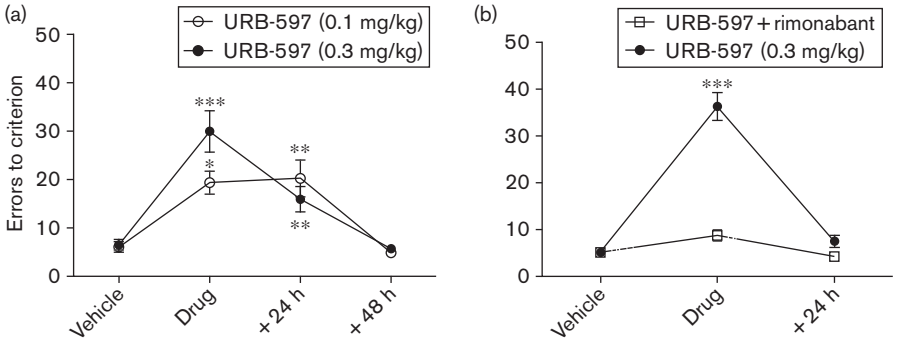
The results from experiment 3 are presented in Fig. 3a and b. Within-subjects analysis showed a significant drug effect relative to baseline on auditory discrimination performance (Bonferroni $F_{ctr} = 6.20$) with URB-597 [0.1 mg/kg; $F(1,15) = 25.03$; $P < 0.001$] and URB-597 [0.3 mg/kg; $F(1,15) = 28.07$; $P < 0.001$]. Analysis also indicated a continuing impairment in performance 24 h after the 0.1 [$F(1,15) = 14.93$; $P < 0.001$] and 0.3-mg/kg URB-597 doses [$F(1,15) = 20.24$; $P < 0.001$ (Fig. 3a)]. The effects of URB-597 on auditory performance were clearly blocked by rimonabant [$F(2,28) = 91.97$; $P < 0.001$ (Fig. 3b)]. Within-subjects analysis indicated a significant decrement in auditory discrimination performance in rats treated with URB-597 (0.3 mg/kg) relative to URB-597 + rimonabant [$F(1,14) = 118.16$; $P < 0.001$].

Experiment 4

The results from experiment 4 are presented in Fig. 4a and b. Between-subjects comparisons showed that 0.3, 1 and 3 mg/kg of Δ^9-THC did not significantly affect acquisition of novel olfactory discrimination tasks (Fs < 1). Similarly, treatment with rimonabant plus Δ^9-THC did not produce any significant impairment in the acquisition of a novel olfactory discrimination (Fs < 1; Fig. 4a). There was a tendency towards enhancement in learning when rimonabant was administered on its own [$t(14) = 2.047$; $P = 0.060$].

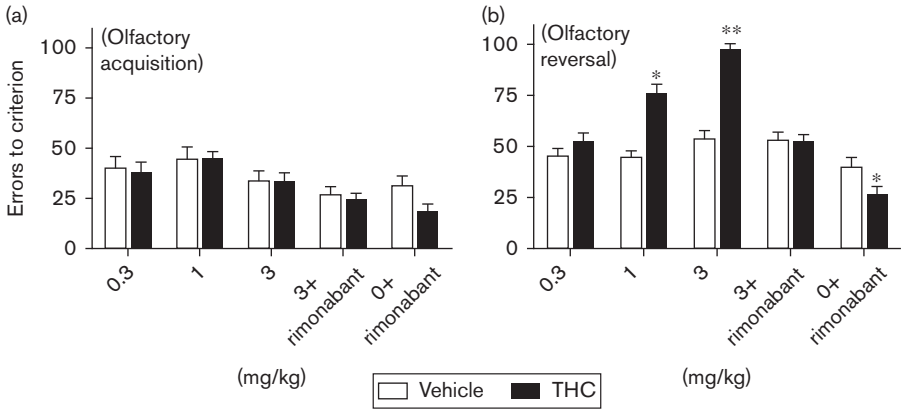
Figure 4b shows the effect of Δ^9-THC on olfactory discrimination reversals. A series of t-tests showed a significant dose-dependent decrement in discrimination

Fig. 3



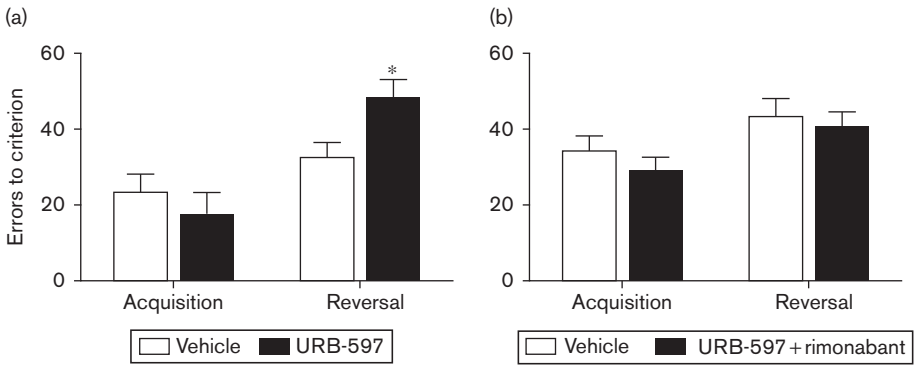
(a) Both 0.1 and 0.3 mg/kg doses of URB-597 impaired auditory discrimination performance. There was a continuing impairment in performance 24 h after administration of these doses (***P<0.001 relative to baseline). (b) The disruptive influence of URB-597 (0.3 mg/kg) on auditory discrimination was prevented by coadministration of rimonabant (3 mg/kg). *P<0.05, **P<0.01 relative to baseline.

Fig. 4



(a) Acquisition of novel olfactory discriminations was unaffected by Δ^9-tetrahydrocannabinol (Δ^9-THC; 0.3, 1 and 3 mg/kg doses) or Δ^9-THC plus rimonabant. (b) Δ^9-THC impaired the reversal of a previously learned olfactory discrimination at doses of 1 and 3 mg/kg and this was prevented by coadministration of rimonabant (3 mg/kg). By itself, rimonabant facilitated the reversal of an olfactory discrimination. *$P<0.05$, **$P<0.01$ relative to vehicle group.

Fig. 5



(a) Acquisition of an olfactory discrimination task was unaffected by URB-597 (0.3 mg/kg); however, the drug impaired the reversal performance. *$P<0.05$. (b) There was no effect on acquisition or reversal of olfactory discrimination when URB-597 was coadministered with rimonabant (3 mg/kg).

reversal accuracy with Δ^9-THC [1 mg/kg; $t(14) = 5.10$; $P < 0.01$], Δ^9-THC [3 mg/kg; $t(13) = 9.02$; $P < 0.01$] but not Δ^9-THC [0.3 mg/kg; $t(14) = 1.51$; not significant]. The effects of Δ^9-THC on the reversal task(s) were prevented by rimonabant, with no difference in errors between the vehicle group and the group treated with rimonabant plus Δ^9-THC (Fs < 1). As with the acquisition task, there was a mild facilitatory effect of rimonabant administered on its own on reversal learning [vehicle vs. 3 mg/kg of rimonabant: $t(14) = 2.52$; $P = 0.02$].

Experiment 5

Between-groups comparisons showed that URB-597 (0.3 mg/kg) did not significantly affect the acquisition of novel olfactory discrimination [$t(14) = 0.58$; not significant (Fig. 5a)]. Similarly, there was no significant difference in acquisition between rats treated with vehicle and those given rimonabant (3 mg/kg) + URB-597 (0.3 mg/kg; Fs < 1; Fig. 5b). However, in the reversal task there was a significant decrement in performance of the drug group, as shown by increased errors in the group treated with URB-507 [0.3 mg/kg; $t(14) = 2.65$; $P < 0.05$ (Fig. 5a)]. These disruptive effects of URB-597 on the reversal task were not observed when it was coadministered with rimonabant [(Fig. 5b); vehicle vs. rimonabant (3 mg/kg) + URB-597 (0.3 mg/kg; Fs < 1)].

Discussion

This study examined the effects of Δ^9-THC, rimonabant and URB-597 on performance of a two-stimulus go/no-go auditory discrimination task and on the performance, acquisition and reversal of an equivalent two-odour olfactory

discrimination task. The use of equivalent auditory and olfactory discrimination tasks allows modality-specific drug effects to be uncovered in a way that controls for nonspecific drug effects on performance elements such as motivation, motor function and behavioural inhibition (Sokolic and McGregor, 2007). Generally, no nonspecific impairment of performance was found with any of the drugs or doses used.

The results from experiments 1 and 3 showed that auditory discrimination performance was powerfully disrupted by the exogenous CB_1 agonist, Δ^9-THC, as well as by the FAAH inhibitor URB-597. In contrast, Δ^9-THC left rats unimpaired in their performance or acquisition of the precisely analogous two-odour go/no-go discrimination task. Thus, the effect on the auditory discrimination task cannot be attributed to sedation, increased impulsivity or altered motivation for the water reinforcer. Rather, some specific disruption of auditory information processing or auditory selective attention seems likely.

Interestingly, in an earlier study from our laboratory, the short-acting benzodiazepine midazolam modestly improved performance on a well-learned auditory task, but impaired olfactory discrimination performance (Sokolic and McGregor, 2007), a pattern opposite to that obtained here with cannabinoid. This suggests that the modality-specific drug effects observed here cannot simply be attributed to one of the tasks (viz. auditory or olfactory) being generally more vulnerable to drug-induced disruption.

A vulnerability of auditory discrimination performance to cannabinoid-induced disruption is supported by findings from previous studies involving both laboratory subjects and humans. Impaired auditory discrimination was seen in monkeys performing a complex operant task in which auditory stimuli served as cues for selection of right or left levers (Elsmore, 1972). Smoked cannabis also increased the number of false alarms in humans, reporting the presence of a tone stimulus under various conditions of concentrated and divided attention (Moskowitz, 1974). Chronic cannabis users, tested in the unintoxicated state, had a lower hit rate and more false alarms in an auditory selective task in which tones of a particular location and duration were to be responded to (Solowij *et al.*, 1991). Cannabis users also showed decreased electroencephalogram power and signal to noise ratio to an auditory click stimulus, again suggestive of abnormal auditory processing (Skosnik *et al.*, 2006). Acute administration of the CB_1 agonist, Δ^9-THC, to healthy subjects significantly decreased auditory P300 amplitude reflecting deficient attentional resource allocation and working memory (Roser *et al.*, 2008). Smoking marijuana also altered the pattern of brain metabolism or regional cerebral blood flow in an auditory attention task (O'Leary *et al.*, 2000, 2002). Taken together, the findings reported here suggest a remarkable sensitivity of tasks in the auditory modality to cannabinoid-induced disruption.

This sensitivity is further emphasized by the finding that auditory performance was also impaired on the day after Δ^9-THC or URB-597 administration (experiments 2 and 3). Some previous studies have reported that high doses of Δ^9-THC have observable behavioural effects for up to 3 days after administration, which may well reflect the slow elimination of Δ^9-THC from the body due to its high lipophilicity and ability to be retained in fatty tissue (Lemberger *et al.*, 1970; Conrad *et al.*, 1972; Gunasekaran *et al.*, 2009). Indeed, the effects of Δ^9-THC on cognitive performance can persist for at least a week after stopping the drug, presumably due to the protracted time required to eliminate accumulated Δ^9-THC in fat stores (Pope *et al.*, 2001, 2002).

The other notable finding was that both Δ^9-THC and URB-597 impaired the reversal, but not the acquisition or performance, of a two-odour discrimination task. Experiments 4 and 5 showed that various doses of Δ^9-THC and URB-597 did not affect the acquisition of a novel two-odour discrimination task. However, when rats were required to reverse the two-odour discrimination task they had recently learned, and performed according to this reversed contingency, both drugs caused a clear impairment. This agrees with previously reported disruptive effects of Δ^9-THC (1 mg/kg) in rats attempting the reversal of an olfactory task in which odours indicated which of two sand-filled beakers contains a food reward (Egerton *et al.*, 2005). It was suggested that this reflects an inability to update affective associations between stimuli and reinforcement value, and that this effect may arise through a cannabinoid effect on orbitofrontal circuitry in which instrumental outcomes are represented (Egerton *et al.*, 2005; Schoenbaum and Shaham, 2008).

The Δ^9-THC-induced and URB-597-induced disruptions in auditory discrimination performance and olfactory reversal learning were prevented by the coadministration of the selective CB_1 receptor antagonist rimonabant, suggesting that these effects are mediated by CB_1 receptors. Reversal by rimonabant of URB-597 effects is consistent with most previous reports (Gobbi *et al.*, 2005; Haller *et al.*, 2006; Solinas *et al.*, 2007; Clapper *et al.*, 2009). By itself, rimonabant tended to improve acquisition of a novel olfactory discrimination and significantly facilitated olfactory discrimination reversals. This latter effect further confirms the sensitivity of reversal learning tasks to modulation by cannabinoids, and also agrees with previous reports of rimonabant facilitating cognitive function in rodents (Terranova *et al.*, 1996; Wolff and Leander, 2003; Takahashi *et al.*, 2005; Varvel *et al.*, 2009). The current literature indicates that rimonabant can exert its own intrinsic actions, including, for example, dose-related increases in the frequency of grooming and scratching behaviour in rodents (Darmani and Pandya, 2000; Jarbe *et al.*, 2002). Although the ability of rimonabant to reverse Δ^9-THC and URB-597 impairment in our study is most likely due to pharmacological

antagonism at CB_1 receptors, a functional antagonism based on the intrinsic nootropic effects of rimonabant (Varvel *et al.*, 2009) cannot be ruled out. Future studies might antagonize Δ^9-THC and URB-597 effects with lower rimonabant doses that have no intrinsic action on discrimination, to provide a definitive answer to this question.

A rather unexpected overall outcome from this series of experiments was the Δ^9-THC-like effects of the FAAH inhibitor URB-597 on both auditory discrimination performance and on reversal learning. Enhancing brain endocannabinoid levels with FAAH inhibitors such as URB-597 represents a novel approach to modulating brain cannabinoid systems and promising preclinical results have been obtained with URB-597 in animal models relevant to the treatment of anxiety, depression and pain (Piomelli *et al.*, 2006; Schlosburg *et al.*, 2009). It has been proposed that subtle modulation of endocannabinoid levels might alter emotional states without producing the psychomotor and cognitive impairment characteristic of exogenous ligands such as Δ^9-THC (Kathuria *et al.*, 2003; Piomelli *et al.*, 2006; Bortolato *et al.*, 2007). However, in this study, URB-597 clearly impaired auditory discrimination performance as well as retarding reversal learning, in both cases to a similar extent to the impairment observed with Δ^9-THC. These effects on reversal learning are somewhat inconsistent with reports, which state that mice with a deletion of the *FAAH* gene (FAAH−/− mice) show intact reversal learning in a Morris water maze, and indeed may show faster acquisition and extinction in spatial learning tasks relative to wild-type controls (Varvel *et al.*, 2001; Niyuhire *et al.*, 2007). A recent study concurs with the latter findings showing that URB-597 improves memory acquisition in a passive avoidance task and that these effects were mediated by peroxisome proliferator-activated receptor-α and CB_1 receptors (Mazzola *et al.*, 2009).

The task-specific nature of cannabinoid interference in cognitive and associative learning paradigms is evident in these results (i.e. auditory vs. olfactory tasks) and in other recent studies showing differential effects on extinction in appetitive versus aversive paradigms (Niyuhire *et al.*, 2007; Varvel *et al.*, 2009) or enhanced acquisition of an aversive, but not of an appetitive-maze task (Wise *et al.*, 2009). In addition, the effects of changing endogenous cannabinoid levels with URB-597 on stress-coping behaviours in rodents seem to be highly sensitive to environmental conditions (Clapper *et al.*, 2009; Haller *et al.*, 2009). We, therefore, hypothesize that auditory and olfactory discrimination reversal tasks may have enhanced sensitivity in detecting URB-597-related impairment relative to some of the other paradigms that have hitherto been used. One important factor may be the water (and therefore) food-deprived state of the subjects in this study: were such deprivation and/or associated stress to alter basal endocannabinoid tone then this might render subjects more sensitive to disruptive effects of URB-597 and other FAAH inhibitors (Rubino *et al.*, 2008; Clapper *et al.*, 2009).

The Δ^9-THC-like effects of URB-597 in this study may have implications for the therapeutic potential of this drug. In particular, our results suggest that URB-597 may indeed have the capacity to disrupt perceptual and cognitive functions in much the same way as a prototypical cannabinoid such as Δ^9-THC, which is consistent with a recent report of URB-597 causing working memory impairment in the *T*-maze (Seillier *et al.*, 2010). Clearly, should the drug reach phase 1 clinical trial, then such disruptive effects may be readily confirmed by the testing of humans.

In summary, it seems that the auditory discrimination and olfactory discrimination reversal tasks are very sensitive to the disruptive effects induced by exogenous cannabinoid and by the altered endocannabinoid tone produced by FAAH inhibitors. Uncovering the neural substrates of these effects is an interesting goal for future research and one that may provide insights into the striking way in which cannabinoid modulates auditory and musical percepts in human beings. Another important point to be addressed in future studies is whether tolerance occurs to auditory discrimination and olfactory reversal impairments when URB-597 or Δ^9-THC are administered chronically.

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