

Effects of various cannabinoid ligands on choice behaviour in a rat model of gambling

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It is estimated that 0.6–1% of the population in the USA and Canada fulfil the *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed. (DSM-5) criteria for gambling disorders (GD). To date, there are no approved pharmacological treatments for GD. The rat gambling task (rGT) is a recently developed rodent analogue of the Iowa gambling task in which rats are trained to associate four response holes with different magnitudes and probabilities of food pellet rewards and punishing time-out periods. Similar to healthy human volunteers, most rats adopt the optimal strategies (optimal group). However, a subset of animals show preference for the disadvantageous options (suboptimal group), mimicking the choice pattern of patients with GD. Here, we explored for the first time the effects of various cannabinoid ligands (WIN 55,212-2, AM 4113, AM 630 and URB 597) on the rGT. Administration of the cannabinoid agonist CB₁/CB₂ WIN 55,212-2 improved choice strategy and increased choice latency in the suboptimal group, but only increased perseverative behaviour, when punished, in the optimal group. Blockade of CB₁ or CB₂ receptors or

inhibition of fatty-acid amide hydrolase did not affect rGT performance. These results suggest that stimulation of cannabinoid receptors could affect gambling choice behaviours differentially in some subgroups of subjects. *Behavioural Pharmacology* 27:258–269 Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

It is estimated that 0.6–1% of the population in the USA and Canada fulfil the *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed. (DSM-5) criteria for gambling disorder (GD) (Kessler *et al.*, 2008; Williams *et al.*, 2011) and neurobiological research in GD supports its characterization as a behavioural addiction (American Psychiatric Association, 2013). Many scientists and clinicians have long believed that patients with GD have common features with patients with substance-use disorders (Dell'Osso *et al.*, 2006; Vilella *et al.*, 2011). Dopamine has long been implicated in addiction processes and early articles postulated a similarly important role for dopamine in GD (Pattij and Vanderschuren, 2008; Zeeb *et al.*, 2013). Drugs that are known to increase dopamine levels consistently increase premature responding in the 5-choice serial reaction time task (5-CSRTT) (van Gaalen *et al.*, 2006). Several studies also support the notion that impulsive behaviour is mediated not only by the dopaminergic system but also by other neurotransmitters including serotonin, noradrenaline and glutamate (Pattij and Vanderschuren, 2008). In recent years, there has been

increasing interest in the study of the involvement of the endocannabinoid system in executive functions in which dopamine plays a crucial role, such as behavioural flexibility and reversal learning (Egerton *et al.*, 2006).

The endocannabinoid system might be of relevance to impulsivity and decision-making. Administration of high doses of CB₁ receptor agonists increases impulsive behaviours, whereas the administration of low doses of CB₁ antagonists improves set-shifting performance and reduces the number of impulsive responses (Hill *et al.*, 2006; Pattij *et al.*, 2007). The synthetic CB_{1/2} cannabinoid agonist WIN 55,212-2, was found to normalize the impulsive profile of adolescent spontaneously hypertensive rats, a strain with lower CB₁ receptor density in the prefrontal cortex compared with control rats (Adriani and Laviola, 2004), suggesting that a reduced cortical density of cannabinoid CB₁ receptors is associated with enhanced impulsivity. Other studies have also shown the effects of the inhibition of the anandamide degradation system or CB₁ antagonism on self-control behaviours (Marco *et al.*, 2007; Pattij *et al.*, 2007). Within the brain, cannabinoid CB₁ receptors are located presynaptically and activation of these receptors inhibits synaptic transmission, which enables the endocannabinoid system to modulate neuronal activity of other

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transmitter systems (Melis and Pistis, 2007). The endocannabinoid system has been implicated in different behaviours, including the reinforcing effects of drugs of abuse (Le Foll and Goldberg, 2005), food intake (Di Marzo and Matias, 2005) and cognitive processing (Takahashi *et al.*, 2005). CB₂, initially described as a peripheral receptor (Maldonado *et al.*, 2006, 2011), has been identified in several brain structures including the striatum, hippocampus and thalamus (Gong *et al.*, 2006), and more recently, into ventral tegmental area neurons (Zhang *et al.*, 2014). It has been suggested that the CB₂ receptor participates in central functions and growing evidence suggests a role in addictive processes (Gamaledin *et al.*, 2012b; Navarrete *et al.*, 2013).

Both marijuana and its principal psychoactive ingredient delta-9-tetrahydrocannabinol (THC) are associated with alterations in response inhibition (McDonald *et al.*, 2003), decision-making (Lane *et al.*, 2005) and behavioural flexibility (Ramaekers *et al.*, 2006) in healthy controls. Chronic cannabis use has also been associated with dysfunctions in decision-making [e.g. in the Iowa gambling task (IGT)] compared with nondrug users (Bolla *et al.*, 2005). However, an obstacle to the rigorous study of factors influencing impulsivity is the large variation present in the human population, which is difficult to both categorize and control. As gambling behaviour is a complex phenomenon involving concurrent activation of different systems, the use of animals is required to study its processes. The complexity of these behaviours is mediated by different neurotransmitters and neuromodulators that work together as a network. Animal models therefore play an increasingly important role in the study of GD-related behaviour as this cannot be mimicked by cellular or molecular models. The prototypical task for studying decision-making in rodents is the rodent gambling task (rGT). An analogue of the IGT used in humans, the rGT allows animals to choose between four options, each associated with varying magnitudes and probabilities of gains and losses (Zeeb *et al.*, 2009). Similar to healthy human volunteers, most rats adopt optimal strategies (optimal group). However, a subset of animals show preference for disadvantageous options (suboptimal group), mimicking the choice pattern of patients with GD.

Altogether, the above studies suggest a role of the endocannabinoid system in executive functions. Endocannabinoids appear to have a negative impact on set-shifting and cognitive flexibility, and CB₁ receptor antagonists can improve such executive functions (Hill *et al.*, 2006). There are no published data on the impact of the endocannabinoid system on the performance on the rGT in outbred optimal versus suboptimal rats. To this end, in the present study, we hypothesized that the CB_{1/2} agonist WIN 55,212-2 would promote greater impulse to select the least optimal choice and that the CB₂ antagonist/inverse agonist, AM 630, would have no effect on this task. We also hypothesized that the neutral CB₁ antagonist AM 4113 and the selective inhibitor of fatty-acid

amide hydrolase, URB 597, would increase self-control and therefore promote the most optimal choice (greatest earnable reward per unit time). Therefore, in this study, we tested the effects of the systemic injection of WIN 55,212-2, AM 4113, AM 630 and URB 597 on decision-making in the rGT.

Methods

Subjects

Male Long-Evans rats ($N=24$; Charles River Laboratories, Lachine, Quebec, Canada) were used in this study. All animals weighed 300–325 g at the start of the experiment. Animals were individually housed in a temperature-controlled colony room under a 12 h reverse light cycle (lights off at 07:00 h). Testing took place between 12:00 and 16:00 h 5 days/week. Water was freely available, except during testing periods. Rats were food restricted (18–20 g of standard rat chow per day) and maintained at 90% of their free-feeding body weight (food was available immediately after behavioural testing). All experiments were conducted in accordance with the Canadian Council of Animal Care and experimental protocols were approved by the Animal Care Committee of the Centre for Addiction and Mental Health.

Apparatus

A detailed description of the testing chambers has been reported previously (Zeeb *et al.*, 2009; Di Ciano *et al.*, 2015). Briefly, testing took place in traditional 5-CSRTT chambers (Med Associates, St Albans, Vermont, USA). A house light was located at the top of the chamber. Within the chambers, five holes were positioned 2 cm above a metal bar floor. A stimulus light was located at the back of each hole. Only the outer four of the five holes were utilized in all experimental procedures (i.e. the middle hole, three of five, was unused). A food tray with a tray light at the top of the opening was located on the opposite wall. Nose-poke responses into the response holes or food tray were detected by a horizontal infrared beam. Rewards consisted of non-sweet-enriched pellets (45 mg; Bioserv, Frenchtown, New Jersey, USA) that were delivered to the food tray from an external pellet dispenser. All operant chambers were contained within a ventilated and sound-attenuating box. The chambers were controlled by software written in Med-PC (Med Associates, St. Albans, Vermont, USA) running on a Dell desktop computer.

Pre-rGT Training

Animals were trained according to previously described methods (Zeeb *et al.*, 2009). Briefly, animals were trained in 30 min daily sessions to make nose-poke responses, within 10 s, into an illuminated response hole, which resulted in a non-sweet-enriched pellet (Bioserv) being delivered to the food receptacle. The illuminated hole changed each trial with equal numbers of presentations of each possible hole over the course of the session. Each of the three training stages continued until 30 trials (for

Table 1 Training parameters across training stages

Training stage	Limited hold (s)	Stimulus duration (s)	Intertrial interval (s)	Criterion to move on to next stage
1	30	30	2	≥ 30 correct trials
2	20	20	2	≥ 30 correct trials
3	10	10	5	

Each of the three training stages continued until 30 trials (for the two first sessions) or 100 trials (for the third session) were completed, or 30 min had elapsed. For the third stage of the training phase, animals were trained until they accomplished at least 50 correct responses. The difficulty of the program through the three stages of the training phase was increased by shortening the stimulus presentation and limited-hold periods, increasing the ITI duration and altering criteria for progression. At the end of the third session, rats had to make at least 80% accuracy [correct/(correct+incorrect)] and fewer than 20% omissions (omissions/trials) before the next stage of training. Animals started and completed training stages, rGT training and testing all at once, which allowed them the same number of sessions during all of the study.

ITI, intertribal interval; rGT, rat gambling task.

the two first sessions) or 100 trials (for the third session) were completed, or 30 min had elapsed, whichever came first. For the third stage of the training phase, animals were trained until they accomplished at least 50 correct responses. The difficulty of the program through the three stages of the training phase was increased by shortening the stimulus presentation and limited-hold periods, increasing the intertrial interval (ITI) and altering criteria for progression (Table 1). The final training program had limited hold and stimulus duration lengths of 10 s and an ITI of 5 s. Animals completing two consecutive sessions (100 trials each), with 80% of trials being correct and less than 20% omissions (i.e. no response made within 10 s), were subjected eight daily sessions (30 min each) of a forced-choice version of the rGT, where only one of the holes was illuminated at each time. During the forced-choice version of the rGT, each of the options was associated only with a particular hole and the probability of reward/punishment (and the reward magnitude) was set at this time and remained unchanged; thus, the rats could learn the association of each hole with the different outcomes. Details of the rewards and punishments delivered at each hole are described below. This procedure ensured that all the animals had an equal training experience in terms of rewards (i.e. 45 mg no-sweet enriched diet pellets), specific duration of punishment (i.e. time-out period) and the respective probabilities of each, for the four different holes, thereby preventing the development of simple biases towards a particular hole. Animals were counter-balanced on two versions of the forced-choice rGT (A or B), which differed only in the hole assignment for each contingency (P_1 – P_4 ; see Fig. 1 for contingencies).

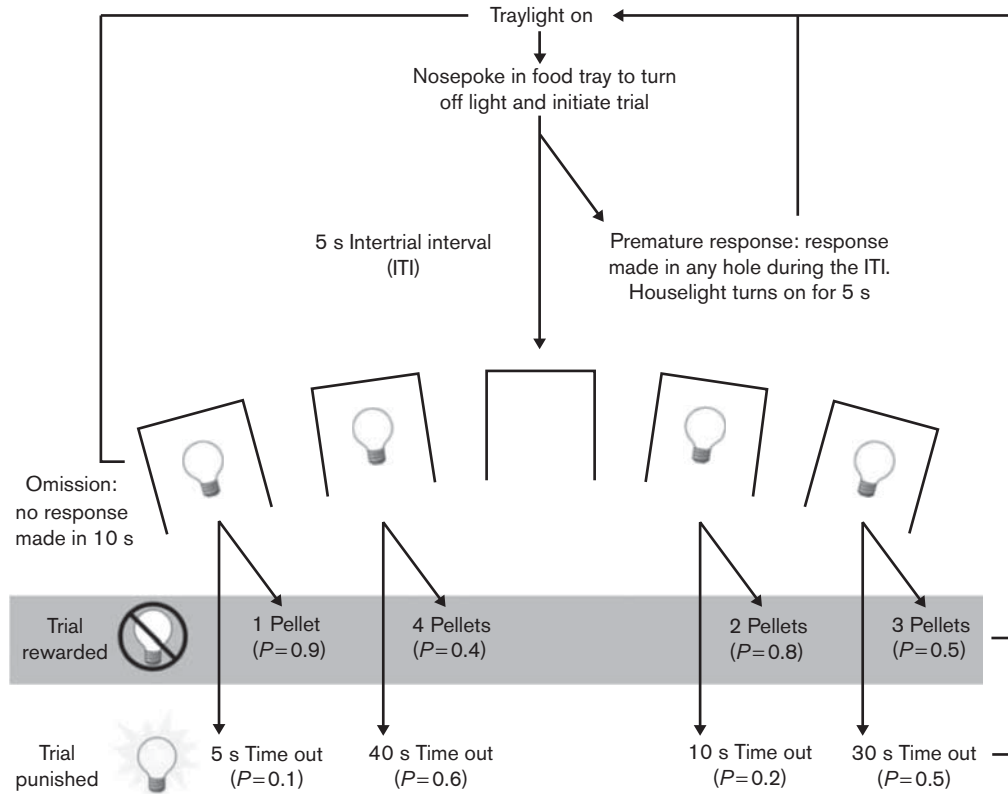
The rGT

The design of the rGT has been described previously (Zeeb *et al.*, 2009) and a diagram of the trial structure and reinforcement schedules is provided in Fig. 1. Briefly, animals were administered 30 daily sessions (30 min each), where they were required to make a nose-poke

response into the illuminated food receptacle to start a trial. Once the response was made, the chamber was darkened for a 5 s ITI, during which responses made were recorded as being premature responses and resulted in a 5 s houselight illumination and a time-out period (during which responding had no effect), followed by an illumination of the food receptacle that allowed the animal to begin another trial. If no premature response was made during the ITI, the four response holes were illuminated and the animal had 10 s to nose-poke into any of the four holes. If no response took place during the 10 s, the lights were turned off and the food receptacle light was turned back on, with the trial being counted as an omission. If the animal responded into one of the four holes before 10 s, the four lights were turned off and the trial was either rewarded [i.e. the food receptacle was illuminated and pellet(s) were delivered according to the contingency for the chosen hole; collection of the reward resulted in initiation of a new trial] or punished (the stimulus light of the chosen hole flashed at 0.5 Hz for the duration of the time-out period, according to the contingency of the chosen hole, following which the food receptacle was illuminated, allowing for a new trial to be initiated). The size of reward and punishment probability/duration are shown in Table 2. Perseverative responses made at the array or food tray following a reward or during the time-out periods were recorded, but not punished.

Responses in the holes were rewarded such that P_1 resulted in one pellet with a probability of 0.9 and the punishment was a 5-s time-out with a 0.1 probability. P_2 was rewarded with two pellets with a probability of 0.8 and the punishment was a 10-s time-out with a 0.2 probability. P_3 was rewarded with three pellets with a probability of 0.5 and a 30-s time-out signalled the punishment with a 0.5 probability. P_4 was rewarded with four pellets with a probability of 0.4 and the punishment was a 40-s time-out with a 0.6 probability. Reinforcement schedules were designed such that the two-pellet choice (P_2) was optimal (i.e. this option resulted in the most rewards earned per unit time). Selection from any other option (one-pellet, three-pellet or four-pellet options) yielded fewer rewards per unit of time as a consequence of the probability of winning or losing and the duration of the punishing time-out periods associated with each option (i.e. the maximum numbers of pellets were $P_1 = 295$, $P_2 = 411$, $P_3 = 135$ and $P_4 = 99$). Thus, P_3 and P_4 would be considered the risky/disadvantageous choices, where the greatest reward would be associated with the highest probability and size of punishment. Selection of P_1 and P_2 represented a more advantageous strategy (Table 2). The location of the response holes remained stable across sessions. A high rGT impulsive choice behaviour reflects persistent choice of high-risk options, which are linked to larger rewards, but ultimately result in fewer pellets earned.

Fig. 1



Design of the rat gambling task (rGT). The task began with illumination of the tray light. A nose-poke response in the food tray extinguished the tray light and initiated a new trial. After an intertrial-interval (ITI) of 5 s, four stimulus lights were turned on in holes 1, 2, 4 and 5, and the animal was required to respond in one of these holes within 10 s. This response was then rewarded or punished depending on the reinforcement schedule for that option (indicated by the probability of a win or a loss in brackets for each option). If the animal was rewarded, the stimulus lights were extinguished and the animal received the corresponding number of pellets in the now-illuminated food tray. A response at the food tray then started a new trial. If the animal was punished, the stimulus light in the corresponding hole flashed at a frequency of 0.5 Hz for the duration of the punishing time-out and all other lights were extinguished. At the end of the punishment period, the tray light was turned on and the animal could initiate a new trial. Failure to respond at the illuminated holes resulted in an omission, whereas a response during the ITI was classified as a premature response and punished by a 5-s time-out during which the house light was turned on. Reproduced with permission from Zeeb *et al.*, 2009.

Table 2 Reward and punishment received for the various response options in the rat gambling task

Group A	Hole 1	Hole 4	Hole 5	Hole 2
Group B	Hole 2	Hole 5	Hole 4	Hole 1
Choice	P_1	P_2	P_3	P_4
Reward (number of pellets)	1	2	3	4
Punishment duration (s)	5	10	30	40
Punishment probability	0.1	0.2	0.5	0.6
Rewards possible	295	411	135	99

Pharmacological treatment

All drugs were obtained from Cayman Chemical (Ann Arbor, Michigan, USA), with the exception of AM 4113, which was obtained through Dr Alexandros Makriyannis' laboratory (Center for Drug Discovery, Northeastern University, Boston, Massachusetts, USA). The neutral CB₁ antagonist AM 4113 was dissolved in Tween-80 (10%), DMSO (10%) and saline (80%). The CB₂ antagonist AM 630 was dissolved in Tween-80 (10%),

DMSO (10%) and distilled water (80%). WIN 55,212-2, a CB_{1/2} agonist was dissolved in Tween-80 (0.3%) and saline (99.7%). URB 597, a selective inhibitor of fatty-acid amide hydrolase, was dissolved in DMSO (20%) and saline (80%). All drugs were administered intraperitoneally at a volume of 1 ml/kg body weight, except for URB 597 and AM 630, which were administered at 2 ml/kg (Table 3). Drug injections were administered on a 3-day cycle, starting initially with a baseline session (rats went into the session, but received no drugs). The following day, rats received a drug or vehicle injection before testing. On the third day, animals were not tested. Treatments commenced with URB 597 (30 min before behavioural testing), followed by WIN 55,212-2 (15 min before behavioural testing), AM 630 (30 min before behavioural testing) and AM 4113 (60 min before behavioural testing) (Table 3). Animals were tested drug free for a minimum of 1 week between compounds to prevent carryover effects. All drugs were prepared fresh daily.

Table 3 Doses, delivery volume and pretreatment time for the cannabinoid drugs used in the study

Drug	Effect	Delivery volume	Pretreatment time (min)		Doses (mg/kg)			
WIN 55,212-2	CB ₁ agonist	1 ml/kg	15	Vehicle	0.1	0.3	1	3
URB 597	Fatty-acid amide hydrolase inhibitor	2 ml/kg	30	Vehicle	0.1	0.3	1	
AM 4113	Neutral CB ₁ antagonist	1 ml/kg	60	Vehicle	1	3	10	
AM 630	CB ₂ antagonist	2 ml/kg	30	Vehicle	1.25	2.5		

All drugs were administered intraperitoneally.

Data analysis

The following formula was used to calculate the percentage of trials on which an animal chose a particular option: number of choices of a particular option/number of total trials made $\times 100$. To determine the percentage of advantageous choices made, the percentages from P_1 and P_2 were summed; disadvantageous choices were calculated as the percentage sum of P_3 and P_4 choices. Rats were divided into optimal and suboptimal groups on the basis of their performance under the vehicle condition; those that made more advantageous choices under vehicle were assigned to the optimal group, whereas rats that made more ($P_3 + P_4$) choices than ($P_1 + P_2$) were then assigned to the suboptimal group. The numbers of animals for the optimal and suboptimal groups (respectively) were as follows: URB 597, $n = 15$ and $n = 9$; WIN 55,212-2, $n = 13$ and $n = 11$; AM 630, $n = 12$ and $n = 12$; and AM 4113, $n = 13$ and $n = 11$.

The percentage of premature responses made was calculated as the number of premature responses made/total number of trials initiated $\times 100$. Perseverative responses made during the punishment period were analysed as a fraction of the total punishment duration experienced. Similarly, perseverative responses (i.e. made after a reward was received) were analysed as a fraction of the total number of trials rewarded (Di Ciano *et al.*, 2015). In addition, the latency to respond at the array and to collect the reward for each choice option was analysed.

During the acquisition phase, the main variables analysed were percentage choice for each option (P_1 – P_4) and percentage of optimal choices ($P_1 + P_2$). Data were analysed using two-way repeated-measures analysis of variance (ANOVA) (session \times choice) or (dose \times choice) separately for each of the optimal and suboptimal groups during the acquisition phase, or three-way ANOVA (dose \times choice \times group) during the test phase, followed by post-hoc Fisher least significant difference tests. Comparisons for the average percentage of advantageous choice during the total 30 sessions of acquisition were performed using Student's *t*-test. Differences were considered statistically significant when P is less than 0.05. All statistical analyses were carried out using statistical package Stat Soft, version 10 (Stat soft, Inc. Tulsa, OK, USA).

Results

Acquisition of the rGT in the optimal and suboptimal groups of rats

The learning curve for the acquisition of choice behaviour by the optimal group of rats ($n = 15$) significantly

differed from that of the suboptimal rats ($n = 9$) (Fig. 2). Two-way ANOVA showed no significant session \times group interaction [$F_{(29,64)} = 1.89$, NS], suggesting that the overall advantageous or disadvantageous choices remained constant for the duration of the training in both groups (Fig. 2a and b). A significant session \times choice \times group interaction was observed [$F_{(87,19)} = 1.32$, $P < 0.05$], indicating the progression of learning rate along sessions, with the optimal group of rats showing a significantly greater preference for the advantageous choice (P_1 and P_2) during the acquisition phase (Fig. 2c), compared with the suboptimal group [$t_{(58)} = 50.44$, $P < 0.001$], as expected. The low choice for P_1 was fully compensated by increased P_2 choice strategy in the optimal group; thus, the advantageous choice strategy ($P_1 + P_2$) remained high and constant in that group. In contrast, in the suboptimal group, the low P_1 choice strategy was not compensated by an increased P_2 choice strategy. The suboptimal group showed a preference for P_3 and P_4 choices (disadvantageous) during the rGT acquisition.

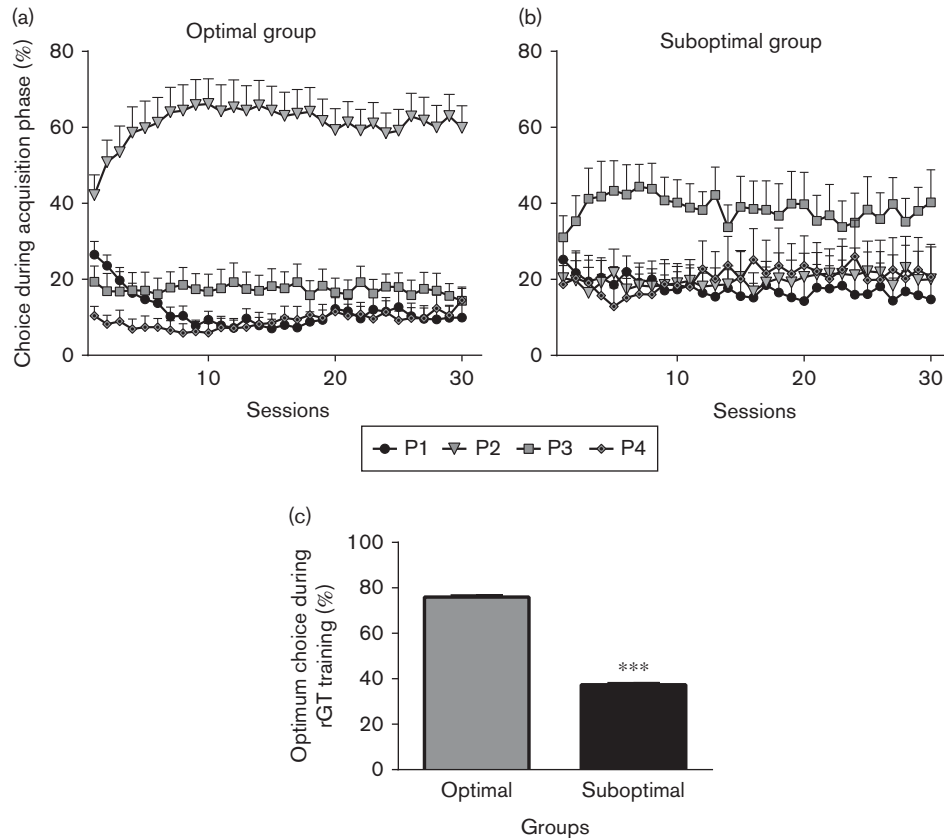
Effects of cannabinoid ligands on the rGT

Administration of the CB_{1/2} agonist WIN 55,212-2 did not modify the number of trials completed. Two-way repeated-measure ANOVA (dose \times group) indicated an overall significant effect of group [$F_{(1,22)} = 12.40$, $P < 0.01$], but no significant effect of dose [$F_{(4,88)} = 1.39$, NS] or dose \times group interaction [$F_{(4,88)} = 1.86$, NS] (Table 4). Administration of WIN 55,212-2 dose dependently increased the advantageous choices (in detriment of disadvantageous choices) in the suboptimal group (Fig. 3a), but had no significant effects in the optimal group of rats (Fig. 3b), with significant choice \times group [$F_{(1,22)} = 47.70$, $P < 0.001$] and dose \times choice \times group [$F_{(4,88)} = 3.51$, $P < 0.05$] interactions. Fisher least significant difference post-hoc analysis showed a significant increase in the preference for the advantageous strategy and a decrease for the disadvantageous strategy ($P < 0.005$ – 0.01) in the Suboptimal group of animals pretreated with WIN 55,212-2 (3 mg/kg) compared with vehicle. No significant effects on choice strategy were observed in the optimal group following the administration of WIN 55,212-2. Pretreatment with URB 597, AM 4113 and AM 630 did not modify the choice strategy [dose \times choice \times group interaction: $F_{(3,66)} = 0.12$, NS, $F_{(3,66)} = 1.42$, NS, $F_{(2,44)} = 1.37$, NS, respectively] (Table 5).

Effects of cannabinoid ligands on choice and collect latencies

The administration of the highest dose of WIN 55,212-2 tested in this study (3 mg/kg) increased the latency to

Fig. 2



Acquisition of the rat gambling task (rGT) in optimal and suboptimal groups of rats during the training phase. In (a) and (b), rats in the optimal group ($n = 15$) showed a different learning acquisition curve for the four choice options compared with the suboptimal group ($n = 9$) during the 30 sessions (of 30 min) corresponding to the training phase. In (c), the optimal group of rats showed a significantly greater preference for optimal choices ($P_1 + P_2$) compared with the suboptimal group of animals. *** $P < 0.001$, Student's t -test.

Table 4 Effects of the CB₁ agonist (WIN 55,212-2) on the number of trials completed during the 30 min period of rGT

Variable	Factor	Doses				
		Vehicle	0.1	0.3	1	3
WIN 55,212-2	Optimal group	102 ± 7	102 ± 7	101 ± 8	89 ± 5	92 ± 7
Number of trials	Suboptimal group	70 ± 4	70 ± 4	72 ± 5	68 ± 5	76 ± 4

Data represent the average number of trials ± SEM in the optimal and suboptimal groups.
rGT, rat gambling task.

make a choice on the suboptimal group of rats (Fig. 4a). Two-way ANOVA analysis indicated a significant effect of latency type [$F_{(1,20)} = 15.23$, $P < 0.001$] and dose [$F_{(4,80)} = 10.49$, $P < 0.001$] and a significant dose × latency type interaction [$F_{(4,80)} = 3.25$, $P < 0.05$]. Post-hoc analyses showed that WIN 55,212-2, at the dose of 3 mg/kg, increased choice latency (advantageous and disadvantageous) compared with the vehicle condition (Fig. 4a). The effects of WIN

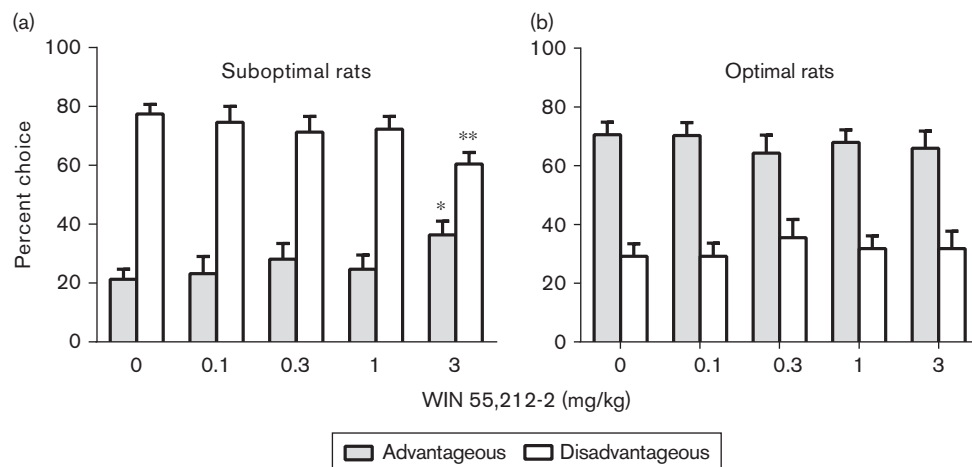
55,212-2 in the optimal group of rats were indicated by two-way ANOVA showing significant main effects of latency type [$F_{(1,24)} = 17.06$, $P < 0.001$] and dose [$F_{(4,96)} = 6.28$, $P < 0.001$], but no significant interactions [$F_{(4,96)} = 1.09$, NS] (Fig. 4b).

Pretreatments with URB 597, AM 4113 or AM 630 had no significant effects on choice or collect latencies in either optimal or suboptimal groups of rats (Table 6).

Effects of cannabinoid ligands on perseverative behaviour

Perseverative responses made during the punishment period were analysed as a fraction of the total punishment duration experienced in the suboptimal group. WIN 55,212-2 pretreatment exerted no significant effects on punishment and reward perseverative behaviour [$F_{(4,80)} = 1.95$, NS] (Fig. 5a). The CB_{1/2} agonist WIN 55,212-2 dose dependently increased punishment perseveration in the optimal group. Two-way ANOVA analysis showed a significant interaction between dose and perseverative response type [$F_{(4,96)} = 1.89$, $P < 0.05$]. Post-hoc analyses showed significant differences between vehicle

Fig. 3



Effects of the $CB_{1/2}$ agonist WIN 55,212-2 on choice behaviour in optimal and suboptimal groups of rats. The figure shows behaviour in the group of rats showing a preference for P_3 and P_4 (disadvantageous choices) under vehicle (suboptimal group) or in the group of rats showing a preference for P_1 and P_2 (advantageous choices) under vehicle (optimal group). Bars represent the average (\pm SEM) percentage of choice for the advantageous strategy (gray bars) and for the disadvantageous strategy (white bars) following the administration of the $CB_{1/2}$ agonist WIN 55,212-2 in suboptimal rats (a) and optimal rats (b). * $P < 0.05$, ** $P < 0.01$, versus vehicle, Fisher LSD post-hoc tests. The maximum number of pellets for each option is as follows: $P_1 = 295$, $P_2 = 411$, $P_3 = 135$ and $P_4 = 99$. The advantageous choice consisted of the percentage sum of responses on P_1 and P_2 , whereas the percentage sum of responses on P_3 and P_4 options was considered the disadvantageous choice. LSD, least significant difference.

Table 5 Effects of the FAAH inhibitor (URB 597), the CB_1 antagonist (AM 4113) and the CB_2 antagonist (AM 630) on decision-making during the rGT

Variable	Group	Factor	Doses (mg/kg)			
URB 597 Choice (%)	Optimal	Advantageous	Vehicle	0.1	0.3	1
		Disadvantageous	69.3 \pm 3.6	71.1 \pm 4.1	70.5 \pm 4.0	68.4 \pm 3.8
	Suboptimal	Advantageous	29.7 \pm 3.4	28.1 \pm 4.1	28.8 \pm 3.9	30.2 \pm 3.8
		Disadvantageous	29.4 \pm 3.8	29.8 \pm 6.6	31.2 \pm 7.1	30.3 \pm 6.4
AM 4113 Choice (%)	Optimal	Advantageous	Vehicle	1	3	10
		Disadvantageous	67.7 \pm 3.9	65.1 \pm 5.3	65.6 \pm 5.8	65.8 \pm 5.8
	Suboptimal	Advantageous	31.2 \pm 4.1	30.5 \pm 5.0	33.1 \pm 5.7	31.8 \pm 5.8
		Disadvantageous	26.3 \pm 4.9	29.8 \pm 4.8	30.6 \pm 4.5	23.5 \pm 4.7
AM 630 Choice (%)	Optimal	Advantageous	Vehicle	1.25	2.5	
		Disadvantageous	70.2 \pm 5.4	75.5 \pm 4.0	68.5 \pm 5.4	
	Suboptimal	Advantageous	28.4 \pm 5.2	23.8 \pm 3.9	29.9 \pm 5.2	
		Disadvantageous	29.9 \pm 4.6	31.2 \pm 5.2	31.5 \pm 4.8	73.8 \pm 4.2

Data represent the percentage of advantageous and disadvantageous strategies \pm SEM in the optimal and suboptimal groups. rGT, rat gambling task.

and the two higher doses of WIN 55,212-2 in the optimal group: under vehicle treatment, rats showed fewer punishment perseverative responses compared with WIN 55,212-2 treatment at the doses of 1 and 3 mg/kg ($P < 0.5$ and $P < 0.01$, respectively, Fig. 5b).

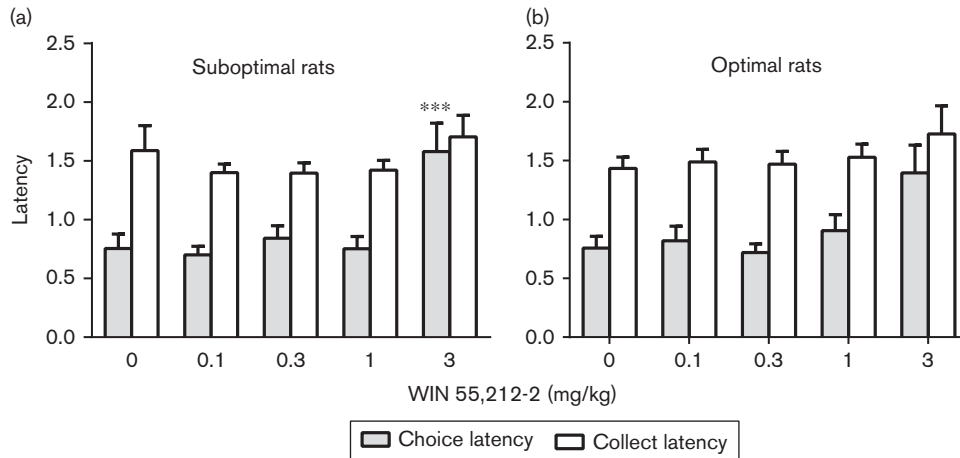
Perseverative responses (i.e. responses made after a reward was received) were analysed as a fraction of the total number of trials rewarded. No significant differences were observed in either the suboptimal or the optimal group (Fig. 5a and b).

Two-way ANOVA analysis showed no significant effects of URB 597, AM 4113 and AM 630 pretreatments on

punishment and reward perseverative behaviour in either group [suboptimal: $F_{(3,48)} = 0.47$, NS; $F_{(3,60)} = 1.07$, NS; $F_{(2,44)} = 1.05$, NS; optimal: $F_{(3,84)} = 1.61$, NS; $F_{(3,72)} = 0.39$, NS; $F_{(2,44)} = 3.03$, NS] (Table 7).

The cannabinoid drugs tested in this study had no significant effects on the premature response behaviour in either the optimal or the suboptimal group. The mean response times across treatments with WIN 55,212-2, URB 597, AM 4113 and AM 630 and vehicle control are shown in Table 8. Two-way ANOVA analysis comparing optimal and suboptimal groups showed no significant dose \times group interactions.

Fig. 4



Effects of the CB_{1/2} agonist WIN 55,212-2 on the latency to make a choice and collect latency in optimal and suboptimal groups of rats. Graphs show mean ± SEM for the latency to make a choice (gray bars) and the latency to collect the food reward (white bars) during the rGT following the administration of the CB_{1/2} agonist WIN 55,212-2 in the suboptimal group (a) (rats showing a preference for the disadvantageous strategy under vehicle treatment) and the optimal group (b) (rats showing a preference for the advantageous strategy under vehicle treatment). ****P* < 0.001 versus vehicle, Fisher LSD post-hoc tests. The advantageous choice consisted of the percentage sum of responses on P₁ and P₂, whereas the percentage sum of responses on P₃ and P₄ options was considered the disadvantageous choice. LSD, least significant difference.

Table 6 Effects of the FAAH inhibitor (URB 597), the CB₁ antagonist (AM 4113) and the CB₂ antagonist (AM 630) on choice and collect latency during the rGT

Variable	Group	Factor	Doses (mg/kg)				
URB 597 Latency	Optimal	Choice latency	Vehicle	0.1	0.3	1	
		Collect latency	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	
		Suboptimal	Choice latency	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
			Collect latency	1.52 ± 0.3	1.32 ± 0.2	1.25 ± 0.3	1.36 ± 0.2
AM 4113 Latency	Optimal	Choice latency	Vehicle	1	3	10	
		Collect latency	1.0 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	
		Suboptimal	Choice latency	1.9 ± 0.5	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.1
			Collect latency	1.00 ± 0.2	1.12 ± 0.2	1.28 ± 0.3	1.05 ± 0.1
AM 630 Latency	Optimal	Choice latency	Vehicle	1.25	2.5		
		Collect latency	0.9 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	1.4 ± 0.1	
		Suboptimal	Choice latency	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.2
			Collect latency	0.91 ± 0.2	0.93 ± 0.2	0.94 ± 0.1	1.68 ± 0.2

Data represent choice latency and collect latency ± SEM in the optimal and suboptimal group. rGT, rat gambling task.

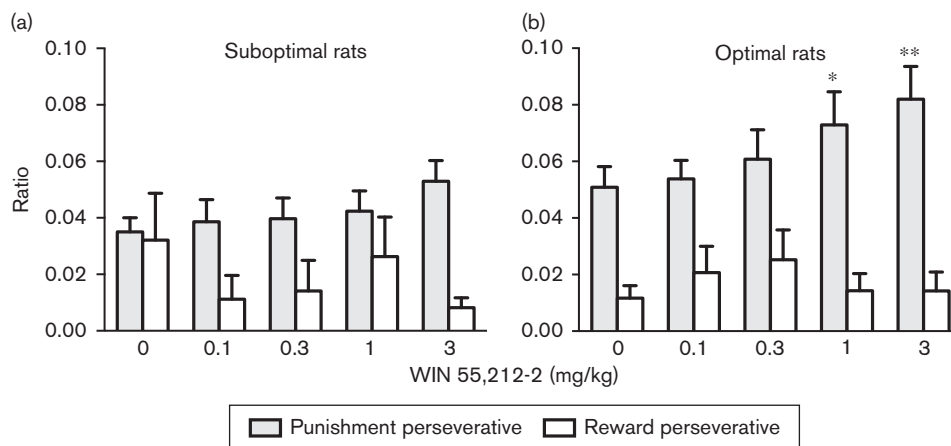
Discussion

In this study, we evaluated the effects of various drugs affecting the endocannabinoid system in a rodent model of decision-making under risk, the rGT, which is similar to the IGT, a human model of decision-making under risk (Zeeb *et al.*, 2009). The results show that the CB_{1/2} receptor agonist WIN 55,212-2 increased the preference for advantageous choices and decreased disadvantageous choices in rats showing Suboptimal response strategies at baseline. However, WIN 55,212-2 had no effect on choice preference in rats formerly showing optimal choice preferences. Administration of WIN 55,212-2 also increased the latency to make a choice in the suboptimal group of rats, and dose dependently increased punishment perseverance behaviour

in the optimal group. The CB₂ receptor antagonist tested (AM 630), the CB₁ antagonist (AM 4113) and the endocannabinoid hydrolysis inhibitor (URB 597) had no effects on choice performance and impulsive behaviour, as evaluated on rGT.

The logic behind the rGT procedure predicts that the advantageous choice produces the highest reward delivery (i.e. most number of pellets in a given time), despite the fact that each rewarded trial produces relatively few pellets. However, the disadvantageous option may appear to be more 'tempting' as it results in a higher number of pellets per trial, but overall, the number of pellets available in a session is fewer. Previous studies using the IGT have observed that pathological gamblers

Fig. 5



Effects of the CB_{1/2} agonist WIN 55,212-2 on punishment and reward perseverative behaviour in optimal and suboptimal groups of rats. Graphs represent mean (\pm SEM) number of punishment perseverative responses (gray bars) and reward perseverative responses (white bars), (a) in the group of rats showing a preference for the disadvantageous strategy under vehicle treatment (suboptimal group) and (b) in the group of rats showing a preference for the advantageous strategy under vehicle treatment (optimal group) following administration of the CB_{1/2} agonist WIN 55,212-2. * $P < 0.05$, ** $P < 0.01$ versus vehicle, Fisher LSD post-hoc tests. The advantageous choice consisted of the percentage sum of responses on P₁ and P₂, whereas the percentage sum of responses on P₃ and P₄ options was considered the disadvantageous choice. LSD, least significant difference.

Table 7 Effects of the FAAH inhibitor (URB 597), the CB₁ antagonist (AM 4113) and AM 630 on perseverative behaviours during the rGT

Variable	Group	Factor	Doses (mg/kg)				
URB 597	Perseverative behaviour	Optimal	Punishment perseverative	Vehicle	0.1	0.3	1
			Reward perseverative	0.07 \pm 0.01	0.06 \pm 0.01	0.06 \pm 0.01	0.06 \pm 0.01
		Suboptimal	Punishment perseverative	0.02 \pm 0.01	0.03 \pm 0.00	0.01 \pm 0.01	0.01 \pm 0.01
			Reward perseverative	0.06 \pm 0.01	0.05 \pm 0.01	0.06 \pm 0.01	0.06 \pm 0.01
AM 4113	Perseverative behaviour	Optimal	Punishment perseverative	Vehicle	1	3	10
			Reward perseverative	0.07 \pm 0.01	0.05 \pm 0.01	0.06 \pm 0.01	0.05 \pm 0.01
		Suboptimal	Punishment perseverative	0.09 \pm 0.08	0.03 \pm 0.01	0.02 \pm 0.01	0.06 \pm 0.03
			Reward perseverative	0.04 \pm 0.01	0.05 \pm 0.01	0.05 \pm 0.01	0.04 \pm 0.01
AM 630	Perseverative behaviour	Optimal	Punishment perseverative	Vehicle	1.25	2.5	
			Reward perseverative	0.06 \pm 0.01	0.07 \pm 0.02	0.05 \pm 0.01	
		Suboptimal	Punishment perseverative	0.02 \pm 0.01	0.02 \pm 0.01	0.03 \pm 0.01	
			Reward perseverative	0.04 \pm 0.01	0.05 \pm 0.01	0.06 \pm 0.01	

Data represent punishment and reward perseverative behaviour time \pm SEM in the optimal and suboptimal groups. rGT, rat gambling task.

Table 8 Effects of the CB₁ agonist (WIN 55,212-2), the FAAH inhibitor (URB 597), the CB₁ antagonist (AM 4113) and the CB₂ antagonist (AM 630) on premature response during the rGT

Variable	Factor	Doses					
WIN 55,212-2	Premature response	Vehicle	0.1	0.3	1	3	
		Optimal group	16.9 \pm 2.0	15.6 \pm 2.4	15.9 \pm 2.6	18.3 \pm 1.9	12.0 \pm 1.6
		Suboptimal group	16.2 \pm 2.1	16.1 \pm 2.7	17.1 \pm 3.0	17.9 \pm 3.1	10.8 \pm 3.1
URB 597	Premature response	Vehicle	0.1	0.3	1		
		Optimal group	12.6 \pm 2.3	13.0 \pm 1.6	12.2 \pm 1.8	12.4 \pm 1.9	
		Suboptimal group	9.7 \pm 2.2	12.7 \pm 2.7	12.9 \pm 3.0	9.9 \pm 2.1	
AM 4113	Premature response	Vehicle	1	3	10		
		Optimal group	12.8 \pm 2.7	7.7 \pm 1.6	9.2 \pm 1.8	7.5 \pm 1.5	
		Suboptimal group	14.7 \pm 2.1	14.3 \pm 2.7	10.4 \pm 2.4	13.1 \pm 3.4	
AM 630	Premature response	Vehicle	1.25	2.5			
		Optimal group	10.6 \pm 1.9	12.8 \pm 2.6	11.0 \pm 2.5		
		Suboptimal group	15.0 \pm 2.9	13.1 \pm 2.4	15.4 \pm 2.9		

Data represent the average premature response time \pm SEM in the optimal and suboptimal groups. rGT, rat gambling task.

show a greater preference for these risky options (Petry, 2001; Linnert *et al.*, 2006). In the present study, we could identify a subset of animals as a suboptimal group on the basis of their preference for disadvantageous versus advantageous choices. These animals may represent a phenotype akin to those at risk for GD.

Pretreatment with WIN 55,212-2 could improve choice strategy during the rGT in the formerly suboptimal group of animals. The improvement consisted of increased choice of smaller immediate gains, despite this suboptimal group of animals still showing a preference for disadvantageous strategies. This suggests that acute WIN 55,212-2 administration may improve the advantageous strategy in the group of rats that are prone to poor decision-making. Previous studies have shown that the effects of cannabinoids on impulsive decision-making are complex and even contradictory: the CB₁ antagonists rimonabant and AM 251 increase impulsive choice (Hernandez *et al.*, 2014), whereas no effects or decreased impulsive choice were observed with THC (McDonald *et al.*, 2003) and WIN 55,212-2 (Pattij *et al.*, 2007; Wiskerke *et al.*, 2011), respectively. Our results strengthen the notion that the acute administration of WIN 55,212-2 in rats prone to poor decision-making had specific benefits in the ability to balance rewards and punishments, which might contribute towards improvement of risky behaviours. These results are consistent with findings from other studies showing that pharmacological manipulations, aimed to enhance the endocannabinoid tone, resulted in increased self-control and reduced novelty-seeking behaviour (Marco *et al.*, 2007).

In humans, chronic marijuana use is associated with deficits in decision-making that impair the ability to make advantageous decisions over time (Whitlow *et al.*, 2004; Hermann *et al.*, 2009). Acute and long-term effects of CB₁ receptor agonists (such as THC) are associated positively with deficits in cognitive flexibility (Pope *et al.*, 2001; Bolla *et al.*, 2002; Lundqvist, 2005; Crean *et al.*, 2011). Specifically, deficits in cognitive flexibility were reported in chronic cannabis users (Pope *et al.*, 2001; Bolla *et al.*, 2002) and seem to be persistent after 28 days of cannabis abstinence (Bolla *et al.*, 2002). Our results provide a new insight in that the acute administration of a CB₁ agonist could improve the performance on the rGT task in rats prone to poor decision-making.

It should be noted that WIN 55,212-2 is a potent synthetic agonist that has some broader effects that could be relevant to these findings. Thus, it has been shown that WIN 55,212-2 has reinforcing effects of its own (Martellotta *et al.*, 1998; Fattore *et al.*, 2001; Lefever *et al.*, 2014) and can reinstate nicotine-seeking behaviour and enhance motivation for nicotine (Gamaledin *et al.*, 2012a). Therefore, the present results should be interpreted with caution. The effects of WIN 55,212-2 on reward and motivation may contribute towards the

findings observed in this study. Indeed, both dopaminergic and endocannabinoid systems have been implicated independently in the temporal control of behaviour (Pattij *et al.*, 2008; Narayanan *et al.*, 2012). The impulsive choice might be influenced by altered sensitivity to reinforcer magnitude (i.e. perceived rewarding properties), inappropriate perception of time (i.e. delay) or both (Ho *et al.*, 1999). Cannabinoid agonists have also been shown to alter time perception in humans (McDonald *et al.*, 2003) and in rats (Crystal *et al.*, 2003), and to enhance an aversive motivational state (Arguello and Jentsch, 2004).

However, we did not find significant changes in the choice preferences of the optimal and suboptimal groups following the blockade of CB₁ receptors with AM 4113. This finding is in agreement with the previously reported absence of effects of the CB₁ inverse agonist rimonabant in the 5-CSRTT (Pattij *et al.*, 2007). Similarly, the blockade of CB₂ receptors with AM 630 exerted no effects on choice preference in either optimal or suboptimal groups of rats.

It is interesting to note that the change in preference (in the suboptimal group) for more advantageous choice strategies, at the highest dose of WIN 55,212-2 tested, was also associated with higher latencies to make the choice. These findings are in agreement with previous studies showing that THC and WIN 55,212-2 increased response and collection latencies (Pattij *et al.*, 2007; Wiskerke *et al.*, 2011) as evaluated using 5-CSRTT. The increase in latency induced by cannabinoid agonists could be produced by effects on the locomotor response. In fact, exogenous cannabinoid receptors agonists such as THC have consistently been found to decrease locomotor activity (Herkenham, 1992). Similarly, increasing the neural levels of the endocannabinoid anandamide decreases locomotor activity, similar to exogenous cannabinoids (Romero *et al.*, 1995; de Lago *et al.*, 2004). It is possible that high doses of WIN 55,212-2 might produce some nonspecific motor impairment. Previous studies have shown that WIN 55,212-2 at the doses of 1 and 3 mg/kg significantly decreased response rates of rats trained under the nicotine discrimination task (Gamaledin *et al.*, 2012a). However, WIN 55,212-2 at the lower range of doses (i.e. 1 mg/kg) did not disrupt responding during reinstatement of nicotine-seeking behaviours (Gamaledin *et al.*, 2012a). The numbers of trials completed for the suboptimal group at the different doses of WIN 55,212-2 tested in this study (70 ± 4; 70 ± 4; 72 ± 5; 68 ± 5 and 76 ± 4 for the doses of 0, 0.1, 0.3, 1 and 3 mg/kg of, respectively) suggest that the effects of WIN 55,212-2 observed in the rGT latencies are not attributable to an impairment in locomotor activity. This notion is supported by the absence of changes in the number of omissions through all doses during the rGT (data not shown). Furthermore, in the present study, the effect of WIN 55,212-2 (3 mg/kg) to increase choice latency in the

suboptimal group was correlated positively with speed of decision-making. With respect to the latency data, the fact that animals showed the same reward-collection latency and that they were uniformly faster to collect larger rewards, both under vehicle and under WIN 55,212-2 (3 mg/kg) treatments, suggests that rats were not hypoactive in the presence of reward or differentially sensitive to changes in reward magnitude.

The absence of effects of URB 597 on choice and collection latencies is in agreement with previous studies showing no effects of URB 597 on locomotor activity (Jayamanne *et al.*, 2006; Adamczyk *et al.*, 2009). None of the cannabinoid drugs tested in this study could modify premature responding.

There are some limitations to the interpretation of the present results. We cannot rule out the possible existence of differences in the expression of CB_{1/2} receptors in the optimal versus suboptimal groups that could explain the differential effects of the drugs tested. Another limitation is the observation of increased latencies, particularly in the suboptimal group, which might be caused by a decreased motivation to obtain this particular reinforcer. A study of the motivation for food (i.e. food self-administration under a progressive ratio schedule in optimal vs. Suboptimal groups) may help to elucidate whether differences in motivation between groups could account for the observed increase in latency. An additional limitation of the present study, in terms of therapeutic relevance, is the use of a potent synthetic cannabinoid (WIN 55,212-2), which has different pharmacokinetic properties compared with THC.

In conclusion, the administration of a CB_{1/2} agonist improves choice performance in a suboptimal group of rats, as evaluated using the rGT. It is premature to propose that the stimulation of CB_{1/2} receptors may provide a treatment for gambling individuals prone to poor decision-making. Our study implicates the cannabinoid system in the processing of cost-benefit decision-making. As we used a systemic administration of cannabinoid agents, the observed effects might be because of the net result of the cannabinoid action in several brain regions expressing cannabinoid receptors. Future studies targeting discrete brain areas might help to further clarify the specific role of these on the effects of cannabinoids on decision-making. Our preliminary findings suggest that such a possibility should be explored further as it could result in a novel therapeutic strategy for GD.

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Conflicts of interest

There are no conflicts of interest.

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