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Cannabidiol reverses the reduction in social interaction produced by low dose Δ^9-tetrahydrocannabinol in rats

Daniel Thomas Malone *, Dennis Jongejan, David Alan Taylor

Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville 3052, Victoria, Australia

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ABSTRACT

While Δ^9-tetrahydrocannabinol (THC) is the main psychoactive constituent of the cannabis plant, a non-psychoactive constituent is cannabidiol (CBD). CBD has been implicated as a potential treatment of a number of disorders including schizophrenia and epilepsy and has been included with THC in a 1:1 combination for the treatment of conditions such as neuropathic pain. This study investigated the effect of THC and CBD, alone or in combination, on some objective behaviours of rats in the open field. Pairs of rats were injected with CBD or vehicle followed by THC or vehicle and behaviour in the open field was assessed for 10 min. In vehicle pretreated rats THC (1 mg/kg) significantly reduced social interaction between rat pairs. Treatment with CBD had no significant effect alone, but pretreatment with CBD (20 mg/kg) reversed the THC-induced decreases in social interaction. A higher dose of THC (10 mg/kg) produced no significant effect on social interaction. However, the combination of high dose CBD and high dose THC significantly reduced social interaction between rat pairs, as well as producing a significant decrease in locomotor activity. This data suggests that CBD can reverse social withdrawal induced by low dose THC, but the combination of high dose THC and CBD impairs social interaction, possibly by decreasing locomotor activity.

1. Introduction

Δ^9-tetrahydrocannabinol (THC) is the major psychoactive constituent of the plant *Cannabis sativa* and was first isolated around 65 years ago (Adams, 1942). There are also a number of non-psychoactive cannabinoids present in reasonable quantities such as cannabidiol (CBD) and cannabinol. Despite having a structure similar to that of THC, CBD does not produce the classic behavioural tetrad of effects associated with cannabinoids which is characterised by hypothermia, catalepsy, hypomotility and antinociception (Compton, 1992). This is not surprising as CBD is devoid of agonist activity at CB_1 and CB_2 receptors, although it does display some antagonist activity at micromolar concentrations (Petitet et al., 1998). A 1:1 mixture of THC and CBD (Sativex®) has been developed recently and has been approved in Canada for the treatment of neuropathic pain associated with multiple sclerosis (Barnes, 2006). This necessitates the need to investigate in more detail the interaction between these 2 cannabinoids in preclinical studies. In addition, potential interactions between CBD and THC could have important implications for understanding consequences of cannabis use.

A number of studies have investigated the behavioural effects of combining THC and CBD in rodents with variable results. In mice, CBD has been shown to potentiate the antinociceptive effects and reduce the cataleptic effects of THC (Karniol and Carlini, 1973). Another mouse study showed that high doses of CBD were required to potentiate the antinociceptive effects of a low dose of THC, with no significant effects on other parameters measured including hypoactivity, catalepsy and hypothermia (Varvel et al., 2006). In rats, the cataleptic and hypothermic effects of THC were potentiated by CBD (Fernandes et al., 1974). More recently it was shown that 10 or 50 mg/kg doses of CBD exacerbated the hypoactivity, hypothermia and impairment of spatial memory effects of a 1 mg/kg dose of THC (Hayakawa et al., 2008). Moreover, CBD (50 mg/kg) with THC (1 mg/kg) enhanced the level of CB_1 receptor expression in the hippocampus and hypothalamus. On the other hand the depressant effects of THC on operant responding have been shown to be inhibited by a high dose of CBD in rats (Davis and Borgen, 1974).

Social interaction is a normal animal behaviour that is displayed when a rat investigates the presence of and communicates with another rat via physical cues. Social interaction can be measured by placing two rats in an open arena and observing events such as time spent in proximity to each other and number of interactions, as well as more interactive events such as climbing and biting. A decrease in this normal social interaction may occur in states such as anxiety and social withdrawal and was initially used to screen for anxiogenic and anxiolytic drug activity (File and Hyde, 1978; File and Seth, 2003). More recently social withdrawal has also become an accepted animal model for negative symptoms of schizophrenia, as it may be induced by psychotomimetic drugs that produce negative symptoms such as

* Corresponding author. Tel.: +61 3 99039576; fax: +61 3 99039638.
E-mail address: daniel.malone@pharm.monash.edu.au (D.T. Malone).

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phencyclidine (PCP) and MK-801 when tested in low light levels (Rung et al., 2005b).

Previous research has shown that acute doses of THC produce differential effects on social interaction depending on the dose given. High doses tend to decrease nearly all social interactions, whilst low doses have been reported to decrease some behavioural measures of social contact but not others (van Ree et al., 1984). In the same study CBD was shown to have no effect on social interaction when given alone. Acute treatment with the synthetic CB_1 receptor agonist CP 55940 decreased social interaction at doses that also decreased locomotor activity, but not at lower doses (Genn et al., 2004). Recently it was reported that in adolescent rats, the CB_1 receptor agonist WIN 55212-2 reduced social play, while interestingly the inhibitor of endocannabinoid hydrolysis, URB 597 enhanced social play (Trezza and Vanderschuren, 2008a). In light of previous research showing THC can alter social interaction and that CBD can modify some effects of THC, the present experiment was conducted to examine the effects of THC and CBD on social interaction in a low anxiety environment (i.e. an environment in which rats had been acclimatised to and which was lit by low levels of light).

2. Material and methods

2.1. Animals and housing

Male Sprague–Dawley rats weighing between 150 and 260 g (aged between 45 and 52 days) were used. The rooms in which the animals were kept were maintained at an ambient temperature of 22 °C on a 12 h reversed light–dark cycle (lights on at 2200). Acclimatization to the reversed light–dark cycle started 4 days prior to the first day of acclimatization of the rats in the open field. Before any of the rats received an injection they were housed in group cages with 6 rats per cage. Food and drinking water were available *ad libitum*. The experimental protocol was approved by the Victorian College of Pharmacy, Monash University Animal Ethics Committee and conforms to the guidelines set out by the National Health and Medical Research Council of Australia and all government regulations.

2.2. Drug treatment

The following drugs were used: THC (Sigma, USA) and CBD (a generous gift from GW Pharmaceuticals, UK). THC was dissolved in a 1:1 mixture of Tween 80® and absolute ethanol, and diluted with saline to a final ratio of 1:1:98 Tween 80®: ethanol: saline (T:E:saline). CBD was dissolved in a 1:1 mixture of cremophor and absolute ethanol, and diluted with saline to a final ratio of 1:1:18 Cremophor®: ethanol: saline (C:E:saline).

Separate groups of rats were taken from their group cages and injected i.p. with CBD (5 or 20 mg/kg) or vehicle (C:E:saline) followed 20 min later by an i.p. injection of THC (1–10 mg/kg) or vehicle (T:E:saline). Following each injection and prior to placement in the open arena, rats were placed in individual cages. All rats were exposed to one treatment combination only and received the same number of injections, ensuring that appropriate vehicle controls were present. In all groups there were 6 pairs of rats used.

2.3. Behavioural testing

Social interaction was measured during the dark phase in a black open arena measuring 78 cm × 79 cm × 18 cm. The arena was in a soundproof ventilated room. A black light fluorescent lamp was suspended 1 m above the arena to provide illumination for videotaping purposes. The light level in the test arena was 6.0 lx. A Sony Digital Video Camera Recorder with a Sony Video Lens/Optical 20× was used to record the session. The camera was placed 226 cm above the open field and connected to the computer via a USB port.

During the dark phase, animals were acclimatised in the open arena for a daily 10 min session on the four consecutive days leading up to the test day. Each day an unfamiliar pair of rats that were not cage mates and had not received prior physical or visual exposure to one another were placed in the arena for 10 min and allowed to move freely. The arena was cleaned with water and ethanol between the placement of each pair of rats. On the fifth day, an unfamiliar, weight-matched pair of rats received two injections. 20 min after the second injection, rats were removed from their individual cages and placed in opposite corners of the open arena. Video recording of the open arena commenced immediately after placement of the rats in the arena, and ceased after 10 min had elapsed.

The recordings were then analysed by "Spontaneous Motor Activity Recording & Tracking" (SMART®) video tracking software (San Diego Instruments, San Diego, U.S.A.). The resulting 10 min tracks describing the rats' position (taken from the centres of gravity of each rat) every 0.04 s (25 times/s) were exported to a Microsoft Excel® spreadsheet for data analysis.

Previously, social interaction has been calculated by an automated tracking system based on the assumption that the level of social behaviour correlates to the time that rats are in close proximity to each other (Sams-Dodd, 1998). In order to determine the distance between rat pairs that should constitute a social encounter, behaviors of rats indicative of social interaction such as mounting, sniffing, following and grooming were scored manually for 4 treatment groups (24 rat pairs). The scoring was performed by an observer who was blind to the treatment groups. Recordings from the same treatment groups were then analysed to determine the value of parameters used to define an interaction which gave the best correlation with results obtained from manual tracking (as determined by a linear regression analysis, see Table 1). The parameter used to define whether rats were in close enough proximity to interact that was altered was the distance between centre of gravity of rat pairs (7.5, 10, 15, 20 or 25 cm). This distance, coupled with the velocity of at least 1 rat (0.1, 0.25, 1, 2 or 5 cm/s) was used to determine whether an interaction was active or passive, based on whether the rats were moving or not. These parameters were measured every 2 s. From this analysis, an encounter was defined as when the video tracking software detected that the centre of gravity of each rat was within 10 cm of the other rat. These encounters were subsequently divided into active of passive encounters based on whether at least one rat had a velocity of greater (active) or less than (passive) 0.25 cm/s. The division of encounters as measured by automated methods into active and passive encounters has been described previously (Sams-Dodd, 1996). The time that rats spent moving towards each other and time that one rat spent moving towards the other stationary rat was also calculated.

2.4. Statistical analysis

A two way ANOVA with two fixed factors, pre-treatment (CBD or vehicle) and THC (THC or vehicle), was used to measure differences in behavioural scores between treatment groups. If a main effect of treatment (CBD or THC) or a CBD × THC interaction was detected

Table 1
Correlation coefficients (r values) of active social interaction encounters as measured by manual tracking of behaviours vs active encounters as measured by an automated method.

		Velocity (cm/s)				
		0.1	0.25	1	2	5
Distance apart (cm)	7.5	0.822	0.808	0.779	0.740	0.701
	10	0.823	0.825	0.805	0.763	0.695
	15	0.745	0.753	0.737	0.697	0.638
	20	0.708	0.716	0.714	0.672	0.611
	25	0.684	0.687	0.698	0.657	0.598

For the automated method, the distance between the centres of gravity of rat pairs that defined the rats as being close enough to interact was varied between 7.5 cm and 25 cm. The velocity at which at least one rat was moving in order to define an active interaction was varied between 0.1 and 5 cm/s.

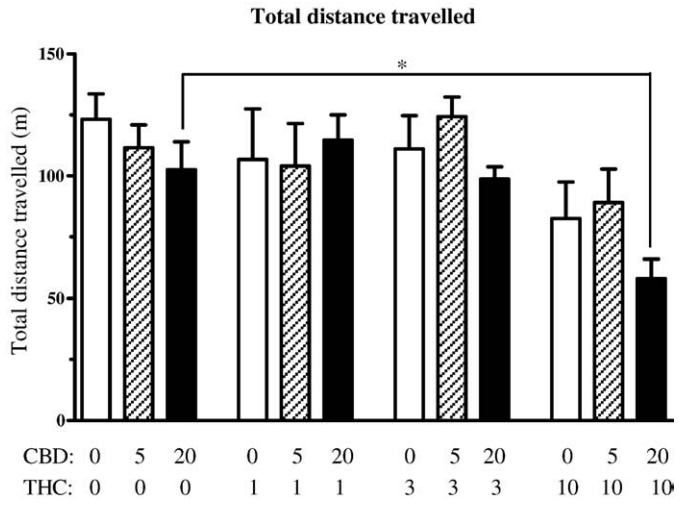

Fig. 1. Effect of CBD (0–20 mg/kg) and THC (0–10 mg/kg) on total distance travelled (*$p<0.05$). Data is expressed as Mean + S.E.M.

($p<0.05$), a one way ANOVA and post-hoc Tukey's multiple comparison test was used to compare differences in the relevant treatment groups.

A Linear Regression analysis was performed in order to determine which automated software parameters to determine active interactions (distance apart of the rats' centres of gravity and the velocity of at least one rat) gave the best correlation with time spent in active interaction as obtained from manual tracking.

3. Results

3.1. Total distance travelled

Over the 10 min observation period the total distance travelled by pairs of Vehicle + Vehicle treated rats was approximately 123 m (Fig. 1). A two way ANOVA revealed a significant effect of THC on total distance travelled ($F(3,60)=5.536$, $p<0.01$). A subsequent one way ANOVA revealed a significant decrease in total distance travelled of rats treated with 20 mg/kg CBD + 10 mg/kg THC compared with rats treated with 20 mg/kg CBD + Vehicle ($F(3,20)=7.455$, post hoc Tukey's test, $p<0.05$). This indicates that the combination of 20 mg/kg CBD and 10 mg/kg THC was able to significantly reduce total distance travelled compared with 20 mg/kg CBD + Vehicle.

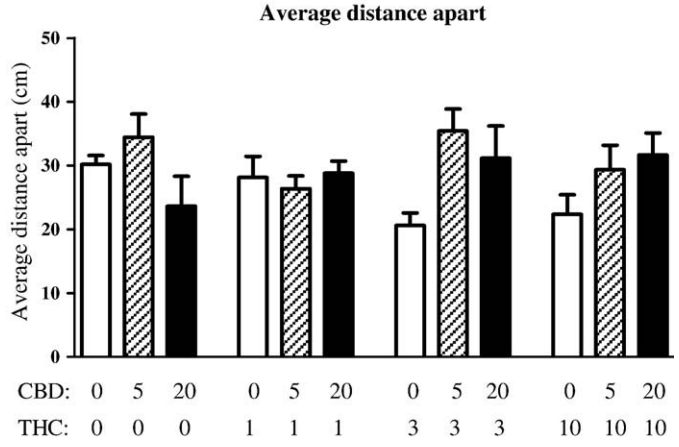

Fig. 2. Effect of CBD (0–20 mg/kg) and THC (0–10 mg/kg) on average distance apart. Data is expressed as Mean + S.E.M.

3.2. Average distance apart

The average distance apart was approximately 30 cm for pairs of Vehicle + Vehicle treated rats (Fig. 2). A two way ANOVA revealed a significant effect of CBD on the average distance apart ($F(2,60)=3.258$, $p<0.05$). Subsequent one way ANOVAs performed revealed no significant differences in CBD treated rats compared with Vehicle treated rats.

3.3. Comparison between manual and automated determination of social interactions

There was a significant positive correlation between the social interaction determined by manually counting interaction events and the time spent in active interaction as determined by the automated video tracking software (Table 1). The best correlation as indicated by the highest correlation coefficient was when the distance between the centres of gravities of each rat pair that defined an interaction was set at 10 cm, and to be considered an active interaction the velocity of at least one rat was greater than 0.25 cm/s ($r=0.825$, $p<0.0001$).

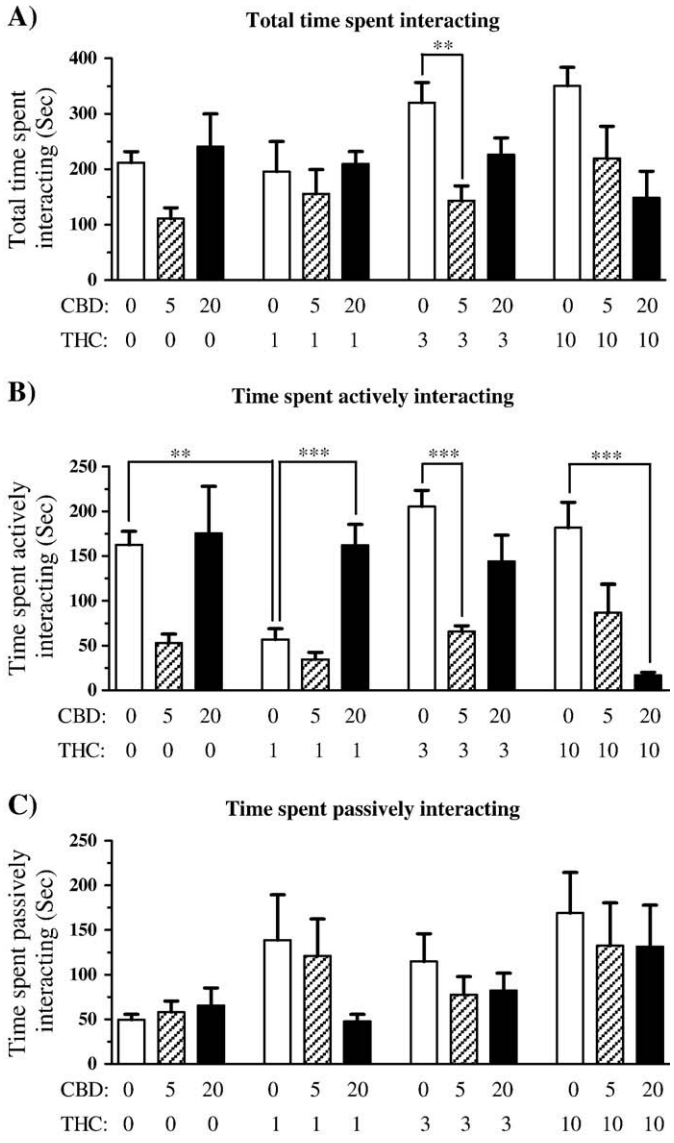

Fig. 3. Effect of CBD (0–20 mg/kg) and THC (0–10 mg/kg) on A) Total time spent interacting, B) Time spent actively interacting and C) Time spent passively interacting (**$p<0.01$, ***$p<0.001$). Data is expressed as Mean + S.E.M.

3.4. Total time spent interacting

A two way ANOVA revealed a significant effect of CBD ($F(2,60) = 7.814$, $p<0.001$; Fig. 3A) on total time spent interacting as well as a significant CBD×THC interaction ($F(6,60) = 2.280$, $p<0.05$). A subsequent one way ANOVA performed at each dose of THC and CBD revealed that rats treated with 5 mg/kg CBD + 3 mg/kg THC spent significantly less time actively interacting than rats treated with Vehicle + 3 mg/kg THC ($F(2,15) = 7.892$, post hoc Tukey's test, $p<0.01$).

3.5. Time spent actively interacting

A two way ANOVA revealed a significant effect of CBD ($F(2,60) = 15.28$, $p<0.001$; Fig. 3B) and THC ($F(3,60) = 3.576$, $p<0.05$) on time spent actively interacting as well as a significant CBD × THC interaction ($F(6,60) = 6.918$, $p<0.001$). A one way ANOVA performed at each dose of THC and CBD revealed that rats treated with Vehicle + 1 mg/kg THC spent significantly less time actively interacting than rats treated with Vehicle + Vehicle ($F(3,20) = 11.28$, post hoc Tukey's test, $p<0.01$). Rats treated with Vehicle + 1 mg/kg THC spent significantly less time actively interacting than rats treated with 20 mg/kg CBD + 1 mg/kg THC ($F(2,15) = 18.26$, post hoc Tukey's test, $p<0.001$). Rats treated with 5 mg/kg CBD + 3 mg/kg THC spent significantly less time actively interacting than rats treated with Vehicle + 3 mg/kg THC ($F(2,15) = 12.02$, post hoc Tukey's test, $p<0.001$). Rats treated with CBD 20 + 10 mg/kg THC spent significantly less time interacting than rats treated with Vehicle + 10 mg/kg THC ($F(2,15) = 11.11$, post hoc Tukey's test, $p<0.001$).

This indicates that rats treated with low dose (1 mg/kg) THC spent significantly less time actively interacting and that this decrease was attenuated by pretreatment with 20 mg/kg CBD. In addition, the combination of 20 mg/kg CBD and 10 mg/kg THC significantly decreased time spent actively interacting.

3.6. Time spent passively interacting

A two way ANOVA revealed a significant effect of THC ($F(3,60) = 3.486$, $p<0.05$; Fig. 3C) on time spent passively interacting. However, when one way ANOVAs and post-hoc Tukey's tests were performed, no significant differences were observed.

3.7. Time rats spent moving towards each other

A two way ANOVA revealed a significant effect of THC ($F(3,60) = 12.79$, $p<0.001$; Fig. 4) on time rats spent moving towards each other as well as a significant CBD × THC interaction ($F(6,60) = 3.616$, $p<0.01$). A one way ANOVA performed at each dose of THC and CBD revealed that rats treated with Vehicle + 1 mg/kg THC and rats treated with Vehicle + 10 mg/kg THC both spent significantly less time moving towards each other than rats treated with Vehicle + Vehicle ($F(3,20) = 5.634$, post hoc Tukey's test, Vehicle + 1 mg/kg THC vs Vehicle + Vehicle $p<0.05$, Vehicle + 10 mg/kg THC vs Vehicle + Vehicle $p<0.01$). Rats treated with 20 mg/kg CBD + 10 mg/kg THC spent significantly less time moving towards each other than rats treated with CBD 20 mg/kg + Vehicle ($F(3,20) = 11.27$, post hoc Tukey's test, $p<0.01$).

This indicates that rats treated with either 1 or 10 mg/kg THC spent less time moving toward each other than Vehicle treated rats, and this effect of THC was not altered by pretreatment with CBD. In addition, rats treated with the combination of high dose CBD (20 mg/kg) and THC (10 mg/kg) spent less time moving toward each other than rats treated with high dose CBD only.

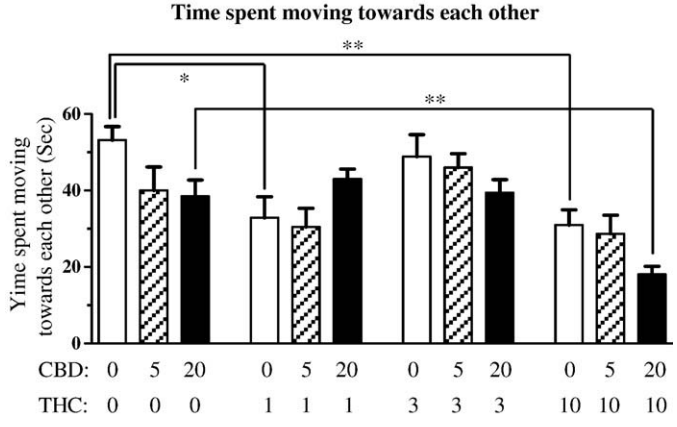

Fig. 4. Effect of CBD (0–20 mg/kg) and THC (0–10 mg/kg) on time rats spent moving towards each other (*$p<0.05$, **$p<0.01$). Data is expressed as Mean + S.E.M.

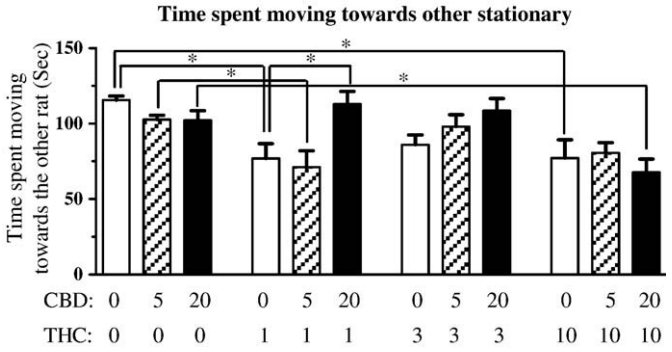

Fig. 5. Effect of CBD (0–20 mg/kg) and THC (0–10 mg/kg) on time one rat spent moving towards the other rats that was stationary (*$p<0.05$). Data is expressed as Mean + S.E.M.

3.8. Time that one rat spent moving towards the other stationary rat

A two way ANOVA revealed a significant effect of THC ($F(3,60) = 8.532$, $p<0.001$; Fig. 5) on the time that one rat spent moving towards the rat that was stationary as well as a significant CBD × THC interaction ($F(6,60) = 3.174$, $p<0.01$). A one way ANOVA performed at each dose of THC and CBD revealed that rats treated with Vehicle + 1 mg/kg THC and rats treated with Vehicle + 10 mg/kg THC both spent significantly less time with 1 rat moving towards the stationary rat than rats treated with Vehicle + Vehicle ($F(3,20) = 4.514$, post hoc Tukey's test, both $p<0.05$). Rats treated with 5 mg/kg CBD + 1 mg/kg THC spent significantly less time with 1 rat moving towards the stationary rat than rats treated with CBD 5 mg/kg + Vehicle ($F(3,20) = 3.702$, post hoc Tukey's test, $p<0.05$). Rats treated with 20 mg/kg CBD + 10 mg/kg THC spent significantly less time of 1 rat was moving towards the stationary rat than rats treated with CBD 20 mg/kg + Vehicle ($F(3,20) = 6.628$, post hoc Tukey's test, $p<0.05$). Rats treated with 20 mg/kg CBD + 1 mg/kg THC spent a significantly greater amount of time of 1 rat moving towards the stationary rat than rats treated with Vehicle + THC 1 mg/kg ($F(3,20) = 5.378$, post hoc Tukey's test, $p<0.05$).

This indicates that rats treated with either 1 or 10 mg/kg THC had a reduced time during which one rat was moving towards the other rat when it was stationary than Vehicle treated rats. This effect was reversed by pretreatment with 20 mg/kg CBD in rats treated with 1 mg/kg THC but not in rats treated with 10 mg/kg THC.

4. Discussion

The present study investigated the effect of the cannabinoids THC and CBD on objective behaviours of rat pairs in the open field such as distance travelled, average distance apart and time spent actively and

passively interacting with each other. Initially, the ability of the automated video tracking software to measure social interactions was correlated with manually scored social interactions (e.g. sniffing, grooming) in order to determine the maximum distance between the centres of gravity of rats that was used to define an interaction, and the velocity of movement of at least one rat that above which should constitute an active interaction. A regression analysis revealed that defining an active interaction between rat pairs as when their centres of gravity were less than 10 cm apart and had a velocity threshold of 0.25 cm/s gave the best correlation with manually scored social interactions (see Table 1). This is in keeping with previous studies that have defined a social interaction as when rats' centres of gravity came within 20 cm of each other (Rung et al., 2005a,b; Sams-Dodd, 1995a,b). Furthermore, in other research by Sams-Dodd, a passive encounter was defined as when the centres of gravity of rats were within 20 cm of each other and they were moving at less than 1–3 cm/s (Sams-Dodd, 1996).

The observation that rats treated with the highest dose of CBD and THC used (20 mg/kg and 10 mg/kg respectively) had a decrease in total distance travelled, indicates a decrease in locomotor activity. In addition, the same rats had a decrease in social interaction, as measured by a dramatic decrease in time spent actively interacting with each other, a significant decrease in time spent moving towards each other and time that one rat spent moving towards the other rat when it was stationary. Neither rats treated with 10 mg/kg THC nor 20 mg/kg CBD alone had such decreases in total distance travelled or in social interaction. It is well known that THC can produce catalepsy in rats (Hayakawa et al., 2008; Lichtman and Martin, 1997; Sano et al., 2008; Tseng and Craft, 2004), an effect which would result in a decrease in locomotor activity. Whilst there are no known reports of CBD producing catalepsy, there have been a number of reports of high doses of CBD potentiating some effects of THC. For example, the cataleptic and hypothermic effects of THC were potentiated by CBD (Fernandes et al., 1974) and CBD (10 or 50 mg/kg) exacerbated the hypoactivity, hypothermia and impairment of spatial memory produced by a 1 mg/kg dose of THC in rats (Hayakawa et al., 2008). Similar potentiation of CBD on THC-induced behaviours have been demonstrated in mice (Carlini et al., 1974; Karniol and Carlini, 1973; Varvel et al., 2006) and humans (Carlini et al., 1974). It has been previously reported that high doses of CBD can increase levels of THC in the brain by inhibiting the metabolism of THC in the liver by inhibiting the cytochrome P-450 oxidative system (Bornheim and Grillo, 1998; Bornheim et al., 1995; Jones and Pertwee, 1972). Thus, it is possible that this could be the reason why in the present study the high dose THC: CBD combination produced a decrease in distance travelled when neither drug produced a significant effect on their own. It is also possible that this could be the reason why a decrease in social interaction was seen in rats treated with high doses of both THC and CBD.

Few significant differences were observed between treatment groups with respect to total time interacting (see Fig. 3A). However, when total time spent interacting was divided into active and passive interactions based on whether at least one rat was moving, a number of significant differences in active interactions between groups became apparent (see Fig. 3B). This highlights the importance of differentiating between active and passive encounters, as only relying upon the parameter of rats coming within a certain distance of each other as being social interaction time may confound results.

Rats treated with the lowest dose of THC used (1 mg/kg) spent less time actively interacting with each other. In addition, these rats spent less time moving towards each other and less time when one rat was moving towards the other stationary rat when compared to vehicle treated rats. The decreased time spent in active interaction is in agreement with previous studies that reported a decrease in social interaction following administration of the CB_1 receptor agonists THC (van Ree et al., 1984), CP 55,940 (Genn et al., 2004) and WIN 55212-2 (Trezza and Vanderschuren, 2008a,b). A decrease in social interaction in rat pairs produced by psychotomimetic drugs has been proposed as an animal model of negative symptoms of schizophrenia (Rung et al., 2005b). Given the propensity of cannabinoids to induce psychotic-like symptoms in rats (Dissanayake et al., 2008; Hajos et al., 2008), a decrease in social interaction was hypothesised from these experiments. However, this was not a dose-dependent effect as a decrease in active interactions was not observed following treatment with the higher doses of THC used (3 and 10 mg/kg).

The decrease in active social interaction produced by injection of 1 mg/kg THC was reversed by pretreatment with 20 mg/kg CBD. In addition, the decrease in time rats spent moving towards each other induced by 1 mg/kg THC was partially reversed by pretreatment with 20 mg/kg CBD, and the decrease in time that one rat was moving towards the stationary rat produced by injection with 1 mg/kg THC was also reversed in rats pretreated with 20 mg/kg CBD. Whilst CBD can potentiate some effects of THC as discussed above, there are also reports of CBD inhibiting some effects of THC. For example, the depressant effects of THC on operant responding have been shown to be reversed by a high dose of CBD in rats (Davis and Borgen, 1974) and suppression of the abdominal constriction response to formic acid induced by THC was reversed by CBD (Welburn et al., 1976). In addition, it has been shown that varying the ratio of THC: CBD can either antagonise or potentiate the effects of THC on variable interval performance (Zuardi and Karniol, 1983). As mentioned above, high doses of CBD have been reported to increase levels of THC in the brain by inhibiting the metabolism of THC in the liver (Bornheim and Grillo, 1998; Bornheim et al., 1995; Jones and Pertwee, 1972). Rats treated with 3 mg/kg THC had no differences from vehicle treated rats with regard to active interactions, time spent moving toward each other or time that one rat spent moving towards the other stationary rat. Thus it is possible that the reason rats treated with 20 mg/kg CBD and 1 mg/kg THC also had no significant effect compared with vehicle treated rats is that brain levels of THC were increased as a result of pretreatment with 20 mg/kg CBD to levels similar to that produced by 3 mg/kg THC. Quantification of the amount of THC in the CNS would be required to confirm this hypothesis.

The present study shows how the decrease in social interaction produced by low dose of THC (1 mg/kg) can be reversed by pretreatment with CBD. Whilst higher doses of THC had no significant effect on social interaction, the combination of 20 mg/kg CBD and the highest dose of THC used (10 mg/kg) produced a synergistic effect in terms of decreasing time spent actively interacting and decreased time spent moving towards each other. This combination also produced a decrease in locomotor activity, which may explain the decrease in social behaviours observed.

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