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by Brittany Ford

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Abbreviations

AC: adenylyl cyclase

cAMP: cyclic adenosine monophosphate

CPA: conditioned place avoidance

CRF: corticotropin-releasing factor

EPM: elevated plus maze

i.p.: intraperitoneally

M-VEH: morphine vehicle (saline)

MOR: morphine

MOR+NAL-WD: naloxone-precipitated morphine withdrawal

N-VEH: naloxone vehicle

NAL: naloxone

PAG: periaqueductal gray

S-VEH: SR141716 vehicle (TWEEN-80/saline)

SR: SR141716

T-VEH: THC vehicle (TWEEN-80/saline)

THC: Δ^9-tetrahydrocannabinol

THC+SR-WD: SR141716-precipitated THC withdrawal

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Abstract

The selective cannabinoid CB1 receptor antagonist SR141716 has been shown to precipitate physical signs of withdrawal in Δ^9-tetrahydrocannabinol (THC)-dependent rats; however, the affective state associated with this withdrawal state has not yet been well characterized. Thus, the aim of present study was to examine the physical and affective consequences of SR141716-precipitated THC withdrawal in male Sprague-Dawley rats. Rats were injected with THC (5 mg/kg, i.p.) or its vehicle twice daily for 13 consecutive days, and challenged with SR141716 (1 mg/kg, i.p.) or its vehicle 1 h later on days 3, 5, 7, 9, 11, and 13. Consistent with previous reports, SR141716 induced signs of physical withdrawal (e.g., increased scratching) in THC-dependent animals. The affective state induced by both SR141716-precipitated THC withdrawal and naloxone-precipitated morphine withdrawal were then assessed using a tactile cue-conditioning paradigm, and withdrawal-induced anxiety was measured using a test battery consisting of the emergence test, elevated plus maze (EPM), and social interaction test. Precipitated morphine withdrawal induced both significant conditioned cue avoidance and anxiogenic-like behaviour; however, precipitated THC withdrawal failed to produce a conditioned cue avoidance, and did not induce anxiety in a manner different from that produced by administration of THC alone. These findings provide novel evidence that unlike opiate withdrawal, cannabinoid withdrawal manifests physical signs of withdrawal, but does not induce anxiety or a dysphoric state. Although there may be overt physical similarities between opiate and cannabinoid withdrawal, these syndromes likely represent distinct emotional and subjective states.

Marijuana is indisputably one of the most widely used illicit drugs, with 32.7% of the Canadian population having tried it more than once (Health Canada, 2008). Its use is especially prevalent among adolescents. A recent Health Canada survey revealed that 60% of 15-24-year-old individuals reported using marijuana at least once, and nearly 1 in 10 of those individuals reported using it on a daily basis (Health Canada, 2007). Despite the large number of regular users, there is contention whether marijuana produces physical dependence in which cessation results in a withdrawal syndrome typified by physical and emotional distress—a characteristic of almost all drugs of abuse. Laboratory and clinical studies have reliably demonstrated withdrawal syndromes using psychostimulants, narcotics, ethanol, and nicotine (Young & Herling, 1986; Yokel, 1987), but studies using Δ^9-tetrahydrocannabinol (THC)—the main psychoactive constituent of marijuana—or other synthetic cannabinoids, have been less convincing.

Currently, a cannabis withdrawal syndrome is not included in the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV*, 2000*)*, which states that this syndrome is of limited clinical significance. However, human studies of cannabis withdrawal provide conflicting evidence. Inpatient studies, dating back more than 60 years, showed a variety of signs following abrupt discontinuation of marijuana smoking or oral THC, including sleep disturbances, mood changes, decreased appetite, and nausea (Haney, Ward, Comer, Foltin, & Fischman, 1999a; Haney, Ward, Comer, Foltin, & Fischman, 1999b; Jones & Benowitz, 1976; Jones, Benowitz, & Bachman, 1976; Williams, Himmelsbach, Wikler, Ruble, & Lloyd, 1946). Nevertheless, other similar inpatient studies did not observe withdrawal effects after abrupt cessation from marijuana, oral THC, or hashish (Greenberg, Mendelson, Kuehnle, Mello, & Babor,

1976; Stefanis, Liakos, Boulougouris, Dornbush, & Ballas, 1976). Retrospective and outpatient studies have yielded more consistent results, reporting many of the same withdrawal signs as inpatient studies, in frequent marijuana users when they abstained from smoking marijuana (Budney, Novy, & Hughes, 1999; Budney, Hughes, Moore, & Novy, 2001; Budney, Moore, Vandrey, & Hughes, 2003; Kouri & Pope, 2000; Wiesbeck, Schuckit, Kalmijn, Tipp, Bucholz, & Smith, 1996). Recent reviews of the cannabis withdrawal literature have attempted to assimilate the findings to better characterize a cannabinoid withdrawal syndrome, but even these articles disagree. Two reviews concluded that a withdrawal syndrome in humans is reliable, valid, and clinically important (Budney, Hughes, Moore, & Vandrey, 2004; Lichtman & Martin, 2002). Moreover, one review likened cannabis withdrawal to tobacco withdrawal and proposed diagnostic criteria for the syndrome (Budney et al., 2004). Smith (2002), on the other hand, concluded that strong evidence base is lacking for a cannabis withdrawal syndrome and that cannabis does not appear to induce a distinct withdrawal pattern in a manner similar to other drugs of abuse. Although they are ultimately opposed, the reviews agree that several relevant areas of cannabis withdrawal have yet to be explored and that more controlled research would be useful. Animal studies offer greater experimental control, but because human studies provide few objective measures of cannabis withdrawal and rely heavily on subjective accounts, animal studies have had little direction for examining somatic withdrawal signs.

Early animal studies of cannabis withdrawal also yielded mixed results. Studies of rhesus monkeys withdrawing from chronic intravenous, intramuscular, or oral THC found transient signs, including aggression, anorexia, irritability, hair-pulling, and

scratching (Beardsley, Balster, & Harris, 1986; Fredericks & Benowitz, 1980; Kaymakcalan, 1973; Stadnicki, Schaeppi, Rosenkrantz, & Braude, 1974). One study using rhesus monkeys, however, failed to observe any withdrawal signs following abrupt discontinuation of repeated intravenous THC (Harris, Waters, & McLendon, 1974). Studies using rats, dogs, and pigeons also failed to observe THC withdrawal signs (Leite & Carlini, 1974; Dewey, Jenkins, O'Rourke, & Harris, 1972; McMillan, Dewey, & Harris, 1971; McMillan, Harris, Frakenheim, & Kennedy, 1970). These inconsistent findings have been attributed to methodological difficulties associated with using an spontaneous withdrawal procedure. THC is highly lipophilic and, accordingly, has a long half-life, causing withdrawal symptoms to be delayed and difficult to quantify (Wall, Sadler, Brine, Taylor, & Perez-Reyes, 1983).

Discovery of the endogenous cannabinoid system and identification of cannabinoid receptors signified a major advancement in cannabinoid research. The involvement of the endogenous cannabinoid system in cognition, appetite, pain perception, and motor regulation has served to clarify the actions of cannabinoids. Two cannabinoid receptors have been cloned: the CB1 cannabinoid receptor (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990), which is abundant in the brain, and the CB2 cannabinoid receptor (Munro, Thomas, & Abu-Shaar, 1993), which is localized primarily outside the central nervous system. Several lines of evidence suggest that the CB1 cannabinoid receptor is responsible for the central effects of cannabinoids, one source of evidence being the development and effectiveness of the selective CB1 receptor antagonist SR141716 (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1 *H*-pyrazole carboxamide HCl; Rinaldi-Carmona et al., 1994). SR141716 has

been shown to attenuate many of the pharmacological effects of cannabinoids in laboratory animals including rodents, dogs, rhesus monkeys, and also in humans (Compton, Aceto, Lowe, & Martin, 1996; Lichtman et al., 1998; Vivian et al., 1998; Huestis et al., 2001). More importantly, the development of SR141716 provided a valuable new model for withdrawal studies. Administering SR141716 to cannabinoid-dependent animals produces an immediate and quantifiable withdrawal syndrome, thus, apparently eradicating the challenges of measuring spontaneous withdrawal. Rats made tolerant to THC and challenged with SR141716 have been found to exhibit a dramatic withdrawal syndrome characterized by wet-dog shakes, facial rubbing, forepaw fluttering, and scratching (Aceto, Scates, Lowe, & Martin, 1995; Tsou, Patrick, & Walker, 1995).

Precipitated withdrawal has proven to be a reliable method for studying opiate withdrawal, a better-established syndrome than cannabis withdrawal. The nonselective opioid receptor antagonist naloxone, when administered to morphine-dependent rats, induces clear behavioural signs of withdrawal including wet-dog shakes, writhing behaviour, self-care, and exploration (Wei, Loh, & Way, 1973; Gellert & Holtzman, 1978; Higgins, Nguyen, Joharchi, & Sellers, 1991; Maldonado, Stinus, Gold, & Koob, 1992; Espejo, Cador, & Stinus, 1995; Frenois, Cador, Caillé, Stinus, & Le Moine, 2002). In addition to somatic signs, morphine withdrawal is characterized by a dysphoric state, described as anxiety, depression, restlessness, and irritability in humans (American Psychiatric Association, 2000; Haertzen & Hooks, 1969). This finding is supported by the demonstration of a robust conditioned place avoidance (CPA) to an environment paired with naloxone-induced precipitated withdrawal in morphine-dependent rodents,

which is indicative of a dysphoric state (Mucha, 1987; Higgins et al., 1991; Higgins & Sellers, 1994; Frenois et al., 2002; Rothwell, Thomas, & Gewirtz, 2009). Studies specifically of anxiogenic-like behaviour during morphine withdrawal, however, have yielded equivocal results in animals.

The elevated plus maze (EPM) is commonly used to measure anxiety in rodents. The administration of naloxone to either morphine-dependent rats, or rats acutely exposed to morphine, dose-dependently suppresses time spent exploring the open arms of the EPM, thus indicating withdrawal-induced anxiety (Schulteis, Yackey, Risbrough, & Koob, 1998; Zhang & Schulteis, 2008). However, recent studies of naloxone-precipitated morphine withdrawal have found the opposite effects in mice on the EPM; that is, mice undergoing opioid withdrawal spent significantly more time exploring the open arms (Hodgson, Hofford, Norris, & Eitan, 2008; Buckman, Hodgson, Hofford, & Eitan, 2009). Withdrawal-potentiated acoustic startle (Rothwell et al., 2009) and defensive probe burying (Emmett-Oglesby, Harris, Lane, & Lal, 1984; Higgins & Sellers, 1994) have also been observed as indicators of anxiety in rats acutely exposed to morphine. Few other behavioural measures of anxiety have been used to examine naloxone-precipitated morphine withdrawal-induced anxiety, and little research exists for anxiogenic-like behaviour during cannabinoid withdrawal. One study using the defensive withdrawal test in rats undergoing SR141716-precipitated withdrawal from the potent synthetic cannabinoid HU-210 (Rodriguez de Fonseca, Rocio, Carrera, Navarro, Koob, & Weiss, 1997) and a recent study of mice undergoing antagonist-precipitated THC withdrawal on the EPM are the only to report anxiety-like effects of cannabinoid withdrawal (Huang, Liu-Chen, & Kirby, 2010).

The present study sought to elucidate both the behavioural effects and affective state associated with THC withdrawal. Rats chronically exposed to THC were assessed for behavioural signs of SR141716-precipitated withdrawal (Experiment 1) and tested in a conditioned cue avoidance task for withdrawal-induced dysphoria (Experiment 2). The conditioned cue avoidance task is similar to the CPA task but, rather than pairing a distinct environment with a drug, the conditioned cue avoidance task used in this study employs tactile differentiation between drug- and vehicle-paired floors within a single compartment. This procedure requires animals to physically touch the drug-paired cue in order to elicit the conditioned motivational response that presumably underlies the preference or avoidance for the cue (Vezina & Stewart, 1987a). Thus, the problem of detecting cues from a distance, as is possible with visual, auditory, and olfactory cues, is eradicated. Tactile cues alone have been repeatedly demonstrated as an effective conditioning procedure in both rats (e.g., Vezina & Stewart, 1987a; 1987b; Cunningham & Niehus, 1993; Roma & Riley, 2005) and mice (e.g., Cunningham, Ferree, & Howard, 2003; Cunningham, Henderson, & Bormann, 1998; Cunningham, & Prather, 1992). In fact, multimodal stimuli have been found to be redundant in place conditioning and of no significant benefit, particularly when a tactile stimulus is included (Cunningham, Patel, & Milner, 2006). Tactile conditioned cue avoidance is produced by naloxone-precipitated morphine withdrawal following repeated conditioning sessions (Parker & Joshi, 1998; Manwell et al., 2009) and following a single conditioning session (Parker, Cyr, Santi, & Burton, 2002). This avoidance procedure is novel to cannabinoid withdrawal studies, and thus the present study sought to determine whether the same phenomenon occurs with SR141716-precipitated THC withdrawal.

An additional advantage of the tactile cue avoidance procedure is that it is not dependent on the hippocampus, unlike the CPA procedure. CB1 receptors are expressed in high abundance within the hippocampus (Glass, Dragunow, & Faull, 1997; Herkenham et al., 1990; Tsou, Brown, Sañudo-Peña, Mackie, & Walker, 1998) and administration of cannabinoids has been shown to reduce hippocampal theta oscillations, implicated in memory encoding (Robbe et al., 2006), thereby impairing hippocampus-dependent memory (e.g., Heyser, Hampson, & Deadwyler, 1993; Lichtman, Dimen, & Martin, 1995; Lichtman & Martin, 1996; Stiglick & Kalant, 1982). Thus, place conditioning procedures that depend on spatial location cues are impractical for measuring the rewarding or aversive effects of cannabinoids. This may explain the lack of CPA in mice undergoing SR141716-precipitated THC withdrawal (Hutcheson et al., 1998). The conditioned cue avoidance task was repeated on rats undergoing naloxone-precipitated morphine withdrawal in order to confirm the validity of the apparatus and procedure, and for comparison with the results of SR141716-precipitated THC withdrawal conditioned cue avoidance.

The subjective effects of precipitated THC-withdrawal were also assessed using an anxiety test battery, which included the emergence test, the EPM, and the social interaction task (Experiment 3). This test battery was again repeated using naloxone-precipitated morphine withdrawing rats, first, for comparison with those measures in rats experiencing a THC withdrawal syndrome and, second, because the emergence test is a novel measure of anxiogenic-like behaviour induced by naloxone-precipitated morphine withdrawal.

Specific hypotheses and expected results for each experiment are presented below.

Methods and Materials

Subjects

Subjects were 156 experimentally naïve male Sprague-Dawley rats. Of these, 102 rats weighed between 201 and 225 g at time of shipment (Charles River Laboratories, St. Constant, Quebec, Canada) and 54 rats were bred in house from Sprague-Dawley rats originating from Charles River Laboratories. Animals bred in house were weaned on postnatal day 23 and pair-housed. They were maintained on a 12-h reverse light-dark cycle (7:00 am–7:00 pm) and given ad libitum access to food and water except during testing. Experimental testing commenced when rats weighed at least 200 g. Rats received from Charles River Laboratories were pair-housed as described for in-house bred rats and allowed to acclimate to the housing conditions for a minimum of 5 days. All animals were gently handled for a minimum of 4 days prior to any testing. This study was reviewed and approved by the Wilfrid Laurier University Animal Care Committee, and all experimental procedures were carried out in accordance with the Canadian Council on Animal Care Guide to the Care and Use of Laboratory Animals (CCAC, vol. 1, 1993).

Drugs

THC (THC Pharm GmbH, Frankfurt, Germany) and SR141716 (Rimonabant; Onbio Inc., Richmond Hill, ON, Canada) were first dissolved in ethanol then mixed with a few drops of TWEEN-80 (polyoxyethylene sorbitan monooleate; ICN Biomedicals, Seven Hills, NSW, Australia). The solution was stirred under a stream of nitrogen gas until all ethanol was evaporated. Physiological saline was then added and the suspension

was well mixed. The final vehicle suspension contained 0.75% TWEEN-80 and 0.9% NaCl. THC and SR141716 were administered at a dose of 5 mg/kg and 3 mg/kg, respectively.

Morphine hydrochloride (CDMV, St. Hyacinth, Quebec, Canada) and naloxone hydrochloride (Tocris, Ellisville, Missouri, USA) were dissolved in 0.9% saline and administered at a dose of 5 mg/kg and 1 mg/kg, respectively.

All drugs were administered intraperitoneally (i.p.) in a volume of 1 ml/kg body weight. Equivalent volumes of either saline or TWEEN-80/saline were used for vehicle control treatments.

Experiment 1: Behavioural Assessment of SR141716-precipitated THC Withdrawal

Animals were examined for somatic signs of SR141716-precipitated THC withdrawal for three reasons: 1) to ensure that the selected doses of THC and SR141716 produced a quantifiable physical withdrawal syndrome, 2) to exclude the possibility that SR141716 alone produces somatic effects to the same degree as THC withdrawal: SR141716 itself has been found to induce behaviour similar to cannabinoid withdrawal in rodents (Aceto et al., 1995; Compton et al., 1996; Rodriguez de Fonseca et al., 1997; Aceto, Scates, Razdan, & Martin, 1998), and 3) to ensure that the timing for measuring the affective state of animals experiencing SR141716-precipitated THC withdrawal would encompass the physical display of withdrawal signs.

It was expected that all animals treated with SR141716 would demonstrate a modest increase in withdrawal-like behaviours, but that animals repeatedly pre-exposed to THC would exhibit pronounced somatic withdrawal signs, characterized by increased scratching, wet-dog shakes, and facial rubbing. Furthermore, because precipitated

withdrawal indicates physical dependence on a drug (Aceto, Scates, & Martin, 2001), it was anticipated that increasing the number of THC exposures would augment the SR141716-precipitated withdrawal.

Apparatus

Behavioural signs of SR-141716-precipitated THC withdrawal were observed in an experimental chamber (61 x 26 x 40 cm) consisting of clear acrylic sides and top, and a black ABS plastic floor, situated in a dimly lit room (37 Lux at apparatus level). Activity was recorded by a video camera positioned 75 cm in front of the apparatus.

Procedure

A procedural timeline is presented in Table 1. Animals were injected twice daily with either TWEEN-80/saline (n=8) or THC (n=8) for 13 consecutive days at approximately 9:00 am and 9:00 pm. The first injection occurred at 9:00 pm on day 1 and the last injection at 9:00 am on day 13. All rats were injected 1 h following the 9:00 am drug injections with TWEEN-80/saline on days 3, 7, and 11 and with SR141716 on days 5, 9, and 13. Rats were then immediately placed individually in experimental chambers for a period of 30 min on both vehicle and SR141716 challenge days. Video recorded activity was manually scored by an observer blind to group allocations using ODLog software (Macropod Software, 2001; www.macropodsoftware.com). Rats were assessed for behavioural signs of withdrawal, including scratching, wet-dog shakes, and facial rubs, for 5 min starting at 10 min into the 30 min trial and for the last 5 min of each trial. These assessment time points were selected based on previous reports that physical signs of SR141716-precipitated THC withdrawal emerge approximately 10 min following SR141716 administration and persist throughout a 1 h period (Aceto et al., 1995; Tsou et

al., 1995). Scratching was defined as hindlimb scratching and was recorded as time (s) spent scratching by animals during each 5 min observation segment. Wet-dog shakes were defined as paroxysmic shuddering of the head, neck, and trunk, reminiscent of purposeful movement in dogs. Facial rubs were defined as the animal wiping any part of its head with both forelimbs. Wet-dog shakes and facial rubs were counted as number of events per each 5 min observation segment.

Statistical Analysis

As shown in Figure 1, the second observation segment yielded more pronounced withdrawal signs relative to the first observation segment. Thus, only the second segment was used for the statistical analysis. Somatic withdrawal signs associated with SR141716-precipitated THC withdrawal were analyzed using a three-factor [group (T-VEH vs. THC) x treatment (S-VEH vs. SR141716) x day] mixed design ANOVA with repeated measures on the second two factors. Each behavioural measure was analyzed individually.

Where significant main effects were found, pairwise comparisons were conducted using Bonferroni-adjusted t-tests. Significant two-way interactions were followed by one-way tests of the simple main effects. All analyses were carried out using PASW 18.0 for Macintosh (SPSS, Chicago, IL, USA) with an alpha level of 0.05.

Results

Data for the physical signs of SR141716-precipitated THC withdrawal are presented in Figure 1. The three-factor mixed design ANOVAs revealed a significant main effect of treatment on all three measures: time spent scratching [$F(1,9)=16.343$, $p=.003$; Fig. 1A], number of wet dog shakes [$F(1,9)=21.522$, $p=.001$; Fig. 1B], and

number of facial rubs [$F(1,9)=6.469$, $p=.032$; Fig. 1C] were increased by SR141716 in all animals. For time spent scratching, there was also a main effect of group [$F(1,9)=5.836$, $p=.039$], such that THC animals spent significantly more time scratching than vehicle control animals. Furthermore, time spent scratching yielded a significant group by treatment interaction [$F(1,9)=5.472$, $p=.040$]. One-way tests of the simple main effects revealed that THC animals spent a significantly more time scratching than vehicle control animals when treated with SR141716 [$F(1,14)=5.811$, $p=.030$; Fig. 2]. No significant difference in time spent scratching was observed between groups when treated with the vehicle for SR141716.

Discussion

The results of Experiment 1 are in agreement with previous studies (e.g., Aceto et al., 1995; Tsou et al., 1995; Compton et al., 1996; Rodriguez de Fonseca et al., 1997; Aceto et al., 1998; Aceto et al., 2001) showing that treatment with SR141716 alone induces limited withdrawal-like behaviour, and that pre-exposure to THC results in a pronounced increase in these physical withdrawal signs. As has been suggested, this effect may be a result of antagonistic effects of SR141716 on the endogenous cannabinoid system, ultimately disrupting its tonic inhibitory action in the presence of THC (Aceto et al., 2001; Sañudo-Peña, Tsou, Delay, Hohman, Force, & Walker, 1997). Alternatively, it has been proposed that SR141716 may act as an inverse agonist on the CB1 receptor (Compton et al., 1996; Richardson, Aanoson, & Hargreaves, 1997). It is important to note also, that rats undergoing spontaneous withdrawal from THC show modest or no physical symptoms of withdrawal (Aceto, Scates, Lowe, & Martin, 1996; Diana, Melis, Muntoni, & Gessa, 1998). Of course, this could be due to the long half-life

of THC as previously mentioned, but a synergistic effect of THC and SR141716 may be a reasonable explanation for the apparent withdrawal symptoms.

Scratching was found to be the most reliable physical sign of SR141716-precipitated THC withdrawal. Although SR141716 alone increased scratching behaviour, time spent scratching also specifically reflected withdrawal from THC, whereas wet-dog shakes and facial rubbing could not be differentiated from an effect of merely SR141716. During SR141716-vehicle challenges, however, facial rubbing was representative of the well-documented locomotor-reducing effects of cannabinoids (Schramm-Sapyta, Young, Chaudhry, Wilson, Swartzwelder, et al., 2007; Oviedo, Glowa, & Herkenham, 1993; Hill, Gorzalka, & Choi, 2004). Slight tolerance to THC-induced locomotor suppression was evident on the second and third vehicle days, which is typical during chronic cannabinoid exposure (Oviedo et al., 1993; Hill et al., 2004). It should be noted that, in addition to the quantified behavioural signs of SR141716-precipitated THC withdrawal, other overt somatic symptoms were observed but were not systematically measured. Stretching, arched back, and diarrhea occurred in THC-treated rats when challenged with SR141716, which have been reported in previous cannabinoid withdrawal studies and were associated with precipitated withdrawal (Aceto et al., 1995, Aceto et al., 2001, Lichtman et al., 1998). These observations suggest that SR141716 produces a fundamentally different effect in THC-dependent rats than in nondependent rats.

As mentioned previously, there was a generally smaller effect of SR141716 on THC-treated rats at the 10-15 min observation segment, the presumed onset of withdrawal, and a more pronounced effect during the 25-30 min segment. There was no

significant effect of day, which suggests that animals were physically dependent on THC by the first day of SR141716 treatment.

Experiment 2: SR141716-precipitated THC Withdrawal-induced Cue-conditioning

Avoidance conditioning is a sensitive measure of the aversive properties of drug withdrawal (Koob, Stinus, Le Moal, & Bloom, 1989; Mucha, 1987; Mucha, 1991; Mucha & Iversen, 1984). Two unbiased cue-conditioning experiments were conducted to investigate whether the physical signs of SR141716-precipitated THC withdrawal were coupled with dysphoria: Experiment 2A examined conditioned cue avoidance following a single vehicle- and drug-cue pairing, and Experiment 2B examined conditioned cue avoidance following three separate vehicle- and drug-cue pairings. The drug-cue pairings conditioned THC-treated animals to the SR141716-precipitated withdrawal state, and THC-vehicle-treated animals to the effects of SR141716 alone. Similarly, in morphine control groups, morphine-treated animals were conditioned to the naloxone-precipitated withdrawal state during drug-cue pairings, and morphine vehicle-treated animals to the effects of naloxone alone. Although naloxone-precipitated morphine withdrawal-induced cue avoidance has been observed following only one vehicle- and one drug-pairing (Parker et al., 2002), both one cycle and three cycle cue-conditioning schedules were used to allow for repeated drug treatment in an attempt to increase the severity of withdrawal and related dysphoria.

It was predicted that an avoidance to the THC withdrawal-paired cue would be observed, indicating that a dysphoric state is associated with cannabinoid withdrawal, and the magnitude of this withdrawal would be greater following three cycles of conditioning relative to a single cycle. In agreement with previous place- and cue-conditioning studies,

it was also anticipated that naloxone-precipitated morphine withdrawal would produce a conditioned avoidance to the withdrawal-paired cue that varied in severity according to the number of conditioning cycles.

Apparatus

The cue-conditioning task was performed in eight identical unbiased chambers (61 x 29 x 30 cm high) in a room dimly illuminated by four 13 W compact fluorescent red lamps (10 Lux measured at the apparatus floor). The chambers were constructed of black UHMW polyethylene walls, black wire mesh tops, and two texturally different interchangeable floor types. One floor was made of ABS plastic with holes arranged in a grid pattern (holes were 1 cm in diameter, spread 1 cm apart with a depth of 2 mm; termed "hole" floor). The other floor was made of textured plastic sheeting typically used for fluorescent ceiling light covers. These provided a rough "bumpy" surface (bumps were 1 mm high, each 2.5 x 2.5 cm square contained 100 bumps; termed "bump" floor). Activity was recorded by four video cameras mounted 120 cm above the chamber floors and images were transmitted via IEEE 1394 interface to a computer in the adjacent room, running the ANY-maze Video Tracking System version 4.50 (Stoetling Co., Wood Dale, IL, USA).

Experiment 2A

Procedure.

Animals were divided into four groups: THC vehicle+SR141716 (T-VEH+SR, n=12), THC+SR141716-precipitated withdrawal (THC+SR-WD, n=12), morphine vehicle+naloxone (M-VEH+NAL, n=8), and morphine+naloxone-precipitated withdrawal (MOR+NAL-WD, n=12). A procedural timeline is shown in Table 2.

Animals were injected twice daily with saline, TWEEN-80/saline, THC, or morphine for 5 consecutive days, at approximately 9:00 am and 9:00 pm. The first injection occurred at 9:00 pm on day 1 and the last injection at 9:00 pm on day 5. Each animal was tested every second day, beginning on day 1 and ending on day 5 with a final test on day 6, at approximately the same time of day, during the dark phase of the light/dark cycle. The cue-conditioning task was conducted in three phases: preconditioning (day 1, prior to first drug injection), conditioning (following the morning injection on days 3 and 5), and test (day 6).

The preconditioning phase consisted of one 15-min undrugged session. During this phase, rats were placed in the chambers equipped with one floor type on either side (27 x 25 cm) separated by a neutral centre floor made of smooth black ABS plastic (25 x 9 cm). A baseline measure of time spent on each floor type was recorded, which did not show a significant difference between seconds spent on the "hole" or "bump" floors indicating that the apparatus provides an unbiased test of conditioned cue preference and avoidance. The conditioning phase consisted of two 30-min sessions in which the chambers were equipped with alternate floor types. During the first conditioning session, a vehicle conditioning session conducted on day 3, THC- and THC-vehicle-treated rats were injected with TWEEN-80/saline and morphine- and morphine-vehicle-treated rats were injected with saline 1 h following the 9:00 am drug injections and placed in the test apparatus immediately afterwards. Two days following the vehicle conditioning session, on day 5, the drug-conditioning session, THC- and THC-vehicle-treated rats were injected with SR141716 and morphine- and morphine-vehicle-treated rats were injected with naloxone 1 h following the 9:00 am drug injections and placed in the test apparatus

immediately afterwards. At the conclusion of this phase, SR141716 and naloxone had each been paired once with one floor texture and their respective vehicles had each been paired once with the other floor texture in a counterbalanced manner, yielding one cycle of cue-conditioning. The drug-associated context ("hole" or "bump" floor) was counterbalanced across groups. Locomotor activity was also assessed during the conditioning phase and was defined by total distance traveled (m), time in motion (s), and absolute turn angle (°) as quantified by ANY-maze. One day after the conditioning phase, each animal received a 15-min test session, identical to the preconditioning phase. Time spent on each floor type was again scored to test the persistence of any cue-conditioned preference or avoidance.

Statistical Analysis.

Cue-conditioning preference scores were computed by subtracting time spent on the SR141716- or naloxone-paired floor during the test day from time spent on the SR141716- or naloxone-paired floor during the preconditioning day. Independent samples t-tests were then used to compare T-VEH+SR and THC+SR-WD groups, and to compare M-VEH+NAL and MOR+NAL-WD groups. Locomotor activity occurring during the conditioning phase was analyzed separately for the aforementioned THC and morphine groups using a two-factor [group (T-VEH vs. THC and M-VEH vs. MOR) x treatment (S-VEH vs. SR and N-VEH vs. NAL)] mixed design ANOVA with repeated measures on the second factor. Each activity measure, including distance traveled, time in motion, and absolute turn angle, was analyzed individually.

Where significant main effects were found, pairwise comparisons were conducted using Bonferroni-adjusted t-tests. Significant two-way interactions were followed by one-

way tests of the simple main effects. All analyses were carried out using PASW 18.0 for Macintosh (SPSS, Chicago, IL, USA) with an alpha level of 0.05.

Results.

The t-test comparing the change in time spent on the drug-paired floor for T-VEH+SR and THC+SR-WD groups yielded no significant differences on cue preference for one cycle of tactile cue-conditioning (Fig. 3).

Data from five animals on the SR141716 conditioning session were lost due to equipment malfunction. Locomotor activity during the conditioning phase for one cycle of cue-conditioning was analyzed accordingly for T-VEH+SR (n=9) and THC+SR-WD (n=10) groups. Data are presented in Figure 4. The two-factor mixed design ANOVAs comparing locomotor activity for THC- and THC-vehicle-treated rats revealed no significant differences for distance traveled or absolute turn angle measures (Fig. 4, A and Fig. 4, C, respectively). The two-factor mixed design ANOVA for time spent in motion yielded a significant main effect of group, such that THC-treated animals spent significantly less time in motion than THC-vehicle-treated animals [$F(1,17)=4.830$, $p=.042$; Fig. 4, B]. The group by treatment interaction was also significant for time spent in motion [$F(1,17)=5.407$, $p=.033$]. One-way tests of the simple main effects for the group by treatment interaction revealed that THC-treated animals spent significantly less time in motion during the SR141716-vehicle conditioning session [$F(1,22)=5.412$, $p=.03$; Fig. 4, B]. There was no significant difference between the two groups for time spent in motion during the SR141716 conditioning session.

The t-test comparing the change in time spent on the drug-paired floor for M-VEH+NAL and MOR+NAL-WD groups yielded no significant differences on cue preference for one cycle of tactile cue-conditioning (Fig. 5).

Locomotor activity data for morphine groups during the conditioning phase of one cycle of cue-conditioning are presented in Figure 6. The two-factor mixed design ANOVAs comparing morphine- and morphine-vehicle-treated rats revealed significant main effects of treatment on all three measures of locomotor activity: distance traveled, time in motion, and absolute turn angle [$F(1,18)=15.602$, $p=.001$; $F(1,18)=27.796$, $p<.001$; and $F(1,18)=14.900$, $p=.001$, respectively] were reduced by naloxone in all animals. Absolute turn angle also yielded a significant main effect of group [$F(1,18)=5.889$, $p=.026$], such that morphine-treated animals had a significantly lower absolute turn angle than morphine-vehicle-treated animals.

Experiment 2B

Procedure.

An additional group of experimentally-naïve animals was used to examine the effects of three conditioning cycles. Animals were divided into four groups identical to those in Experiment 2A: T-VEH+SR (n=8), THC+SR-WD (n=8), M-VEH+NAL (n=8), and MOR+NAL-WD (n=8). A procedural timeline is shown in Table 3. This procedure was identical to that used in Experiment 2A, except that the conditioning phase consisted of six sessions (following the morning injection on days 3, 5, 7, 9, 11, and 13) such that SR141716 or naloxone had each been paired with one floor texture and their respective vehicles each with the other floor texture three times, yielding three cycles of cue-conditioning. Accordingly, two daily injections of saline, TWEEN-80/saline, THC, or

morphine began after preconditioning at 9:00 pm on day 1 and ended at 9:00 am on day 13. The test followed on day 14.

Statistical Analysis.

Cue-conditioning preference scores were calculated and analyzed identical to that of Experiment 2A. Locomotor activity occurring during the conditioning phase was analyzed separately for T-VEH+SR and THC+SR-WD groups, and for M-VEH+NAL and MOR+NAL-WD using a three-factor [group (T-VEH vs. THC and M-VEH vs. MOR) x treatment (S-VEH vs. SR and N-VEH vs. NAL) x day] mixed design ANOVA with repeated measures on the second two factors. Activity measures were analyzed individually as per Experiment 2A and significant main effects and two-way interactions were followed up identical to that of Experiment 2A. All analyses were carried out using PASW Statistics 18.0 for Macintosh (SPSS, Chicago, IL, USA) with an alpha level of 0.05.

Results.

The t-test comparing the change in time spent on the SR141716-paired floor for T-VEH+SR and THC+SR-WD groups yielded no significant difference on cue preference following three cycles of tactile cue-conditioning (Fig. 7).

The data for locomotor activity occurring during the conditioning phase of three cycles of SR141716-precipitated THC withdrawal-induced cue-conditioning are presented in Figure 8. The three-factor mixed design ANOVAs comparing locomotor activity for THC-vehicle- and THC-treated animals revealed significant differences on all activity measures. Distance traveled (Fig. 8, A) yielded a significant main effect of group [$F(1,14)=15.296$, $p=.002$], such that THC-treated animals traveled significantly less,

treatment [$F(1,14)=19.369$, $p=.001$], whereby SR141716 significantly reduced distance traveled, and day [$F(2,28)=8.949$, $p=.001$]. Bonferroni-adjusted pairwise comparisons of the day factor revealed that animals traveled a significantly greater distance on the first vehicle- and SR141716-conditioning days than on the second and third conditioning days ($p=.027$ and $p=.007$, respectively). Time in motion (Fig. 8, B) yielded a significant main effect of group [$F(1,14)=10.187$, $p=.007$], revealing that THC-treated animals spent significantly less time in motion, and day [$F(2,28)=3.870$, $p=.033$]. Bonferroni-adjusted pairwise comparisons of the day factor revealed that animals spent significantly more time in motion on the first vehicle- and SR141716-conditioning day as compared with the third conditioning day ($p=.03$). Absolute turn angle (Fig. 8, C) yielded a significant main effect of group [$F(1,14)=25.979$, $p<.001$], revealing that THC-treated animals had a significantly lower absolute turn angle, treatment [$F(1,14)=7.452$, $p=.016$] such that SR141716 significantly reduced absolute turn angle, and day [$F(2,28)=6.458$, $p=.005$]. Bonferroni-adjusted pairwise comparisons of the day factor revealed that absolute turn angle was significantly greater on the first vehicle- and SR141716-conditioning days as compared with conditioning days two and three ($p=.049$ and $p=.041$, respectively).

The t-test comparing the change in time spent on the naloxone-paired floor for M-VEH+NAL and MOR+NAL-WD groups revealed a significant difference on cue preference for three cycles of tactile-conditioning [$t(14)=3.165$, $p=.003$; Fig. 9]. Animals treated with morphine spent significantly less time on the naloxone-paired floor during the test day than on the preconditioning day as compared with vehicle control animals.

The data for locomotor activity occurring during the conditioning phase of three cycles of naloxone-precipitated morphine withdrawal-induced cue-conditioning are

presented in Figure 10. The three-factor mixed design ANOVAs comparing locomotor activity for morphine-vehicle- and morphine-treated animals revealed significant differences on all activity measures. Distance traveled (Fig. 10, A) yielded a significant main effect of group [$F(1,14)=7.413$, $p=.017$], such that morphine-treated animals traveled significantly greater distance, treatment [$F(1,14)=76.222$, $p<.001$], whereby naloxone significantly reduced distance traveled, and day [$F(2,28)=5.088$, $p=.013$]. Bonferroni-adjusted pairwise comparisons of the day factor revealed that distance traveled was significantly greater on the first vehicle- and naloxone-conditioning days as compared with the second conditioning days ($p=.024$). Distance traveled also yielded a significant group by treatment interaction [$F(1,14)=39.823$, $p<.001$] and group by day interaction [$F(2,28)=6.162$, $p=.006$]. One-way tests of the simple main effects for the group by treatment interaction revealed that morphine-treated animals traveled a significantly greater distance than vehicle control animals during vehicle-conditioning sessions [$F(1,14)=18.005$, $p=.001$]. There was no difference between groups on distance traveled during naloxone-conditioning sessions. For the group by day interaction, tests of the simple main effects revealed that morphine-treated animals traveled a significantly greater distance on the third vehicle- and naloxone-conditioning days as compared with vehicle control animals [$F(1,14)=16.964$, $p=.001$]. No significant differences on distance traveled existed between groups on the first or second conditioning days. Time in motion (Fig. 10B) yielded a significant main effect of group and treatment [$F(1,14)=4.840$, $p=.045$ and $F(1,14)=68.328$, $p<.001$, respectively], consistent with the morphine-induced hyperactivity and naloxone-induced hypoactivity findings of distance traveled. The group by treatment interaction [$F(1,14)=25.318$, $p<.001$] and group by day interaction

[F(2,28)=9.740, p=.001] were also significant for time in motion. One-way tests of the simple main effects for the group by treatment interaction revealed that morphine-treated animals spent significantly more time in motion during vehicle-conditioning sessions than vehicle control animals [F(1,14)=21.331, p<.001]. No difference was observed between groups during naloxone-conditioning sessions. Tests of the simple main effects for time in motion revealed a significant difference between groups on the third vehicle- and naloxone-conditioning days such that morphine-treated rats spent significantly more time in motion than vehicle control rats [F(1,14)=16.797, p=.001]. Absolute turn (Fig. 10, C) angle yielded a significant main effect of group [F(1,14)=5.270, p=.038], revealing that morphine-treated animals had a significantly greater absolute turn angle, treatment [F(1,14)=60.810, p<.001], whereby naloxone significantly reduced absolute turn angle, and day [F(2,28)=4.261, p=.024]. Bonferroni-adjusted pairwise comparisons of the day factor revealed that absolute turn angle was significantly greater on the first vehicle- and naloxone-conditioning days as compared with the second conditioning days (p=.042). Absolute turn angle also yielded a significant group by treatment interaction [F(1,14)=39.823, p<.001] and group by day interaction [F(2,28)=5.745, p=.008]. One-way tests of the simple main effects revealed that morphine-treated animals demonstrated a significantly larger absolute turn angle than vehicle control animals during vehicle-conditioning sessions [F(1,14)=13.689, p=.002], but not during naloxone-conditioning sessions. For the group by day interaction, tests of the simple main effects revealed that morphine-treated animals had a significantly greater absolute angle than vehicle-treated animals on the third vehicle- and naloxone-conditioning days [F(1,14)=17.681, p=.001], but not the first or second conditioning days.

Discussion

SR141716-precipitated THC withdrawal failed to produce a conditioned cue avoidance following both one and three conditioning cycles, suggesting the absence of a dysphoric state. This finding agrees with the previous study of place preference in mice withdrawing from THC (Hutcheson et al., 1998), implying that the hippocampal aspect of place conditioning is not responsible for the absence of a preference or avoidance. SR141716 elicited neither a cue avoidance nor preference in THC naïve rats, which is consistent with past studies using the place conditioning procedure (Singh, Verty, McGregor, & Mallet, 2004; Chaperon, Soubrié, Puech, & Thiébot, 1998; Hutcheson et al., 1998); however, other studies have reported a place preference for SR141716 (Cheer, Kendall, & Marsden, 2000; Sañudo-Peña et al., 1997). Since SR141716 had no motivational effects in the paradigm used in this study, it is unlikely that the antagonist blocked any aversive consequences of THC withdrawal. Thus, an opposing rewarding action of SR141716 could not account for the absence of a withdrawal associated cue avoidance. Consistent with past research (Parker et al., 2002; Parker & Joshi, 1998; Manwell et al., 2009), naloxone-precipitated morphine withdrawal produced a distinct conditioned cue avoidance, but only following three cycles of conditioning. Naloxone produced a slight avoidance in morphine naïve rats, though this is a common effect of naloxone and it is reliably enhanced by pre-treatment with morphine (Mucha & Herz, 1985; Mucha & Iversen, 1984; Parker & Rennie, 1992; Parker & Joshi, 1998).

It was evident after both one and three cycles of cue-conditioning that THC suppressed locomotor activity, which is consistent with previous findings (Singh, McGregor, & Mallet, 2005; Norwood, Cornish, Mallet & McGregor, 2003; Arévalo, de

Miguel, & Hernández-Tristán, 2001). SR141716 also slightly reduced locomotor activity irrespective of drug history. This became apparent only during three cycles of cue-conditioning as locomotor activity was elevated on the first conditioning day, likely a result of novelty-seeking behaviour. Previous studies, however, have demonstrated that SR141716 typically does not influence locomotor activity (Arévalo et al., 2001; Gardner & Mallet, 2006; Verty, McFarlane, McGregor, & Mallet, 2004a; Verty, McFarlane, McGregor, & Mallet, 2004b; Verty, McGregor, & Mallet, 2004; Singh et al., 2004). This unusual effect of SR141716 may be explained by the order in which animals were conditioned to vehicle and SR141716: SR141716 conditioning trials followed vehicle conditioning trials by one session and thus, novel exploratory behaviour would be generally lower on those days than on vehicle conditioning days. Morphine generally stimulated locomotor activity, which escalated with repeated exposure and completely abolished the novelty-seeking effect observed in morphine-naïve rats. Naloxone attenuated morphine-induced hyperactivity and reduced locomotor activity in drug naïve animals. These findings are in agreement with previous studies of opiate-induced activity (Kuribara, 1995; Singh et al., 2004).

Experiment 3: SR141716-precipitated THC Withdrawal-induced Anxiety

The dysphoric state typically associated with drug withdrawal often incorporates a feeling of anxiety. Although a dysphoric state was not observed in THC-withdrawing rats on the conditioned cue avoidance task, evidence exists for the involvement of corticotropin-releasing factor (CRF) systems and other stress-related hormones during cannabinoid withdrawal (Rodriguez de Fonseca et al., 1997). Furthermore, results from a recent study of opiate withdrawal suggest that dysphoric and anxiogenic manifestations

may be mediated by distinct neural systems (Rothwell et al., 2009). Thus, it remains possible that cannabinoid withdrawal may produce anxiety-like behaviour without producing dysphoria and, as such, anxiety associated with precipitated THC withdrawal was examined using a test battery comprising the emergence test, EPM, and social interaction test (Morley, Gallate, Hunt, Mallet, & McGregor, 2001).

The emergence test involves conflict between the desire to explore and the desire to avoid the anxiogenic stimuli of open space (Crawley & Goodwin, 1980). Similarly, the EPM involves conflict between the desire for exploration and for avoidance of open and high spaces (Lister, 1990). Both of these tasks are considered to be measures of generalized anxiety since agents used to alleviate generalized anxiety disorder symptoms modify defensive behaviours evoked by the models (Pellow, Chopin, File, & Briley, 1985). The social interaction test involves the anxiety rats display towards an unfamiliar conspecific, wherein anxiety is indicated by reduced contact or defensive behaviour. The test has been extensively validated with different classes of drugs for both anxiogenic and anxiolytic effects (File, 1980; File, 1985). These three anxiety measures were selected and used in conjunction to obtain reliable observations and to control for possible drug effect interference. For example, both the emergence test and EPM rely on locomotor activity and since THC suppresses locomotor activity these measures may not be suitable for examining the associated anxiety. Thus, the social interaction test may provide a more reliable measure of anxiety in THC groups. Furthermore, it is possible that the emergence test and EPM are hippocampal-dependent, which would be disturbed by THC, and therefore the hippocampal-independent social interaction test may again be a better measure.

Few studies have examined cannabinoid withdrawal-induced anxiety. As mentioned, a very recent study demonstrated anxiety-like effects of SR141716-precipitated THC withdrawal in mice on the EPM (Huang et al., 2010), but this has not been shown with rats nor corroborated with other behavioural measures of anxiety. Anxiety associated with opioid withdrawal has been studied more closely, and yielded fairly consistent results. Increased anxiogenic-like behaviour has been observed on the EPM in rats experiencing spontaneous and naloxone-precipitated morphine withdrawal following both acute or repeated morphine exposure (Schulteis et al., 1998; Zhang & Schulteis, 2008). Limited research exists on the emergence test and social interaction test for morphine withdrawing animals. One study found increased social interaction between morphine withdrawing rats paired with control rats; however, this observation was deduced to be a result of behaviour associated with morphine withdrawal rather than anxiety (Grasing, Wang, & Schlussman, 1996).

Despite the absence of a dysphoric state in Experiment 2, it was predicted that the physical symptoms exhibited by rats withdrawing from THC may instigate a feeling of anxiety and thus, anxiogenic-like behaviour would be observed on the emergence test, EPM, and social interaction test. Precipitated morphine withdrawal was expected to induce generalized anxiety on all three measures in accordance with previous research and with the dysphoric state demonstrated in Experiment 2.

Emergence Test

The emergence test was conducted in a dimly lighted room illuminated by one 13 W compact fluorescent red lamp (4 Lux at apparatus floor level) within an apparatus consisting of a 120 x 120 x 45 cm white melamine arena with a black ABS plastic floor

and a 40 x 24 x 17 cm black melamine hide box. The rat was placed in the hide box at the beginning of the test period. Activity was recorded by a video camera mounted 225 cm above the apparatus, using the ANY-maze Video Tracking System, as previously described. Scored behaviours included latency to emerge from the hide box, number of open-field entries, and time spent in the open field.

Elevated Plus Maze

The EPM consisted of two open (52 x 12 cm) and two closed (52 x 12 x 40 cm) black melamine arms arranged in a cross-elevated position, 53 cm above the floor. The EPM was conducted in a dark room illuminated by one 13 W compact fluorescent red lamp (2 Lux at maze floor level) and activity was recorded by one camera mounted 140 cm above the apparatus, using ANY-maze. Scored behaviours included percent number of entries to open arms, percent time spent in open arms, and number of entries to closed arms.

Social Interaction

The social interaction test was conducted in a room dimly illuminated by white lights (37 Lux at apparatus level). This test was performed in an experimental chamber (61 x 26 x 40 cm) consisting of Plexiglas sides and top, and a black ABS plastic floor. Rats were placed in the apparatus for 10 min with a treatment-matched unfamiliar conspecific of approximately the same body weight. Activity was recorded by a video camera positioned 75 cm in front of the apparatus. An observer blind to group allocations manually scored trials using ODLog software. Scored behaviours included sniffing the other rat, following the other rat, grooming the other rat, and rearing.

Procedure

Animals were divided into eight groups: THC vehicle+SR141716 vehicle (T-VEH+S-VEH, *n*=8), THC vehicle+SR141716 (T-VEH+SR, *n*=8), THC+SR141716 vehicle (THC+S-VEH, *n*=8), THC+SR141716 (THC+SR-WD, *n*=8), morphine vehicle+naloxone vehicle (M-VEH+N-VEH, *n*=8), morphine vehicle+naloxone (M-VEH+NAL, *n*=8), morphine+naloxone vehicle (MOR+N-VEH, *n*=8) and morphine+naloxone (MOR+NAL-WD, *n*=8). A procedural timeline is shown in Table 4. Rats were injected twice daily with vehicle, THC, or morphine for 5 consecutive days, at approximately 9:00 am and 9:00 pm. The first injection occurred at 9:00 pm on day 1 and the last injection at 9:00 am on day 5. Rats were injected with vehicle, SR141716, or naloxone 1 h following the 9:00 am injection on day 5. Each rat was placed in either the emergence test or EPM 15 min later. This delay was selected such that the brief duration of these anxiety measures captured animals in a state of withdrawal, as per the onset of somatic symptoms. The order in which the emergence test and EPM were conducted was counterbalanced across groups. Following both tests, rats were placed in the social interaction test.

Statistical Analysis

Data for all behavioural measures of emergence test activity (number of open field entries, percent of time spent in open field, and latency to exit hide box), EPM activity (percent number of open arm entries, percent time spent in open arms, and number of entries to closed arms), and social interaction (sniffing, following, grooming, and rearing) were analyzed separately for THC and morphine groups by one-way ANOVAs, followed by post hoc Tukey tests when significant. All analyses were carried

out using PASW 18.0 for Macintosh (SPSS, Chicago, IL, USA) with an alpha level of 0.05.

Results

Emergence Test.

The one-way ANOVA comparing THC groups revealed a significant difference on number of open field entries [$F(3,28)=7.579$, $p=.001$]. As shown in Figure 11 (B), post hoc analyses revealed that animals receiving THC alone and animals receiving both SR141716 and THC made significantly fewer entries to the open field as compared with vehicle control animals ($p=.002$ for both). No significant differences on percent time spent in open field (Fig. 11, A) or latency to exit hide box (Fig. 11, C) were observed between any THC groups.

The one-way ANOVA comparing morphine groups revealed significant differences on time spent in open field and number of open field entries [$F(3,28)=5.479$, $p=.004$ and $F(3,28)=3.714$, $p=.023$, respectively]. As shown in Figure 12 (A), post hoc analyses revealed that animals receiving both morphine and naloxone spent significantly less time in the open field as compared with vehicle control animals, animals receiving naloxone alone, and animals receiving morphine alone ($p=.009$; $p=.014$; $p=.019$, respectively). There were no significant differences between any of the other groups for time spent in open field. As shown in Figure 12 (B), post hoc analyses for number of open field entries revealed that animals receiving both morphine and naloxone made significantly fewer entries to the open field than animals receiving naloxone alone ($p=.033$). No other significant differences were observed between groups on number of

open field entries. No significant differences on latency to exit the hide box (Fig. 12, C) were observed between any morphine groups.

Elevated Plus Maze.

The one-way ANOVA comparing THC groups revealed no significant differences on all three EPM measures: percent time spent in open arms (Fig. 13, A), percent number of open arm entries (Fig. 13, B), and number of closed arm entries (Fig. 13, C).

The one-way ANOVA comparing morphine groups revealed significant differences on percent number of open arm entries [$F(3,28)=5.613$, $p=.004$]. As shown in Figure 14 (B), animals receiving both morphine and naloxone made significantly fewer entries to the open arms (as a function of total entries to open and closed arms) than vehicle control animals and animals receiving naloxone alone ($p=.002$ and $p=.043$, respectively). No significant differences were observed between any morphine groups on percent time spent in open arms (Fig. 14, A) or number of entries to closed arms (Fig. 14, C).

Social Interaction.

The one-way ANOVA comparing THC groups revealed significant differences on time spent sniffing the other rat [$F(3,28)=9.992$, $p<.001$; Fig. 15, A], time spent following the other rat [$F(3,28)=4.869$, $p=.008$; Fig. 15, B], and number of rears [$F(3,28)=6.788$, $p=.001$; Fig. 15, D]. No significant differences were observed between any THC groups on time spent grooming the other rat (Fig. 15, C). Post hoc analyses revealed that animals receiving THC alone and animals receiving both THC and SR141716 spent significantly less time sniffing the other rat as compared with vehicle control animals ($p=.002$ and $p<.001$, respectively) and compared with animals receiving SR141716 alone ($p=.034$ and

p=.010, respectively). Time spent following the other rat was significantly lower in animals treated with both THC and SR141716 compared with vehicle control animals and animals receiving SR141716 alone (p=.011 and p=.045, respectively). Number of rears significantly differed between animals receiving SR141716 alone and animals receiving THC alone, such that THC-treated animals performed significantly fewer rears (p=.001).

The one-way ANOVA comparing morphine groups revealed significant differences on time spent sniffing the other rat [F(3,28)=4.753, p=.008; Fig. 16, A], following the other rat [F(3,28)=3.074, p=.044; Fig. 16, B], and grooming the other rat [F(3,28)=6.441, p=.002; Fig. 16, C]. No significant differences were found on number of rears (Fig. 16, D). Post hoc analyses revealed that animals receiving both morphine and naloxone spent significantly less time sniffing the other rat compared with vehicle control animals and animals receiving naloxone alone (p=.007 and p=.042, respectively). Time spent following the other rat significantly differed between vehicle control animals and animals treated with both morphine and naloxone, such that morphine-naloxone-treated animals showed reduced following time (p=.035). Post hoc analyses for time spent grooming revealed that animals treated with naloxone alone, morphine alone, and with both morphine and naloxone spent significantly less time grooming the other rat compared with vehicle control animals (p=.002, p=.031, and p=.007, respectively).

Discussion

SR141716-precipitated THC withdrawal produced some level of anxiety-related behaviour, but was not exclusively associated with these behaviours. Rather, THC *per se* appeared to be anxiogenic, which is in agreement with previous reports of cannabinoid-

associated anxiety (Schramm-Sapyta et al., 2007; Arévalo et al., 2001; Onaivi, Green, & Martin, 1990), and this effect was neither reversed nor augmented by subsequent SR141716 exposure. Past research has demonstrated that pre-treatment with SR141716 fails to inhibit the anxiogenic effects of cannabinoids (Arévalo et al., 2001); however, SR141716 on its own has also been shown to produce anxiety (Navarro et al., 1997; Arévalo et al., 2001), which was not observed in the current study. These findings are in accordance with Experiment 2 that SR141716 has no motivational effects *per se* nor blocked the consequences of THC. Furthermore, comparable levels of anxiety induced by THC and withdrawal from THC may explain the lack of a withdrawal associated dysphoric state in the cue-conditioning procedure.

In contrast to the locomotor assessment in Experiment 2, THC did not reliably suppress activity on the anxiety test battery. Although incidence of rearing was reduced in THC-treated animals on the social interaction test, the number of entries to the closed arms was not altered on the EPM. Interestingly, SR141716 was found to attenuate the reduction in rearing induced by THC. This observation is in accordance with previous studies that show reversal of CP 55,940- and WIN 55,212-2-induced locomotor effects by SR141716 (Arévalo et al., 2001; Rinaldi-Carmona et al., 1994), but contradicts the locomotor results of Experiment 2 in the current study.

Naloxone-precipitated morphine withdrawal clearly induced anxiety-related behaviour on all three measures. Neither morphine nor naloxone on its own elicited behaviour indicative of anxiety. These findings are consistent with past research of precipitated morphine withdrawal-induced anxiety (Schulteis et al., 1998; Zhang & Schulteis, 2008). No alterations of locomotor activity were observed in animals treated

with morphine, naloxone, or morphine and naloxone based on closed arm entries on the EPM and incidence of rearing during social interaction. Although morphine-induced hyperactivity was recorded in Experiment 2, this discrepancy is likely attributable to the short duration and single trials of the anxiety tests. Nonetheless, this finding agrees with previous similar studies (Schulteis et al., 1998; Zhang & Schulteis, 2008).

General Discussion

Results of the present study can be summarized as follows: (1) consistent with past research, administration of SR141716 produced physical signs of withdrawal in rats chronically exposed to THC; (2) as previously observed, SR141716 induced withdrawal-like behaviour in THC-naïve rats, but to a lesser extent than in dependent rats; (3) precipitated THC withdrawal failed to produce a conditioned cue avoidance; (4) increased anxiety was observed in rats withdrawing from THC, but this increase was not dissimilar to anxiety induced by THC on its own; (5) naloxone-precipitated morphine withdrawal produced a significant conditioned cue avoidance following three conditioning trials and induced clear anxiogenic-like behaviour, confirming the validity of the tests.

Collectively, these results present novel evidence that SR141716-precipitated THC withdrawal induces a somatic manifestation of drug withdrawal in the absence of a clear dysphoric or anxiogenic state. This withdrawal pattern is peculiar as cessation of other drugs of abuse produces distinct and conjunct somatic and dysphoric signs. Interestingly, however, adaptive neurophysiological responses to THC withdrawal are similar to those produced by withdrawal from opiates.

An increase of adenylyl cyclase (AC) activity is observed in the cerebellum during SR141716-precipitated THC withdrawal, which could be due to a compensatory response to the persistent inhibition of AC during chronic THC treatment (Hutcheson et al., 1998). Similarly, naloxone-precipitated morphine withdrawal is expressed at the cellular level by the upregulation of AC activity in brain regions with high opioid receptor populations, such as the locus coeruleus, and upregulation of the cyclic adenosine monophosphate (cAMP) pathway has been directly related to behavioural expression of precipitated morphine withdrawal (Duman, Tallman, & Nestler, 1988; Matthes et al., 1996; Rasmussen, Beitner-Johnson, Krystal, Aghajanian, & Nestler, 1990). Neuroadaptive changes in the AC system may also help to clarify the lack of dysphoric and anxiogenic effects of THC withdrawal. In fact, the absence of adaptive changes of the AC system in brain structures other than the cerebellum, such as the mesolimbic system or the autonomic areas, may explain the lack of dysphoric effects of SR141716-precipitated THC withdrawal (Hutcheson et al., 1998). Morphine withdrawal, on the other hand, induces changes in the striatum and periaqueductal gray (PAG; Matthes et al., 1996), which correlates with the aversive properties of naloxone-precipitated morphine withdrawal.

Elevations in CRF and *c-fos* expression in the amygdala and other stress-responsive brain sites during precipitated cannabinoid withdrawal (Rodriguez de Fonseca et al., 1997) are also common to withdrawal from opiates (Beckmann, Matsumoto, & Wilce, 1995). Furthermore, these increases have been correlated with the progression of physical signs of cannabinoid withdrawal, primarily implicating the basal ganglia in the motor component of cannabinoid withdrawal (Rodriguez de Fonseca et al., 1997).

Interestingly, however, the study demonstrating anxiety-like responses on the EPM in mice undergoing SR141716-precipitated THC withdrawal reported barely detecting somatic signs of withdrawal (Huang et al., 2010). This suggests that the physical and affective effects of cannabinoid withdrawal may be mediated by different neural mechanisms, which could explain the results of the current study.

If the physical and affective aspects of cannabinoid withdrawal are mediated by distinct neural mechanisms, it is possible that these mechanisms are progressively engaged as withdrawal unfolds. Thus, concurrently examining affective manifestations of withdrawal when physical signs are exhibited may actually serve to overlook dysphoria and anxiety. And, although anxiety is often included under the umbrella of a negative affective state, it is possible that even dysphoric and anxiogenic aspects of cannabinoid withdrawal are mediated by separate systems and temporally misaligned. This has recently been suggested for morphine withdrawal (Rothwell et al., 2009). Systematic examination of the time course of peak neural changes associated with physical and affective states of withdrawal may provide helpful direction.

Aside from the possibility that somatic and affective signs of cannabinoid withdrawal are mediated by temporally exclusive mechanisms, the neurophysiological consequences of cannabinoid withdrawal are similar to those of drugs exerting a negative motivational drive during withdrawal. This has been suggested to account for the continued use of cannabis in humans (Rodriguez de Fonseca et al., 1997). Of course, as demonstrated in the current study, behavioural effects of withdrawal from cannabinoids and opioids are distinct *in vivo*. These discrepant findings could result simply from the magnitude of the withdrawal induced by discordant dosing regimens of THC and

morphine and their respective receptor antagonists. Indeed, THC and SR141716 independently produce differential effects at small and large doses (Hutcheson et al., 1998; Sañudo-Peña et al., 1997). Furthermore, downregulation of CB1 receptors associated with tolerance during repeated exposure to cannabinoids (Dill & Howlett, 1987) may require increasing doses over time in order to observe an aversive state induced by SR141716-precipitated withdrawal. However, interrupting cannabinoid treatment with antagonist challenges—as in conditioning trials of the cue avoidance task—may interfere with the effects of chronic cannabinoid exposure, and perhaps reduce the severity of withdrawal.

Finally, although SR141716 provides a valuable animal model of cannabis withdrawal, and there is general agreement that this antagonist produces a valid physical withdrawal syndrome (Aceto et al., 1995; Diana et al., 1998; Moranta, Esteban, & García-Sevilla, 2009; Tsou et al., 1995), there are important considerations for its use in these types of studies and the generalizability of the results. As previously discussed, SR141716 appears to have intrinsic activity, which may modify the true manifestations of cannabinoid withdrawal and explain the disconnect between physical and affective withdrawal characteristics. Whether the action of SR141716 is by inverse agonistic effects on the CB1 receptor or by antagonistic effects on the endogenous cannabinoid system remains to be determined. Further research is required to elucidate the biochemical and behavioural functions of SR141716 and its role in precipitated cannabinoid withdrawal. It would be prudent to investigate spontaneous cannabinoid withdrawal, despite the methodological difficulties, to corroborate results from

precipitated withdrawal studies. After all, SR141716 produces withdrawal-like effects that otherwise do not occur in spontaneous withdrawal.

In conclusion, the present study is the first to demonstrate a dissociation between the physical and affective signs of cannabinoid withdrawal in rats, contributing to the divergent literature on the existence and clinical significance of a cannabis withdrawal syndrome. The behavioural model of precipitated cannabinoid withdrawal was confirmed, though the motivational effects of THC withdrawal are lacking. Although there are some similarities between THC and morphine withdrawal, results generally indicate that the consequences of abstaining from both drugs of abuse are fundamentally different and that withdrawal from THC is less intense. Based on these results, continued use of cannabis cannot be explained by the simple desire to alleviate negative aspects of withdrawal. However, future research on the relationship between amount or duration of cannabis use and the associated severity of withdrawal as well as the potential for relapse is necessary to resolve the clinical importance of a cannabis withdrawal syndrome. These findings could reveal the need for pharmacological and behavioural treatments to abate cannabis withdrawal.

References

Aceto, M. D., Scates, S. M., Lowe, J. A., & Martin, B. R. (1995). Cannabinoid precipitated withdrawal by the selective cannabinoid receptor antagonist SR141716A. *European Journal of Pharmacology, 282*, R1-R2.

Aceto, M. D., Scates, S. M., Lowe, J. A., & Martin, B. R. (1996). Dependence on delta 9-tetrahydrocannabinol: studies on precipitated and abrupt withdrawal. *The Journal of Pharmacology and Experimental Therapeutics, 278*, 1290-1295.

Aceto, M. D., Scates, S. M., & Martin, B. R. (2001). Spontaneous and precipitated withdrawal with a synthetic cannabinoid, WIN 55212-2. *European Journal of Pharmacology, 416*, 75-81.

Aceto, M. D., Scates, S. M., Razdan, R. K., & Martin, B. R. (1998). Anandamide, an endogenous cannabinoid, has a very low physical dependence potential. *Journal of Pharmacology and Experimental Therapeutics, 287*, 598-605.

American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders: DSM-IV-TR*, 4th edition. American Psychiatric Association: Washington, DC, xxxvii, 943 p.pp.

Beardsley, P. M., Balster, R. L., & Harris, L. S. (1986). Dependence on tetrahydrocannabinol in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics, 239*, 311-319.

Beckmann, A. M., Matsumoto, L., & Wilce, P. A. (1995). Immediate early gene expression during morphine withdrawal. *Neuropharmacology, 34*, 1183-1189.

Buckman, S. G., Hodgson, S. R., Hofford, R. S., & Eitan, S. (2009). Increased elevated plus maze open-arm time in mice during spontaneous morphine withdrawal. *Behavioural Brain Research, 197*, 454-456.

Budney, A. J., Novy, P. L., & Hughes, J. R. (1999). Marijuana withdrawal among adults seeking treatment for marijuana dependence. *Addiction, 94*, 1311-1322.

Budney, A.J., Hughes, J. R., Moore, B. A., & Novy, P. L. (2001). Marijuana abstinence effects in marijuana smokers maintained in their home environment. *Archives of General Psychiatry, 58*, 917-924.

Budney, A. J., Hughes, J. R., Moore, B. A., & Vandrey, R. (2004). Review of the validity and significance of cannabis withdrawal syndrome. *American Journal of Psychiatry, 161*, 1967-1977.

Budney, A. J., Moore, B. A., Vandrey, R., & Hughes, J. R. (2003). The time course and significance of cannabis withdrawal. *Journal of Abnormal Psychology, 112*, 393-402.

Chaperon, F., Soubrié, P., Puech, A. J., & Thiébot, M. H. (1998). Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. *Psychopharmacology, 135*, 324-332.

Cheer, J. F., Kendall, C. A., & Marsden, C. A. (2000). Cannabinoid receptor and reward in the rat: a conditioned place preference study. *Psychopharmacology, 151*, 25-30.

Compton, D., Aceto, M., Lowe, J., & Martin, B. (1996). In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of Δ^9-tetrahydrocannabinol-induced responses and apparent agonist activity. *Journal of Pharmacology and Experimental Therapeutics, 277*, 586-594.

Crawley, J. & Goodwin, F. K. (1980). Preliminary report of a simple animal behaviour model for the anxiolytic effects of benzodiazepines. *Pharmacology, Biochemistry and Behaviour, 13*, 167-170.

Cunningham, C. L., Ferree, N. K., & Howard, M. A. (2003). Apparatus bias and place conditioning with ethanol in mice. *Psychopharmacology, 170*, 409-422.

Cunningham, C. L., Henderson, C. M., & Bormann, N. M. (1998). Extinction of ethanol-induced conditioned place preference and conditioned place aversion: Effects of naloxone. *Psychopharmacology, 139*, 62-70.

Cunningham, C. L. & Niehus, J. S. (1993). Drug-induced hypothermia and conditioned place aversion. *Behavioural Neuroscience, 107*, 468-479.

Cunningham, C. L., Patel, P., & Milner, L. (2006). Spatial location is critical for conditioning place preference with visual but not tactile stimuli. *Behavioural Neuroscience, 120*, 1115-1132.

Cunningham, C. L. & Prather, L. K. (1992). Conditioning trial duration affects ethanol-induced conditioned place preference in mice. *Animal Learning & Behaviour, 20*, 187-194.

Dewey, W. L., Jenkins, J., O'Rourke, T. & Harris, L. S. (1972). The effects of chronic administration of trans-delta-9-tetrahydrocannabinol on behaviour and the cardiovascular system of dogs. *Archives of International Pharmacodynamic Therapy, 198*, 118-131.

Diana, M., Melis, M., Muntoni, A. L., & Gessa, G. L. (1998). Mesolimbic dopaminergic decline after cannabinoid withdrawal. *Proceedings of the National Academy of Sciences, 95*, 10269-10273.

Dill, G. A., & Howlett, A. C. (1987). Regulation of adenylate cylcase by chronic exposure to cannabimimetic drugs. *Journal of Pharmacology and Experimental Therapeutics, 244*, 1157-1163.

Duman, R. S., Tallman, J. F., & Nestler, E. J. (1988). Acute and chronic opiate regulation of adenylyl cyclase in brain: specific and acute effects in locus coeruleus. *Journal of Pharmacology and Experimental Therapeutics, 246*, 1033-1039.

Emmett-Oglesby, M. W., Harris, C. M., Lane, J. D., & Lal, H. (1984). Withdrawal from morphine generalizes to a pentylenetetrazol stimulus. *Neuropeptides, 5*, 37-40.

Espejo, E. F., Cador, M., & Stinus, L. (1995). Ethopharmacological analysis of naloxone-precipitated morphine withdrawal syndrome in rats: a newly-developed "etho-score". *Psychopharmacology, 122*, 122-130.

File, S. E. (1980). The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *Journal of Neuroscience Methods, 2*, 219-238.

File, S. E. (1985). Animal models for predicting clinical efficacy of anxiolytic drugs: social behaviour. *Neuropsychobiology, 13*, 55-62.

Fredericks, A. B. & Benowitz, N. (1980). An abstinence syndrome following chronic administration of delta-9-tetrahydrocannabinol in rhesus monkeys. *Psychopharmacology, 71*, 201-202.

Frenois, F., Cador, M., Caillé, S., Stinus, L., & Le Moine, C. (2002). Neural correlates of the motivational and somatic components of naloxone-precipitated morphine withdrawal. *European Journal of Neuroscience, 16*, 1377-1389.

Gardner, A. & Mallet, P. E. (2006). Suppression of feeding, drinking, and locomotion by a putative cannabinoid receptor 'silent antagonist'. *European Journal of Pharmacology, 530*, 103-106.

Gellert, V. F. & Holtzman, S. G. (1978). Development and maintenance of morphine tolerance and dependence in the rat by scheduled access to morphine drinking solutions. *Journal of Pharmacology and Experimental Therapeutics, 205*, 536-546.

Glass, M., Dragunow, M., & Faull, R. L. M. (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience, 77*, 299-318.

Grasing, K. Wang, A., & Schlussman, S. (1996). Behavioral measures of anxiety during opiate withdrawal. *Behavioural Brain Research, 80*, 195-201.

Greenberg, I., Mendelson, J. H., Kuehnle, J. C., Mello, N., & Babor, T. F. (1976). Psychiatric and behavioral observations of casual and heavy marijuana users in a controlled research setting. *Annual New York Academy of Sciences, 282*, 72-84.

Haertzen, C. A. & Hooks Jr., N. T. (1969). Changes in personality and subjective experience associated with the chronic administration and withdrawal of opiates. *Journal of Nervous and Mental Disease, 148*, 606-614.

Haney, M., Ward, A. S., Comer, S. D., Foltin, R. W., & Fischman, M. W. (1999a). Abstinence symptoms following oral THC administration to humans. *Psychopharmacology, 141*, 385-394.

Haney, M., Ward, A. S., Comer, S. D., Foltin, R. W., & Fischman, M. W. (1999b). Abstinence symptoms following smoked marijuana in humans. *Psychopharmacology, 141*, 395-404.

Harris, R. T., Waters, W. & McLendon, D. (1974). Evaluation of reinforcing capability of delta-9-tetrahydrocannabinol in rhesus monkeys. *Psychopharmacologia, 37*, 23-29.

Health Canada. (2007). Canadian addiction survey (CAS). Government of Canada, Retrieved April 11, 2008 from http://www.hc-sc.gc.ca/hl-vs/alt_formats/hecs-sesc/pdf/pubs/adp-apd/cas-etc/youth-jeunes/youth-jeunes_e.pdf

Health Canada. (2008). Canadian Tobacco Use Monitoring Survey (CTUMS). Government of Canada, Retrieved December 3, 2009 from http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/research-recherche/stat/_ctums-esutc_2008/ann-table10-eng.php

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S.,..., Rice, K. C. (1990). Cannabinoid receptor localization in brain. *Proceedings of the National Academy of Sciences of the United States of America, 87*, 1932-1936.

Heyser, C. J., Hampson, R. E., & Deadwyler, S. A. (1993). Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. *Journal of Pharmacology and Experimental Therapeutics, 264*, 294-307.

Higgins, G. A., Nguyen, P., Joharchi, N., & Sellers, E. M. (1991). Effects of 5-HT_3 receptor antagonists on behavioural measures of naloxone-precipitated opioid withdrawal. *Psychopharmacology, 105*, 322-328.

Higgins, G. A. & Sellers, E. M. (1994). Antagonist-precipitated opioid withdrawal in rats: evidence for dissociations between physical and motivational signs. *Pharmacology Biochemistry and Behaviour, 48*, 1-8.

Hill, M. N., Gorzalka, B. B., & Choi, J. W. (2004). Augmentation of the development of behavioural tolerance to cannabinoid administration through Pavlovian conditioning. *Neuropsychobiology, 49,* 94-100.

Hodgson, S. R., Hofford, R. S., Norris, C. J., & Eitan, S. (2008). Increased elevated plus maze open-arm time in mice during naloxone-precipitated morphine withdrawal. *Behavioural Pharmacology, 19*, 805-811.

Huang, P., Liu-Chen, L. Y., & Kirby, L. G. (2010). Anxiety-like effects of SR 141716-precipitated delta-9-tetrahydrocannabinol withdrawal in mice in the elevated plus-maze. *Neuroscience Letters, in press.*

Huestis, M. A., Gorelick, D. A., Heishman, S. J., Preston, K. L., Nelson, R. A., … Frank, R. A. (2001). Blockade of effects of smoked marijuana by CB1-selective cannabinoid receptor antagonist SR141716. *Archives of General Psychiatry, 58*, 322-328.

Hutcheson, D., Tzavara, E., Smadja, C. T., Valjent, E., Roques, B., Hanoune, J., Maldonado, R. (1998). Behavioural and biochemical evidence for signs of abstinence in mice chronically treated with Δ^9-tetrahydrocannabinol. *British Journal of Pharmacology, 125*, 1567-1577.

Jones, R. T. & Benowitz, N. (1976). The 30-day trip: clinical studies of cannabis tolerance and dependence. *Pharmacology of Marihuana,* 627-642.

Jones, R. T., Benowitz, N, & Bachman, J. (1976). Clinical studies of cannabis tolerance and dependence. *Annual New York Academy of Science, 282*,221-239.

Kaymakcalan, S. (1973). Tolerance to and dependence on cannabis. *Bulletin on Narcotics, 25*, 39-47.

Koob, G. F., Stinus, L., Le Moal, M., & Bloom, F. E. (1989). Opponent process theory of motivation: neurobiological evidence from studies of opiate dependence. *Neuroscience & Biobehavioural Reviews, 13*, 135-140.

Kouri, E. M. & Pope, H. G. (2000). Abstinence symptoms during withdrawal from chronic marijuana use. *Experimental and Clinical Psychopharmacology, 8*, 483-492.

Kuribara, H. (1995). Modification of morphine sensitization by opioid and dopamine receptor antagonists: evaluation by studying ambulation in mice. *European Journal of Pharmacology, 275*, 251-258.

Leite, J. R. & Carlini, E. A. (1974). Failure to obtain "cannabis-directed behaviour" and abstinence syndrome in rats chronically treated with cannabis sativa extracts. *Psychopharmacologia, 36*, 133-145.

Lichtman, A. H., Dimen, K. R., & Martin, B. R. (1995). Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology, 119*, 282-131.

Lichtman, A. H. & Martin, B. R. (2002). Marijuana withdrawal syndrome in the animal model. *Journal of Clinical Pharmacology, 42*, 20S-27S.

Lichtman, A. H. & Martin, B. R. (1996). Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. *Psychopharmacology, 126*, 125-131.

Lichtman, A. H., Wiley, J. L., LaVecchia, K. L., Neviaser, S. T., Arthrur, D. B., ... Martin, B. R. (1998). Effects of SR 141716A after acute or chronic cannabinoid administration in dogs. *European Journal of Pharmacology, 357*, 139-148.

Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. *Pharmacology & Therapeutics, 46*, 321-340.

Maldonado, R., Stinus, L., Gold, L. H., & Koob, G. F. (1992). Role of different brain structures in the expression of the physical morphine withdrawal syndrome. *Journal of Pharmacology and Experimental Therapeutics, 261*, 669-677.

Manwell, L. A., Satvat, E., Lang, S. T., Allen, C. P., Leri, F., & Parker, L. A. (2009). FAAH inhibitor, URB-597, promotes extinction and CB1 antagonist, SR141716, inhibits extinction of conditioned aversion produced by naloxone-precipitated morphine withdrawal, but not extinction of conditioned preference produced by morphine in rats. *Pharmacology, Biochemistry and Behaviour, 94,* 154-162.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature, 346*, 561-564.

Matthes, H. W. D., Maldonado, R., Simoninin, F., Valverde, O., Kitchen, I.,..., Keiffer, B. L. (1996). Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature, 383*, 819-823.

McMillan, D. E., Dewey, W. L., & Harris, L. S. (1971). Characteristics of synthetic tetrahydrocannabinol tolerance. *Annual New York Academy of Sciences, 191*, 83-99.

McMillan, D. E., Harris, L. S., Frankenheim, J. M., & Kennedy, J. S. (1970). Delta-9-trans-tetrahydrocannabinol in pigeons: tolerance to the behavioural effects. *Science, 169*, 501-503.

Moranta, D., Esteban, S., & García-Sevilla, J. A. (2009). Chronic treatment and withdrawal of the cannabinoid agonist WIN 55,212-2 modulate the sensitivity of presynaptic receptors involved in the regulation of monoamine syntheses in rat brain. *Naunyn-Schmiedeberg's Archives of Pharmacology, 379*, 61-72.

Morley, K. C., Gallate, J. E., Hunt, G. E., Mallet, P. E., & McGregor, I. S. (2001). Increased anxiety-like behaviors and impaired memory in rats three months after 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") but not *d*-amphetamine. *European Journal of Pharmacology, 433*, 91-99.

Mucha, R. F. (1987). Is the motivational effect of opiate withdrawal reflected by common somatic indices of precipitated withdrawal? A place conditioning study in the rat. *Brain Research, 418*, 214-220.

Mucha, R. F. (1991). What is learned during opiate withdrawal conditioning? Evidence for a cue avoidance model. *Psychopharmacology, 104*, 391-396.

Mucha, R. F. & Herz, A. (1985). Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. *Psychopharmacology, 82*, 241-247.

Mucha, R. F. & Iversen, B. D. (1984). Reinforcing properties of morphine and naloxone revealed by conditioned place preference: a procedural examination. *Psychopharmacology, 82*, 241-247.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Naure, 365*, 61-64.

Navarro, M., Hernández, E., Muñoz, R. M., del Arco, I., Villanúa, M. A., Carrera, M. R. A., & Rodriguez de Fonseca, F. (1997). Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induced anxiety-like responses in the rat. *NeuroReport, 8*, 491-497.

Norwood, C. S., Cornish, J. E., Mallet, P. E., & McGregor, I. S. (2003). Pre-exposure to the cannabinoid receptor agonist CP 55,940 enhances morphine behavioral sensitization and self-administration in Lewis rats. *European Journal of Pharmacology, 465*, 105-114.

Onaivi, E. S., Green, M. R., & Martin, B. R. (1990). Pharmacological characterization of cannabinoids in the elevated plus-maze. *Journal of Pharmacology and Experimental Therapeutics, 253*, 1002-1009.

Oviedo, A., Glowa, J., & Herkenham, M. (1993). Chronic cannabinoid administration alters cannabinoid receptor binding in rat brain: A quantitative autoradiographic study. *Brain Research, 616,* 293-302.

Parker, L. A., Cyr, J. A., Santi, A. N., & Burton, P. D. (2002). The aversive properties of acute morphine dependence persist 48 h after a single exposure to morphine. Evaluation by taste and place conditioning. *Pharmacology, Biochemistry and Behaviour, 72,* 87-92.

Parker, L. A. & Joshi, A. (1998). Naloxone-precipitated morphine withdrawal induced place aversions: Effect of naloxone at 24 hours postmorphine. *Pharmacology, Biochemistry and Behaviour, 61,* 331-333.

Parker, L. A. & Rennie, M. (1992). Naltrexone-induced aversions: Assessment by place conditioning, taste reactivity and taste avoidance paradigms. *Pharmacology, Biochemistry and Behaviour, 41*, 559-565.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods, 14*, 149-167.

Rasmussen, K., Beitner-Johnson, D., Krystal, J. H., Aghajanian, G. K., & Nestler, E. J. (1990). Opiate withdrawal and the rat locus coeruleus: behavioural, electrophysiological, and biochemical correlates. *Journal of Neuroscience, 349*, 2308-2317.

Richardson, J. D., Aanoson, L., & Hargreaves, K. M. (1997). SR 141716A, a cannabinoid receptor antagonist produces hyperalgesia in untreated mice. *European Journal of Pharmacology, 319*, R3-R4.

Rinaldi-Carmona, M., Barth, F., Héaulme, M., Shire, D., Calandra, B., ... Le Fur, G. (1994). SR 141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Letters, 350*, 240-244.

Robbe, D., Montgomery, S. M., Thome, A., Rueda-Orozco, P. E., McNaughton, B. L., & Buzsaki, G. (2006). Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nature Neuroscience, 9*, 1526-1533.

Rodriguez de Fonseca, F. R., Rocio, M., Carrera, A., Navarro, M., Koob, G. F., & Weiss, F. (1997). Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science, 276*, 2050-2054.

Roma, P. G. & Riley, A. L. (2005). Apparatus bias and the use of light and texture in place conditioning. *Neurobiology of Learning and Memory, 78*, 625-636.

Rothwell, P. E., Thomas, M. J., & Gewirtz, J. C. (2009). Distinct profiles of anxiety and dysphoria during spontaneous withdrawal from acute morphine exposure. *Neuropsychopharmacology, 34*, 2285-2295.

Sañudo-Peña, M. C., Tsou, K., Delay, E. R., Hohman, A. G., Force, M., & Walker, J. M. (1997). Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. *Neuroscience Letters, 223*, 125-128.

Schramm-Sapyta, N. L., Young, C. M., Chaudhry, S., Wilson, W. A., Swartzwelder, H. S., & Kuhn, C. M. (2007). Differential anxiogenic, aversive, and locomotor effects of THC in adolescent and adult rats. *Psychopharmacology, 191*, 867-877.

Singh, M. E., McGregor, I. S., & Mallet, P. E. (2005). Repeated exposure to Δ^9-tetrahydrocannabinol alters heroin-induced locomotor sensitization and Fos-immunoreactivity. *Neuropharmacology, 49,* 1189-1200.

Singh, M. E., Verty, A. N. A., McGregor, I. S., & Mallet, P. E. (2004). A cannabinoid receptor antagonist attenuates conditioned place preference but not behavioural sensitization to morphine. *Brain Research, 1026*, 244-253.

Smith, N. T. (2002). A review of the published literature into cannabis withdrawal symptoms in human users. *Addiction, 97*, 621-632.

Stefanis, C., Liakos, A., Boulourgouris, J. C., Dornbush, R. L., & Ballas, C. (1976). Experimental observations of a 3-day hashish abstinence period and reintroduction of use. *Annual New York Academy of Sciences, 282*, 113-120.

Stiglick, A. & Kalant, H. (1982). Learning impairment in the radial-arm maze following prolonged cannabis treatment in rats. *Psychopharmacology, 77*, 117-123.

Stadnicki, S. W., Schaeppi, U., Rosenkrantz, U., & Braude, M. C. (1974). Crude marihuana extract: EEG and behavioural effects of chronic oral administration in rhesus monkeys. *Psychopharmacologia, 37*, 225-233.

Tsou, K., Brown, S., Sañudo-Peña, M. C., Mackie, K., & Walker, J. M. (1998). Immunohistochemical distribution of cannabinoid CB_1 receptors in the rat central nervous system. *Neuroscience, 83*, 393-411.

Tsou, K., Patrick, S. L., & Walker, M. (1995). Physical withdrawal in rats tolerant to Δ^9-tetrahydrocannabinol precipitated by a cannabinoid receptor antagonist. *European Journal of Pharmacology, 280*, R13-R15.

Verty, A. N. A.,McFarlane, J. R., McGregor, I. S., Mallet, P. E. (2004a). Evidence for an interaction between CB1 cannabinoid and melanocortin MCR-4 receptors in regulating food intake. *Endocrinology, 145*, 3224-3231.

Verty, A. N. A.,McFarlane, J. R., McGregor, I. S., Mallet, P. E. (2004b). Evidence for an interaction between CB1 cannabinoid and oxytocin receptors in food and water intake. *Neuropharmacology, 47*, 593-603.

Verty, A. N. A., McGregor, I. S., & Mallet, P. E. (2004). A cannabinoid receptor antagonist in non-deprived rats equally suppresses consumption of high carbohydrate, high fat, and normal chow. *Neuroscience Letters, 354*, 217-230.

Vezina, P. & Stewart, J. (1987a). Morphine conditioned place preference and locomotion: The effect of confinement during training. *Psychopharmacology, 93*, 257-260.

Vezina, P. & Stewart, J. (1987b). Conditioned locomotion and place preference elicited by tactile cues paired exclusively with morphine in an open field. *Psychopharmacology, 91*, 375-380.

Vivian, J. A., Kishioka, S., Butelman, E. R., Broadbear, J., Lee, K. O., & Woods, J. H. (1998). Analgesic, respiratory and heart rate effects of cannabinoid and opioid agonists in rhesus monkeys: antagonist effects of SR 141716A. *Journal of Pharmacology and Experimental Therapeutics, 286*, 697-703.

Wall, M. E., Sadler, B. M., Brine, D., Taylor, H., & Perez-Reyes, M. (1983). Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women. *Clinical Pharmacology & Therapeutics, 34*, 352.

Wei, E., Loh, H. H., & Way, E. L. (1973). Quantitative aspects of precipitated abstinence in morphine dependent rats. *Journal of Pharmacology and Experimental Therapeutics, 184*, 398-403.

Wiesbeck, G. A., Schuckit, M. A., Kalmijn, J. A., Tipp, J. E., Bucholz, K. K., & Smith, T. L. (1996). An evaluation of the history of a marijuana withdrawal syndrome in a large population. *Addiction, 91*, 1469-1478.

Williams, E., Himmelsbach, C., Wikler, A., Ruble, D., & Lloyd, B. Studies on marihuana and pyrahexyl compound. *Public Health Reports, 61*, 1059-1083.

Table 1

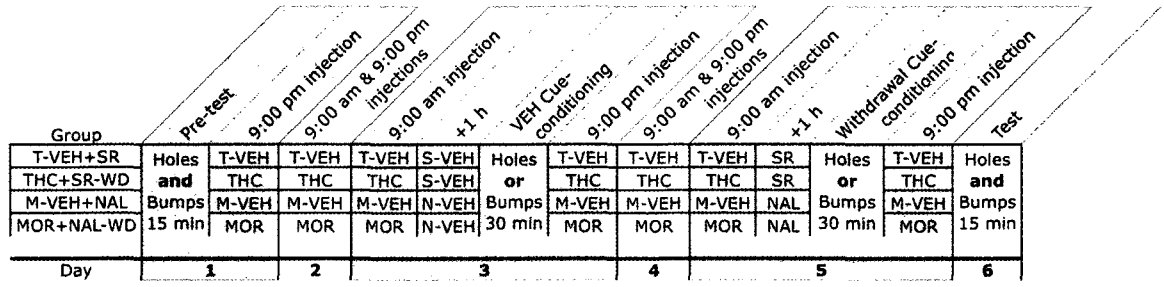
Procedural timeline for Experiment 1: Behavioural Assessment of SR141716-precipitated THC Withdrawal

Group	9:00 pm injection	9:00 am & 9:00 pm injections	9:00 am injection	+1 h	VEH Behavioural Assessment	9:00 pm injection	9:00 am & 9:00 pm injections	9:00 am injection	+1 h	Withdrawal Behavioural Assessment	9:00 pm injection
T-VEH+SR	T-VEH	T-VEH	T-VEH	S-VEH	30 min	T-VEH	T-VEH	T-VEH	SR	30 min	T-VEH
THC+SR-WD	THC	THC	THC	S-VEH		THC	THC	THC	SR		THC
Day	1	2	3				4	5			
		6	7				8	9			
		10	11				12	13			

T-VEH = THC vehicle; S-VEH = SR141716 vehicle; SR = 3 mg/kg SR141716; THC = 5 mg/kg THC; WD = withdrawal.

Table 2

Procedural timeline for Experiment 2A: SR141716-precipitated THC Withdrawal-induced Cue-conditioning, One Cycle



Group	Pre-test	9:00 pm injection	9:00 am & 9:00 pm injections	9:00 am injection	+1 h	VEH Cue-conditioning	9:00 pm injection	9:00 am & 9:00 pm injections	9:00 am injection	+1 h	Withdrawal Cue-conditioning	9:00 pm injection	Test
T-VEH+SR	Holes	T-VEH	T-VEH	T-VEH	S-VEH	Holes	T-VEH	T-VEH	T-VEH	SR	Holes	T-VEH	Holes
THC+SR-WD	**and**	THC	THC	THC	S-VEH	**or**	THC	THC	THC	SR	**or**	THC	**and**
M-VEH+NAL	Bumps	M-VEH	M-VEH	M-VEH	N-VEH	Bumps	M-VEH	M-VEH	M-VEH	NAL	Bumps	M-VEH	Bumps
MOR+NAL-WD	15 min	MOR	MOR	MOR	N-VEH	30 min	MOR	MOR	MOR	NAL	30 min	MOR	15 min
Day	1		2	3				4	5				6

T-VEH+SR = T-VEH = THC vehicle; S-VEH = SR141716 vehicle; SR = 3 mg/kg SR141716; THC = 5 mg/kg THC; M-VEH = morphine vehicle; N-VEH = naloxone vehicle; NAL = 1 mg/kg naloxone; MOR = 5 mg/kg morphine; WD = withdrawal.

Table 3

Procedural timeline for Experiment 2B: SR141716-precipitated THC Withdrawal-induced Cue-conditioning, Three Cycle

Group	Pre-test	9:00 pm injection	9:00 am & 9:00 pm injections	9:00 am injection	+1 h	VEH Cue-conditioning	9:00 pm injection	9:00 am & 9:00 pm injections	9:00 am injection	+1 h	Withdrawal Cue-conditioninc	9:00 pm injection	Test
T-VEH+SR	Holes	T-VEH	T-VEH	T-VEH	S-VEH	Holes	T-VEH	T-VEH	T-VEH	SR	Holes	T-VEH	Holes
THC+SR-WD	**and**	THC	THC	THC	S-VEH	**or**	THC	THC	THC	SR	**or**	THC	**and**
M-VEH+NAL	Bumps	M-VEH	M-VEH	M-VEH	N-VEH	Bumps	M-VEH	M-VEH	M-VEH	NAL	Bumps	M-VEH	Bumps
MOR+NAL-WD	15 min	MOR	MOR	MOR	N-VEH	30 min	MOR	MOR	MOR	NAL	30 min	MOR	15 min
	1		**2**		**3**			**4**		**5**			
Day			**6**		**7**			**8**		**9**			
			10		**11**			**12**		**13**			**14**

T-VEH = THC vehicle; S-VEH = SR141716 vehicle; SR = 3 mg/kg SR141716; THC = 5 mg/kg THC; M-VEH = morphine vehicle; N-VEH = naloxone vehicle; NAL = 1 mg/kg naloxone; MOR = 5 mg/kg morphine; WD = withdrawal.

Table 4

Procedural Timeline for Experiment 3: SR141716-precipitated THC Withdrawal-induced Anxiety

Group	9:00 pm injection	9:00 am & 9:00 pm injections			9:00 am injection	+1 h	+15 min Anxiety Battery
T-VEH+S-VEH	T-VEH	T-VEH			T-VEH	S-VEH	Emergence Test Elevated Plus Maze Social Interaction
T-VEH+SR	T-VEH	T-VEH			T-VEH	SR	
THC+S-VEH	THC	THC			THC	S-VEH	
THC+SR-WD	THC	THC			THC	SR	
M-VEH+N-VEH	M-VEH	M-VEH			M-VEH	N-VEH	
M-VEH+NAL	M-VEH	M-VEH			M-VEH	NAL	
MOR+N-VEH	MOR	MOR			MOR	N-VEH	
MOR+NAL-WD	MOR	MOR			MOR	NAL	
Day	1	2	3	4	5		

T-VEH = THC vehicle; S-VEH = SR141716 vehicle; SR = 3 mg/kg SR141716; THC = 5 mg/kg THC; M-VEH = morphine vehicle; N-VEH = naloxone vehicle; NAL = 1 mg/kg naloxone; MOR = 5 mg/kg morphine; WD = withdrawal.

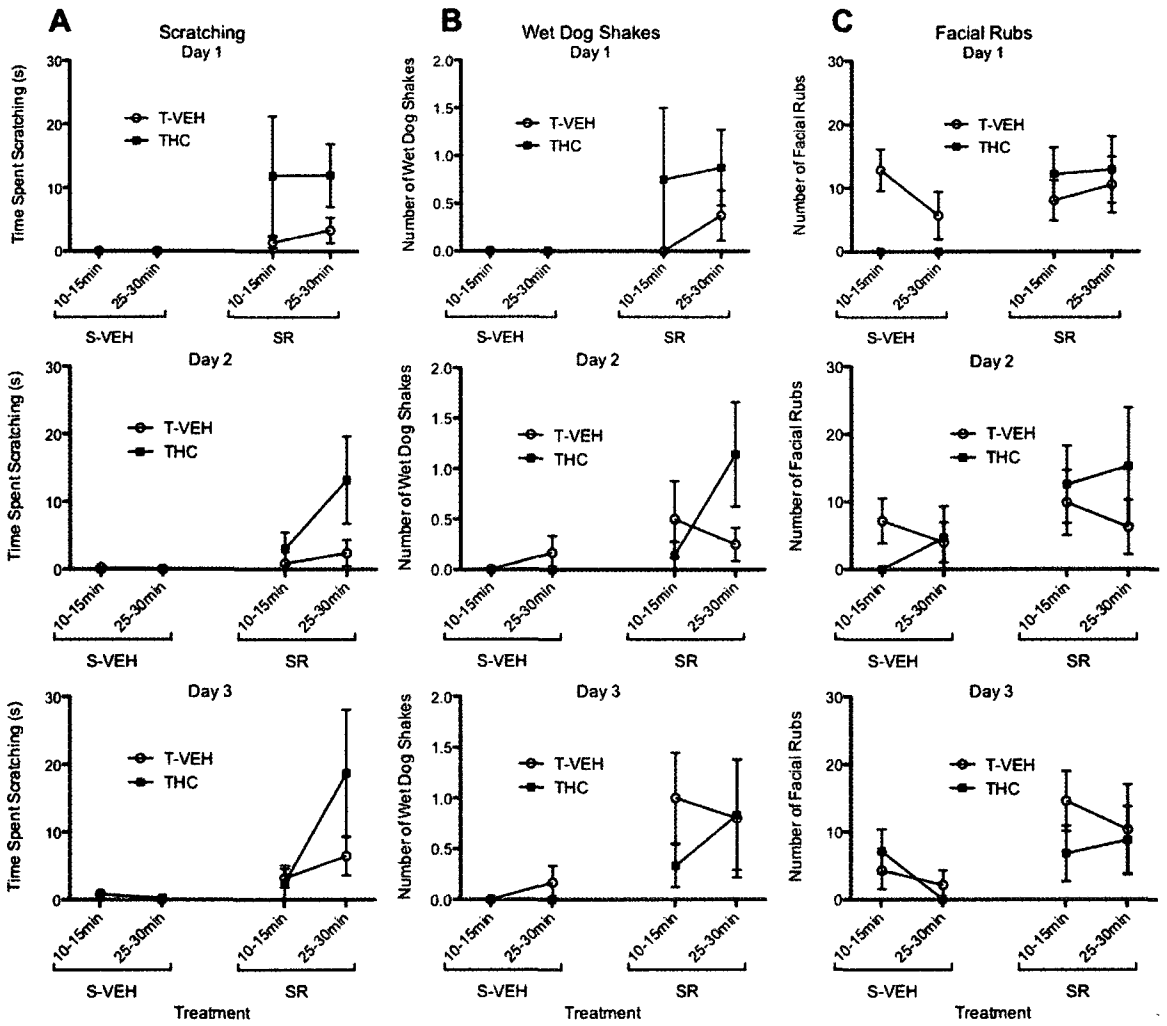


Figure 1. Data for physical symptoms of SR141716-precipitated THC withdrawal. A: mean (±SEM) time spent scratching. SR141716 significantly increased time spent scratching in all animals (p=.003), and THC rats spent significantly more time scratching than T-VEH rats (p=.039). B: mean (±SEM) number of wet-dog shakes. SR141716 significantly increased the number of wet dog shakes (p=.001). C: mean (±SEM) number of facial rubs. SR141716 significantly increased the number of facial rubs (p=.032).

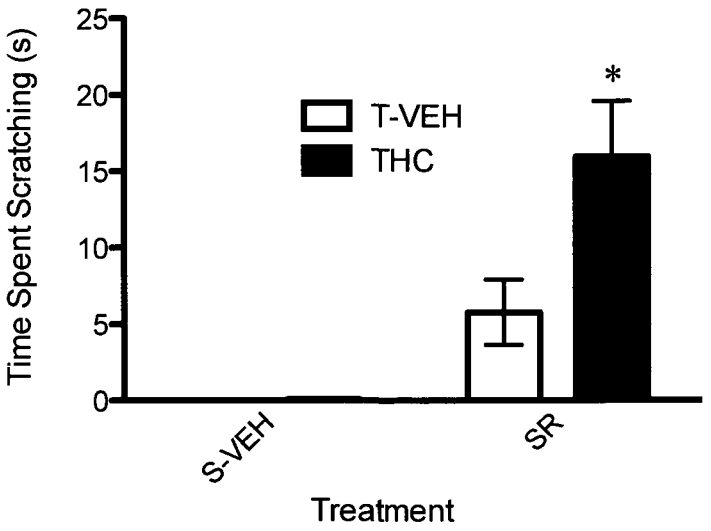


Figure 2. SR141716-precipitated THC withdrawal significantly increased scratching behaviour. Mean (±SEM) time spent scratching. *p<.05, significantly different from T-VEH.

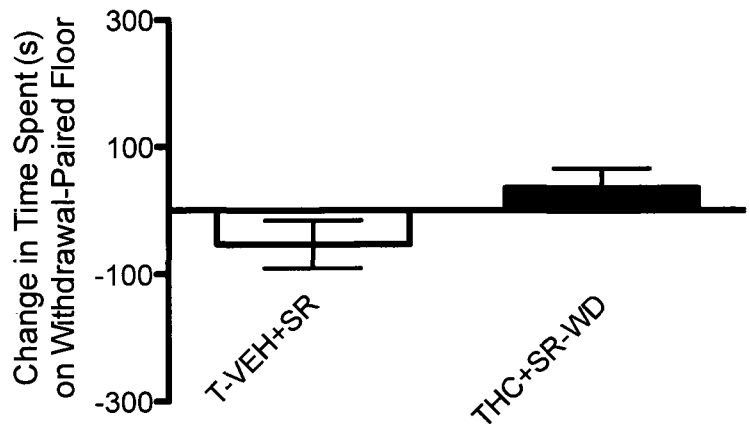


Figure 3. SR141716-precipitated THC withdrawal did not significantly alter conditioned cue preference after one cycle of cue-conditioning. Mean (±SEM) change in time spent on SR141716-paired side between pre-test and test phase of one cycle cue-conditioning.

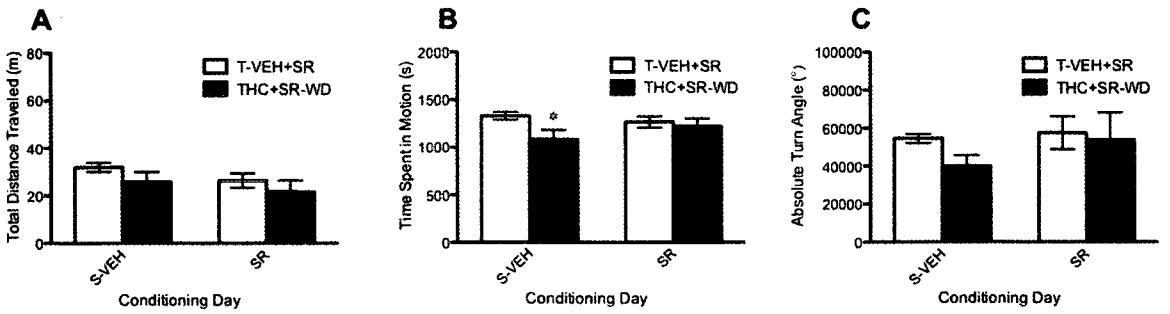


Figure 4. Locomotor activity data for THC groups during conditioning phase of one cycle cue-conditioning. A: mean (±SEM) distance traveled. B: mean (±SEM) time in motion. *p<.05, significantly different from T-VEH. THC-treated animals spent significantly less time in motion than T-VEH-treated animals (p=.042). C: mean (±SEM) absolute turn angle.

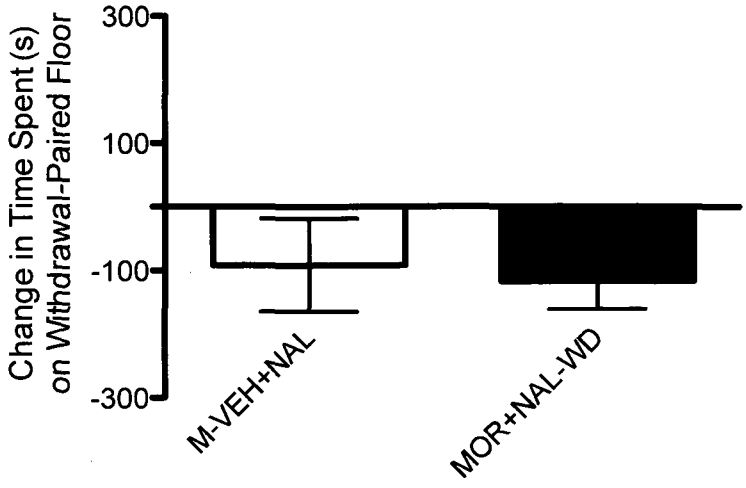


Figure 5. Naloxone-precipitated morphine withdrawal did not significantly alter conditioned cue preference after one cycle of cue-conditioning. Mean (±SEM) change in time spent on naloxone-paired side between pre-test and test phase of one cycle cue-conditioning.

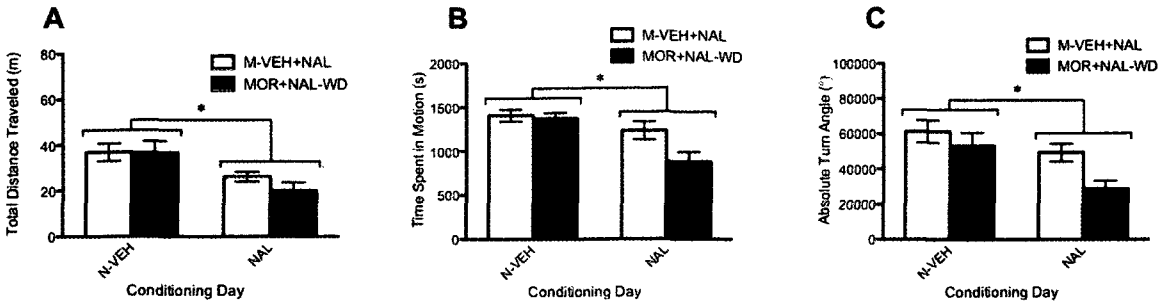


Figure 6. Locomotor activity data for morphine groups during conditioning phase of one cycle cue-conditioning. A: mean (±SEM) distance traveled. *p<.005, significantly different from N-VEH. B: mean (±SEM) time in motion. *p<.001, significantly different from N-VEH. C, mean (±SEM) absolute turn angle. *p<.005, significantly different from N-VEH. MOR-treated animals had a significantly lower absolute turn angle than M-VEH-treated animals (p=.026).

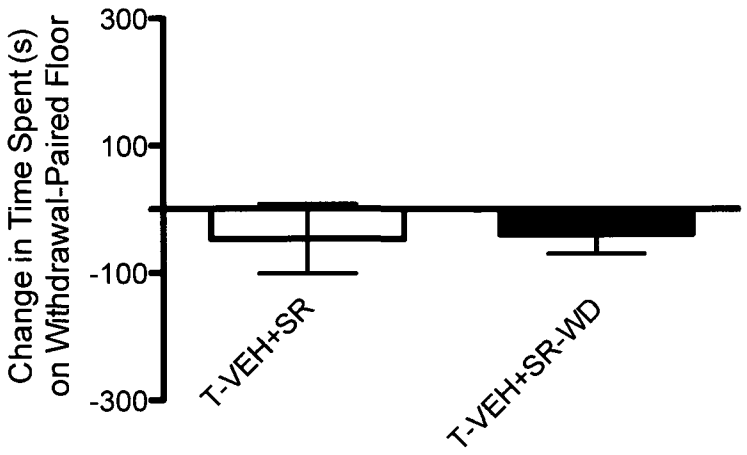


Figure 7. SR141716-precipitated THC withdrawal did not significantly alter conditioned cue preference after three cycles of cue-conditioning Mean (±SEM) change in time spent on SR141716-paired side between pre-test and test phase of three cycle cue-conditioning.

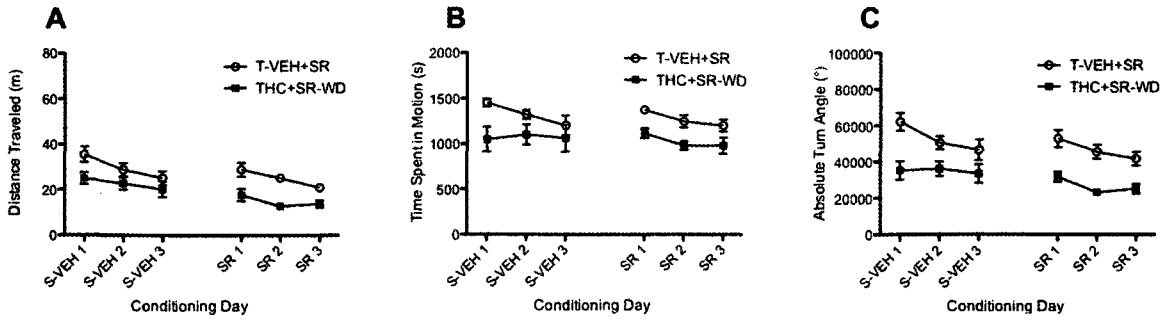


Figure 8. Locomotor activity data for THC groups during conditioning phase of three cycle cue-conditioning. A: mean (±SEM) total distance traveled. THC-treated animals traveled significantly less distance than T-VEH-treated animals (p=.002); treatment with SR141716 significantly reduced distance traveled (p=.001); and all animals traveled a significantly greater distance on day one than on day two (p=.027) and day three (p=.007). B: mean (±SEM) time in motion. THC-treated animals spent significantly less time in motion than T-VEH-treated animals; and all animals spent significantly more time in motion on day one than on day three (p=.03). C: mean (±SEM) absolute turn angle. THC-treated animals had a significantly lower absolute turn angle than T-VEH-treated animals (p<.001); treatment with SR141716 significantly reduced absolute turn angle (p=.016); and absolute turn angle was significantly greater on day one than on day two (p=.049) and day three (p=.041).

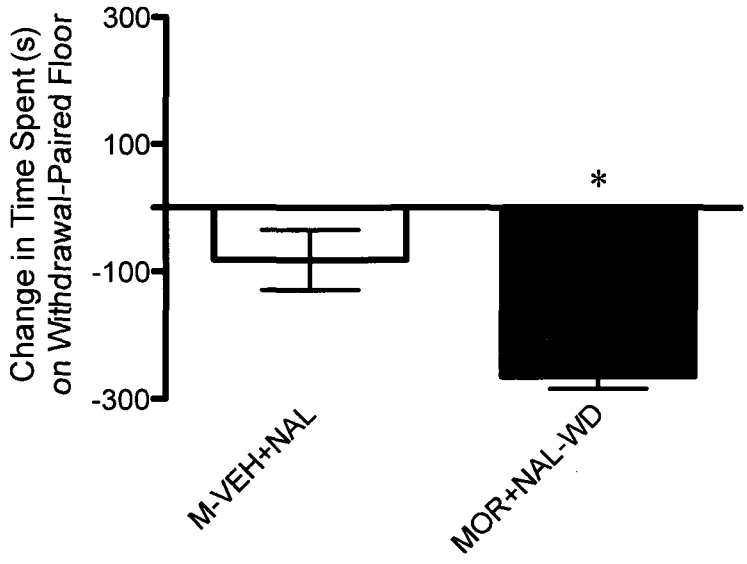


Figure 9. Naloxone-precipitated morphine withdrawal produced a significant conditioned cue avoidance after three cycles of cue-conditioning. Mean (±SEM) change in time spent on naloxone-paired side between pre-test and test phase of three cycle cue-conditioning. *p<.005, significantly different from M-VEH+NAL.

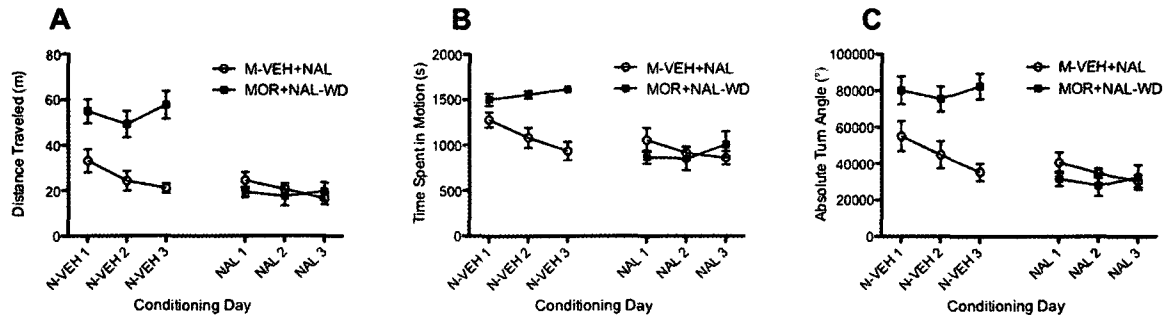


Figure 10. Locomotor activity data for morphine groups during conditioning phase of three cycle cue-conditioning. A: mean (±SEM) total distance traveled. MOR-treated animals traveled significantly greater distance than M-VEH-treated animals ($p=.017$); treatment with NAL significantly reduced distance traveled in all animals ($p<.001$); all animals traveled a significantly greater distance on day one than on day two ($p=.024$); MOR-treated animals traveled a significantly greater distance than M-VEH-treated animals during N-VEH-conditioning sessions; and MOR-treated animals traveled a significantly greater distance than M-VEH-treated animals on day 3 ($p=.001$). B: mean (±SEM) time in motion. MOR-treated animals spent significantly more time in motion than M-VEH-treated animals; treatment with NAL significantly reduced time spent in motion in all animals; MOR-treated animals spent significantly more time in motion than M-VEH-treated animals during N-VEH-conditioning sessions ($p<.001$); and MOR-treated animals spent significantly more time in motion than M-VEH-treated animals on day 3 ($p=.001$). C: mean (±SEM) absolute turn angle. MOR-treated animals had a significantly greater absolute turn angle than M-VEH-treated animals ($p=.038$); treatment with NAL significantly reduced absolute turn angle in all animals ($p<.001$); absolute turn angle was significantly larger on day one than day two ($p=.042$); MOR-treated animals had a significantly greater absolute turn angle than M-VEH-treated animals during N-

VEH-conditioning sessions; and MOR-treated animals had a significantly greater absolute turn angle than M-VEH-treated animals on day 3 ($p=.001$).

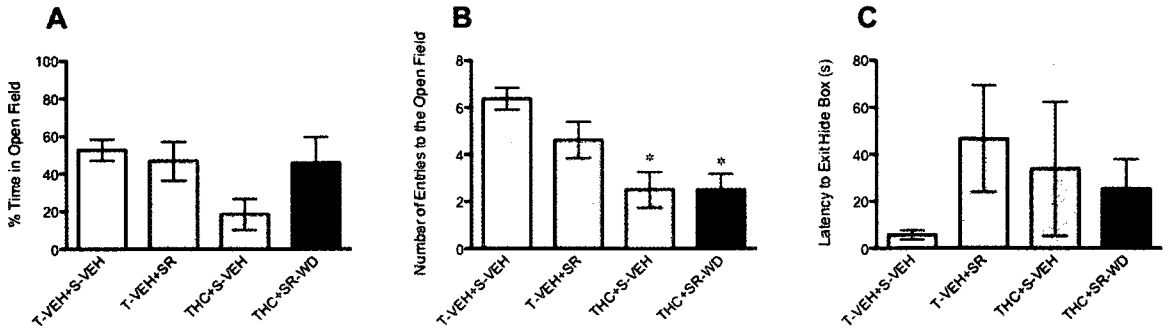


Figure 11. SR141716-precipitated THC withdrawal produced some anxiety-related behaviour on the emergence test, but it was not dissimilar to the behaviour induced by THC on its own. A: mean (±SEM) percent time spent in open field. B: mean (±SEM) number of entries to open field. *p<.005, significantly different from T-VEH+S-VEH. C, mean (±SEM) latency to first exit hide box.

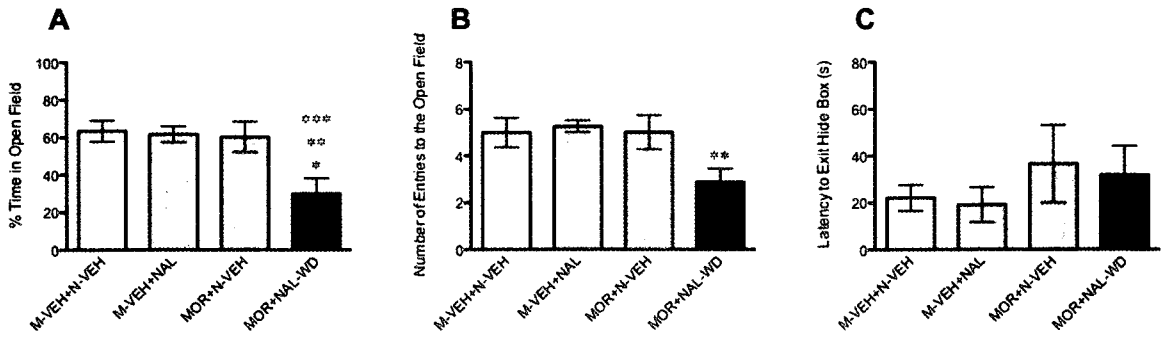


Figure 12. Naloxone-precipitated morphine withdrawal significantly increased anxiety-related behaviour on the emergence test. A: mean (±SEM) percent time spent in open field. *p<.01, significantly different from VEH+VEH; **p<.05, significantly different from M-VEH+NAL; ***p<.05, significantly different from MOR+N-VEH. B: mean (±SEM) number of entries to open field. *p<.05, significantly different from M-VEH+NAL. C: mean (±SEM) latency to first exit hide box.

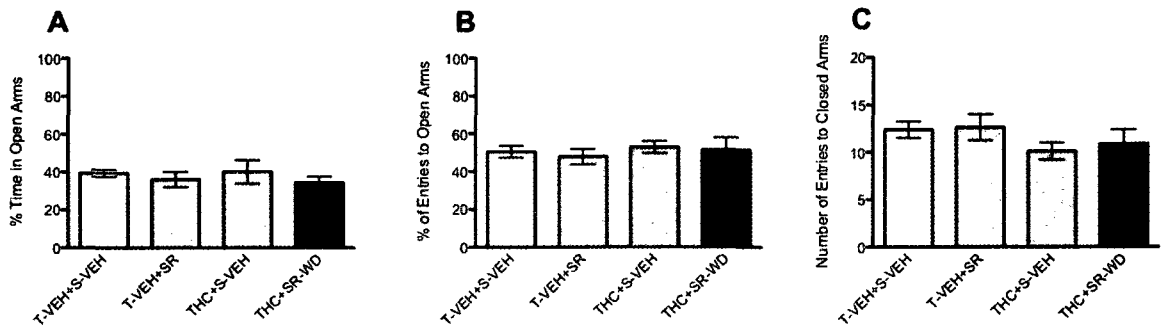


Figure 13. SR141716-precipitated THC withdrawal did not affect anxiety-related behaviour on the EPM. A: mean (±SEM) percent time spent in open arms. B: mean (±SEM) percent number of entries to open arms. C: mean (±SEM) number of entries to closed arms.

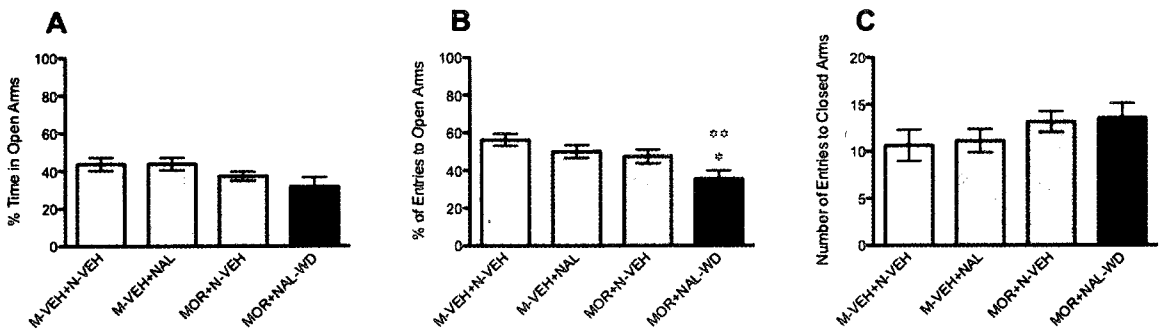


Figure 14. Naloxone-precipitated morphine withdrawal significantly increased anxiety-related behaviour on the EPM. A: mean (±SEM) percent time spent in open arms. B: mean (±SEM) percent number of entries to open arms. *p<.005, significantly different from M-VEH+N-VEH; **p<.05, significantly different from M-VEH+NAL. C: mean (±SEM) number of entries to closed arms.

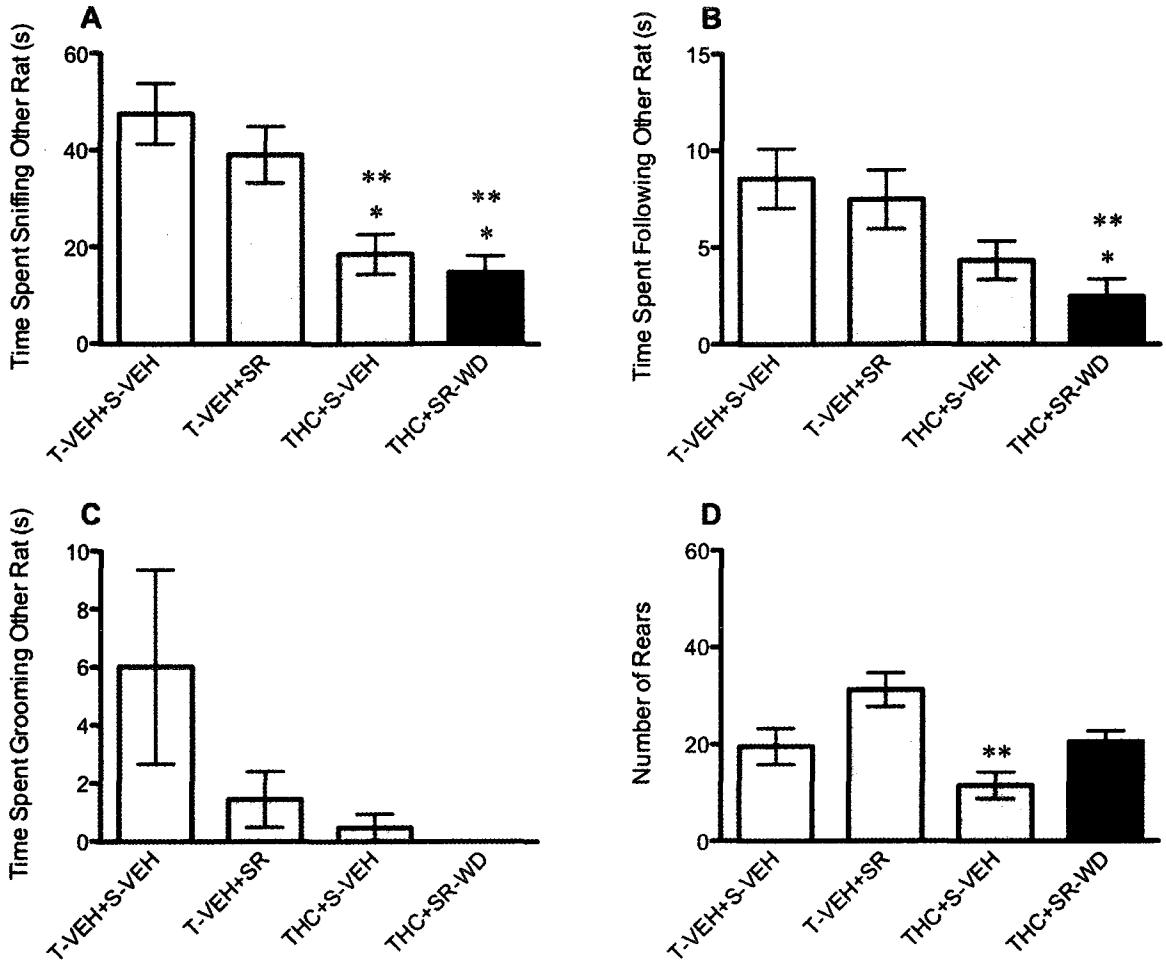


Figure 15. SR141716-precipitated THC withdrawal produced some anxiety-related behaviour during social interaction with an unfamiliar conspecific, but it was generally similar to anxiety-related behaviour induced by THC on its own. A: mean (±SEM) time spent sniffing other rat. *p<.005, significantly different from T-VEH+S-VEH; **p<.05, significantly different from T-VEH+SR. B: mean (±SEM) time spent following other rat. *p<.05, significantly different from T-VEH+S-VEH; **p<.05, significantly different from T-VEH+SR. C: mean (±SEM) time spent grooming other rat. D: mean (±SEM) number of rears. **p<.001, significantly different from T-VEH+SR.

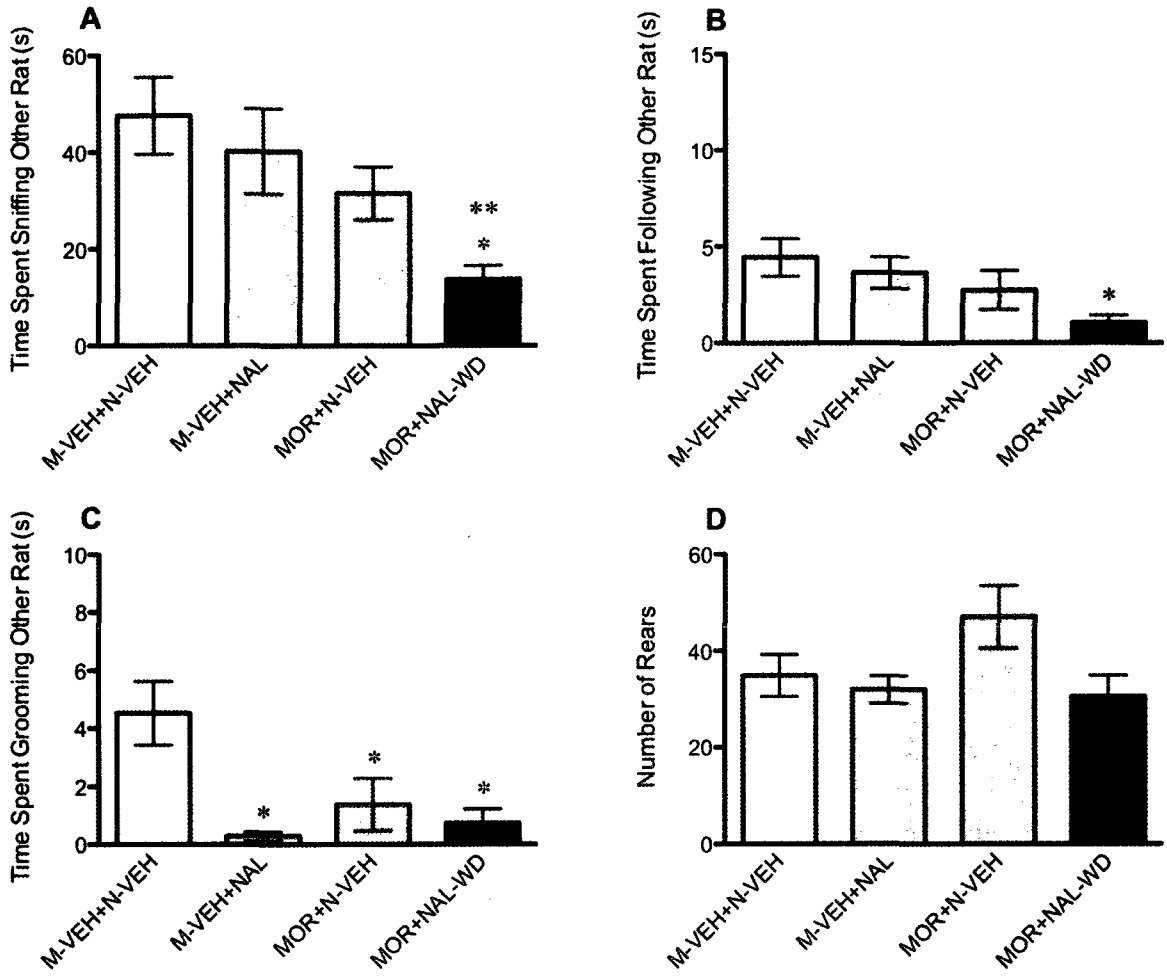


Figure 16. Naloxone-precipitated morphine withdrawal significantly increased anxiety-related behaviour during social interaction with an unfamiliar conspecific. A: mean (±SEM) time spent sniffing other rat. *p<.01, significantly different from M-VEH+N-VEH; **p<.05, significantly different from M-VEH+NAL. B: mean (±SEM) time spent following other rat. *p<.05, significantly different from M-VEH+N-VEH. C: mean (±SEM) time spent grooming other rat. *p<.01, significantly different from M-VEH+N-VEH. D: mean (±SEM) number of rears.