NIH Public Access
Author Manuscript
Pharmacol Biochem Behav. Author manuscript; available in PMC 2015 March 01.

Published in final edited form as:
Pharmacol Biochem Behav. 2014 March ; 118: 30–35. doi:10.1016/j.pbb.2014.01.002.

Evaluation of WIN 55,212-2 self-administration in rats as a potential cannabinoid abuse liability model

Timothy W. Lefever, **Julie A. Marusich**, **Kateland R. Antonazzo**, and **Jenny L. Wiley**
RTI International, Research Triangle Park, NC 27709-2194, USA

Abstract

Because Δ^9-tetrahydrocannabinol (THC) has been a false negative in rat intravenous self-administration procedures, evaluation of the abuse potential of candidate cannabinoid medications has proved difficult. One lab group has successfully trained self-administration of the aminoalkylindole WIN55,212-2 in rats; however, their results have not been independently replicated. The purpose of this study was to extend their model by using a within-subjects design, with the goal of establishing a robust method suitable for substitution testing of other cannabinoids. Male Long-Evans rats were trained to self-administer WIN55,212-2 (0.01 mg/kg/infusion) on a fixed ratio 3 schedule. Dose-effect curves for WIN55,212-2 were determined, followed by vehicle substitution and a dose-effect curve with THC. WIN55,212-2 self-administration was acquired; however, substitution with THC did not maintain responding above vehicle levels. Dose-dependent attenuation by rimonabant confirmed CB_1 receptor mediation of WIN55,212-2's reinforcing effects. Vehicle substitution resulted in a session-dependent decrease in responding (i.e., extinction). While this study provides systematic replication of previous studies, lack of substitution with THC is problematic and suggests that WIN55,212-2 self-administration may be of limited usefulness as a screening tool for detection of the reinforcing effects of potential cannabinoid medications. Clarification of underlying factors responsible for failure of THC to maintain self-administration in cannabinoid-trained rats is needed.

Keywords

abuse liability; aminoalkylindole; cannabinoids; methods; rats; reinforcing effects; self-administration; Δ^9-tetrahydrocannabinol; WIN55,212-2

1.0 Introduction

Self-administration, an animal model of the reinforcing effects of drugs, has high predictive validity for drugs that are abused by humans for their euphoric effects (Ator and Griffiths, 1987; Johanson and Balster, 1978). Consequently, intravenous (i.v.) self-administration has become the "gold standard" in preclinical assessment of abuse liability and is a primary method recommended by the U.S. Food and Drug Administration for use in screening novel compounds (Food and Drug Administration, 2010). However, not all drugs abused by

© 2013 Elsevier Inc. All rights reserved.

To whom correspondence should be addressed: Jenny L. Wiley, Ph.D., RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709-2194, USA, Phone: (1) 919-541-7276, Fax: (1) 919-541-6499, jwiley@rti.org.

Conflicts of interest: There are no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

humans are self-administered by animals. Until a little over a decade ago, Δ^9-tetrahydrocannabinol (THC), the major psychoactive substituent of the marijuana plant, and other cannabinoids were considered to be false negatives in this procedure (e.g., Mansbach et al., 1994). In the early 2000s, Steven Goldberg's group reported success in training squirrel monkeys to self-administer THC (Justinova et al., 2003; Tanda et al., 2000); and later, the endocannabinoids, anandamide (Justinova et al., 2005b) and 2-arachidonoylglycerol (Justinova et al., 2011). Despite success in training nonhuman primates to self-administer THC, robust i.v. self-administration of THC in rodents has not been reported (although see Takahashi and Singer, 1979; 1980). Investigators have demonstrated intracerebroventricular (i.c.v.) self-administration of THC and the bicyclic synthetic cannabinoid, CP 55,940 (Braida et al., 2004a; Braida et al., 2001), and i.v. self-administration of the aminoalkylindole cannabinoid, WIN 55,212-2, in rodents (Fattore et al., 2001; Martellotta et al., 1998). Further, CB_1 receptor mediation of the reinforcing effects of these cannabinoids was suggested by reversal by the CB_1 receptor antagonist rimonabant (Braida et al., 2004a; Braida et al., 2001; Fattore et al., 2001). Conditioned place preference does not offer a viable alternative, as results of this type of experiment have been inconsistent with reports of both cannabinoid-induced preference (Lepore et al., 1995; Valjent and Maldonado, 2000) and aversion/no effect (Chaperon et al., 1998; Cheer et al., 2000). Although cannabinoids have not been widely investigated in intracranial self-stimulation procedures, extant studies also report mixed results: both facilitation/rewarding effects (Gardner et al., 1988) and anhedonic or no effect (Vlachou et al., 2005; Vlachou et al., 2007).

Although a number of subsequent studies have reported use of WIN55,212-2 self-administration to investigate various aspects of the reinforcing effects of cannabinoids (Deiana et al., 2007; Fadda et al., 2006; Fattore et al., 2001; Fattore et al., 2007a; Fattore et al., 2010; Fattore et al., 2007b; Martellotta et al., 1998; Mendizábal et al., 2006; Solinas et al., 2007), all but one of these studies (Mendizábal et al., 2006) appear to have originated from one prolific lab group at the University of Cagliari, Italy. Similarly, successful attempts to train nonhuman primates to self-administer cannabinoids have been reported primarily by one intramural lab group at the U.S. National Institute of Drug Abuse (Justinova et al., 2003; Tanda et al., 2000). Hence, widespread availability of methods for evaluation of the reinforcing effects of potential cannabinoid medications is lacking.

Establishment of self-administration as a robust model of the reinforcing effects of cannabinoids requires replication by a number of lab groups and extension of the methods, such that substitution testing of candidate medications can be undertaken in animals that have a proven history of self-administration of the control cannabinoid. The need for such a model is emphasized by two developments: renewed interest in investigation of plant-based products and inhibitors of endocannabinoid metabolic enzymes as potential medications (Hill et al., 2012; Mulvihill and Nomura, 2012; Pryce and Baker, 2012), and emergence of synthetic cannabinoids as an abuse problem (Hu et al., 2011). The present study represents an extension of seminal work by the Italian group on development of a WIN55,212-2 self-administration procedure.

2.0 Materials and methods

2.1 Subjects

Eight male Long-Evans rats (PND50) were surgically implanted with indwelling jugular catheters and attached to Quick Connect Harnesses (QCH-23, SAI Infusion Technologies) at the vendor (Harlan, Dublin, VA). This strain of rat was chosen because it has been the most commonly used strain in previous investigations of cannabinoid self-administration (Deiana et al., 2007; Fadda et al., 2006; Fattore et al., 2001; Fattore et al., 2007a; Solinas et al.,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

2007). Upon arrival, the rats were individually housed in clear polycarbonate cages in a temperature-controlled (20–22°C) environment with a 12h light-dark cycle (lights on at 6 a.m.). Catheters were flushed with 0.2ml sterile saline USP prior to each test session and with 0.2ml post-flush solution (0.01% gentamicin, 0.03% heparin, 99.6% sterile saline USP) post testing. Throughout the procedure, rats were maintained at ~90% of their free feeding weight and were fed 15–20g of rat chow (Purina® Certified 5002, Barnes Supply, Durham, NC) per day following testing. Rats had ad libitum access to water in their home cages at all times. All experiments were carried out in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and were approved by the Institutional Animal Care and Use Committee at RTI.

2.2 Apparatus

Rats were trained and tested in standard operant conditioning chambers (ENV-007CT, Med Associates, St. Albans, VT) housed in sound-attenuating cubicles (ENV-018V, Med Associates). Each chamber had two retractable levers, with a stimulus light over each lever and a house light mounted on the opposite wall near the top of the chamber. Fans provided ventilation for each chamber and speakers provided ~80db of white noise. Infusion pumps (PHM-100VS, Med Associates) were located outside the cubicle. A luered extension line (EXT-36, SAI) was attached to a 20ml syringe at the infusion pump and fed through a small hole in the cubicle where it was attached to a tether (QCT-12, SAI), supported by a swing arm (PHM-110-SAI, Med Associates) and swivel (QCS-D, SAI) at the top of the operant chamber. The tether was then connected directly to the quick connect harness on each rat. The swing arm, swivel, tether and quick connect harness allowed the rats to move freely inside the operant chamber. Infusion lines and tethers were flushed at the end of each test day with 2.0 ml 80% ETOH (VWR, Radnor, PA), followed by 2.0 ml distilled water. MED-PC software (Med Associates version 4.31) was used to control experimental events and record data.

2.3 Drugs

Δ^9-Tetrahydrocannabinol [THC] [National Institute on Drug Abuse (NIDA), Bethesda, MD] and WIN55,212-2 (Cayman Chemical, Ann Arbor, MI) were suspended in a vehicle of 1% Polysorbate 80 N.F. (VWR, Radnor, PA) and sterile saline USP (Butler Schein, Dublin, OH). Rimonabant [SR141716] (NIDA) was suspended in a vehicle of 7.8% Polysorbate 80 and sterile saline. Concentrated stock solutions of WIN55,212-2 and THC were suspended in a vehicle of 1% Polysorbate 80 and sterile saline, and this same vehicle of 1% Polysorbate 80 and sterile saline was used to dilute the stock solutions. When formulating THC, all ethanol was evaporated off prior to making the concentrated stock solutions. Syringes were kept in a dark refrigerator when not in use during the self-administration sessions to avoid exposure to light. Solutions of all drugs were used for a maximum of one month after initial formulation to avoid degradation. Doses of all drugs are expressed as mg/kg of the base. WIN55,212-2 and THC were administered to the rats intravenously by infusion. Rimonabant was injected intraperitoneally (i.p.) at a volume of 1 ml/kg 30 min prior to the start of the session. Pre-session injection time was based upon our previous experience with this compound. Post-flush solution components, gentamicin USP and heparin USP, were purchased from Butler Schein.

2.4 Procedure

After one week of acclimation, rats were food deprived overnight and operant training began. The rats were trained to lever press for WIN55,212-2 (0.01mg/kg/infusion). The first four days of acquisition consisted of a 1-h autoshaping session followed approximately 90 min later by a 1-h self-administration fixed ratio 1 (FR1) schedule of reinforcement session. Autoshaping was utilized in the present study to help initiate lever-pressing and to provide a

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

controlled baseline of behavior (Brown and Jenkins, 1968; Campbell et al., 2002). During the autoshaping session, the active lever was extended at random intervals (30, 40, 50, 60, 70, 80 or 90 s). If the rat pressed the lever, or after 15 s passed, an infusion of the training dose (0.1 ml of 0.01 mg/kg WIN55,212-2) was delivered and the active lever was retracted. Autoshaping continued in this way for 15 min, with 10 infusions delivered during the session. For the remaining 45 min, the active lever was retracted. Subsequently, rats were removed from the chamber and placed in their home cages for 90–120 min until the start of the self-administration session. During the self-administration session, the active lever remained extended and each lever press resulted in delivery of an infusion. Throughout all autoshaping and self-administration sessions, the inactive lever was extended. Presses on the inactive lever were recorded, but had no consequence.

After five session of acquisition, autoshaping sessions were discontinued, and the daily self-administration session was lengthened to 3 h for the remainder of the study. The value of the FR remained at 1 for 3 additional sessions, followed by 3 sessions at FR2 and 10 sessions at FR3 (the terminal FR). During each self-administration session, both levers were extended. Whereas responses on the inactive lever had no programmed consequence, completing the required number of responses (FR) on the active lever resulted in an infusion. Each infusion delivered 0.1 ml of drug to the rat over a period of 3.4 s. Simultaneously, the stimulus lights above both levers turned on. These lights remained on for 20 s. During this time, responses on both levers were recorded, but an additional infusion could not be earned. Starting on the 6th session of FR3, 3 priming infusions were delivered before the start of each session for the remainder of the study.

Following 10 sessions of self-administration training on FR3, dose-effect curves were determined. Each dose was tested for five sessions, during which the session parameters were identical to those described in the preceding paragraph. Throughout the course of the study, assessment of the training dose of WIN55,212-2 was conducted periodically for a total of eleven times. First, a dose-effect curve with WIN55,212-2 was determined followed by tests with vehicle. Responding was allowed to stabilize during vehicle self-administration. After completion of the first WIN55,212-2 dose-effect curve and vehicle substitution/extinction procedure, self-administration of the training dose (0.01 mg/kg/infusion WIN55,212-2) was reinstated for 5–7 sessions. Subsequently, rats were tested with various doses of THC. The effects of pre-treatment with rimonabant, the prototypic CB_1 receptor antagonist, on responding maintained by 0.01 mg/kg/infusion WIN55,212-2 was evaluated next. Each antagonist dose (and controls) was evaluated twice. At the end of the study, the dose-effect curve for WIN55,212-2 was re-determined. Finally, patency tests were conducted at the conclusion of the study to confirm that catheters were still viable. For these tests, 0.2 ml of 15 mg/ml morphine HCl, followed by 0.2 ml sterile saline USP, was administered through the i.v. port and rats were observed for immediate effects. One rat died before completing the initial dose-effect curve, and two rats never acquired WIN55,212-2 self-administration. The data from these rats were omitted from all analyses, resulting in n = 5 for all results.

2.5 Data Analysis

For each operant session, the mean (± SEM) number of infusions and numbers of responses on the active and inactive levers were calculated. Individual subject data for control tests with WIN55,212-2 are shown as the mean (± SEM) number of infusions/session for the last two sessions of each evaluation of the training dose (Figure 1, bottom panel). For the dose-effect curve functions for WIN55,212-2 and THC, data are presented as mean (± SEM) number of infusions/session and/or mean (± SEM) number of presses on the active and inactive levers for each of the five exposures to each drug concentration. Dose-effect functions for infusions of WIN55,212-2 and THC were analyzed using separate two-way

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(dose X exposure) repeated-measures analysis of variance (ANOVA) across dose and vehicle (Figure 2, left top and bottom panels, respectively). Active and inactive lever presses were analyzed for WIN55,212-2 and THC dose-effect curves with separate within-subject factorial (dose X lever X exposure) ANOVAs (Figure 2, right top and bottom panels, respectively). Results for the vehicle substitution/extinction test were analyzed with a repeated measures factorial (exposure day X lever) ANOVA (Figure 3, top panel). For the antagonist tests (and controls), data are presented as mean (± SEM) number of infusions/session for each rat, averaged over the two exposures to each condition. Results of antagonist tests were analyzed with a two-way (infusion condition X pre-session injection) repeated ANOVA (Figure 3, bottom panel). For each experimental unit, significant ANOVAs were further analyzed with Tukey post hoc tests ($\alpha = 0.05$) to specify differences between means.

3.0 Results

The top panel of Figure 1 shows responding on the active and inactive levers during acquisition of self-administration of 0.01 mg/kg/infusion WIN55,212-2. At the beginning of the experiment, responding on both levers was very low. The number of presses on the inactive lever was minimal across the entire acquisition period, averaging 11 or fewer lever presses per session. Differential increases in responding on the active lever began on session 10, and daily pre-session priming infusions began on session 16. Increased responding on the active lever was maintained throughout the FR3 segment of the acquisition period, although individual variability in the magnitude of responding was considerable. Further, between-subject variability remained high throughout the study, as seen in subsequent tests with the training dose (Fig. 1, bottom panel). This dose of WIN55,212-2 (0.01 mg/kg) was assessed eleven times over the course of the study. The mean number of infusions during the last two sessions of WIN55,212-2 availability served as data for each of the tests. With the exception of one rat (#514), the number of infusions was relatively stable across tests; however, considerable variability in this measure was observed between the rats, with four of the rats infusing substantially more WIN55,212-2 than the fifth rat during most sessions (bottom panel). This between rat variability was also reflected in responding for vehicle injections (left side, bottom panel).

Figure 2 shows results of determination of a dose-effect function for WIN 55,212-2 on two occasions (top panels) and a dose-effect function for THC in the same rats (bottom panels). Left panels show number of infusions of each compound and right panels show number of presses on the active and inactive levers. Each dose was evaluated five times, with data presented for each test. The first WIN55,212-2 dose-effect function (top panels, center sections of X-axes) was conducted at the beginning of the study whereas the second one (top panels, right sections of X-axes) was performed at the end of the study. In the first WIN 55,212-2 dose-effect function, the number of infusions was elevated by the 0.0056 and 0.01 mg/kg doses compared to the number of vehicle infusions (V1) on the corresponding exposure. Significant increases occurred during the first exposure to each dose and during the last 2–3 exposures for the 0.01 and 0.0056 mg/kg doses, respectively [dose X exposure interaction: $F(12,48) = 2.36$, $p<0.05$, $1-\beta = 0.92$]. In addition, a significant main effect of dose was obtained for 0.0056 mg/kg versus vehicle [$F(3,12) = 5.26$, $p<0.05$, $1-\beta = 0.82$]. At a higher dose (0.03 mg/kg), the number of infusions did not differ from vehicle levels. Because the descending limb of an inverted U-shaped function typically shows this pattern (i.e., increased responding at lower doses and decreases at higher doses), lower WIN 55,212-2 doses were tested during the second dose-effect function in an attempt to capture the ascending limb of the function. This attempt was successful, as the second WIN 55,212-2 dose-effect function exhibited the common inverted U-shaped curve, with peak number of infusions occurring at the 0.003 mg/kg dose during the last four exposures

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(compared to corresponding vehicle exposure) [dose X exposure interaction: $F(16,64) = 2.69$, $p<0.05$, $1-\beta = 0.99$]. Although significant increases in the number of infusions of each of the other doses were also observed, these increases were confined to the final exposure session and were primarily the result of decreases in the number of infusions of vehicle over exposure session (i.e., extinction). As expected, number of presses on the active lever mirrored results with number of infusion (top right panel). Statistically significant differences between mean numbers of presses on the active lever (across all exposures for each treatment condition) were observed at doses of 0.0056 and 0.01 mg/kg for the first WIN55,212-2 dose-effect curve [dose X lever interaction: $F(3,12) = 5.52$, $p<0.05$, $1-\beta = 0.84$] and at a dose of 0.003 mg/kg for the second WIN55,212-2 dose-effect curve [dose X lever interaction: $F(4,16) = 3.99$, $p<0.05$, $1-\beta = 0.80$]. In addition, the mean numbers of presses on the active lever also exceeded those on the inactive lever at these doses, as well as at the 0.001 and 0.01 mg/kg WIN55,212-2 doses in the second dose-effect curve. In contrast, the number of infusions of THC did not significantly exceed the number of vehicle infusions at any dose or for any exposure (Fig. 2, bottom left panel) [dose X exposure interaction: $F(20,80) = 1.32$, $p>0.05$, $1-\beta = 0.83$] nor did the number of presses on the active lever at any THC dose exceed the number of active lever presses during vehicle sessions (Fig. 2, bottom right panel). The overall number of presses on the active lever was significantly greater than the overall number of presses on the inactive lever only at the 0.01 mg/kg dose of THC [dose X lever interaction: $F(5,20) = 3.37$, $p<0.05$, $1-\beta = 0.80$].

The top panel of Figure 3 shows the results of substitution with vehicle for the WIN55,212-2 training dose over a period of 10 consecutive sessions. Extinction resulted in a session-dependent decrease in responding on the active lever (beginning during session 2 of extinction, compared to responding for WIN55,212-2 0.01 mg/kg/infusion) [exposure X lever interaction: $F(10,40) = 6.39$, $p<0.05$, $1-\beta = 1.0$] without alteration of responding on the inactive lever across sessions. The number of active lever responses significantly exceeded the number of presses on the inactive lever over the first three sessions of extinction and on sessions 5 and 7. The bottom panel of Figure 3 shows the effects of rimonabant on the number of infusions of vehicle or the training dose of WIN55,212-2 (left and right side of panel, respectively). When pre-treated with vehicle, rats self-administered significantly more infusions of WIN55,212-2 (0.01 mg/kg/infusion) than infusions of vehicle [pre-session injection X infusion condition interaction: $F(2,8) = 6.03$, $p<0.05$, $1-\beta = 0.72$]. Pre-treatment with rimonabant dose-dependently attenuated responding for the training dose of WIN55,212-2, with 1 mg/kg rimonabant significantly reducing the number of WIN55,212-2 infusions.

4.0 Discussion

Acquisition of WIN55,212-2 self-administration in naïve rats was successful, albeit associated with considerable within-group variability in number of infusions/session that continued (to a lesser extent) throughout the course of the study. Hence, self-administration was not as robust as for other classes of self-administered drugs such as stimulants (e.g., see Marusich et al., 2013). Nevertheless, when other WIN55,212-2 doses were substituted, orderly dose-effect functions were obtained, with a descending limb evident in the first determination and a full inverted U-shaped function observed in the second determination. Maximal number of infusions for each dose tended to occur during later exposures, albeit the concomitant decrease in vehicle infusions during later exposures (i.e., decrease in comparison condition) may also have been a contributory factor to these results. Dose-dependent reversal of WIN55,212-2's reinforcing effects by pre-treatment with rimonabant suggests CB_1 receptor mediation. These results are consistent with those reported in previous studies in which WIN55,212-2 self-administration was maintained under an FR1 schedule (Deiana et al., 2007; Fattore et al., 2001). However, the maximum mean number of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

WIN55,212-2 infusions in the current study exceeded those seen in previous cannabinoid self-administration studies in rats, which may have been the result of the use of a higher FR (i.e., FR3). In support of this hypothesis, previous research with other drugs of abuse has shown that an increase in the FR requirement from FR1 to FR2, FR3, or FR5 is associated with an increase in response rate and with maintenance or increase in the number of infusions (Celentano et al., 2009; Chaudhri et al., 2006; Marusich and Bardo, 2009; Weeks, 1962). The same result was found for WIN55,212-2 in the present study, as shown by the increase in responding and number of infusions as the FR value increased during acquisition.

The number of vehicle infusions in the present study was also larger than reported previously in at least one study (Fattore et al., 2001) [although not as great as the maximal number of WIN22,212-2 infusions]. Not all of the other previous studies reported rates of vehicle infusion, but rather, responding on the WIN55,212-2-associated lever was compared to responding on the inactive lever (Deiana et al., 2007; Fattore et al., 2007a). For this reason, an examination of responding on active versus inactive levers was undertaken in the current study. Consistent with previous results, WIN55,212-2 produced more responding on the active lever than on the inactive lever (see top right panel of Figure 2). Interestingly, however, the number of responses on the active lever for vehicle was also higher than responding on the inactive lever for 5 of 10 consecutive sessions during an extinction procedure (Figure 3, top panel), suggesting that comparisons between active and inactive lever presses (as used in the cited previous studies) is not the most appropriate measure of self-administration of a compound. Rates of responding on the active lever may be affected by factors other than the reinforcing effects of the drug, including non-drug cues (Marusich et al., 2011), context (Alvers et al., 2012; Gipson et al., 2011), and the internal state of the animal (e.g., stress) (Conrad et al., 2010). Therefore, a comparison of responding for drug compared to vehicle is a more useful measure than inactive lever presses because this comparison holds all associated variables constant except for the drug.

In this study, substitution tests with THC also serve to illustrate this point. Although the overall number of active lever presses for the 0.01 mg/kg dose was significantly higher than inactive lever presses at the same dose, the number of THC infusions was not greater than the number of vehicle infusions at any dose, suggesting that THC was not reinforcing. The failure of THC to maintain self-administration in rats, even with use of a cannabinoid as a training drug, has several possible implications. Since humans and nonhuman primates readily self-administer THC under certain experimental conditions (Justinova et al., 2005a; Justinova et al., 2003), these results may suggest a species difference in sensitivity to the reinforcing effects of THC. These results may also suggest that full and partial CB_1 receptor agonists (i.e., WIN55,212-2 and THC, respectively) (Breivogel and Childers, 2000) differ in their reinforcing efficacies in rats, although THC was self-administered via i.c.v. infusion in this species (Braida et al., 2004b). On the other hand, training conditions for i.v. cannabinoid self-administration in rats may not yet be optimized. In nonhuman primates, this process required research over a number of years before successful THC self-administration was reported (reviewed in Justinova et al., 2005a).

In summary, successful i.v. self-administration of WIN55,212-2, an aminoalkylindole cannabinoid, was acquired and maintained over the course of this study. Substitution of other doses of WIN55,212-2 produced an inverted U-shaped dose-effect function which resembled that obtained with cocaine in rats trained to self-administer this stimulant (e.g., see Marusich et al., 2013). Rimonabant dose-dependently attenuated the number of infusions of the 0.01 mg/kg/infusion WIN55,212-2 training dose, suggesting CB_1 receptor mediation of WIN55,212-2's reinforcing effects. In contrast, the partial agonist THC failed to maintain self-administration above vehicle levels in the WIN55,212-2-trained rats. Vehicle

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

substitution resulted in session-dependent decreases in responding on the active lever (i.e., extinction), with reinstatement upon substitution with WIN55,212-2. While cannabinoid self-administration in rodents has been used fruitfully to examine factors that may affect the reinforcing effects of cannabinoids (e.g., strain, sex, dose), it currently has limited usefulness as a screening procedure for assessment of cannabinoid abuse liability in the medication development process, as demonstrated by its failure to "detect" a positive control (THC) in the present study and lack of substitution testing in previous studies. Clarification of underlying factors responsible for failure of THC to maintain self-administration in cannabinoid-trained rats will require additional research and is crucial for development of this procedure as a screening tool for medications development.

Acknowledgments

Research supported by RTI Internal Research and Development funds and by NIH/NIDA Grants DA-03672 and DA-031988. The funding agencies did not have any role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

References

Ator N, Griffiths R. Self-administration of barbiturates and benzodiazepines: A review. Pharmacol Biochem Behav. 1987; 27:391–398. [PubMed: 2888136]

Braida D, Iosue S, Pegorini S, Sala M. Delta-9-tetrahydrocannabinol-induced conditioned place preference and intracerebroventricular self-administration in rats. Eur J Pharmacol. 2004a; 506:63–69. [PubMed: 15588625]

Braida D, Iosue S, Pegorini S, Sala M. Delta-9-tetrahydrocannabinol induced conditioned place preference and intracerebroventricular self administration in rats. Eur J Pharmacol. 2004b; 506:63–69. [PubMed: 15588625]

Braida D, Pozzi M, Parolaro D, Sala M. Intracerebral self-administration of the cannabinoid receptor agonist CP 55,940 in the rat: interaction with the opioid system. Eur J Pharmacol. 2001; 413:227–234. [PubMed: 11226397]

Breivogel CS, Childers SR. Cannabinoid agonist signal transduction in rat brain: comparison of cannabinoid agonists in receptor binding, G-protein activation, and adenylyl cyclase inhibition. J Pharmacol Exp Ther. 2000; 295:328–336. [PubMed: 10991998]

Chaperon F, Soubrie P, Puech A, Thiebot M. Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. Psychopharmacology (Berl). 1998; 135:324–332. [PubMed: 9539255]

Cheer JF, Kendall DA, Marsden CA. Cannabinoid receptors and reward in the rat: a conditioned place preference study. Psychopharmacology (Berl). 2000; 151:25–30. [PubMed: 10958113]

Conrad KL, McCutcheon JE, Cotterly LM, Ford KA, Beales M, Marinelli M. Persistent increases in cocaine-seeking behavior after acute exposure to cold swim stress. Biol Psychiatry. 2010; 68:303–305. [PubMed: 20494337]

Deiana S, Fattore L, Spano MS, Cossu G, Porcu E, Fadda P, Fratta W. Strain and schedule-dependent differences in the acquisition, maintenance and extinction of intravenous cannabinoid self-administration in rats. Neuropharmacology. 2007; 52:646–654. [PubMed: 17098261]

Fadda P, Scherma M, Spano MS, Salis P, Melis V, Fattore L, Fratta W. Cannabinoid self-administration increases dopamine release in the nucleus accumbens. Neuroreport. 2006; 17:1629–1632. [PubMed: 17001282]

Fattore L, Cossu G, Martellotta CM, Fratta W. Intravenous self-administration of the cannabinoid CB1 receptor agonist WIN 55,212-2 in rats. Psychopharmacology (Berl). 2001; 156:410–416. [PubMed: 11498718]

Fattore L, Spano MS, Altea S, Angius F, Fadda P, Fratta W. Cannabinoid self-administration in rats: sex differences and the influence of ovarian function. Br J Pharmacol. 2007a; 152:795–804. [PubMed: 17891164]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Fattore L, Spano MS, Altea S, Fadda P, Fratta W. Drug- and cue-induced reinstatement of cannabinoid-seeking behaviour in male and female rats: influence of ovarian hormones. Br J Pharmacol. 2010; 160:724–735. [PubMed: 20590575]

Fattore L, Vigano D, Fadda P, Rubino T, Fratta W, Parolaro D. Bidirectional regulation of mu-opioid and CB1-cannabinoid receptor in rats self-administering heroin or WIN 55,212-2. Eur J Neurosci. 2007b; 25:2191–2200. [PubMed: 17419755]

Food and Drug Administration. Guidance for industry: Assessment of abuse potential of drugs. U.S. Department of Health and Human Services; Rockville, MD: 2010.

Gardner EL, Paredes W, Smith D, Donner A, Milling C, Cohen A, Morrison D. Facilitation of brain stimulation reward by Δ^9 -tetrahydrocannabinol. Psychopharmacology (Berl). 1988; 96:142–144. [PubMed: 2852376]

Hill AJ, Williams CM, Whalley BJ, Stephens GJ. Phytocannabinoids as novel therapeutic agents in CNS disorders. Pharmacol Ther. 2012; 133:79–97. [PubMed: 21924288]

Hu X, Primack BA, Barnett TE, Cook RL. College students and use of K2: an emerging drug of abuse in young persons. Subst Abuse Treat Prev Policy. 2011; 6:16. [PubMed: 21745369]

Johanson C, Balster R. A summary of results of drug self-administration studies using substitution procedures in rhesus monkeys. Bull Narc. 1978; 30:43–54. [PubMed: 36945]

Justinova Z, Goldberg SR, Heishman SJ, Tanda G. Self-administration of cannabinoids by experimental animals and human marijuana smokers. Pharmacol Biochem Behav. 2005a; 81:285–299. [PubMed: 15932767]

Justinova Z, Solinas M, Tanda G, Redhi GH, Goldberg SR. The endogenous cannabinoid anandamide and its synthetic analog R(+)-methanandamide are intravenously self-administered by squirrel monkeys. J Neurosci. 2005b; 25:5645–5650. [PubMed: 15944392]

Justinova Z, Tanda G, Redhi GH, Goldberg SR. Self-administration of delta-9-tetrahydrocannabinol (THC) by drug naive squirrel monkeys. Psychopharmacology (Berl). 2003; 169:135–140. [PubMed: 12827345]

Lepore M, Vorel SR, Lowinson J, Gardner EL. Conditioned place preference induced by delta 9-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. Life Sci. 1995; 56:2073–2080. [PubMed: 7776834]

Mansbach RS, Nicholson KL, Martin BR, Balster RL. Failure of Δ9-tetrahydrocannabinol and CP 55,940 to maintain intravenous self-administration under a fixed-interval schedule in rhesus monkeys. Behav Pharmacol. 1994; 5:219–225. [PubMed: 11224271]

Martellotta MC, Cossu G, Fattore L, Gessa GL, Fratta W. Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. Neuroscience. 1998; 85:327–330. [PubMed: 9622233]

Marusich, JA.; Lefever, TW.; Novak, SP.; Blough, BE.; Wiley, JL. RTI Press publication No. OP-0014-1307. RTI Press; Research Triangle Park, NC: 2013. Prediction and prevention of prescription drug abuse: Role of preclinical assessment of substance abuse liability.

Mendizábal V, Zimmer A, Maldonado R. Involvement of kappa/dynorphin system in WIN 55,212-2 self-administration in mice. Neuropsychopharmacology. 2006; 31:1957–1966. [PubMed: 16292318]

Mulvihill MM, Nomura DK. Therapeutic potential of monoacylglycerol lipase inhibitors. Life Sci. 2012; 92:492–497. [PubMed: 23142242]

National Research Council. Guide for the care and use of laboratory animals. National Academies Press; Washington, D.C: 2011.

Pryce G, Baker D. Potential control of multiple sclerosis by cannabis and the endocannabinoid system. CNS Neurol Disord Drug Targets. 2012; 11:624–641. [PubMed: 22583441]

Solinas M, Scherma M, Fattore L, Stroik J, Wertheim C, Tanda G, Fratta W, Goldberg SR. Nicotinic alpha 7 receptors as a new target for treatment of cannabis abuse. J Neurosci. 2007; 27:5615–5620. [PubMed: 17522306]

Takahashi RN, Singer G. Self-administration of Δ9-tetrahydrocannabinol by rats. Pharmacol Biochem Behav. 1979; 11:737–740. [PubMed: 231789]

Takahashi RN, Singer G. Effects of body weight levels on cannabis self-injection. Pharmacol Biochem Behav. 1980; 13:877–881. [PubMed: 6259669]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Tanda G, Munzar P, Goldberg SR. Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nature Neurosci. 2000; 3:1073–1074. [PubMed: 11036260]

Valjent E, Maldonado R. A behavioural model to reveal place preference to delta -9-tetrahydrocannabinol in mice. Psychopharmacology (Berl). 2000; 147:436–438. [PubMed: 10672638]

Vlachou S, Nomikos GG, Panagis G. CB1 cannabinoid receptor agonists increase intracranial self-stimulation thresholds in the rat. Psychopharmacology (Berl). 2005; 179:498–508. [PubMed: 15821959]

Vlachou S, Nomikos GG, Stephens DN, Panagis G. Lack of evidence for appetitive effects of Delta-9-tetrahydrocannabinol in the intracranial self-stimulation and conditioned place preference procedures in rodents. Behav Pharmacol. 2007; 18:311–319. [PubMed: 17551324]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Highlights

- Procedure for evaluation of reinforcing effects of cannabinoids in rodents is needed.
- Rats self-administered WIN55,212-2 (WIN), with inverted U-shaped dose-response curve.
- WIN self-administration was attenuated by the CB_1 receptor antagonist rimonabant.
- Δ^9-Tetrahydrocannabinol (THC) was not self-administered by WIN-trained rats.
- Results suggest limited usefulness of this procedure for abuse liability assessment.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

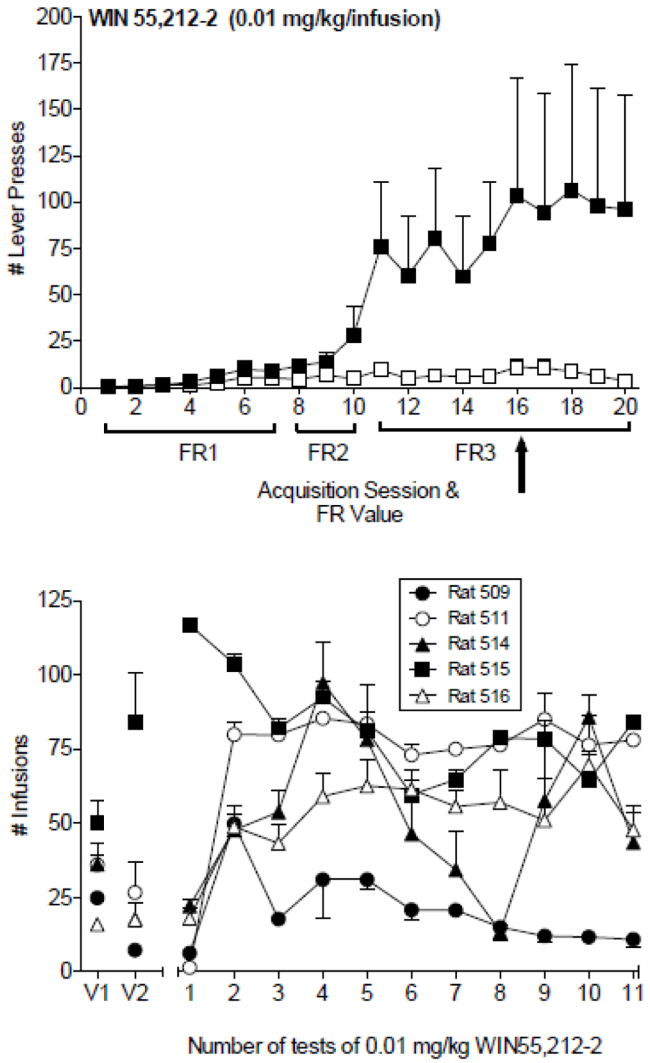

Figure 1.

Top panel: Acquisition of i.v. self-administration of 0.01 mg/kg/infusion WIN55,212-2 in adult male Long Evans rats. Number of presses on the active (filled symbols) and inactive (unfilled symbols) levers is shown as a function of number of acquisition sessions and value of the fixed ratio. Daily pre-session priming with 3 infusions of the training dose was initiated on session 16, as indicated by the arrow, and continued throughout the remainder of the experiment. Each point represents the mean (± SEM) number of lever presses for 5 rats. Bottom panel: Number of infusions of 0.01 mg/kg/infusion WIN55,212-2 for individual subjects during tests with the training dose or vehicle (left side of panel) throughout the study. Each point represents the mean number of infusions (± SEM) for a single rat during five sessions of WIN55,212-2 (0.01 mg/kg) or vehicle availability at 11 different times across the study. Points above V1 and V2 represent mean number of infusions (± SEM) of vehicle during separate exposures that occurred at two different time points during the study.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

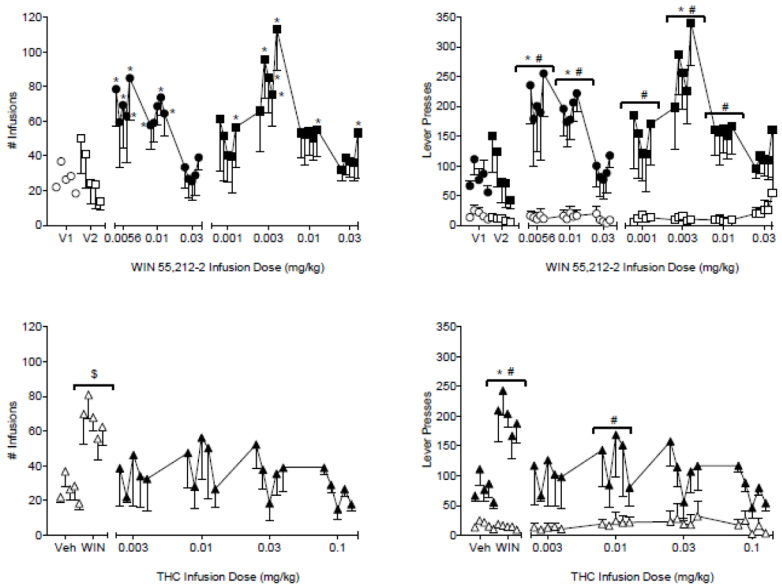

Figure 2.

Substitution tests with various doses of WIN55,212-2 (top panels) and THC (bottom panels). For both drugs, each of 5 substitution tests with each dose (occurring on 5 consecutive sessions) is shown. The top panels show number of infusions (left panel) and active and inactive lever presses (right panel) as a function of WIN55,212-2 dose at the beginning (center section, circles) and end of the experiment (right section, squares). Points above V1 and V2 (left sections of each top panel) represent the results of control tests with vehicle conducted prior to the start of the first and second dose-effect curve determinations, respectively. The bottom panels show number of infusions of THC (left panel) and active and inactive lever presses (right panel) as a function of THC dose (right section). Points above Veh and WIN (left sections of each bottom panel) represent the results of control tests with vehicle and the training dose (0.01 mg/kg/infusion WIN55,212-2), respectively, conducted prior to the start of the dose-effect curve determination. In both right panels, filled symbols represent responses on the active lever and unfilled symbols represent responses on inactive lever. All values represent the mean (±SEM) of data from 5 rats. *P < 0.05 indicates significant dose X day (top left panel) or significant dose X lever (both right panels) interaction and post hoc difference compared to mean rates of infusions or active lever responding during the corresponding control test with vehicle. # P < 0.05 indicates significant dose X lever interaction and post hoc difference compared to presses on the inactive lever at the same dose. $ P < 0.05 indicates a significant main effect of dose.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

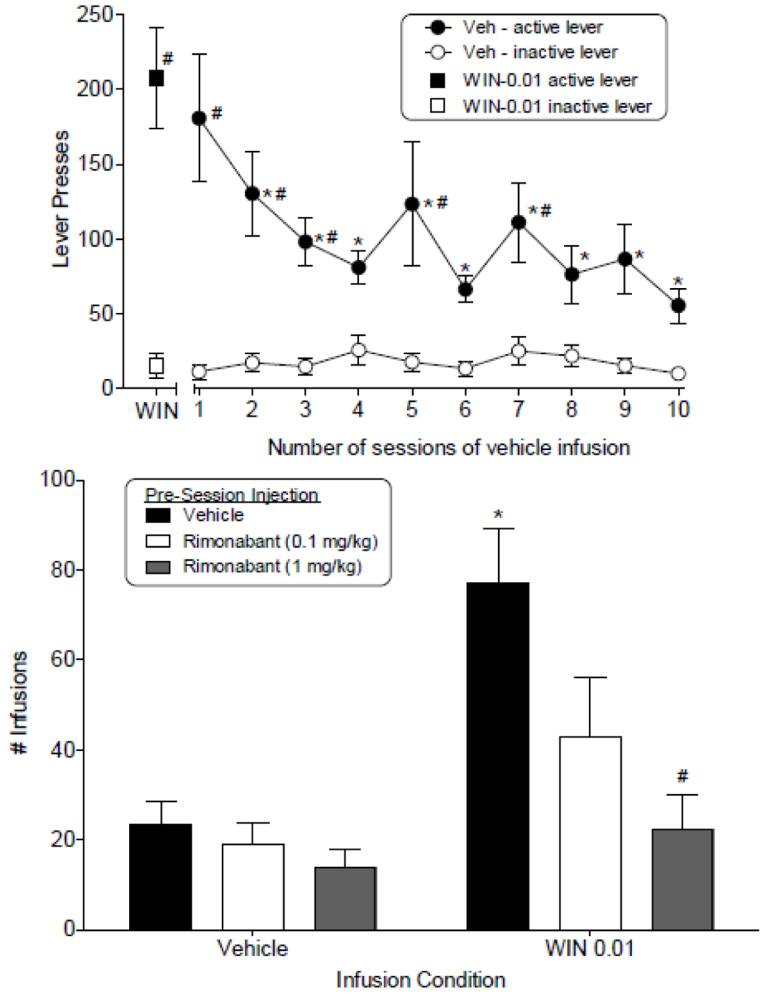

Figure 3.

Top panel: Effects of substitution of vehicle for the WIN55,212-2 training dose over a period of 10 consecutive sessions on lever presses on the active and inactive levers. At the left side of the panel, responding during test sessions with WIN55,212-2 (0.01 mg/kg/infusion) is shown for comparison purposes. All values represent the mean (±SEM) of data from 5 rats. ANOVA revealed a significant interaction. Post-hoc analysis of the simple effects: * indicates a significant difference ($p < 0.05$) in number of lever presses on the active lever on a vehicle session compared to active lever presses during WIN55,212-2 sessions. # designates a significant difference ($p < 0.05$) between number of presses on the active lever compared to the inactive lever during the same session. Bottom panel: Effects of pre-session administration of rimonabant (0.1 and 1 mg/kg) on responding on the active lever in rats trained to self-administer 0.01 mg/kg/infusion WIN55,212-2. The left side of the top panel shows results of pre-session injections on number of infusions when vehicle was available for infusion. The right side of the top panel shows results of the injections on number of infusions when 0.01 mg/kg WIN55,212-2 was available for infusion. Values represent the mean (±SEM) of data from 5 rats. ANOVA revealed a significant interaction. Post-hoc analysis of the simple effects: * designates a significant ($p < 0.05$) difference compared to the corresponding test with vehicle and # indicates a significant ($p < 0.05$) difference compared to the training dose of WIN55,212-2.