Psychopharmacology (2017) 234:1029–1043
DOI 10.1007/s00213-017-4548-2

ORIGINAL INVESTIGATION

Differential effects of cannabinoid CB1 inverse agonists and antagonists on impulsivity in male Sprague Dawley rats: identification of a possibly clinically relevant vulnerability involving the serotonin $5HT_{1A}$ receptor

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Received: 28 November 2016 / Accepted: 21 January 2017 / Published online: 1 February 2017

Abstract
Rationale Cannabinoid CB1 inverse agonists hold therapeutic promise as appetite suppressants but have produced suicidal behaviors among a small subpopulation in clinical trials. Anatomical and pharmacological evidence implicate the $5HT_{1A}$ serotonin receptor in suicide in humans and impulsivity in humans and animals.
Objective The objective of the study is to assess whether $5HT_{1A}$ blockade is necessary for CB1 ligands to produce impulsivity.
Methods Sprague Dawley rats were administered the CB1 inverse agonist AM 251, the CB1 antagonist AM 6527, or the peripherally restricted antagonist AM 6545, with or without pretreatment with the $5HT_{1A}$ antagonist WAY 100,635 (WAY) on the paced fixed consecutive number (FCN) task, which measures choice to terminate a chain of responses prematurely. As FCN is sensitive to changes in time perception, which have been demonstrated with CB1 blockade, a novel variable consecutive number task with discriminative stimulus (VCN-S_D) was also performed and proposed to be less sensitive to changes in timing.
Results Pretreatment with WAY enabled mild but significant reductions in FCN accuracy for AM 251 and AM 6527. No effects were found for AM 6545. On the VCN-S_D task, substantial impairments were found for the combination of WAY and AM 251.
Conclusions AM 251, but not the antagonists AM 6527 or AM 6545, produced impulsivity only following systemic $5HT_{1A}$ blockade. Although preliminary, the results may indicate that disrupted serotonin signaling produces a vulnerability to undesirable effects of CB1 inverse agonists, which is not evident in the general population. Furthermore, neutral CB1 antagonists do not produce this effect and therefore may have greater safety.

Keywords Cannabinoid · Impulsivity · Individual differences · Individualized medicine · Rimonabant · Serotonin · Suicide

Electronic supplementary material The online version of this article (doi:10.1007/s00213-017-4548-2) contains supplementary material, which is available to authorized users.

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Introduction

Rimonabant and other cannabinoid CB1 inverse agonists are effective at reducing appetite, producing weight loss, and improving markers of metabolic disorders such as diabetes (Van Gaal et al. 2005; Scheen et al. 2006; McLaughlin 2012; Thompson et al. 2016). They may also have promise in assisting in cessation of nicotine use (Le Foll et al. 2008) and possibly in enhancing certain learning domains (Dillon

et al. 2011; Boggs et al. 2012). However, symptoms such as depression and anxiety became apparent in a minority of participants (Christensen et al. 2007; Nissen et al. 2008; Johansson et al. 2009; Moreira and Crippa 2009), culminating most notably in a small number of suicide attempts (Topol et al. 2010). The reasons for such troubling side effects as suicidality in these and other psychotropic medications are uncertain and have led to reduced enthusiasm for the development of CB1 antagonists, in spite of therapeutic potential they would possess if these adverse events were better understood and minimized.

Prior to these results, only a few preclinical studies in animals may have indicated such deleterious effects on mood and suicidality (e.g., Deroche-Gamonet et al. 2001). There may be several reasons for this, one of which is the inherent difficulty in modeling suicidality in animals. Furthermore, depression and suicidality may be apparent only in a vulnerable clinical subpopulation, while animal models of depression require a strong enough effect in most (typically wild type and drug naïve) subjects to produce statistical significance. Analogously, even though rimonabant caused 3.0% of participants to discontinue treatment due to depression, there were no overall group differences on the Hospital Anxiety and Depression Scale (Christensen et al. 2007), and rimonabant was reported to increase quality of life in the RIO-Europe study (Van Gaal et al. 2005). There is therefore a clear need to identify individual differences that predispose certain patients to deleterious side effects and to screen novel therapies for similar effects.

In spite of the difficulty in modeling suicidal behavior, some insight may be gained by investigating the mechanism of behaviors that may contribute to suicidality, such as impulsivity (Dougherty et al. 2004; Seo et al. 2008; Malkesman et al. 2009; Dalley and Roiser 2012). Impulsivity is not a unitary phenomenon. In animals, it has been functionally divided using several taxonomies (Evenden 1999; Dellu-Hagedorn 2006; Pattij et al. 2007; Dalley et al. 2011; Dalley and Roiser 2012). Analysis of human behavior and personality also supports the general distinction between impulsive response and impulsive choice (Reynolds et al. 2006), although other types have been identified (Evenden 1999), and such distinctions are not always employed in inquiries into its relevance in suicide attempts (e.g., Brezo et al. 2006; Gvion et al. 2014). While it is therefore difficult to predict which type of animal impulsivity is most relevant to suicide, the fixed consecutive number (FCN) task may be appropriate for several reasons. In this two-choice design, animals must inhibit one type of response until a learned sequence of another type of response is complete. Animals are required to respond on one (counting) lever a number of times before transitioning to a second (reinforcing) lever (Mechner and Guevrekian 1962). FCN has been used to model impulsivity because selecting the reinforcing lever prematurely (i.e., switching to a reinforcing response before the count criterion is attained) results in reset of the count at zero (Dellu-Hagedorn 2006; Rivalan et al. 2007).

By employing a single reinforcer, FCN reduces the possibility that alterations in choice behavior are due to CB1 antagonism-induced decreases in sensitivity to differences in reinforcer magnitude, which is relevant in tasks such as delay discounting or delayed reinforcement. Nevertheless, it has been classified with impulsive choice tasks a model of cognitive impulsivity (Dellu-Hagedorn 2006). These types of tasks have been suggested for the preclinical modeling of suicide (Malkesman et al. 2009). Furthermore, lithium, but not valproate, is both clinically effective at reducing suicidal behavior and decreases impulsive choice (Halcomb et al. 2013). Of great importance, $5HT_{1A}$ agonism reduces impulsive FCN responding on a paced version (responding is paced by retracting the counting lever between presses, to reduce the influence of motor slowing), while antagonism enhances impulsive responding (Evenden 1998). This is particularly relevant because the $5HT_{1A}$ receptor has been found to be abnormally expressed in the brains of suicide victims (Hsiung et al. 2003; Thompson et al. 2012). Therefore, while likely not the only relevant assay, the FCN task may be of use in preclinically modeling aspects of impulsivity that are relevant in suicide. It is proposed here that interrupted $5HT_{1A}$ signaling may produce vulnerability to effects of CB1 inverse agonism on suicidality, mediated in part via increases in impulsive behavior.

In the present study, separate groups of animals were first treated with three types of CB1 ligands: AM 251, an inverse agonist and rimonabant analog; the putative neutral antagonist AM 6527; and the peripherally restricted neutral antagonist AM 6545. Because the therapeutic potential of these compounds is related to their hypophagic effects, doses were selected that have been shown to induce significant hypophagia. AM 251 significantly inhibits feeding and food-motivated responding in the range of 2.0–8.0 mg/kg, without overt signs of motor slowing or the complete abolition of feeding behavior (McLaughlin et al. 2003; Tallett et al. 2007; Hodge et al. 2008; McLaughlin et al. 2010), as does AM 6545 (Randall et al. 2010). The neutral antagonist AM 6527 was found to produce this profile of behavior at doses of 1.0 to 4.0 mg/kg (Thompson et al. 2016). Neutral antagonists, especially peripherally restricted compounds, exhibit fewer side effects than inverse agonists (Limebeer et al. 2010). Initial assays of AM 6545 demonstrated not only null effects on cAMP release (indicating neutral antagonism) but also low brain penetrability in rats and mice over a range of five or more hours, relative to the neutral CB1 antagonist AM 4113 (Cluny et al. 2010). It was predicted that all compounds would have minimal negative effects on the paced FCN task, as has been shown for the unpaced FCN task (Mansbach et al. 1996) and the delayed reinforcement model of impulsive choice (Pattij et al. 2007).

Importantly, a variation was then conducted in which animals were pretreated with the $5HT_{1A}$ antagonist WAY 100,635 (WAY) and then administered the same CB1 ligand over a slightly lower dose range. Dose range, route, and pretreatment time for WAY used in the present design replicate that which produced impulsive behavior in this task on its own (Evenden 1998). In the coadministration designs, we hypothesized that animals would respond impulsively, particularly those treated with AM 251, and possibly AM 6527 and AM 6545 to a lesser extent, due to a generally safer profile in these two compounds. This would suggest that disrupted $5HT_{1A}$ signaling permits CB1 antagonist-mediated impulsive behavior.

Methods (experiments 1–3)

Subjects

Twenty-four adult male Sprague Dawley rats (Hilltop Labs, Scottdale, PA, USA) were used. Rats were pair-housed in a colony room with a 12-h light-dark cycle (lights on 0800–2000 h), and procedures were carried out at approximately 1300 h. After arrival, animals were allowed to feed ad libitum and acclimate to the colony room until weights reached at least 250 g and then placed on food restriction to a minimum of 85% free-feeding body weight. Water was available at all times except during operant conditioning sessions. Animal protocols were approved by the Institutional Animal Care and Use Committee of Edinboro University of Pennsylvania (Protocol No. 20111) and were in accord with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Research 2011).

Apparatus and procedure

Habitest™ experimental chambers (Coulbourn Instruments, Whitehall, PA, USA) contained one retractable (counting) and one fixed (reinforcing) lever on either side of a food tray. Location of the levers to the left or right of the food tray was alternated between chambers. A computer running Windows XP controlled experimental contingencies and recorded lever presses and nose pokes into the food tray.

Training sessions were conducted 30 min per day, 5 days per week. After one session of magazine training, the counting lever was retracted and animals were subjected to a continuous reinforcement schedule on the reinforcing lever. After a minimum of 100 responses in a session, animals began an alternation protocol in which successive responses on the counting and reinforcing levers were reinforced, and the counting lever was retracted after each response and reinserted after a reinforcing lever press. The number of counting lever responses required before a reinforcing lever response would result in pellet delivery was gradually increased, and a 1-s interresponse interval (IRI; i.e., presses of the counting lever caused it to retract for 1 s) was introduced at FCN-2. Also beginning with this schedule, reinforcing lever responses made before the criterion was achieved on the counting lever led to a reset of the count at zero and a 5-s timeout signaled by the house light. This included responses made after other reinforcing lever presses, which were termed *zero chains*. The IRI increased to a final value of 2.6 s at FCN-4. At each training level, criterion to advance to the next level was at least 10 chains with an accuracy (ratio of reinforced chains to total chains) of 70%.

In the final protocol, on which all drug testing occurred, all chains that met or exceeded eight were reinforced (i.e., FCN-8). All counting lever responses retracted the lever for 2.6 s. As soon as the eighth response in a chain was emitted, any subsequent response on the reinforcing lever would result in a single food pellet delivered to the recessed food tray in between the two levers. At that point, subsequent responses on the counting lever would continue to retract the lever for 2.6 s and were recorded for analysis, but had no other programmed consequence. As in training, responding on the reinforcing lever prior to the eighth counting lever press resulted in a 5-s timeout signaled by the house light and a reset of the count at zero. Responses on either lever during the timeout had no programmed consequence. Test procedures began when no rat had more than three consecutive sessions of increasing accuracy on FCN-8.

Drugs

All compounds were injected systemically in a counterbalanced, repeated-measures design with at least two baseline FCN training days prior to each test and with at least 3 days between injections. Data from intervening baseline days were used to ensure no carryover of drug effect. The CB1 ligands AM 251, AM 6527, and AM 6545 were synthesized at the Center for Drug Discovery, Northeastern University and were dissolved in 15% DMSO, 15% Tween-80, and 70% saline and delivered via i.p. injection. WAY (Abcam, Cambridge, MA, USA, and Tocris, Minneapolis, MN, USA) was dissolved in 0.9% saline and administered s.c.

Experiments 1a and 1b

Eight animals were administered the CB1 inverse agonist AM 251 in doses of 2.0, 4.0, and 8.0 mg/kg or vehicle control, 30 min prior to FCN testing. After 6 weeks of task performance to allow for drug washout, a co-administration study of AM 251 and WAY commenced, in which subjects were pretreated with WAY s.c. 15 min prior to testing and AM 251 i.p. 10 min prior. Conditions were (dose WAY-dose AM 251) as follows: saline-vehicle; 0.1 mg/kg-vehicle; 0.1 mg/kg-

2.0 mg/kg; 0.1 mg/kg-4.0 mg/kg; 0.3 mg/kg-vehicle; 0.3 mg/kg-2.0 mg/kg; 0.3 mg/kg-4.0 mg/kg.

Experiments 2a and 2b

Eight animals were administered AM 6527 (CB1 neutral antagonist) in doses of 1.0, 2.0, and 4.0 mg/kg or vehicle control 30 min prior to testing. Four weeks later, they were co-administered WAY prior to AM 6527. Conditions were (dose WAY-dose AM 6527) as follows: saline-vehicle; 0.1 mg/kg-vehicle; 0.1 mg/kg-1.0 mg/kg; 0.1 mg/kg-2.0 mg/kg; 0.3 mg/kg-vehicle; 0.3 mg/kg-1.0 mg/kg; 0.3 mg/kg-2.0 mg/kg.

Experiments 3a and 3b

Subjects (n = 8) were given the peripherally restricted neutral antagonist AM 6545, administered i.p. 30 min before testing at doses of 2.0, 4.0, and 8.0 mg/kg and vehicle. Four weeks later, experiment 3b commenced, which included pretreatment with 0.1 and 0.3 mg/kg WAY (s.c. 15 min before testing) prior to AM 6545 doses of 4.0 and 8.0 mg/kg or vehicle.

Statistical analysis

The main measure of impulsivity was *accuracy*, defined as reinforced chains/sum of all chains, both reinforced and unreinforced, and expressed as a percentage. Reinforced chains were those that reached a minimum of eight responses before a reinforcing lever press. Other response measures were *chain length*, number of *chains*, and *counting* and *reinforcing lever responses*, as well as *response rate* on the counting lever. Chain length was the mean number of counting lever responses per chain in each session. On the other hand, number of chains is a count of all completed chains in the session, as counting and reinforcing lever responses are a count of all such responses, regardless of consequence. Response rate is defined as average time (ms) from lever insertion to the subsequent response on counting lever presses within chains, excluding the initial response that began the chain. The three counting measures, as well as response rate, are taken as gross measures of motivation or activity. Accuracy, chain length, and chains exclude zero chains, defined as trials that began and ended with a single reinforcing lever response. Analysis of zero chains produced no effects in any experiment (p-s > 0.05) and therefore are not shown in the results.

One-way repeated-measures ANOVA was conducted on all measures in sessions with at least seven chains. Significant ANOVAs in experiments 1a, 2a, and 3a were followed by nonorthogonal paired comparisons (Keppel and Wickens 2004) of each dose to vehicle. In experiments 1b, 2b, and 3b, comparisons were planned between the vehicle-vehicle control condition and both WAY-alone treatments (0.1 and 0.3 mg/kg). Four more post hoc comparisons were made between the CB1 antagonist-treatment conditions and the combination of vehicle and WAY treatment, to determine if the combination of WAY and CB1 ligand impairs performance relative to WAY alone. To further elucidate differential effects of CB1 ligands in the presence of different concentrations of WAY, a 3 (WAY condition) × 3 (CB1 ligand dose) repeated-measures ANOVA was also conducted for each experiment. The 2.0 and 4.0 mg/kg conditions of AM 251, 1.0 and 2.0 mg/kg conditions of AM 6527, and 4.0 and 8.0 mg/kg conditions of AM 6545, along with the vehicle for each, were entered as one factor and compared in WAY pretreatment conditions of no pretreatment (i.e., part A of each experiment), 0.1, and 0.3 mg/kg. Although some dose conditions are excluded from this analysis, a significant interaction would indicate differential effects of the CB1 ligand on accuracy, depending upon pretreatment with WAY.

Results (experiments 1–3)

Three animals failed to complete at least seven chains when administered 4.0 mg/kg of AM 6527; this dose was therefore removed for analyses of percentage and session average data, including accuracy and chain length in experiment 2a. One rat in the AM 6545 group failed to meet training criteria, resulting in n = 7 for experiment 3. Figure 1 depicts the main measure of accuracy, which is equivalent to the percentage of chains of at least eight responses; full survival plots of all chain lengths are shown in Supplementary Fig. S1. For some repeated-measures ANOVAs, assumption of sphericity was violated; in those cases, results are presented with a Greenhouse-Geisser correction. AM 251 (Fig. 1a), AM 6527 (Fig. 1b), and AM 6545 (Fig. 1c) did not significantly impair accuracy per se nor did they significantly affect chain lengths. For measures of overall activity, there was a reduction of chains for AM 251 ($F(3, 21) = 3.12$, $p = 0.048$, $\eta_p^2 = 0.308$) and for AM 6527 a decrease in chains ($F(3, 21) = 12.63$, $p < 0.001$, $\eta_p^2 = 0.643$) and in counting ($F(3, 21) = 15.24$, $p < 0.001$, $\eta_p^2 = 0.685$) and reinforcing ($F(3, 21) = 9.84$, $p < 0.001$, $\eta_p^2 = 0.584$) lever presses. No other effects were significant (ps > 0.05). These results are displayed in Table 1.

In contrast, AM 251 impaired accuracy under conditions of $5HT_{1A}$ blockade with 0.3 mg/kg WAY ($F(6, 42) = 3.26$, $p = 0.010$, $\eta_p^2 = 0.318$) at both the 2.0 and 4.0 mg/kg doses (Fig. 2a). A similar effect was found with AM 6527 (Fig. 2b; $F(6, 42) = 3.50$, $p = 0.007$, $\eta_p^2 = 0.334$). In this case, effects of AM 6527 were more mild, as the 4.0 mg/kg AM 6527 dose reduced accuracy only when animals were administered the higher 0.3 mg/kg WAY dose, compared with animals only treated with this dose of WAY, and the vehicle for AM 6527 (survival plots shown in Supplementary Fig. S2). On the other hand, when directly comparing the experiments with and

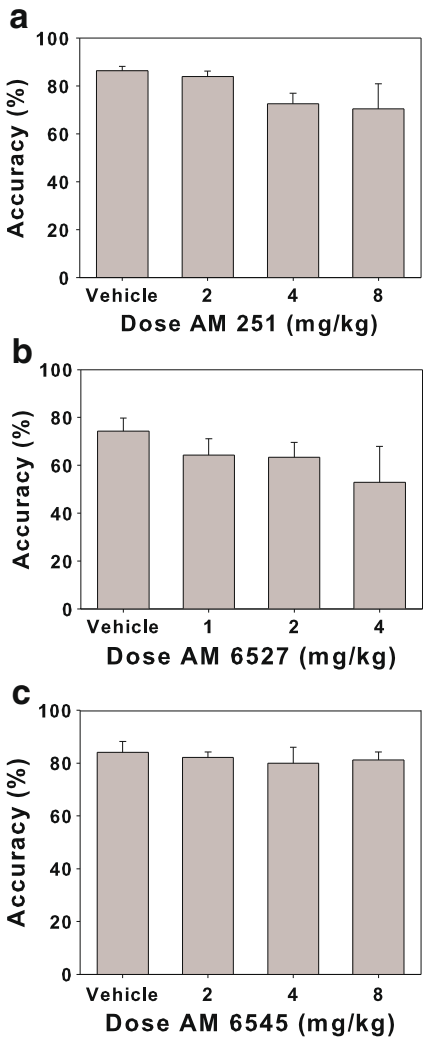

Fig. 1 Accuracy is shown for **a** AM 251, **b** AM 6527 (4.0 mg/kg represents data from $n = 5$ subjects), and **c** AM 6545 in the FCN task

without WAY using a 3 (WAY condition) × 3 (CB1 ligand dose) ANOVA, no interaction was found for AM 251 or AM 6527.

Figure 2c displays accuracy for dose combinations of WAY and the peripherally restricted antagonist AM 6545. Although there was a significant dose effect of accuracy ($F(6, 36) = 2.71$, $p = 0.028$, $\eta_p^2 = 0.311$), planned comparisons revealed a significant decrease only in the 0.1 mg/kg WAY-AM 6545 vehicle condition, relative to both the vehicle-vehicle control and the 0.1 mg/kg WAY-4.0 mg/kg AM 6545 condition, suggesting that AM 6545 reversed the WAY-induced deficit. Using a 3 × 3 ANOVA across experiments, a significant interaction ($F(4, 24) = 3.19$, $p = 0.031$, $\eta_p^2 = 0.347$) was found, suggesting that AM 6545 affected accuracy differently depending on dose of WAY. A reduction in chain length was also found (Table 2; $F(6, 36) = 2.82$, $p = 0.023$, $\eta_p^2 = 0.320$), with a decrease in the 0.1 mg/kg WAY condition, and a lengthening with the combination of 0.1 mg/kg WAY and 4.0 mg/kg AM 6545, relative to this condition. The combination of WAY and AM 251 or AM 6527 did not significantly affect chain length.

Both AM 251 ($F(6, 42) = 2.76$, $p = 0.024$, $\eta_p^2 = 0.283$) and AM 6527 ($F(6, 42) = 7.32$, $p < 0.001$, $\eta_p^2 = 0.511$), in combination with WAY, decreased the number of chains and also counting lever presses (AM 251 $F(6, 42) = 3.98$, $p = 0.003$, $\eta_p^2 = 0.362$; AM 6527 $F(6, 42) = 10.20$, $p < 0.001$, $\eta_p^2 = 0.593$), as shown in Table 2. In the WAY pretreatment designs, AM 251 ($F(6, 42) = 3.00$, $p = 0.016$, $\eta_p^2 = 0.300$), AM 6527 ($F(6, 42) = 4.66$, $p = 0.001$, $\eta_p^2 = 0.400$), and AM 6545 ($F(6, 42) = 3.38$, $p = 0.008$, $\eta_p^2 = 0.326$), all reduced reinforcing lever responses, but only AM 6527 led to increases in response latency ($F(6, 42) = 4.95$, $p = 0.001$, $\eta_p^2 = 0.414$). All other analyses were nonsignificant.

Brief discussion

AM 251, AM 6527, and AM 6545, respectively, a CB1 inverse agonist, antagonist, and antagonist that putatively does not penetrate the brain, had no effect on accuracy (percentage of reinforced chains) in the FCN task alone. In contrast, both AM 251 and AM 6527 significantly decreased accuracy when serotonergic activity was blocked at the $5HT_{1A}$ receptor with WAY. This is in spite of the fact that, for both CB1 ligands, the highest doses given per se (experiments 1a and 2a) were not used, because it was predicted that WAY would permit impulsivity-driven responding at lower doses. Indeed, both lower doses of AM 251 decreased accuracy in the presence of 0.3 mg/kg WAY, while up to 8.0 mg/kg had no effect on its own (experiment 1a). When preceded by 0.1 mg/kg WAY, the 4.0 mg/kg dose of AM 251 narrowly missed the criterion ($p = 0.053$) for a significant decrease in accuracy. The lower dose of 2.0 mg/kg is approximately the minimal effective dose to significantly inhibit food intake in rats (McLaughlin et al. 2003; Tallett et al. 2007; Hodge et al. 2008; McLaughlin et al. 2010). AM 6527 also decreased accuracy, but only at the highest combination of 0.3 mg/kg WAY and 4.0 mg/kg AM 6527. This dose of AM 6527 is above the ED_{50} for suppression of food intake (0.58 mg/kg; Sink et al. 2009). Effects in the presence of WAY are in comparison with WAY and the vehicle of the CB1 ligand, suggesting that they do not result from WAY alone. On the other hand, when doses of AM 251 and AM 6527 in experiments 1b and 2b were compared to the same doses in experiments 1a and 2a, respectively, no significant interaction was found. Taken together, effects of the CB1 inverse agonist AM 251, and antagonist AM 6527, are mild, even following WAY pretreatment, and have no effect alone.

On the other hand, AM 6545 did not affect accuracy, either when given alone or in the presence of WAY; if anything, low doses of AM 6545 may have even reversed the impulsivity-like effect of 0.1 mg/kg WAY, as supported by a significant interaction of AM 6545 dose and WAY pretreatment condition. This is

Table 1 Secondary FCN performance measures in groups treated with CB1 ligands

	CB1 ligand	Vehicle	Low dose	Medium dose	High dose
Chain length	AM 251	9.7 (0.2)[a]	9.9 (0.5)	9.2 (0.3)	9.6 (1.2)
	AM 6527	9.6 (0.9)	9.2 (0.8)	8.7 (0.5)	8.3 (1.5)[b]
	AM 6545	9.5 (0.6)	9.9 (1.0)	10.1 (1.2)	9.4 (0.3)
Chains	AM 251*	41.8 (1.8)	38.5 (3.6)	36.4 (2.5)	30.6 (3.4)**
	AM 6527**	33.8 (5.1)	27.8 (5.3)	25.8 (5.8)	7.6 (1.2)**
	AM 6545	43.3 (2.0)	43.3 (2.9)	42.0 (3.9)	39.9 (3.6)
Counting responses	AM 251	426.5 (29.2)	395.6 (38.1)	346.4 (28.7)	311.5 (46.6)
	AM 6527**	314.4 (44.7)	244.0 (39.0)*	225.6 (50.9)**	59.6 (12.4)**
	AM 6545	421.0 (18.2)	422.6 (22.9)	418.3 (28.5)	383.7 (36.2)
Reinf. responses	AM 251	60.5 (8.5)	52.3 (6.0)	57.4 (9.0)	47.5 (6.9)
	AM 6527**	44.4 (4.6)	37.1 (5.9)	35.9 (6.6)	14.1 (2.5)**
	AM 6545	57.9 (7.6)	48.9 (3.0)	56.3 (9.4)	51.1 6.2)
Response rate (ms)	AM 251	823.8 (70.0)	987.1 (157.4)	1089.8 (107.6)	1032.5 (84.8)
	AM 6527	1222.0 (265.1)	1394.3 (254.7)	1456.6 (251.9)	1608.2 (108.1)[b]
	AM 6545	951.7 (156.1)	941.3 (152.4)	936.6 (152.0)	812.5 (57.4)

[a] Standard error of the mean is shown in parentheses

[b] Average of $n = 5$; group mean not included in analysis due to low activity in three of eight subjects

*$p < 0.05$, **$p < 0.01$ significant dose effect and comparison with vehicle condition

in spite of the fact that, because AM 6545 was completely ineffective when given alone (whereas AM 251 and AM 6527 produced nonsignificant trends of decreasing accuracy), the two higher doses (4.0 and 8.0 mg/kg) were used in experiment 3b. The fact that AM 6545 reversed the impulsive effect of WAY (but was ineffective per se) was unexpected but interestingly may reveal other possible, even peripheral sites of action, because AM 6545 has limited brain penetrability. Low baseline sympathetic activation is related to increased impulsive behavior (Takahashi et al. 2007; Wang et al. 2013). CB1 receptors are found on the terminals of postganglionic noradrenergic fibers and modulate the sympathetic nervous system (Szabo et al. 2001); CB1 antagonism may therefore lead to an increment in noradrenergic activity. In this scenario, it is conceivable that AM 251 and AM 6527 have similar effects on peripheral CB1 receptors, but these are masked by effects produced by binding to central sites. On the other hand, as the unexpected effect of WAY and AM 6545 only occurred in one condition, confidence in any interpretation should be tempered.

Overall, these results are consistent with the interpretation that AM 251 and AM 6527 produce little to no impulsive behavior alone, but do so to a higher degree in a state of disrupted $5HT_{1A}$ receptor signaling, while AM 6545 produces no relevant impairments, even in the presence of WAY.

Confound of time perception

Critically, however, examination of task performance after the conclusion of experiments 1–3 strongly called into question the interpretation that the effects of AM 251 and AM 6527 were due to impulsive responding. After the conclusion of the experiments, the IRI was adjusted to longer or shorter delays, in order to determine whether animals were counting responses during chains or using a timing strategy by maintaining a consistent interval between the start and end of a chain. In this procedure (data not shown), average chain length (number of responses per chain) was substantially altered, while the time to complete a chain was relatively more stable in the face of altered task parameters. This suggests that animals likely relied upon timing mechanisms, rather than counting, to perform the FCN, in accordance with evidence that rats prefer a strategy of timing to counting in tasks where either is permitted (Davis and Memmott 1983; Breukelaar and Dalrymple-Alford 1998).

This means that performance in the paced FCN task was sensitive to changes in time perception, which in rats is altered by CB1 ligands, including THC and rimonabant (Mathew et al. 1998; Han and Robinson 2001; Sewell et al. 2013); therefore, it is possible that the impairments found with AM 251 and AM 6527 were due to changes in time perception, not impulsivity. Because of this, adjustments were made to the task, and AM 251 and AM 6527 were reassessed in experiments 4 and 5, respectively.

Variable consecutive number with discriminative stimulus

The FCN task typically proceeds without programmed feedback to indicate when animals reach the criterion number; however, a discriminative stimulus (FCN-S_D) has been used to set the occasion for switching to the reinforcing lever

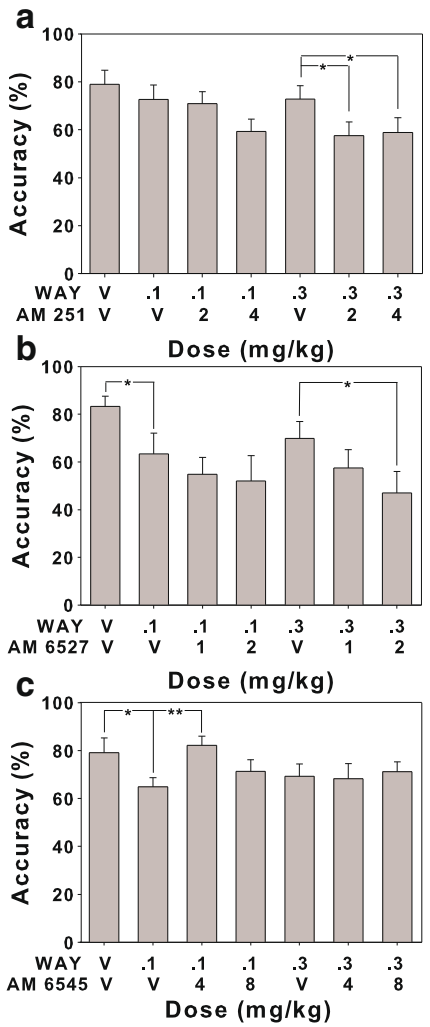

Fig. 2 Effects of combinations of WAY and **a** AM 251, **b** AM 6527, or **c** AM 6545 on FCN accuracy. *$p < 0.05$, **$p < 0.01$ group differences via planned comparisons

(Laties 1972; Rivalan et al. 2007). Not surprisingly, performance is superior in the signaled condition, sometimes requiring a higher FCN criterion to produce similar accuracy (Rivalan et al. 2007). However, it is still possible that subjects may rely upon timing or counting strategies, even with the programmed stimulus control of the S_D. Therefore, in experiments 4 and 5, the criterion number shifted on each trial (hence the change in nomenclature to *variable*), and a tone indicated that the criterion had been reached. In so doing, any change in accuracy would be more likely due to impulsive behavior, rather than to alteration of timing processes necessary for task performance.

AM 251 and AM 6527 were tested on the variable consecutive number task with discriminative stimulus (VCN-S_D) task, again with and without WAY. AM 6545 was not included; because the FCN task may have been sensitive to changes in either impulsivity or timing, results of experiment 3 indicated that AM 6545 did not affect either.

Methods (experiments 4 and 5)

Subjects

Sixteen adult male Sprague Dawley rats were acquired and housed as described above.

Apparatus and procedure

Habitest™ experimental chambers were set up as described above; training was similar with a few exceptions. Early in training, when pellets were delivered, a 1.5 kHz tone sounded through a speaker above the food tray for 2 s. Later, an alternation protocol (i.e., FCN-1) was put in place in which the 2-s cue followed each counting lever response to signal availability of reinforcement. As above, an FCN-2 task was then used with a 1-s IRI. Starting with this task, the S_D, still a 1.5 kHz tone, began after the criterion counting lever response and continued until a reinforcing response was made. Animals were permitted to make subsequent counting lever presses in the presence of the S_D. The variable consecutive number task was introduced by selecting a criterion of either two or three counting responses on a per-trial basis, using the S_D to indicate that reinforcement was available by a single reinforcing lever response. The variable criterion was increased over several weeks until accuracy stabilized in the range of 75–85%, similar to that of the FCN task.

The final schedule used criterion values of 12, 15, 18, 21, and 24, randomly selected each trial. As with the FCN task, when the counting lever response that reached criterion was made, the lever not only retracted, but any subsequent reinforcing lever response ended the trial with the delivery of a pellet. Counting lever presses that matched or exceeded the criterion continued to retract the lever for 2.6 s. However, unlike the FCN experiments, the stimulus tone initiated with the criterion counting lever press and only ended with a reinforcing lever press; therefore, all activity (including counting lever presses) after criterion was reached occurred in the presence of the S_D. Other task parameters were identical to the FCN task.

When performance on the final schedule stabilized, the 16 animals were split randomly into two groups (n = 8) for testing with AM 251 and AM 6527, using the preparation, dose ranges, and regimen from experiments 1 and 2, respectively. Statistical analysis was also identical, except that dose effects on accuracy and chain length were also analyzed by a dose × criterion repeated-measures ANOVA to determine whether drugs affected performance differently at longer or shorter criterion values.

Table 2 Secondary FCN performance measures in groups treated with WAY and CB1 ligands

	Dose WAY	Vehicle	0.1 mg/kg			0.3 mg/kg		
	Dose cannabinoid	Vehicle	Vehicle	Low dose	High dose	Vehicle	Low dose	High dose
Chain length	AM 251	9.6 (0.7)[a]	9.0 (0.5)	9.3 (0.6)	8.4 (0.4)	9.3 (0.6)	9.0 (0.9)	8.6 (0.6)
	AM 6527	9.5 (0.4)	8.4 (0.7)	8.0 (0.4)	7.9 (0.8)	8.9 (0.6)	8.0 (0.5)	7.3 (0.7)
	AM 6545*	9.8 (0.6)	8.4 (0.3)**	9.4 (0.4)*	9.2 (0.5)	8.9 (0.5)*	8.7 (0.4)	8.5 (0.3)
Chains	AM 251*	39.4 (3.3)	34.9 (2.0)	34.1 (2.0)	27.0 (3.8)	34.8 (2.7)	24.1 (3.9)*	28.5 (5.3)
	AM 6527**	42.4 (4.0)	39.5 (6.0)	29.4 (5.0)	25.8 (4.3)**	42.9 (5.0)	32.8 (5.5)*	27.8 (5.7)**
	AM 6545	40.0 (3.5)	39.8 (3.7)	35.5 (3.7)	34.9 (4.7)	38.3 (4.3)	36.3 (4.8)	38.6 (5.1)
Counting responses	AM 251**	374.8 (21.9)	319.0 (18.5)	325.9 (19.1)	240.9 (38.5)	331.8 (33.0)	217.4 (38.4)**	247.9 (43.4)*
	AM 6527**	405.4 (30.0)	329.4 (41.1)*	245.5 (45.7)*	226.1 (48.0)**	389.1 (37.2)	265.6 (44.8)**	215.9 (45.5)**
	AM 6545	391.1 (27.3)	350.4 (28.7)	345.0 (40.2)	322.6 (40.1)	339.3 (32.8)	321.4 (41.1)	341.6 (43.8)
Reinf. responses	AM 251*	50.6 (3.9)	57.6 (6.1)	52.0 (4.0)	37.4 (6.1)**	49.3 (7.0)	34.0 (4.4)*	41.0 (7.1)
	AM 6527**	51.4 (5.0)	57.8 (10.4)	36.4 (6.3)**	35.0 (6.4)**	54.1 (7.2)	42.6 (6.2)	40.4 (6.4)*
	AM 6545**	55.5 (6.0)	57.3 (5.3)	45.5 (5.1)**	41.8 (6.6)**	48.3 (6.2)	50.6 (7.1)	51.6 (8.1)
Response rate (ms)	AM 251	1037.1 (71.5)	1139.5 (93.4)	1122.0 (82.4)	1282.6 (107.0)	1120.5 (113.3)	1306.4 (142.0)	1200.3 (106.5)
	AM 6527**	976.0 (341.5)	1110.4 (516.0)	1365.5 (472.4)*	1403.8 (609.5)*	1082.1 (362.9)	1389.7 (614.2)**	1362.2 (494.6)*
	AM 6545	999.7 (183.1)	1092.5 (249.6)	1184.8 (287.6)	1079.0 (202.1)	1124.0 (197.4)	1156.9 (198.5)	1143.9 (250.1)

[a] Standard error of the mean is shown in parentheses

*$p < 0.05$, **$p < 0.01$ significant dose effect and comparison with vehicle condition

Experiments 4a and 4b

Subjects were administered AM 251 once per week in counterbalanced fashion, followed 3 weeks later by AM 251 with WAY pretreatment.

Experiments 5a and 5b

AM 6527 was administered to the other eight subjects, followed 3 weeks later by AM 6527 with WAY pretreatment on VCN-S_D.

Results (experiments 4 and 5)

AM 251 once again had no effect on accuracy, nor did its effects on accuracy interact with criterion, although effect of criterion on accuracy approached significance ($p = 0.079$), suggesting more impulsive behavior on chains with larger response criteria. Mean accuracy is displayed in Fig. 3a, with full survival plots of chains in Supplementary Fig. S3. In the AM 6527 study, one animal ceased completing at least seven chains per session reliably and was removed from the study. Neither main effect nor the interaction of dose and criterion were significant for AM 6527 on impulsive behavior (Fig. 3b). As shown in Table 3, AM 251 also significantly reduced the number of chains ($F(3, 21) = 6.14$, $p = 0.004$, $\eta_p^2 = 0.467$) and counting lever responses ($F(3, 21) = 3.85$, $p = 0.024$, $\eta_p^2 = 0.355$) and increased response latency ($F(3, 21) = 4.13$, $p = 0.019$, $\eta_p^2 = 0.371$). AM 6527 had similar effects on chains ($F(3, 18) = 14.48$, $p < 0.001$, $\eta_p^2 = 0.707$), counting lever presses ($F(3, 18) = 9.95$, $p < 0.001$, $\eta_p^2 = 0.624$), and response latency ($F(3, 18) = 9.33$, $p = 0.001$, $\eta_p^2 = 0.609$).

In contrast to the overall lack of effect that AM 251 had on impulsivity at up to 8.0 mg/kg, pretreatment with WAY led to significant drug effects of AM 251 on accuracy ($F(6, 42) = 5.59$, $p < 0.001$, $\eta_p^2 = 0.444$). Figure 4a and Supplementary Fig. S4 indicate significant deficits in performance at 4.0 mg/kg AM 251 in subjects treated with both doses of WAY. The criterion of each trial also influenced accuracy ($F(4, 28) = 5.77$, $p = 0.002$, $\eta_p^2 = 0.452$), and dose interacted with criterion ($F(24, 168) = 1.78$, $p = 0.019$, $\eta_p^2 = 0.203$). The interaction suggests that accuracy effects were particularly evident for longer criterion values (Fig. 4b). Simple main effects of dose were conducted for each of the five criterion values; the combination of WAY and AM 251 substantially impaired accuracy over WAY alone on chains in which the criterion was 24 responses ($F(6, 42) = 6.97$, $p < 0.001$, $\eta_p^2 = 0.499$). As with the overall analysis, planned comparisons revealed impairments at 4.0 mg/kg AM 251 for both WAY doses. Comparing effects of AM 251 under different WAY pretreatment conditions directly, a

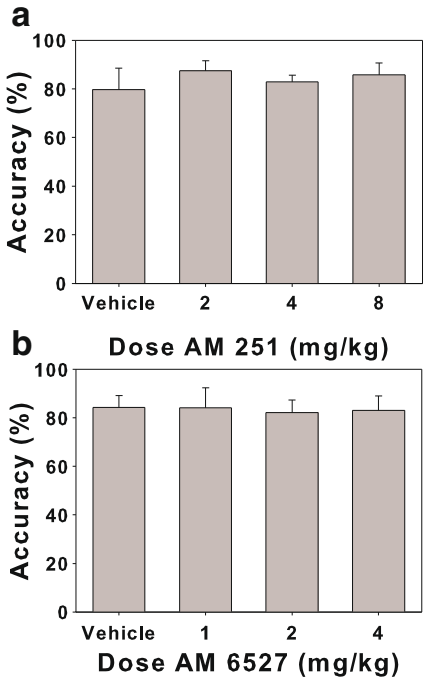

Fig. 3 VCN-S_D task accuracy, i.e., percentage of chains reaching criterion, for animals treated with **a** AM 251 or **b** AM 6527

significant interaction was found ($F(4, 28) = 3.29$, $p = 0.025$, $\eta_p^2 = 0.319$), suggesting that the relationship between AM 251 and accuracy was altered at different WAY pretreatment conditions.

Similarly, analysis indicated decreases, relative to WAY alone, for chains ($F(6, 42) = 6.22$, $p < 0.001$, $\eta_p^2 = 0.471$) and counting responses ($F(6, 42) = 5.92$, $p < 0.001$, $\eta_p^2 = 0.458$). WAY, both alone and in combination with AM 251, increased response latency ($F(6, 42) = 9.11$, $p < 0.001$, $\eta_p^2 = 0.566$). Means of these measures are shown in Table 4.

Pretreatment with WAY did not engender AM 6527-related changes in impulsivity (Fig. 5 and Supplementary Fig. S5), an effect of criterion, or the interaction of dose and chain criterion. The 3 (WAY pretreatment condition) × 3 (AM 6527 dose) interaction was not significant. As shown in Table 4, the combination of WAY and AM 6527 significantly reduced reinforcing responses ($F(6, 36) = 2.53$, $p = 0.038$, $\eta_p^2 = 0.296$) but had no effect on other measures.

Discussion

The VCN-S_D version of the task was designed to eliminate the confounding effects of time perception that may have influenced the unsignaled FCN task. The S_D altered task performance from a smoother function of chain lengths (Supplementary Figs. S1 and S2), to a step-like function, with the majority of chains terminated just after criterion was obtained (Supplementary Figs. S3–S5), indicating that it was salient enough to control behavior, as predicted. With these parameters in place, the CB1 inverse agonist AM 251 significantly impaired accuracy when the $5HT_{1A}$ receptor was blocked with either 0.1 or 0.3 mg/kg WAY. These effects were not evident when AM 251 was administered alone, even at higher doses. Effect sizes were even stronger for the combination of WAY and AM 251 in the VCN-S_D, relative to the FCN task, and an interaction was found between AM 251 doses and WAY pretreatment condition in the VCN-S_D, but not the FCN task. Importantly, the neutral CB1 antagonist AM 6527 was devoid of effects with or without WAY pretreatment, at doses that reduce food-motivated behavior (Thompson et al. 2016). This suggests that the decrease in accuracy caused by the combination of WAY and AM 6527 on the FCN task (experiment 2b) was not likely related to impulsivity, but rather to time estimation or other processes necessary to complete the task. Time estimation is known to be sensitive to CB1 agonists and inverse agonists (Mathew et al. 1998; Han and

Table 3 Secondary performance measures on the VCN-S_D task following administration of AM 251 or AM 6527 alone

		Vehicle	Low dose	Medium dose	High dose
Chain length	AM 251	16.0 (1.5)[a]	17.9 (0.9)	16.5 (0.5)	17.2 (1.1)
	AM 6527	18.1 (1.1)	18.1 (1.3)	18.0 (0.7)	19.8 (1.6)
Chains	AM 251**	40.5 (2.9)	32.3 (3.2)*	30.9 (3.9)*	24.3 (3.6)**
	AM 6527**	32.9 (4.4)	27.0 (3.7)*	32.9 (3.4)	21.0 (3.4)**
Counting responses	AM 251*	640.4 (54.2)	579.0 (59.8)	514.8 (514.8)	417.8 (62.7)*
	AM 6527**	634.7 (63.2)	521.4 (62.2)*	578.9 (45.4)	390.4 (56.8)**
Reinf. responses	AM 251	51.5 (7.7)	37.3 (3.3)	35.5 (4.6)	30.3 (4.9)
	AM 6527	41.8 (2.3)	39.8 (6.2)	43.9 (6.4)	31.5 (7.2)
Response rate (ms)	AM 251*	943.6 (55.7)	1096.3 (101.5)	1185.8 (104.0)**	1208.8105.0)**
	AM 6527**	888.8 (82.4)	1212.3 (107.5)**	1042.3 (58.0)*	1236.9 (135.2)**

[a] Standard error of the mean is shown in parentheses

*$p < 0.05$, **$p < 0.01$ significant dose effect and comparison with vehicle condition

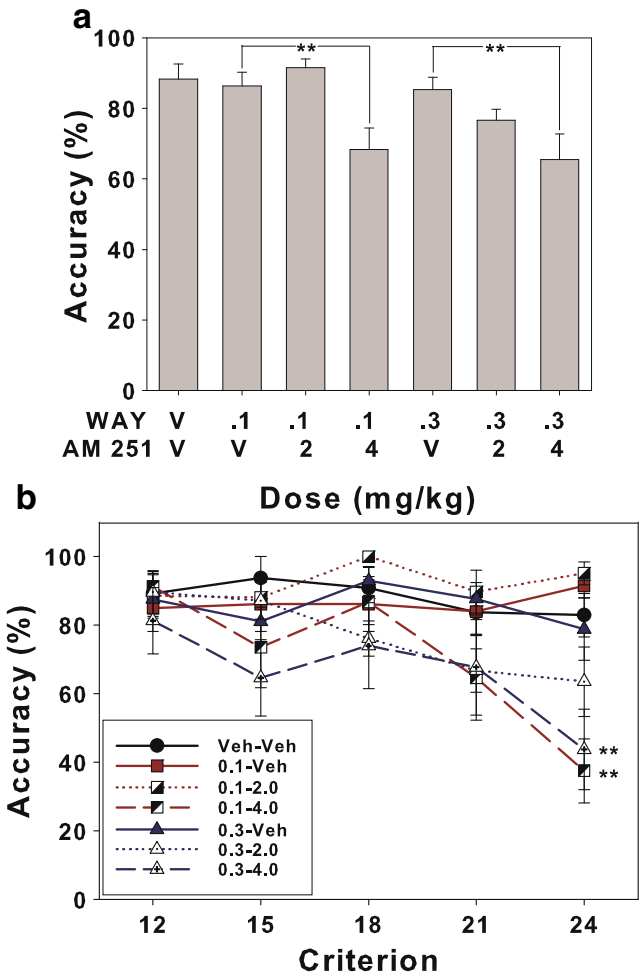

Fig. 4 Effects of the combination of WAY and AM 251 on accuracy in the VCN-S_D task. Shown are both **a** overall accuracy and **b** accuracy by criterion value, due to a significant dose × criterion interaction. $^{**}p < 0.01$ group differences via planned comparisons

Robinson 2001; Sewell et al. 2013), although effects of neutral antagonists (such as AM 6527) have not been examined previously. Because AM 6527 affected the unsignaled, but not the signaled task, it is suggested that time estimation is mediated by endocannabinoid tone that can be blocked by a neutral CB1 antagonist, while impulsivity requires inverse agonism of the CB1 receptor. Meanwhile, AM 6545 produced no impairments on the FCN task, indicating no increases in impulsive behavior or changes in time estimation, in line with its putative limited brain penetrability (Cluny et al. 2010).

It is possible that effects on accuracy reflected not impulsivity but rather reduced food motivation or motor control, as has been described for several of these compounds (McLaughlin et al. 2003; Cluny et al. 2010; Randall et al. 2010; Thompson et al. 2016). Secondary performance measures (e.g., number of chains and responses and response rate) were altered in all experiments, most likely reflecting changes to motivation or motor control. However, accuracy was only affected in a small number of experiments, suggesting that accuracy is unrelated to overall activity levels or motivation. Accuracy in the VCN-S_D task could also be impaired by a manipulation that decreased stimulus control. This is also unlikely to underlie the present results, given that both WAY (Carli and Samanin 2000) and AM 251 (McLaughlin et al. 2005) do not affect stimulus control in tasks using very brief stimuli (although these studies did not examine effects of their coadministration). Furthermore, the VCN-S_D task was designed to place minimal demand on stimulus control by using an auditory cue that terminated only with the successful completion of a chain. That effects were strongest at longer criterion values (Fig. 4b) also implicates increased impulsivity in the deficits demonstrated.

Impulsivity has been identified as an important predictor for suicide attempts (Klonsky and May 2010; Dvorak et al. 2013), and animal models of impulsivity may therefore be relevant in elucidating the biology of suicide (Malkesman et al. 2009). Just as WAY pretreatment was required for AM 251 to produce impulsive responding, CB1 inverse agonism may produce unwanted side effects only in a vulnerable clinical subpopulation. While clinical trials of rimonabant indicated an increase in depression (Christensen et al. 2007), and suicide attempts were noted (Topol et al. 2010), rimonabant also increased self-esteem and quality of life in the RIO-Europe trial (Scheen et al. 2006). These seemingly incongruous findings may represent dispositional differences within the clinical sample. In the current paper, CB1 ligands had minimal effect per se, other than decreases in secondary measures, which likely represented decreased motivation for food reinforcement. These effects were in line with a lack of effect of rimonabant on the traditional, unpaced FCN task (Mansbach et al. 1996) and also of rimonabant and the putative neutral CB1 antagonist O-2050 in the delayed reinforcement task, another two-response choice model of impulsivity (Pattij et al. 2007; Wiskerke et al. 2011; cf. Boomhower et al. 2013). At the same time, rimonabant and O-2050 decreased premature responding in the five-choice serial reaction time task (Pattij et al. 2007; Wiskerke et al. 2011), a model of impulsive action, suggesting that CB1 blockade can reduce, rather than increase, certain types of impulsivity. Nevertheless, the present set of results strongly suggest that reduced signaling at the $5HT_{1A}$ receptor permits the deleterious side effects of CB1 inverse agonists. As certain $5HT_{1A}$ gene polymorphisms are related to suicide (Lemonde et al. 2003; Samadi Rad et al. 2012) and impulsivity (Bagdy et al. 2012), it is possible that genetic factors that alter $5HT_{1A}$ expression or function play a role in these effects of CB1 inverse agonism.

WAY has been found to affect paced FCN performance at the doses tested (Evenden 1998), an effect partially replicated in the current study. We found that 0.1 and 0.3 mg/kg tended to reduce FCN accuracy slightly, achieving significance at the 0.1 mg/kg dose in the AM 6527 and AM 6545 (but not AM 251) designs, with no difference from vehicle at the 0.3 mg/kg dose. Because accuracy at the 0.1 and 0.3 doses was similar, this may represent a limit to the efficacy of WAY in this type of

Table 4 Secondary performance measures of the combination of WAY and CB1 ligands on the VCN-S_D task

	Dose WAY	Vehicle	0.1 mg/kg			0.3 mg/kg		
	Dose cannabinoid	Vehicle	Vehicle	Low dose	High dose	Vehicle	Low dose	High dose
Chain lengths	AM 251	16.8 (1.2)[a]	16.8 (0.9)	17.8 (0.3)	15.8 (0.5)	17.1 (0.6)	16.5 (0.4)	15.9 (2.1)
	AM 6527	18.5 (0.9)	17.8 (0.6)	16.9 (0.2)	18.3 (1.4)	17.6 (0.7)	17.7 (0.4)	17.2 (0.9)
Chains	AM 251**	29.1 (3.4)	27.0 (4.7)	23.3 (4.6)	18.1 (3.3)**	27.0 (3.8)	23.3 (3.4)	14.9 (2.3)**
	AM 6527	32.4 (3.8)	35.7 (3.2)	28.7 (3.4)	29.1 (3.8)	27.6 (4.2)	27.0 (4.0)	27.4 (3.1)
Counting responses	AM 251**	490.8 (63.5)	464.8 (79.2)	414.8 (79.8)	289.8 (58.2)**	467.3 (68.1)	384.6 (53.4)	246.9 (50.7)**
	AM 6527	599.9 (71.6)	636.0 (53.4)	497.0 (64.1)	513.6 (59.5)	488.9 (81.8)	476.1 (63.5)	485.4 (69.5)
Reinf. responses	AM 251	40.6 (6.4)	34.5 (5.3)	29.8 (3.3)	30.9 (5.2)	35.6 (3.8)	30.3 (4.4)	27.4 (5.2)
	AM 6527*	44.4 (3.3)	48.4 (5.7)	40.9 (5.9)	34.9 (4.4)**	33.3 (3.7)*	34.0 (4.5)	37.3 (4.4)
Response rate (ms)	AM 251**	1153.0 (171.0)	1292.7 (188.2)*	1375.1 (160.0)	1447.2 (154.1)*	1243.1 (159.7)	1369.5 (176.2)*	1543.8 (168.6)**
	AM 6527	1022.9 (157.4)	1024.7 (104.9)	1176.9 (104.7)	1222.6 (89.6)	1178.4 (130.1)	1243.5 (92.3)	1258.6 (155.6)

[a] Standard error of the mean is shown in parentheses

*$p < 0.05$, **$p < 0.01$ significant dose effect and comparison with vehicle condition

task. Indeed, WAY per se was not effective in the VCN-S_D task, except for an increase in response rate in experiment 4 and a reduction in reinforcing lever responses in experiment 5 (Table 4). This can be contrasted with the stronger effects of AM 251, in the presence of WAY, in this task, relative to FCN performance in experiment 1. These findings may indicate that the S_D decreased task difficulty in the VCN-S_D, and in the FCN task, greater potency of WAY was found in the present study, compared with previous findings (Evenden 1998; Evenden and Meyerson 1999). In addition to these effects in animal models of impulsivity, WAY was employed more importantly because of the relevance of the cortical $5HT_{1A}$ receptor in impulsivity and suicide (Soubrié 1986; Seo et al. 2008) and depression (Bhagwagar et al. 2004). In addition to decreased binding, a blunted intracellular $5HT_{1A}$ response has also been found in suicide victims relative to controls (Hsiung et al. 2003), although upregulation of the receptor has been found (Underwood et al. 2012).

On the other hand, high levels of $5HT_{1A}$ are found not only in cortex and hippocampus but also as an inhibitory autoreceptor in raphe nuclei (Ito et al. 1999), and activation of this receptor population is believed to contribute to depression and impulsivity via a global decrease in serotonergic activity (Celada et al. 2013). Indeed, raphe $5HT_{1A}$ binding is positively related to suicide and lethality of suicide attempts (Stockmeier et al. 1998; Sullivan et al. 2015; cf. Arango et al. 2001). Therefore, a limitation of the present study is that the systemic administration of WAY does not permit elucidation of the mechanism of the deficits induced by AM 251. Rather, the purpose of these findings is to confirm that the $5HT_{1A}$ receptor mediates part of the side effects of CB1 inverse agonism. It should also be noted that the agonist 8-OH-DPAT also enhances delay discounting (Winstanley et al. 2005), which may indicate that presynaptic and postsynaptic $5HT_{1A}$ receptors are relevant in different forms of impulsive behavior. In humans, self-reported impulsivity was also correlated more with a blunted response to a $5HT_{1A}$ agonist in postsynaptic but not presynaptic receptors (Minzenberg et al. 2005), further implicating a receptor population other than autoreceptors in raphe nuclei for the present findings. A proposed mechanism for the antidepressant effect of selective serotonin reuptake inhibitors (SSRIs) is in the desensitization of raphe $5HT_{1A}$ receptors (Blier and de Montigny 1994), an effect also found with rimonabant (Aso et al. 2009), suggesting that the effect of WAY to potentiate AM 251 in impulsivity is not likely to involve presynaptic raphe $5HT_{1A}$ receptors. Rather, antidepressant-like interactions between CB1 agonists and the serotonergic system may be related to activity in prefrontal cortex or hippocampus (Sagredo et al. 2006; Bambico et al. 2007, 2009), where rimonabant decreases serotonin release (Beyer et al. 2010). Conversely, the SSRI fluoxetine enhanced CB1-activated adenylyl cyclase in prefrontal cortex, an effect blocked by WAY (Mato et al. 2010). Similarly,

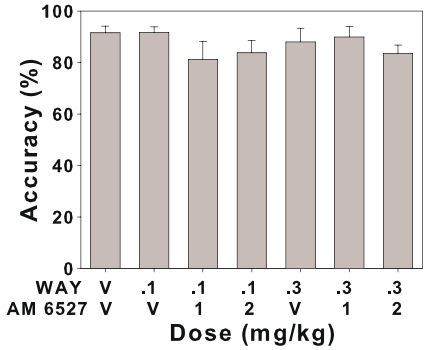

Fig. 5 Accuracy on the VCN-S_D task is shown for the combination of WAY and AM 6527

administration of WAY into prefrontal cortex inhibited antidepressant-like effects of cannabidiol on the forced swim test (Sartim et al. 2016). These effects are more in line with the deficits in the current study that occurred via blockade of both $5HT_{1A}$ and CB1 receptors. Further research employing localized injection is needed to fully understand the precise population of $5HT_{1A}$ receptors that permit the performance deficit seen in animals treated with WAY and AM 251.

It is also important to note that decreased $5HT_{1A}$ signaling is not the only possible risk factor that would permit CB1 inverse agonism to produce impulsive behavior; other receptor subtypes, including $5HT_{2A}$ and $5HT_{2C}$, are also implicated in suicide (van Heeringen et al. 2003; Di Narzo et al. 2014) and impulsive behavior (Evenden 1998; Fletcher et al. 2007; Blasio et al. 2012; Fink et al. 2015), as is the serotonin transporter (Courtet et al. 2001; Lindström et al. 2004). SSRIs such as fluoxetine also modulate CB1 receptor function (Malone and Taylor 1998, 1999; Hill et al. 2008). Indeed, others have already proposed testing for particular variants of the serotonin transporter in assessing patients for possible rimonabant-induced risk (Lazary et al. 2011). Moreover, norepinephrine-endocannabinoid interactions have been proposed to be relevant in the effects of rimonabant on mood (Kirilly et al. 2013). Dopamine is also involved in impulsive responding in animals (Dalley and Roiser 2012). However, it appears that rimonabant and O-2050 reverse, rather than potentiate, amphetamine-induced increases in impulsive action and decreases in impulsive choice (Wiskerke et al. 2011), indicating that not all manipulations that alter impulsive responding enhance the AM 251-related effects shown presently. Taken together, there are several possible interactions with other neurotransmitter systems that would permit effects of CB1 inverse agonism on depression and suicidality, which are not mutually exclusive (Beyer et al. 2010).

However, the AM 251 dose-dependent impulsive behavior in the presence of $5HT_{1A}$ blockade shown here suggests a functional interrelationship between cannabinoid and serotonin systems that leads to maladaptive behavior. The present study may be the first demonstration of CB1 inverse agonism producing suboptimal behavior in the presence of disrupted serotonin signaling. These results highlight possible differences between individuals who are or are not at heightened risk for suicidal behavior, while acknowledging that impulsivity is but one component of suicidal risk and that neurotransmission involving the $5HT_{1A}$ receptor may not be the only critical variable in the side effects of CB1 inverse agonism. Nevertheless, the present study adds to literature on the relative safety of neutral CB1 antagonists and lays groundwork for a novel approach to predicting the safety of centrally acting pharmaceuticals in general.

Acknowledgements The authors are grateful to Rachel E. Drozdowski, Rachel M. Hardy, Caitlin M. Luskin, Felicia M. Myers, Marigny C. Normann, Taylor M. Proper, and Emily E. Thompson for assistance in conducting this project.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Arango V, Underwood MD, Boldrini M et al (2001) Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. Neuropsychopharmacology 25:892–903. doi:10.1016/S0893-133X(01)00310-4

Aso E, Renoir T, Mengod G et al (2009) Lack of CB1 receptor activity impairs serotonergic negative feedback. J Neurochem 109:935–944. doi:10.1111/j.1471-4159.2009.06025.x

Bagdy G, Juhasz G, Gonda X (2012) A new clinical evidence-based gene-environment interaction model of depression. Neuropsychopharmacol Hungarica 14:213–220. doi:10.5706/nph201212001

Bambico FR, Katz N, Debonnel G, Gobbi G (2007) Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. J Neurosci 27:11700–11711. doi:10.1523/JNEUROSCI.1636-07.2007

Bambico FR, Duranti A, Tontini A et al (2009) Endocannabinoids in the treatment of mood disorders: evidence from animal models. Curr Pharm Des 15:1623–1646

Beyer CE, Dwyer JM, Piesla MJ et al (2010) Depression-like phenotype following chronic CB1 receptor antagonism. Neurobiol Dis 39:148–155. doi:10.1016/j.nbd.2010.03.020

Bhagwagar Z, Rabiner E, Sargent P et al (2004) Persistent reduction in brain serotonin1A receptor binding in recovered depressed men measured by positron emission tomography with [11C]WAY-100635. Mol Psychiatry 9:386–392. doi:10.1038/sj.mp.4001401

Blasio A, Narayan AR, Kaminski BJ et al (2012) A modified adjusting delay task to assess impulsive choice between isocaloric reinforcers in non-deprived male rats: effects of 5-HT_2A/C and 5-HT_1A receptor agonists. Psychopharmacology 219:377–386. doi:10.1007/s00213-011-2517-8

Blier P, de Montigny C (1994) Current advances and trends in the treatment of depression. Trends Pharmacol Sci 15:220–226

Boggs DL, Kelly DL, McMahon RP et al (2012) Rimonabant for neurocognition in schizophrenia: a 16-week double blind

randomized placebo controlled trial. Schizophr Res 134:207–210. doi:10.1016/j.schres.2011.11.009

Boomhower SR, Rasmussen EB, Doherty TS (2013) Impulsive-choice patterns for food in genetically lean and obese Zucker rats. Behav Brain Res 241:214–221. doi:10.1016/j.bbr.2012.12.013

Breukelaar JW, Dalrymple-Alford JC (1998) Timing ability and numerical competence in rats. J Exp Psychol Anim Behav Process 24:84–97. doi:10.1037/0097-7403.24.1.84

Brezo J, Paris J, Turecki G (2006) Personality traits as correlates of suicidal ideation, suicide attempts, and suicide completions: a systematic review. Acta Psychiatr Scand 113:180–206. doi:10.1111/j.1600-0447.2005.00702.x

Carli M, Samanin R (2000) The 5-HT1(A) receptor agonist 8-OH-DPAT reduces rats' accuracy of attentional performance and enhances impulsive responding in a five-choice serial reaction time task: role of presynaptic 5-HT1(A) receptors. Psychopharmacology 149:259–268. doi:10.1007/s002139900368

Celada P, Bortolozzi A, Artigas F (2013) Serotonin 5-HT1A receptors as targets for agents to treat psychiatric disorders: rationale and current status of research. CNS Drugs 27:703–716. doi:10.1007/s40263-013-0071-0

Christensen R, Kristensen PK, Bartels EM et al (2007) Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet 370:1706–1713. doi:10.1016/S0140-6736(07)61721-8

Cluny NL, Vemuri VK, Chambers AP et al (2010) A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents. Br J Pharmacol 161:629–642. doi:10.1111/j.1476-5381.2010.00908.x

Courtet P, Baud P, Abbar M et al (2001) Association between violent suicidal behavior and the low activity allele of the serotonin transporter gene. Mol Psychiatry 6:338–341. doi:10.1038/sj.mp.4000856

Dalley JW, Roiser JP (2012) Dopamine, serotonin and impulsivity. Neuroscience 215:42–58. doi:10.1016/j.neuroscience.2012.03.065

Dalley JW, Everitt BJ, Robbins TW (2011) Impulsivity, compulsivity, and top-down cognitive control. Neuron 69:680–694. doi:10.1016/j.neuron.2011.01.020

Davis H, Memmott J (1983) Autocontingencies: rats count to three to predict safety from shock. Anim Learn Behav 11:95–100. doi:10.3758/BF03212314

Dellu-Hagedorn F (2006) Relationship between impulsivity, hyperactivity and working memory: a differential analysis in the rat. Behav Brain Funct 2:10. doi:10.1186/1744-9081-2-10

Deroche-Gamonet V, Le Moal M, Piazza P, Soubrié P (2001) SR141716, a CB1 receptor antagonist, decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. Psychopharmacology 157:254–259. doi:10.1007/s002130100804

Di Narzo AF, Kozlenkov A, Roussos P et al (2014) A unique gene expression signature associated with serotonin 2C receptor RNA editing in the prefrontal cortex and altered in suicide. Hum Mol Genet 23:4801–4813. doi:10.1093/hmg/ddu195

Dillon GM, Lubbers LS, Ferguson MT et al (2011) MK-7128, a novel CB1 receptor inverse agonist, improves scopolamine-induced learning and memory deficits in mice. Behav Pharmacol 22:91–100. doi:10.1097/FBP.0b013e3283423d7e

Dougherty DM, Mathias CW, Marsh DM et al (2004) Laboratory measured behavioral impulsivity relates to suicide attempt history. Suicide Life Threat Behav 34:374–385. doi:10.1521/suli.34.4.374.53738

Dvorak RD, Lamis DA, Malone PS (2013) Alcohol use, depressive symptoms, and impulsivity as risk factors for suicide proneness among college students. J Affect Disord 149:326–334. doi:10.1016/j.jad.2013.01.046

Evenden JL (1998) The pharmacology of impulsive behaviour in rats IV: the effects of selective serotonergic agents on a paced fixed consecutive number schedule. Psychopharmacology 140:319–330. doi:10.1007/s002130050773

Evenden JL (1999) Varieties of impulsivity. Psychopharmacology 146:348–361. doi:10.1007/PL00005481

Evenden J, Meyerson B (1999) The behavior of spontaneously hypertensive and Wistar Kyoto rats under a paced fixed consecutive number schedule of reinforcement. Pharmacol Biochem Behav 63:71–82. doi:10.1016/S0091-3057(98)00222-6

Fink LH, Anastasio NC, Fox RG et al (2015) Individual differences in impulsive action reflect variation in the cortical serotonin 5-HT2A receptor system. Neuropsychopharmacology 40:1–45. doi:10.1038/npp.2015.46

Fletcher PJ, Tampakeras M, Sinyard J, Higgins GA (2007) Opposing effects of 5-HT2A and 5-HT2C receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. Psychopharmacology 195:223–234. doi:10.1007/s00213-007-0891-z

Gvion Y, Horresh N, Levi-Belz Y et al (2014) Aggression-impulsivity, mental pain, and communication difficulties in medically serious and medically non-serious suicide attempters. Compr Psychiatry 55:40–50. doi:10.1016/j.comppsych.2013.09.003

Halcomb ME, Gould TD, Grahame NJ (2013) Lithium, but not valproate, reduces impulsive choice in the delay-discounting task in mice. Neuropsychopharmacology 38:1937–1944. doi:10.1038/npp.2013.89

Han CJ, Robinson JK (2001) Cannabinoid modulation of time estimation in the rat. Behav Neurosci 115:243–246. doi:10.1037/0735-7044.115.1.243

Hill MN, Ho WSV, Hillard CJ, Gorzalka BB (2008) Differential effects of the antidepressants tranylcypromine and fluoxetine on limbic cannabinoid receptor binding and endocannabinoid contents. J Neural Transm 115:1673–1679. doi:10.1007/s00702-008-0131-7

Hodge J, Bow JP, Plyler KS et al (2008) The cannabinoid CB1 receptor inverse agonist AM 251 and antagonist AM 4113 produce similar effects on the behavioral satiety sequence in rats. Behav Brain Res 193:298–305. doi:10.1016/j.bbr.2008.06.010

Hsiung SC, Adlersberg M, Arango V et al (2003) Attenuated 5-HT1A receptor signaling in brains of suicide victims: involvement of adenylyl cyclase, phosphatidylinositol 3-kinase, Akt and mitogen-activated protein kinase. J Neurochem 87:182–194. doi:10.1046/j.1471-4159.2003.01987.x

Institute of Laboratory Animal Resources (2011) Guide for the care and use of laboratory animals, 8th edn. National Academy Press, Washington DC

Ito H, Halldin C, Farde L (1999) Localization of 5-HT1A receptors in the living human brain using [carbonyl-11C]WAY-100635: PET with anatomic standardization technique. J Nucl Med 40:102–109

Johansson K, Neovius K, Desantis SM et al (2009) Discontinuation due to adverse events in randomized trials of orlistat, sibutramine and rimonabant: a meta-analysis. Obes Rev 10:564–575. doi:10.1111/j.1467-789X.2009.00581.x

Keppel G, Wickens TD (2004) Design and analysis: a researcher's handbook, 4th edn. Prentice-Hall, Englewood Cliffs

Kirilly E, Hunyady L, Bagdy G (2013) Opposing local effects of endocannabinoids on the activity of noradrenergic neurons and release of noradrenaline: relevance for their role in depression and in the actions of CB1 receptor antagonists. J Neural Transm 120:177–186. doi:10.1007/s00702-012-0900-1

Klonsky ED, May AM (2010) Rethinking impulsivity in suicide. Suicide Life Threat Behav 40:612–619. doi:10.1521/suli.2010.40.6.612

Laties VG (1972) The modification of drug effects on behavior by external discriminative stimuli. J Pharmacol Exp Ther 183:1–13

Lazary J, Juhasz G, Hunyady L, Bagdy G (2011) Personalized medicine can pave the way for the safe use of CB1 receptor antagonists. Trends Pharmacol Sci 32:270–280. doi:10.1016/j.tips.2011.02.013

Le Foll B, Forget B, Aubin HJ, Goldberg SR (2008) Blocking cannabinoid CB1 receptors for the treatment of nicotine dependence: insights from pre-clinical and clinical studies. Addict Biol 13:239–252. doi:10.1111/j.1369-1600.2008.00113.x

Lemonde S, Turecki G, Bakish D et al (2003) Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J Neurosci 23:8788–8799

Limebeer CL, Vemuri VK, Bedard H et al (2010) Inverse agonism of cannabinoid CB 1 receptors potentiates LiCl-induced nausea in the conditioned gaping model in rats. Br J Pharmacol 161:336–349. doi:10.1111/j.1476-5381.2010.00885.x

Lindström MB, Ryding E, Bosson P et al (2004) Impulsivity related to brain serotonin transporter binding capacity in suicide attempters. Eur Neuropsychopharmacol 14:295–300. doi:10.1016/j.euroneuro.2003.11.001

Malkesman O, Pine DS, Tragon T et al (2009) Animal models of suicide-trait-related behaviors. Trends Pharmacol Sci 30:165–173. doi:10.1016/j.tips.2009.01.004

Malone DT, Taylor DA (1998) Modulation of delta9-tetrahydrocannabinol-induced hypothermia by fluoxetine in the rat. Br J Pharmacol 124:1419–1424. doi:10.1038/sj.bjp.0701980

Malone DT, Taylor DA (1999) Modulation by fluoxetine of striatal dopamine release following Delta9-tetrahydrocannabinol: a microdialysis study in conscious rats. Br J Pharmacol 128:21–26. doi:10.1038/sj.bjp.0702753

Mansbach RS, Rovetti CC, Winston EN, Lowe JA (1996) Effects of the cannabinoid CB1 receptor antagonist SR141716A on the behavior of pigeons and rats. Psychopharmacology 124:315–322. doi:10.1007/BF02247436

Mathew RJ, Wilson WH, Turkington TG, Coleman RE (1998) Cerebellar activity and disturbed time sense after THC. Brain Res 797:183–189

Mato S, Vidal R, Castro E et al (2010) Long-term fluoxetine treatment modulates cannabinoid type 1 receptor-mediated inhibition of adenylyl cyclase in the rat prefrontal cortex through 5-hydroxytryptamine 1A receptor-dependent mechanisms. Mol Pharmacol 77:424–434. doi:10.1124/mol.109.060079

McLaughlin PJ (2012) Reports of the death of CB1 antagonists have been greatly exaggerated: recent preclinical findings predict improved safety in the treatment of obesity. Behav Pharmacol 23:537–550. doi:10.1097/FBP.0b013e3283566a8c

McLaughlin PJ, Winston K, Swezey L et al (2003) The cannabinoid CB1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. Behav Pharmacol 14:583–588. doi:10.1097/00008877-200312000-00002

McLaughlin PJ, Brown CM, Winston KM et al (2005) The novel cannabinoid agonist AM 411 produces a biphasic effect on accuracy in a visual target detection task in rats. Behav Pharmacol 16:477–486

McLaughlin PJ, Winston KM, Swezey LA et al (2010) Detailed analysis of food-reinforced operant lever pressing distinguishes effects of a cannabinoid CB1 inverse agonist and dopamine D1 and D2 antagonists. Pharmacol Biochem Behav 96:75–81. doi:10.1016/j.pbb.2010.04.009

Mechner F, Guevrekian L (1962) Effects of deprivation upon counting and timing in rats. J Exp Anal Behav 5:463–466. doi:10.1901/jeab.1962.5-463

Minzenberg MJ, Grossman R, New AS et al (2005) Blunted hormone responses to ipsapirone are associated with trait impulsivity in personality disorder patients. Neuropsychopharmacology 2:197–203. doi:10.1038/sj.npp.1300853

Moreira FA, Crippa JAS (2009) The psychiatric side-effects of rimonabant. Rev Bras Psiquiatr 31:145–153. doi:10.1590/S1516-44462009000200012

Nissen SE, Nicholls SJ, Wolski K et al (2008) Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. JAMA 299:1547–1560. doi:10.1001/jama.299.13.1547

Pattij T, Janssen MCW, Schepers I et al (2007) Effects of the cannabinoid CB1 receptor antagonist rimonabant on distinct measures of impulsive behavior in rats. Psychopharmacology 193:85–96. doi:10.1007/s00213-007-0773-4

Randall PA, Vemuri VK, Segovia KN et al (2010) The novel cannabinoid CB1 antagonist AM6545 suppresses food intake and food-reinforced behavior. Pharmacol Biochem Behav 97:179–184. doi:10.1016/j.pbb.2010.07.021

Reynolds B, Ortengren A, Richards JB, de Wit H (2006) Dimensions of impulsive behavior: personality and behavioral measures. Pers Individ Dif 40:305–315. doi:10.1016/j.paid.2005.03.024

Rivalan M, Grégoire S, Dellu-Hagedorn F (2007) Reduction of impulsivity with amphetamine in an appetitive fixed consecutive number schedule with cue for optimal performance in rats. Psychopharmacology 192:171–182. doi:10.1007/s00213-007-0702-6

Sagredo O, Ramos JA, Fernández-Ruiz J et al (2006) Chronic Δ9-tetrahydrocannabinol administration affects serotonin levels in the rat frontal cortex. Naunyn Schmiedeberg's Arch Pharmacol 372:313–317. doi:10.1007/s00210-005-0026-1

Samadi Rad B, Ghasemi A, Seifi M et al (2012) Serotonin 1A receptor genetic variations, suicide, and life events in the Iranian population. Psychiatry Clin Neurosci 66:337–343. doi:10.1111/j.1440-1819.2012.02333.x

Sartim AG, Guimarães FS, Joca SRL (2016) Antidepressant-like effect of cannabidiol injection into the ventral medial prefrontal cortex-possible involvement of 5-HT1A and CB1 receptors. Behav Brain Res 303:218–227. doi:10.1016/j.bbr.2016.01.033

Scheen AJ, Finer N, Hollander P et al (2006) Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. Lancet 368:1660–1672. doi:10.1016/S0140-6736(06)69571-8

Seo D, Patrick CJ, Kennealy PJ (2008) Role of serotonin and dopamine system interactions in the neurobiology of impulsive aggression and its comorbidity with other clinical disorders. Aggress Violent Behav 13:383–395. doi:10.1016/j.avb.2008.06.003

Sewell RA, Schnakenberg A, Elander J et al (2013) Acute effects of THC on time perception in frequent and infrequent cannabis users. Psychopharmacology 226:401–413. doi:10.1007/s00213-012-2915-6

Sink KS, Vemuri VK, Wood J et al (2009) Oral bioavailability of the novel cannabinoid CB1 antagonist AM6527: effects on food-reinforced behavior and comparisons with AM4113. Pharmacol Biochem Behav 91:303–306. doi:10.1016/j.pbb.2008.07.013

Soubrié P (1986) Reconciling the role of central serotonin neurons in human and animal behavior. Behav Brain Sci 9:319. doi:10.1017/S0140525X00022871

Stockmeier CA, Shapiro LA, Dilley GE et al (1998) Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression: postmortem evidence for decreased serotonin activity. J Neurosci 18:7394–7401

Sullivan GM, Oquendo MA, Milak M et al (2015) Positron emission tomography quantification of serotonin(1A) receptor binding in suicide attempters with major depressive disorder. JAMA Psychiatry 72:169. doi:10.1001/jamapsychiatry.2014.2406

Szabo B, Nordheim U, Niederhoffer N (2001) Effects of cannabinoids on sympathetic and parasympathetic neuroeffector transmission in the rabbit heart. J Pharmacol Exp Ther 297:819–826

Takahashi T, Ikeda K, Fukushima H, Hasegawa T (2007) Salivary alpha-amylase levels and hyperbolic discounting in male humans. Neuro Endocrinol Lett 28:17–20

Tallett AJ, Blundell JE, Rodgers RJ (2007) Grooming, scratching and feeding: role of response competition in acute anorectic response to rimonabant in male rats. Psychopharmacology 195:27–39. doi:10.1007/s00213-007-0880-2

Thompson PM, Cruz DA, Olukotun DY, Delgado PL (2012) Serotonin receptor, SERT mRNA and correlations with symptoms in males

with alcohol dependence and suicide. Acta Psychiatr Scand 126: 165–174. doi:10.1111/j.1600-0447.2011.01816.x

Thompson EE, Jagielo-Miller JE, Vemuri VK et al (2016) CB1 antagonism produces behaviors more consistent with satiety than reduced reward value in food-maintained responding in rats. J Psychopharmacol 30:482–491. doi:10.1177/0269881116639287

Topol EJ, Bousser M-G, Fox KAA et al (2010) Rimonabant for prevention of cardiovascular events (CRESCENDO): a randomised, multicentre, placebo-controlled trial. Lancet 376:517–523. doi:10.1016/S0140-6736(10)60935-X

Underwood MD, Kassir SA, Bakalian MJ et al (2012) Neuron density and serotonin receptor binding in prefrontal cortex in suicide. Int J Neuropsychopharmacol 15:435–447. doi:10.1017/S1461145711000691

Van Gaal LF, Rissanen AM, Scheen AJ et al (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. Lancet 365:1389–1397. doi:10.1016/S0140-6736(05)66374-X

van Heeringen C, Audenaert K, Van Laere K et al (2003) Prefrontal 5-HT2a receptor binding index, hopelessness and personality characteristics in attempted suicide. J Affect Disord 74:149–158

Wang TS, Huang WL, Kuo TBJ et al (2013) Inattentive and hyperactive preschool-age boys have lower sympathetic and higher parasympathetic activity. J Physiol Sci 63:87–94. doi:10.1007/s12576-012-0238-3

Winstanley CA, Theobald DEH, Dalley JW, Robbins TW (2005) Interactions between serotonin and dopamine in the control of impulsive choice in rats: therapeutic implications for impulse control disorders. Neuropsychopharmacology 30:669–682. doi:10.1038/sj.npp.1300610

Wiskerke J, Stoop N, Schetters D et al (2011) Cannabinoid CB1 receptor activation mediates the opposing effects of amphetamine on impulsive action and impulsive choice. PLoS One. doi:10.1371/journal.pone.0025856