

# Activation of CB1 Receptors May Provide an Effective Treatment for Obsessive Compulsive Disorder

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Obsessive compulsive disorder (OCD) is an anxiety disorder characterized by mental obsessions and compulsions stemming from chemical imbalances in the brain. Drugs that facilitate serotonin transmission are commonly used to treat OCD, but they can become ineffective with prolonged use. It has been suggested that CB1 (cannabinoid type 1) receptors are an alternative drug target that could provide effective treatment. The aims of this study were to investigate more successful longer-term drug treatment options for anxiety-based symptoms of OCD and to better understand serotonin's interaction with CB1. This study used an 11-day marble burying behavioral model with three groups of mice treated respectively with saline, WIN 55,212-2 (CB1 agonist), or Tianeptine (5-HT antagonist; Tianeptine and WIN 55,212-2 days 6-10). Mice receiving the CB1 agonist buried fewer marbles than did the control with no deterioration of effect over ten days. Mice receiving both Tianeptine and WIN 55,212-2 also buried fewer marbles. These results indicate that WIN 55,212-2 has anxiolytic properties that could be an effective treatment for the compulsive symptoms of OCD. It also suggests that CB1 receptors are situated downstream of serotonin receptors.

Abbreviations: 5-HT – Serotonin; CB1 – Cannabinoid 1 (Receptor Type); OCD – Obsessive Compulsive Disorder; SSRI – Selective Serotonin Reuptake Inhibitor

Keywords: OCD; Marble Burying; CB1 agonist; WIN 55,212-2 mesylate; anxiety

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## Introduction

According to the National Institute of Health, obsessive compulsive disorder (OCD) affects about 1% of the adult U.S. population. OCD is classified as an anxiety disorder, the most commonly diagnosed type of mental disorder (Kessler et al., 2005). The average age of onset is 19, and approximately 50% of all cases are classified as severe.

There is a grouping of brain structures deemed the “worry circuit” model of OCD. The worry circuit refers to a system of brain structures that have been found to be altered in OCD patients (Schwartz and Begley, 2003). The worry circuit contains the caudate nucleus, which helps in switching gears from one thought or behavior to another; the cingulate gyrus, which aids in the regulation and activation of the

sympathetic nervous system; and the thalamus, which processes sensory input. These worry circuit structures are altered in volume and neurotransmission and there is much more cross talk between them in OCD patients (Szeszko et al., 2004; Soriano-Mas et al., 2007). This increased cross talk and activity in the worry circuit results in a repetitive feedback loop that barrages the rest of the brain with feelings that something is wrong (Lind-Kyle, 2009). As a result, this circuit of brain structures over-activates one another in an infinite loop.

Within these brain structures there are also neurotransmitter alterations in patients diagnosed with OCD. OCD is most often treated through daily medication that facilitates serotonin transmission (Muscatello et al., 2011).

Drugs such as fluvoxamine, fluoxetine, and citalopram are some of the more common selective serotonin reuptake inhibitors (SSRIs) that are used in the treatment of OCD (Ichimaru et al., 1995; Sugimoto et al., 2007). However, these drugs do not always maintain their efficacy in the long term. After prolonged use of the drug over the course of months, patients notice a decrease in efficacy. The patients experience mild symptoms returning even in the presence of the drug (Hembree et al., 2003). With the vast number of people suffering from this disorder, a more reliable and long-lasting treatment is necessary.

Though results are still somewhat equivocal, CB1 endocannabinoid receptor agonists can lower anxiety in both mice and humans (Casarotto et al., 2010; Gomes et al., 2011; Umathe et al., 2011, 2012). CB1 receptors are an appropriate target because they are located in the amygdala, basal ganglia, hippocampus, hypothalamus, and cingulate gyrus. These structures have been implicated in the worry circuit model of OCD (Witkin et al., 2005; Marinelli et al., 2007). CB1 receptors are ligand-gated pre-synaptic modulators of glutamate, GABA, dopamine, serotonin, acetylcholine and norepinephrine release (Darmani et al., 2003; Morales et al., 2004). In addition, CB1 and 5HT<sub>3A</sub> receptors have been found to co-localize on GABA neurons (Morales et al., 2004). Recently, studies have demonstrated that these CB1 receptors interact with serotonergic neurons in murine behavioral assessments of head twitching, scratching, and marble burying anxiety models of OCD (Darmani et al., 2003; Umathe et al., 2011). Marble burying is a firmly established model for gauging OCD behavior. It is successful because it is an unconditioned defensive action in mice, it is not associated with physical danger or novelty, but it is a measure of compulsive repetition and the mice do not habituate when used repeatedly in trials over many days (Njung'e and Handley, 1991; Umathe et al., 2011).

Individually, drugs that facilitate either serotonin or endocannabinoids have been shown overwhelmingly to decrease marble burying behavior (Ichimaru et al., 1995; Sugimoto et al., 2007; Gomes et al., 2011; Umathe et al., 2011).

However, a very recent study has effectively demonstrated that CB1 receptor activation lowers marble burying because the endocannabinoid and serotonergic systems interact with each other (Umathe et al., 2011). This was determined by a pharmacologically based study in which both CB1 and 5HT agonists and antagonists were given individually and co-administered. Alone SSRIs and CB1 agonists decreased compulsive marble burying. However, a CB1 antagonist blocked the effects of the SSRI, thus demonstrating that the CB1 receptors are in some way interacting with the serotonergic system (Umathe et al., 2011). Had the results indicated a decrease in marble burying behavior in the SSRI+CB1 antagonist, it would be evident that the SSRI was working independent of the cannabinoid system. This is well supported by histological data demonstrating co-localization of CB1 and serotonin receptors (Morales et al., 2004).

However, the question remains: What is the biochemical role of CB1 in anxiety, compulsion, and OCD, as there seems to be confusion about its relationship to 5-HT. If CB1 receptors are found on the pre-synaptic buttons of serotonergic neurons, why are they producing behavioral results of decreasing anxiety, when normally serotonergic facilitation brings about that change (Ichimaru et al., 1995)? If CB1 receptors are performing their previously described functions, then why have we not seen an increase in anxiety as CB1 agonists inhibit vesicle release of serotonin? This is a puzzling situation because while it has been shown that CB1 agonists decrease marble burying and CB1 antagonists coupled with serotonin agonists show no change (Umathe et al., 2011), others have demonstrated that serotonin activates endocannabinoid release, which then retrogradely inhibits other neurotransmitter release such as glutamate (Best and Regehr, 2008). This interaction could manifest itself as serotonin evoking the endocannabinoid system and then retrogradely suppressing excitatory synapses, or serotonin activating CB1 release (Best and Regehr, 2008; Umathe et al., 2011). Inversely, CB1 could be a downstream target of 5-HT. As the exact nature of these cellular interactions has yet to be fully illuminated, the current experiment attempts to clarify the

relationship between cannabinoid and serotonin releasing neurons on anxiety in mice.

Considering the findings of Umathe et al. (2011), who demonstrated the anticomulsive effects of CB1 activation through serotonin agonists and CB1 antagonists, this study set out to further clarify the 5-HT/CB1 relationship by co-administering a CB1 agonist and serotonin antagonist. We hypothesized that a CB1 agonist alone would significantly and consistently reduce marble burying behavior in mice over 10 days. In addition, we hypothesized that the same CB1 agonist, co-administered with a serotonin antagonist, would show a change in marble burying behavior when compared to a control. If there is a significant difference between the co-administered group and the control group, then it will imply that CB1 is having a direct impact on marble burying and has a direct relationship with compulsive behavioral symptoms. This would demonstrate that activation of CB1 could be an effective treatment for OCD. Umathe et al. (2011) demonstrated initial support for this relationship, in that a CB1 antagonist blocked the impact of an SSRI on marble burying. By reversing which neurotransmitter systems are blocked and facilitated we can confirm CB1's role in anxiety reduction, as well as its relationship with 5-HT.

## Material and Methods

The College of Wooster Institutional Animal Care and Use Committee (IACUC) approved all methods, and animals were treated in accordance with the Policy on Humane Care and Use of Laboratory Animals.

### Marble Burying Test

#### *Subjects*

Forty C57BL/6J female mice were tested in adulthood. Female mice were used for consistency of the marble burying protocol. These mice were provided through breeding at the College of Wooster. There were three independent treatment conditions: Control (n=10), WIN 55,212-2 (n=15), and Tianeptine (and Win 55,212-2 on days 6-10; n=15). The dependent variable was the number of marbles buried for each subject. The mice were pair

housed in normal caging conditions with 12:12 light/dark cycles and ad libitum food and water.

#### *Apparatus*

The marble burying assay is a test of the anxiety-related behaviors of compulsion, repetition, and perseveration in which a mouse is placed in a box containing uncovered marbles, with stronger symptoms correlating with more marbles buried (Deacon, 2006). The chamber was a 29x10x11.6 cm standard housing cage with 12 marbles inside, spaced 5cm apart from one another. The marbles were 1.5cm in diameter, black, made of glass, and provided by www.megaglass.com. The box had an even 5 cm coating of pine shaving bedding at the bottom of the box, which was tamped down before the marbles were added. Marbles covered 2/3rds of the way or more were counted as buried following the 15-minute exposure.

#### *Drugs*

The following drugs were used: saline as the control solution (5 mg/kg, 10 ml/kg) mixed with 1% Tween 80; WIN 55,212-2, non-selective CB1 agonist (3 mg/kg, 10ml/kg); and Tianeptine, enhancer of 5-HT transporter (5 mg/kg, 10 ml/kg) provided by Fisher Scientific (Waltham, MA). All drugs were injected i.p. Tianeptine is a 5-HT antagonist and is widely used in rodent trials. Its mechanism is as a selective serotonin reuptake enhancer (SSRE). The WIN 55,212-2 was suspended in physiological saline with 1% Tween 80. The half-life of WIN 55,212-2 is 24 hours and the half life of Tianeptine is 3 hours.

#### *Procedure*

There were three groups: The control group received saline injections on all 11 days. The second group received WIN 55,212-2 on the first 10 days and then received an injection of both WIN 55,212-2 and tianeptine on day 11. The primary purpose of this group was to examine the longer-term efficacy of using a CB1 agonist to treat OCD. The third group received only the tianeptine for the first 5 days. On days 6 through 11, they received an injection of tianeptine, followed by an injection of WIN 55,212-2 5 minutes later (See Table 1). The primary purpose of this group was to identify the interaction between the CB1 and 5-HT receptor systems as well as the effects of tianeptine when administered alone.

Testing began 30 minutes following injection to allow the drug(s) to take effect. The mouse was placed in the center of the marble burying task for 15 minutes with 12 marbles. Afterwards, the mouse was removed and the number of marbles that had more than 2/3 of their surface covered in sawdust was recorded.

Mice were tested daily for 11 days. After this period elapsed, the mice were tested on a probe trial 2 days after the last injection to allow the drugs to metabolize. They were tested without drug administration so it could be determined if there were any lasting effects of the drugs. There were 12 total days of marble burying testing.

Table 1

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Probe
Control	Saline											No Drug
WIN 55,212-2 (CB1 Agonist)	WIN 55,212-2										WIN 55,212-2 + Tianeptine	
Tianeptine (5-HT Antagonist)	Tianeptine					Tianeptine+ WIN 55,212-2						

Open Field Maze

Previous research indicated that CB1 agonists generally do not impact activity (Umathe et al., 2011, 2012), however, subjective observation of the mice during marble burying necessitated an exploration of activity levels and the impact of WIN-55,212-2.

Subjects

Twelve C57BL/6J female mice, all previously tested in marble burying, were assessed in open field. This was completed to determine the impact of CB1 agonists on locomotion. Therefore, three groups were assessed as follows: one group of 4 were controls, the second group of 4 received WIN 55,212 for the first time at open field testing (naïve treatment), and the final 4 had been receiving WIN 55,212-2 for 11 days in the marble burying experiment and were treated again before open field testing (habituated treatment). The small group size is due to the need to use the mice that had been drug naïve and a software malfunction.

Apparatus

The open field apparatus is a 36x36x12 inch wooden box painted uniformly white. Each mouse was permitted to move freely for 5 mins.

Drugs

The same drugs were used in the open field as the marble burying except that Tianeptine was not tested. This is because the open field testing was performed to assess WIN 55,212-2 effects on locomotion.

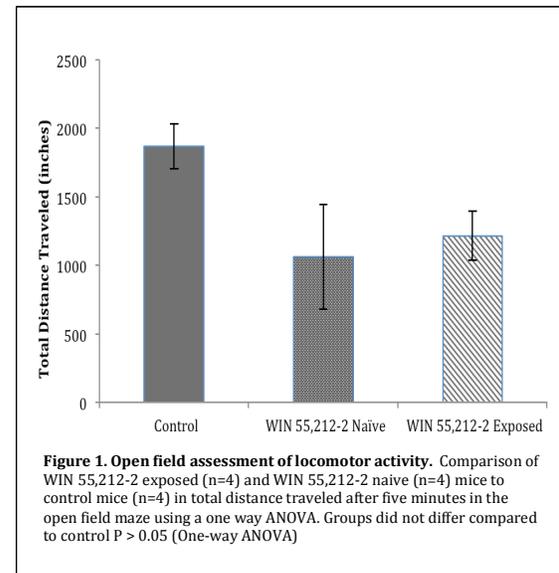
Procedure

Open field testing was performed 72 hours after marble burying was completed. A total of 12 mice were used in open field testing. There were 3 groups: control, WIN 55,212-2 naïve, and WIN 55,212-2 habituated. Thirty minutes after injection, the mouse was placed in the open field. Each mouse was allowed to traverse the box freely for 5 minutes, one time. The computer program Noldus Ethovision XT (Leesburg, VA) tracked the total distance traveled by the animal via an overhead camera.

**Results**

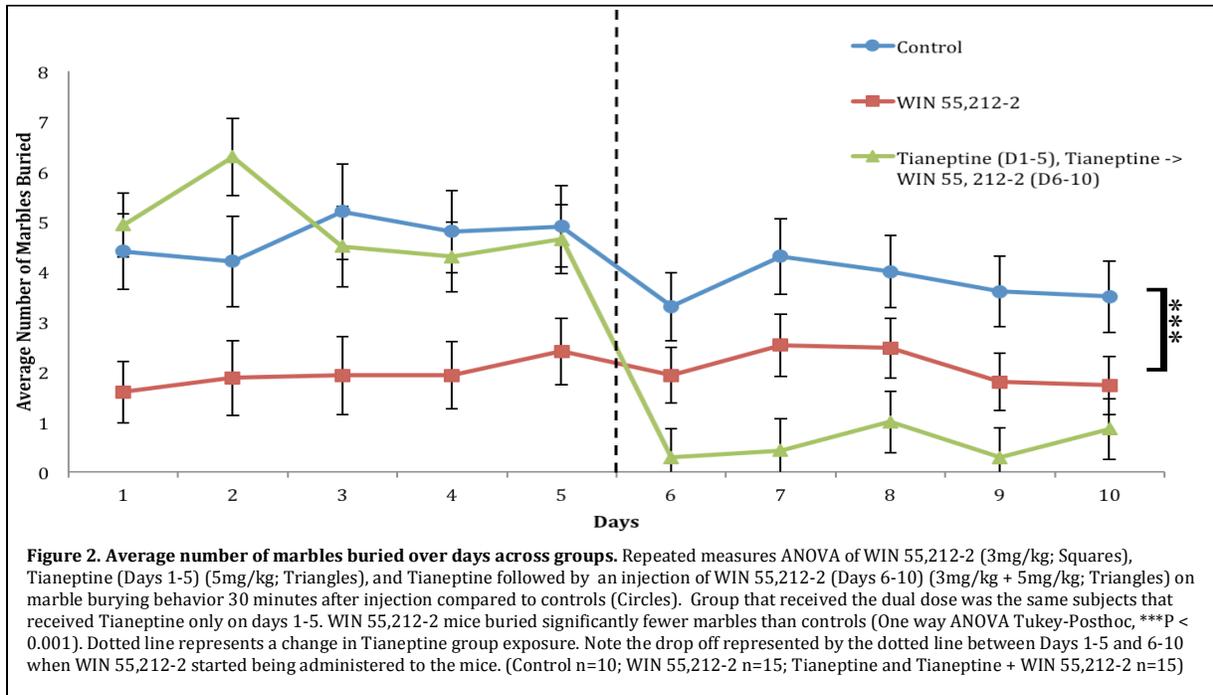
*Effect of WIN 55,212-2 on locomotor activity*

A one-way ANOVA revealed that there were no statistically significant differences in locomotor activity between the three groups [ $F(2,9)=2.682 P > 0.05$ ] (Fig. 1).



*Anxiolytic Properties of WIN 55,212-2*

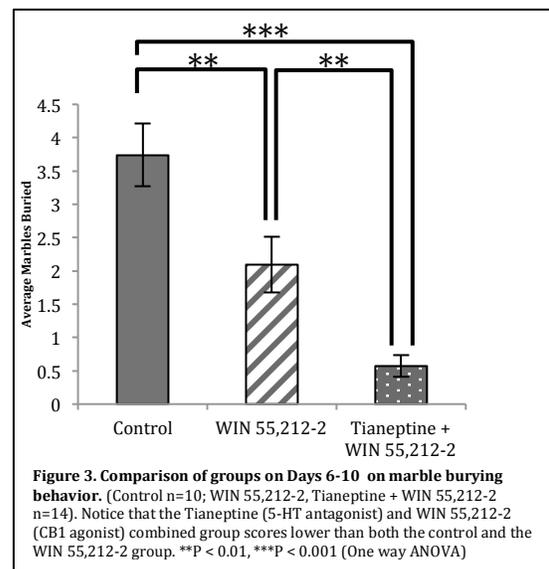
Two separate repeated measures ANOVAS were run for days 1-5 and days 6-10. The ANOVA on days 1-5 revealed significant differences between groups [ $F(2,37)=13.774 P$



< 0.001] and no differences between days or interaction effects (Fig 2). Tukey post-hoc testing revealed that the WIN 55,212-2 group buried significantly fewer marbles than both control group and Tianeptine group [P < 0.001] (Fig. 2.). The WIN 55,212-2 treated mice demonstrated a 52% decrease in marble burying, cutting number of marbles buried by more than half. There was no significant difference between the control and tianeptine group on days 1-5. The ANOVA for days 6-10 revealed a significant difference between groups [ $F(2,36)=17.318$  P < 0.001] with no difference between days or interaction effects. Tukey post-hoc testing revealed a significant difference between WIN 55,212-2 and control [P < 0.01]. WIN 55,212-2 stays consistently and statistically significantly low on all ten days of testing (Fig. 2).

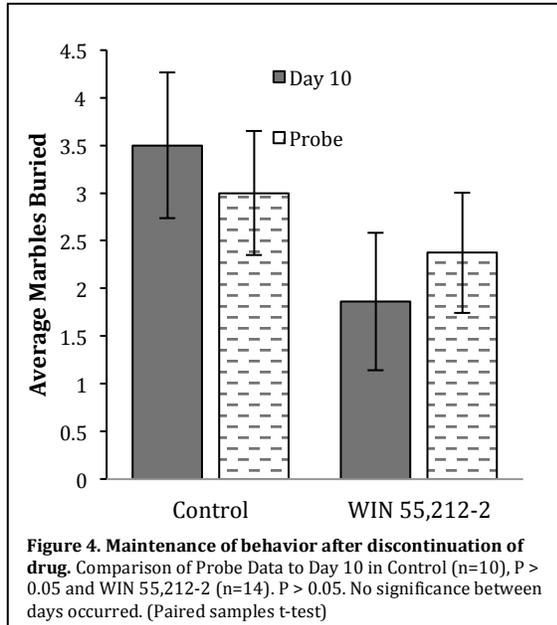
#### Synergy between CB1 and 5-HT systems

For days 6-10, post-hoc analyses also revealed a statistical significance between Tianeptine+WIN and both remaining groups [P < 0.01] (Figs. 2 and 3). The double dose group demonstrated the lowest level of marble burying.



#### Analysis of Probe Data

Two paired samples t-tests were conducted for Control and WIN 55,212-2 groups to evaluate marble burying behavior between the last day of drug treatment and the probe day. The paired samples t-tests revealed no significant differences between Day 10 and the probe trial for either group [P>0.05, Fig. 4].



## Discussion

There were two primary purposes for this study: to investigate the anxiolytic properties of a CB1 agonist and to better understand its involvement with serotonergic neurons. Following the same line of logic of Umathe and colleagues (2011), the anti-compulsive effects of a CB1 agonist with a mouse marble burying model were measured. Our marble burying data indicate that there is no significant habituation within groups in number of marbles buried over the course of the experiment (Fig. 2), replicating previous findings (Njung'e and Handley, 1991; Umathe et al., 2011).

If there was a possibility that WIN 55,212-2 inhibited locomotion, it would be difficult to make any inferences about WIN 55,212-2's anxiolytic effects if the mice were not burying marbles due to locomotor inhibition rather than lowered compulsive behavior. During the first two days of experimental trials, it was observed that the mice receiving WIN 55,212-2 were less active during marble burying. It was thought that the mice were possibly encumbered by locomotor inhibiting effects, but then came to tolerate it over time. In order to test the potential inhibitory effects of WIN 55,212-2 on locomotion, open field

activity was assessed 72 hours after marble burying was completed. There was a control group, a habituated WIN 55,212-2 group (WIN 55,212-2 throughout the experiment), and a WIN 55,212-2 naïve group to assess initial drug effects. The average distance traveled between groups was not statistically significant (Fig. 1), suggesting that this particular CB1 agonist does not have a large inhibitory effect on locomotion. It should be noted, however, that both groups of WIN 55,212-2 treated mice did travel less than controls, and WIN 55,212-2 inhibited naïve mice more than habituated mice. As the number of animals in this analysis was small and the variance high, this finding should be replicated. Nonetheless, it may indicate that pretreating the mice with CB1 agonists for a day or two before using the burying model would give them time to build a tolerance to any possible locomotor hindering effects in future studies.

Based on the open field data, along with qualitative assessments of the mice throughout the experiment, we interpret the effects of WIN 55,212-2 to be anxiolytic. Mice administered WIN 55,212-2 buried significantly fewer marbles than control mice throughout all days of behavioral testing (Figs. 2 and 3). This demonstrates that WIN 55,212-2 has a meaningful anxiolytic effect on compulsive behavior. Not only did WIN 55,212-2 have an anti-compulsive effect, but the effect was overwhelmingly apparent with a 50% reduction in measured compulsive behavior (Fig. 3). It demonstrates that WIN 55,212-2 should be a candidate for reducing compulsive behavior as a function of irrational anxiety. The burying, in this case, acts as evidence of an irrational anxiety because the marbles pose no threat to the mouse's routine or life, yet the mice still pay attention to them. This CB1 agonist allows the mice to execute their normal behavior without being hindered by the presence of something that, while harmless, is unordinary.

In addition to WIN 55,212-2's success as an anti-compulsive drug, its efficacy persisted in this longer-term treatment. Marble burying experiments typically span four to seven days (Casarotto et al., 2010) or they are just done on one day (Gomes et al., 2011; Umathe et al., 2011). Over the course of ten days, marble burying in WIN 55,212-2 treated mice did not

increase significantly from the starting day (Fig. 2). This demonstrates that the efficacy over days is maintained and that this drug may be a potential candidate for long-term OCD treatment. Often SSRI efficacy decreases over time (Lesch et al., 1991) and symptoms can start to reappear after long-term use of the medication in human trials (Hembree et al., 2003). Substituting or co-administering a CB1 agonist with other pharmacological agents could offer more sustainable treatment options.

These findings add to the strong support that WIN 55,212-2 has anxiolytic, anticomulsive effects, but by what means does this CB1 agonist have these effects? Several studies had indicated that CB1 and serotonin interact at both the behavioral and biochemical levels, but the direction of the cellular contact remains unclear (Morales et al., 2004; Umathe et al., 2011). Umathe and colleagues demonstrated that blocking CB1 receptors somehow blocks the anti-compulsive effects of fluoxetine. It was hypothesized that serotonin activated cannabinoid receptors in the brain and by this mechanism down regulated glutamate release, bringing about a relief in anxiety (Best and Regehr, 2008; Umathe et al., 2011). Our experiment provides further evidence for this relationship. We demonstrated that activating CB1 receptors, even in the presence of a serotonin antagonist effectively lowered marble burying behavior (Fig. 3). These results indicate that decreasing serotonin activity is essentially having no effective and that the main impact on anticomulsive behavior occurs via CB1 receptors. This result reflects the idea that not only are cannabinoid and serotonin receptors activating one another, but that cannabinoid neurons are situated downstream from serotonergic neurons in structures responsible for compulsive and anxiety-related behavior measured with marble burying, verifying Umathe et al.'s prediction (2011).

Interestingly, mice receiving the co-administration of the CB1 agonist and the 5-HT antagonist buried significantly fewer marbles than the group receiving only the CB1 agonist (Fig. 2, 3). There are a few possible reasons for the enhanced impact of co-administration. The first could be methodological. It is possible that the handling of the animal to administer two

injections combined with the presence of more exogenous drug in the system could have caused the animals to bury fewer marbles. This could especially be the case where the WIN 55,212-2 animals used to receiving only one injection each day for ten days, received two injections on day 11. However, as can be seen in Figure 2, the Tianeptine mice receive a double injection with WIN 55,212-2 during days 6-10 and show no habituation to this handling that would be demonstrated by a decrease in marbles buried.

A physiological possibility is that this behavioral effect is due to Tianeptine starving the system of serotonin. Serotonin is involved in a large number of cognitive functions and the injected Tianeptine is acting systemically. Serotonin's many pathways may have been inhibited. It becomes more puzzling once one examines that the Tianeptine-only mice are burying on par with the controls (Fig. 2). The monoamine hypothesis of affective disorders posits that a decrease in neurotransmitter leads to symptoms. However, this study did not demonstrate that effect; depleting serotonin from the synapse did not increase marble burying. Another possibility is that the interaction is much more complicated than originally anticipated and that feedback mechanisms could be involved. CB1 could be modulating serotonin neurons in a way that balances the impact of these two neurotransmitters. CB1 is often associated with feedback mechanisms and it would not be surprising if this were the case. More studies are required to determine the seemingly synergistic effect of activating CB1 while antagonizing serotonin.

CB1 agonists, specifically WIN 55,212-2, have demonstrated positive anti-compulsive effects, do not demonstrate behavioral tolerance over 10 days and seem to have minimal side effects as far as could be recognized. Also, there was interest in WIN 55,212-2's lasting effects on compulsive behavior even after drug treatment had stopped. A probe trial was conducted, allowing mice to bury marbles without a drug injection. There was no difference between the number of marbles buried on Day 10 of treatment and the probe day for the WIN 55,212-2 group (Fig. 4), indicating either a long half-life or slight learning effect of

the drug. This persistence may provide for the possibility of intermittent dosing.

WIN 55,212-2 appears to be a promising CB1 agonist for the treatment of OCD-related behaviors and warrants further investigation on its anticomulsive and anxiolytic properties. Future investigations should include histological examination of brain structures, neurotransmitter level alterations, and electrical excitability changes in the presence of WIN 55,212-2. In addition to its positive effects on OCD, WIN 55,212-2 could hold potential as a treatment option for other general anxiety disorders. It is clear that this drug is working downstream of serotonergic neurons that have an influence on cannabinoid receptors, but it appears that the effect of serotonin can be overridden by the introduction of a CB1 agonist.

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