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JWH-018 and Selective Serotonin Reuptake Inhibitors: Drug-Drug Interactions and Implications on Toxicity

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SENIOR THESIS APPROVAL

This Honors thesis entitled

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JWH-018 and Selective Serotonin Reuptake Inhibitors:

Drug-Drug Interactions and Implications on Toxicity

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Abstract

Drug abuse has expanded from more well-known substances, such as cocaine and marijuana, to relatively new novel psychoactive substances. A group of these substances called synthetic cannabinoids have been increasing in usage throughout the 2000's, and these compounds carry significant and varying risks depending on the dose and composition of the synthetic cannabinoid. Patients have been observed having symptoms associated with high doses of synthetic cannabinoids when they take lower doses of the synthetic cannabinoid in addition to their antidepressant medication. In order to test the effects of co-administration of selective serotonin reuptake inhibitors (SSRIs) and synthetic cannabinoids, mice were observed in a marble burying assay under the influence of the different drugs. Mice were given either fluoxetine (10-mg/kg), citalopram (20-mg/kg), or JWH-018 (0.1-mg/kg or 0.3-mg/kg) or a combination of the drugs. Mice without being injected with any of the drugs buried 6.375 marbles on average in the twenty-minute test. Mice injected with fluoxetine or citalopram alone buried 4.25 marbles and 4.0 marbles respectively. The JWH-018 doses were chosen to be ineffective so that the marbles buried at 0.1-mg/kg was 6.125 and the 0.3-mg/kg dose resulted in 6.375 marbles buried. Fluoxetine in combination with JWH-018 resulted in 3.0 marbles being buried for the lower dose and 1.875 marbles were buried with the high dose. Citalopram in combination with JWH-018 resulted in 3.625 marbles buried for the lower dose and 2.875 marbles buried for the high dose. When the SSRIs were taken with an ineffective dose of JWH-018 a greater than anticipated drug effect occurred, since it occurred in both combinations it points to a pharmacodynamic effect instead of a pharmacokinetic effect.

JWH-018 and Selective Serotonin Reuptake Inhibitors:

Drug-Drug Interactions and Implications on Toxicity

Background

There are approximately two hundred scheduled drugs internationally, but there are over seven hundred other substances that are available. These drugs are called new psychoactive substances and are largely unregulated (National Institute of Abuse, 2015). These substances include but are not limited to both synthetic cathinones and synthetic cannabinoids. Both of these groups are becoming increasingly prevalent in drug abuse and are significant risks to public health, especially in young people (Office of Drug Control Policy).

In the past few years synthetic drugs have been in the news more frequently. The use of synthetic cathinones, also known as bath salts, has led to dramatized news reports of individuals involved in grisly acts of violence against others (ABC News, 2012). Synthetic cannabinoids on the other hand have not seen as many sensationalized news articles, which adds to a perception of safety that abusers see in the drugs. Abusers also see that marijuana is beginning to be legalized and used medically, so people think the synthetic cannabinoids are "synthetic marijuana", a safe and legal alternative. In a 2012 Monitoring the Future Survey, 11.3% of high school seniors reported using some type of synthetic cannabinoids (National Institute of Abuse, 2015). On the other hand, bath salts are perceived as more dangerous so only 1.3% of high school seniors reported having used bath salts (Seeley et al., 2013).

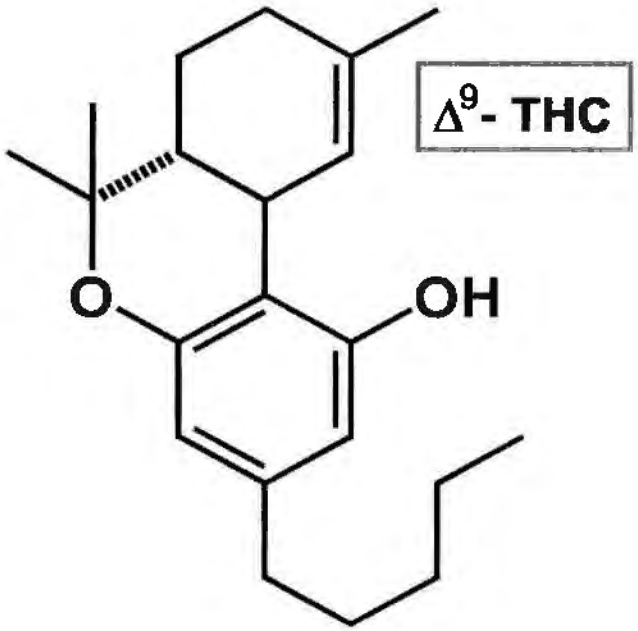


Figure 1: Active Ingredient in Marijuana, Δ^9-THC

One of the most dangerous aspects of synthetic cathinones is the different effects that can occur from different derivatives. Synthetic cathinones have been known to mimic the effects of several big name illicit drugs for example methylenedioxypyrovalerone (MDPV) mimics the effects of cocaine. Other cathinones seem to be similar to MDMA (ecstasy) and methamphetamine. Synthetic cannabinoids on the other hand are seen only as "fake weed" but it has varying effects similar to synthetic cathinones.

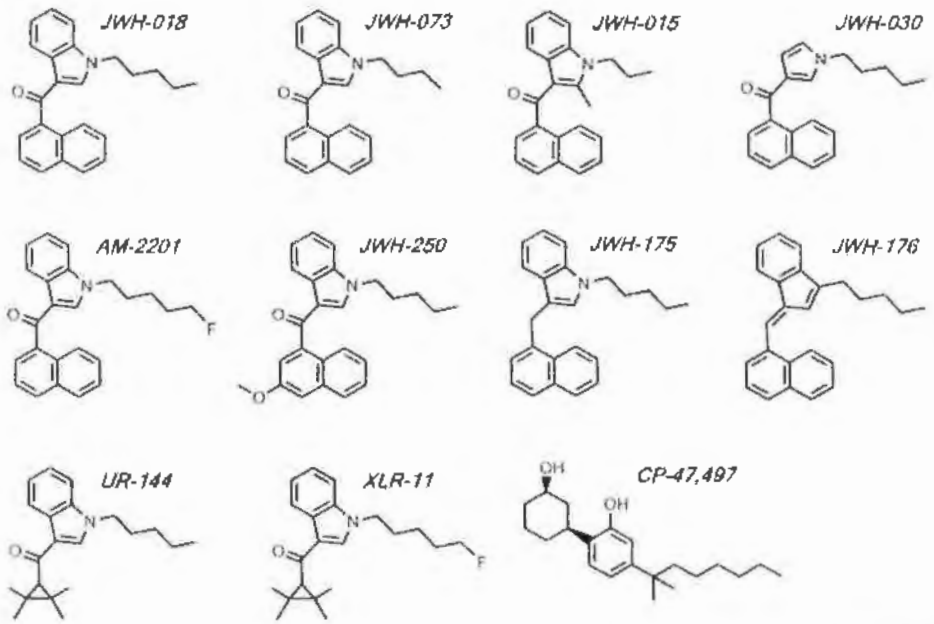
The ability of synthetic cannabinoids to have multiple effects is problematic for healthcare. For example, in Brunswick, Georgia during late August and early September 2013 twenty-two patients were admitted to emergency departments for synthetic cannabinoid use. Patients experienced a variety of symptoms from the drug abuse including hyperglycemia, hyperkalemia, acidosis, tachycardia, nausea, vomiting, confusion or disorientation, aggression, unresponsiveness and seizures. Some of these patients experienced complications of pneumonia (two), rhabdomyolysis (one), and myocardial infarctions (one). Certain patients were admitted to

intensive care units and some required assisted ventilation. The huge variety of effects that resulted from synthetic cannabinoid use for these patients resulted from the same product, called "Crazy Clown", that was bought from the same smoke shop (Dreznek, 2013).

This example is only the effects of one synthetic cannabinoid product from one smoke shop in one state. The United Kingdom National Poisons Information Service (UKNPIS) used a survey that lasted for eight years to identify different effects that have resulted from synthetic cannabinoid abuse. In addition to the variety of effects that were seen in Georgia, the UK survey uncovered symptoms such as abdominal pain, chest pain, hypotension, arrhythmia, speech disorders, and more (Waugh, et al, 2016). The increase in symptoms found by the UKNPIS may be a function of different products, since the most popular synthetic cannabinoid products in the survey were Black Mamba, Clockwork Orange, and Pandora's Box, not Crazy Clown (Waugh, et al, 2016).

Synthetic cannabinoid products that are sold, such as K2, Spice, or Crazy Clown, are unpredictable because they can contain one or many different synthetic cannabinoids. The reason synthetic drugs can be easily altered by changing only side groups on the original chemical resulting in many compounds that have varying effects. Figure 2 shows how many different synthetic cannabinoids, each of which has a slightly different structure and slightly different effects (Tai and Fantegrossi, 2016). The ease in which the drug composition can change helps people to avoid drug enforcement. The tests for drugs are typically done by taking blood or urine samples followed by immunoassays and mass spectrometry assays. The problem is that assays are too specific to identify all of the new compounds. Antibodies for immunoassays are too specific and even if we had less specific antibodies that could identify the main components of synthetic cannabinoids the mass spectrometry would still need to be specific. The mass

spectrometry needs the initial structure and its metabolites to work which with many of these synthetic cannabinoids isn't possible. The mass spectrometry is required as a confirmation in case the immunoassay results in a false positive. Without it the whole test is ineffective (Strickland and Bazydlo, 2018).



Pharmacological and Toxicological Effects of Synthetic Cannabinoids and Their Metabolites. Tai and Fantegrossi 2016.

Figure 2: Variety of Synthetic Cannabinoids

Another problem that occurs with synthetic cannabinoids is that they can be more efficacious and potent than marijuana. Figure 3 illustrates how two synthetic cannabinoids are more efficacious than marijuana. Efficacy is the ability of a compound to bind the receptor, because for a drug to be effective it has to be able to bind the receptor. In Figure 2 the lines of best fit for JWH-018 and JWH-073 are both shifted to the left of the line of best fit for THC, which is the active compound in marijuana. The shift to the left means that it takes less substrate (on the horizontal access) to have the same amount of receptor binding as THC, therefore the two synthetic cannabinoids are more efficacious than THC.

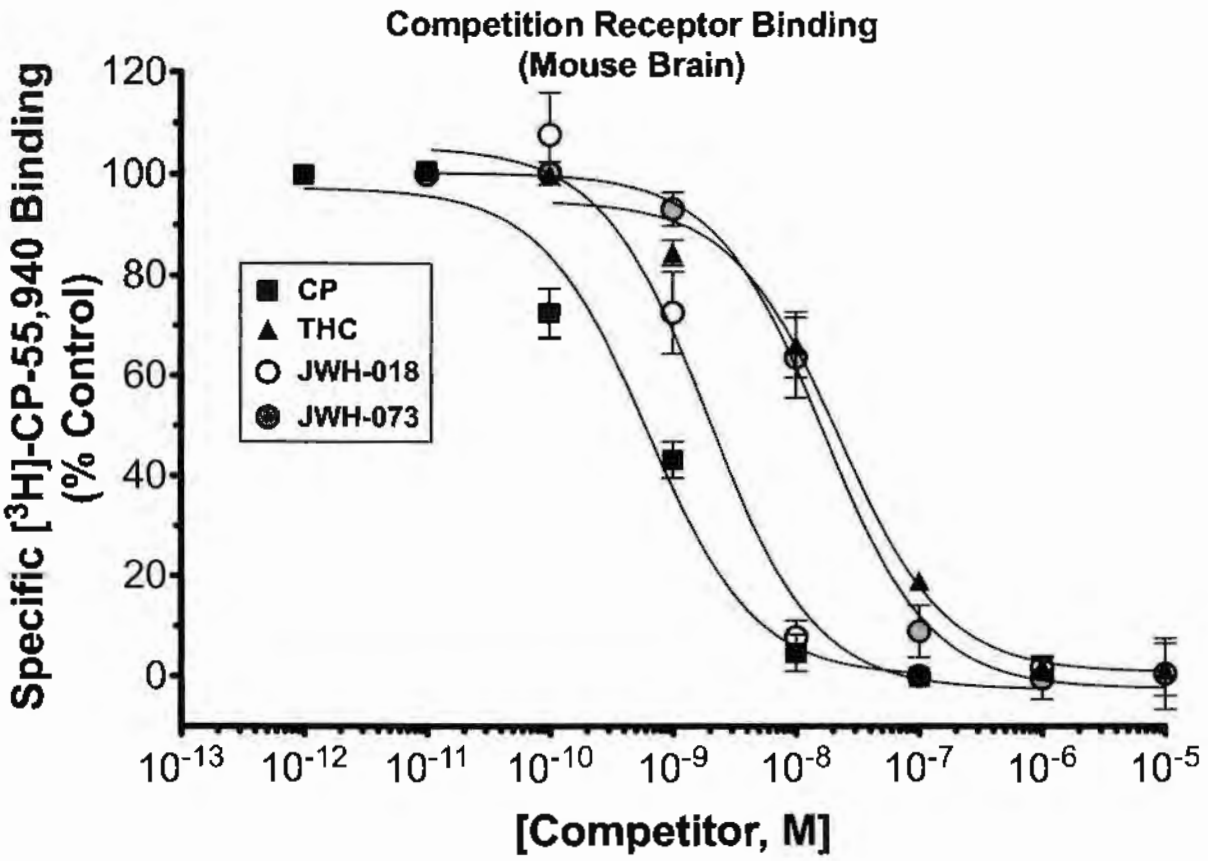


Figure 3: Comparative Receptor Binding Affinity

Figure 4 also shows how the same two drugs are more efficacious, but in a different way. Once the cannabinoid binds to the receptor it activates a G-protein that then causes a signal cascade in the cell. The G-protein activation of JWH-018 and JWH-073 are shifted left of THC, but JWH-073 is much less obvious in this graph. The shift left again means that less substrate is needed for the synthetic cannabinoids to bind to the cannabinoid receptors and therefore means it is more efficacious. The lines of best fit for G-protein activation for the two synthetic cannabinoids are also shifted vertically on the graph, which means that for the same amount of substrate the synthetic cannabinoid has a greater effect. This increase in effect is referred to as the potency of the drug, so the synthetic cannabinoids are more potent than THC.

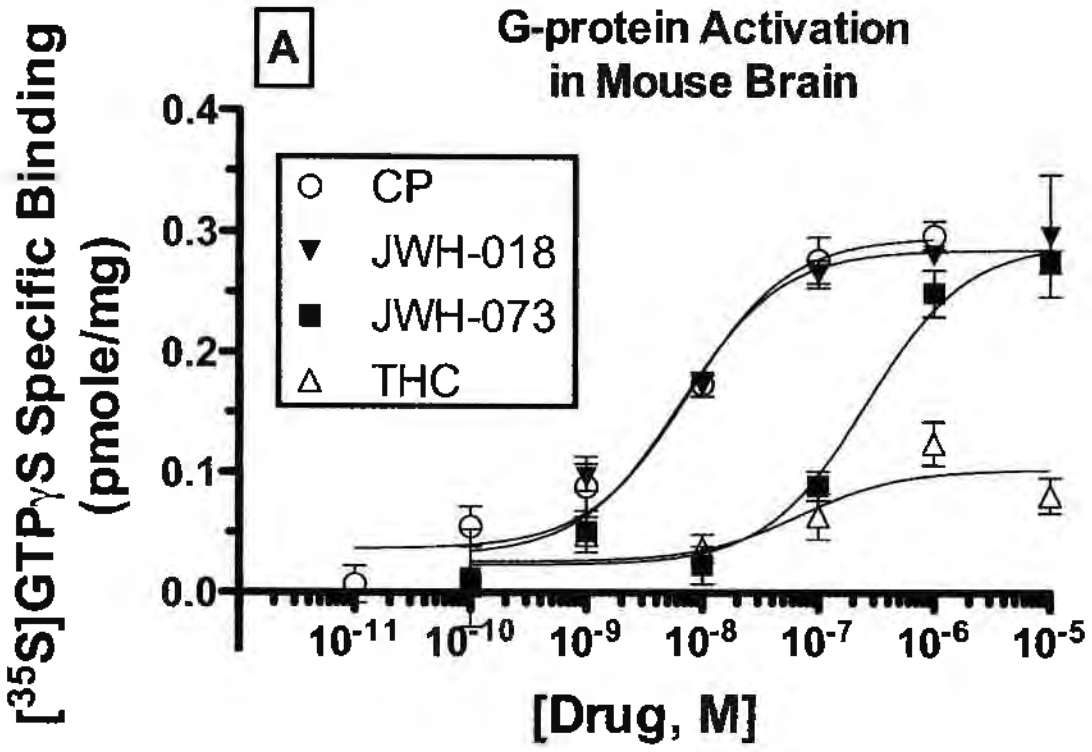


Figure 4: Comparative G-Protein Activation

The increase in efficacy and potency of synthetic cannabinoids as opposed to THC is where the increased likelihood of negative health effects lies. The effect of marijuana and many medicines has been tested and is mostly well known, but synthetic cannabinoids could interact with drugs that marijuana does not. Rumors of patients coming into hospitals with high dose effects of synthetic cannabinoids when taking low doses of synthetic cannabinoids with selective serotonin reuptake inhibitors (SSRIs), a group of antidepressant medications, resulted in the following experiments.

Experiments

Hypotheses

General Hypotheses. Synthetic cannabinoids when co-administrated with SSRIs will result in a larger than anticipated drug effect. The larger than anticipated drug effect resulting from the co-administration will be due to a pharmacokinetic effect involving the enzymatic breakdown of the two compounds.

Experimental Hypotheses. An ineffective dose of JWH-018 when co-administered with an effective dose of the SSRI fluoxetine will result in less marbles buried than with only fluoxetine. The larger than anticipated drug effect will be due to a pharmacokinetic effect involving the cytochrome 2C9 that breaks down both fluoxetine and JWH-018.

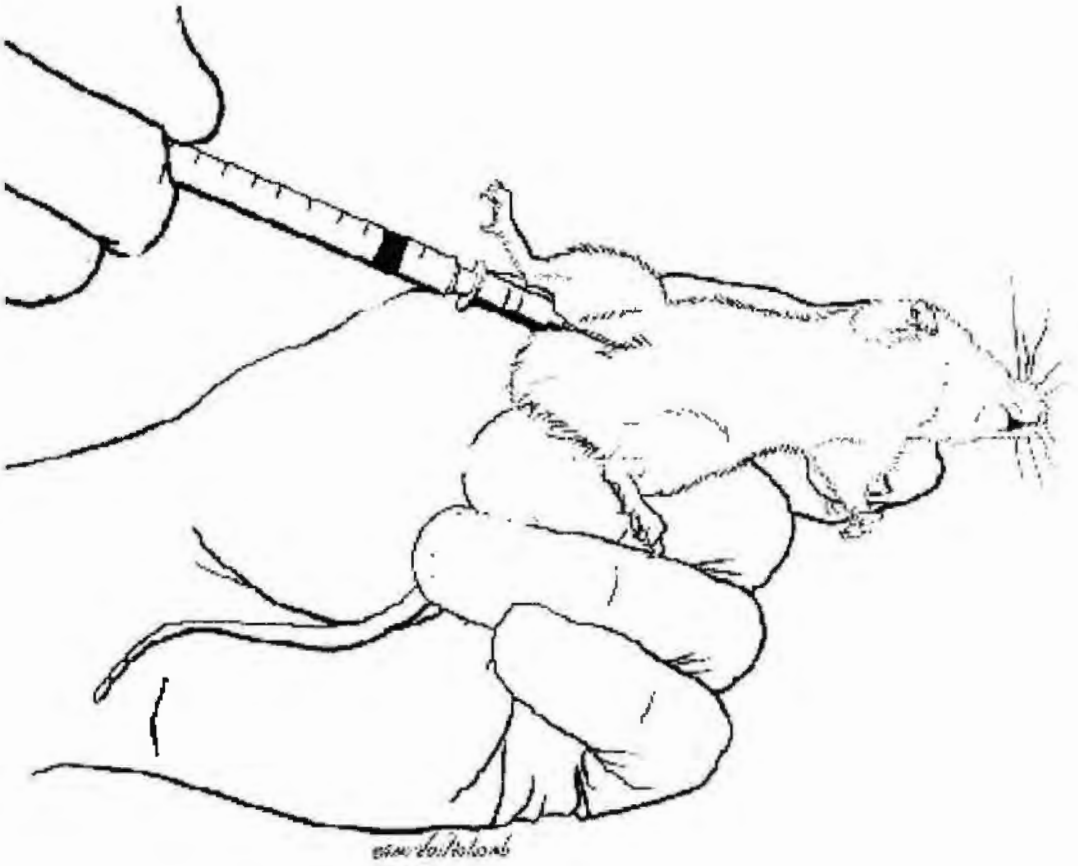
Methods

All methods were carried out as recommended by the Public Health Service Policy on Humane Care and Use of Laboratory Animals of the National Institute of Health. The protocols were approved by the Department of Laboratory Animal Medicine at the University of Arkansas for the Medical Sciences.

Protocols.

1. Standard plastic mouse cages were used for the marble burying test. Fresh standard bedding added to the cages from 3-cm to 5-cm in depth. Plastic filter tops were placed on cages to prevent mice from escaping during the tests. Wire racks that hold food and water were withheld during the tests.
2. Ten standard glass marbles arranged on top of the bedding approximately equidistant in the same pattern for every cage (see Figure 6). Before the marbles were placed in the cages, they were cleaned and dried to remove scents of earlier experiments.

3. Stopwatches were set for twenty minutes, but results were checked and recorded every five minutes.
4. Each mouse was injected intraperitoneally before the test with the dose depending on the compound(s) being tested. Then the mouse was given a treatment time to allow the effects of the drug to occur, which was also dependent on the compound injected.



The Laboratory Mouse. University of Illinois at Chicago, 2014.

Figure 5: Intraperitoneal Injection of Mouse

5. A single mouse was then set into each cage and left undisturbed for twenty minutes. After the twenty minutes the mice were returned to their home cages.
6. The marbles were then examined and those that were buried over half their height were counted.

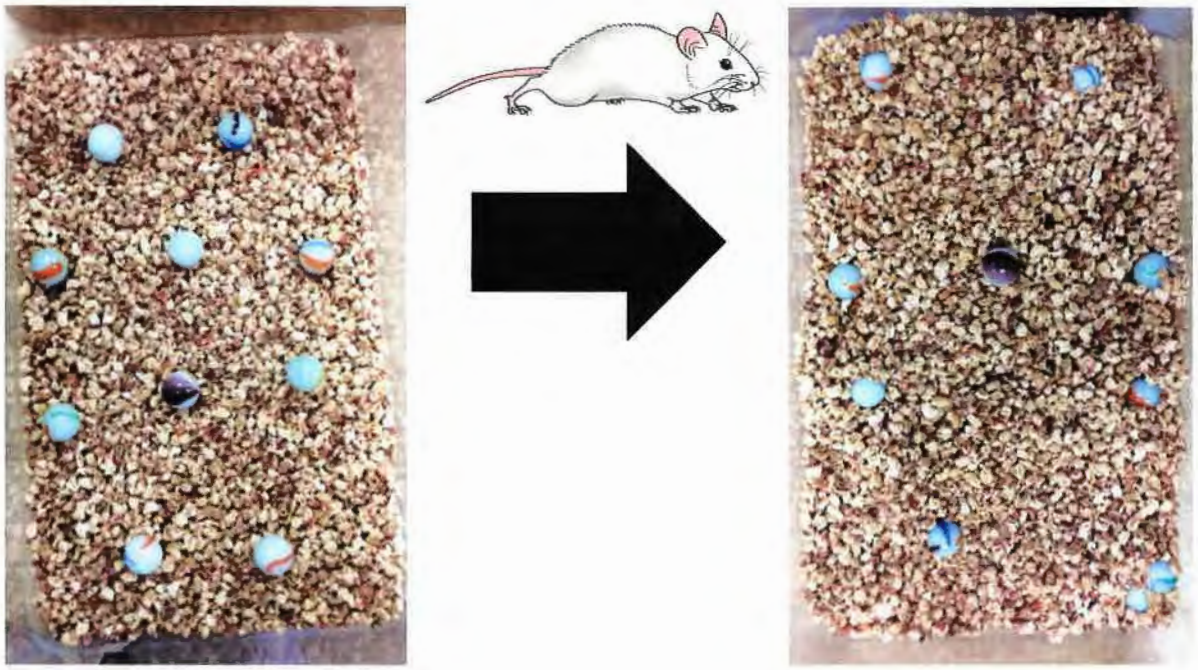


Figure 6: Example Marble Burying Test with Saline

Figure 7: Example Marble Burying Test with SSRI

Pre-Experiment. Before the main experiments began there were several tests to ensure that the marble burying assay would work for the planned experiment. Firstly, the SSRIs fluoxetine was tested at the dose 10-mg/kg with a thirty-minute treatment time to give a baseline effect of the SSRIs. Then citalopram was tested at 10-mg/kg and 20-mg/kg with treatment times of thirty-minute and one-hour treatment times in order to find an effect that was similar to the effect of fluoxetine at 10-mg/kg. The 20-mg/kg dose was chosen to be used for the later tests with a treatment time of thirty-minutes. The chosen doses of fluoxetine and citalopram had nearly equivalent effects on marble burying, but if the JWH-018 interacted in later tests we would still be able to see change in either direction (agonistic or antagonistic interaction effects).

Then tests were run to confirm the ineffective doses of JWH-018 from 0.1-mg/kg, 0.3-mg/kg and 1.0-mg/kg with a thirty-minute treatment time. Both 0.1-mg/kg and 0.3-mg/kg were found to have no effect on the marble burying test, hut the 1.0-mg/kg dose was eliminated due to interference with the ability of the mice to hury marbles.

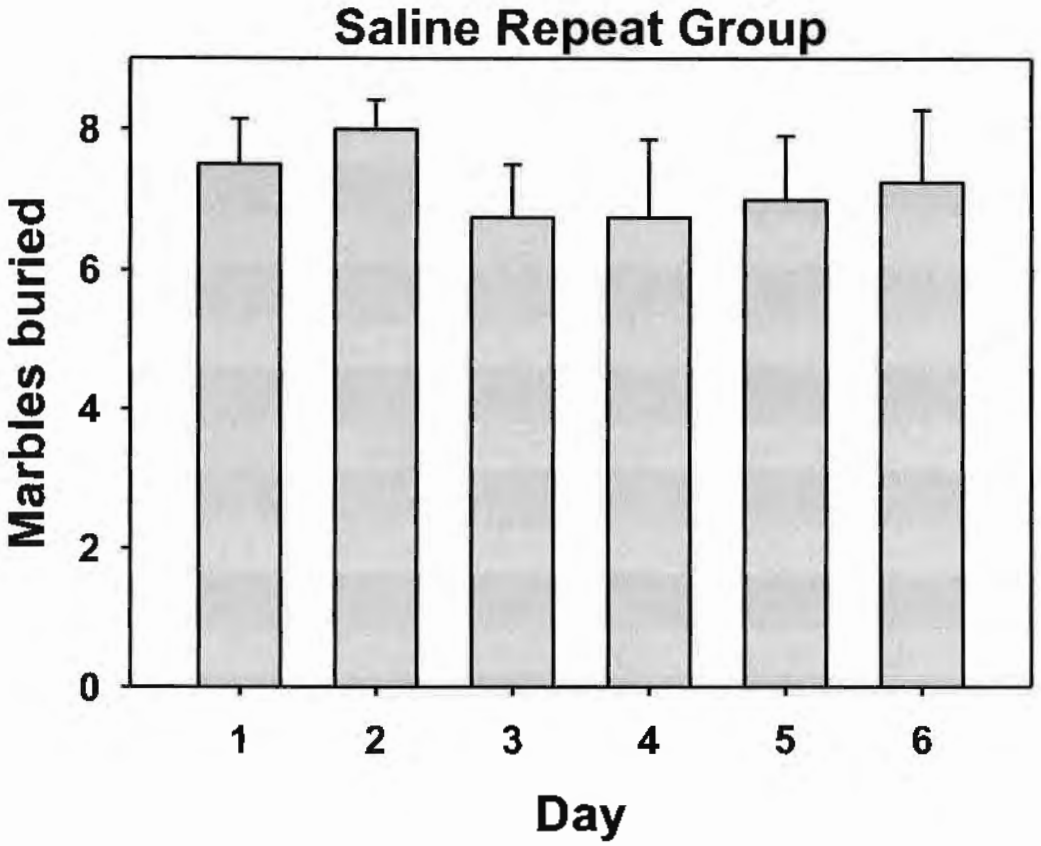


Figure 8: Repeated Saline Trials

The final pre-experiment test was to find if the marble burying test could be used repeatedly without having an effect on results. This would determine if the same mice could be used in a longitudinal experiment, so that they could be used as controls against themselves. In order to test that we gave a group of 8 mice saline injections, and then they were subjected to the marble burying test. The same group of 8 mice repeated this test with saline from between 10:00 am and 12:00 pm every day for six days. The results of all 8 mice were averaged for each day and the results were recorded in Figure 8. There was no significant difference in the results of tests over the course of the six days, so we decided to continue with the longitudinal experiment design.

Experiment. The main experiment was run with eight mice, which were included in all of the tests. The design of the experiment was longitudinal, meaning that the same mice were used over a relatively long period of time so that the results of earlier tests can be used as controls for the future tests. The longitudinal experiment design is illustrated in Figure 9.

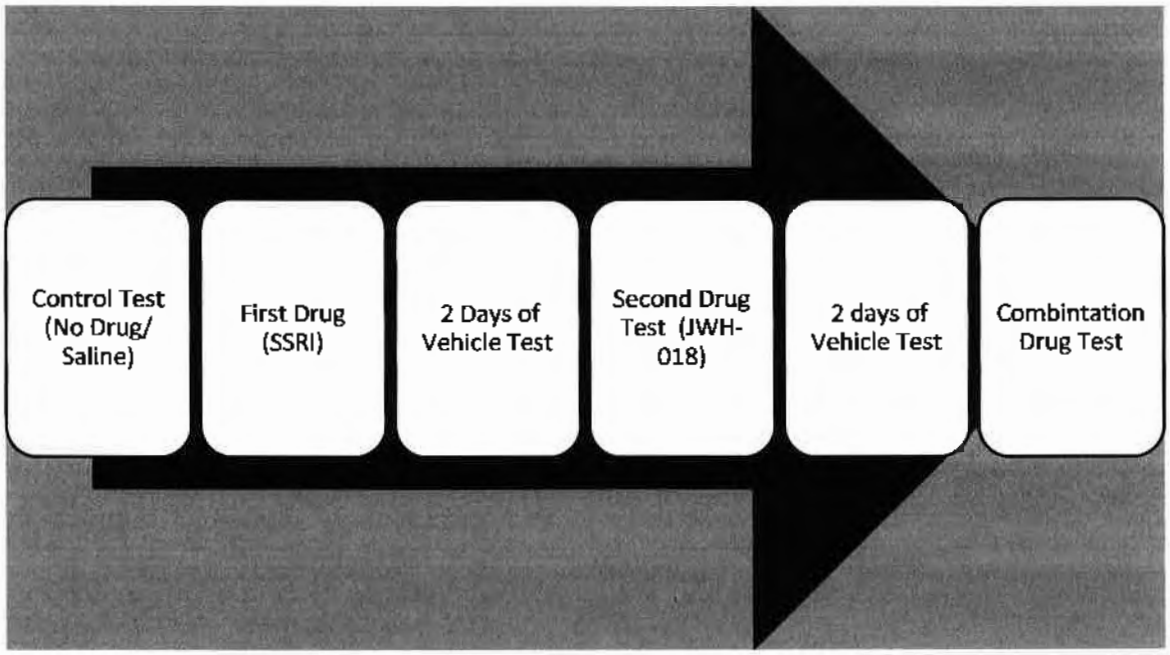


Figure 9: Longitudinal Experiment Design

First the mice went through two control tests. The first control test was with no drug to see what a mouse would normally do when presented with the marbles. Then the second test was with saline in order to see if discomfort or aggravation from the injection would change the results of the marble burying test. These tests were used as a baseline to compare the results of the drugs later tested as can be seen in Figure 10.

The day after the control testing was finished, the SSRI drugs were tested. The first test was with the 10-mg/kg dose of fluoxetine with a thirty-minute treatment time, and then the mice were tested with 20-mg/kg of citalopram with a thirty-minute treatment time. These tests allowed us to have a baseline drug effect for SSRIs to compare with our interaction tests. Between the

tests with the SSRI injections two days of saline tests were run in order to allow each drug time to washout. This prevented residual drugs in the mouse from influencing the results of later experiments.

After the SSRI tests and the final two-day washout period for them, the mice began the two JWH-018 tests. The first test was with JWH-018 at 0.3-mg/kg with a thirty-minute treatment time. Two days of saline tests were then administered. After the washout period the second test was run with JWH-018 at 0.1-mg/kg with a thirty-minute treatment time. The two synthetic cannabinoid tests were run in order to confirm that the doses did not affect the marble burying test in the same way as it did in previous tests.

After the final JWH-018 test the mice were again given a two-day washout period before beginning the final portion of the experiments. The next tests conducted were the JWH-018 0.3-mg/kg dose with the two doses of SSRIs. First the SSRI was injected and immediately afterward the JWH-018 dose was injected into the mouse. After the mouse was injected a thirty-minute treatment time was observed before the mice began the marble burying test. Between each combination test the same two-day washout period was observed. After the high dose of JWH-018 was tested, the same was done for the 0.1-mg/kg dose.

Post Experiment. After the tests in the main experiment were completed an additional test was run to give additional data on whether an interaction discovered would be pharmacokinetic or pharmacodynamic. In the test, the mice were given the 0.3-mg/kg dose of JWH-018, a 10-mg/kg dose of fluoxetine, and a 10-mg/kg dose of rimonabant. The antagonist rimouabant was added to the test to block any pharmacodynamic effect, so if there was no change to marble burying the interaction was pharmacokinetic, but if there was a change with the rimonabant it would be pharmacodynamic.

Results

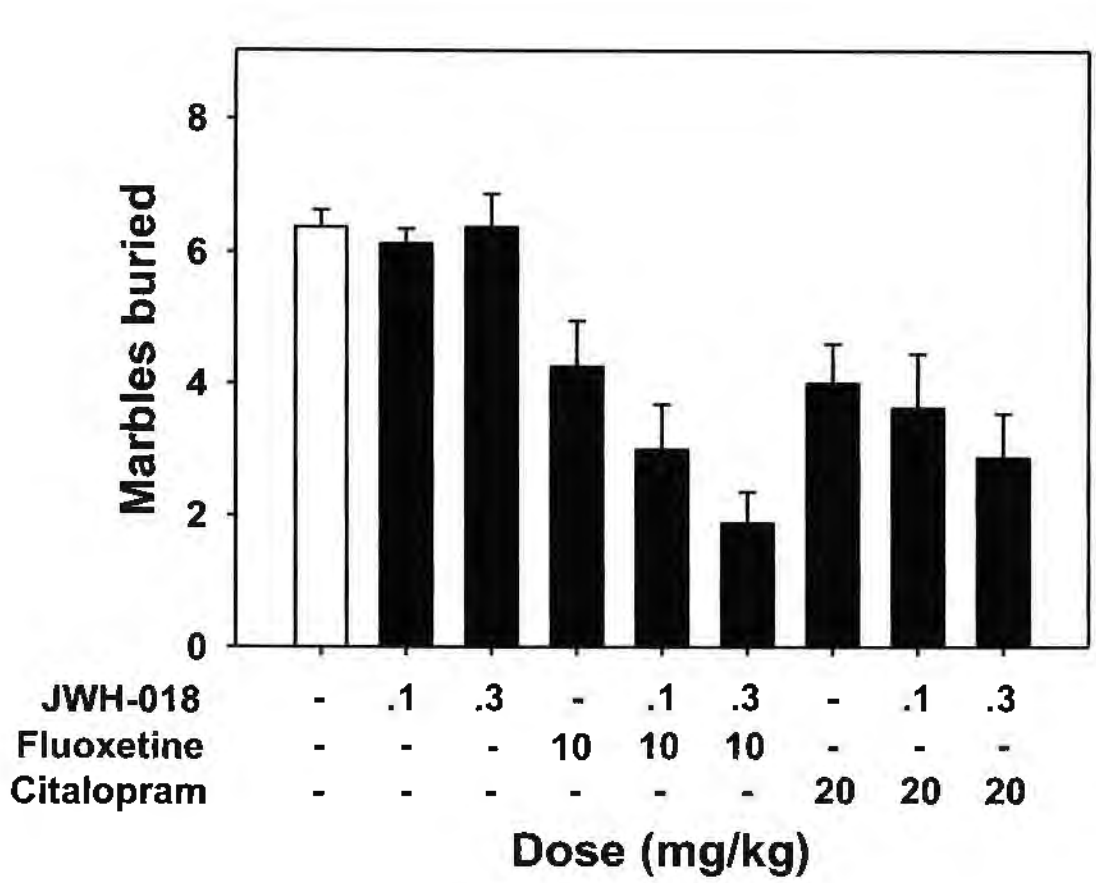


Figure 10: Average Marbles Buried for Various Treatments

When the mice were given only saline the mice buried on average 6.375 marbles. When mice were injected with the 0.1-mg/kg dose of JWH-018 6.125 marbles were buried on average and at 0.3-mg/kg 6.375 marbles were buried on average. Neither of these values reflects a significant difference from the saline control group. The fluoxetine dose of 10-mg/kg resulted in an average of 4.25 marbles buried, and the citalopram dose of 20-mg/kg resulted in an average of 4.0 marbles buried. Both of the SSRIs caused the marble burying to decrease significantly from the control group. The SSRI effects are not significantly different than each other. Fluoxetine in combination with both doses of JWH-018 caused a decrease in marble burying. The combination of fluoxetine with the 0.1-mg/kg dose of JWH-018 resulted in 3.0 marbles being buried on

average, and the 0.3-mg/kg dose of JWH-018 resulted in 1.875 marbles being buried on average. The citalopram combination was not as successful as the fluoxetine combination, but a decrease also occurred. The citalopram and 0.1-mg/kg dose of JWH-018 caused an average of 3.625 marbles to be buried, which is not significantly different the citalopram test. The 0.3-mg/kg of JWH-018 combination was significantly different at 2.875 marbles buried. These results can be seen graphically in Figure 10.

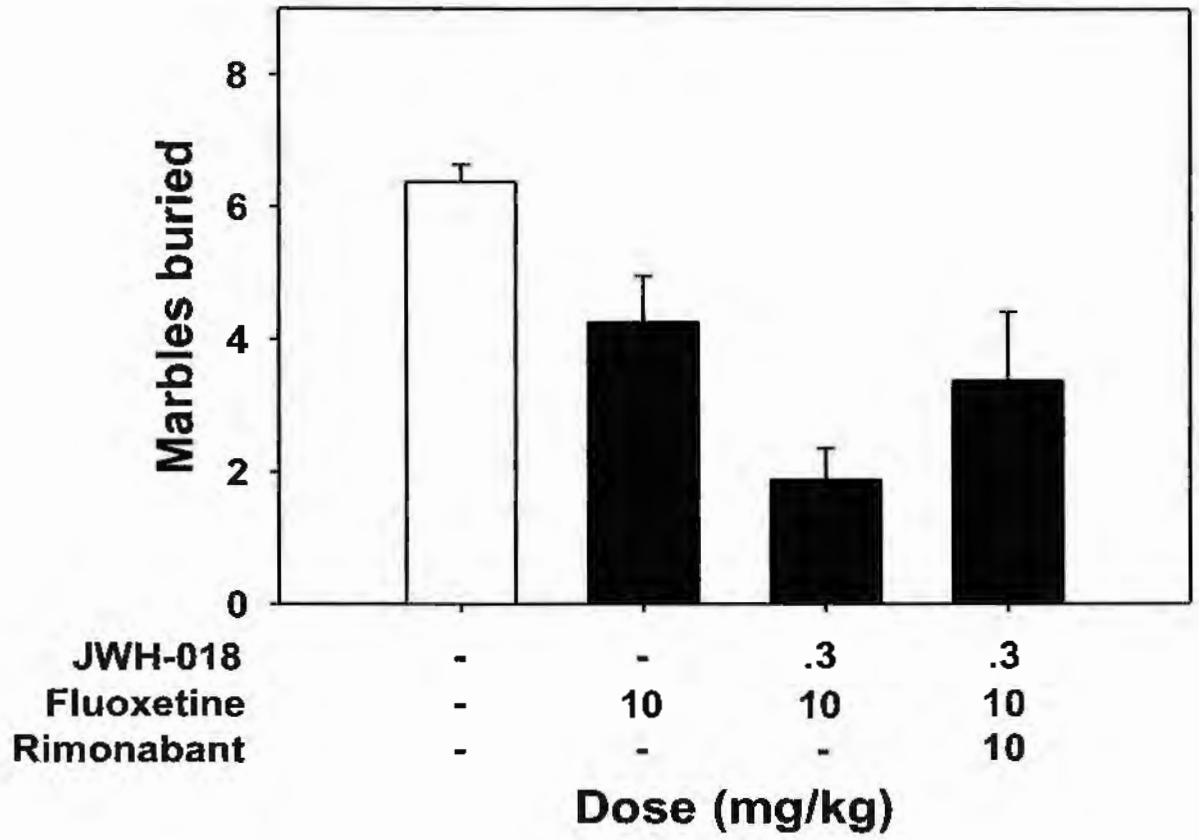


Figure 11: Marbles Buried for Rimonabant Combination

Figure 11 shows the results of the last test that occurred after the main experiment had ended. The first bar is the same coutrol as was used in the original experiment, 6.375 marbles buried. The second bar is the 10-mg/kg fluoxetine result from the previous experiment (4.25

marbles buried), and the third bar is the combination of 0.3-mg/kg JWH-018 and fluoxetine from the previous experiment (1.875 marbles buried). The final bar was the same 0.3-mg/kg JWH-018 and 10-mg/kg fluoxetine combination as before, but 10-mg/kg rimonabant was added to the combination. When rimonabant was added to the experiment, the amount of marbles buried was 3.375.

Conclusions

Co-administration of JWH-018 and both SSRIs, fluoxetine and citalopram, resulted in a greater than anticipated drug effect as was hypothesized. The increased drug effect with the synthetic cannabinoid and fluoxetine was expected, but it was not expected to occur with citalopram. Citalopram was a positive control, since it did not share the same enzyme as the other two compounds, which means that there is likely a pharmacodynamic aspect to the increased drug effect. The evidence that the effect is pharmacodynamic goes against the initial hypothesis. Also, the results of the rimonabant experiment support a pharmacodynamic interaction, since the inclusion of rimonabant reduced the additional effect of the combination.

These results are significant since they illustrate how co-administration of the two types of compounds can result in high dose effects at low doses. The greater breadth of increased effect than expected will also warrant more tests with a variety of SSRIs and other anti-depressant medication. It will also mean that the increase in synthetic cannabinoid abuse with common prescription drugs may result in more hospitalizations or potential deaths.

There are many future experiments that could be useful in examining the interaction between these drugs. First, having more trials of the current tests would help to validate what has already been done, but changing from a longitudinal experiment design could be helpful. Mice could be used in a control test and then two other tests before being replaced. This would

help to eliminate any changes in marble burying that occurred due to tolerance, sensitivity or dependence. Mice being used for a shorter time would also help increase the sample size, which would help prevent outliers from skewing any results.

Tests that examined the effects of chronic use of these compounds could also be interesting. Either JWH-018 or an SSRI could be given chronically then an acute injection of the other drug could be given with a treatment time before the marble burying test is administered. SSRIs are taken daily, so it would be relevant to see the chronic effects. Synthetic cannabinoids can also be abused chronically, so giving either drug chronically before an acute injection would be relevant.

More testing is imperative so that more information can be observed regarding the effects of synthetic cannabinoids and SSRIs together. SSRIs are already one of the most commonly prescribed types of antidepressants, and synthetic cannabinoids abuse has been increasing for years. The use of these drugs together can cause many negative health effects, but continued tests such as these can help prepare for abuse of these drugs in the future.

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