Abstract:

One of the most studied neurotransmitter systems in addiction is the mesolimbic dopamine pathway, which primarily uses dopamine to build a reward and learning mechanism. Glutamate and NMDA receptors are also used in the pathway. MK-801 is a NMDA receptor antagonist and is thought to disrupt the mesolimbic pathway, thus slowing or stopping addiction. The present experiment assessed the effects of MK-801 on cocaine conditioned place preference in adolescent female rats. Rats were injected with either saline or MK-801, and 30 minutes later were placed them in the conditioning chamber for 20 minutes. Changes in chamber preference were assessed to detect changes in behavior. The present preliminary data indicates that rats which received saline/saline or MK-801/saline injections treated the chambers neutrally after conditioning, demonstrating that MK-801 alone did not affect chamber preference differently from saline/saline treated rats. At the time of submission no data for the MK-801/cocaine or saline/cocaine injections have been collected, however it is expected a change in chamber preference to be observed in rats administered only cocaine, while seeing little to no change in chamber preference in rats treated with Mk-801 and cocaine. This would demonstrate that MK-801 plays a role in blocking cocaine sensitization in rats.
Introduction:

Cocaine is one of the most widely abused drugs in the entire world yet the mechanism for cocaine addiction is not as well understood as the actual effects of cocaine. When introduced to the body, cocaine affects the neurotransmitter dopamine directly, causing a sharp rise in the concentration of the chemical (Tanda et al. 2009). This uptick in dopamine concentration causes an increase in the user’s mood and overall feeling, making the experience feel rewarding to the user (Tanda et al. 2009). Through earlier research on the subject, it has been concluded that conditioned place preference testing is an effective model of measuring how much behavioral change the subjects go through during drug testing (Brown et al. 2008).

We are interested in the mesolimbic dopamine pathway, which is considered one of the primary dopamine pathways in the brain, and also involved in the reward and learning mechanism in the brain (Jerlhag et al. 2010). This pathway involves the use of glutamate and NMDA receptors as well to carry along the signal (Kretschmer 1999). Glutamate is another neurotransmitter which is actively involved in the learning process in the brain (Kretschmer 1999). MK-801, the substance utilized in the present work, is a known effective NMDA receptor antagonist (Kretschmer 1999). This means that MK-801 will interfere with glutamate’s ability to bind to the NMDA receptors and effectively carry the signal.

In order to test and gauge the possible effects of MK-801 on cocaine conditioned place preference in adolescent female rats, MK-801 was administered with and without cocaine to test changes in preference for environmental cues paired with the drug. The conditioned place preference paradigm relies primarily on environmental cues paired with the physical effects of the drug to test the subjects’ preference and learning behavior. The conditioned place preference
system has shown to be effective when testing the addiction patterns of various substances, such as cocaine, alcohol and nicotine (Badanich et al., 2006). This paradigm should serve well to display the effects of cocaine under normal circumstances, and identify if the NMDA receptor mediates this change in behavior in adolescent female rats.

Four groups of subjects were utilized in the present experiment, organized in the table below:

<table>
<thead>
<tr>
<th>Group 1: Saline/Saline (Control)—n=10</th>
<th>Group 2: MK-801/Saline (Control)—n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 3: Cocaine/Saline (Control)—n=10</td>
<td>Group 4: MK-801/Cocaine (Test)—n=10</td>
</tr>
</tbody>
</table>

The doses that were administered include 0.2 mg/kg of MK-801 and 20 mg/kg of cocaine. All of the animals were adolescent female Sprague Dawley rats. Adolescent animals are utilized in the present work due to the fact they have been shown to be more sensitive to the effects of cocaine compared to adults and their brains are also undergoing dramatic neuronal development at this stage. Female rats were used in the present experiment, to assess if 1) adolescent female rats respond differently to the conditioning effect of cocaine during the adolescent developmental period and 2) to identify if the NMDA is one of the mechanisms which may mediate this effect. No change in the subjects’ behavior is expected for Groups 1 and 2, indicating that MK-801 does not have an effect on chambers in chamber preference in an isolated setting. For Group 3, a shift in the subject behavior and bias is expected as cocaine will be tested under isolated conditions. For Group 4, no change in the subject behavior is expected, given it is hypothesized that the NMDA receptor mediates the behavioral effects of cocaine.

**Materials/Methods:**
The conditioned place preference paradigm will be employed to test MK-801 and its effectiveness on blocking cocaine conditioned place preference in adolescent female rats. Animals underwent the exact same conditioning procedure as indicated below:

<table>
<thead>
<tr>
<th>Day(s)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>Handling</td>
</tr>
<tr>
<td></td>
<td>PND 28-29</td>
</tr>
<tr>
<td>3</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>PND 30</td>
</tr>
<tr>
<td>4-11</td>
<td>Drug conditioning</td>
</tr>
<tr>
<td></td>
<td>PND 31-38</td>
</tr>
<tr>
<td>12</td>
<td>Test</td>
</tr>
<tr>
<td></td>
<td>PND 39</td>
</tr>
</tbody>
</table>

The apparatus that was a Plexiglas box divided into two chambers. One chamber had a sandpaper floor with horizontal stripes and the other chamber had a wire mesh floor with vertical stripes. On the baseline test, the rats were into the apparatus for a total of fifteen minutes and initial chamber preference was assessed. The chamber that the subject spent less time in was noted as the least preferred chamber, and thus the drug-conditioning chamber.

The conditioning phase of the testing followed a schedule of alternating the least preferred and preferred chamber for 8 days. The rats were given their first injection, and then placed into their home cage for thirty minutes, followed by their second injection, then being placed into their conditioning chamber for twenty minutes. Following the eight days of conditioning, the animals were tested for a change in chamber preference using the same conditions as the baseline test mentioned above. Once again, the time the animals spent in each drug-paired chamber was assessed and compared the results to the animal’s baseline results.

Results/Data:
At the time of submission, the only data that is available is the data from Groups 1 & 2, the saline/saline animals and the MK-801/saline animals. By the time of the symposium, the data for the other two groups will be collected and presented. For the groups that were tested, it was confirmed that the rats administered MK-801 did not differ from those administered saline alone, indicating MK-801 alone did not influence behavior differently from saline-treated rats.
In the above graph, no differences between the groups in terms of how much time they spent in each chamber were observed. In both groups, there was an initial strong chamber bias at baseline. At the post conditioning test, there was a shift in chamber preference with the subjects spending approximately equivalent time in both the initially least preferred and initially most-preferred chamber. This indicates the rats tested thus far treat the chambers neutrally after conditioning.

Again, at the time of this submission, there are no data available for the other groups being tested, but they will be available by the date of the Symposium.

**Discussion:**

Examining the data available to date, there are a few conclusions that can be drawn and a few expectations for the data that will be available. The first major conclusion that can be drawn is that running conditioned place preference will not impact the animals’ behavior, insofar as they will not change their chamber preference. Although we did see an increase in the amount of time that the subjects spent in their less preferred chamber from baseline to final, they did not spend enough time in their less preferred chamber during final testing to show a change in their chamber bias.

Another major conclusion that can be drawn from the data that is available is the effects of MK-801 by itself on the subjects. We wanted to affirm that MK-801 did not play a role in chamber bias and therefore would not impact the animals’ choices. There were similar changes in the time spent in the less preferred chamber between the two groups. This indicates MK-801 does not effectively change the animals’ chamber preference and bias. This helps to affirm that
any major change which does take place in the preference for the other groups can be attributed to the cocaine that was given.

Although there are no data for groups 3 and 4, it is expected for group 3, which is the group being given saline/cocaine injections, to see a large change in the chamber preference and behavior of the animals, above that of the control groups. Because cocaine will be paired with the initially less preferred chamber during conditioning testing, the animals must overcome the initial bias and spend significantly more time in the drug-paired chamber after conditioning. By having a shift in their chamber preference, the rats have shown that they have overcome their natural bias and have undergone a reward and learning mechanism.

The most important group to be tested is group 4, which is given MK-801/cocaine injections while undergoing conditioning. It is expected this group will elucidate the role of the NMDA receptor in cocaine place conditioning in adolescent female rats. Little to no change in the chamber preference for the animals is expected for this group, indicating MK-801 has interfered with the reward and learning mechanism that impacted the animals in group 3. Because the group 3 animals should show a large change in the chamber preference, it is expected the animals of group 4 spend the same amount of time in their less preferred chamber during both baseline and final testing. Again, this would show that MK-801 did sufficiently disrupt the mesolimbic dopamine pathway in the brain.

If there were changes in the chamber preferences, this would show that glutamate and NMDA receptors do play a significant role in the carrying of signals for the mesolimbic pathway (Kretschmer 1999). We hope to see group 3 change chamber preference as we noted above (due to exposure to solely cocaine) and we hope to see changes in Group 4 chamber preference as we
have seen in some of the other literature that has been reviewed in this topic, such as by Brown and colleagues in 2008. However, one major topic that we would like to keep our eyes on is the comparison of results from our tests and the results from the Brown 2008 article. Brown 2008 affirmed that MK-801 can interrupt cocaine conditioned place preference for subjects. We would also expect to see these changes taking place with adolescent rats, as described by Badanich and colleagues (2006).
Literature Cited


