The Effect of a Neurotoxic Dose of Methamphetamine on Attention and Response Learning in the Rat

Anja K. Kremer

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Approved by:

_____
Advisor: Dr. Karen Sabol

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Reader: Dr. Michael Allen

_____
Reader: Dr. John Young

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Abstract.

High doses of methamphetamine (neurotoxic doses) have been shown to have an effect on both attention and response learning. In previous literature, meth rats were found to have higher reaction times, and therefore, it has been concluded that meth may cause a deficit in response learning. Our study, using modal initiation time as a measure of response learning, found no effects of methamphetamine or training condition on mode. Studies have also shown positively skewed reaction time distributions, which have been defined as attentional lapses. Our experiment used DevMode to measure these lapses. Although meth did not, training condition did have a main effect on DevMode. Therefore, one may conclude that stimulus salience did have an effect on attention. It is still unclear what the effects of neurotoxic meth are on attention.

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Methamphetamine (meth) is a relatively inexpensive drug with a very high abuse potential, the use of which has unfortunately become an epidemic. Meth is now a health concern among youth around the world (Fast et al., 2014). It is important to understand exactly what the physiological and behavioral effects are of meth, especially now that it is so commonly abused. Through trying to understand these effects, scientists have found neurochemical and cognitive impairments in humans (Salo et al., 2007). However, human studies are difficult to execute, so animal studies are often used to test for any behavioral and physiological effects occurring as a result of a high dose of methamphetamine. In these studies, which usually use rats, it has been found that high dose meth exposure causes monoamine depletions in experimental animals, just like it did in the humans (Chapman et al., 2002). In both of the human and animal models, behavioral impairments were found in the subjects who had been exposed to methamphetamine. The further study of how neurotoxic doses of meth affect behavioral deficits was our goal.

Humans.

The main focus of human research has been of abstinent meth users, usually after at least about 3 weeks of abstinence, and of people who have had a lifetime dependence on methamphetamine. Usually the humans used for the study are actively being urine tested for drugs to make sure that they are truly in abstinence. Sometimes researchers use fMRI's or other imaging techniques to get an idea of the amount of monoamine

depletions present. The studies also test for several facets of behavior, including impulsivity and attention.

Impulsivity. A commonly accepted definition for impulsivity is the preference of a small, immediate reward over a large reward that one would have to wait for (Schwartz et al., 2010). Schwartz et al. identified impulsivity as a major contributor to drug-seeking behavior in humans. Schwartz et al. used a delay-discounting task, which asks participants to choose between an immediate small monetary reward or a large monetary reward for which they would have to wait a period of time to receive. It was found that the ex-meth abusing subjects were significantly more likely to choose the smaller, sooner reward, ergo more impulsive.

Attention. Methamphetamine may also have an effect on the ability of an organism to pay attention. In 2007, Ruth Salo and cohorts carried out a study attempting to measure the difference between attentional control in former meth-abusing humans and the drug-free controls. Subjects participated in a Stroop task experiment. The Stroop task was performed on a computer and consisted of words being presented one at a time, sometimes conflicting in nature and sometimes non-conflicting. An incongruent word was defined as a word that was the name of a color written in a different color font, for example the word “purple” written in green font. A congruent word was the name of a color written in font matching the word. Participants were asked to name the color of the font and not read the word aloud. “Stroop interference” was a measure which was calculated by subtracting the mean reaction time in neutral trials from the mean reaction time during incongruent trials. In this study, it was found that meth subjects had significantly higher Stroop interference values, and therefore had an attention deficit

(Salo et al., 2007). The same task was used in 2009, and it was found that control subjects were more accurate when an incongruent trial was followed by another incongruent trial. However, former methamphetamine abusing subjects were *not* any better at the incongruent-to-incongruent trials than they were at the congruent-incongruent (Salo et al., 2009). Humans who have been exposed to high doses of meth may not learn from previous trials as well as controls.

Performing these experiments with humans produces valuable data; however whenever humans are used in such trials, there are many uncontrollable factors that could confound the results of the experiment. For instance, it is difficult to know how much of a drug each human has actually used, and it is also difficult to control for environmental factors.

Rats

Studies have been conducted that attempt to determine the effects of a neurotoxic dose of methamphetamine on attention, learning, memory, and movement in the rat. Typically, to study the long term effects of a high dose of methamphetamine, researchers inject rats with the drug (for example, four 20 mg/kg injections, at two hour intervals) and then wait around three weeks before beginning behavioral training.

Researchers have investigated the effect of a high dose of methamphetamine on animal motor skills. In rat studies, results indicated that rats that had been previously given a neurotoxic dose of meth had a deficit in performing a balance beam task (Walsh and Wagner, 1992).

When an animal is given a small dose of meth, one of the commonly observed behavioral effects during intoxication is an increase in stereotypic movement. Wallace et

al. (1999) treated rats with a high dose meth regimen or saline, to begin with. After the initial injections and recovery, rats were given a lower dose of either meth or saline. It was found that meth treated rats showed significantly more stereotypic movement during the drug intoxication than control rats did (Wallace et al., 1999).

There are also many studies examining the effects of a neurotoxic dose of meth on memory in the rat. Inhibitory avoidance and escape tasks can be considered measures of memory or learning. So to measure the effect of meth on memory, Walsh and Wagner (1992) tested rats on their ability to escape an unpleasant electrical shock. Rats were placed in a chamber, which was bisected by a plexi-glass wall. The floor on the starting side of the box delivered an electric shock to the rat after ten seconds from the start of a tone. Therefore, a rat needed to escape to the safe side within ten seconds. Meth pre-treated rats were significantly impaired on the time it took them to cross to the safe side of the box. For the inhibitory avoidance task, the model was the same except the goal was to stop oneself from entering the other side of the cage, where the shock would take place. The inhibitory avoidance task yielded no significant impairment due to the methamphetamine treatment. The deficits in the avoidance task do not appear to be an inability to learn, because if that were true, they would have been replicated in the inhibitory avoidance task (Walsh and Wagner, 1992). It is important to recognize, however, that any impairments on the escape task could be due to a motor deficit.

Many researchers have attempted to measure the effects of high doses of meth on a rat's ability to recognize a novel objet, another common memory test. A Novel Object Recognition task involves an animal being presented with two objects, one familiar and one novel. The amount of time investigating each object is recorded. Rats who recognize

the familiar object should spend more time investigating the novel object, and animals who do not recognize the familiar object should spend an equal amount of time with the novel object as the familiar object, because both will be novel to them. In the Novel Object Recognition tasks, methamphetamine rats spent less time investigating novel objects, indicating a lack of recognition of the familiar object (Bisagno et al., 2002, Herring et al., 2008, O'dell et al., 2011).

Researchers have also studied what the effects are of a high dose of methamphetamine on an animals ability to use spatial learning. Distal cues are used by animals when they are remembering how to complete a task that is placement-oriented. A common paradigm, the Morris Water Maze (MWM) involves a pool filled with opaque water and a hidden platform that the animal is striving to reach, because the animal does not want to swim. The MWM is considered a test of spatial learning because, due to the opaque liquid, the only tool the rat has to find the platform is its ability to use the surrounding environment as a visual map to reach its goal. Friedman et al. (1998) used variations of the Morris Water Maze to identify any deficits in place learning that may be a result of a high dose of meth. Friedman and associates found that it took the methamphetamine-exposed rats longer to get on the platform during the MWM task, which they named as a deficit in place learning (Friedman et al. 1998). However, after three blocks of training, the methamphetamine rats did get back to the same level of achievement as the control rats. In contrast, Herring et al. (2008) found no difference between methamphetamine and control rats in performing the Morris Water Maze task. It is unclear, based on the current evidence, whether high doses of methamphetamine are a cause for decreased spatial memory ability.

Purpose

Research has identified deficits in movement and novel object recognition in rats who had been previously exposed to a neurotoxic dose of meth. There were also significant deficits in escape behaviors, but not in the avoidance task, which could possibly be explained by the deficits found in movement. As far as spatial memory, some studies have shown an impairment due to meth, and some have not shown a significant impairment.

Two other constructs that researchers investigate in relation to high dose meth exposure are response learning and attention. The present experiment was designed to assess the behavioral effects of a high dose of methamphetamine on a reaction time task that is a test of both response learning and attention in rats.

Response Learning

*Egocentric Responses S*cientists have studied whether or not high doses of meth would cause deficits in response learning. The Cincinnati Water Maze (CWM) is a task that requires the use of egocentric cues, which is a feature of response learning. Egocentric is a term used to describe the cues that come from within the animal itself. This can mean body position, or bodily sensations. The CWM was performed in a room lit only with infrared lights, to remove the option of using distal cues to solve the maze. The maze was a complex T-Maze. Each time the rat came to the end of the hallway it had only two choices, to go right or to go left. This means that the rats could only use egocentric cues, as opposed to distal cues, to complete the maze. The deficits in the

CWM were presumed to be non-spatial and labeled “path integration” (Herring et al., 2008). Herring showed that path integration was significantly impaired in the meth rats during the Cincinnati Water Maze. In another study of egocentric learning, Chapman et al. (2000) gave rats a neurotoxic regimen of methamphetamine, and then tested them in a radial arm maze. Each rat began in the center compartment, which had eight closed doors. The doors surrounded the center and they led to eight corridors, which extended outward from the center. One at a time, doors opened to allow the rats to pass into the corridor behind it to reach the food reward. As soon as the rat left the first hallway, timing began and ceased when the rat had entered the second hallway and reached the second pellet of food, which yielded the dependent variable, reaction time. First, all of the rats were tested with a constant pattern of door-openings repeatedly. After this, rats performed the same task, except the pattern of door-openings was randomized. After these randomized pattern trials, rats were again tested in the original pattern. After data analysis, it was shown that the rats that had received methamphetamine were slower during both of the trials in which the fixed pattern of door-openings was used. However, saline rats were slower during random pattern trials (Chapman et al., 2000). These two studies both demonstrated impairments in egocentric behavior in rats treated with high doses of meth.

Reaction Time. Richards et al. (1993) attempted to measure the effect meth has on the performance of a reaction time task, which was similar to our own. The task involved an operant chamber with a lever, a stimulus light, and a method of delivery for the reward: water. Rats had to hold down the lever at the front wall of the operant chamber until the stimulus light located directly above the lever turned on. Once the stimulus light

turned on, the rat could go to the water station. After 7 weeks, it became apparent through data analysis that the rats that had been subjected to meth were significantly slower than the saline rats. Richards et al. named learning deficits, motor deficits, a decreased ability to process sensory information, and inattention as possible causes for the difference.

Action-Outcome vs. Stimulus-Response Habit. Action-outcome (A-O) behavior is behavior displayed by an animal when the animal is concerned primarily with a goal, such as a reward. In fact, action-outcome behavior has also been referred to as "goal-directed behavior" (Son et al., 2011). A rat in the A-O phase of learning will be easily deterred from continuing an action if the reward has been devalued. If the same rat suddenly started receiving a devalued reward, it would quickly stop working for it.

Stimulus response (S-R) habit behavior is based on the association between a stimulus and the response. S-R habit is when an animal responds to the conditioned stimulus only, and does so habitually (Son et al., 2013). If the reward has been devalued, a rat in S-R will likely continue pushing the lever out of habit. S-R rats show a difference when compared to A-O rats, as mentioned above, because the A-O rat would be focusing on the reward and quickly discontinue the behavior if the reward was devalued. A-O responses are typically seen in the beginning of training, and are usually replaced by stimulus-response (S-R) habit some time after that.

Researchers have set out to determine how neurotoxic methamphetamine might affect A-O and S-R behavior. In Son et al.'s (2011) experiment, rats learned to push a lever to receive a drop of sucrose solution. Later, Son et al. used lithium chloride (LiCl) to devalue the food rewards. LiCl was given to the rats while they had free access to the sucrose. The LiCl treatment made the rats sick, rendering the sucrose no longer

rewarding. When returned to the test chamber, meth rats who had received LiCl injections showed a great decrease in response rates compared to their pre-treatment rates, whereas the control rats who had received LiCl did not have a significant difference in response rate compared to before the LiCl treatment. The reward was devalued for the rats, and since the meth rats stopped responding, one can suggest that they were most likely in the goal-directed (A-O) phase of learning, while the control rats had moved to the S-R habit phase. Son and cohorts believe that this means that meth pretreated rats have impaired S-R habit formation (Son et al., 2011).

Attention. In the Richards et al.'s (1993) study discussed above, they attempted to determine whether neurotoxic doses of meth would have an effect on reaction time. The positive skew of the distribution of reaction times was one of the most intriguing of results. In human research Leth-Steensen et al. (2000) found that children with ADHD weren't slower but had a significantly longer distribution skew, and he called these attentional lapses (Leth-Steensen et al., 2000). This new lens on attention led to Sabol et. al's 2003 study in rats which attempted to separate these brief lapses in attention from the trials in which the rats were genuinely paying attention to the stimulus light.

In Sabol et al.'s study (2003), the reaction time was split into two separate processes: initiation time and movement time. Initiation time was the time between the stimulus light (either the left or right) illuminating until the moment the rat removes its nose from the center nose poke hole. Movement time was defined as the time between the rat removing its nose from the center hole, and inserting it into the reward hole on either the left or right. The researchers focused on two initiation time measures: mode and DevMode. Sabol et al. examined the value referred to as "DevMode," which was meant

to quantify the positive skew of the initiation time distributions, and determine exactly how long and frequent these lapses in attention were. DevMode was calculated to be difference between the mode initiation time of all 100 trials and the mean of the intiation times. The other main measure of interest, the mode, was interpreted as representing sensory motor processing.

Response Learning and Attention: Analysis

Response Learning. The studies by Herring et al. (2008), Chapman et al. (2000) and Son et al. (2011) as discussed above report a number of different effects methamphetamine has on response learning. Son et al. divided response learning behavior into two categories: A-O and S-R (Son et al., 2011). A-O behavior, also referred to as goal-directed behavior, is usually how an animal first attempts a task. Animals eventually switch into the S-R type of behavior in which they are acting no longer with the reward in mind, but purely out of habit (Son et al., 2011). Son et al. found that rats exposed to methamphetamine decreased their responses when their reward was devalued, showing that they were acting in a goal-directed manner (A-O), whereas the control rats did not change their response rate and were therefore probably already in the habit of responding (S-R) (Son et al., 2011). Chapman et al. (2000) found that methamphetamine treated rats were significantly slower than control rats in the radial arm task. This version of the radial arm task is a response learning task because the rat is encouraged to memorize the body positions it needed to follow in order to reach the food in the fastest manner possible. There are several possible explanations for the deficit in the meth rat's

performance. It may be due to impaired egocentric learning, or perhaps the slowness of the meth rats could be due to them not switching from A-O to S-R habit.

Richards et al. (1993) measured the effect a high dose of meth would have on the ability of a rat to perform a reaction time task. The results showed that meth did cause an increase in time taken to react to a visual stimulus. Several interpretations for this deficit exist, such as a decrease in the ability to switch into the S-R habit, response learning impairments, sensory impairment or a possible attention deficit.

Attention. There has been little research done to investigate the effects of a high dose of methamphetamine on attentional lapses in the animal literature. However, in the human data, Salo et al. (2007) found that methamphetamine exposed humans had a significantly larger Stroop Interference (mean reaction time during incongruent trials minus the mean reaction time during congruent trials), suggesting deficits in attention.

Referring to the reaction time task from Richards et al. (1993), the distribution of the data has a positive skew, showing that there were some very long latencies, which can be interpreted as lapses in attention. With these lapses in mind, Sabol et al. designed a study which measured the variable DevMode as a measure of distribution skew (Sabol et al., 2003).

Using a similar reaction time task as the one Richards et al. (1993) and Sabol et al. (2003) used, the present study aims to identify any effects high doses of meth might have on the rat's ability to perform a response learning task, as well as its ability to maintain attention. Rats were treated with high dose meth, after two weeks of recovery, they were trained on the reaction time task. Once they had reached stable performance,

they were trained in four more conditions in which stimulus salience and the presence of distracting stimulus were manipulated.

Hypotheses.

In Richards et al.'s (1993) study, rats who had been subjected to methamphetamine had significantly slower reaction times than control rats. Son et al. (2011) showed that meth rats were not leaving the A-O phase to enter S-R. Based on these two findings, we might expect that meth rats would have significantly higher initiation time modes than control rats. This increase could be due to one of two possible mechanisms: an impairment in response learning or an inability of rats to switch from A-O to S-R habit.

It is also possible that meth rats will be more easily distracted than control rats. This is based on the results of the Salo study in which meth-exposed humans were impaired on their performance during the Stroop attention task (Salo et al., 2007). It is predicted that meth-treated rats will have higher initiation time DevMode values because they should be more likely to have attentional lapses. In addition, it is suspected that meth rats will have significantly higher DevMode values in training conditions in which a distractor is present and/or stimulus salience is diminished, indicating lapses of attention.

Methods

Procedures carried out in this experiment were part of a graduate student's dissertation project.

Animals.

Sixteen male Sprague-Dawley Rats were obtained from Harlan, Indianapolis, USA. The rats had free access to food, but water was restricted to only 20 minutes per day. The rats did have free access to water for a period of 24 hours on the weekend. The rats weighed between 250 and 275 grams when first received. The rats lived one to a cage. Lights were on in the room for twelve hours, 7:00 am to 7:00 pm.

Drug Treatment.

There were two different treatments: eight experimental rats received four injections, with a two-hour interval between injections, of 20 mg/kg of methamphetamine per injection. The meth was dissolved in saline. The drug was injected intraperitonealy. Eight control rats received biologically inert saline injections at the same time.

Surgery.

Before undergoing surgery, rats were anaesthetized with 80 mg/kg of ketamine, 10 mg/kg of xylazine, and 0.54 mg/kg of atropine. Devices that measure temperature (Mini-mitter, model #VM-FH disc) were implanted into the abdominal cavity. To ease post-operation pain, rats were given Ketoprofen (5.0 mg/kg). The rats were finally placed into warming chambers, and monitored for stress. When the rat began to show movement, they were put back in their home cages.

Apparatus.

Rats were trained in operant chambers. There were three nose poke holes: one in the center, one on left and one on the right. Directly above the center nose poke hole was

a distractor light bulb. Above the right and left holes each, was a stimulus light. Water was dispensed, as a reward, by a dropper behind both the left and right nose poke holes. There was a house light on the back wall of the chamber. The distractor was the light directly above the center nose poke hole, and on distractor trials the light would blink on and off (1 flash/sec; 0.25 s flash duration). An infrared detector monitored nose poke holes and head entries. The chambers were enclosed into sound-attenuating boxes. Each operant chamber was 22.5 cm x 22.5 cm x 20 cm (length x width x height).

Training Sequence

In preliminary training, rats were trained to poke their nose into the center hole to start a trial, wait for a stimulus light to turn on, and make the correct response to earn a drop of water. Once the rat poked its nose into the center hole, the stimulus light above the left or right water delivery holes was illuminated. The stimulus light remained illuminated until the rat put its nose in the corresponding nose poke hole. Each trial was concluded when the rat responded to the left or the right. This was done with the chamber light off (salient condition); a rat was ready to continue to the next phase of training when he had completed 100 trials in 45 minutes, and got a minimum of 70% correct. If rats couldn't figure out the task by the second session, the researchers used hand-shaping to facilitate the task.

In the next phase of training, rats needed to hold their nose in the center nose poke hole for a variable amount of time (foreperiod) before one of the two stimulus lights turned on. The latency was variable because the researchers did not want the rats predicting when the light would turn on, as this would have an effect on their reaction

time. This value was gradually increased from 0.1 to 6 seconds. A minimum reaction time requirement was also imposed to insure that the rats responded as quickly as possible. If they failed to meet this requirement, they did not receive a reinforcer for the correct response. This criterion was was adjusted to control for individual differences. If rats responded within the criterion reaction time, the criterion reaction time was shortened. If the rat was too slow on one trial, the RT criterion was increased.

Rats reached stable performance after five consecutive trials in which the mean initiation time of the rats did not differ by more than 4%. The rats were then moved through a series of 5 training conditions. In the first training condition the house light was off, and there was no distractor present (salient, no distractor). The second training condition was that in which there was a salient stimulus, but with a distractor. The third training condition was a non-salient stimulus paired with no distractor. The fourth was non-salient with a distractor. In the fifth training condition, non-salient, no distractor, was repeated.

Dependent Variables.

Reaction time was defined as the time between the onset of the stimulus light, up until the rat broke the infrared beam by inserting its nose into the hole under one of the side stimulus lights. Reaction time was sectioned into two parts: initiation time and movement time. Initiation time was the time between the stimulus onset and the moment the infrared beam senses that the rat has removed its nose from the center nose poke hole. Movement time picks up when the animal removes its nose from the center hole and ends when the rat pokes its nose into the left or the right water hole (note that movement time

will not be reported on in this project). Initiation time was of special importance because this was supposed to be the time in which attentional lapses are likely to happen. If a rat does not respond quickly to the stimulus light the rat might have had a lapse in attention and therefore did not notice the stimulus light come on.

Central tendency and distribution skew were measured for the initiation time distribution. The measure of central tendency we used was mode, and distribution skew was estimated by the deviation from the mode (DevMode). Recall that DevMode was calculated as the mode subtracted from the mean. Thus, the effects of a neurotoxic dose of meth on the mode initiation time and the DevMode of the initiation time were the main measures of this study. The mode of the initiation times was a measure of response learning. The DevMode of the initiation time was used as a measure of lapses of attention because it measured the skew of the distribution.

Omissions were the trials during which rats took longer than 2 seconds to respond. Incorrect omissions were trials in which the rat took longer than 2 seconds to respond, and chose the wrong stimulus hole. Correct omissions were defined as trials in which the rat took longer than 2 seconds, but did end up selecting the hole under the correct stimulus light. Correct omissions can be interpreted as moment of inattention because, since the animal did successfully complete the task, the extra long pause during reaction time is most likely a lapse in attention. The extra long reaction times were also what pulled the mean up from the mode. Therefore correct omissions are closely linked to DevMode, as they are another way to view lapses in attention.

Premature initiations were moments in which the rat removed its nose from the center hole before the stimulus light appeared. Premature responses were defined as

moment when the rat removes its nose from the center hole before the stimulus appeared and goes to one of the side holes and inserts its nose.

Procedure

After the rats were received, they were quarantined for one week, at which point they underwent surgery to implant the temperature sensor. After another two weeks for the rats to recuperate, methamphetamine and saline treatments were administered in temperature controlled chambers. Temperature was monitored telemetrically during the treatment regimen to insure that the animals' core temperature did not become too high. If their temperature reached 39.5 degrees, the chamber was cooled until they fell below this temperature. After the injections, rats rested for a week, then water restriction treatment began. Rats were then trained in the operant chamber one week later, and this training period lasted for 8 weeks.

At the end of the experiment, brain tissue was assayed and monoamine depletions were measured. These depletions will not be reported in this report.

Data Analysis

There were two independent variables to this experiment. For the first factor, all rats were either subjected to meth treatment or saline treatment. The training condition was the second factor. The two groups, meth and control, were both taken through five different conditions: salient/no-distractor, salient/distractor, non-salient/no-distractor, non-salient/distractor and the non-salient/no-distractor. Comparing the meth rats to the saline rats was between subjects, but then the five conditions were analyzed within

subjects. Data was analyzed with a repeated measures ANOVA with a between subject factor. Data analysis was done with IBM SPSS Statistics 22. Both between and within subject effects were tested. When significant main or interaction effects were identified, contrasts were made.

Results

Mode.

There were no significant effects of treatment or training condition on modal initiation time. Greenhouse-Geisser values were used. See Figure 1.

Total Omissions.

There was a significant main effect of training condition [$F(4,56) = 19.472$; $p<0.01$], but no significant effect of treatment. Contrast analysis showed that training conditions 3 and 5 (both non-salient, no distractor) as well as training condition 4 (non-salient, with distractor), were significantly different than training condition 1 ($p<0.05$, $p<0.t05$, $p<0.05$). There was a significant interaction effect on total omissions between training condition and meth treatment on training conditions 3 and 5 when compared to training condition 1. See Figure 2.

Correct Omissions.

There was a significant main effect of training condition ($F(4,56)= 16.641$; $p<0.001$), but no significant effect of treatment. Contrast analysis revealed that training conditions 3, 4 and 5 were significantly different from training condition 1 ($p<0.001$,

$p<0.05$, $p<0.05$). There was no significant interaction effect between treatment and training condition. See Figure 3.

DevMode.

Although there was no main effect of treatment, there was a significant main effect of training condition ($F(4, 56)= 13.43$; $p<0.001$). Training conditions 2, 3, 4 and 5 were significantly different from Condition 1 ($p<0.05$, $p<0.001$, $p<0.001$, $P<0.01$). There was no interaction between treatment and training condition. See Figure 4.

Premature Initiations per second.

There was no significant main effect of treatment on premature initiations per second. However, there was a main effect of training condition ($F(4, 56)= 3.511$; $p<0.05$), and contrast analysis revealed a significant difference between condition 5 and condition 1 ($p< 0.05$). There was no significant interaction between treatment and training condition. See Figure 5.

Premature Responses per second.

Greenhouse-Geisser values were used. There was a main effect for training condition ($F(4,56)= 11.556$; $p<0.001$). Contrast analysis revealed that condition 3 and condition 4 were significantly different from condition 1 ($p<0.05$, $p<0.05$). See Figure 6.

Discussion

There was a main effect of training condition on premature initiations per second, premature responses per second, correct omissions, total omissions and DevMode, but

not mode. There were no main effects of drug treatment, showing that the rats were not significantly affected by the high doses of meth alone. There was a significant interaction of training condition and treatment on total omissions on the reaction time task.

Mode.

Mode remained unaffected by both training condition and treatment. This shows that neither training condition nor drug treatment affected response learning in our reaction time task.

DevMode.

As for DevMode, there was a main effect of training condition. The contrasts revealed that training conditions 2 (salient, with distractor), 3 (non-salient, no distractor), 4 (non-salient, with distractor), and 5 (non-salient, no distractor) were all significantly different from training condition 1 (salient, no distractor). This suggests that the presence of a distractor and a decrease in salience both cause lapses of attention in animals.

Total Omissions.

Total omissions were any trials in which the rat took longer than 2 seconds to complete the trial. The total omissions were affected by an interaction between methamphetamine treatment and training condition. Animals performing the reaction time task under training condition 3 or 5 (both non-salient, no distractor) were significantly impacted by meth treatment.

Premature Initiations.

Premature Initiations were also significantly affected by training condition. Training condition 5 (non-salient, no distractor) yielded significant contrast results, again, when compared to training condition 1. During training condition 5 rats had a less difficult time inhibiting themselves from responding before the stimulus actually signaled that it was time to initiate the trial. Because the difference did not occur until training condition 5, and did not occur in training condition 3 (both non-salient, no distractor), the only difference being time during training sequence, one must infer that rats were more able to inhibit responses after going through four training conditions and then returning to one of the training conditions.

Premature Responses.

When rats were in training condition 3 (non-salient, no distractor) or 4 (non-salient, with distractor), premature responses were significantly increased compared to training condition 1 (salient, no distractor).

Comparison to our Hypothesis.

Initiation Time Mode. We hypothesized that rats who had been subjected to neurotoxic methamphetamine would have significantly higher mode initiation times than the control subjects, which we interpreted to be a deficit in response learning. Our results showed no effect of either training condition or meth treatment on modal initiation time. This suggests that, in negation to the hypothesis, the rats did not suffer from a response learning deficit, due to meth treatment.

Initiation Time DevMode. It was hypothesized that high doses of meth would cause a significant increase in DevMode initiation time compared to controls, suggesting an impairment in attentional lapses. Finally, it was hypothesized that while the stimulus lights are less salient (house light is on) or there was a distractor present, the rat would have an increase in attentional lapses. It was found that meth had no main effect on DevMode, showing that meth did not cause an increase in attentional lapses. Training condition did have an effect on DevMode, showing that stimulus salience and the presence of a distractor both did have an effect on attention

Comparison to the Literature: Meth Effects.

Initiation Time Mode. The previous literature on response learning is consistent in finding a significant impairment in meth rats when compared to controls. Herring et al., using the Cincinnati Water Maze as a measure of response learning, found that high doses of meth had a significant effect on performance in the task (Herring et al., 2008). Chapman et al. also found a significant impairment in response learning using a radial arm maze task (Chapman et al., 2000). Our data were inconsistent with the findings of Chapman et al. and Herring et al. because it did not reveal any impairment in modal initiation time in meth rats when compared to control rats.

One study that used a measure with a different perspective of response learning, was Son et al.'s (2011) study. It was found that rats that had received high doses of meth were more easily deterred from responding to a stimulus when the reward was devalued, compared to controls. This led Son et al. to conclude that the meth rats were not transitioning from A-O behavior into S-R habit behavior. Our findings were again

inconsistent, as we found no effect of meth on a response learning task (initiation time mode), but Son et al.'s experiment reveals possible avenues for interpreting our data (Son et al., 2011). Using a reaction time task similar to ours, Richards et al. found a difference in response learning between meth and control rats. However, it did not occur until weeks 7-9 of training. Over the training sessions control rats steadily decreased in reaction time, as they were getting better at the task. However, meth rats remained at a fairly consistent rate. Perhaps when one views the data of the control rats over the 9 weeks of training, the decline in reaction time shows switching from A-O to S-R habit. Perhaps it takes this long for a rat to switch from A-O to S-R habit, and we didn't see a significant main effect of treatment in our study because we didn't give the rats enough time in each condition to transition into S-R habit. This prevented the meth rats from separating from the control rats. This would shed a new light on reaction time tasks, and at what time the transition from A-O to S-R response learning tends to fall. In a future study, we would allow at least 7-9 weeks per condition, in order to ensure that we give the rats enough time with each training condition to have the chance to switch from A-O to S-R habit. It is important to note, however, that the results of Herring et al. (2008) and Chapman et al. (2000) do not support this theory. Herring et al. saw differences in latencies between meth and saline rats after only a couple days, and Chapman et al. saw significant differences starting from the beginning of training. Therefore, more research is required to understand why the rats in our study were not affected like the rats of previous studies were by high doses of methamphetamine.

Total Omissions. The interaction between the meth treatment and training conditions 3 and 5 (both non-salient, no distractor), when compared to training condition

1(salient, no distractor), is difficult to interpret. This is because omissions were defined as any trial in which the rat took longer than 2 seconds to respond, which includes both correct and incorrect omissions. In training condition 1 meth rats had worse performance than the controls, but in training conditions 3 and 5 (both non-salient, no distractor), they actually performed better during the non-salient training conditions than control rats. Now we see that the effect of meth on rats was different depending on stimulus salience

Initiation Time DevMode. DevMode, our measure of attentional lapses, was not significantly different in the meth rats compared to the control rats. This finding is not consistent with Salo et al. 2007, who studied attention in human meth users. It was found that meth did cause a significant impairment in performance on the stroop task, i.e. a higher Stroop Interference (Salo et al., 2007).

Initiation Time DevMode and Omissions. One interpretation of both DevMode and omissions is that they both represent attentional lapses. In our present study, we found an interaction effect between meth and training condition on total omissions. Although there were no significant effects of meth on DevMode, the meth results for total omissions and DevMode are consistent with each other (Figure 2, Figure 4). They were consistent in the fact that in total omissions, during training conditions 3 (non-salient, no distractor), 4 (non-salient, with distractor) and 5 (non-salient, no distractor), meth rats had fewer omissions than saline rats. During training conditions 4 and 5, although the effect of meth on DevMode is not significant, one should note that there is again a trend for the meth rats to have less distribution skew under low saliency conditions. It is possible that methamphetamine actually decreases attentional lapses, which is the opposite of our

hypothesis. Similar to response learning, further research is needed to explore the possibility of neurotoxic methamphetamine decreasing attentional lapses.

Comparison to the Literature: Training Condition Effects.

Initiation Time Mode. Our experiment did not result in a significant main effect of training condition on initiation time mode. This is inconsistent with the findings of Sabol et al. (2003), in which an increase of stimulus salience resulted in a decrease in initiation time modes.

Total Omissions. There was a significant effect of training condition on total omissions. In all three of the non-salient conditions, rats were slower in comparison to their performance in condition 1 (salient, no distractor). Our finding is consistent with Sabol et al.'s (2003) study, which found a borderline ($p=0.053$) significant effect of stimulus salience on omissions.

Initiation Time DevMode. The training conditions either had salient or non-salient stimuli, and either a distractor or no distractor. When there was no distractor, but the stimulus wasn't salient (training conditions 3 & 5), rats had significantly higher initiation time DevMode than they did in training condition 1, where there was still no distractor, but the stimulus was salient. Since there was an increase in DevMode, one can suggest that decreasing the stimulus saliency increases attentional lapses. These results are consistent with Sabol et al. (2003), who found that increasing stimulus salience decreased the skew of the initiation time, DevMode.

Future Research.

In order to further explore the effects of methamphetamine on performance in a response learning and attention task, each training condition should be measured for a longer period of time. It may also be necessary to control for the age of the rats. Since all of the rats in the present experiment ran through the training conditions in the same order at the same time, perhaps randomizing this could control for the age of the rat possibly contributing to deficits. Therefore, to control for age as well as time as confounding variables, the training conditions would each need more time and the order of the training conditions should be randomized. Further research using response learning and attention tasks could discern whether the A-O/S-R habit theory applies to this response learning task, as well as the effect of meth on frequency of attentional lapses.

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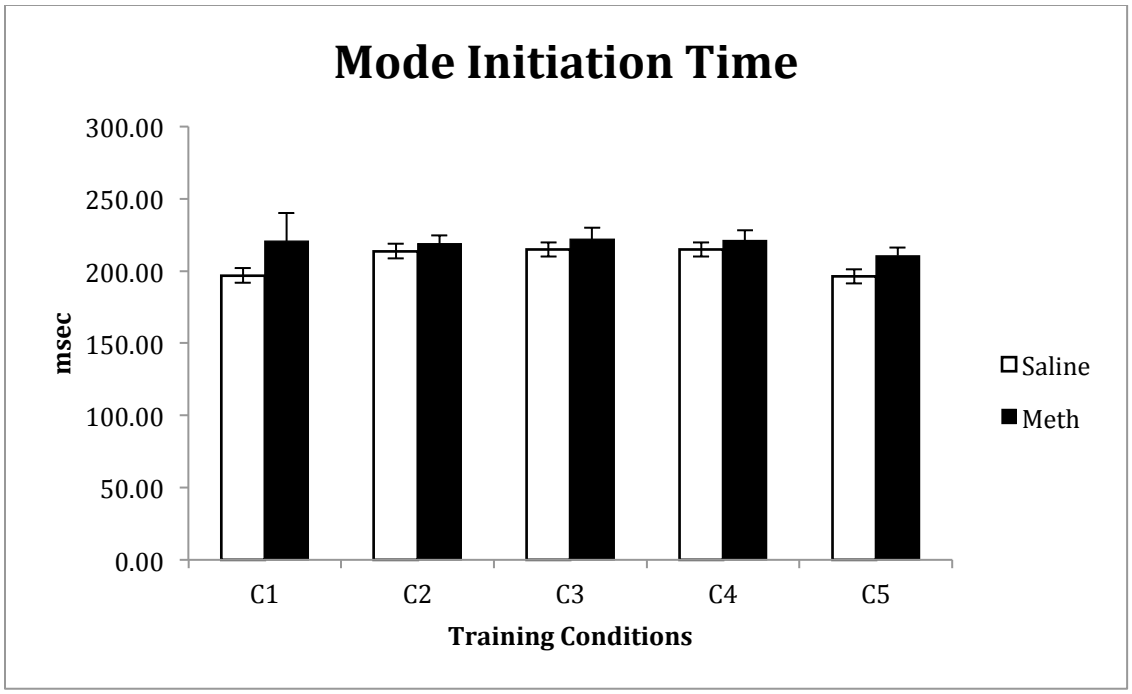


Figure 1. Values represent mean (+ Sem).

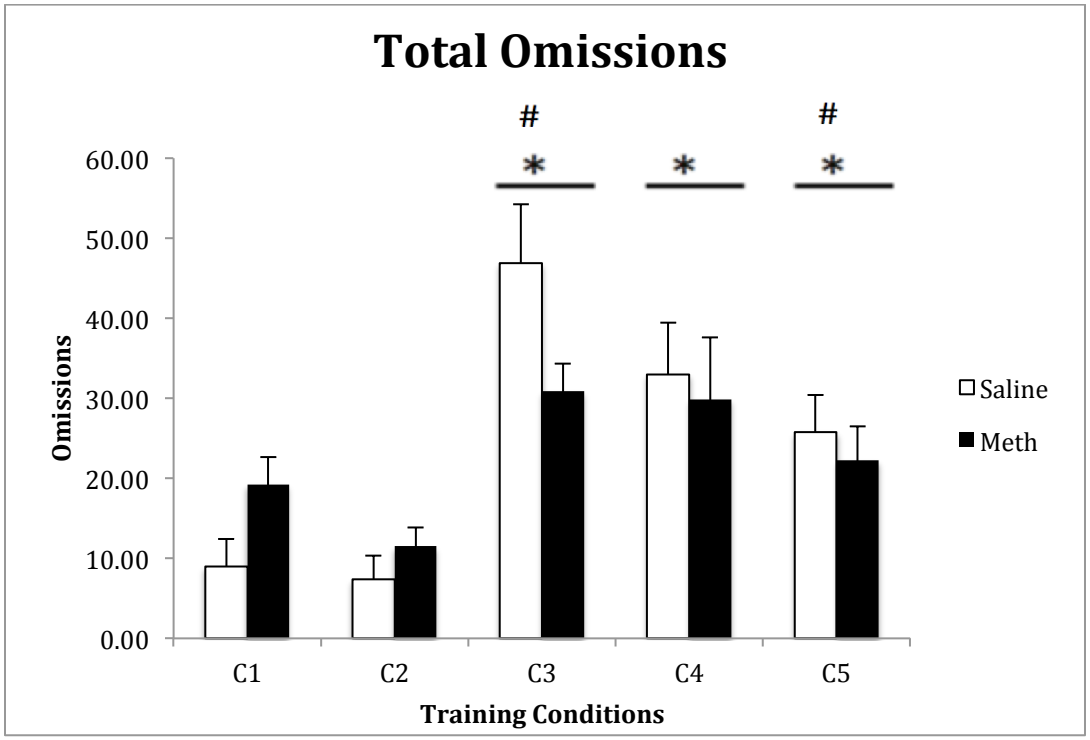
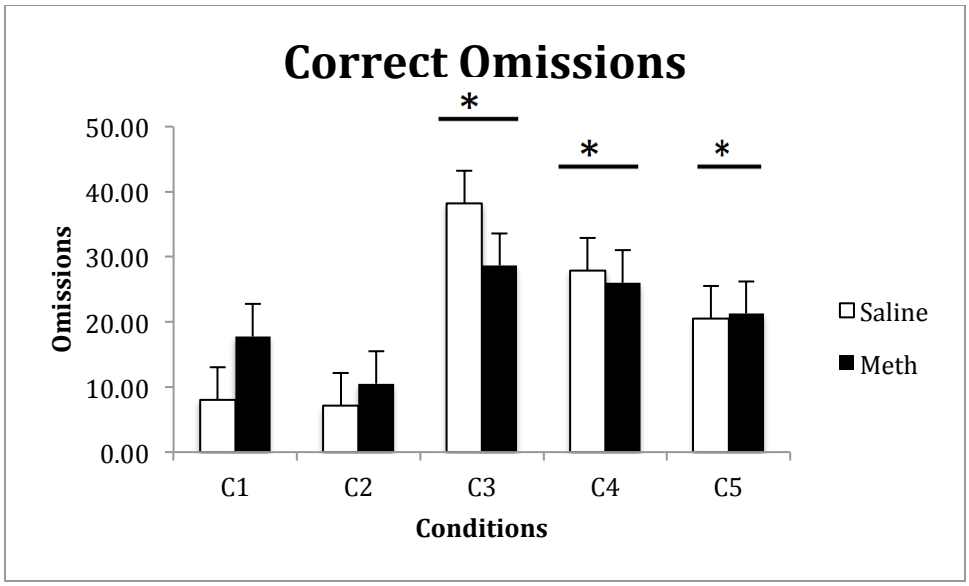


Figure 2. Values represent mean (+ Sem). * = significant difference compared to condition 1 ($p<0.05$). # = significant interaction between treatment and training condition, C1 v. C3 and C5 ($p<0.05$).



F*igure 3*. Values represent mean (+ Sem). * = significant difference compared to condition 1 ($p<0.05$).

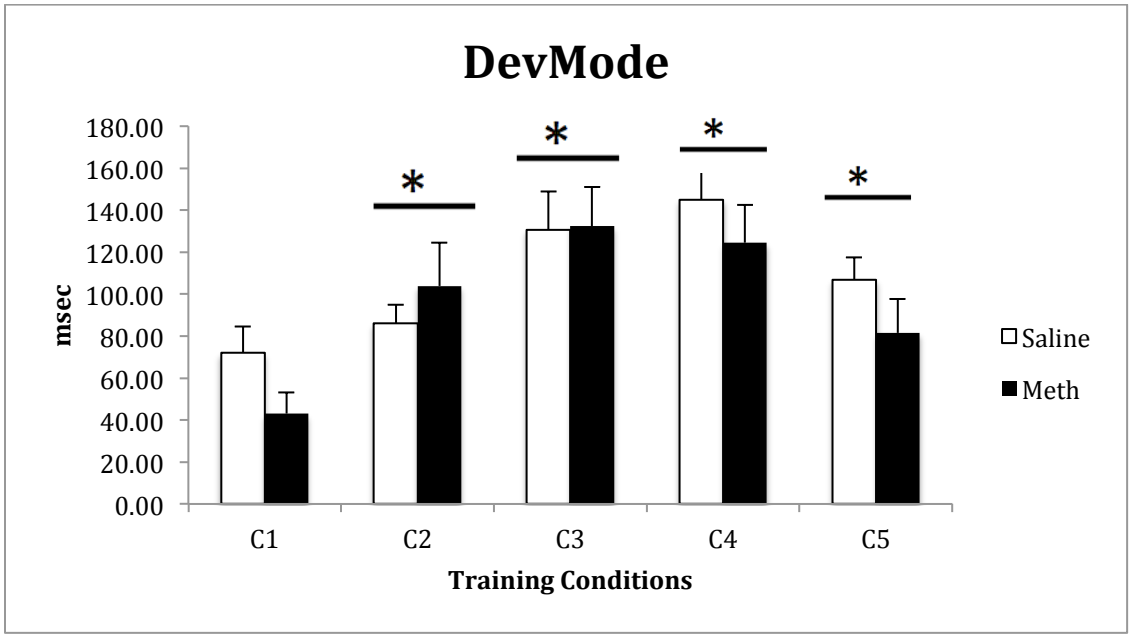


Figure 4. Values represent mean (+ Sem). * = significant difference compared to condition 1 ($p<0.05$).

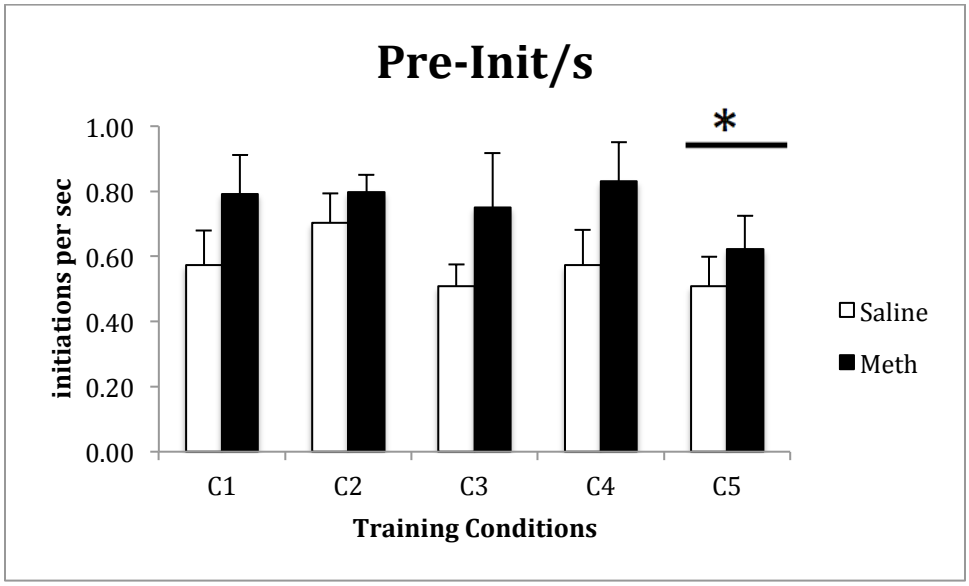


Figure 5. Values represent mean (+ Sem). * = significant difference compared to condition 1 ($p<0.05$).

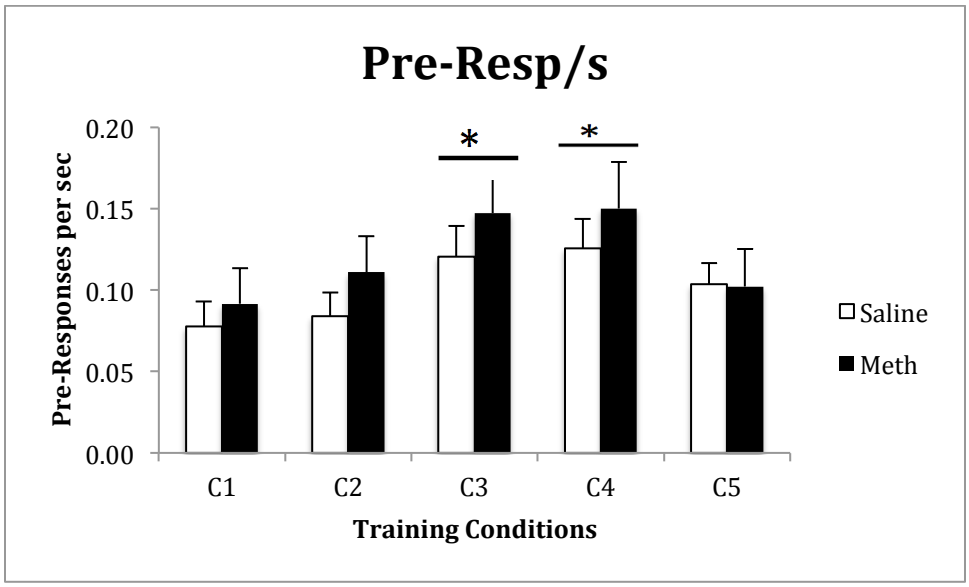


Figure 6. Greenhouse-Geisser Values used. Values represent mean (+ Sem). * = significant difference compared to condition 1 ($p<0.05$).