The Co-Use of Methamphetamine and Alcohol: Behavioral and Neurobiological Effects

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ABSTRACT

Alcohol is a drug commonly co-administered with other drugs like the psychostimulant methamphetamine. Both alcohol and methamphetamine target the mesocorticolimbic reward system of the brain through distinct mechanisms of action and the concurrent use of methamphetamine and alcohol likely has complex neurobiological consequences that could cause a variety of negative side effects. The present study serves to examine the way these two powerful drugs of abuse influence the consumption of one another. Alcohol-preferring rats or “P rats” were used as an animal model and given access to a two-bottle alcohol choice procedure in the home cage to measure alcohol consumption and preference. Methamphetamine intake was measured by training animals to self-administer methamphetamine in an operant conditioning chamber. Our study found that while alcohol consumption has no significant effect on the self-administration of methamphetamine, methamphetamine self-administration decreases the consumption and preference for alcohol. This effect was observed through all procedural variations and suggests an antagonistic effect of methamphetamine use on alcohol reward. Adenosine signaling serves as a modulator for dopamine in the reward pathway and helps to attenuate reward signaling at physiological levels. To identify a possible mechanism behind the behavioral effects we’d found, we examined the presence of adenosine signaling proteins such as ENT1 proteins and A1 receptors in animals taking methamphetamine. Our study found an increase in ENT1 protein and a decrease in A1 receptors in animals taking methamphetamine which suggests a role for adenosine signaling in the effect of methamphetamine on alcohol consumption and preference. Our experimental findings are some of the first insights into the neurobiological aspects behind the drug interaction of methamphetamine and alcohol, and are contributions to the knowledge of the chemical basis of drug addiction.
INTRODUCTION AND BACKGROUND

Polydrug use is defined by the use of two or more psychoactive drugs. Prolonged use of multiple substances can result in polysubstance abuse and dependence, which is characterized by a physical or psychological dependence on a group of substances. Polydrug use and abuse can be quite dangerous given the various negative physical and psychological effects that have been associated including decreased cognitive functioning, the development of psychiatric disorders, seizures, heart problems, liver damage, and death (Grov et al., 2009).

Alcohol is a substance that is commonly co-administered with other drugs. In 2008, 18.3% of admissions to rehab facilities were for alcohol and another drug (NIDA, 2011). In a 2006 study, the prevalence for the abuse of alcohol and prescription drugs across a year-long period was 12.1% of students at a University with 6.9% being the simultaneous ingestion of drugs and alcohol. Similarly, in 2004 the Drug Abuse Warning Network states that the majority of emergency department visits involving prescriptions like benzodiazepines and opioids also involved the use of another substance, the most frequent additional substance being alcohol (McCabe et al., 2006). More recent data from the Drug Abuse Warning Network (DAWN) report (2011) showed that 33% of emergency room visits involved both alcohol and other drugs and were substantially higher in prevalence than the 12% of emergency room visits that involved only alcohol. Another study of US college students found that users of nonmedical prescription stimulants were at least six times more likely to report frequent heavy drinking than those students who did not use nonmedical prescription stimulants (McCabe et al., 2005).

Cocaine is a popular psychostimulant that is often abused with alcohol. A survey of the general US population estimated that 5 million people had concurrently used alcohol and cocaine within the last month and 12 million had done so within the last year (Grant et al., 1990). The
popular concurrent use of cocaine and ethanol is attributed to its formation of cocaethylene, an active metabolite that produces enhanced drug effects like increased blood cocaine levels, deficits in psychomotor performance, and detriment to heart rate (Pennings et al., 2002). Cocaethylene has been implicated in increased cardiotoxic effects, and the deficits in psychomotor performance and learning caused by alcohol consumption are worsened by the addition of cocaine consumption (Pennings et al., 2002). The idea that cocaine and alcohol have synergistic effects continues to be supported. In 2014, a study found that the administration of cocaine increases alcohol seeking and relapse in laboratory animals, providing further evidence for the proposed synergistic effects that come from the simultaneous ingestion of cocaine and alcohol (Hauser et al., 2014).

There are numerous studies that examine the co-use of alcohol and cocaine, however there are a significantly smaller number that examine the co-use of alcohol and other psychostimulant drugs such as methamphetamine. Unlike cocaine, the combination of methamphetamine and alcohol is not known to produce an active metabolite, suggesting that these drugs may interact differently. According to NSDUH, in 2014 over half a million people used nonmedical methamphetamines while 130 million people used alcohol. These numbers suggest that the majority of the population abusing nonmedical methamphetamines is also co-using alcohol (SAMHSA, 2014). Despite the prevalence of this combinatorial drug use and its detriment to psychological and physical health, much of the neurobehavioral effects and neurobiological consequences remain unknown.

Drugs of abuse, including methamphetamine and alcohol, are highly addictive due to their ability to influence the brain’s reward systems. Dopamine is a catecholamine neurotransmitter that is a key player in motivation and goal-directed behavior associated with
survival behaviors such as feeding, sex and social behaviors. The majority of dopamine in the brain is derived from the mesocorticolimbic system where dopamine neurons of the ventral tegmental area (VTA) supply dopamine input to forebrain regions such as the nucleus accumbens (NAc) and prefrontal cortex (Gilpin and Koob, 2008).

The stimulation of these pathways provokes the release of dopamine in areas like the NAc, to establish incentive salience, or, a motivation associated with rewarding stimuli. Under normal physiological conditions, γ-Aminobutyric Acid (GABA) interneurons provide potent inhibition on the dopaminergic neurons of the VTA. This attenuates dopamine neuron activity and keeps the reward pathways inactive at rest. Upon the presentation of a reward or rewarding stimulus, GABA interneurons lose their inhibitory control of dopamine neurons and dopamine neurons increase their firing to induce the release of dopamine into the NAc. Dopamine stimulation in the NAc is linked to the establishment of reward learning by associating the reward with the initiating stimulus. Nearly all drugs of abuse activate the dopamine system in ways that exceed these normal patterns of activation and produce aberrant reward learning that overly incentivizes drug-related behaviors (Gilpin and Koob, 2008).

Methamphetamine enhances dopamine neurotransmission in the mesolimbic system by binding to the vesicular monoamine transporter (VMAT) that normally serves to package dopamine into synaptic vesicles for storage or release. Methamphetamine binding to the transporter induces a conformational change in VMAT causing it to reverse its natural transport direction leading to a depletion of vesicular dopamine and higher levels of cytosolic dopamine. Similarly, methamphetamine also binds to dopamine transporters (DATs) in the cell membrane that are responsible for reuptake of dopamine that has been released into the synapse. Methamphetamine also induces a conformational change allowing the reverse transport of
dopamine from the cytosol and into the synaptic cleft. VMAT, which is now pumping dopamine out of vesicles and into the cytosol, subsequently reverses the concentration gradient in the cell and supplies a driving force for dopamine to be transported into the synaptic cleft through DATs. Dopamine’s presence in the synapse continues to increase producing continuous activation of dopamine receptors on the post synaptic membrane and activating the reward-based pathways in the brain (Nickell et al., 2014).

Alcohol also enhances dopamine neurotransmission, although the mechanisms are altogether different. Alcohol administration increases the release of endorphins, natural opioid ligands that bind to opioid receptors. In the VTA, these endorphins bind to inhibitory mu opioid receptors on the GABA interneurons neurons and prevent them from inhibiting the VTA dopamine neurons. The dopaminergic neurons of the VTA are therefore disinhibited and become excited, releasing dopamine onto the neurons of the NAc. Simultaneously, the endorphins from alcohol are binding to opioid receptors on the neurons of the NAc and further activating the mesolimbic reward pathway. The activation of this mesolimbic pathway leads to an establishment of reward to the associated stimuli, in this case, alcohol (Gilpin and Koob, 2008).

Alcohol also produces its reinforcing effects through the neurotransmitter adenosine. Adenosine is a purine nucleoside that regulates the signaling of other neurotransmitters such as dopamine and glutamate. Adenosine is important in regulating mesolimbic dopamine and glutamate signaling and is thus, important in alcohol preference and sensitivity. Under physiological levels, adenosine binds to $A_1$ and $A_{2A}$ receptors on post-synaptic neurons that negatively modulate the activity of dopamine $D_1$ and $D_2$ receptors, respectively. Adenosine-dependent modulation of $D_2$ receptors attenuates the activation of the reward pathways in a manner similar to the GABAergic VTA neurons. Levels of adenosine in the synapse are
regulated by equilibrative nucleoside transporters, specifically type 1 (ENT1), which is present on astrocytes in the VTA and NAc. Acute alcohol exposure has been shown to inhibit ENT1 and leads to decreased extracellular concentrations of adenosine. The decreased presence of adenosine in the synapse reduces its ability to negatively regulate D2 receptors, whose disinhibition causes an increased excitation of the mesocorticolimbic pathways and further reward associated with the consumption of alcohol. Studies show that dopamine antagonists injected into the NAc inhibit alcohol consumption, which further supports the role of alcohol’s dopamine-targeted effects in the motivation-reward system (Gilpin and Koob, 2008).

Methamphetamine and alcohol are two powerful drugs of abuse that influence the mesolimbic dopamine system through distinct mechanisms. We sought to determine how co-use of methamphetamine and alcohol influence the consumption of one another. Based on research involving the concurrent use of alcohol and other psychostimulants, and the fact that methamphetamine and alcohol employ different mechanisms, suggests that the consumption of the two would have synergistic effects on the consumption of one or the other. However, the idea that their co-consumption has antagonistic effects on the consumption of one or the other is still a possibility as well as the idea that no interaction occurs. To identify how these drugs of abuse influence the consumption of one another, we chose to use rats as an animal model. Standard experimental rat strains generally consume little alcohol voluntarily. However, rats have been selectively bred over 100 generations to prefer alcohol, resulting in high alcohol consumption. Therefore, our studies will utilize the selective like of high alcohol preferring, P rats, to explore the interactive effects of methamphetamine self-administration and alcohol drinking.

Interestingly, our initial studies showed that P rats showed less preference for, and consumed less alcohol during the periods of methamphetamine self-administration. Given the
surprising nature of these initial findings, we performed several additional experiments with similar premises to ensure consistency of results, and found that the results held true through all of the procedural variations. To identify possible mechanisms associated with these behavioral effects, we assessed how methamphetamine self-administration alters the expression of A$_1$, A$_{2A}$, and ENT1 receptor proteins which are associated with adenosine signaling. Based on previous work, we hypothesized that methamphetamine self-administration would upregulate the adenosine transporter, ENT1, in the NAc, and decrease the expression of adenosine A$_{2A}$ receptors.

MATERIALS AND METHODS

Animals: Male alcohol-preferring rats (P rats) were acquired from Indiana University School of Medicine, Indianapolis, IN. Rats (300+ grams) were housed individually and fed with ad libitum food and water. All experiments were conducted during the light period of a 12 hr light/dark cycle and in accordance with the Guide for the Care and Use of Animals and approved the Institutional Animal Care and Use Committee at the University of Colorado Boulder.

General Procedures

Intravenous Catheter Surgery: Catheters were implanted into the jugular vein under halothane anesthesia (1–2.5%) according to previously published procedures (Kavanagh et al., 2014). Rats were allowed 3-5 days to recover in their home cage and catheters were flushed daily with 0.1 ml of 0.9% heparinized and 0.26 mg/ml gentamicin saline to ensure they retained function and were unobstructed before experimental procedures began.
**Two-Bottle Choice Alcohol Drinking Procedure:** Rats were given *ad libitum* food and access to two water bottles upon arrival. After one week of habituation alcohol drinking procedures were initiated by introducing a 10-20% alcohol bottle or second water bottle. That is, the experimental animals had a choice between a water bottle or a 10-20% alcohol bottle whereas control animals had a choice between two water bottles. The animals were exposed to this two-bottle alcohol drinking procedure for 5 consecutive days followed by 2 days of only water access to establish baseline. This cycle was repeated over the course of each experiment for a variable amount of time.

**Self-Administration and Extinction Procedures:** Self-administration procedures were conducted in operant conditioning chambers (Med-Associates, St. Albans, VT, USA) equipped with two response levers, a sucrose hopper, and an infusion pump system. Animals were trained to self-administer intravenous delivery of methamphetamine (0.1 mg/kg/100 µl injection) on a fixed-ratio 1 (FR1) schedule in daily 2-hour sessions for 5 days/week using the response lever-infusion pump system. Methamphetamine injections were delivered over 5 seconds and were synchronized with a light cue above the active lever. Drug and cue delivery were followed by a 15 second time out period, where the house light remained off and responding produced no consequence. Some experiments implemented control animals that received infusions of saline for each lever press. Over the weekend the animals had one day recovery to restore baseline. These baseline days are not included in schematics or figures.

After the period of self-administration animals went through a 24-hour withdrawal period from methamphetamine and the proceeded to the extinction period. During this period, animals were subjected to the same self-administration procedure except that the lever presses occurring
during this time were inactive and inactive lever responses produced no consequence throughout testing.

**Immunoblotting:** Bilateral 1 mm³ tissue punches were taken from chilled tissue slices and were homogenized immediately in lysis buffer. Samples were stored at -80°C until protein levels were quantified by a Lowry protein assay (BioRad, Hercules, CA). Samples (15 µg/well) from each animal were separated by SDS-PAGE and transferred by electrophoresis to PVDF membranes. Blots were run with equal numbers of control and methamphetamine SA samples per gel and loaded in an alternating fashion. The membranes were blocked overnight in 5% bovine serum albumin at 4°C and incubated in primary antibody for adenosine A₁ (1:500 in 5% BSA Calbiochem/EMD Millipore, Billerica, MA), adenosine A₂A (1:2000 in 5% BSA Millipore, Billerica, MA) receptors, and equilibrative nucleoside transporter (1:100 in 5% BSA) for 24 hours at 4°C. The membranes were washed and labeled with species-specific peroxidase-conjugated secondary (1:5-25 K; Bio-Rad) for one hour at 25°C. Following chemiluminescence detection (SuperSignal Dura West; Thermoscientific), blots were stripped for 20 minutes at room temperature (Restore, Thermo Scientific, Rockford, IL, USA) and re-probed. All blots were stripped and re-probed for the loading control protein, β-Tubulin (1:1000 in 5% BSA Millipore, Billerica, MA). Immunoreactivity was quantified by densitometry (ImageJ) under conditions linear over at least a threefold concentration range. The optical density for the proteins was normalized to β Tubulin and a percentage change from control was derived.
Experimental Designs

Experiment 1: Effect of 10% Ethanol Drinking on Methamphetamine Self-Administration

Experimental animals (n=8) drank 10% ethanol while control animals (n=8) drank water for 5 days prior to starting self-administration sessions, in which all animals (n=16) self-administered methamphetamine (0.1mg/kg/infusion). During the self-administration phase, the animals continued to drink either 10% ethanol or water. For the first six days, methamphetamine was self-administered on a fixed-ratio 1 schedule of reinforcement. For the last 5 days of self-administration, the animals were tested on a fixed-ratio 5 schedule of reinforcement. The animals then extinguished for 3 days after a 24-hr withdrawal period. Extinction sessions were administered identically to the self-administration sessions except lever responding on either the drug-paired or inactive lever had no programmed consequences.

Experiment 2: The effect of methamphetamine self-administration on 10% and 20% ethanol consumption

Experimental animals (Exp. 2a: n=19; Exp. 2b: n=9) and control animals (Exp. 2a: n=12; Exp. 2b: n=9) were subjected to the two-bottle alcohol drinking procedure (Exp. 2a: 10% alcohol; Exp. 2b 20% alcohol) for 5 days prior to starting self-administration sessions. Experimental animals were trained to self-administer methamphetamine (0.1mg/kg/infusion) on a fixed ratio 1 schedule while control animals self-administered saline. Following self-administration, animals went through 24-hours of forced withdrawal in the home cage, followed by 3 days of extinction. Animals continued to have a free choice between ethanol or water throughout the experiment.
Experiment 3: The effect of methamphetamine self-administration on extended ethanol drinking

Animals were subjected to the two-bottle alcohol procedure for 3 days with 10% ethanol and then 19 days of 20% ethanol. The animals then began the nine-day self-administration phase where experimental animals (n=14) self-administered methamphetamine (0.1mg/kg/infusion) and control animals (n=10) self-administered saline. The self-administration was on fixed-ratio 1 conditions, after which the animals went through a 24 hours withdrawal and then extinguished for 3 days.

Experiment 4: The effect of methamphetamine on subsequent ethanol consumption

First, alcohol-naive animals self-administered methamphetamine (0.1mg/kg/infusion, n=11) or saline (n=9) for 7 days. Afterwards the animals were subjected to the two-bottle alcohol procedure with 10% ethanol for eight days while continuing to self-administer. The animals extinguished for a subsequent 5 days while still having access to alcohol bottles.

Experiment 5: Expression of ENT, A2A, and A1 Receptors in the Nucleus Accumbens Core

These animals went through eight days of self-administration of either methamphetamine (n=10) or saline (n=8) and neither group consumed alcohol. After the self-administration period the animals were sacrificed and a 24-hour tissue punch was done.

Experiment 6: The effect of methamphetamine self-administration on 0.1% sucrose consumption

Experimental animals and control animals were subjected to the two-bottle drinking procedure with a 0.1% sucrose solution instead of ethanol. This occurred for 3 days prior to starting self-administration sessions, in which experimental animals (n=10) self-administered
methamphetamine infusions (0.1mg/kg/infusion) while control animals (n=10) infused saline. During the seven-day self-administration phase, the animals continued to drink either sucrose or water. The self-administration was on fixed-ratio 1 conditions, and no extinction period occurred.

Statistical Analyses:

The numbers of animals in each group ranged from 8 to 19 and are reported for each experiment in the corresponding results section. All self-administration data (dependent variables: ethanol consumption, ethanol preference, and drug infusions) were analyzed by a mixed design two-way ANOVA. Significant main effects were followed by appropriate post-hoc analyses and significant interactive effects were subsequently analyzed by simple main effects analyses and post-hoc tests.

RESULTS

Experiment 1: Effect of 10% Ethanol Drinking on Methamphetamine Self-Administration

We first examined the effects of alcohol drinking on methamphetamine self-administration. Animals were separated into control and experimental groups in which controls had access to two water bottles while experimental animals had access to a water bottle and 10% ethanol-containing bottle. Animals in both groups self-administered methamphetamine. There was no significant main effect of alcohol consumption the self-administration of methamphetamine (Figure 1B). Thus, the consumption of alcohol had no significant effect on the self-administration of methamphetamine infusions. We also explored whether alcohol consumption would influence methamphetamine seeking during extinction. Here, animals were
tested under extinction conditions where drug-paired lever responding did not result in the delivery of a methamphetamine infusion. We observed no significant effect of alcohol consumption during the extinction procedure (Figure 1C). These results suggest that methamphetamine self-administration is not dependent on alcohol drinking.

**Exp. 1 Methamphetamine Infusions and Extinction**

![Diagram](image)

**Figure 1**: Alcohol consumption had no effect on the self-administration of methamphetamine. (A) Animals consumed 10% ethanol in the two-bottle choice procedure prior to and during the self-administration procedure and during the extinction period. There was no statistical difference in (B) methamphetamine self-administration or (C) extinction responding between the alcohol-consuming group and water-consuming control group.

**Experiment 2: The effect of methamphetamine self-administration on 10% and 20% ethanol consumption**

**10% Ethanol Experiment**

To examine the effects of methamphetamine self-administration on alcohol drinking, animals were separated into control and experimental groups in which control animals self-
administered saline while experimental animals self-administered methamphetamine. All animals had access to a 10% ethanol bottle in a two-bottle choice procedure. Prior to methamphetamine self-administration, the difference in alcohol consumption and preference between the control and experimental groups was not significantly different. Figure 2 illustrates that during methamphetamine self-administration, animals that self-administered methamphetamine showed a significant decrease in alcohol consumption ($F_{1,28}=11.48, p<0.01$) and alcohol preference ($F_{1,28}=9.697, p<0.01$). This significant main effect of methamphetamine on alcohol consumption and preference suggests that self-administration of methamphetamine causes a decreased preference and intake of 10% ethanol. However; methamphetamine self-administration did not alter total fluid consumption (data not shown). There was also a significant difference in alcohol consumption, but not preference, between groups during the extinction period. This suggests that the effects of methamphetamine self-administration were persistent because they remained for several days even after the self-administration period had ended.

20% Ethanol Experiment

To examine the effects of methamphetamine self-administration on 20% alcohol drinking, animals were separated into control and experimental groups in which controls self-administered saline while experimental animals self-administered methamphetamine. All animals had access to a 20% ethanol bottle. Prior to methamphetamine self-administration, the difference in alcohol consumption and preference between the control and experimental groups was not significantly different. Figure 2 illustrates that during methamphetamine self-administration, there was a significant decrease in alcohol consumption ($F_{1,16}=6.746, p<0.05$)
and alcohol preference ($F_{1,16}=6.215$, $p<0.05$). This significant main effect of methamphetamine on alcohol consumption and preference suggests that self-administration of methamphetamine causes a decreased preference and intake of 20% ethanol. Methamphetamine self-administration did not alter total fluid consumption (data not shown). There was also a significant difference in alcohol consumption ($F_{1,16}=6.905$, $p<0.05$) and preference ($F_{1,16}=5.888$, $p<0.05$) between groups during the extinction period. This suggests that the effects of methamphetamine self-administration were persistent because they remained for several days even after the self-administration period had ended.
Figure 2: Methamphetamine Self-Administration Decreases Alcohol Drinking and Preference. Animals consumed either 10% (A) or 20% (D) ethanol in the two-bottle choice procedure prior to and during the self-administration procedure. The difference in alcohol consumption (C, F) and preference (B, E) in the methamphetamine self-administration and saline self-administration groups during the self-administration procedure was significant; methamphetamine self-administration decreases alcohol drinking and preference. The gray areas on the graphs signify the period of methamphetamine self-administration. One asterisk represents a \( p < 0.05 \) and two asterisks represents a \( p < 0.01 \).
**Experiment 3: The effect of methamphetamine self-administration on extended ethanol drinking**

This experiment examined the effects of methamphetamine self-administration on alcohol drinking after an extended period of alcohol drinking. All animals were given access to an alcohol bottle for 36 days in both the pre-methamphetamine period and the period of methamphetamine self-administration. Prior to starting methamphetamine self-administration, animals were housed with a 10% ethanol bottle available for the initial 3 days and a 20% ethanol bottle for 19 days (Figure 3A). However, during the self-administration phase of eleven days, control animals self-administered saline while experimental animals self-administered methamphetamine. As shown in Figure 3, there was a significant main effect of methamphetamine self-administration on alcohol consumption ($F_{1,20}=34.39$, $p<0.0001$) and alcohol preference ($F_{1,20}=20.64$, $p<0.001$). Thus, animals who drank alcohol for an extended period of time prior to self-administering methamphetamine had decreased alcohol consumption (Figure 3B) and alcohol preference (Figure 3C) during the period of methamphetamine self-administration. In addition, there was a significant main effect of time on alcohol preference both prior to ($F_{23,460}=38.68$, $p<0.0001$) and during the self-administration procedure ($F_{8,160}=9.597$, $p<0.0001$). Similarly, there was a significant main effect of time on alcohol consumption prior to ($F_{23,460}=33.08$, $p<0.0001$) and during the self-administration procedure ($F_{8,160}=9.781$, $p<0.0001$). However, methamphetamine self-administration had no effect on total fluid consumption.
Figure 3: Methamphetamine Self-Administration Effects Alcohol Drinking and Preference

Exp. 3 Extended EtOH Consumption and Preference

A

B

C

after an Extended Period of Alcohol Consumption. Animals consumed ethanol in the two-bottle choice procedure prior to and during the self-administration procedure (A). The difference in alcohol consumption (C) and preference (B) in the methamphetamine self-administration and saline self-administration groups during the self-administration procedure was significant; methamphetamine self-administration decreases alcohol drinking and preference after an extended period of alcohol consumption. The gray areas on the graphs signify the period of methamphetamine self-administration. Three asterisks represent a p<0.001.
Experiment 4: The effect of methamphetamine on initiation of ethanol consumption

This experiment examined the effects of methamphetamine self-administration on subsequent alcohol consumption. Experimental animals began self-administering methamphetamine while control animals self-administered saline (Figure 4A). Seven days after this self-administration phase began, all the animals were given access to alcohol bottles and consumption was measured. Animals self-administering methamphetamine had a decreased preference for initial 10% alcohol drinking ($F_{1,17}=4.664, p<0.05$) and a decreased consumption of alcohol ($F_{1,17}=6.02, p<0.05$) during the period of self-administration (Figure 4). After self-administration, however; the experimental animals showed no significant difference from the control animals in alcohol drinking or preference. Thus, these animals had a recovery of alcohol preference and consumption to the same magnitude of control animals after they stopped self-administering methamphetamine. Methamphetamine self-administration did not alter total fluid consumption (data not shown).
Figure 4: Methamphetamine Self-Administration Decreases Subsequent Alcohol Consumption. (A) Animals self-administered methamphetamine prior to and during the consumption of 10% ethanol in the two-bottle choice procedure. The difference in alcohol consumption (C) and preference (B) in the methamphetamine self-administration and saline self-administration groups during the self-administration procedure was significant; methamphetamine self-administration decreases subsequent alcohol drinking and preference. The gray areas on the graphs signify the period of methamphetamine self-administration. One asterisk represents a p<0.05.
Experiment 5: Methamphetamine-induced Alterations in the Expression of Adenosine Signaling Proteins in the Nucleus Accumbens

To identify possible mechanisms associated with the previously observed behavioral changes in alcohol consumption, we examined brain regions associated with reward systems and looked for changes in expression of adenosine signaling proteins. The protein expression of adenosine signaling proteins were quantified in the nucleus accumbens, and while expression of A1 receptors were significantly upregulated ($t_{14}=2.559$, $p<0.05$), ENT1 proteins were significantly downregulated in experimental animals ($t_{16}=3.645$, $p<0.01$). There was no significant difference in A2A receptor expression between experimental and control animals. This data suggests that methamphetamine has a role in the expression of adenosine signaling proteins.

Exp. 5 Protein Expression in the NAcc

![Bar charts showing A1, A2A, and ENT1 expression](image)

Figure 5: Methamphetamine Self-Administration Associated with an Upregulation of A1 Receptors and a Downregulation of ENT1 proteins

Experimental animals self-administered methamphetamine prior to sacrifice and tissue punching of the NAc. The expression of A1 receptors (A) was upregulated in experimental animals while the expression of ENT1 proteins (C) was downregulated, suggesting a role for methamphetamine in the expression of adenosine signaling proteins. No significant change in A2A receptor expression (B) was observed. One asterisk represents a $p<0.05$ and two asterisks represents a $p<0.01$. 
Experiment 6: The effect of methamphetamine self-administration on 0.1% sucrose consumption

To examine the effects of methamphetamine self-administration on another reward-based pathway, this experiment tested the effects of methamphetamine self-administration on sucrose consumption. Sucrose preference in all groups was high, and there were no significant changes in sucrose consumption or preference when those animals self-administered methamphetamine. Similarly, methamphetamine administrations were consistently higher than that of saline administrations. Methamphetamine self-administration did not alter total fluid consumption (data not shown).

Figure 6: Methamphetamine Self-Administration has No Effect on Sucrose Preference or Consumption. (A) Experimental animals consumed 0.1% sucrose in the two-bottle choice procedure prior to and during the self-administration procedure. The difference in sucrose consumption (C) and preference (B) in the methamphetamine self-administration and saline self-administration groups during the self-administration procedure was not significant; methamphetamine self-administration has no effect on sucrose drinking or preference.
DISCUSSION:

Methamphetamine and alcohol are powerful drugs of abuse that target the mesocorticolimbic reward system and are widely known for their addictive qualities. While there are many studies on the combined consumption of alcohol and other psychostimulants like cocaine, there is little research on the effects of concurrent use of methamphetamine and alcohol. Thus, we sought to explore the neurobiological aspects behind the drug interaction between methamphetamine and alcohol, and how the consumption of each would effect the consumption of the other. In our initial study, animals that drank water had no significant difference in methamphetamine self-administration than the animals that drank alcohol, suggesting that the consumption of alcohol has no effect on the consumption of methamphetamine. However, in our second study, animals that self-administered methamphetamine had a lower consumption of and preference for alcohol than animals who self-administered saline. This same trend was observed when animals had a phase of extended alcohol drinking and when animals were exposed to methamphetamine self-administration before the alcohol drinking period began. Together, these results suggest that the self-administration of methamphetamine has an antagonistic effect on the consumption of alcohol. To identify a potential mechanism for these effects, we looked at the expression of proteins associated with adenosine signaling within the mesocorticolimbic reward pathway. Our results showed that methamphetamine self-administration produced no change in the expression of adenosine A$_{2A}$ receptors, downregulation of ENT1 protein expression, and upregulation of adenosine A$_1$ receptor expression. These findings suggest that methamphetamine may alter adenosine signaling and the changes may relate to methamphetamine-induced decreases in consumption and preference for alcohol.
The behavioral effects of methamphetamine and alcohol co-use were quite surprising given previous work illustrating synergistic effects of alcohol co-use with other substances. For example, literature suggests that the combined consumption of cocaine and alcohol has an additive effect through the active metabolite cocaethylene (Pennings et al., 2002). It is also well documented that alcohol and nicotine are often co-used and that a large population of individuals develop co-dependence on these two drugs (SAMHSA, 2014). Similar to cocaine and alcohol co-use, studies on nicotine and alcohol co-consumption have provided evidence that their concurrent use has synergistic effects. For example, the consumption of nicotine causes the reinstatement of alcohol seeking behavior and the increase of alcohol consumption (Lê et al., 2003 & 2014). The idea that alcohol often has synergistic effects when abused with other reward-targeting drugs and the fact that methamphetamine and alcohol employ different mechanisms, suggests that the consumption of the two would also have synergistic effects on the consumption of one or the other.

There are several possible explanations for the observed decrease in alcohol consumption and preference during periods of combined alcohol and methamphetamine use. The first explanation is that methamphetamine’s action on the mesocorticolimbic reward pathway is inhibiting the reward effects associated with alcohol. Our findings of the decrease in ENT1 expression and increase in A₁ expression play well into a mechanistic hypothesis that supports this line of reasoning. Activation of A₁ receptors has been shown to decrease the affinity of D₂ receptors for their endogenous ligand, dopamine (Salim et al., 2000). The upregulation of A₁ receptors seen in our study suggests an increase in adenosine receptor signaling in the nucleus accumbens. In addition, the downregulation of ENT1, adenosine’s regulatory transporter, suggests that adenosine has an increased activity in the synapse due the decreased regulation.
The modified presence of these two adenosine signaling proteins could easily cause an increase in adenosine receptor signaling. The combination of decreased adenosine regulation through ENT1 and the increased presence of A<sub>1</sub> receptors could be causing an increased A<sub>1</sub> receptor activity and thus, decrease the affinity of D<sub>2</sub> receptors for dopamine. This decreased affinity would dampen dopamine signaling in the reward pathway and subsequently impair the reward associated with alcohol. The impairment of reward associated with alcohol consumption would cause animals to both consume less alcohol and have a lower preference for alcohol, similar to what we saw in this study.

Other researchers investigating the mechanisms behind alcohol abuse and the function of ENT1 have produced findings inconsistent with this potential explanation. In a 2004 study, ENT1 knockout mice were shown to have an increased preference and consumption of alcohol than control mice which was attributed to increased adenosine signaling (Choi et al., 2004). While the idea that adenosine signaling is increased with alcohol consumption remains consistent, our study differs in concluding the function of ENT1 in the pathway and how its expression is regulated. However, the ENT1 gene knockout used in this study are not developmentally-regulated and is a global knockout having no neuroanatomical specificity. The complete loss of this important adenosine regulator insinuates an instability in the health of ENT1 knockout mice and could explain the difference in results between our study that of the ENT1 knockout study (Choi et al., 2004).

Another plausible explanation for the methamphetamine-induced decrease in alcohol preference and consumption is that methamphetamine’s effects on the dopaminergic reward system are substantial enough to “prime” the reward system, and that smaller amounts of alcohol are sufficient to generate a rewarding experience. As previously stated, methamphetamine acts
on the reward pathway by targeting vesicular and membrane monoamine transporters to increase the presence of dopamine in synapses. In addition, methamphetamine self-administration can cause decreased function and immunoreactivity of dopamine transporters (DATs) in the striatum at least 30 days after the last self-administration session, and a decreased presence of DAT altogether (McFadden et al., 2012 and Hirth et al., 2016). Because DATs generally reuptake dopamine into surrounding cells, this decrease in DAT presence and functionality could cause an extended presence of dopamine in the neural synapses. This additional mechanism of increasing dopamine presence in the synapse combined with the latency at which these effects were seen supports the idea that the reward pathway is overexcited by methamphetamine administration. 

The methamphetamine-induced release of dopamine and the decrease in its reuptake could cause an excess of dopamine in synapses and the saturation of post-synaptic dopamine receptors. This methamphetamine “priming” allows increases in synaptic dopamine concentrations to be stimulated by consuming smaller amounts of alcohol, and this “priming” idea can be seen in nicotine and alcohol concurrent use studies as well (Lê et al., 2003). The priming of the reward system by methamphetamine would allow for rewarding effects after the consumption of smaller amounts of alcohol, and would cause animals to consume less alcohol and have a lower preference for alcohol similar to what we saw in our study.

Future studies should aim to uncover the pathways involved with methamphetamine and alcohol co-use, and to examine how methamphetamine co-use decreases alcohol consumption and preference. For example, the idea that methamphetamine administration is saturating post-synaptic dopamine receptors and priming the reward pathway is a testable hypothesis. The concentration of dopamine in the mesocorticolimbic pathway could be determined in experimental and control groups where experimental animals are administered
methamphetamine and subjected to microdialysis measures of dopamine concentrations. An experiment using western blotting could identify changes in expression of post-synaptic dopamine receptors where upregulation of these receptors onto post-synaptic membranes would suggest a neuronal compensatory mechanism for dopamine receptor saturation. In addition, future studies should examine the idea that increased adenosine signaling through changes in the expression of adenosine receptor proteins causes a decrease in dopamine receptor activity and an attenuation of alcohol’s reward. An experiment using an ENT1 antagonist and an A1 agonist could be done where the exogenous ligands mimic the changes seen in adenosine signaling after the administration of methamphetamine. Similar results in alcohol consumption and preference after the administration of the ENT1 antagonist and A1 agonist would suggest that the increase in adenosine signaling is correlated to the changes in alcohol’s reward.

Conclusions

The concurrent use of methamphetamine and alcohol is a significant problem in society that has complex neurobiological and chemical mechanisms. Our findings suggest that methamphetamine administration has an antagonistic effect on the consumption of alcohol, and that this effect could be linked to changes in molecular signaling in the dopaminergic reward pathways of the brain. The changes we observed in the expression of adenosine signaling proteins suggest that adenosine signaling is involved in the concurrent use of methamphetamine and alcohol and that is has some bearing on the antagonistic effect between the two drugs of abuse.

The experimental findings from this study and from future studies on this topic will contribute to knowledge of the chemical basis of drug addiction and the molecular interactions
that occur between drugs that are often co-abused in humans. Exploring the neurobiological aspects behind the drug interactions of methamphetamine and alcohol is important in discovering new treatments, medications, and therapies to help addicts recovering from drug abuse. Polydrug abuse is a relatively understudied problem and our findings could provide some of the first insights into the phenomena.
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