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TEMPORALLY DISTINCT IMPAIRMENTS IN COGNITIVE FUNCTION FOLLOWING A SENSITIZING REGIMEN OF METHAMPHETAMINE

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ABSTRACT

Janetsian, Sarine Sona. M.S., Purdue University, December 2013. Temporally Distinct Impairments in Cognitive Function Following a Sensitizing Regimen of Methamphetamine. Major Professor: Christopher C. Lapish.

Methamphetamine (MA) is a widely abused psychostimulant that has been shown to evoke an array of neurobiological abnormalities and cognitive deficits in humans and in rodent models (Marshall & O'Dell, 2012). Alterations in cognitive function after repeated drug use may lead to impaired decision-making, a lack of behavioral control, and ultimately the inability to abstain from drug use. Human studies have shown that alterations in neurobiology resulting from prolonged MA use may lead to a number of cognitive deficits, including impairments in executive function, learning, memory, and impulsivity. These impairments, specifically those that engage the prefrontal cortex (PFC) or hippocampus (HC), may persist or recover based on the duration of abstinence. In rodents, repeated intermittent injections of MA yield protracted changes in neurobiology and behavior, which have been shown to effectively model a number of the biological and cognitive abnormalities observed in addiction. In order to assess the temporal evolution of impaired cognitive function throughout abstinence, sensitization was first induced in rats (7 x 5.0 mg/kg MA over 14 days). MA-treated rats initially exhibited a robust increase in locomotion that

transitioned to stereotypy as the induction phase progressed. Then, the effects of MA sensitization on social interaction (SI), temporal order recognition (TOR) and novel object recognition (NOR) was assessed at one-day and 30-days post induction. No differences were observed in SI in either group or after a single injection of MA. However, an acute injection of 5.0 mg/kg of MA 30-minutes prior to testing dramatically reduced SI time. Impairments in TOR and NOR were observed in MA-treated rats after one day of abstinence, and impairments in TOR, but not NOR, were observed on day 30 of abstinence. No differences in TOR and NOR after a single injection of MA or saline were observed. These data establish that after 30 days of abstinence from a sensitizing regimen of MA, the ability to recall the temporal sequence that two stimuli were encountered was impaired and that was not attributable to impaired novelty detection. These data also suggest that at least some of the neurocognitive abnormalities caused by chronic MA administration may normalize after prolonged abstinence, since the ability to detect novelty recovered after 30 days of abstinence. These data provide compelling support that, since MA-sensitization caused temporal deficits in memory, PFC and HC function may be differentially impaired throughout the time course of abstinence.

INTRODUCTION

Prevalence

Methamphetamine (MA) is an addictive Schedule-II psychostimulant that is widely abused around the world. With the number of abusers on the rise, the United Nations Office on Drugs and Crime (2011) reports that MA and other types of amphetamines are the second most widely used illicit drugs in the world, even exceeding cocaine and opiates, with an estimate of 13.7 to 56.4 million users worldwide. The National Survey on Drug Use and Health (2010) reports that in the United States, 353,000 people aged 12 and older have used MA and 105,000 people aged 12 and older initiated MA use for the first time in 2010. In addition, 2.5% of 10^{th} graders have used MA at least once in their lifetime (United Nations Office on Drugs and Crime, 2011). It is even popular among adults; with 11% of 18-year-old high school graduates and 52% of 50-year-old individuals having reported use of amphetamine at least once (Johnson et al., 2009). In fact, the percentage of individuals being admitted to a U.S. state licensed or certified substance abuse facility for MA treatment has increased from 1% in 1992 to 5% in 2010 (TEDS, 2011).

The high potential for continued MA use and abuse may be due to the ability for MA to cause impairments in cognition and decision making, in addition to the hedonic properties of the drug and the inexpensive nature to manufacture this substance using over-the-counter ingredients (Nordahl et al., 2003). Manufacturing MA has become an epidemic, with 10,287 MA lab incidents in 2011 alone (US DEA, 2011). Also, its production has increased and prices have decreased from 2008 (U.S. Department of Justice National Drug Intelligence Center, 2011), making it more available and affordable to the population. Increased production not only leads to an increase in the number of the drug users, but the illicit trade of the drug poses a larger threat to security, society, and health (UNODC, 2011). According to the National Institute on Drug Abuse (2010), MA not only causes cognitive deficits and neurophysiological changes, but it can cause physical changes including extreme weight loss and severe dental problems, as well as anxiety, confusion, insomnia, paranoia, hallucinations, violent behavior and some changes in mood. Given the substantial number of users and manufacturers, MA continues to be a major public health concern due to its highly addictive and potentially neurotoxic nature that can cause cognitive and neurological abnormalities arising from acute or repeated use. Elucidating the neural substrates of MA-induced cognitive impairments will allow for the evaluation of recovery prognosis as well as potential pharmacological treatments that may assist in the process.

Mechanism of Action

The mechanisms of action of MA have been extensively studied and are critical to inform our understanding of the persistent changes in neurobiology, neurochemistry and cognitive impairments brought upon by repeated use of the drug. MA acts primarily as an indirect dopamine (DA) agonist (Bannon, 1987). MA is highly lipophilic and crosses the blood brain barrier (BBB) faster than other psychostimulants (Cho, 1990). It enters the DA neuron via the DA transporter (DAT) and by passive diffusion (Kajitani et al., 1989). After entering the synapse, it increases extracellular DA, serotonin (5-HT), and norepinephrine (NE) levels in several different ways. Two competing models exist with opposing views on how MA elicits increases in extracellular monoamine levels.

(1) In the exchange diffusion model (Fischer & Cho, 1979), MA acts primarily on plasma membrane monoamine uptake transporters. Specifically, extracellular MA binds to DAT where it is substituted or exchanged with intracellular DA and is transported via DAT into the cell. However, a concentration-dependent mechanism of DA release has also been suggested (Liang & Rutledge, 1982). At lower concentrations of extracellular MA, MA binds to DAT and is exchanged for cytosolic DA, which is transported along its concentration gradient via accelerated exchange diffusion, causing increases in extracellular DA concentration. At higher concentrations of extracellular MA, MA diffuses through the plasma membrane because it is highly lipophilic and accumulates inside the presynaptic terminal, where it releases DA from binding

to intracellular binding sites, including monoamine oxidase (MAO) or other transporters, causing DA to exit the cell along its concentration gradient from the intracellular field to the extracellular field via reversal of normal DAT, 5-HT transporter (SERT) and in higher doses, norepinephrine transporter (NET) function.

(2) The weak base model states that the primary site of action of amphetamines is at secretory vesicles (Sulzer et al., 1990 & 1992). Based on this model, accumulation of monoamines in synaptic vesicles found in presynaptic terminals depends on the interior-acidic pH gradient. After MA enters neurons by lipophilic diffusion and by the DAT, it penetrates the vesicles filled with monoamines via the vesicular monoamine transporter (VMAT-2) and reduces the intracellular pH gradient to more basic. Since psychostimulants are weak bases, it increases the pH of the vesicles to 5.6 from a normal of 5.5 and also leads to a pH increase in the external medium to 7.4 from a normal 7.25. MA accumulates in the vesicle and displaces monoamines from the vesicles via the VMAT-2. Since the vesicle's interior-acidic pH gradient is reduced, the buffering capacity of the vesicle is exceeded and the driving force for monoamine uptake back into the vesicle is suppressed, causing monoamine release and build-up in the cytosol, which is then released into the extracellular field via reverse transport of DAT.

Other mechanisms include (3) blocking reuptake of DA, NE, and at higher levels, 5-HT (Kokoshka et al., 1998), also resulting in increases in monoamine levels in the synapse. Lastly, (4) MA can bind to MAO in DA neurons and

prevents the degradation of DA, which leaves free DA in the presynaptic terminal, which can then be taken into extracellular fluid by reverse transport.

Although there is a compelling support for the two competing hypotheses and mechanisms of action for MA, these mechanisms are not mutually exclusive. For example, the exchange diffusion model and the weak base model both agree that MA is transported into the cell via DAT and by passive diffusion. However, it is at the primary site of action where there is disagreement between the two models. The exchange diffusion model proposes that the primary site of action for DA release is at the transporter level, whereas the weak base model suggests that it is at the secretory vesicles. However, it is possible that the release of DA from the secretory vesicles (weak base model) is necessary for DA to be released into the cytosol before DA binds to DAT (exchange diffusion model). Furthermore, both models are in agreement when they propose that MA is transported via passive diffusion and DAT, but after MA enters the cell and penetrates DA-filled vesicles that then release DA is when DA accumulates in the cytosol. After accumulation of DA, additional extracellular MA binds to DAT and DA is then substituted for MA and released into the extracellular field. In support of this speculation, Jones et al. (1998) reports that reversal of DAT and release of vesicular DA are both necessary for extracellular release of DA, although depletion of DA in the vesicle is the rate-limiting step in the extent to which DA is released. This suggests that both models are critical for the release of extracellular DA, although the weak base model may be fundamental in this process.

Although these models are crucial to our understanding of the immediate mechanisms of action of MA, it is important to consider neurotoxicity caused by heavy use, which is exemplified by increases in markers of oxidative stress and apoptosis in animal models (Yamamoto & Zhu, 1998; Cubells & Sulzer, 1994; Jayanthi et al., 2004; Nakato et al., 2011). MA-induced neurotoxicity is observed in animal models of binging, which consists of multiple injections of MA in a single day. MA can produce free radicals such as reactive oxygen species (ROS) and nitric oxide (NO), which can both result in neuronal cell death. When MA enters the cell, it enters vesicles (weak base model) and mitochondria. Then, DA and 5-HT are released from vesicles and are oxidized via MAO. Oxidative stress causes mitochondria permeability transition (MPT) pores to open, which results in an increase in calcium and an increase in glutamate release. An increase in calcium can either cause low ROS or high ROS, which results in apoptosis or necrosis, respectively (Davidson et al., 2001). An increase in glutamate can cause excitotoxicity, which can also result in neuronal cell death (Stout et al., 1998).

In conclusion, the mechanisms of action of MA described above not only pertain to animal models of MA use, but also occur in human MA users. Additionally, it is thought that acute overdose of MA in humans may result in neurotoxicity because it parallels animal binge models of MA administration (Davidson et al., 2001).

Human Studies

Previous human and rodent literature has heavily concentrated on the neurobiological and neurochemical effects induced by MA use. In humans, most of the literature encompasses techniques including Magnetic Resonance Imaging (MRI), functional MRI (fMRI), Magnetic Resonance Spectroscopy (MRS), and Positron Emission Tomography (PET) to observe tissue density, neurotransmission, neuronal deterioration, etc. (Salo et al., 2011). Cognitive function after MA use is also assessed using various behavioral assays including the Wisconsin Card Sort Test (WCST), Attentional Network Task (ANT), Stroop Task, etc.

As mentioned above, individuals who chronically abuse MA exhibit a wide array of cognitive impairments (Marshall & O'Dell, 2012). These include impaired time perception (Wittmann et al., 2007), impaired decision-making (Paulus et al., 2003), poor recall memory (Simon et al., 2000), increased impulsivity (Hoffman et al., 2006), and deficits in attention (Salo et al., 2009). Impairments in cognitive function are paralleled by a number of persistent changes in neurobiology. In human immunoreactivity studies, direct effects of MA are observed on glutamate (GLUT), DA, and 5-HT systems which point to a broad and complex array of neuroadaptive changes in the brain, including reductions in DA content and in DAT density (Wilson et al., 1996), decreases in 5-HT and tyrosine hydroxylase (TH) levels (Kish et al., 2009), and reductions in SERT density (Sekine et al., 2006).

Neurobiological Effects After Abstinence

The long-term behavioral, neurobiological and neurochemical effects of MA abuse are not static, but rather appear to change with both the duration and the period of abstinence following MA use. For example, individuals abstinent for more than six months show increased gray matter density in the right medial prefrontal cortex (mPFC) compared to individuals abstinent for shorter periods (Kim et al., 2006). Alterations in this brain region may be particularly important as it plays a central role in executive function (Habets et al., 2008). These studies suggest that there is partial recovery of grey matter density, which consists of neuronal cell bodies, glial cells, and capillaries, after prolonged abstinence. This suggests that longer periods of abstinence from MA can potentially normalize brain function. Additionally, reductions in neurotransmitter transporter expression associated with long-term MA use seem to recover with extended abstinence. Volkow et al. (2001) have shown that DAT expression is significantly increased in the caudate and putamen in individuals abstinent for nine months when compared to individuals abstinent for less than six months, suggesting remediation of this crucial neurobiological factor that controls the bioavailability of DA in the brain after prolonged abstinence. However, in the same study, motor and memory function did not seem to recover in these individuals to the same extent as did DAT levels, suggesting that recovery of DAT alone is not sufficient for full normalization of motor and memory function.

Neurochemical Effects After Abstinence

Neurochemical differences have also been observed following abstinence from MA. GLUT and glutamine levels are reduced in the frontal cortex in individuals abstinent for less than one month, with less reduction in individuals abstinent for longer periods (Ernst & Chang, 2009). In the same study, individuals with symptoms of craving had lower levels of GLUT and glutamine compared to levels of those who had no cravings. Therefore, since reductions in GLUT and glutamine are suspected to play a role in craving in rodents using self-administration models (Hasler et. al, 2007; Baker et al. 2003), the aforementioned study suggests that prolonged abstinence can potentially minimize or reduce the propensity to crave the drug and possibly relapse. Taken together, these studies show that although MA causes alterations in neurobiology and neurochemistry after repeated use, there is potential for an individual to exhibit increases in cell density and DAT availability, which play a role in crucial brain functions, after prolonged periods of abstinence. Additionally, since altered brain function such as a decrease in GLUT and glutamine levels is associated with craving and cognitive functions (Kim et al., 2006), partial or full recovery of these neuronal alterations can possibly mitigate the propensity to relapse or the cognitive deficits otherwise observed in MA-addicted individuals.

Cognitive Effects After Abstinence

Similar to findings of the neurobiological effects of MA after prolonged abstinence, a number of cognitive abnormalities persist following chronic MA use; however, some are ameliorated with time. Two studies by Salo et al. (2009 & 2011) examined alerting, orienting, and executive function in a task called the Attentional Network Task (ANT). The ANT identifies specific deficits, such as alerting, orienting, and executive function by presenting participants with a target item (>) that is surrounded by congruent (>>>>>), incongruent (<<><<), or neutral stimuli (-->--). The participants have to identify the target stimulus and these tasks measure executive control and attention shifting. The authors found differences in short-term (less than one year) vs. long-term (more than one year) abstinent individuals in the ability to ignore the distracting stimuli associated within the ANT, suggesting that individuals abstinent from MA for a shorter period of time had more profound deficits in shifting attention compared to MA users abstinent for longer periods. Other studies also support the hypothesis of partial or full recovery of cognitive abilities. Amongst these is a study by Kim et al. (2006) that examined a positive correlation between scores on the Wisconsin Card Sort Test (WCST), a test that examines the participant's ability to display behavioral flexibility when asked to shift attention to different stimuli, and grey matter density, known to be associated with executive function. Also, a study by Chang et al. (2002) shows that abstinent MA users had slower reaction times on tasks that require working memory, such as the one-back cued response and

three sequential number task, when compared to control subjects. These studies support the hypothesis that some aspects of cognition can recover after certain periods of abstinence, with the most cognitive recovery occurring after prolonged periods. Lastly, it is possible that these improvements may be associated with increases in cell density, DAT availability, and other neurobiological effects stated above.

Animal Studies

In order to assess the behavioral and cognitive abnormalities found in addiction, animal models are commonly used to control for genetic, environmental, and pharmacologic factors that may be impossible to control for in human addicts. In particular, rodents treated with MA via contingent (non-experimenter administered) or non-contingent (experimenter administered) methods are used to facilitate a better understanding of the brain regions that underlie MA addiction and to examine the effects of MA on tasks that assess cognitive domains that parallel human MA addicts. As mentioned above, animal studies have examined behavioral, neurobiological, and neurochemical changes after MA administration using a few different models. The common contingent model is self-administration of MA, which is specifically used to examine the reinforcing properties of the drug, including drug-seeking and drug-taking behavior. In this model, animals are allowed to self-administer for specific periods of time, such as one hour or 12 hours a day for up to two weeks. The self-

administration method has more face validity than the non-contingent methods because humans self-administer MA; however, both self-administration and experimenter-administered methods have construct validity because the neural circuitry underlying the mechanisms of action of MA for both methods is reliably predicted (Steketee & Kalivas, 2011).

The most common human pattern of MA use is using 1-6 times a day (Homer et al., 2008). To model this, “binge” methods consist of repeatedly injecting a rodent with MA several times within a short amount of time (Cho et al., 2000). After completing this bingeing method, which is usually meant to induce neurotoxicity, many studies have looked at transporter and neurotransmitter levels and neurobiological changes including grey and white matter density. However, both binge and chronic regimens (such as behavioral sensitization: see behavioral sensitization), mimic human patterns of addiction, with one exemplifying bouts of injections and the other resembling repeated and long-term use of the drug (Belcher et al., 2008). Although there is a substantial amount of data on neurobiological and neurochemical alterations as well as cognitive impairments induced by MA after binge administration in rodents, chronic administration experiments are not frequently employed and there is a lack of literature on how chronic administration affects neurobiology and cognition, especially in a long-term manner. Additionally, since the “binge” pattern is most common in humans, chronic intermittent MA use might precede the transition to full-blown addiction, which makes exploring chronic use very important. Recent data suggests that MA-induced cognitive impairments may be a particularly

important feature of the addicted phenotype as impairments in this domain may reflect altered neurocognitive control of behavior, thus contributing to increased probability of relapse (Tapert et al., 2004; Reichel et al., 2011). However, only a few studies have examined possible recovery of cognitive functions after varying periods of abstinence after chronic administration regimens. Therefore, by characterizing the time course of changes in cognition that occur after chronic MA, we will better understand the brain regions and neural systems that are progressively impaired by MA and how they recover.

Behavioral Sensitization

Behavioral sensitization is a useful method to examine how brain function is altered after repeated exposure to a substance. It is defined as an augmented psychomotor response with repeated administration of a pharmacological agent (Pierce & Kalivas, 1997). Sensitization yields a number of protracted changes in neurobiology and behavior, and has been shown to effectively model a number of the cognitive abnormalities found in addicted individuals (Fletcher et al., 2007). Sensitization can be separated into three phases: acute, which is the effects of the first injection of the drug, induction, which is defined as the neural events that occur immediately after the first few injections, and expression, which is defined as the long-term changes that occur after the injection (Robinson et al., 1998). However, initiation and expression can manifest during different times depending on the number of injections, the dose

of drug, the number of session, the pattern of injections, and the length of the withdrawal period from the drug (Phillips & Di Ciano, 1996).

A few methods, including conditioned place preference (CPP) or alterations in locomotor activity, are used to examine if an animal is sensitized to a drug (Steketee & Kalivas, 2011). In CPP, rats are sensitized to the drug if they spend more time on the side of the chamber that was previously paired with the drug. This behavior identifies if the rat is sensitized to the motivational effects of the drug. Moreover, to explore motor activity, rats are placed in locomotor chambers and activity is recorded for a period of time. Then, locomotion and stereotypy are measured and used as indices of behavioral sensitization. Previous research shows that administering chronic doses of MA induces locomotor sensitization in rodents, suggesting that they have increased locomotor activity to the same dose of drug throughout drug administration compared to the animals that did not receive MA (Wang et al., 2012; Lee et al., 2011). This phenomenon is reported to last at least a year (Paulson et al., 1991). With chronic treatment, MA shifts from inducing hyperactivity to stereotypy (Kuczenski et al., 2009; Fujiwara et al., 1987). Although dosing regimens differ from one study to the next, increased locomotor activity, and stereotypy (with higher doses), has been observed using various doses in rats, including 1.0, 2.0, 2.5, 3.0, 5.0, and 10.0 mg/kg (Slamberova et al. 2011; Fujiwara et al. 1987; McDaid et al. 2006; Belcher et al., 2006; Brady et al. 2003; McGuire et al., 2011).

Both increases in locomotor activity and stereotypy are observed after high doses of MA administration. It is thought that these two behaviors compete

with one another, suggesting that there might be separate neurocircuits underlying each (Joyce et al., 1984; Segal & Mandell, 1974). It is important to consider the neurocircuitry involved in behavioral sensitization to determine the different pathways that mediate the expression of these two behaviors. Previous studies show that the mPFC, hippocampus (HC), ventral tegmental area (VTA), nucleus accumbens (NAcc), ventral pallidum (VP), amygdala, laterodorsal tegmentum, and parts of the thalamus are involved in the development of sensitization (Steketee & Kalivas, 2011). According to Pierce and Kalivas (1997a), a rodent that has never received a drug normally transmits DA from the VTA to the NAcc (mesolimbic pathway) and from the VTA to the PFC (mesocortical pathway). When a rodent is sensitized to a drug, there are changes in neurocircuitry, which include increases in dopaminergic transmission in these areas. Although there are other known projection pathways that likely contribute to behavioral sensitization, the ones mentioned above are the primary pathways driving sensitization.

Studies have observed that increases in locomotor activity are predominantly due to psychostimulant-induced neuroplasticity of DA neurotransmission and DA receptors within the mesolimbic DA system. After injections of a psychostimulant, increased neurotransmission of mesolimbic DA, specifically to the ventral striatum, is observed (Koob & Volkow, 2010). Therefore, the dopaminergic projection via the mesolimbic pathway is what drives increases in locomotor activity. When inducing stereotypy, the basal ganglia (BG), which includes the caudate, putamen, and globus pallidus (GP), is

the most important mediator of the expression of stereotyped behaviors. Additionally, functions of the NAcc and olfactory tuberculum, as well as an overactive DA system, are involved in stereotyped behaviors (Nishikawa et al., 1983; Costall et al, 1977). The BG consists of parallel cortico-basal ganglia loops that give rise to the limbic (ventral striatal pathway) and associative and sensorimotor (dorsal striatal pathway) circuits. In the ventral striatal pathway, there are direct and indirect pathways that disinhibit or inhibit movement, respectively (Alexander et al., 1986; Parent & Hazrati, 1995; Wolgin et al., 2012). Furthermore, GABA neurotransmission drives these differences in movement. In the direct pathway, GABAergic inputs from the GP to the thalamus disinhibit glutamatergic neurotransmission to the motor cortex, thereby increasing thalamocortical output and disinhibiting movement. In the indirect pathway, GABAergic inputs from the GP to the thalamus inhibit glutamatergic transmission, causing a decrease in thalamocortical output and an inhibition in movement. Taken together, GABAergic transmission, specifically from the GP, is involved in stereotyped behaviors. Furthermore, the direct pathway disinhibits movement whereas the indirect pathway inhibits movement, and this can possibly be associated with the differences in the expression of stereotypy.

Although there is support that locomotor activity is driven by the mesolimbic pathway and stereotypy is driven by the BG, Kelley et al. (1975) proposes that the mesolimbic DA pathway induces locomotor activity, and stereotypy is mediated by the nigrostriatal DA pathway. Since DA neurons in the nigrostriatal pathway prevent full functioning of mesolimbic neurons, locomotor

activity and stereotypy compete with one another (Joyce and Iverson, 1984). Taken together, the hypotheses of the expression of locomotor activity and stereotypy mentioned above by Wolgin et al. and Kelley et al. provide insight into the different pathways contributing to sensitization. It is important to consider this transition to stereotypy and the underlying neurocircuitry associated with it, because these changes in neurocircuitry after chronic intermittent use may model the transitional period from recreational use to binging in humans. Described below are the neurobiological and cognitive effects that MA induces after abstinence in rodents. With the exception of the neurobiological effects stated subsequently, the studies below all employed a sensitization regimen.

Neurobiological Effects After Abstinence

Previous research suggests that the impairments of cognitive function following MA use in humans are caused by long-term or permanent changes in the neurobiology of the brain and all likely contribute to the neurophysiological changes observed in MA addicts. Many of these changes are also seen in the rodent after binge regimens, including reductions in brain DA and DAT levels in the ventral-caudate and putamen of the striatum (Chapman et al., 2001; Gross et al., 2011), and depletions in 5-HT and SERT levels in cortical, hippocampal, and amygdalar regions (Fukumara et al., 1998; Gross et al., 2011). Although few studies have observed neurobiological changes after employing sensitization regimens, a few studies have found reductions in DA and 5-HT in the PFC after

chronic MA administration (Ago et al., 2012 & 2007). It is unclear whether the cognitive deficits are due to the acute pharmacological effects of the drug, due to the neurotoxic changes brought upon by binge administration methods, due to long-term changes caused by sensitization, or if cognitive impairments can occur without the regimen causing neurotoxicity or long-term alterations in the brain. However, these changes in neurotransmission and neurobiology possibly contribute to the differences observed in cognition after repeated administration of MA in animals.

Cognitive Effects After Abstinence

Similar to MA users, animals sensitized to MA exhibit impaired cognitive functions in tasks that examine attentional set-shifting (Parsegian et al. 2011), reversal learning (Kosheleff et al., 2012), and recognition memory (Kamei et al., 2006; Belcher et al., 2008; Reichel et al., 2011). Although there are few studies to date that examine the cognitive effects of MA in rodent models after chronic use, it is important to examine each of the studies in detail since repeated use of the drug is a common pattern used in humans and understanding the brain regions that underlie the cognitive tasks where impairments are found is an active step in understanding addiction.

Set-Shifting

Set-shifting and behavioral flexibility tasks are used to identify deficits in the ability to switch from one cognitive domain to another and these tasks involve selective PFC functioning. A behavioral task commonly used in animals to examine behavioral flexibility is the attentional set-shifting task (ASST) that examines a series of cognitive functions such as working memory, procedural memory, and flexibility in performance strategy. Impairments in set-shifting is demonstrated in an animal study by Parsegian et al. (2011), who observed that after a short-access (1-hour access for 7 days) followed by a long-access (6-hour access for 14 days) period of MA self-administration, rodents were impaired in the extradimensional shift portion of an attentional set shift task, which is a domain that measures flexibility in performance strategy, and involves the functioning of the mPFC.

Novel Object Recognition (NOR)/Temporal Order Recognition (TOR)

Impairments have also been observed in recognition memory. To examine the effects on NOR, the ability to detect novelty when given a novel or a previously encountered object, a study by Kamei et al. (2006) showed that mice that received 1.0 mg/kg of MA for seven consecutive days showed no impairments in NOR one hour after the last injection, but did 24 hours after the last injection. Since NOR was intact one hour after injection, this suggests that repeated administrations of MA may impair long-term retention of an object

without necessarily affecting short-term memory. These impairments lasted up to 28 days. Additionally, Belcher et al. (2006) observed that rats that received 3.0 mg/kg of MA every other day for 10 days showed a decrease in exploratory behavior toward the novel object in comparison to control subjects, although these differences were not significant. This took place one week after the last day of drug-administration. Conversely, Clark et al. (2007) examined rats that received a progressively increasing dose of MA for 14 days, followed by four daily injections of 6.0 mg/kg every two-hours for 11 days. They showed no deficit in NOR when compared to control animals. As suggested by the previous findings, this may be due to the ability of MA exposure prior to a binge regimen to attenuate some of the neurochemical and neurophysiological consequence of binge injections, including neurotoxicity and hyperthermia, by developing tolerance to the toxic effects of the drug during extended administration of MA (Riddle et al., 2002; Segal et al., 2003).

It is important to consider the brain regions involved in recognition memory to understand the neuroanatomical alterations that are driving the deficits in novelty detection after chronic MA use. Previous research suggests that rodents receiving bilateral lesions of the HC are impaired in the ability to detect novelty (Broadbent et al., 2010). Temporal memory, the ability to sequence events in time, is also a common way to measure cognitive impairments in memory in animals (DeVito & Eichenbaum, 2011). However, there are no animal studies that have examined impairments in temporal memory after a sensitizing regimen of MA. It is known that transiently inactivating the prelimbic regions of the PFC

(Hanneson et al., 2004) may impair temporal memory, whereas administering a D1 agonist peripherally may improve the ability of rodents to sequence the order in which objects were encountered and impair the ability to detect novelty (Hotte et al., 2005). Therefore, the aforementioned studies provide support that these two tasks require the proper functioning of the HC and PFC, and both involve the DA system. Also, the impairments found in NOR in the studies by Kamei et al. (2006) and Belcher et al. (2006) suggests that there may be dysfunctions in the HC that are mediating the impairments in novelty detection in these animals after chronic MA administration.

Social Interaction (SI)

Lastly, there are currently no papers published on the effects of MA on SI after a sensitizing regimen. Humans engage in SI, the mutual actions of two or more people oriented towards one another (Rummel, 1976), on a daily basis. Impairments in SI can be seen in individuals with depression, anxiety, autism or phobias. Decreases in SI are also seen in acute and chronic human abusers of psychostimulants (Miczek & Tidey, 1989). Since social interactions are a common part of everyday life, it is important to determine if MA has an effect on SI in rodents, and if it does have an effect, then it is important to examine if these decreases in SI are persistent or if they recover after abstinence.

Regardless of the cause of these disturbances, whether or not they are due to social anxiety, depression, or autism, a decrease in SI may be due to

disruptions of different neuroanatomical sites, including the cerebral cortex, limbic system, and cerebellum (Adolphs et al., 2002; Bachevalier & Malkova, 2006; Baron-Cohen et al., 2000). In animals, the efferent and afferent connections from the amygdala in the limbic system to the orbito-frontal cortex (OFC) and the mPFC are known to be the key substrates involved in social behaviors (Truitt et al., 2007). Therefore, impairments seen in SI in rats after MA administration may be due to disruptions of efferent and afferent connections between the amygdala and OFC and mPFC, although none of the above studies have examined these disruptions. Looking at SI in rodents after abstinence is crucial since it is speculated that the changes in social functioning that remain after abstinence in humans may be due to the long-term or irreversible structural changes that occur in the brain following MA use (Homer et al., 2008), and as mentioned above, these changes may be due to disruptions in the limbic system, cerebral cortex, and cerebellum.

In conclusion, these findings in animal literature suggest that based on the dose, administration regimen, and period of abstinence from the drug, there may be different results when examining set-shifting and NOR. The next step is to observe if there are impairments in temporal memory and SI after a sensitizing regimen of MA to further explore the underlying substrates that are altered after MA use.

Statement of Purpose

MA is a dangerous drug that is highly prevalent and its use is on the rise, however, not much is known about the chronic effects of MA use, especially after early and prolonged abstinence. In these sets of studies, I first developed a regimen of MA that lead to robust sensitization. I hypothesized that chronically injecting 5.0 mg/kg of MA every other day for 14 days would generate an initial increase in locomotor activity transitioning into stereotypy as the sensitization regimen progresses. Secondly, I hypothesized that rats receiving 5.0 mg/kg of MA after 40 days of abstinence will have high stereotypy scores comparable to the scores on the last day of injection, which will suggest that rats are sensitized. After sensitization, I employed a set of behavioral and cognitive tasks that examined different domains of PFC and HC function after varying periods of abstinence to determine if there are MA-induced impairments in cognition. These tasks included TOR, NOR, and SI. TOR and NOR were selected as indices of PFC and HC mediated cognitive function, respectively. The SI task allowed the observation of social withdrawal that is also observed in human MA addiction. These tasks were chosen because, collectively, similar tasks that are mediated by the same brain regions are used to examine impairments across a range of behavioral and neurocognitive domains observed in human MA users (Kalechstein et al., 2003). I hypothesized that rats will have decreased SI after short and prolonged abstinence. Also, an acute injection of 5.0 mg/kg of MA 30-minutes prior to experimentation will decrease SI in drug naïve animals.

Additionally, rats will be impaired in TOR and NOR after short and prolonged abstinence, suggesting that MA sensitization possibly causes impairments in PFC, HC, and perirhinal cortex functioning which persists up to 30 days. Lastly, these cognitive impairments will not be present after a single injection of MA in a group of drug naïve animals. By employing these tasks after varying periods of abstinence, I was able to determine if there were any type of recovery in cognition or if these impairments persisted chronically in these rodents and possibly, for MA addicts. Lastly, based on the cognitive impairments observed after employing cognitive tasks mediated by the PFC or HC, there is support that these regions, along with the DA system, may be differentially impaired throughout the time course of abstinence due to the impairments seen while undergoing these cognitive tasks.

METHODS

Subjects

110 adult male (PND 63) Sprague-Dawley rats were purchased from Harlan Research Laboratories (Indianapolis, IN). At the time of arrival, animals weighed approximately 250-300 g. Since the SI task required two animals to interact with one another, animals were individually housed in polycarbonate cages (10”x18”x8”) throughout all experimentation to control for the effects that MA has on interacting with another rodent. These cages were filled with pine chips bedding and were kept in a climate-controlled room. Housing was maintained on a reverse 12:12 light/dark schedule (0700-1900 hours) so that testing occurred during the normal active (dark phase) of the awake/sleep cycle. Animals had ad libitum access to food and water throughout the experiments. Animals were handled one week for approximately 10-minutes per day to lessen the possibility of anxiety and to familiarize animals to human contact before all experiments. All experimentation was carried in accordance with Indiana University-Purdue University Indianapolis School of Science IACUC.

Drug Treatments

On the days of injection, rats were administered either Methamphetamine Hydrochloride (MA) or 0.9% saline solution (MA; Sigma Chemical Co., St Louis, Mo., USA) via intraperitoneal (i.p.) injection. MA solution was prepared on each day of experimentation. A slightly higher dose (5.0 mg/kg) of MA hydrochloride than Belcher et al. (2006) was used in this study to strengthen the probability of creating cognitive impairments. Lastly, although Brady et al. (2005) administered 5.0 mg/kg of MA for five days and observed sensitization, more injections of MA was administered and for a longer period of time (two weeks) in these sets of experiments to generate robust sensitization.

Behavioral Measures

Methamphetamine Behavioral Sensitization

In Experiment 1, the induction of behavioral sensitization began one week after the animals arrived. Animals were administered either 5.0 mg/kg of MA (n = 20) or 0.9% saline solution (n = 18) via i.p. injection every other day for 13 days (5.0 mg/kg x 7/13 days; induction phase). This regimen was chosen based on other studies that have shown that an every other day dosing regimen overcomes the neurotoxicity and long-term monoamine terminal deficits in the brain otherwise caused by binge regimens (Kuczenski & Leith, 1981; Nishikawa et al., 1983). Additionally, 13 days was chosen based on two studies that

observed an increase in locomotor activity and stereotypy after administering 4.0 mg/kg (Ujike et al., 1989) or 6.0 mg/kg (Nishikawa et al., 1983) of MA for 13 days. On the days of injection (days 1, 3, 5, 7, 9, 11, 13), animals were first acclimated to the open circular locomotor chambers (21.5” diameter x 16.5” H) for 60 minutes (acclimation). Animals were then injected with either MA or saline and placed back into the chamber for 60 minutes (testing). One week following the last day of induction, animals were given an injection of MA (5.0 mg/kg) or saline to verify sensitization (Figure 2). After all cognitive testing, rats received counter balanced MA (0.5 mg/kg, n = 12; 5.0 mg/kg, n = 8) or saline (n = 18) on two consecutive days (MA Challenge). Either 0.5 mg/kg or 5.0 mg/kg of MA was administered to examine whether or not rats would exhibit locomotor activity to the lower dose or stereotypy to the higher dose. On all days of injection, locomotor activity was recorded (ANY-maze, Wood Dale, IL) via video camera mounted above the chambers and the distance travelled (meters) was collected. Additionally, two observers manually scored stereotypy using a stereotypy rating scale adopted from Ellinwood Jr. & Balster (1974).

Social Interaction (SI)

In Experiment 2, to examine the effects of MA on SI, rats were tested after one day (MA, n = 10; Saline, n = 8; Partner, n = 7) and 30 days (MA, n = 10; Saline, n = 9; Partner, n = 6) of abstinence after the induction phase described in Experiment 1. Two days before the experiment, all animals were individually

habituated in the open field chamber for 10 minutes. The chamber was illuminated with a red light similar to the light in their home cage during their dark cycle. SI was assessed using partner rats that were age, gender, and weight matched. Each partner rat was also was used in no more than three test sessions and had at least 40 minutes between running each session. On testing day, one partner rat was placed in the open field chamber followed by the experimental rat. The two animals were left in the open field chamber for 10 minutes and interaction was recorded using ANYmaze via a video camera mounted above the chamber. After 10 minutes of recording, animals were placed back into their home cages. Each video was manually scored using the methods of Truitt et al. (2007). To assess test validity on SI, naïve adult Sprague-Dawley rats (n=22; received on PND 90) were used as a positive control to ensure that an effect would also be produced with the 5.0 mg/kg dose of MA, as seen with the 0.5 mg/kg-1.5 mg/kg doses in the studies by Šlamberová et al. (2010 & 2011). Rats were handled for 10 minutes each day for one week. Two days before the experiment, animals were individually habituated in the chambers as described above. On testing day, rats were injected once with MA (n = 8) or 0.9% saline solution (n = 8). Six of the remaining rats were used as partner animals and paired with an experimental rat based on weight. Thirty-minutes after the injection, SI was assessed as described above.

Temporal Order Recognition (TOR)

In Experiment 3, rats were tested for temporal order memory (the ability for animals to remember the order of objects in which they had experience)- one day (MA, n = 8; Saline, n = 7) or 30 days (MA, n = 10; Saline, n = 9) following the induction phase in the same open field chamber used in the SI experiments. Velcro was applied on two opposite sides of the chamber, approximately 6.5 inches from the corner of the box. The objects included two rubber ducks (3.2" x 2.5"), two Rubik's cubes (2.3" x 2.3"x 2.3"), and two circular open white cups (3" x 1.8"). The order that animals experienced the objects was randomized to control for differences in object effects. On testing day, animals were placed in the open-field chamber for 4 minutes and were able to explore two identical objects. After a 1-hour inter-trial interval, they were placed in the chamber with two identical objects different than the ones experienced previously and rats were able to explore for another 4 minutes. After a 45-minute inter-trial interval, rats were exposed to two objects, one from each previous trial for another 4 minutes. The amount of time (seconds) that an animal interacted with an object was recorded.

Novel Object Recognition (NOR)

Immediately after TOR, recognition memory (the ability for animals to discriminate between novel and familiar objects) was examined. Testing took place in the same open-field chamber as the previous experiment after a 45-minute inter-trial interval. Animals were placed in the chamber with the last object

encountered along with a novel object, and then were aloud to explore for four minutes. The amount of time each object was explored (in seconds) was also examined as described above (See Temporal Order Recognition). To examine whether or not the impairments seen after one day of abstinence were due to the sensitizing regimen, a naïve group of rats were injected with 5.0 mg/kg of MA (n = 8) or 0.9% saline solution (n = 11) and locomotor activity was recorded 60 minutes pre- and post-injection. The next day, TOR and NOR were observed. If rats that received an acute injection of MA had impairments in TOR and NOR, this would suggest that an injection of MA one day before testing, whether it being the last day of the induction phase or an acute injection, may contribute to the impairments seen in the two cognitive tasks.

Euthanasia

Animals were properly sacrificed in accordance with NIH guidelines and methods approved by the IUPUI School of Science IACUC. After animals were done with experimentation, they were individually placed in CO_2 chambers for approximately 10-minutes while still in their cages. After 10 minutes, animals were removed and reflexes (including toe/tail pinch and shallow breathing) were examined. Once animals were dead due to the CO_2, they were decapitated using a guillotine to physically ensure death. They were then transferred into the freezer. The methods are well established and shown to be painless and quick, causing little or no discomfort to the animals.

Analyses

For Experiment 1, the distance travelled (meters) and counts of stereotypy were analyzed using repeated-measures ANOVA with treatment as a between-subjects factor and days as the within-subjects factor. For stereotypy, the first minute of every five- minute bin was collected and averaged between the two scorers (12 bins for each day). Then, the average of the 12 bins for each animal was calculated. Lastly, the stereotypy score for that day was calculated by taking the average score for each animal on that day. Repeated-measures ANOVA was used to examine differences between treatment groups at 5-minute bins on Day 1 vs. Day 21. A two-factor ANOVA was used to examine locomotor and stereotypy data from the MA challenge experiment. In Experiment 2, SI was analyzed using unpaired t-tests comparing the MA-treated groups with the saline-treated groups at the three different time points (1-day abstinence, 30-day abstinence, acute injection). In order to analyze the data for TOR and NOR (Experiment 3), total time spent with each object was examined using a two-factor ANOVA. Bonferroni post-hoc comparisons or Tukey's LSD were used to examine sub-group differences when applicable.

RESULTS

Experiment 1: Methamphetamine Behavioral Sensitization

Intermittent treatment with MA significantly increased locomotor activity post-injection compared to saline-treated rats (main effect of treatment ($F_{(1, 238)}$ = 15.94, $p < 0.0005$) and day ($F_{(7,238)}$ = 5.853, $p < 0.0001$), as well as a treatment by day interaction, ($F_{(7,238)}$ = 3.176, $p < 0.005$). Bonferroni post-hoc analyses revealed significant differences in locomotor activity on days 1, 3, 5, and 9 in saline versus MA treated animals (Figure 3A). However, as the induction phase progressed, MA-treated rats exhibited a decrease in locomotor activity and an increase in stereotypy (Figure 3B), with a main effect of treatment ($F_{(1, 138)}$ = 1170, $p < 0.0001$) and day ($F_{(7,138)}$ = 4.53, $p = 0.0001$), and a treatment by day interaction ($F_{(7,138)}$ = 5.282, $p < 0.001$). Bonferroni post-hoc analyses revealed significant differences in stereotypy in saline versus MA treated animals on all days of the induction phase. Additionally, Bonferroni post-hoc comparisons also revealed significant increases in stereotypy on Days 7, 9, 11, 13, and 21 compared to stereotypy on Day 1 in the MA-treated rats.

When examining locomotor activity in 5-minute bins on Day 1 of treatment, rats that received MA significantly increased locomotor activity compared to saline-treated rats (main effect of treatment, $F_{(1,110)} = 7.431$, $p < 0.05$). Bonferroni post-hoc analyses revealed significant differences in locomotor activity in saline versus MA during the 45-minute bin (Figure 4A). When examining 5-minute bins on Day 21 of treatment, MA rats exhibited an initial increase in locomotor activity that transitioned into a decrease in locomotor activity compared to saline-treated rats (time by treatment interaction, $F_{(11,110)} = 2.815$, $p < 0.005$). Bonferroni post-hoc analyses revealed significant differences between MA and saline only during the first time point at 5 minutes (Figure 4B). Lastly, when comparing locomotor activity in MA-treated rats on Day 1 and Day 21, MA-treated rats showed a significant increase in locomotor activity on Day 1 compared to the same rats on Day 21 (time by day interaction, $F_{(11,110)} = 2.998$, $p < 0.005$ (Figure 4C). Bonferroni post-hoc analyses revealed significant differences in locomotor activity between Day 1 and Day 21 during the 40, 45, 50, 55, and 60-minute time points.

Figure 5 shows the path of the animal for a saline-treated animal (top) and a MA-treated animal (bottom) on day 1 (left) and day 21 (right) of the induction phase. It seems that the MA-treated animal had robust locomotor activity initially on day 1, but after the injection on day 21, locomotor activity substantially subsided. The saline animal remained similarly activity on day 1 and day 21 of the induction phase. Although not shown, this suggests that the decrease in

locomotor activity depicted by the activity path in the MA animal on day 21 may be due to the fact that the animal is exhibiting stereotypy.

Approximately 30 days after the induction phase, rats received counterbalanced injections of MA (0.5 mg/kg or 5.0 mg/kg) or saline. Following 0.5 mg/kg of MA, both chronic saline-treated and chronic MA-treated rats showed an increase in locomotor activity compared to rats that received saline (Figure 6A), (main effect of treatment, $F_{(1,42)} = 25.67$, $p < 0.0001$). Bonferroni post-hoc analyses revealed significant differences in injection for chronic MA-treated ($p < 0.001$) or saline-treated ($p < 0.05$). There were no differences in stereotypy in either of the treatment groups when given this dose (Figure 6C). When rats received 5.0 mg/kg of MA, there was a significant increase in locomotor activity only in the chronic saline- treated group that received MA, with a main effect of treatment ($F_{(1,26)} = 8.845$, $p < 0.05$), main effect of acute injection ($F_{(1,26)} = 11.65$, $p < 0.005$), and a treatment by injection interaction ($F_{(1,26)} = 8.993$, $p < 0.05$). Bonferroni post-hoc analyses revealed significant differences in injection for chronic saline-treated ($p < 0.001$) group (Figure 6B). Significant differences were also observed when examining stereotypy in both treatment groups, (main effect of treatment, $F_{(1,24)} = 46.39$, $p < 0.0001$ and injection ($F_{(1,24)} = 8.682$, $p < 0.05$). Bonferroni post-hoc analyses revealed significant differences in injection for chronic MA-treated ($p < 0.001$) and chronic saline-treated ($p < 0.001$) rats (Figure 6D).

Experiment 2: Social Interaction

No significant differences in SI were observed between MA- and saline-treated rats after one day (Figure 7A) or 30 days (Figure 7B) of abstinence following the induction of behavioral sensitization. With acute treatment, rats receiving an injection of MA (5.0 mg/kg) exhibited a significant decrease in the amount of SI time compared to the rats that received a single injection of saline (Figure 7C, unpaired t-test, $t(14) = 7.649$, $p < 0.0001$).

Experiment 3: Temporal Order and Novel Object Recognition

One-Day Abstinence

In Experiment 3, MA-treated, but not saline-treated rats were significantly impaired in TOR main effect of order, $F_{(1,26)} = 4.443$, $p < 0.05$) (Figure 8A), and in NOR (main effect of order, $F(1,32) = 4.920$, $p < 0.05$) (Figure 9A), and a treatment by order interaction, ($F_{(1,32)} = 5.878$, $p < 0.05$) after one day of abstinence. This suggests that MA-treated rats were not able to sequence events in time and were not able to detect novelty after one day of abstinence from MA. Tukey's LSD revealed significant differences in order in the saline-treated group during TOR, $t(6) = 6.094$, $p = 0.0004$) and during NOR, $t(7) = 3.272$, $p = 0.0068$. This suggests that there was only a significant difference in the saline-treated animals in the amount they spent with one object over the other.

Thirty-Day Abstinence

After 30 days of abstinence, MA-treated, but not saline-treated rats, were significantly impaired in TOR (main effect of order, $F_{(1,34)} = 10.03$, $p < 0.005$) (Figure 8B). One-tailed, paired t-tests revealed an order effect in the saline-treated group, $t(8) = 4.507$, $p = 0.001$. This indicates that saline-treated rats preferred the older object significantly more than the recent object. Since this effect was not seen in MA-treated animals, it suggests that chronic MA animals still have impairments in TOR after extended abstinence. Both MA- and saline-treated rats exhibited NOR (Figure 9B) after 30 days of abstinence (main effect of order, $F_{(1,34)} = 22.33$, $p < 0.0001$). Tukey's LSD revealed significant differences in the order of the object in both the saline, $t(8) = 2.716$, $p = 0.0132$and MA, $t(9) = 3.738$, $p = 0.0023$.

Single Injection

One day after a single injection of saline or MA, an increase in time spent exploring the older object relative to the recent was observed in both treatment groups, indicating that rats receiving either a saline or MA injection exhibited TOR (main effect of order, $F_{(1,34)} = 14.04$, $p < 0.005$) (Figure 10A). When examining NOR, both groups spent more time with the novel object versus the recent object, (main effect of order, $F_{(1,34)} = 6.60$, $p < 0.05$) (Figure 10B). Lastly, a main effect of treatment ($F_{(1,34)}= 7.256$, $p < 0.05$) was observed in the NOR

experiments indicating that while a bias in exploring the novel object existed, the MA animals spent less time exploring either object overall.

DISCUSSION

The goal of this study was to first induce behavioral sensitization in rats via repeated intermittent injections of MA, and then determine whether or not chronic MA administration caused temporally distinct impairments in cognitive function after short-term and long-term abstinence. In the behavioral sensitization studies, there was an initial increase in locomotor activity that eventually transitioned into stereotypy. An examination of the short-term impairments in cognition revealed that rats were impaired in temporal sequencing and novelty detection. Rats were still impaired in temporal sequencing, but not novelty detection, after extended abstinence. The impairments seen after short-term abstinence were not due to the acute effects of MA, but due to the sensitizing regimen and possibly the long-term neuronal changes caused by chronic intermittent injections of the drug. When examining SI, there were no differences between saline-treated and MA-treated rats after short or extended abstinence, but there were significant decreases in SI in the MA-treated rats after an acute injection of MA 30 minutes before the SI task. Lastly, rats were still sensitized to the drug ~40 days after the end of the induction phase, which supports that the

impairments seen in temporal memory after extended abstinence may be due to the long-term and persistent effects of MA.

Locomotor Activity and Stereotypy During the Induction Phase

The hyperlocomotion observed in this study is similar to what others have observed after varying doses of peripherally delivered MA. However, studies that used lower doses have only seen increased locomotor activity, but have not reported increases in stereotypy. For example, in a study by Zakharova et al. (2009), rats that received 0.5 mg/kg once daily for 5 days showed an increase in locomotor activity compared to saline rats, but rats that received 0.125 mg/kg of MA showed locomotion similar to those that received saline. This suggests that a slightly higher dose of MA increased locomotor activity in the rats chronically treated with MA. Hyperlocomotion is also observed when administering moderately higher doses of MA. For example, rats that received 1.0 mg/kg of MA every day for 10-14 days showed elevated locomotor activity as the injections progressed (Wang et al. 2012; Meyer et al., 2011; Futamura et al., 2011). There were no reports of stereotypy in any of the studies. Taken together, these studies suggest a dose-dependent manner in which locomotor activity is expressed. When looking at the locomotor data in the experiments that I have conducted, MA induces locomotor activity initially, but unlike the aforementioned studies, transitions into stereotypy. The differences between our studies may be due to differences in the length of drug administration and dose administered. When

considering the experiments below that examine locomotor activity and stereotypy, it is apparent that the two behaviors begin to compete when MA is administered at higher doses. It is important to assess how various doses differentially affect locomotor activity and stereotypy to reach a better understanding of what is driving decreased locomotor activity and increased stereotypy in the experiments that I have conducted.

Moderate to higher doses of MA induce stereotypy. This is apparent in a study by Ujike et al. (1989) who observed progressively augmented locomotor activity and stereotypy in rats that received 4.0 mg/kg of MA for 14 days. To examine what was driving stereotypy, rats were administered a selective D_1 or D_2 antagonist, which were both shown to reverse the effects of stereotypy while inducing hyperlocomotion, suggesting that the DA system is crucial to induce stereotypy. This further supports the hypothesis that the nigrostriatal pathway may be involved in stereotypy and the mesolimbic pathway may be involved in the expression of locomotor activity, since D_1 receptors are most abundantly expressed in the nigrostriatal pathway (Neve et al., 2004), and antagonizing the receptors in this region may decrease basal ganglia functioning, which includes motor control and stereotypy.

Studies have also shown differences in the onset of stereotypy within a sensitizing regimen when administering 4.0 mg/kg of MA for 7 days (Szumlinski et al., 2000) or 6.0 mg/kg of MA for 14 days (Nishikawa et al., 1983) to rats. When going back to Figure 4C, rats that received MA on day 21 have a significant decrease of locomotor activity after 5-minutes compared to locomotor

activity on day 1. This is when stereotypy possibly dominates locomotor activity, suggesting a shorter onset of latency of stereotypy compared to day 1 of injections. Lastly, Tsukamato et al. (2001) observed a dose response function when rats exhibited locomotor activity or stereotypy. In this study, rats that received 2.0 mg/kg of MA showed the highest locomotor activity compared to saline or to rats that received 1.0 mg/kg, 4.0 mg/kg, or 8.0 mg/kg of MA. When examining stereotypy, rats that received 8.0 mg/kg of MA showed the highest stereotypy scores. These scores decreased with lower doses. These data clearly support that the differences observed (locomotor activity vs. stereotypy) in the aforementioned studies are due to the difference in doses between the studies. For example, the higher doses of MA induce stereotypy, a phenomena that is not seen in the lower doses.

Locomotor Activity and Stereotypy During the MA Challenge

Similar results have been observed in response to a MA challenge. For example, rats that received 3.0 mg/kg of MA every 3 days for a total of 5 injections, followed by a challenge dose of 0.5 mg/kg, had increased locomotor activity compared to chronic saline-treated rats that received 0.5 mg/kg of MA on the challenge day (Yang et al., 2006). Additionally, Brady et al. (2005) administered 5.0 mg/kg of MA every day for 5 days followed by 0.5 mg/kg of MA as a challenge dose, and observed that the MA-sensitized rats had increased locomotor activity compared to the chronic saline-treated rats that received a MA

challenge dose. This was also observed in the experiments that I conducted. Chronic MA rats that received 0.5 mg/kg of MA during the MA challenge had higher locomotor activity compared to the chronic saline-treated rats that received 0.5 mg/kg of MA. However, when administered a higher 5.0 mg/kg challenge dose, the MA rats had decreased locomotor activity and higher stereotypy. This was also seen in rats that received 6 mg/kg of MA once daily for 14 days, followed by a challenge dose of 1.0 or 1.75 mg/kg 65 days after the sensitization regimen (Nishikawa et al., 1983). The results showed that 5% of animals receiving 1.0 mg/kg showed stereotypy and 100% of rats that received 1.75 mg/kg showed stereotypy. This suggests that the closer the challenge dose to the original dose used during sensitization, the more likely rats would exhibit stereotypy.

As a conclusion, although dosing regimens differ from one study to the next, increased hyperlocomotion, and stereotypy (with higher doses) have been observed using various doses in rats, including 1.0, 2.0, 2.5, 3.0, 5.0, and 10.0 mg/kg (Slamberova et al., 2011; Fujiwara et al., 1987; McDaid et al., 2006; Belcher et al., 2006; Brady et al., 2003; McGuire et al., 2011). Both hyperlocomotion and stereotypy were also observed in these studies, which confirm that rats were behaviorally sensitized to MA.

Social Interaction

To date, there are no studies published on chronic MA administration and SI in animals. This may be due to the observations in these experiments that SI is not reduced in MA-treated animals after repeated 5.0 mg/kg injections one day and 30 days after abstinence. For example, after one day of abstinence, saline-treated rats spent ~120 seconds and MA-treated rats spent ~115 seconds out of 300 seconds interacting with partner rats. There were no differences in SI time between the two groups. The same pattern is seen after 30 days of abstinence. Saline-treated rats spent ~115 seconds and MA-treated rats spent ~100 seconds interacting with a partner rat. Again, no significant differences were seen between the two treatment groups in SI after prolonged abstinence.

Although I did not find differences in SI after repeated injections of MA, a binge study by Clemens et al. (2004) observed decreases in SI in MA-treated rats 4 weeks after the treatment. Rats were injected with saline or MA (2.5 mg/kg or 5.0 mg/kg) every two hours for a total of four injections. Four weeks later, rats were assessed on SI. They found that both doses of MA decreased SI significantly more than in the saline-treated animals. Also, based on the results of an emergence test in the same study, MA-treated rats had increased anxiety after four weeks of treatment compared to saline-treated rats. The caveat in this experiment was that during SI, a MA-treated rat was paired with another MA-treated rat and a saline-treated rat was paired with another saline-treated rat, which introduces the possibility that when an anxious MA rat was paired with

another anxious MA rat, that there was an additive effect on anxiety toward one another, thereby decreasing SI in the MA-treated groups.

When examining the data presented herein, SI was not significantly different or decreased compared to the saline-treated rats after abstinence, although SI was decreased after 30 days of abstinence in the binge study described above. The differences in results collectively suggest a few possibilities; that the brain regions thought to be responsible for SI, specifically the OFC and mPFC, may not be altered after long periods of chronic MA administration, but may only be altered after binge regimens that are thought to cause neurotoxicity to the brain. Lastly, it is possible that regions other than the OFC and mPFC, such as the amygdala (Amaral, 2006), contribute to social interactions, and these structures are altered, causing decreases in SI after binge administrations. It is important to note that these are all speculations, given that I have only conducted behavioral experiments in these animals and have not simultaneously measured the biological or neurochemical underpinnings associated with SI. Future studies may potentially include examining protein levels of DA, SERT, NE, and GLUT using immunohistochemistry and transmitter levels using microdialysis.

When examining SI 30 minutes after an acute injection of MA, there was a significant decrease in SI compared to the animals that received saline. During SI, rats were still under the influence of MA since the half-life of MA is one hour. These results suggest that a rodent exhibits decreases in SI while under the influence of 5.0 mg/kg of MA. This has also been observed in two studies by

Slamberova et al. (2010 & 2011), who administered 0.5, 1.0, or 1.5 mg/kg of MA 30 minutes prior to a SI task and found a decrease in SI after a MA injection compared to rats injected with saline. The differences in SI may be due to the anxiogenic effects that are brought upon by stimulant drugs (Ettenberg and Geist, 1991). When watching the videos, the partner rats were trying to interact with the MA-treated rats, but the MA-treated rats would quickly escape from interacting. If MA-treated rats were experiencing anxiety, then it is very likely that they did not want to interact. However, the saline-treated rats may be experiencing anxiety as well, since they interacted for an average of ~70 seconds, which is less than the interaction time of saline-treated rats after one day and 30 days of abstinence. SI time in these animals was decreased compared to one day and 30 days of abstinence, possibly due to the fact that animals have only been exposed to human handling for one week. The rats in the one-day and 30-day experiments were sensitized and not only handled during the one-week handling period, but also had contact with humans during the sensitization phase. It may be possible that the animals in the single injection group were merely stressed due to handling or to a situation that they were not used to, such as being in an open field, although they were exposed to the open field for 10 minutes a day for two days. Lastly, it is important to note that all SI experiments took place in the same open field so there were no contextual differences from one experiment to the next.

In conclusion, SI was not decreased in chronically-treated MA rats after one day or 30 days of abstinence, but was decreased 30 days after a binge

regimen (Clemens et al., 2004). This could be due to the difference in administration, such that the chronic intermittent injections of MA may not be causing neurotoxicity that is otherwise caused by binge regimens. Lastly, SI was decreased 30 minutes after an acute injection of MA, which was also seen by Slamberova et al. (2010 & 2011). It is speculated that the acute decreases may be due to the anxiogenic effect that MA has on SI.

Temporal Order Recognition

There are no previous studies that have examined TOR after repeated MA exposure. In these sets of experiments, impairments in TOR were observed after one day and 30 days of abstinence following a sensitizing regimen of MA. As mentioned previously, the prelimbic region of the PFC is critical in mediating temporal memory, since inactivating this region impairs temporal sequencing in rodents. Additionally, PFC damage in rats (Kesner & Holbrook, 1987) and in monkeys (Petrides, 1991) can disrupt temporal memory. Collectively, those findings suggests that the impairments observed in this study could be due to alterations in PFC functioning, specifically in the prelimbic area.

Impairments in TOR were also observed after up to 30 days of abstinence in the MA-treated rats, which suggests that the sensitization regimen had a persistent effect on the PFC. The long-lasting effect in this region impaired the MA-treated rats' ability to sequence events in time, thereby impairing temporal memory. Although the specific neurochemical and neurobiological underpinnings

contributing to these impairments after inactivating (by lesioning or antagonizing) the prelimbic area is unclear, a recent article suggests that inactivation of the prelimbic region was paralleled with exaggerated synaptic plasticity, the phenomenon of synaptic circuits being strengthened or reorganized (Baudin et al., 2012). Changes in synaptic plasticity have also been observed in the HC-mPFC pathway by Ishikawa et al. (2005), who administered 4.0 mg/kg of MA to mice for four consecutive days. MA caused a decrease in D_1 receptors due to its ability to increase long-term extracellular DA. Since the mPFC plays a crucial role in working memory (Kesner et al., 2005) and D_1 receptors are essential for cognitive function (Seamans et al., 1998), these studies collectively suggest that the DA system plays a major role in synaptic plasticity within the mPFC. In the mPFC, synaptic plasticity is also demonstrated after repeated injections of amphetamine. For example, Morshedi et al. (2009) observed an increase in the number of excitatory synapses onto dendritic spines. Also, Robinson and Kolb (1999) observed increases in the length of dendrites and increases in spine density in pyramidal neurons in the mPFC, which increases the ability for neurons to communicate with one another since there is more room on the neuron for additional synaptic contact. Collectively, these studies support the idea that amphetamines induce changes in synaptic plasticity or changes in the morphology of neurons, which in turn allows neurons to be more available to receive excitatory or inhibitory input from other cells since there are more available areas on a neuron (such as on the spines) for additional excitatory synaptic contact.

Another possibility contributing to the impairments seen in temporal memory after both time periods of abstinence may be MA-induced changes in neuronal firing rate. When observing the acute effects of MA, Jang et al. (2007) observed that a single dose of 4.0 mg/kg of MA showed a bidirectional and transient effect on firing rate. Shortly after the MA injection, neurons in the mPFC had an increase in firing rate; however, after 60 minutes, cells either increased or decreased firing rate. These different effects may be due to simultaneous inhibitory input to this region, as well as an increase in DA in this area (Jang et al., 2007). Also, Jodo et al. (2003) observed that after injecting rats acutely with 1.5 mg/kg of MA, there was no apparent increase or decrease in firing rate in the mPFC compared to saline controls. When observing the effects of chronic MA on firing rate, the results are different. For example, Parsegian et al. (2011) observed that rats that self-administered MA for 21 days showed an increase in basal firing frequency of pyramidal cells in the mPFC 11 days after abstinence compared to control rats that received yoked saline. Chronic MA not only altered firing rate of pyramidal cells in these animals, but these neuronal changes were paralleled with impairments in behavioral flexibility demonstrated by the Attentional Set-Shift Task (ASST). This suggests that chronic MA caused deficits in cognition, which was also associated with changes in basal firing rate. Although the aforementioned studies found different results when examining MA-induced firing rate in the mPFC, it is important to note that they all used drastically different administration paradigms before recording neuronal activity. For example, Parsegian et al. (2011) looked at firing rate after 21 days of MA

self-administration, which is very different than the effects that MA has on the brain and on firing rate after a single injection. Also, the other two studies observed firing rate after a single injection of MA, one study was using a fairly low dose of MA, whereas the other was using a high dose of MA.

Although much is unknown about firing rate in the PFC after acute or chronic MA exposure in an anesthetized or awake-behaving animal, there is much more consistent support for alterations in firing rate in rats that have received amphetamine. For example, Gulley and Stanis (2010) have observed that awake-behaving rats exhibited decreases in firing rate of PFC neurons after a single injection of 1.0 mg/kg of amphetamine. These inhibitory responses were also observed in the same rats after repeated exposure to amphetamine. Decreased firing rate in the PFC has also been observed in anesthetized rats that had received 1.0 or 2.0 mg/kg of amphetamine (Mora et al., 1976). However, when injected with a smaller dose such as 0.5 mg/kg of amphetamine, excitatory responses have been observed (Homayoun & Moghaddam, 2006). Collectively, these studies suggest that amphetamines cause changes in firing rate, whether be it an increase or a decrease, based on the administration paradigm and dose administered. These changes in firing rate are also associated with deficits in cognition, which may be contributing to the impairments seen in temporal memory after a sensitizing regimen of MA.

To examine if the impairments in cognition observed after one day of abstinence were due to the acute effects of MA administration or due to the long-term neurochemical or neurobiological changes caused by the sensitizing

regimen, I injected a naïve group of rats with a single injection of 5.0 mg/kg of MA and tested them on TOR one day after the injection. The single-injection effect on TOR supports the speculation that chronic administration of MA caused these alterations in PFC functioning. Since there were no differences in TOR after a single injection of MA, this suggests the impairments seen in cognition were not due to the acute effects of MA after one day of abstinence, but were actually due to the sensitizing regimen itself. Furthermore, this suggests that a single injection of MA is not enough to induce synaptic plasticity in the prelimbic area, thereby not causing impairments in temporal memory. Additionally, changes in firing rate are not expected at this time point since the studies mentioned above examined MA-induced firing rate while the animals were under the influence of MA or after repeated exposure of the drug, which does not coincide with when I examined TOR in this experiment. This provides further support for my findings that the cognitive impairments observed after the sensitizing regimen were not due to the acute effects of MA, but due to the long-term neuronal changes caused by MA.

In conclusion, it is possible that the impairments in temporal memory seen after one day or 30 days of abstinence are due to MA-induced changes in the functioning of the prelimbic area. Additionally, the sensitizing regimen may be causing long-lasting changes in synaptic plasticity and alterations in firing rate of PFC neurons, which could also be contributing to the memory deficits observed in these rodents. However, since we have not examined neurotransmission or the underlying mechanisms that are contributing to the impairments in these

areas, it is difficult to make a statement on the neurobiological changes that are driving these impairments in cognition. In conclusion, it is possible that the long-term cognitive deficits seen in humans who chronically abuse MA (Salo et al., 2009 & 2011) may be due to functional alterations in the mPFC, including synaptic plasticity or changes in the firing rate of PFC neurons, although these hypotheses need to be further assessed.

Novel Object Recognition

To examine if there were impairments in recognition memory, I tested NOR in rats after one day and 30 days of abstinence following sensitization. Interestingly, impairments in NOR were observed in MA-treated rats after one day of abstinence, but not after 30 days of abstinence. Saline-treated rats were able to detect novel objects at the two time points, and, therefore, had no impairment in NOR throughout experimentation.

There are no studies that examine short-term effects on novelty detection associated with chronic MA administration. However, a few studies have observed impairments in NOR after an abstinence period from MA. Belcher et al. (2006) looked at NOR after one week of abstinence and found decreased NOR in the MA-treated rats compared to the saline rats, although differences were not significant. Although we did see impairments in NOR after one day of abstinence, impairments did not persist for 30 days. It is important to note that unlike Belcher et al., I only tested NOR after one day and at 30 days of abstinence, but did not

examine novelty detection at one week. It is possible that they did not find significant effects in their experiments because the rats were starting to regain their recognition memory a week after abstaining from MA and the effect was not strong enough to induce significant impairments in this domain. It is also difficult to compare my experiments to the experiments by Belcher et al. because there were a few differences in our studies. For example, they employed a shorter sensitization regimen with a lower dose of MA. They administered 3.0 mg/kg of MA every day for 10 days, whereas I administered 5.0 mg/kg of MA every other day for 14 days. Perhaps the dose they administered was not potent enough to induce significant impairments in novelty detection after one week of abstinence. Taken together, it is difficult to ascertain at exactly what point the impairments in cognition started to recover in my set of experiments because I did not test any days that fell between one day and 30 days of abstinence from MA.

As mentioned previously, MA-treated rats showed a recovery of recognition memory after 30 days of abstinence. These findings were different than the findings by Kamei et al. (2006) who showed that after receiving 1.0 mg/kg of MA for seven consecutive days, mice were impaired in novelty detection after one day and 28 days of abstinence. Although I did not find differences in NOR after extended abstinence, there are many differences between our studies that may lead to the differences in results. First, the sensitization regimen was different. They administered MA consecutively for one week, whereas I administered MA intermittently for two weeks. I employed an every other day injection paradigm to allow for MA to be cleared from the system

substantially before the next injection, because accumulation of residual MA can cause neurotoxicity. However, and as mentioned previously, I cannot be sure if the sensitization regimen did not induce neurotoxicity. Another difference between our studies was that I tested NOR in rats, whereas they tested novelty detection in mice. Rats and mice have different metabolic rates, considering that the LD_{50} for MA in mice is 57.0 mg/kg and 55.0 mg/kg in rats (Kiyatkin & Sharma, 2009). Although not significant, this suggests that it takes different amounts of drug for MA to be considered a lethal dose in the two species. Therefore, it is important to consider that my studies were done in rats whereas their studies were done in mice, and the differences in the doses of drug could have led to differences in cognitive impairments.

It is important to explore the different possibilities that are contributing to the impairments seen in novelty detection after one day of abstinence, but not after 30 days of abstinence. As mentioned above, lesioning the HC bilaterally impairs novelty detection (Broadbent et al., 2010). Damage to the HC has also been associated with impaired recognition memory in humans (McKee & Squire, 1993) and in monkeys (Pascalis & Bachevalier, 1999). These studies suggest that since damage to the HC produces deficits in recognition memory, it is possible that there may be initial alterations in the HC preventing the expression of NOR, but these alterations do not persist for up to 30 days after abstinence. This suggests that HC functioning was altered shortly after chronic intermittent injections, but functioning recovered after prolonged abstinence. Recovery of recognition memory after 30 days of abstinence may be due to the fact that the

HC is extremely prone to neuroplasticity (McEwen, 1990). Since the HC is undergoing neuroplasticity quicker than other regions, it is possible that MA initially causes impairments in HC functioning, but with the process of synaptic plasticity, begins to recover after some amount of time. Another possible reason for HC-mediated recovery may be due to the recent findings that the dentate gyrus, which is a part of the hippocampal formation, is one of two regions in the brain that generates new cells (Schmidt-Hieber et al., 2004). It is possible that the initial impairments seen in NOR may be due to MA-induced cell death in the hippocampus (Deng et al., 2001); however, after prolonged abstinence, the HC undergoes neurogenesis, which contributes to a reversal of impairments seen in cognition. Furthermore, impairments in NOR could be due to disruptions in DA transmission after long-term MA administration. In the study by Kamei et al. mentioned above, they observed that antagonizing D_1 receptors via peripheral injections prevented MA-treated rats from exhibiting impairments in NOR.

The DA system is not only involved in TOR as mentioned previously, but also involved in novelty detection. However, it is possible that alterations in DA are affecting the regions involved in mediating TOR and NOR differently. For example, it is possible that alterations in DA transmission in the mesolimbic pathway or in the mesocortical pathway differentially contribute to the impairments seen in NOR and TOR, respectively. The mesolimbic pathway, which sends neuronal projections primarily containing DA from the VTA to the limbic system, could be altered acutely after prolonged MA administration, but may be recovering after extended abstinence. Since the HC is a part of the limbic

system, MA could be causing changes in DA transmission in the areas receiving DA, such as the limbic system (HC) and thereby causing impairments in recognition memory. Moreover, the mesocortical pathway, which sends DA projections from the VTA to cortical areas (such as the PFC), may be involved in TOR. MA could be altering DA transmission in this pathway and causing long-term alterations in the PFC, thereby contributing to the persistent impairments observed in TOR after chronic MA sensitization. However, these are all speculations since I have only examined behavioral differences in TOR and NOR after MA administration.

It is important to mention that the impairments seen in my studies were due to the sensitizing regimen and not due to the acute effects of MA. As mentioned previously in the TOR section, MA-treated rats showed no impairments in TOR after a single injection of 5.0 mg/kg of MA. Similarly, they did not show impairments in NOR after an injection 5.0 mg/kg of MA. In both cases, saline-treated rats also had no impairments in novelty detection. These findings suggest that impairments seen in NOR after sensitization were likely due to the long-term neuronal changes induced by chronic MA administration. These results also suggest that one injection did not induce alterations to the functioning of the HC, as well as changes synaptic plasticity or in neuronal firing rate, therefore not contributing to impairments in recognition memory. Lastly, it is possible that the DA system was not significantly altered after only one injection of MA. Perhaps at this point, the mesolimbic projection neurons were still intact and sending dopaminergic projections to the HC.

These animal findings of recovery in recognition memory after MA administration are interesting because Salo et al. (2009 & 2011) has recently observed that humans who chronically abuse MA have impairments in cognition which may recover after prolonged abstinence. The results in NOR recovery after prolonged abstinence is important since these studies have found that there may be initial deficits in cognition, but after prolonged abstinence, these deficits may start to recover. Since I also observed recovery in specific cognitive functions after extended abstinence, this animal model provides a good tool to start examining the neurobiological and neurochemical differences that contribute to the impairments that persist or recover after MA administration. Also, this animal model could be used for pharmacological manipulations to better understand the underlying neuronal mechanisms contributing to these cognitive impairments and the alterations in these mechanisms that eventually lead to a recovery in cognition.

Collectively, these studies suggest that the functioning of the PFC is altered after short-term and long-term abstinence, and therefore, causes chronically sensitized MA rats to be impaired in temporal memory. Additionally, HC functioning is altered after short-term abstinence but seems to recover after long-term abstinence, suggesting recovery of HC functioning. This further suggests that novelty detection is impaired initially, but recovers after prolonged abstinence. These findings with recognition memory are similar to the human findings of Salo et al. who shows that there may be recovery in cognitive function

after prolonged abstinence. This sensitization model provides a good tool to examine the underlying mechanisms involved in the recovery process.

Future Studies

Future studies will assess the electrophysiological underpinnings of mPFC and HC functioning while undergoing TOR and NOR in awake, behaving rodents that self-administer MA to get a better understanding of the extent to which these regions are causing impairments in cognition after one day and prolonged abstinence. Additionally, synchrony and firing patterns would be assessed to get a better understanding of communication between these two regions to see if they are communally affecting cognition. Lastly, $_{D}$-Govadine, which is shown to be a cognitive enhancer by increasing DA efflux (Lapish et al. in preparation), would be administered after inducing MA impairments in temporal and recognition memory to examine if this stereoisomer would remediate the impairments in cognition caused by MA. This would provide a treatment strategy for those affected with long-term MA addiction and will hopefully decrease or alleviate the cognitive deficits associated with chronic MA use.

Summary

In conclusion, these findings demonstrate that high, chronic doses of MA cause behavioral sensitization and short-term impairments in temporal sequencing and novelty detection, which suggests alterations in the mPFC and

HC, respectively. Further, the MA-induced mPFC dysfunction is static after 30 days of abstinence, although HC functioning is remediated with prolonged abstinence. This suggests two different mechanisms in which MA affects cognitive substrates.

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FIGURES

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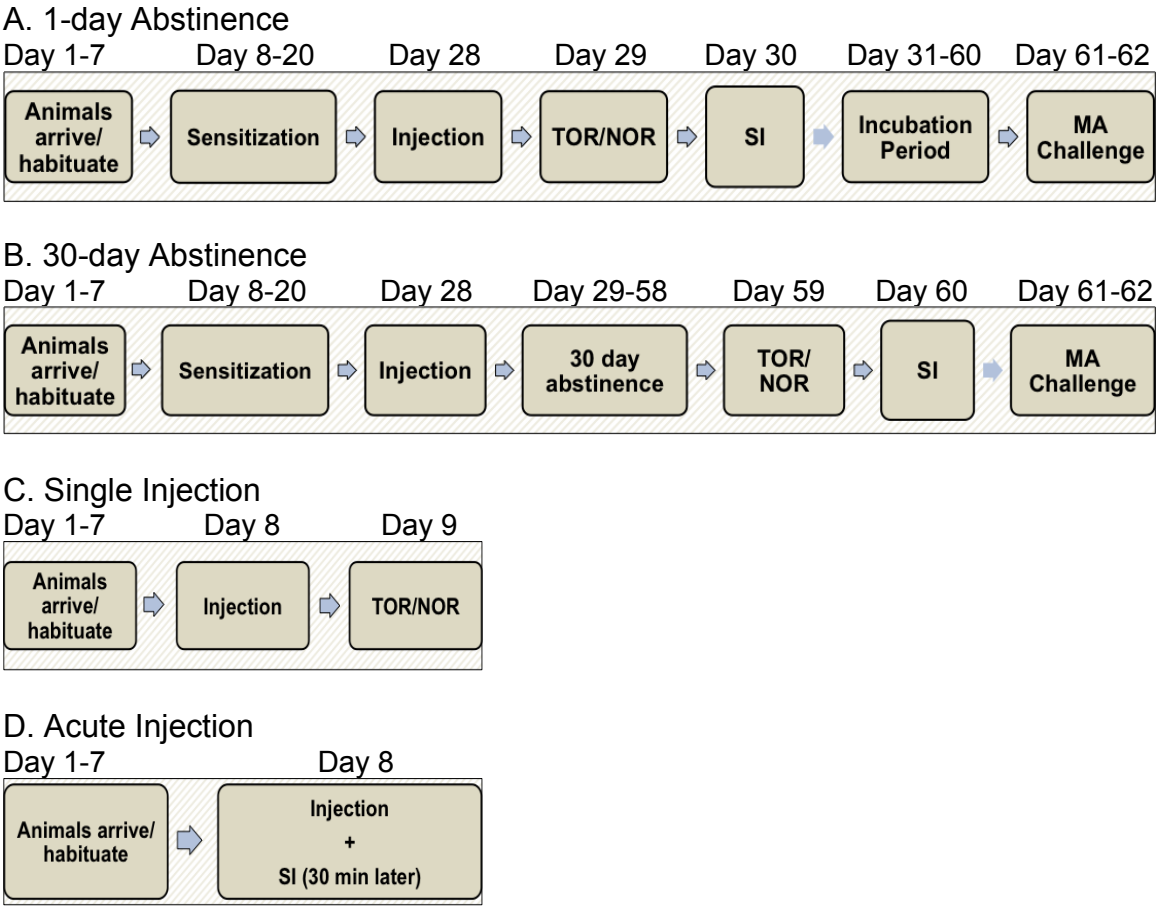


Figure 1: Timeline of Experiments: A timeline of the experiments during 1-day abstinence (A), 30-day abstinence (B), single injection (C) and acute injection (D).

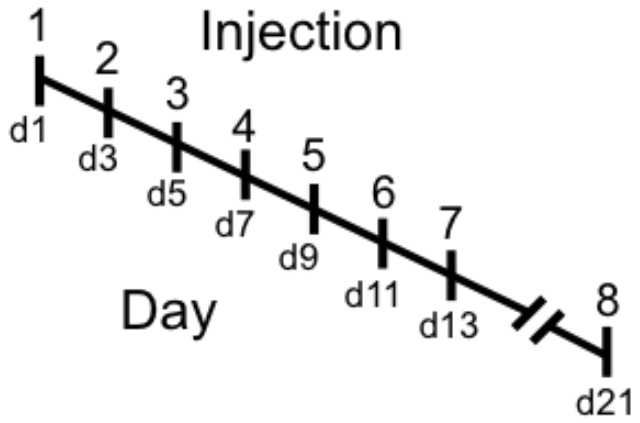
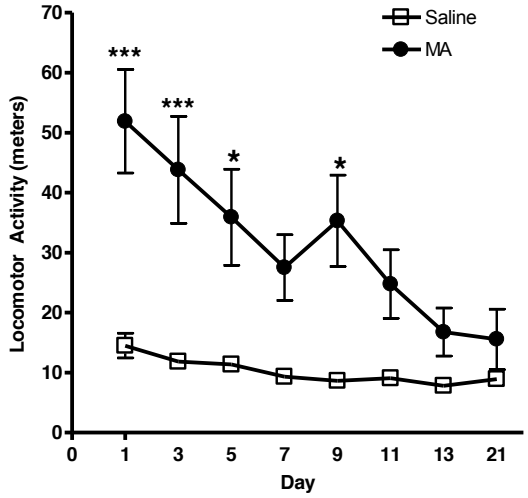


Figure 2: Timeline of Behavioral Sensitization. Animals were injected with either 5.0 mg/kg of MA or saline every other day for 13 days, for a total of 7 injections (Day 1-13, induction phase). 1 week after the induction phase, rats were injected again to confirm sensitization (Day 21).

A.



B.

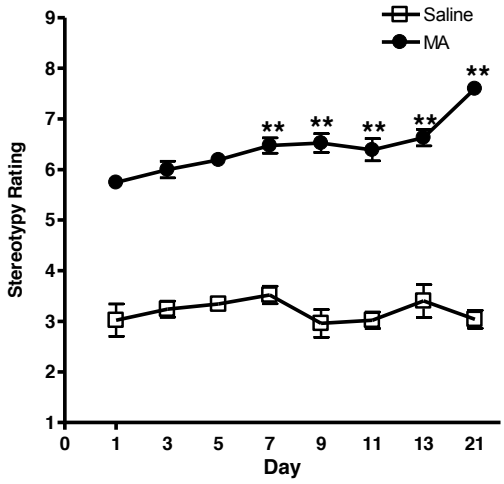


Figure 3: Locomotor Activity & Stereotypy: Induction Phase. Locomotor activity and stereotypy during the induction phase. (A) Locomotor activity (mean ± SEM) post-injection. Initially, MA-treated rats increased locomotor output when

Figure 3: continued, compared to saline-treated rats, but after repeated administration, MA-treated rats exhibited a decrease in locomotor activity; *(Bonferroni post-hoc, $p < 0.05$), ***(Bonferroni post-hoc, $p < 0.005$), significantly different than saline-treated rats. (B) Stereotypy rating (mean ± SEM) post-injection during the induction phase. MA-treated rats increased stereotypy throughout the induction phase when compared to saline-treated rats; **(Bonferroni post-hoc, $p < 0.005$), significantly different from MA-treated rats on Day 1.

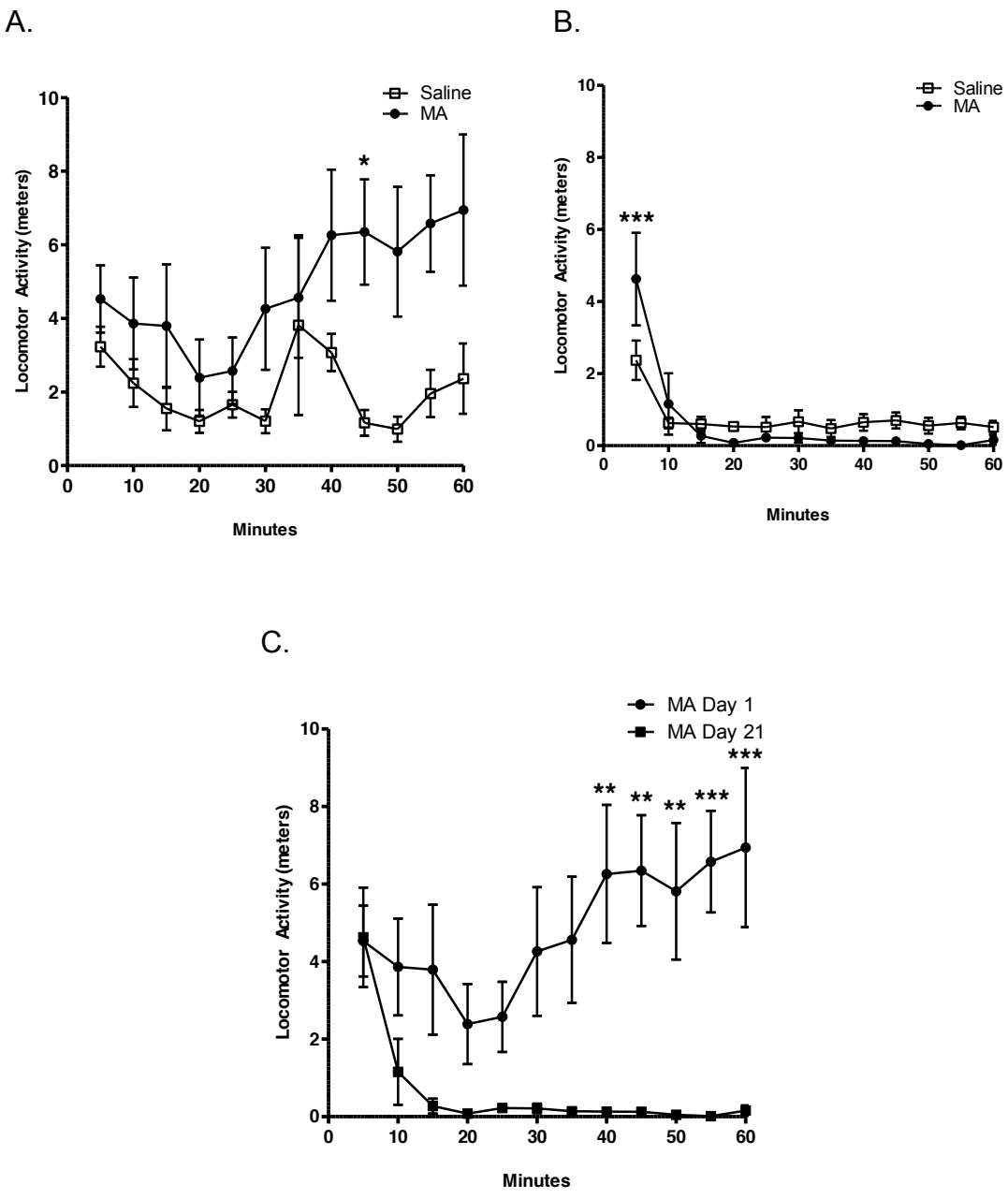


Figure 4: Locomotor Activity 5-Minute Bins: Day 1 Vs. Day 21. Locomotor activity in 5-minute bins during the induction phase. (A) Locomotor activity (mean ± SEM) post-injection on Day 1. As the induction phase progressed, MA-treated rats had an increase in locomotor activity; *(Bonferroni post-hoc, $p < 0.05$),

Figure 4: continued, significantly different than saline-treated rats. (B) Locomotor activity (mean ± SEM) post-injection on Day 21. Soon after the injection, there were no differences in locomotor activity between MA-treated and saline-treated rats; ***(Bonferroni post-hoc, $p < 0.001$), significantly different than saline-treated rats. (C) Locomotor activity (mean ± SEM) post-injection on Day 1 vs. Day 21 in the MA-treated rats. On Day 1 of the injection, MA-treated rats had an increase in locomotor activity, with a significant decrease in locomotor activity on Day 21 as the induction phase progressed; **(Bonferroni post-hoc, $p < 0.01$), ***(Bonferroni post-hoc, $p < 0.001$), significantly different than locomotor activity scores on Day 21.

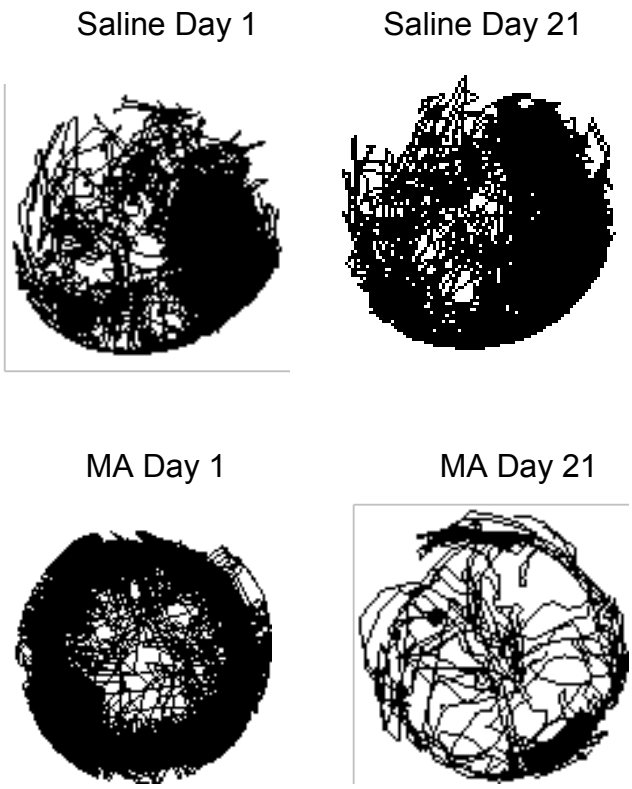


Figure 5: Locomotor Activity Animal Path: Day 1 Vs. Day 21. Animal paths post-injection of saline-treated (top) and MA-treated (bottom) rats on Day 1 (left) and Day 21 (right) of the induction phase.

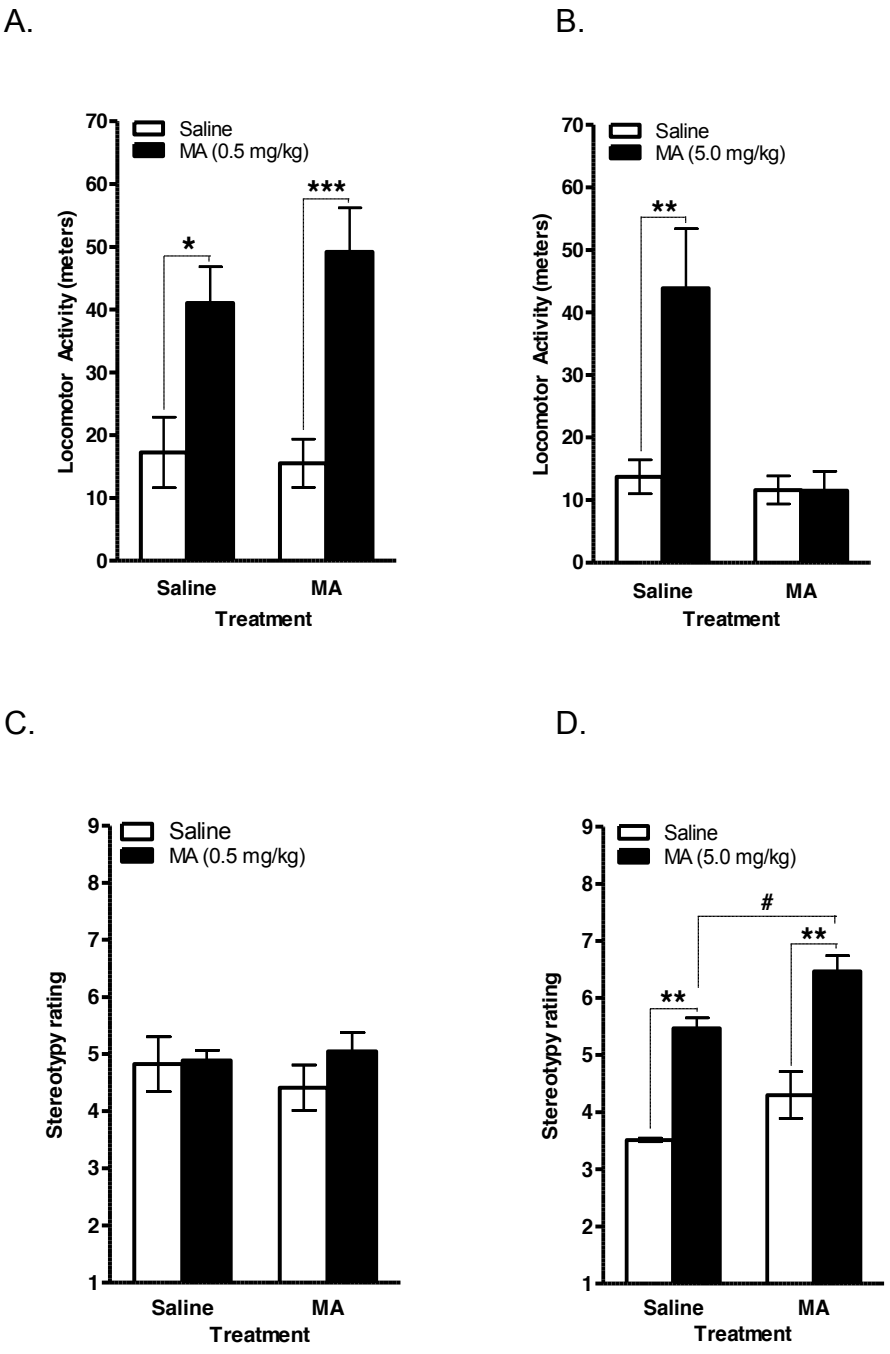


Figure 6: Locomotor Activity and Stereotypy: MA Challenge. Locomotor activity (mean ± SEM) and stereotypy (mean ± SEM) during MA challenge. (A) All rats

Figure 6: continued, that received MA (0.5 mg/kg) showed increased locomotor activity compared to the rats that received saline; *(Bonferroni post-hoc, $p < 0.05$), ***(Bonferroni post-hoc, $p < 0.0005$), significantly different than saline injection. (B) Chronic saline-treated rats that received MA (5.0 mg/kg) showed increased locomotor activity; **(Bonferroni post-hoc, $p < 0.005$), significantly different from all other groups. (C) There were no significant differences in stereotypy scores between either of the groups or treatments after a 0.5 mg/kg MA or saline injection. (D) MA injections (5.0 mg/kg) evoked stereotypy in both chronic saline-treated and chronic MA-treated rats when compared to rats that received a saline injection, but was more robust in chronic MA-treated rats; #(Tukey's post-hoc, $p < 0.05$), chronically treated MA rats injected with MA significantly greater than chronically treated saline rats injected with MA; **(Bonferroni post-hoc, $p < 0.005$), MA injection significantly greater than saline injections across treatments.

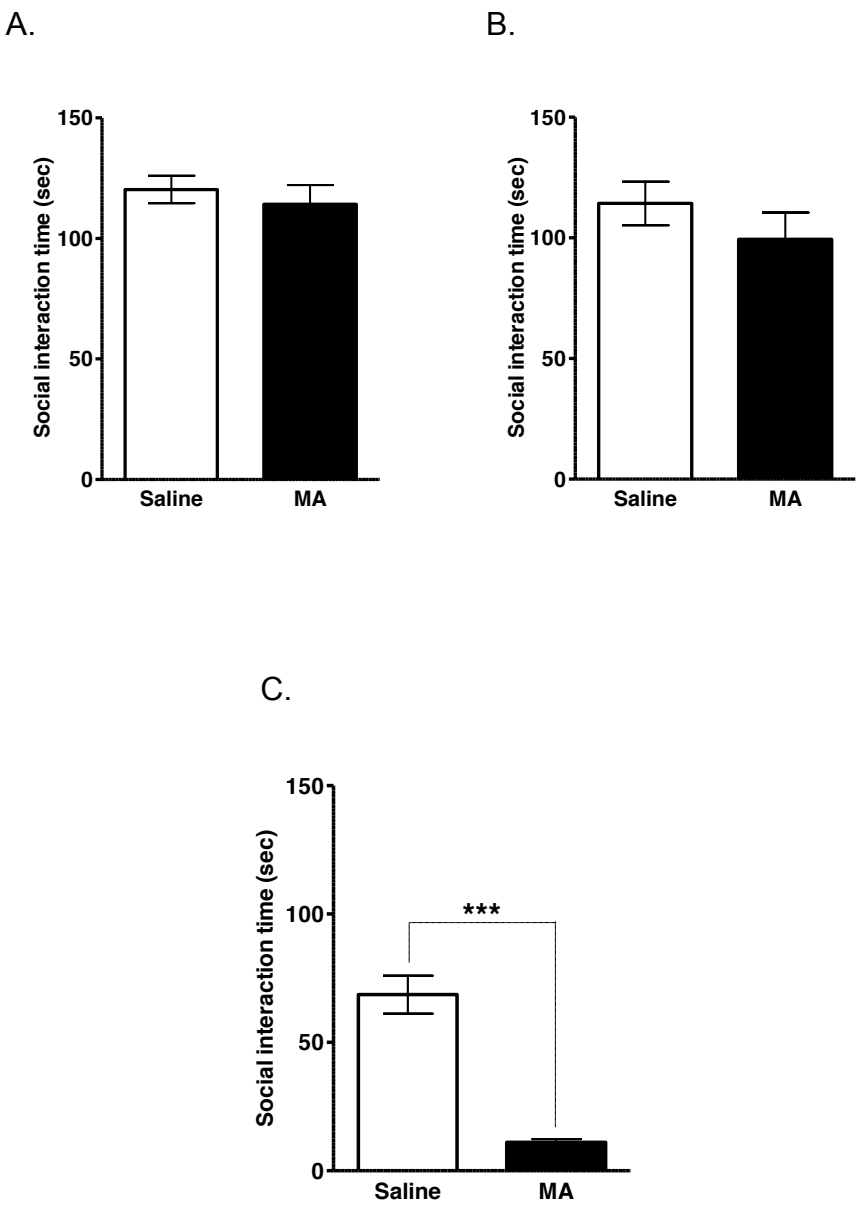


Figure 7: Social Interaction. Social interaction time (mean ± SEM) during the SI task. There were no significant differences in social interaction time between the chronic saline-treated and chronic MA-treated rats during the SI task 1 day (A) or 30 days (B) following abstinence. (C) Rats injected with 5.0 mg/kg of MA

Figure 7: continued, interacted significantly less 30 minutes after the injection compared to the rats that received saline; ***(unpaired t-test, $p < 0.0005$), significantly greater than saline-treated rats.

A. B.

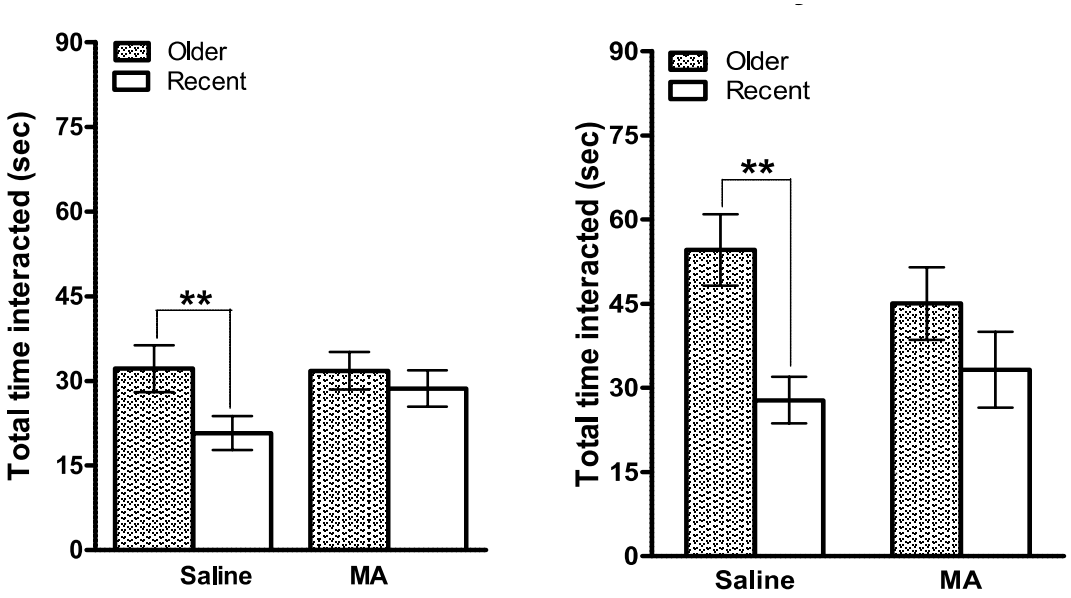
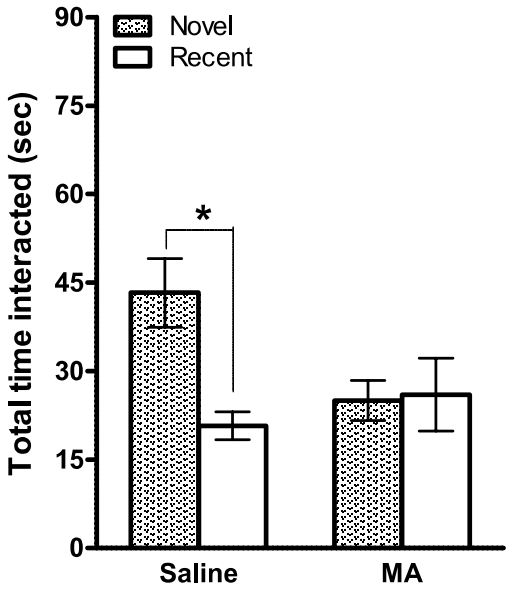


Figure 8: Temporal Order Recognition: 1-Day & 30-Day Abstinence. TOR after 1-day or 30-days of abstinence. (A) Total time interacted (mean ± SEM) 1 day following the last day of the induction phase. Saline-treated rats, but not MA-treated rats, exhibited TOR after 1-day of abstinence(B) Total time interacted (mean ± SEM) 30 days following the last day of the induction phase. Saline-treated rats, but not MA-treated rats, exhibited TOR after 30 days of abstinence; **(paired t-test, $p < 0.005$).

A.



B.

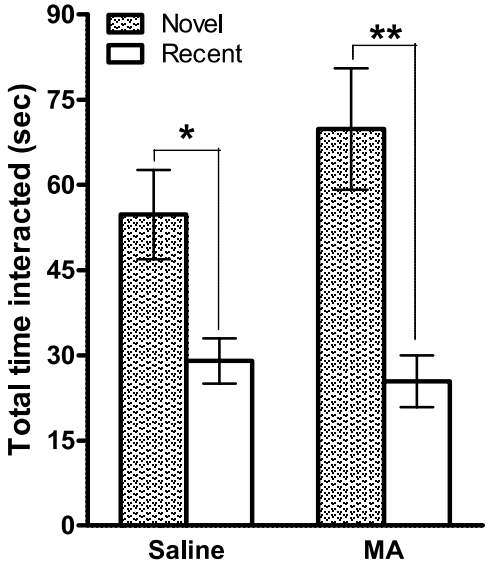


Figure 9: Novel Object Recognition: 1-Day & 30-Day Abstinence. NOR after 1-day or 30-days of abstinence. (A) Total time interacted (mean ± SEM) 1 day following the last day of the induction phase. Saline-treated rats, but not MA-treated rats NOR after 1-day of abstinence(B) Total time interacted (mean ± SEM) 30 days following the last day of the induction phase. Both saline-treated and MA-treated rats exhibited NOR after 30 days of abstinence; *(paired t-test, $p < 0.05$), *(paired t-test, $p < 0.005$)

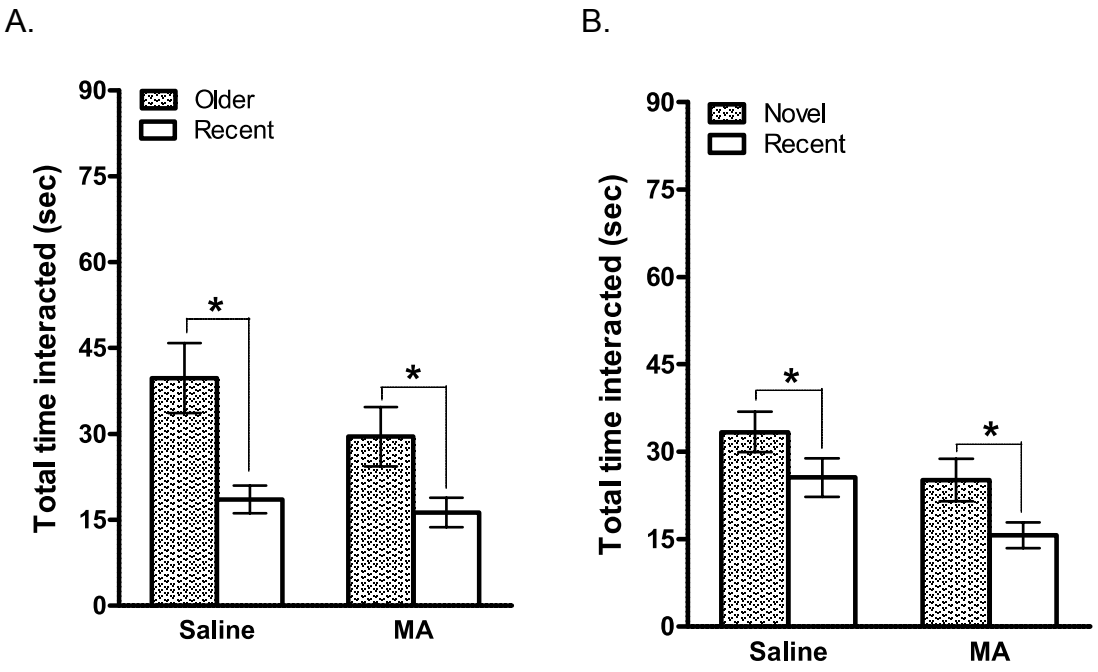


Figure 10: Single Injection. TOR and NOR after a single injection. Total time interacted (mean ± SEM) 1 day following a single injection of MA or saline. Both treatment groups exhibited TOR (A) and NOR (B) after a single injection of MA or saline; *(paired t-test, $p < 0.05$).