Published in final edited form as:

Synapse. 2013 May ; 67(5): . doi:10.1002/syn.21635.

Chronic Methamphetamine Exposure Produces a Delayed, Long-Lasting Memory Deficit

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Abstract

Methamphetamine (METH) is a highly addictive and neurotoxic psychostimulant. Its use in humans is often associated with neurocognitive impairment. Whether this is due to long-term deficits in short-term memory and/or hippocampal plasticity remains unclear. Recently, we reported that METH increases baseline synaptic transmission and reduces LTP in an *ex vivo* preparation of the hippocampal CA1 region from young mice. In the current study, we tested the hypothesis that a repeated neurotoxic regimen of METH exposure in adolescent mice decreases hippocampal synaptic plasticity and produces a deficit in short-term memory. Contrary to our prediction, there was no change in the hippocampal plasticity or short-term memory when measured after 14 days of METH exposure. However, we found that at 7, 14, and 21 days of drug abstinence, METH-exposed mice exhibited a deficit in spatial memory, which was accompanied by a decrease in hippocampal plasticity. Our results support the interpretation that the deleterious cognitive consequences of neurotoxic levels of METH exposure may manifest and persist after drug abstinence. Therefore, therapeutic strategies should consider short-term as well as long-term consequences of methamphetamine exposure.

Keywords

long-term potentiation; neurotoxicity; psychostimulant; synaptic transmission; cognition

INTRODUCTION

Methamphetamine (METH) is a powerful psychostimulant with a high abuse potential. The latest contemporary drug, "bath salts" (Joksovic et al., 2012; Rasimas, 2012), now

penetrating our society with cruel effects, contains mephedrone, which has strong amphetamine-like psychoactive properties (Joksovic et al., 2012; Spiller et al., 2011). METH abuse is prevalent in young adults (2005) and may lead to long-lasting abnormalities in brain structure, chemistry, and function (Chang et al., 2007; Sekine et al., 2003, 2006; Volkow et al., 2001b) that can persist even after prolonged periods of drug abstinence (McCann et al., 2008; Sekine et al., 2003; Simon et al., 2002; Wilson et al., 1996). Thus, METH intake produces cognitive impairments, including deficits in episodic memory as well as decreases in hippocampal volume in human subjects with METH psychosis (Orikabe et al., 2011). These functional and structural impairments remain evident even after prolonged drug abstinence (Akiyama et al., 2011).

The hippocampus is a limbic structure that is involved in learning and memory (Kensinger et al., 2001), and is a target for psychostimulants (Vorel et al., 2001). Memory circuits can be influenced by monoamine signaling in the brain (Everitt and Robbins, 2005). The hippocampus receives neural projections from the dopaminergic ventral tegmental area (VTA) (Gasbarri et al., 1997; Scatton et al., 1980), and sends glutamatergic efferents to the nucleus accumbens (French and Totterdell, 2003; French et al., 2003). Neurochemical stimulation of the hippocampus (with either *N*-methyl-D-aspartate (NMDA) or muscarinic agonists (carbachol)) causes dopamine release in the nucleus accumbens (Moss et al., 2003). Theta burst stimulation of the subiculum of the hippocampus reinstates cocaine-seeking behavior in rats (Vorel et al., 2001). Clinical data suggest that long-term METH exposure induces a profound deficit in memory tasks such as verbal, working, and episodic memory (Gonzalez et al., 2007; Hoffman et al., 2006; Simon et al., 2004). Similarly, noncontingent METH exposure via injections impairs recognition memory in rats (Belcher et al., 2008, 2005, 2006; Bisagno et al., 2002; Schroder et al., 2003). Although some METH-mediated neuronal deficits appear to recover with time (Volkow et al., 2001a), the long-term consequence of METH on short-term memory after drug abstinence is not well understood.

Recently, we reported that *ex-vivo* METH increases baseline synaptic transmission and reduces LTP in the CA1 region of the hippocampus (Swant et al., 2010). In the current study, we tested the hypothesis that adolescent male mice exposed to a repeated neurotoxic regiment of METH (24 mg/kg/daily for 14 days) would exhibit impairment in hippocampal synaptic plasticity in parallel with a deficit in short-term memory. Contrary to our prediction, we found that the deleterious consequences of METH exposure on hippocampal neurotransmission and short-term memory did not occur during active drug exposure. Rather, these untoward consequences (deficits in spatial memory and decreased hippocampal plasticity) are manifested after drug abstinence. Our results suggest that the deleterious consequences of neurotoxic levels of METH exposure in adolescent male mice manifest long after drug abstinence. Thus, therapeutic strategies should consider long-term as well as short-term METH exposure.

MATERIALS AND METHODS

Animals

C57BL/6J male mice at 4 to 5 weeks of age were obtained from the Jackson Laboratory (Bar Harbor, ME). These animals were within the adolescent age range for rodents (postnatal days 28–42) (Spear, 2000, 2004). All of the animals were housed in the AALAC accredited animal care facility at Meharry Medical College, under constant temperature and a 12-h light/dark cycle (lights on: 7 A.M. to 7 P.M.), with food and water provided ad libitum. All experimental procedures complied with the NIH Guide for the Care and Use of Laboratory Animals, and were conducted with the approval of Meharry Medical College Institutional Animal Care and Use Committee. All efforts were made to keep the stress and discomfort at a minimum and to decrease the number of animals utilized in this study. To decrease stress-

induced responses (Khoshbouei et al., 2002), the animals were gently handled every day for 5 days before experimentation, and throughout the study.

Drug treatment

METH addicts may abuse large quantities of the drug over a few hours for months and years (55–640 mg METH) (Bell, 1973). In the United States, arrest reports of drivers show METH blood levels can be above 300 mg/L (Logan, 1996). To examine METH-induced neurotoxicity in animal models, various non-contingent high doses of METH have been used. For example, METH regimens of 60 mg/kg/day (Fumagalli et al., 1998), 40 mg/kg/day (Wallace et al., 1999), 32 mg/kg/day (Armstrong and Noguchi, 2004), and 30 mg/kg/day (Cadet et al., 2011; O'Callaghan and Miller, 1994; Sonsalla et al., 1996), have been used to study the neurotoxic consequence of METH exposure. We selected a daily dose of 24 mg/kg/day METH, which is a lower dose when compared with other studies. We used a lower dose because we administered METH for 14 days longer than the previous reports (Cadet et al., 2011; Fumagalli et al., 1998; O'Callaghan and Miller, 1994; Sonsalla et al., 1996). The animals were gently handled every day for 5 days before experimentation, and received 14 days of intraperitoneal (i.p.) injection of 24 mg/kg/day METH, followed by 21 days of drug abstinence. The animals were randomly assigned to five major groups.

Additional considerations for drug/vehicle exposure strategies

We selected daily METH/saline injections because we observed one injection per day does not produce detectable signs of stress, as compared with multiple daily injections. Nonetheless, even one per day injections can induce some level of stress in both experimental groups (METH/saline).

Group 1—This is the drug naïve (no injection) control group that is used for establishment of behavioral testing ($N = 20$). These animals did not receive METH or saline injection. They were used for establishing the protocol for the measurement of short-term memory (Supporting Information Fig. 3) in the absence of METH/saline. After establishing a reproducible behavioral protocol in this drug naïve group, the reliability of the resulting behavioral protocol (i.e., the measurement of short-term memory) was examined before and after scopolamine pretreatment (0.5 mg/kg, and 1 mg/kg, i.p.) (Akkerman et al., 2012; Sambeth et al., 2007; van Goethem et al., 2012) (see experimental procedure section).

Group 2—The animals in this group were equipped with telemetry transmitters (DSI; available at: www.datasci.com) to establish the optimum time for behavioral assessment following METH exposure ($N = 8$; 4 METH, 4 saline) (Supporting Information Figs. 1 and 2). These animals were not used for electrophysiology, behavioral, or biochemical analysis. The surgery procedure is described in Cesarovic et al. (2011). Briefly, the mice were anesthetized in a small Perspex chamber using sevoflurane (8%) or isoflurane (5%) in pure oxygen (600 mL/min). The animal's abdominal hair was clipped. A small incision was made in the abdominal region of the mouse. Then, the abdominal wall was opened at the linea alba and the body of the telemetric transmitter was placed into the abdominal cavity of the mouse. The positive electrode was sutured to the xiphoid process with silk sutures in the left upper abdominal region. The muscle layers of the abdominal region were closed with absorbable sutures (VICRYL 6-0, Ethicon, Norderstedt, Germany). Finally, the skin of the abdominal region is restored with staples (3 mm, Precise, 3 M Health Care, St. Paul, MN). During the recovery period the animals' general condition and body weight, as well as food and water consumption, were monitored.

After 2 weeks of recovery period, the animals received 14 days of METH/saline treatment followed by a drug abstinence period. The telemetry analysis replicated previous findings

that METH injection increases locomotor activity for 4 to 6 hr after injection (Cadet et al., 2011; O'Callaghan and Miller, 1994; Sonsalla et al., 1996). Furthermore, this analysis revealed that at 20- to 23-hr postinjection, the locomotor activity of METH exposed animals is similar to saline control levels (Supporting Information Figs. 1 and 2). Therefore, the behavioral and electrophysiology experiments in groups 3 and 4 (please see below) were performed at 11 AM, which is 23 hr post-drug/saline injection.

Group 3—The animals in this group ($N=24$; 12 METH, 12 saline) received 14 days of daily METH/saline injections. Twenty-three hours after the last injection they were subjected to biochemical, behavioral, and electrophysiological analysis. The short-term memory of these animals was examined at baseline, on day 7, and on day 14 after METH/saline injection (see Fig. 1 for experimental protocol). After the behavioral test on day 14, the animals were anesthetized with isoflurane and decapitated. The brains were either used for *ex vivo* electrophysiology recording (Fig. 8), or the tissue was collected and stored at -80°C for biochemical analysis (Fig. 3).

Group 4—The animals in this group ($N=16$; 8 METH, 8 saline) received 14 days of METH/saline injection followed by 21 days of drug abstinence. At the end of the drug abstinence period, the animals were subjected to behavioral and electrophysiological analysis. The short-term memory of these animals was examined at day 0 (baseline), during drug exposure period (day 7 and 14) and during drug abstinence period (day 21, 28, and 35) (see Fig. 1). After the behavioral test on day 35, the animals were anesthetized with isoflurane and decapitated. The brains were used for electrophysiology recording.

Group 5—A separate group of animals ($N=7$), were used to determine the level of METH in the blood 23 hr postinjection. Twenty-three hours after the last METH/saline injection, the animals were anesthetized with isoflurane (USP, Baxter International Inc., Deerfield, IL) and trunk blood was collected (23 hr postinjection) and assayed for METH and its metabolite amphetamine (AMPH) levels by Aegis Sciences Corporation (Nashville, TN). The average trunk blood levels contained 23.2 ± 4.5 ng/mL METH with an undetectable level of amphetamine. Thus, the daily injection of the drug did not accumulate in the blood over the course of 14 days.

Western blot analysis

The total tissue lysate of the samples from the cortex, striatum, and hippocampus was prepared on ice in cell lysis buffer (150 mM NaCl, 1 mM Na_2EDTA , 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na_3VO_4 , 1 $\mu\text{g/mL}$ leupeptin; 0.1 mL/100 mg tissue) (pH 7.5), which was obtained from Cell Signaling Technology, Inc. (Danvers, MA), and contained 2% SDS with protease inhibitor cocktail tablets (Roche Diagnostics Corporation, Indianapolis, IN) and halt protease and phosphatase inhibitor cocktail with EDTA (Thermo Fisher Scientific, Rockford, IL). The samples were then sonicated for 30 s using an ultrasonic processor (Sonics and Materials, Inc., Newtown, CT) and centrifuged for 20 min at 4°C and 13,000 rpm. The amount of total protein from the supernatant in each sample was quantitatively determined using the Bradford protein assay (Biorad, Hercules, CA). The absorbance was read at 595 nm on a NanoDrop 2000 microvolume spectrophotometer (Thermo Fisher Scientific, Rockford, IL). Protein concentrations were calculated against a BSA standard curve. For Western blotting, equal amount of protein for each sample was subjected to SDS-PAGE (Biorad; 4–20% Mini-PROTEAN[®] TGX[™] precast polyacrylamide gels; 200 V for 1 hr). Protein bands were transferred onto a Immobilon-P PVDF transfer membrane (Millipore, Billerica, MA), blocked for 1 hr with 10% nonfat dry milk, washed three times at 10 min each with $1 \times$ Tris-Buffered Saline and Tween 20 (TBST) (pH 7.4), and then probed with goat polyclonal

primary antibody for dopamine transporter (DAT (C-20), 1:1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), rabbit polyclonal primary antibodies for α -actin (1:5000; Sigma-Aldrich, St. Louis, MO), which was used as a control for sample loading and transfer efficiency, and tyrosine hydroxylase (TH, 1:5000; Millipore, Billerica, MA), overnight at 4°C. After four consecutive washes, the membranes were incubated with horseradish peroxidase (HRP) conjugated rabbit anti-goat (1:5000; Abcam, Cambridge, MA) or goat anti-rabbit (1:5000; Promega Corporation, Madison, WI) secondary antibodies for 2 hr, washed seven times with 1× TBST, developed using SuperSignal West Pico/Dura/Femto chemiluminescent substrate (Thermo Fisher Scientific), and exposed to HyBlot CL[®] autoradiography film (Denville Scientific, Inc., Metuchen, NJ). Densitometry of the protein bands from the scanned autoradiography films was performed using the Image Processing and Analysis in Java Software (ImageJ, National Institutes of Health, Bethesda, MD; available at: <http://rsb.info.nih.gov/ij/>).

Analysis of Western blot data

All data obtained was analyzed using GraphPad Prism software (Version 5.01, Graphpad Software, Inc., La Jolla, CA). Differences between groups were determined by unpaired, two-tailed Student's *t*-test. A *P* value of <0.05 was considered significant. Data were plotted as mean ± SEM.

Electrophysiology recording

Slice preparation—As described previously (Swant et al., 2011), acutely prepared hippocampal slices (400 μ m) were obtained from anesthetized (isoflurane) animals after 14 days of METH/saline exposure, and after 21 days of drug abstinence. Transverse brain slices were dissected in ice cold, oxygenated (95% O₂/5% CO₂) artificial cerebrospinal fluid (aCSF) containing (mM): NaCl (125), KCl (2.5), KH₂PO₄ (1.25), MgSO₄ (1.2), CaCl₂ (2), NaHCO₃ (25), and dextrose (10) adjusted to pH 7.35 with a final osmolality of 290 mosm. The CA3 region was surgically removed from all slices to minimize epileptiform activity. Slices were then transferred to a submerged recording chamber, with continuously superfused aCSF (saturated with 95% O₂/5% CO₂) at a rate of 1.5 mL/min. The temperature was maintained at 30°C. The slices were allowed to recover from dissection for at least one hour in the recording chamber before experiments were begun.

Extracellular recording—Extracellular recording electrodes (borosilicate glass, ~1 μ m tip) filled with aCSF were placed in the stratum radiatum of CA1. We chose to assess field excitatory postsynaptic potentials (fEPSPs) because they provide a reliable and stable measure of excitatory synaptic transmission (Swant et al., 2010). Dendritic fEPSP responses were evoked with a bipolar tungsten stimulating electrode (Rhodes, Inc.) placed on either the CA3 or the subicular side of the recording electrode in the stratum radiatum. Responses were amplified using the Axoclamp-2A amplifier (Molecular Devices, Inc). The electrical stimulus consisted of a single square waveform of 0.3 ms duration given at intensities of 10 to 130 μ A generated by a Grass S88 stimulator equipped with stimulus isolation unit PSIU6.

Data acquisition and analysis—Data were acquired with Clampex 10 and analyzed with Clampfit 10 software (Molecular Devices). The initial slope of the fEPSP (which provides a measure of the strength of excitatory synaptic transmission) was measured by fitting a straight line to the first millisecond of the fEPSP immediately following the fiber volley, and was monitored in real-time in every experiment. A stimulus-response curve was then determined using stimulation intensities between 10 and 130 μ A. Baseline stimulation parameters were selected to evoke a response of 40 to 60% of the maximum slope. Baseline stimulation was then commenced at a frequency of 0.033 Hz for the entire length of the experiment. The paired-pulse facilitation (PPF) protocol consisted of two pulses at baseline

intensity separated by 50 ms. After acquisition of PPF, baseline stimulation was then commenced at a frequency of 0.033 Hz for the entire length of the experiment. The LTP tetanus event consisted of three 100 Hz pulses with a 30 s interstimulus interval. Synaptic responses were normalized by dividing all slopes by the average of the 10 fEPSP slopes 5 min pretetanus. LTP was quantified as the normalized fEPSP response at 55 to 60 min post-tetanus.

Test of short-term memory

In preclinical animal models, episodic and spatial memory can be assessed by novel object and novel spatial recognition tests of short-term memory (Aggleton and Mishkin, 1985; Aggleton et al., 1988; Ennaceur, 2010; Ennaceur and Delacour, 1988). Object recognition and novel spatial recognition tests of short-term memory are based on the natural tendency of rodents to spend more time interacting with novel objects relative to familiar objects/space (Anderson et al., 2008; Berlyne, 1950). This behavioral test provides a one-trial recognition memory assessment (Ennaceur and Delacour, 1988). Therefore, the assessment requires little training and is, by far, less stressful and arousing than tasks based on negative reinforcement of behavior, such as the hidden platform version of the water maze, the inhibition and active avoidance, or fear conditioning tasks, which are widely used to study the neurobiology of learning and memory in rodents (Anderson et al., 2008; Dere et al., 2005; Ennaceur and Delacour, 1988; Hannesson et al., 2004; Kart-Teke et al., 2006, 2007; Mitchell and Laiacina, 1998; Mumby et al., 2002). Therefore, we used the novel object recognition (NOR) and spatial recognition memory (NSR) tasks to evaluate the consequence of chronic METH exposure on short-term memory.

Establishing the behavioral test in the drug naive, no injection control group for behavioral testing

Before examining the consequence of METH exposure on NOR and NSR tests of short-term memory, a group of untreated animals ($N=20$) was used to determine the reproducibility of this behavioral test. The novel object recognition test was performed in a 50 cm $\times$ 50 cm open field arena made of clear white Plexiglas. Exploration time was recorded for an object if the mouse's nose touched or was within 2 cm of the object. All pairs of objects were placed in the opposite quadrants of the open field 5 cm from each wall (Ferguson et al., 2010; Tan et al., 2012). The light intensity was equal in the different parts of the apparatus (about 30 lx). The behaviors were recorded via digital camera mounted above the Plexiglas boxes. To prevent the effect of odor cues, for all behavioral experiments, each box (total of six boxes) was used only once/day. The boxes were thoroughly cleaned daily with water and 70% ETOH, and air-dried overnight in a ventilated area.

Objects

To reduce potential biases, the order of objects and location were randomly selected. The objects were either red plastic squares made with LEGO® or black plastic triangles made with LEGO®. The total surface of the square and triangle objects were similar, but the height and the shape were different. The size of all of the square objects were identical. Each object was used only once/day. At the end of each day, the objects were thoroughly cleaned and dried. To reduce potential biases due to preferences for a particular object, the order of the objects used per subject per session was determined randomly. Supporting Information Figure 3 shows that the animals exhibited no preference for the objects themselves. Exploration was defined as the animal directing its nose within 2 cm of the object while looking at, sniffing, or touching it (Bertaina-Anglade et al., 2006; Ferguson et al., 2010; Tan et al., 2012). The animals' exploratory activity were recorded and subsequently scored by two different experimenters who were blind to the treatment. Exploration time was defined

as the total time spent with an object and was analyzed with paired student's *t*-test. A *P* value of <0.05 is considered significant.

Experimental procedure

First we established the behavioral protocol in drug free, no METH/saline injection animals (group 1). (a) Habituation period: the animals were habituated for thirty minutes. No object is present during habituation. (b) Familiarization period: the animals were familiarized for 12 min with two identical objects. (c) Retention time: the animals were returned to their home cage for 1 h. (d) Novel Spatial recognition (NSR): the animals were returned to the box for 3 min; the location of the object is new. This was followed by (e) retention time. The animals were returned to their home cage for 1 hr; (f) test of novel object recognition (NOR): the animal is returned to the box for 3 min; one of the objects is new. The animals exhibited no preference for a specific shape or location of the objects, and had higher exploration of novel object, novel location (note: these animals are drug free). To ascertain the reproducibility of this protocol in drug free, control animals, the NOR and NSR were determined at multiple retention times. The recorded NOR and NSR values remained significantly different (paired *t*-test, $P < 0.05$) for the retention times of 12 min ($N = 5$), 30 min ($N = 5$), 45 min ($N = 5$), and 60 min ($N = 5$). Finally, we asked whether this test of short-term memory is sensitive to scopolamine pretreatment. Consistent with previous reports, the animals' preference for novel location and novel objects were blocked by scopolamine pretreatment (0.5 mg/kg, and 1 mg/kg, i.p.) (Akkerman et al., 2012; Sambeth et al., 2007; van Goethem et al., 2012). After establishing a reproducible behavioral protocol, these animals were humanely euthanized and were not used for any other experiment. Therefore, for measurement of NOR and NSR in groups 3 and 4 (METH/saline injections) the following protocol was used: 30 min habituation, 12 min familiarization, 60 min retention followed by 3 min test of NOR or NSR as described above (Fig. 2).

Statistical analysis

Data were analyzed using GraphPad Prism software (Version 5.01, Graphpad Software, Inc., La Jolla, CA). Differences between groups were determined by paired, two-tailed Student's *t*-test. A *P* value of <0.05 was considered significant. Biochemical data were analyzed using repeated measures ANOVA, followed by Newman-Keuls analysis. The electrophysiology data was analyzed for statistical significance with an unpaired *t*-test.

RESULTS

The protocol shown in Figure 1 was used to study the effects of noncontingent METH exposure on short-term memory and hippocampal physiology in juvenile mice

Four- to 5-week-old C57BL/6J male mice were handled for 5 days and received daily intraperitoneal (i.p.) injection of a neurotoxic regiment of 24 mg/kg/day METH or saline for 14 days followed by 21 days of drug abstinence. The NOR and NSR were measured on day 0 (baseline) as well as after 7 and 14 days of drug exposure as described in the Materials and Methods. On day 14, some of the animals were sacrificed for LTP measurement ($N = 12$; 6 METH, 6 saline) and biochemical analysis ($N = 12$; 6 METH, 6 saline) as described in the Materials and Methods. The rest of the animals ($N = 16$) continued to the drug abstinence phase of the study where the NOR and NSR were measured on days 21, 28, and 35 followed by LTP measurement ($N = 16$; 8 METH, 8 saline) analysis.

One of the indicators of METH-induced neurotoxicity is a decrease in the tyrosine hydroxylase (TH) protein levels in the brain (Armstrong and Noguchi, 2004; Cadet et al., 2011; Fumagalli et al., 1998; O'Callaghan and Miller, 1994; Sonsalla et al., 1996; Wallace et al., 1999). Consistent with these reports, the noncontingent neurotoxic METH exposure

produced signs of neurotoxicity as shown by a decrease in tyrosine hydroxylase (TH) protein levels in the cortex, hippocampus, and striatum of METH exposed animals (saline compared to METH exposed animals $P<0.05$) (Fig. 3, top panel) (Armstrong and Noguchi, 2004; Cadet et al., 2011; Fumagalli et al., 1998; O'Callaghan and Miller, 1994; Sonsalla et al., 1996; Wallace et al., 1999).

It has been shown that METH exposure alters DAT protein levels in the brain (Baucum et al., 2004; Hastrup et al., 2003). In the striatum of METH exposed animals, we measured a decrease in the low molecular weight of DAT protein species, 75 KDa, (low MW of DAT), and an increase in the high molecular weight of DAT protein species, ~100 KDa, (high MW of DAT) (saline compared with METH exposed animals $P<0.05$) (Fig. 3, lower panel).

In the cortex of METH exposed animals, we detected an increase in the high MW of DAT protein species, but no change in the low MW of DAT protein species (saline compared with METH exposed animals $P<0.05$) (Fig. 3, lower panel). In the hippocampus of METH exposed animals, a decrease in the low MW of DAT protein species was detected without a change in the high MW of DAT protein species (saline compared with METH exposed animals $P<0.05$) (Fig. 3, lower panel). Therefore, consistent with the literature, it seems, the METH exposure paradigm used in this study produces similar signs of neurotoxicity. Next, we examined the consequence of METH exposure on short-term memory and hippocampal neurotransmission.

Fourteen days of daily neurotoxic dose of METH does not alter short-term memory as measured by NOR and NSR

The initial exploration of the animals did not differ during the familiarization session ($N=12$ group mean $\pm$ SE) (Figs. 4 and 5). In addition, the exploration ratios of mice exposed to daily noncontingent neurotoxic METH regimen (mean $\pm$ SEM) or saline control ($N=12$ per group, mean $\pm$ SEM) were similar (novel+familiar) during familiarization or the test phase of NOR and NSR tests (Figs. 4 and 5). Surprisingly, at 7 and 14 days of daily saline or noncontingent neurotoxic METH regimen, both groups of animals displayed similar object and spatial recognition memory. The percent total exploration time for novel and familiar object and location was significant for both treatment groups at baseline (no drug treatment) as well as after 7 and 14 days of daily saline or METH exposure (Figs. 4 and 5A–5C, $N=10$ –12 paired t -test, $P<0.05$). These results are consistent with the interpretation that active noncontingent neurotoxic METH exposure does not immediately affect novel object and novel spatial recognition memory.

Spatial but not novel object memory is disrupted after METH abstinence

Figures 6 and 7 compare percent total exploration time for novel and familiar object and novel and familiar location of the object during drug abstinence period (NOR and NSR respectively). The NOR and NSR of animals were examined following METH/saline abstinence.

Drug abstinence did not affect the novel object memory. METH- and saline-exposed animals differentiated between the novel versus familiar object during the drug abstinence period as indicated by the significant difference between percent total explorations for novel vs. familiar object (Figs. 6A–6C, $N=8$, *denotes paired t -test, $P<0.05$). However, after 7 days of drug abstinence the METH-exposed animals could not differentiate between the novel versus familiar location of the object. There is no difference between the percent exploration time for novel and familiar locations of the object (Figs. 7A–7C) ($N=8$ per group, *paired t -test, $P<0.05$). This disruption in the spatial recognition memory continued up to 3 weeks of

drug abstinence (day 35) (i.e. 3 weeks of drug abstinence) (Figs. 7A–7C, $N = 8$, *paired t -test, $P < 0.05$).

Synaptic plasticity is unaltered after 14 days of noncontingent METH exposure; however, 21 days after METH abstinence synaptic dysfunction occurs

Previously, we have shown that *ex vivo* METH exposure reduces synaptic transmission in the CA1 subregion of mouse hippocampus. Therefore, in parallel experiments we investigated whether the measured METH-mediated disruption of spatial memory correlates with METH-modulation of hippocampal synaptic transmission. The possible METH-induced changes in the synaptic transmission in the CA1 hippocampal subregion was evaluated via field potential recordings (fEPSPs) evoked by stimulation of Schaffer collateral/commissural fibers in mice exposed to either METH or saline for 14 days ($N = 6$ per group) and after 21 days of drug abstinence ($N = 8$ per group) as described in Figure 1 (Figs. 8A and 8B).

Fourteen days of noncontingent METH exposure did not alter either the LTP or paired-pulse facilitation in the CA1 hippocampal subregion (Figs. 8C and 8E, $N = 6$ per group METH/saline). The results correlate with the finding that 14 days of noncontingent METH exposure does not alter short-term memory as measured by novel object and novel spatial recognition tasks. The LTP and paired-pulse facilitation in the CA1 hippocampal subregion were altered after 21 days of drug abstinence (Figs. 8D and 8F, $N = 8$ per group METH/saline). The changes in the hippocampal synaptic transmission long after drug abstinence correlate with the disruption of spatial memory reported in METH-exposed animals.

DISCUSSION

Chronic METH exposure can lead to long-lasting cognitive deficits in humans addicted to METH and in animal models of METH addiction. The biological elements involved in the development and recovery from METH-induced cognitive deficits are less understood. Here, we examined the impact of noncontingent METH exposure on hippocampal electrophysiology and short-term memory performance using novel object recognition (NOR) and novel spatial recognition (NSR) tasks. We found that the untoward cognitive consequences of METH exposure on hippocampal neurotransmission and short-term memory likely did not occur during daily drug exposure (Figs. 4, 5, and 8C). Remarkably, we found that after drug abstinence, METH-exposed mice exhibited a deficit in spatial memory and a decrease in hippocampal neurotransmission (Figs. 7 and 8D). Therefore, our data suggest that the deleterious consequences of neurotoxic METH exposure on short-term memory and hippocampal neurotransmission manifest during drug abstinence period.

Accumulating preclinical evidence indicates that neurotoxic levels of METH exposure induces longterm changes in brain chemistry (Belcher et al., 2008; Boger et al., 2007; Cadet et al., 2007; O'Callaghan et al., 2008; Volz et al., 2007). The measured METH-induced biochemical changes found in the current study are consistent with previous reports (Belcher et al., 2005, 2006, 2008; Bisagno et al., 2002; Schroder et al., 2003). Therefore, regardless of the experimental design, the long-term neurotoxic METH exposure results in profound neurochemical deficits such as cortical and striatal reductions in DAT and tyrosine hydroxylase, accompanied by elevated glial fibrillary acidic protein in these regions (Keller et al., 2011; Krasnova et al., 2010; O'Callaghan et al., 2008; Sailasuta et al., 2010; Schwendt et al., 2009; Segal et al., 2003).

The hippocampus is a critical structure in methamphetamine modulation of the reward circuit (Hori et al., 2010; Keleta and Martinez, 2012; Onaivi et al., 2002; Ricoy and Martinez, 2009). The hippocampus receives neural projections from the VTA (Gasbarri et

al., 1997; Scatton et al., 1980) and sends glutamatergic efferents to the nucleus accumbens (French and Totterdell, 2003). Recent studies support the possibility that METH-induced neurotoxicity may not lead to METH-mediated cognitive deficits. For example, a 2-week neurotoxic regimen of METH significantly decreases striatal dopamine levels without evidence of any cognitive impairment (Marshall et al., 2007). Consistently, Marshall et al. (Belcher et al., 2005) have shown that even a 65% decrease in striatal DA nerve terminals following an acute METH exposure paradigm fails to disrupt visual recognition memory (Belcher et al., 2005). Therefore, the finding that 14 days of daily neurotoxic dose of METH does not alter hippocampal synaptic transmission and short-term memory essentially confirms these observations and opens the possibility that METH-induced impairments to memory function may require a longer than expected time to manifest. One can hypothesize that either monoamine terminal injury did not reach a critical threshold to cause a measurable decrease in METH-induced cognitive impairments, or it is possible that during active drug exposure undefined compensatory mechanisms are triggered, which masked or alleviated the overall decline in behavioral and electrophysiological measures. The latter hypothesis is supported by the reported tolerance to the neurotoxic effect of METH. For example, Fleckenstein's group has shown that a high-dose of METH administration (40 mg/kg/injection) decreased striatal dopamine uptake and DAT ligand binding. These effects were blocked if animals received six biweekly methamphetamine pretreatments (15 mg/kg; s.c.). This neuroprotection was not due to pharmacokinetic tolerance (Riddle et al., 2002). The pre-exposure to METH also attenuated the hyperthermia caused by the neurotoxic regimen of the drug (Riddle et al., 2002; Segal et al., 2005; Volz et al., 2007).

Our finding supports the idea that METH exposure can cause a persistent neurocognitive deficit that directly correlates with a decrease in hippocampal neurotransmission, as we have shown in operationally- and temporally-matched fashion using the same animal. We measured a persistent decrease in novel spatial recognition task after 7, 14, and 21 days of drug abstinence. This impairment in spatial memory was accompanied with a decrease in the LTP of the CA1 subfield of hippocampus and alteration of the paired pulse facilitation, suggesting a presynaptic mechanism. These findings are supported by previous reports that methamphetamine can cause longlasting changes in neuronal systems (Cadet, 2001, 2005) and alter synaptic plasticity (Kauer and Malenka, 2007; Thompson et al., 2002). There have been a number of reports on the effects of cocaine on hippocampal synaptic transmission (Del Olmo et al., 2006a,b; Guan et al., 2009; Thompson et al., 2004), and there are some reports showing that METH alters synaptic transmission in the striatum (Nishioku et al., 1999) and hippocampus (Onaivi et al., 2002; Swant et al., 2010). In this study, the pairedpulse facilitation (PPF) protocol consisted of two pulses at baseline intensity separated by 50 ms, followed by baseline stimulation, and three 100 Hz pulses with a 30-s interstimulus interval for LTP induction. Future studies need to examine whether other patterns of stimulation can produce similar results as presented in the current study.

In summary, this work provides evidence that longterm neurotoxic regimen of methamphetamine may manifest long after drug abstinence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Contract grant sponsor: NIH; contract grant numbers: NIDA DA026947; NINDS NS071122.

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Fig. 1.

The protocol used to study the consequences of non-contingent METH exposure on short-term memory and hippocampal physiology in juvenile mice. Four- to 5-week-old C57BL/6J male mice received daily intraperitoneal (i.p.) METH or saline injection for 14 days followed by 21 days of drug abstinence. The arrows indicate the time points for short-term memory and LTP measurements.

Fig. 2. Schematic drawing of novelty preference paradigm used in this study. The circle indicates higher exploration of novel object and novel location.

Fig. 3.

Fourteen days exposure of neurotoxic levels of METH alters DAT and TH protein levels in the cortex, hippocampus and striatum. Representative immunoblots show TH and DAT protein levels in the cortex, hippocampus and striatum after 14 days of METH/saline exposure. Data presented as mean±SEM. ** $P < 0.05$ versus control. Data are analyzed by ANOVA followed by Newman-Keuls analysis, $N = 6$ per group.

Fig. 4.

Active METH exposure does not alter short-term memory as measured by NOR. **A:** On day 0 (baseline), untreated mice explored the novel object more than the familiar object suggesting the animals can remember the novel object. **B** and **C:** This preference continued after 7 (B) and 14 (C) days of METH and saline treatment. ($N=12$ group, exploration time with the novel object compared with the familiar object, *paired t -test, $P<0.05$).

Fig. 5.

Active METH exposure does not alter short-term memory as measured by NSR. **A:** On day 0 (baseline), untreated mice explored the novel location of the object more than the familiar location suggesting the animals can remember the novel location. **B and C:** This preference continued after 7 (B) and 14 (C) days of METH and saline treatment. ($N = 12$ per group, exploration time for the novel location compared to the familiar location, *paired t -test, $P < 0.05$).

Fig. 6.

Drug abstinence does not disrupt novel object memory. **A–C:** Both METH and saline treated animals preferred the novel object as measured by a significantly longer exploration time of the novel object at 7 (A), 14 (B), and 21 (C) days after METH abstinence ($N = 8$ per group, exploration time with the novel object compared to the familiar object, *paired t -test, $P < 0.05$).

Fig. 7.

Drug abstinence disrupts novel spatial memory. After 7 (**A**), 14 (**B**), and 21 (**C**) days of drug abstinence, the METH-exposed animals did not remember novel versus familiar location of the object and explored both locations similarly ($N = 8$ per group, exploration time for the novel location compared with the familiar location, *paired t -test, $P < 0.05$).

Fig. 8. Synaptic plasticity is unaltered after 14 days of noncontingent METH exposure; however, 21 days after METH abstinence synaptic dysfunction occurs. **A** and **B**: There is no difference in the stimulus-response of fEPSP slope measurements recorded in the CA1 region of the hippocampus after 14 days of METH/saline exposure and after 21 days of METH/saline abstinence. In all panels, the red triangles represent METH-treated animals and the blue circles denote saline-treated animals. Error bars depict $\pm$ SEM in all panels. **(C)**: LTP is unaffected after 14 days of noncontingent METH exposure as compared with saline-treated animals. Data shown as normalized fEPSP slope measurements recorded in the CA1 region

of the hippocampus. Baseline stimulus intensity adjusted to elicit ~50% of maximal response; tetanus was given at 25 min. C: Lower panel depicts 10 averaged representative responses (40 ms), thin line represents response from baseline, thick line represents response during LTP, 1 mV between lines (blue sweeps denote saline, red sweeps denote METH). (D): LTP is decreased in METH-exposed animals after 21 days of METH abstinence. Measurements performed as described above in (C). D: Lower panel depicts 10 averaged representative responses (40 ms), thin line represents response from baseline, thick line represents response during LTP, 1 mV between lines (blue sweeps denote saline, red sweeps denote METH). (E): There is no change in paired-pulse facilitation after 14 days of noncontingent METH exposure. (F): Paired-pulse facilitation is altered after 21 days of METH abstinence. ($N = 6$ per group for 14 days of METH/saline exposure groups; $N = 8$ per group for METH/saline abstinence groups, *unpaired t -test, $P < 0.05$).