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Methamphetamine Causes Persistent Immune Dysregulation: A Cross-Species, Translational Report

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Abstract

Methamphetamine (MA) dependence causes serious cognitive impairments that can persist during abstinence and negatively affect recovery outcomes. Evidence suggests that immune factors, such as cytokines, chemokines, and cellular adhesion molecules, contribute to MA-induced immune dysfunction, neuronal injury, and persistent cognitive impairments, yet the role of MA-induced brain inflammation remains unclear. To address this question, we used a cross-species, translational approach. Thirty-two male C57BL/6J mice were administered MA (1 mg/kg) or saline subcutaneously for seven consecutive days. Mice were euthanized at 72 h or 3 weeks after the last drug dose, and blood and brain samples were collected. In addition, 20 adults in remission from MA dependence and 20 non-dependent controls completed neuropsychological assessments and a blood draw. Multiplex assays were used to measure cytokine, chemokine, and intercellular adhesion molecule (ICAM-1) expression in mouse and human samples. A number of significant MA-induced changes in neuroimmune factors were observed. Of particular interest were the chemokine monocyte chemoattractant protein 1 (MCP-1) and the cellular adhesion molecule ICAM-1, which were similarly increased in the plasma of MA exposed mice as well as humans. In human participants, MA-induced changes in the cytokine and chemokine milieu were accompanied by increased cognitive impairments. Mice showing MA-induced changes in peripheral immune molecule expression also had significant brain-region specific changes in pro-

inflammatory cytokines, chemokines, and ICAM-1. This cross-species, translational study suggests that chronic CNS immune dysregulation may in part contribute to the longstanding neuropsychiatric consequences of MA dependence.

Keywords

Methamphetamine; Substance abuse; Adhesion molecules; Cytokines; Neuroinflammation; Chemokines

Introduction

Methamphetamine (MA) is a highly addictive psycho-stimulant that causes long-term structural damage to regions of the brain that regulate mood and cognitive function (Berman et al. 2008). Patients seeking treatment for MA abuse and dependence often experience serious neuropsychiatric complications, including cognitive impairments, which can remain after discontinuation of use (Volkow et al. 2001) and negatively affect recovery outcomes (London et al. 2004). In 1975, Schuster and Fischman published the first report demonstrating long-term toxicity due to MA exposure, yet the mechanisms contributing to this sustained damage remains speculative. Recent research shows that neuroimmune factors, such as cytokines, chemokines, and cellular adhesion molecules, may play a critical role in perpetuating MA-induced neuronal injury and neuropsychiatric impairments (Narita et al. 2008; Tocharus et al. 2010; Loftis et al. 2009).

At the cellular level, MA use can (1) compromise the blood brain barrier and increase its permeability (Volkow et al. 2001; Bowyer and Ali 2006; Kiyatkin et al. 2007); (2) generate toxic monoamine metabolites [i.e., dopamine (DA)-quinones] (London et al. 2004; Miyazaki and Asanuma 2009); (3) increase the activation of microglia and astrocytes producing pro-inflammatory cytokines and chemokines (Schuster and Fischman 1975; Sekine et al. 2008; Yamamoto and Raudensky 2008; Gonçalves et al. 2010); (4) cause oxidative stress and the over-production of both reactive oxygen and reactive nitrogen species (Narita et al. 2008; Cadet et al. 2007; Yamamoto et al. 2010); (5) damage myelin/white matter tracks (Berman et al. 2008; Tocharus et al. 2010); (6) and alter gray matter density (Schwartz et al. 2010). To date, however, there is limited evidence describing the effects of MA on brain region-specific chemokine or cytokine expression.

Multiple lines of evidence indicate that activated microglia and astrocytes contribute to neurodegeneration through pro-inflammatory processes, including the production of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6, or through oxidative mechanisms (Yamamoto and Raudensky 2008; Kuhn et al. 2006). Methamphetamine administration in rodents induces microglial activation in neostriatum, hippocampus, and other brain regions and contributes to neurodegeneration (Bowyer and Ali 2006; Thomas et al. 2004). At pathophysiologically elevated levels, TNF- α and IL-1 β have both been shown to impair normal neuronal plasticity and to inhibit long-term potentiation (LTP) (Butler et al. 2004; Cunningham et al. 1996).

Chemokines [e.g., monocyte chemotactic protein (MCP-1), macrophage inflammatory protein-1 α (MIP-1 α), and MIP-1 β] also play a critical role in brain function, interact with other neurotransmitter and neuropeptide systems, and influence inflammatory processes and neurogenesis. Importantly, the chemokine system can alter the actions of drugs of abuse, including opioids and cannabinoids (Adler and Rogers 2005; Benamar et al. 2008).

A number of studies in humans have shown that increases in peripheral levels of pro-inflammatory cytokines and chemokines are associated with depression (Pucak et al. 2007; Loftis et al. 2008), anxiety (Figueiredo-Braga et al. 2009), and cognitive impairments (van Exel et al. 2003; Meyers et al. 2005; Hilsabeck et al. 2010). Cytokines and other immune molecules impact neuropsychiatric functions such as mood and cognition in part through their modulation of neuronal anatomy and function. Recently, Sekine et al. (2008), observed a global pattern of microglial activation and microgliosis in the brains of MA abusers that persisted for at least 2 years into abstinence. Our group and others have shown that these neuronal and neuroanatomical changes are associated with significant cognitive impairments that persist even after remission (Berman et al. 2008; Schwartz et al. 2010; Scott et al. 2007; Hoffman et al. 2008; Loftis and Huckans 2010). Collectively, these findings suggest that MA may exert its negative effects on neuropsychiatric functioning, in part, via immune dysregulation and neuroinflammatory processes.

This translational study was designed to test the hypothesis that mice treated with MA will show peripheral and CNS immune dysregulation. Specifically, we predicted plasma and brain-region-specific alterations in the expression of cytokines (i.e., IL-1 β , IL-2, IL-6, IL-10, and TNF- α), chemokines (i.e., MCP-1, MIP-1 α , and MIP-1 β), and the cellular adhesion molecule, ICAM-1. We additionally hypothesized that human adults in early recovery from MA dependence would show a similar pattern of peripheral immune dysregulation and that these MA-induced changes in immune factor expression would be accompanied by increased cognitive impairments.

Methods and Materials

Mice and Drug Treatment Regime

Thirty-two male, C57BL/6J mice (body weight 20–26 g) (Jackson Laboratory, Bar Harbor, ME) were randomized to one of four groups: (1) MA-treated followed by 72 h of abstinence [MA-early recovery (MA-ER) group], (2) saline (SAL)-treated followed by 72 h without injections (SAL-ER group), (3) MA-treated followed by 3 weeks of abstinence [MA-late recovery (MA-LR) group], and (4) SAL-treated followed by 3 weeks without injections (SAL-LR). MA (1 mg/kg) or SAL (0.09%) was administered subcutaneously to mice for 7 consecutive days. This drug treatment schedule was selected because it consistently induces cognitive impairments in mice (Kamei et al. 2006; Ito et al. 2007; Mizoguchi et al. 2008; Lu et al. 2009). The 3-week period of withdrawal from MA was used to approximate the average duration of abstinence reported by our human research participants (53.3 ± 24.4 days). The animals were housed under controlled environmental conditions with food and water ad libitum.

Seventy-two hours or 3 weeks after the last drug dose, mice were euthanized by carbon dioxide inhalation and rapid decapitation. Blood samples were immediately collected, centrifuged for plasma isolation, and stored at -80°C until assayed for multiplex determination of inflammatory markers. All the experiments using mice were reviewed and approved by the Institutional Animal Care and Use Committee at the Portland VAMC.

Human Research Participants

Forty research participants (20 adults in early recovery from MA dependence and 20 non-dependent controls) gave informed consent and were enrolled in this study. The protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the Institutional Review Boards at the Portland Veteran's Affairs Medical Center (VAMC) and Oregon Health & Science University.

Participants were recruited through the Portland Veteran's Affairs Medical Center (VAMC) or the community. General exclusion criteria included: (1) history of a major medical illness that was likely to be associated with serious neurologic, cognitive, or immune dysfunction (e.g., stroke, traumatic brain injury, hepatitis C, and HIV); (2) on the day of testing, use of alcohol, illicit substances, or medications with acute cognitive effects such as sedation or intoxication (e.g., benzodiazepines, opiates, muscle relaxants, and anticholinergic); (3) history of schizophrenia or schizoaffective disorder, current psychiatric or manic episode or currently severe or unstable Axis I psychiatric disorder; (4) positive urinalysis drug screen performed by the Portland VAMC at the time of the study visit. Additional exclusion criteria for the non-dependent control group included Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition-Text Revision (DSM-IV-TR) (American Psychiatric Association 2000) criteria for substance dependence (other than nicotine or caffeine dependence) in the past year. Additional inclusion criteria for the MA group included (1) DSM-IV-TR criteria for MA dependence; (2) use of at least 0.5 g/day for >5 days a week for >2 years; (3) abstinence from MA for >2 weeks and <160 days; and (4) no dependence within the past 5 years for any other substance besides nicotine, caffeine, or MA. All of the participants were compensated with cash (\$30) or a grocery store voucher (\$50) for their participation in the study.

The MA-dependent and non-dependent control groups were not significantly different in terms of age, gender, race, education, or body mass index (BMI) (Table 1). The MA group was dependent on MA for a mean of 11.7 ± 8.1 years, with an average of 53.3 ± 24.4 days of abstinence. Age of first MA use was an average of 18.2 ± 4.8 years.

Human Experimental Procedures

Cognitive function was assessed using a battery of well-validated and widely available neuropsychological subtests. Because participants were from either of two parent studies with different neuropsychological batteries, subtests with similar content and administration procedures [e.g., Digit Span from the Wechsler Adult Intelligence Test, Third Edition (WAIS-III) (Wechsler 1997) and Digit Span from the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph 1998)] were analyzed together in the present analyses, and all subtest scores were converted to z scores using well-validated norms as summarized by cognitive domain in Supplementary Table 1 (S1). Global and domain-specific average z scores were calculated, with domains adapted from those used in other studies with MA users (Scott et al. 2007; Letendre et al. 2005).

Blood samples were collected on the same day as the neuropsychological assessments [average time of day 1:14 pm (1.21 SD)]. Blood was drawn by venipuncture for plasma determinations of cytokines, chemokines, and cellular adhesion molecule levels (BD Vacutainer Systems, Franklin Lakes, NJ). The blood was centrifuged at 2000 RCF for 20 min, and plasma was collected, immediately aliquoted in polypropylene tubes (Phenix Research Products, Hayward, CA), and frozen at -80°C until assayed.

Determination of Peripheral Immune Factor Levels in Mouse and Human Plasma

To assess peripheral immune status, levels of cytokines (i.e., IL-1 β , IL-2, IL-6, IL-10, and TNF- α), chemokines (i.e., MCP-1, MIP-1 α , and MIP-1 β), and the intercellular adhesion molecule (i.e., ICAM-1) were measured in duplicate by commercial assay services (AssayGate, Inc., Ijamsville, MD) using bead-based suspension protein arrays and the Bio-Plex 200 Bead Reader System.

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Assessment of Mouse Brain Neuroimmune Factors

Brains from euthanized mice were rapidly collected and microdissected on dry ice to collect frontal cortex, neostriatal, and hippocampal samples. These regions were selected as they are critical for cognitive functioning and have been shown to sustain longlasting neurochemical and neuroanatomical changes following MA exposure (Bowyer and Ali 2006; Thomas et al. 2004).

Brain tissue was stored at -80°C until further analysis for the determination of inflammatory markers. Levels of cytokines (i.e., IL-1 β , IL-2, IL-6, IL-10, and TNF- α), chemokines (i.e., MCP-1, MIP-1 α and MIP-1 β), and ICAM-1 were measured in duplicate by commercial assay services (AssayGate, Inc., Ijamsville, MD) using the same method that was used for the plasma samples. The protein concentrations for the tissue homogenates were similarly determined by AssayGate, Inc. Data are reported as mean pg per mg total protein.

Data Analysis

Mixed-effects models were used to estimate treatment effects of MA over time in plasma and different brain regions. A random intercept was included in mixed-effects models to account for potential correlations among all molecule expressions within a mouse or a human participant.

Spearman rho correlations were computed between immune factors and cognitive function scores in human participants. Student's *t*-tests were used to assess group differences on the cognitive measures. A two-sided *P*-value less 0.05 was defined as statistical significance; *P*-values less than 0.10 were noted as trends.

Results

Methamphetamine Exposure Altered the Expression of Peripheral Immune Factors in Mice and Humans

In the murine ER groups, a significant effect of MA was observed for plasma levels of IL-6 ($P = 0.003$) and IL-10 ($P = 0.049$) (Table 2), with IL-6 significantly lower and IL-10 significantly higher in MA-exposed mice. A nonsignificant trend was also observed for MIP-1 α ($P = 0.062$) and for IFN- α ($P = 0.063$), as mice administered with MA had higher plasma levels of MIP-1 α and IFN- α compared to controls. Recovery time-dependent changes in the expression of immune markers were also observed in mouse plasma. In the murine LR groups, MA-exposed mice showed significantly higher ICAM-1 ($P = 0.007$) and MCP-1 ($P = 0.002$) levels, as compared to the SAL groups (Table 2).

Human research participants in remission from MA showed a similar pattern of altered cytokine expression as the murine MA-LR group. MA-dependent adults evidenced non-significant trends toward higher levels of ICAM-1 ($P = 0.090$) and MCP-1 ($p = 0.092$) compared with non-dependent controls (Table 2).

The Frontal Cortex, Neostriatum and Hippocampus Showed Significant Changes in Cytokines, Chemokines and ICAM-1 Expression During Abstinence from MA

To understand the effects of MA-induced peripheral immune dysregulation on brain, multiplex assays were performed. Frontal cortex, neostriatal and hippocampal tissue from mice treated with MA or SAL were analyzed for recovery time-dependent changes in the expression of neuroimmune markers. A number of brain region-specific changes in neuroimmune molecule expression were identified (Fig. 1). In the frontal cortex, for the ER but not the LR groups, significant treatment effects of MA were found for IL-1 β ($P = 0.045$) (Fig. 1a) and MCP-1 ($P = 0.035$) (Fig. 1b), with a trend for IL-10 ($P = 0.053$) (*data not*

shown). MA exposure resulted in decreases in these three molecules in the MA-ER group, compared to the SAL-ER group. The neostriatum also evidenced a significant effect of MA on cytokine expression for the ER groups but not the LR groups, with the MA-ER group showing significantly higher IL-2 ($P = 0.009$) levels as compared to the SAL-ER group (Fig. 1c).

In the hippocampus, a number of significant treatment effects of MA on cytokine (Fig. 1d–f), chemokine (Fig. 1g, h) and adhesion molecule (Fig. 1i) expression in the ER groups were found. Unlike the frontal cortex and neostriatum, the hippocampus also evidenced sustained changes in neuroimmune molecule expression at 3 weeks after the last drug dose (Fig. 1j–l). Specifically, IL-1 β ($P = 0.020$) and IL-10 ($P = 0.002$) were significantly increased, and IFN- α ($P = 0.003$) was significantly decreased in the MA-LR group, as compared with the SAL-LR group.

Former MA Users had Increased Cognitive Impairments

In human participants in remission from MA dependence, peripheral immune dysregulation (Table 2) was accompanied by significant increases in cognitive impairment (Table 3). Specifically, compared with the control group, the MA group performed significantly worse in terms of Global Cognition ($P = 0.0004$) as well as Attention ($P = 0.0065$), Memory ($P < 0.0001$), and Learning ($P = 0.0018$).

To test whether MA-induced changes in cytokine, chemokine, and adhesion molecule expressions were associated with cognitive impairments, Global Cognition scores were correlated with the peripheral immune markers (Table 4). Worse Global Cognition was significantly correlated ($r = -0.51$, $P = 0.043$) with higher IFN- α levels in the MA group but not in the control group. Within the control group, no significant correlations between Global Cognitive Function and immune factors were found.

Table 5 further summarizes correlations by cognitive domain within the MA group. Consistent with its effects on Global Cognition, IFN- α was significantly correlated with a number of cognitive domains including Language Fluency, Executive Function, and Learning. Worse performance on Language Fluency was additionally significantly ($P < 0.05$) correlated with higher levels of IL-1 β , IL-6, MCP-1, MIP-1 α , MIP-1 β , and TNF- α . Worse performance on Executive Function was significantly correlated with higher levels of IL-2, with a trend ($p < 0.10$) for higher levels of MIP-1 β . Collectively, these findings suggest that higher level of peripheral pro-inflammatory cytokines (e.g., IFN- α , IL-1 β , and TNF- α) and chemokines (e.g., MCP-1, MIP-1 α , and MIP-1 β) may contribute to the cognitive impairments observed in adults in remission from MA dependence.

Discussion

This cross-species, translational study showed that chronic MA use is associated with significant and longlasting cognitive impairments and immune system abnormalities, suggesting that chronic peripheral and CNS immune dysregulation may in part contribute to these MA-induced behavioral effects. In both mice and humans previously exposed to MA, similar changes in plasma cytokine and chemokine levels were observed (Table 2). For example, in the MA-LR murine treatment group, MCP-1 and ICAM-1 levels were significantly increased relative to controls, and MA-dependent humans showed similar, although non-significant, increases in MCP-1 and ICAM-1. Moreover, in MA-ER mice, plasma levels of IL-6 were decreased, and IL-10 levels were increased compared with SAL-ER mice. These latter data are generally consistent with the suppressive effects of MA on peripheral immune function (Martinez et al. 2009; Saito et al. 2006; In et al. 2005; Yu et al. 2002) as well as the inhibitory effects of MA on transcription factors critical for the

expression of proinflammatory cytokines (Ye et al. 2008), but since these effects were only significant in the ER group, this suggests that the immunosuppressive effects of MA may be most pronounced during active use or early in remission. The question of how MA-induced peripheral immune dysregulation relates to persistent alterations in brain neuroanatomy and function is complex and one that requires a translational research approach to comprehensively address.

As a first step toward answering this question, we administered MA to mice and measured neuroimmune molecule expression at two time points during abstinence (i.e., ER and LR murine treatment groups). Mice in the LR group had 21 days of abstinence from MA, which was comparable with human participants (53.3 ± 24.4 days of abstinence from MA). Using a MA treatment regime that consistently induces cognitive impairments in mice, we found that MA administration caused alterations in the expressions of T-helper cell type 1 (Th1) and Th2 cytokines, chemokines and ICAM-1 in the hippocampus, frontal cortex, and neostriatum of mice (Fig. 1). Early in recovery (i.e., 72 h after the last dose of MA), peripheral immune dysregulation was accompanied by alterations of cytokine and chemokine expression in the neostriatum and frontal cortex that appeared to resolve after 3 weeks of abstinence. However, more enduring changes in neuroimmune molecule expression were seen in the hippocampus, a brain region shown to sustain long-term microglial activation due to MA exposure (Gonçalves et al. 2010) as well as express high concentrations of pro-inflammatory cytokine receptors, specifically receptors for IL-1 β and IL-6 (Cunningham et al. 1992; Parnet et al. 1994; Schöbitz et al. 1993).

The hippocampus may be particularly sensitive to cytokine-induced injury (Swant et al. 2010) and explain why more enduring changes in neuroimmune factor expression were not observed in the neostriatum and frontal cortex of mice in the MA-LR group. Several studies show that regions of the caudate-putamen (e.g., nucleus accumbens and medial aspects of the striatum) exhibit remarkable resistance to MA-induced microglial activation and neurotoxicity (Fukui et al. 1986; Hirata et al. 1996; Harvey et al. 2000; Thomas et al. 2004; Eisch et al. 1992; Cass 1997; Haughey et al. 2000). We additionally found that in MA-treated mice, the hippocampus was characterized by a more pro-inflammatory cytokine profile (e.g., increased IL-1 β , IL-2, and IL-6 levels) as compared with the frontal cortex (e.g., reduced IL-1 β levels).

Although this study is the first to describe brain region-specific differences in the expression of pro-inflammatory cytokines after MA, regional heterogeneity in CNS inflammatory responses is observed with other drugs of abuse (Fitting et al. 2010), in response to lipopolysaccharide (LPS) exposure (Kipp et al. 2008), and in inflammatory disease models (Archambault et al. 2006). This phenomenon may result from regionally specific differences in glial cell activation and function (e.g., heterogeneous expression of cytokines and chemokines). For example, murine astrocytes cultured from different parts of the CNS (e.g., cortex, cerebellum, and spinal cord) secrete different patterns of inflammatory proteins in response to morphine (Fitting et al. 2010). Similarly, and consistent with our findings in MA-exposed mice, midbrain astrocytes challenged with LPS express higher levels of pro-inflammatory cytokines (e.g., IL-1 α), as compared to LPS-treated cortical astrocytes (Kipp et al. 2008).

Increasing evidence demonstrates that low physiological levels of immune factors such as cytokines are required for normal neuronal plasticity and function, and that disruptions in the balance of these immune factors, including either elevations or reductions from normal physiological levels, can lead to long-term impairments in cognition, mood, and behavior in a variety of conditions (for reviews, see McAfoose and Baune 2009; de Araujo et al. 2009; Khairova et al. 2009; Loftis et al. 2010). In line with this literature, we found that adults in

remission from MA dependence experienced significant cognitive deficits, as compared with adults without a history of MA dependence (Table 3). Moreover, in the MA-dependent adults, higher plasma levels of IFN- α significantly correlated with worse Global Cognition, Language Fluency, Executive Function, and Learning. Higher levels of pro-inflammatory cytokines, IL-1 β , IL-6, and TNF- α also correlated with worse Language Fluency. Consistent with these findings, Thompson et al. (2004) showed that MA use leads to cognitive deficits, which are correlated with decreased hippocampal volumes and white-matter hypertrophies, possible markers of inflammation in the region.

While the cellular source of neuroimmune factors was not determined in this study, it may be that, in addition to glial activation (Yamamoto and Raudensky 2008; Gonçalves et al. 2010; Mizoguchi et al. 2008), immune cells from the periphery also play a role in perpetuating neuroinflammation. Given that MA use alters blood-brain barrier permeability (Bowyer and Ali 2006; Kiyatkin et al. 2007), thereby exposing CNS antigens, it is hypothesized that this can facilitate the development of anti-CNS responses. Consequently, chemokine-stimulated infiltration of circulating macrophages/mononuclear phagocytes might be a factor in mediating the sustained increases in pro-inflammatory cytokine expression and neuropsychiatric symptoms observed following MA. Consistent with this theory, we found that mice exposed to MA had significantly higher levels of MIP-1 β in the neostriatum and of MIP-1 α , MIP-1 β , and MCP-1 in the hippocampus, when compared with SAL-treated mice. MCP-1 is a potent mediator of mononuclear phagocyte recruitment into brain. To date, there is no published information regarding the effects of MA on MCP-1 in brain; however, He and Crews (He and Crews 2008) have observed increased MCP-1 in various regions of the human alcoholic brain, including the hippocampus. Further, MCP-1 is positively correlated with the severity of dementia in patients with HIV (Kelder et al. 1998; Sevigny et al. 2004). In humans recovering from MA dependence, we found that higher plasma levels of MIP-1 α , MIP-1 β , and MCP-1 were significantly correlated with worse Language Fluency.

There exist several limitations to this study. First, our sample size was relatively small, limiting our power to detect significant results, particularly in the case of human participants. Further, although all the multiplex immunoassay samples were run in duplicate, the experiments were conducted only once. Thus, these results should be replicated, ideally using a larger human sample and including groups of female mice. An additional limitation of our study was that the murine and human MA groups were not matched on drug dose or recovery period. Human MA addicts use an average of 1 g of MA per day. Applying the body surface area normalization method to calculate dose translation from humans to animals, 1 g of MA for a human (weighing 60 kg) is equivalent to approximately 206 mg/kg of MA per day for a mouse (Regan-Shaw et al. 2007). The LD50 for MA in mice is 67.8 mg/kg (Numachi et al. 2007). Thus, it was not feasible to equate the doses of MA administered to mice in our study with the amounts used by MA addicts. For our human participants, the average duration of abstinence from MA was 53.3 ± 24.4 days, which may have contributed to greater variance in immune molecule expression and limited our ability to detect significant drug effects. Indeed, mice exposed to 21 days (LR group) as compared with 3 days (ER group) of abstinence from MA evidenced fewer enduring changes in cytokine expression. To delineate acute versus chronic MA-induced mechanisms in immune dysregulation and neuropsychiatric impairments, we are currently conducting a follow-up translational study that includes a group of active MA users, as well as early recovery (>1 month and <1 year abstinence from MA) and non-dependent control groups. Despite these differences in MA dose and recovery period, mice and humans displayed similar changes in immune molecule expression due to MA exposure (Table 2). Further, it was recently found that in humans, MA use parameters (e.g., self-reported age at first use, total years of use,

route of consumption, or length of abstinence) was unrelated to MA-induced impairments in cognitive domains (Cherner et al. 2010).

Taken together, this cross-species, translational study suggests that chronic CNS immune dysregulation may in part contribute to the longlasting neuropsychiatric consequences of MA dependence. More research utilizing larger arrays is needed to determine the respective contributions of the key immune signaling pathways involved.

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Fig. 1.

Seventy-two hours and 3 weeks following drug administration, neuroimmune factors were significantly altered in the frontal cortex, neostriatum and hippocampus of MA- as compared to SAL-treated mice. Mixed-effects models were used to estimate treatment effects of MA over time and in different brain regions. Significant treatment effects of MA were found for IL-1 β ($P = 0.045$) and MCP-1 ($P = 0.035$) in the frontal cortex (**a, b**), for IL-2 ($P = 0.009$) in the neostriatum (**c**), and for IL-1 β ($P = 0.018$) (**d**), IL-2 ($P = 0.001$) (**e**), IL-6 ($P = 0.007$) (**f**), MIP-1 β ($P = 0.021$) (**g**), MIP-1 α ($P = 0.025$) (**h**) and ICAM-1 ($P = 0.000$) (**i**) in the hippocampus at 72 h following the last drug dose. Panels **j–l** show the cytokines that were significantly changed in MA-LR as compared to SAL-LR groups [IL-1 β ($P = 0.020$), IL-10 ($P = 0.002$) and IFN- α ($P = 0.003$)]. * Denotes two-sided P -values less than 0.05

Table 1

Characteristics of research participants

	Control group (<i>n</i> = 20)	Methamphetamine group (<i>n</i> = 20)
Age in years, Mean (SEM)	33.8 (2.65)	33.3 (1.84)
Gender, % males	70%	70%
Education in years, mean (SEM)	13.6 (0.34)	11.8 (0.43)
Ethnicity, % Caucasian	85%	100%
Tobacco use in cigarettes/day (SEM)	4.6 (1.5)	11.3 (1.3)
Body mass index (BMI), mean (SEM)	26.9 (1.16)	27.6 (1.35)

Table 2

Human and murine plasma immune factor levels

Immune markers	Human participants					Mice									
	Control	SD	MA	SD	P	SAL-ER	SD	MA-ER	SD	P	SAL-LR	SD	MA-LR	SD	P
IL1 β	7.1	19.2	3.5	7.7	0.441	5.2	5.3	3.9	3.1	0.688	6.8	11.0	3.2	2.7	0.280
IL2	10.8	21.6	19.5	58.5	0.533	9.6	6.0	31.3	61.3	0.160	7.8	3.9	2.6	3.1	0.737
IL6	30.5	48.5	34.9	82.8	0.838	45.7	37.2	6.4	4.7	0.005***	31.8	29.9	21.3	21.8	0.440
IL10	2.3	4.7	13.7	48.8	0.297	16.3	13.1	38.5	34.7	0.049**	16.8	17.1	24.7	18.3	0.481
IFN- α	106.5	182.6	87.7	133.6	0.712	4.5	0.4	5.1	0.8	0.063*	4.8	0.6	4.7	0.5	0.777
ICAM-1	100701.3	28952.1	117287.0	32553.6	0.090*	30721.6	4797.6	29143.7	4475.6	0.591	26328.4	7816.3	34256.7	5798.5	0.007***
MCP-1	465.0	241.5	606.3	284.6	0.092*	176.8	109.5	312.5	96.6	0.212	201.7	84.1	545.1	400.6	0.002***
MIP-1 α	70.6	85.5	74.5	79.6	0.880	213.5	58.5	355.1	284.6	0.062*	237.6	61.1	240.2	57.2	0.973
MIP-1 β	189.3	230.1	114.2	97.7	0.180	272.2	131.1	393.5	250.7	0.119	288.2	119.9	323.8	46.1	0.646
TNF- α	9.4	12.8	5.6	2.3	0.191	6.9	7.5	15.3	13.2	0.323	17.6	29.5	8.4	8.2	0.284

Values shown are means and standard deviations (SD) in pg/ml;

* $P \leq 0.100$ (trend).

** $P \leq 0.050$.

*** $P \leq 0.010$

Table 3

Between group comparisons of neuropsychological functioning

	MA (<i>n</i> = 20)	Control (<i>n</i> = 20)	<i>P</i> -value
Language fluency	−0.09 ± 1.14	0.26 ± 0.82	0.331
Learning	−0.71 ± 1.18	0.63 ± 0.94	0.001***
Memory	−0.82 ± 0.77	0.55 ± 0.71	<0.001***
Attention/information processing	−0.24 ± 0.93	0.62 ± 0.72	0.006**
Executive function	−0.25 ± 0.66	0.27 ± 0.81	0.058
Global cognition	−0.52 ± 0.73	0.51 ± 0.61	<0.001***

By study group, cognitive functioning (average *z* scores by cognitive domain). Data are expressed as the mean ± standard deviation. *p* values reflect between group comparisons using *t* tests for cognitive domains,

* $P \leq 0.050$.

** $P \leq 0.010$.

*** $P \leq 0.001$

Table 4
Correlations between plasma immune factor levels and global cognition by study group

	ICAM-1	IFN- α	IL-1 β	IL-2	IL-6	IL-10	MCP-1	MIP-1 α	MIP-1 β	TNF- α
MA group ($n = 20$)										
Global cognition	0.36	-0.51 (0.043)*	-0.12	0.21	-0.35	0.12	-0.24	-0.29	-0.02	-0.16
Control group ($n = 20$)										
Global cognition	-0.19	0.27	0.15	-0.41	-0.38	0.04	-0.27	-0.25	-0.28	-0.37

Values are reported as Spearman rho correlations with significant P values in parentheses.

*
 $P < 0.050$

Table 5
Correlations between plasma immune factor levels and cognitive domains in early recovery from MA dependence

	ICAM-1	IFN- α	IL-1 β	IL-2	IL-6	IL-10	MCP-1	MIP-1 α	MIP-1 β	TNF- α
MA group ($n = 20$)										
Language fluency (z score)	0.35	-0.57 (0.028)**	-0.60 (0.017)**	-0.30	-0.62 (0.014)**	-0.34	-0.57 (0.026)**	-0.62 (0.015)**	-0.57 (0.025)**	-0.56 (0.029)**
Memory (z score)	0.37	-0.33	-0.10	0.25	-0.18	0.21	-0.10	-0.15	0.07	-0.006
Executive function (z score)	0.10	-0.55 (0.034)**	-0.30	-0.52 (0.048)**	-0.18	-0.19	-0.18	-0.22	-0.48 (0.074)*	-0.0009
Attention (z score)	-0.09	-0.23	0.16	0.16	-0.25	0.34	-0.13	-0.19	0.12	0.16
Learning (z score)	0.42	-0.58 (0.019)**	-0.02	0.29	-0.30	0.15	-0.12	-0.12	0.10	-0.21

Values are reported as Spearman rho correlations with trending and significant p values in parentheses.

* $P < 0.100$ (trend).

** $P < 0.050$.

*** $P < 0.010$