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Review

Extracellular Vesicles in Methamphetamine-Induced Neurotoxicity: Emerging Implications for Addiction, Withdrawal and Neurological Disorders

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Highlights

- Methamphetamine (METH) alters extracellular vesicle biogenesis, release, and cargo, affecting cell-to-cell communication in the brain.
- METH-associated extracellular vesicles contribute to neuroinflammation, oxidative stress, impaired autophagy, synaptic dysfunction, and neuronal injury.
- Changes in extracellular vesicle (EV) proteins and miRNAs during METH withdrawal and abstinence may contribute to neurobehavioral changes and relapse risk.
- METH-HIV interactions may alter EV cargoes and inflammatory signaling, viral cargo transfer, and HIV-related neuropathogenesis.
- Extracellular vesicle cargo may serve as minimally invasive biomarkers and potential therapeutic targets for METH use disorder and related neurological complications.

Abstract

Methamphetamine (METH) is a highly addictive psychostimulant, and its misuse remains a major public health challenge, with substantial physiological, neuropsychiatric, and behavioral consequences. Recovery is further complicated by withdrawal effects, persistent mental health disturbances, and high risk of relapse. METH induces profound neurobiological changes involving monoaminergic dysregulation, maladaptive synaptic plasticity, oxidative and mitochondrial stress, impaired autophagy, neuroinflammation, and blood–brain barrier dysfunction. Growing evidence suggests that extracellular vesicles (EVs) play an important role in these pathological processes during METH dependence, withdrawal, and relapse. METH can alter EV biogenesis, release, and molecular cargo, including proteins, lipids, and microRNAs. By transferring this bioactive cargo between cells, EVs can influence inflammation, oxidative stress, autophagy, synaptic function, and neuronal viability. This review elaborates on the emerging role of EV-mediated mechanisms in METH addiction, neurological dysfunction, withdrawal, relapse, and comorbid HIV infection. Finally, we discuss the translational potential of EV-associated molecular signatures as minimally invasive biomarkers of METH-related disease states, as well as EV-mediated pathways as potential therapeutic targets. A better understanding of EV signaling may therefore provide new opportunities for monitoring and treating METH use disorder and associated neurological comorbidities.

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1. Introduction

Methamphetamine (METH) is a potent and highly addictive psychostimulant that continues to pose a major public health challenge because of its widespread misuse, severe neuropsychiatric consequences, and growing association with polysubstance use and infectious comorbidities [1,2]. In the United States, METH use has remained closely linked to stimulant-related morbidity and mortality, and this burden appears particularly relevant in populations with unstable access to healthcare and in people living with HIV [3]. Although METH retains limited approved medical use, its strong misuse and the absence of FDA-approved medications for METH use disorder have made it one of the most difficult stimulant addictions to manage clinically [1,2]. The pathological effects of METH extend far beyond its acute psychostimulant properties. At the neurochemical level, METH profoundly disrupts monoaminergic signaling, especially in dopaminergic pathways that regulate reward, motivation, reinforcement, and executive function [1]. By entering presynaptic terminals and disrupting dopamine transporter- and vesicular monoamine transporter-dependent monoamine signaling, METH promotes excessive extracellular dopamine accumulation and triggers maladaptive neuroplastic changes within the mesocorticolimbic system [1]. Repeated exposure alters signaling in the nucleus accumbens (NAc), ventral tegmental area (VTA), and prefrontal cortex, thereby driving compulsive drug-seeking behavior, impaired inhibitory control, and a high risk of relapse [1,4]. These circuit-level abnormalities are accompanied by broader cellular pathology, including oxidative stress, excitotoxicity, mitochondrial dysfunction, endoplasmic reticulum stress, blood–brain barrier (BBB) disruption, impaired axonal transport, and dysregulated autophagy [1].

Alongside its effects on neurotransmission, METH exerts substantial inflammatory and neurotoxic effects in the central nervous system (CNS). Neuroinflammation is now recognized as a core feature of METH-induced brain injury rather than a secondary byproduct of neuronal damage [1,2,5–9]. Both in vitro and in vivo studies show that METH activates resident microglia and astrocytes, promoting the release of pro-inflammatory cytokines, chemokines, reactive oxygen species (ROS), and nitric oxide (NO) that amplify tissue injury and disrupt synaptic homeostasis [9–16]. Several signaling pathways contribute to this response, including Toll-like receptor 4 (TLR4), nuclear factor-kappa B (NF-KB), mitogen-activated protein kinase (MAPK) cascades, and inflammasome-associated mechanisms [2,17–22]. Among these, the NLR Family Pyrin Domain containing 3 (NLRP3) inflammasome has emerged as a key mediator linking cellular stress to caspase-1 activation, interleukin-1 beta (IL-1β) maturation, pyroptotic signaling, and progressive neurodegeneration under chronic METH exposure [23–25]. As a result, METH-induced neuroinflammation is increasingly viewed as a mechanistic bridge between addiction, neuronal dysfunction, and cognitive deficits.

This broader view of METH pathology has generated strong interest in intercellular communication pathways that propagate injury across neural and immune networks. Among these, extracellular vesicles (EVs) have emerged as especially important mediators of intercellular communication. EVs are membrane-bound particles released by all cell types and are broadly categorized into exosomes, microvesicles, and related vesicular subtypes on the basis of size characteristics [26–29]. These vesicles carry proteins, lipids, messenger RNAs, microRNAs, and other bioactive molecules that can be transferred to recipient cells, thereby influencing transcriptional responses, metabolic programs, inflammatory signaling,

and synaptic function [30–33]. Despite several advances in the EV field, careful consideration of nomenclature and methodology is required when EV-based studies are interpreted. Under the current MISEV2023 recommendations, the general term "extracellular vesicle" is preferred when vesicle biogenesis has not been directly established, and classification as exosomes or microvesicles based solely on size, molecular markers, or separation method is not established; further, when cellular origin cannot be deciphered, operational terms such as small EVs (sEVs) and large EVs (lEVs) are recommended [34]. Additional methodological variability is introduced by EV isolation techniques, as differences in recovery and specificity are associated with commonly used approaches, including ultracentrifugation, size-exclusion chromatography, precipitation, and affinity-based methods, as well as proteins, lipoproteins, and other non-vesicular particles that may be co-enriched. Therefore, no single separation method is regarded as universally optimal, and method selection should be determined by sample type, biological question, and downstream application. For EV characterization, the use of complementary approaches is recommended, whereby particle properties and EV-associated components are assessed alongside potential non-vesicular contaminants rather than vesicular identity being inferred from particle size or a limited number of canonical markers alone. Particular caution is required when biological effects are attributed to EVs, as comparisons across studies may be confounded by differences in sample processing, purity, and characterization. Thus, adherence to standardized nomenclature, rigorous characterization, and transparent methodological reporting is required for EV-mediated effects to be interpreted reliably and reproduced across studies [34]. With these methodological considerations recognized, the biological significance of EV-mediated communication within the CNS, where neurons, astrocytes, microglia, endothelial cells, and infiltrating immune cells release EVs that function as versatile mediators of both physiological communication and disease propagation, is now well-recognized [28,29,35–38]. EVs have gained increasing attention in neurodegenerative and neuroinflammatory disorders as they can disseminate pathogenic cargo between cells and across anatomical compartments [39–43]. In several neurological diseases, EVs have been implicated in spreading toxic proteins, amplifying inflammation, altering BBB function, and reshaping neuronal-glial interactions [28,32,44–47]. In the setting of substance use, EVs are particularly relevant because drug exposure can modify both the quantity and molecular composition of vesicles released from brain and peripheral tissues [28,48–51]. Accordingly, EVs are no longer regarded merely as passive byproducts of cellular injury; rather, they are increasingly understood as active participants in pathological signaling networks that may sustain addiction-related plasticity, inflammatory responses, and tissue damage [28,48,52].

Emerging evidence suggests that METH profoundly alters EV biology [53–55]. Human studies have shown that plasma EVs from individuals with METH exposure display altered abundance and distinct microRNA (miR) cargo profiles, including shifts in vesicle-associated RNA cargoes linked to inflammatory and neurobehavioral deficits [53,55–58]. Experimental studies further demonstrate that brain-derived EVs (BEVs) can contribute directly to METH-associated neurotoxicity. For example, chronic METH exposure (intravenous 0.05 mg/kg/infusion by self-administration for 2 h per day for 25 days) has been associated with increased levels of EV-associated miR-29a-3p, which can activate TLR- 7 signaling and promote synaptodendritic injury and neuroinflammatory responses in METH-administered rats [55]. Additional work indicates that EV cargo altered by METH may influence autophagy, oxidative stress, neuronal survival, and glial activation, supporting the concept that vesicle-mediated signaling is a central component of METH neuropathogenesis rather than an epiphenomenon [28,55]. Since EVs are detectable in accessible biofluids such as plasma, they also hold substantial promise as minimally invasive biomarkers for

identifying METH-mediated disorders and monitoring therapeutic response [55,59,60]. EV-mediated communication becomes even more pronounced in HIV infection [61–64]. Despite major advances in antiretroviral therapy (ART), people living with HIV remain susceptible to chronic immune activation, low-grade neuroinflammation, and HIV-associated neurocognitive disorders (HAND) [28,64]. METH use can aggravate this vulnerability through both behavioral and biological mechanisms [65,66]. Clinically, METH use has been associated with worsening HIV-related health outcomes, including enhanced viral load even in the presence of ART and disrupted continuity of ART [3]. Mechanistically, METH can amplify viral and host inflammatory processes, alter frontostriatal circuitry, and worsen neuroimmune dysfunction that accelerates HIV-associated brain injury [67,68]. Notably, studies show that METH exposure can induce HIV-infected cells to release proadhesive EVs enriched in factors such as ICAM-1 and Nef, promoting syncytia formation and providing a plausible vesicle-mediated mechanism for enhanced HIV neuropathogenesis [54,69]. These findings place EVs at a critical intersection between stimulant exposure, viral persistence, and neuroinflammatory damage. Beyond active drug exposure, EVs may also contribute to later phases of METH use disorder, including withdrawal, abstinence, and relapse. Recent evidence from human clinical cohorts of METH use and withdrawal, complemented by human plasma EV-to-mouse transplantation and microglial-neuronal models, suggests that METH use and withdrawal are associated with dynamic alterations in circulating EV (cEV) cargo, including inflammatory proteins and miRNAs [53,70,71]. EV-associated Serum Amyloid A1, an inflammatory protein, is markedly elevated during acute withdrawal and declines during protracted withdrawal. Also, these altered EV miRNA profiles are associated with alterations in neurobiological pathways related to neuronal function, microglial activation, and neuroinflammation, leading to anxiety and depressive symptoms associated with METH withdrawal [53,70,71]. Such findings raise the possibility that EVs not only reflect ongoing neuropathology but may also participate in the persistence of maladaptive brain states after cessation of drug use [52,72,73]. This concept is particularly important for METH use disorder, where relapse risk remains high, and long-term cognitive and affective disturbances continue even after abstinence [1,2].

Taken together, current evidence supports a model in which METH-induced brain injury is shaped not only by direct monoaminergic toxicity but also by EV-mediated communication between neurons, glia, endothelial cells, and peripheral immune compartments. In this framework, EVs may serve simultaneously as conveyors of pathogenic molecules, amplifiers of neuroinflammation, biomarkers of disease state, and potential therapeutic targets. Understanding how METH reshapes EV biogenesis, cargo sorting, and functional signaling may therefore provide critical mechanistic insight into addiction, neurodegeneration, and HIV-associated neuropathology. Accordingly, this review examines the emerging literature on EVs in METH-mediated addiction and neurological disorders, with particular emphasis on neuroinflammatory signaling, withdrawal-related alterations, HIV comorbidity, and the translational potential of EV-based biomarkers and interventions.

2. Epidemiology of METH Use in the USA, with a Focus on Nebraska

METH is an energy- and performance-enhancing substance, or psychostimulant, which was first synthesized in Japan in 1893 and later documented for its use during World War II by both the Allies and Axis powers to keep troops awake. Following World War II, large wartime supplies of METH entered the civilian market in Japan, contributing to the first major epidemic-level METH use [74–76]. METH is a listed Schedule II psychostimulant with high addiction potential according to the United Nations Convention on Psychotropic Substances of 1971 [77]. METH has also been approved by the FDA as a prescription drug for the treatment of attention deficit hyperactivity disorder (ADHD) and short-term

intervention for obesity. Moreover, it has been infamous for its emerging misuse along with opioids like fentanyl [78]. METH has been a leading drug contributing to stimulant-related overdose deaths along with cocaine, accounting for an emerging "fourth wave" of drug-related overdose mortality in the US [79–81]. There are multiple complications associated with METH use, including general symptoms of increased blood pressure, heart rate, loss of appetite, skin sores, hallucinations, aggressive behavioral displays, sleep disturbances, and depression, as well as anxiety, indicating production of a nexus of inflammatory and neurotoxic signals in the brain [82–85]. Thus, METH emerges as one of the most commonly used illicit drugs in the US. The challenge of this menace is compounded due to the lack of any FDA-approved treatment for METH use disorders [86].

Specifically, in the Midwest states of Iowa and Nebraska, METH is regarded as the top unregulated substance [86–88]. Furthermore, METH misuse has been assigned as the "number one" drug threat by the Attorney General of Nebraska, associated with a huge concern for public health in 2022 [88]. An astounding figure from the Drug Enforcement Administration's Omaha field office is that between January and July 2025, Nebraska enforcements seized 138% more METH than in 2024 [86,89]. Under the Fentanyl Free America initiative, the U.S. Drug Enforcement Administration's Omaha Field Division seized an astounding 715 pounds of METH along with 2234 METH pills during a 30-day operation in January-February 2026 [90]. Additionally, according to data reported by the Nebraska Foster Care Review Office, approximately 18% of foster care placements are attributable to parental METH use, indicating the extent of the misuse and worsening societal outcomes [86,91].

3. METH-Induced Addiction, Neurological Deficits, and Its Role in Inflammation

3.1. METH-Induced Addiction

METH is a potent psychostimulant with a high potential for penetration across the BBB and its profound effects on the mesocorticolimbic dopaminergic reward system [92,93]. METH enters presynaptic neurons via the dopamine transporter (DAT) and disrupts vesicular monoamine transporter-2 (VMAT2), resulting in excessive cytoplasmic dopamine accumulation and reverse transport of dopamine into the synaptic cleft. The resulting surge in extracellular dopamine within the NAc, VTA, and prefrontal cortex produces intense euphoria and reinforcement, driving the transition from recreational use to compulsive drug-seeking behavior and addiction [94,95]. Neuroimaging studies in chronic METH users demonstrate reduced striatal DAT density and dopamine D2 receptor availability, alterations in other monoaminergic markers such as VMAT2 and serotonin transporter (SERT), as well as region-specific abnormalities in cerebral glucose metabolism, with some metabolic changes correlating with psychiatric symptom severity [96]. These changes correlate with executive dysfunction, impaired impulse control, persistent craving, and increased relapse risk. The severity of METH-induced toxicity is influenced by multiple factors, including route of administration, dosage, duration of exposure, pharmacological characteristics, genetic polymorphisms affecting drug metabolism, and polysubstance use [97]. Intranasal administration is one of the most common routes among recreational users, which rapidly increases heart rate, blood pressure, and body temperature, contributing to systemic sympathomimetic and hyperthermic toxicity [8,13,98–100]. Acute METH exposure in an in vivo rat model induced metabolic activation, brain hyperthermia, BBB disruption, and structural abnormalities like perineuronal edema, sponginess, and expansion of the cortex, along with damaged neuronal structures (cell body shrinkage, condensed nuclei and cytoplasm) [101], while even sub-neurotoxic doses (0.5–5 mg/kg) significantly elevate extracellular dopamine concentrations in rodents [102].

In a rat behavioral-sensitization model, METH (2.5 mg/kg, once daily for 5 days) produced locomotor sensitization that persisted for 3–14 days after exposure. METH increased phospho-cAMP response element-binding protein (pCREB) in the frontal cortex at day 3, decreased pCREB in the NAc and ventral pallidum at day 14, and increased ΔFosB (a splice variant of protein fosB) in the NAc and ventral pallidum, with the latter persisting until day 14 in the ventral pallidum. These findings link repeated METH exposure to persistent, region-specific CREB/ΔFosB transcriptional adaptations accompanying behavioral sensitization [103]. In another study using a repeated-METH behavioral-sensitization mouse model, Yan et al. found that METH increased phosphorylated extracellular signal-regulated kinase1/2 (pERK1/2), phospho- c-Jun N-terminal kinase (pJNK), and ΔFosB in the caudate-putamen. Luteolin suppressed these molecular changes and reduced both the development and expression of behavioral sensitization, implicating particularly the ERK1/2–ΔFosB pathway in persistent METH-induced behavioral plasticity [104]. Miyazaki et al. (2013) [105] demonstrated that converging dopaminergic and glutamatergic signaling in the NAc plays an important role in METH-induced behavioral sensitization, psychosis-related deficits, and conditioned reward, providing a mechanistic basis for the development of dependence-related and drug-seeking behaviors. Tyrosine hydroxylase heterozygous (TH+/−) and N-methyl-D-aspartate (NMDA) receptor NR2A-deficient (NR2A−/−) mice received METH [1 mg/kg, subcutaneous (s.c.)] once daily for 7 days. Repeated METH produced locomotor sensitization and impaired startle response, assessed by prepulse inhibition (PPI) in mice, whereas these effects were absent in TH+/− and NR2A−/− mice. In the conditioned place preference (CPP) paradigm (a Pavlovian measure in which preference for a drug-paired environment reflects the motivational value of drug-associated cues) a lower METH dose (0.3 mg/kg, s.c.; three 20-min conditioning sessions on days 11, 13, and 15, alternating with saline on days 12, 14, and 16) produced significant drug-seeking behavior on the drug-free day-17 in mice that had previously received METH for 7 days, indicating enhanced METH-seeking behavior even with METH-abstinence; this response was attenuated in both mutant strains. Repeated METH exposure also increased NAc PKA activity as well as calcium/calmodulin-stimulated protein kinase II (CaMKII) and CREB phosphorylation in wild-type mice, but not in either mutant strain. Together, these findings suggest that dopamine and NMDA receptor signaling converge on the PKA/CaMKII/CREB pathway in the NAc to contribute to METH-induced sensorimotor-gating deficits and enhanced METH-seeking behavior, linking repeated METH exposure to neuroplastic changes associated with dependence-related behaviors [105]. In FosB-null mice, METH (four injections of 10 mg/kg METH at 2-h intervals) demonstrated greater neuronal degeneration and BBB dysfunction, indicating that FosB exerts a protective effect against METH-induced neurotoxicity through intracellular feedback signaling and supportive astroglial function [106]. Huang et al. (2018) [107] investigated the role of thioredoxin-1 (Trx-1) in the NAc in METH relapse using male C57BL/6 mice. CPP, which measures learned preference for a drug-associated environment, was established with METH (2.5 mg/kg, i.p.; four 15-min conditioning sessions on days 4, 6, 8, and 10), tested on day 12, and reduced to baseline levels through daily drug-free sessions from days 13–18. Following reduction to baseline levels, Trx-1 expression in the NAc was knocked down using AAV-shRNA-mTrx-1 on day 20. On day 25, METH at 1.0 mg/kg reinstated CPP in all groups, whereas a lower dose (0.5 mg/kg) reinstated CPP only in Trx-1-knockdown mice, suggesting greater sensitivity to METH-induced relapse. This low-dose reinstatement was accompanied by increased GluN2B expression and ERK and CREB phosphorylation in the NAc, changes that were not observed in control mice at the same dose. Together, these findings suggest that reduced Trx-1 in the NAc lowers the threshold for METH-primed reinstatement by engaging GluN2B/ERK/CREB signaling,

highlighting this pathway as a potential mechanism underlying METH relapse-related behavior [107].

Together, these preclinical studies using repeated METH exposure, CPP, behavioral sensitization, and reinstatement models suggest that METH alters key intracellular plasticity pathways, including ERK/MAPK, CaMKII, CREB, and FosB-family transcription factors, across corticostriatal and mesolimbic circuits. These molecular changes, together with METH-induced disruptions in dopaminergic and glutamatergic signaling, reinforce drug-seeking behavior and contribute to persistent behavioral sensitization [108,109]. These transcriptional programs also intersect with neuroimmune signaling, as chronic METH exposure enhances glial activation and pro-inflammatory mediator expression, further modulating synaptic plasticity and reward processing, contributing to persistent neuroimmune remodeling within mesocorticolimbic circuits [9,13,100,110].

Collectively, these findings suggest that METH addiction involves the combined effects of excessive dopaminergic signaling, maladaptive synaptic and transcriptional plasticity, neurotoxicity, and neuroimmune activation within mesocorticolimbic and corticostriatal circuits. Importantly, many of these molecular and cellular changes can persist beyond acute METH exposure, potentially contributing to long-lasting drug-associated memories, behavioral sensitization, compulsive drug seeking, and susceptibility to reinstatement. Thus, METH addiction reflects not only persistent changes in neural plasticity but also broader disruption of neural homeostasis. While these molecular pathways are well established in METH addiction, whether EV-mediated intercellular communication directly contributes to these addiction-related processes remains unclear and warrants further investigation.

3.2. METH-Induced Neurological Deficits

Beyond its addictive properties, METH produces extensive neurotoxicity affecting both neuronal and glial populations through multiple interconnected mechanisms, including oxidative stress, excitotoxicity, mitochondrial dysfunction, endoplasmic reticulum (ER) stress, impaired axonal transport, dysregulated autophagy and mitophagy, neuroinflammation, BBB disruption, and apoptotic cell death [83,111,112]. In humans, a single oral dose (0.42 mg/kg) significantly enhances both positive and negative mood states compared with placebo [113]. Mood was assessed in subjects with the Positive and Negative Affect Schedule (PANAS) before dosing and at 3, 4.5, and 24 h after METH dosing. After the single 0.42 mg/kg oral METH dose, participants not only reported significantly greater positive affect (pleasurable/activating emotional state) than placebo, but they also showed significantly greater negative affect (unpleasant emotional state); i.e., the single METH dose produced a mixed affective response, significantly increasing both positive and negative mood ratings relative to placebo, consistent with heightened psychostimulant-induced emotional arousal rather than a purely euphoric effect [113]. Chronic METH exposure produces more diverse, progressive, and persistent neurobiological, structural, and neuropsychiatric abnormalities. Clinically, chronic METH users exhibit impairments in attention, working memory, executive function, and learning, accompanied by affective disturbances, depression, sleep disruption, increased risk of movement disorders and cerebrovascular events, persistent craving, and a markedly elevated incidence of psychotic symptoms, including hallucinations and schizophrenia-like disorders [8,13,114,115]. In a prospective longitudinal study including 278 chronic METH users aged ≥16 years across four-month observation periods, frequent METH use was associated with a progressive increase in positive psychotic symptoms, including hallucinations, paranoia, and unusual or delusional thoughts. Importantly, these symptoms were more common during periods of METH use than during periods of abstinence within the same individuals, supporting an association between METH exposure and worsening psychotic symptoms [114]. Human neuroimaging studies

have provided important insight into the lasting effects of chronic METH use on the brain. One of the most consistent findings is disruption of the dopaminergic system. PET studies in abstinent METH users have shown reduced striatal DAT availability, which is associated with poorer psychomotor and memory performance. Interestingly, DAT availability substantially recovered after approximately 12–17 months of abstinence, suggesting that at least some of these dopaminergic changes may be reversible [116,117]. Reduced striatal dopamine D2-receptor availability has also been associated with altered orbitofrontal metabolism, further suggesting disruption of communication between striatal and prefrontal regions [118]. Beyond the dopaminergic system, ^{1}H-MRS studies have reported reduced N-acetyl aspartate (NAA) and other metabolic alterations in chronic METH users, consistent with compromised neuronal integrity [119,120]. Structural changes have also been observed, including cortical and limbic gray-matter deficits, reduced hippocampal volume associated with poorer memory, and white-matter abnormalities in recently abstinent chronic METH users [121]. Enlargement in striatal volume has similarly been reported during abstinence [122]. Evidence also points to a persistent neuroimmune response, with PET imaging of 12 abstinent METH users showing increased ^{11}C-PK11195 binding across several brain regions, interpreted as a marker of microglial activation [123]. Taken together, these studies suggest that chronic METH use in humans is associated with persistent changes in dopamine signaling, neuronal integrity, brain structure, and neuroimmune activity, although such imaging and metabolic abnormalities do not necessarily indicate irreversible neuronal loss. In contrast, in post-mortem brain tissue from chronic human METH users, no significant evidence of persistent microgliosis or astrogliosis was found. Thus, despite evidence of dopaminergic abnormalities associated with chronic METH use, this study did not support persistent gliosis as a characteristic neuropathological feature of chronic METH exposure in humans, also indicating that neuronal injury and circuit dysfunction can occur in the absence of glial activation [124].

In mice, a single intraperitoneal (i.p.) METH dose (30 mg/kg) causes basal ganglia dysfunction characterized by reduced locomotor activity together with decreased striatal glutamate, glutamine, and γ-aminobutyric acid (GABA) concentrations, indicating acute disruption of both motor function and excitatory-inhibitory neurotransmission [125]. Acute intravenous administration (0.025–0.2 mg/kg) also alters cerebral glucose utilization and central glucose regulation in Wistar rats, highlighting the immediate metabolic stress imposed on neural tissue [126]. In adult rats with prior neonatal lipopolysaccharide (LPS) exposure, METH exposure (0.5 mg/kg) revealed an underlying vulnerability of disruption in the dopaminergic system, resulting in greater damage to nigrostriatal dopaminergic neurons than in control animals. This increased neurotoxicity was accompanied by more pronounced motor and behavioral impairments, suggesting that early-life neuroinflammation may increase susceptibility to METH-induced neurological damage later in life [127]. In adult male rats, acute METH exposure (0.5–5 mg/kg, i.p.) disrupted dopamine signaling in the NAc in a dose-dependent manner, altering both dopamine release and clearance. These changes were accompanied by progressively increased locomotor activity until METH dosing of 2 mg/kg and then a drastic reduction in locomotion at 5 mg/kg, accompanied by an increase in stereotypic behaviors (repetitive sniffing, head movements, licking, biting or gnawing, and grooming, or repetitive movements in a restricted location), suggesting a close relationship between altered accumbal dopamine signaling and METH-induced behavioral dysfunction [102]. Mechanistically, excess cytosolic dopamine can promote oxidative stress and neurodegeneration, providing a potential mechanism for dopaminergic neuronal injury following METH exposure [128].

In vivo animal model studies further show that prolonged METH exposure disrupts dopaminergic neurotransmission and monoaminergic signaling [129] and induces

depression-like behaviors through alterations in dopamine homeostasis. In mice, a single neurotoxic dose of METH (30 mg/kg, i.p.) induced persistent depressive-like behavior and long-lasting disturbances in dopaminergic homeostasis. Despite these sustained affective and neurochemical changes, motor function and procedural learning remained largely intact, suggesting that acute METH neurotoxicity selectively affects certain behavioral and neurochemical domains rather than causing generalized functional impairment [130]. Kesby et al. (2018) [131] showed in mouse models of chronic (6 days of moderate METH dosing at 2 mg/kg per injection) and binge (4 injections of METH at 4 mg/kg per injection, administered at 2-h intervals over a single day) METH exposure that during withdrawal for 6 days, both exposure patterns produced persistent, but regimen-dependent, changes in brain reward function measured by intracranial self-stimulation (ICSS), along with alterations in dopaminergic and serotonergic levels, thus indicating that different patterns of METH exposure can have lasting effects on monoaminergic systems involved in reward processing [131]. In a chronic oral METH self-administration model, adult male ICR mice (Swiss-derived outbred mice established at the Institute for Cancer Research, Philadelphia, USA) received METH (0.05 mg/mL, days 1–9; 0.1 mg/mL, days 10–21) in drinking solution. During withdrawal, METH-exposed mice showed impaired cognitive flexibility, accompanied by mitochondrial dysfunction, impaired mitophagy, altered oxidative-stress pathways, and reduced synaptic plasticity-related proteins. These findings suggest that cognitive deficits following chronic METH exposure are accompanied by lasting disruptions in mitochondrial homeostasis and synaptic plasticity [132]. In another study, 3.5-month-old male and female C57BL/6J mice received chronic low-dose METH (1.4 mg/kg, i.p., twice daily, 5 days/week for 1 month), a regimen designed to mimic therapeutic stimulant exposure in humans. Chronic METH administration altered subsequent sensitivity to METH reward and resulted in sex- and brain region-dependent changes in astrocyte activation and epigenetic modulation, suggesting that repeated METH exposure can produce lasting glial and molecular adaptations alongside altered reward-related behavior [133]. Furthermore, findings from a chronic low-dose METH mouse model were consistent with observations in post-mortem brain tissue from chronic human METH users. In this model, 3–4-month-old male C57BL/6J mice received METH (5 mg/kg, i.p.) once daily for 10 consecutive days. METH exposure significantly reduced microglial activation in the hypothalamic arcuate nucleus. In contrast to the pronounced microglial activation commonly reported after high-dose binge METH exposure, this lower-dose regimen suppressed the local microglial response, suggesting that the neuroimmune effects of METH may vary with dose, exposure pattern, and brain region [134].

Structural and vascular abnormalities also become increasingly evident with prolonged exposure. In rats, four months of i.p. METH administration at both low (4 mg/kg) and high (8 mg/kg) doses increased striatal volume, potentially reflecting edema and neuroinflammatory tissue swelling, together with microglial and cerebrovascular abnormalities [135]. Chronic METH further compromises BBB integrity by increasing vascular permeability to circulating neurotoxic molecules [135,136], thereby exacerbating neuronal injury. Several studies have reported that long-term METH exposure can produce persistent impairments in hippocampal- and cortical-dependent learning and memory [137]. In adult male Wistar rats, extended intravenous METH self-administration (0.05 mg/kg/infusion, 6 h/day for 22 sessions) impaired hippocampal-dependent spatial and working memory and reduced dentate-gyrus neural progenitors, where lower progenitor levels were associated with greater memory deficits and subsequent METH-seeking behavior, linking excessive METH intake to impaired hippocampal neuroplasticity and relapse vulnerability [138]. Also, in another METH self-administration model, male Sprague-Dawley rats were given short access to METH (1 h/day for 7 days), followed by extended access (6 h/day for 14 days; 20 μg

METH per infusion). Extended METH exposure impaired novel-object recognition and was associated with reduced GluN2B-containing NMDA receptor expression and loss of long-term depression (LTD) in the perirhinal cortex. Importantly, restoring GluN2B signaling rescued both LTD and recognition memory, directly linking METH-induced disruption of glutamatergic synaptic plasticity to LTD [139]. Adolescent mice exposed to METH (2 mg/kg, i.p., once daily) from postnatal days 35–42 showed anxiety-like behavior and cognitive deficits when assessed in adulthood (8 weeks of age). These behavioral changes were accompanied by hippocampal neuroinflammation, altered microglial responses, and disrupted neurogenesis, which suggest that METH exposure during adolescence can have lasting effects on hippocampal neuroimmune and neurogenic processes, contributing to cognitive and emotional dysfunction later in life [5]. Similarly, adolescent C57BL/6J mice exposed to METH (1 or 2 mg/kg, i.p., once daily) from P35-P48 showed impaired spatial learning, memory, and novel-object recognition in adulthood. These cognitive deficits were accompanied by lasting alterations in synaptic plasticity within the dorsal hippocampus, suggesting that repeated METH exposure during adolescence can disrupt hippocampal maturation and contribute to memory dysfunction later in life [140]. Qiu et al. reported that 10-week-old mice receiving escalating doses of METH (1–10 mg/kg/day, i.p.) for 28 days, divided into two daily injections 12 h apart, showed deficits in working memory, spatial cognition, learning, and recognition memory. Single-cell RNA sequencing of the hippocampus revealed widespread, cell-specific changes involving neuroinflammation, BBB dysfunction, impaired neuronal support, altered microglial synaptic remodeling, oligodendrocyte dysfunction, and disrupted neurogenesis, indicating that chronic METH exposure produces lasting changes across multiple hippocampal cell populations that accompany cognitive impairments [141]. Collectively, these findings demonstrate that chronic METH exposure initiates a complex pathological cascade leading to progressive neuronal cell death.

In conclusion, chronic METH exposure can lead to lasting disruptions in dopaminergic signaling, mitochondrial function, neuroimmune responses, and synaptic plasticity, which may increase susceptibility to neurodegenerative disorders. This association is particularly relevant to Parkinson's Disease (PD), as several consequences of METH exposure, including dopaminergic dysregulation, oxidative stress, and mitochondrial dysfunction, overlap with mechanisms implicated in PD pathology. Consistent with this link, epidemiological studies have reported a higher subsequent risk of PD among individuals with a history of METH or amphetamine use [142–145].

3.3. METH-Induced Neuroinflammation

Neuroinflammation has emerged as a central mechanism linking METH exposure to addiction, neurotoxicity, cognitive dysfunction, and neurodegeneration. Accumulating evidence demonstrates that METH activates innate immune signaling within the CNS, primarily through resident microglia and astrocytes, resulting in sustained production of pro-inflammatory cytokines, chemokines, ROS, and NO, which collectively exacerbate neuronal injury and synaptic dysfunction, although the absence of significant reactive gliosis in the striatum of chronic METH users reported by Kitamura et al. (2010) suggests that these glial responses may vary across experimental conditions and brain regions [9,10,146,147]. These inflammatory responses are mediated by multiple interconnected pathways, including TLR4, NF-κB, MAPK, phosphoinositide 3-kinase (PI3K)/Akt, and inflammasome signaling, all of which amplify oxidative stress, mitochondrial dysfunction, excitotoxicity, impaired glutamate homeostasis, and maladaptive synaptic plasticity associated with METH addiction [9,18]. Although the magnitude and characteristics of neuroinflammation vary according to METH dose, route of administration, duration of exposure, and brain regions examined, both human and pre-clinical studies consistently demonstrate glial acti-

vation and increased production of inflammatory mediators following METH exposure at higher doses.

Among these inflammatory pathways, inflammasomes have emerged as pivotal mediators of METH-induced neurotoxicity. Human post-mortem studies showed significant upregulation of the NLRP3 inflammasome in the hippocampus (CA1 region) of chronic METH users (average consumption duration of more than 5 years), accompanied by neuronal loss, increased ionized calcium binding adaptor molecule 1 (Iba-1)-positive microglia and glial fibrillary acidic protein (GFAP)-positive astrocytes, together with upregulation of TLR4, caspase-1, and IL-1β, consistent with activation of NLRP3 inflammasome-associated inflammatory signaling contributing to METH-associated neurodegeneration [148]. Likewise, post-mortem prefrontal cortex samples from HIV-positive METH users exhibited stronger evidence of microglial activation and pyroptosis, as evidenced by increased expression of Absent in Melanoma 2 (AIM2), caspase-1, IL-1β, IL-18, and the pyroptotic effector gasdermin D (GSDMD), thus indicating that METH use in HIV-infected individuals may be associated with enhanced AIM2-associated neuroinflammation driven by microglial pyroptosis [149].

Similarly, inflammasome activation has been observed in simian immunodeficiency virus (SIV)-infected macaques, in which chronic METH administration was initiated at 19 weeks post-infection using a 5-week escalating regimen (5 days/week, twice daily) to a final dose of 2.5 mg/kg twice daily (25 mg/kg/week) and was maintained for an additional 18 weeks. Significantly increased caspase-1 and PYCARD (apoptosis-associated Speck-like protein containing a CARD) expressions were observed in the macaque brain-derived microglia, supporting enhanced inflammasome signaling during chronic METH exposure in a clinically relevant non-human primate model [150].

In rodent studies, a single neurotoxic dose of METH (30 mg/kg, i.p.) in 3-month-old male C57BL/6J mice induced a robust inflammatory response in the hippocampus, characterized by microglial activation, astrocytic reactivity, and altered TNF signaling. Pre-treatment with the anti-inflammatory drug indomethacin reduced these inflammatory changes and associated neuronal dysfunction, suggesting that glial-mediated neuroinflammation contributes to METH-induced hippocampal neurotoxicity [6]. Rodent studies have further elucidated the molecular mechanisms underlying inflammasome activation. In rat primary LPS-primed microglial cells, METH administration (50 μM, 12 h) induced robust activation of the NLRP3 inflammasome, characterized by increased ASC recruitment, caspase-1 cleavage, and IL-1β production without changes in total NLRP3 expression, suggesting that METH primarily promotes activation and assembly of pre-existing NLRP3 inflammasome components in LPS-primed microglia rather than inducing NLRP3 expression itself [21].

In BV2 microglial cells exposed to 150 μM METH for 12 h, the miR-143/PUMA signaling axis was identified as an upstream regulator of NLRP3 activation, while pre-treatment of the BV2 cells with the pharmacological inhibitor of NLRP3, MCC950, significantly attenuated METH-induced iNOS expression and thus effectively suppressed subsequent microglial activation [151]. In contrast, mouse primary astrocytes preferentially activated the NLRP6 inflammasome following chronic METH exposure (50 μM for 24 h), resulting in ASC oligomerization, caspase-1 activation, GSDMD cleavage, and maturation of IL-1β and IL-18. These responses were attenuated by NLRP6 knockdown or restoration of miR-152, identifying the miR-152/NLRP6 axis as a key regulator of astrocytic pyroptosis and neuroinflammation [14]. In another study involving mouse primary astrocytes, it was shown that METH-induced (0 to 2.5 mM METH in primary astrocytes for 24 h) astrocytic inflammation was further amplified through TLR4/caspase-11 signaling, which promoted ASC/caspase-1 activation and downstream cytokine maturation despite minimal changes in NLRP3 expression, suggesting that METH primarily enhances downstream

inflammasome signaling in astrocytes [20]. Complementary in vitro studies further support these findings by demonstrating that METH (10–100 μM for 24–48 h) directly activates inflammasomes in human microglia cells (HTHμ), increasing NLRP3, apoptosis-associated speck-like protein (ASC), caspase-1 expression, and ROS production in a time-dependent manner [152]. Primary rat microglia similarly exhibit ASC speck formation and caspase-1 activation following METH exposure, whereas inhibition of caspase-1, lysosomal cathepsin B, or mitochondrial ROS markedly reduces IL-1β maturation, indicating that lysosomal destabilization and oxidative stress are principal triggers of NLRP3 activation rather than increased NLRP3 transcription [21]. Human cerebral organoid models further demonstrate increased NLRP1 expression in astrocytes following METH exposure, suggesting that METH-induced neuroinflammation involves multiple inflammasome complexes beyond NLRP3 [7].

The pathogenic significance of inflammasome activation is underscored by therapeutic intervention studies. Pharmacological inhibition of NLRP3 with MCC950 in chronic METH-administered C57BL/6J mice attenuated cerebellar degeneration, Purkinje cell loss, astrocyte and microglial activation, α-synuclein and phosphorylated tau accumulation, motor deficits, and the expression of NLRP3, ASC, cleaved caspase-1, and mature IL-1β following chronic METH administration [153]. Likewise, betaine ameliorated METH-induced depressive-like behavior and cognitive impairment by suppressing NLRP3 activation, caspase-1 cleavage, IL-1β, and TNF-α expression [154]. Beyond the CNS, METH also activates the NLRP3 inflammasome in intestinal epithelial cells, leading to increased caspase-1, ASC, IL-1β, and IL-18 expression that is reversible by MCC950, indicating that inflammasome-mediated inflammation contributes to both central and peripheral METH toxicity [23]. Collectively, these findings identify NLRP3 as the predominant inflammasome driving METH-induced neuroinflammation while also implicating AIM2, NLRP6, and NLRP1 in cell type-specific inflammatory responses, highlighting inflammasome signaling as a promising therapeutic target for mitigating METH-associated neurological deficits and addiction.

Overall, METH-induced addiction and neurological dysfunction arise from a complex interplay of monoaminergic dysregulation, maladaptive circuit plasticity, metabolic stress, structural brain injury, and persistent neuroinflammatory signaling (summarized in Table 1). Additionally, METH has been shown to alter the biogenesis, release, and molecular cargo of EVs, with evidence from microglial and peripheral immune-cell models as well as altered cEV populations and EV-associated miRNAs in individuals using METH, facilitating the spread of inflammatory cytokines, proteins, lipids, and non-coding RNAs that propagate neuroinflammation and contribute to oxidative stress, mitochondrial dysfunction, autophagy dysregulation, synaptic impairment, and neurodegeneration [53–55,70]. Therefore, elucidating EV-mediated intercellular communication may provide important mechanistic insights into METH-associated neurodegenerative pathology while identifying novel biomarkers and therapeutic targets for METH-induced neurological disorders.

Table 1. METH dosages and their effects in vitro and in vivo.

Experimental Models	METH Concentrations	Exposure Durations	Key Findings
LPS-primed rat microglial cells	50 μM	12 h	Priming and activation of NLRP3 inflammasome complex [21]
BV2 microglial cells	150 μM	12 h	Enhanced microglial activation [151]

Table 1. *Cont.*

Experimental Models	METH Concentrations	Exposure Durations	Key Findings
Human neuroblastoma cell line SH-SY5Y	2 mM	24 h	METH exposure reduced PTEN/Rab7a signaling and impaired autophagosome–lysosome fusion in SH-SY5Y cells; METH-induced neuronal exosomes exhibited altered miRNA profiles (122 upregulated, 151 downregulated); GO/KEGG analyses linked differentially expressed miRNAs to autophagy pathways and predicted PTEN regulation [155]
SH-SY5Y (human neuroblastoma cells) and human glioblastoma cells (U87MG); SH-SY5Y/U87MG Transwell co-culture	1–2 mM	24 h	Increased neuronal exosome release and phosphorylated α-synuclein (p-α-syn) cargo, promoting its transfer to astrocytes and increasing TNF-α, IL-1β, and IL-6 expression [156]
Mouse primary astrocytes	50 μM	24 h	Activation of the NLRP6 inflammasome complex [14]
Mouse primary astrocytes	0–2.5 mM	24 h	Enhanced activation of NLRP3 inflammasome signaling [20]
C57BL/6J mice	2 mg/kg, intraperitoneal injections	Once daily for 5 days	Increased phosphorylated extracellular signal-regulated kinase1/2phospho- c-Jun N-terminal kinase, and ΔFosB in the caudate-putamen associated with hyperactivity and behavioral sensitization [104]
C57BL/6J mice	10 mg/kg, 4 subcutaneous injections at 2-h intervals	Single day	Neuronal degeneration and BBB dysfunction [106]
C57BL/6 mice	30 mg/kg, intraperitoneal injections	Single day	Decreased striatal glutamate, glutamine, and γ-aminobutyric acid concentrations and reduced locomotor activity [125]
C57BL/6J mice	30 mg/kg, intraperitoneal injections	Single day	Depressive-like behavior and reduced dopamine levels [130]
Outbred Albino mice from Institute of Cancer Research, Philadelphia (ICR mice)	0.05 mg/mL, days 1–9; 0.1 mg/mL, days 10–21 in drinking solution for oral self-administration	21 days	Disruptions in mitochondrial homeostasis and synaptic plasticity, accompanied by cognitive deficits [132]
C57BL/6J mice	5 mg/kg, intraperitoneal injections	Once daily for 10 days	Reduced microglial activation in the hypothalamic arcuate nucleus [134]

Table 1. *Cont.*

Experimental Models	METH Concentrations	Exposure Durations	Key Findings
C57BL/6J mice	2 mg/kg, intraperitoneal injections	Once daily for 8 days	Hippocampal neuroinflammation and disrupted neurogenesis, accompanied by anxiogenic behavior and cognitive deficits [5]
C57BL/6J mice	1 and 2 mg/kg, intraperitoneal injections	Once daily for 14 days	Reduced hippocampal maturation and synaptic plasticity, accompanied by impaired spatial learning, memory, and novel-object recognition [140]
C57BL/6 mice	Escalating 1–10 mg/kg/day intraperitoneal injections (divided into two daily injections 12 h apart)	28 days	Dysregulation across multiple hippocampal cell populations, including neuroglial, stromal, vascular, and immune cells, accompanied by cognitive impairments [141]
C57BL/6 mice	1.5 mg/kg, once daily 4.5 mg/kg, once daily 7.5 mg/kg, once daily 10 mg/kg per injection Four times daily, 2-h intervals	Days 1–2 Days 3–4 Days 5–6 Days 7–8	METH increased brain miR-143 expression, BBB permeability, and monocyte infiltration while reducing the expression of tight junction proteins (claudin-5, occludin, and ZO-1). Genetic or lentiviral silencing of miR-143 attenuated BBB disruption, with protection dependent on PUMA [157]
C57BL/6 J mice	10 mg/kg/d, twice daily, i.p.	7 days	METH induced cortical microglial activation, upregulated TLR4/TRIF/Peli1 signaling, and increased IL-1β, IL-6, and TNF-α expression, accompanied by reduced tyrosine hydroxylase levels [158]
BALB/c mice	5 mg/kg/day, i.p.	30 days	Impaired spatial and recognition memory, increased anxiety-like behaviors, reduced hippocampal DCX^+ and $NeuN^+$ cells (neuronal markers), and elevated $Iba\text{-}1^+$ microglia (microglia activation marker) and TNF-α/NF-κB expression (neuroinflammation marker) [159]
Sprague-Dawley rats	0.05 mg/kg intravenous injections	2 h per day for 25 days	Activates TLR-7 signaling pathway and promotes synaptodendritic injury as well as neuroinflammatory responses [55]
Wistar rats	4 mg/kg (i.p.)	1, 7, or 14 days	7- and 14-day METH exposure increased circulating endothelial-cell-derived EVs (Annexin $V^+/CD144^+/CD31^+/CD41^-$), plasma CRP and MDA levels, and locomotor activity in rats [57]

Table 1. *Cont.*

Experimental Models	METH Concentrations	Exposure Durations	Key Findings
Sprague-Dawley rats	0.5–5 mg/kg, intraperitoneal injections (two doses: ≤2 mg/kg and 5 mg/kg)	Two doses given cumulatively 25 min apart on a single day	Enhanced extracellular dopamine concentrations and locomotor activity in the lower dose (≤2 mg/kg), whereas with the higher dose (5 mg/kg), enhanced stereotypic behavior and reduced horizontal locomotor activity were demonstrated [102]
Rats	2.5 mg/kg, intraperitoneal injections	Once daily for 5 days	Persistent locomotor sensitization occurs for 3–14 days, with transcriptional increase in CREB/ΔFosB-associated with behavioral sensitization [103]
Wistar rats	0.025–0.2 mg/kg, acute intravenous injections	Single day	Reduction in cerebral glucose utilization and dysregulated central glucose regulation [126]
Sprague-Dawley rats	4 mg/kg (low) and 8 mg/kg (high) intraperitoneal injections	4- months	Increased striatal volume, potentially reflecting edema and neuroinflammatory swelling, together with microglial activation [160]
Wistar rats	0.05 mg/kg, intravenous injections for 6 h/day	22 sessions per day	Reduced dentate-gyrus neural progenitors, associated with greater memory deficits and drug-seeking behavior [138]
Sprague Dawley rats	20 µg (1 h/day for 7 days as short access, followed by 6 h/day for 14 days) infusion	21 days	Reduced GluN2B-NMDA receptor expression and impaired novel-object recognition [139]
Humans	0.42 mg/kg, oral administration	Acute administration by a combination of capsules: 0, 2, 5, 10, or 20 mg METH	Shows significant increases in positive (pleasurable/activating emotional state) moods [113]
Humans	METH-self-administration	Chronic use: ~13 years; assessed over four non-consecutive one-month periods.	Exhibits an increase in the occurrence of psychotic symptoms [114]

Abbreviations: LPS: Lipopolysaccharide; NLRP: Nucleotide-binding oligomerization domain, Leucine-rich Repeat and Pyrin domain-containing; ΔFosB: FBJ murine osteosarcoma viral oncogene homolog B; BBB: blood–brain barrier; TLR: Toll-like receptor; DCX: doublecortin; TNF: tumor necrosis factor; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; GluN2B: Glutamate receptor ionotropic, NMDA 2B; NMDA: N-methyl-D-aspartate receptor; CRP: C-reactive protein; MDA: malondialdehyde.

4. EVs as Mediators of METH-Induced Neurotoxicity

EVs are conduits for intercellular communication and signaling and play a pivotal role in modulating pathobiological outcomes in substance-use disorders [28,32,48,49,51,52,161]. Zhao et al. reported substantial changes in circulating exosomal miRNAs in abstinent individuals with METH use disorder (MUD), identifying 113 differentially expressed miRNAs, including 94 upregulated and 19 downregulated, compared with healthy controls. Pathway enrichment analysis associated these altered miRNAs with biological processes related to

synaptic plasticity. Among these, miR-184-3p and miR-4433a-5p were significantly reduced in the blood-derived exosomes from MUD patients, and a dual-luciferase assay further confirmed that miR-184-3p directly targets CRTC1, a co-activator of CREB-dependent transcription factor involved in long-term synaptic plasticity. Together, these findings identify CRTC1 as a molecular target of miR-184-3p and suggest a potential association between altered exosomal miR-184-3p-targeted pathways that may contribute to METH-associated neuroadaptation; although its direct role in neuronal dysfunction or neurotoxicity remains to be experimentally established [162]. An exploratory study on METH-dependent subjects demonstrated the release of CD9+, CD81+, and CD63+ EVs in plasma, carrying 169 miRNA cargoes, including miR-301a-3p, miR-382-5p, and miR-628-5p, which are predicted to target neuroinflammatory pathways; several EV-associated miRNAs were also correlated with clinical measures such as onset age, percentage of lifetime spent using METH, and frequency of METH use [53]. These observations identify candidate miRNAs and pathways associated with METH exposure but do not establish that these EV-associated miRNAs directly regulate neuroinflammation via experimental evidence. Furthermore, the presence of exosome-derived miRNAs, namely hsa-miR-143-3p, hsa-miR-200a-3p, hsa-miR-363-3p, and hsa-miR-125b-5p, in the plasma of METH-dependent subjects was shown to be significantly reduced in comparison to healthy controls. These miRNAs were also associated with pathways relevant to anxiety and depression, suggesting that altered exosomal miRNA profiles may be linked to the neuropsychiatric features associated with METH dependence [163]. Similarly, another study highlighted the enrichment of miR-9-3p in serum-derived exosomes from METH users compared to controls [164]. This differential expression should primarily be regarded as biomarker signatures unless direct EV-miR-mediated functional effects are demonstrated. In METH-dependent rats (2 mg/kg, s.c., days 4–7 and sacrificed on day 10), plasma EV miRNA profiling identified ~301 differentially expressed miRNAs, including miR-218b, miR-194-5p, and miR-152-3p [165]. Although bioinformatic analyses can associate such miRNA profiles with systemic or neuroimmune pathways, these predictions alone do not demonstrate that the identified EV-associated miRNAs regulate those pathways in vivo. METH exposure has also been shown to be associated with BBB dysfunction. Chronic METH administration (i.p., 1.5 mg/kg on days 1–2, 1 time/day; 4.5 mg/kg on days 3–4, 1 time/day; 7.5 mg/kg on days 5–6, 1 time/day; and 10 mg/kg on days 7–8, 4 times/day) in C57BL/6 mice increases expression of miR-143 via a sigma-1 receptor-dependent pathway and disrupted BBB integrity by increasing endothelial permeability and altering tight-junction proteins; while silencing of miR-143 protects this METH-induced BBB damage, indicating that miR-143 may be a potential therapeutic target for BBB damage-related vascular dysfunction [157]. Although EVs can facilitate intercellular transfer of regulatory miRNAs, whether METH-induced EVs deliver miR-143 to brain endothelial cells and thereby contribute to BBB disruption remains experimentally unverified. Further studies directly examining EV uptake, cargo transfer, and BBB permeability are required to establish an EV-dependent mechanism of METH-induced BBB dysfunction. In rats exposed to 4 mg/kg METH (i.p.) for 1, 7, and 14 days, Annexin V^+/$CD144^+$/$CD31^+$/$CD41^-$ endothelial-cell-derived EV release (isolated from platelet-free plasma) was increased in the case of METH administration for 7 and 14 days. METH administration for 7 and 14 days also increased oxidative stress (malondialdehyde) and inflammation (C-reactive protein), with a concomitant increase in locomotor activity, suggesting a vascular endothelial-cell response to METH exposure and highlighting the potential of circulating EVs as biomarkers of METH-associated vascular injury [57]. Although EVs can mediate intercellular communication within the neurovascular unit, direct experimental evidence demonstrating that METH-induced EVs disrupt BBB integrity remains limited. A similar study showed that METH (2 mg/kg, s.c.) administered to Sprague-Dawley rats

increased both the time spent and distance traveled in the METH-paired compartment, while rhynchophylline (60 mg/kg, i.p.) reduced these behavioral effects in CPP. METH also altered miRNA profiles in the hippocampus and serum exosomes, with 50 exosomal miRNAs upregulated and 2 downregulated, while rhynchophylline reduced the expression of 23 METH-upregulated miRNAs. This study demonstrated that miR-1b, miR-181a-5p, miR-200a-3p, and miR-375-3p were enriched in both the hippocampus and serum exosomes of METH-administered rats. Computationally predicted targets of these miRNAs include synaptic transmission pathways [166]. Overall, current evidence suggests that METH exposure can substantially alter EV abundance and molecular cargo, and that EV-enriched preparations can produce biological effects across several experimental models. However, the strength of mechanistic evidence varies considerably between studies. In particular, changes in miRNA expressions, together with pathway-enrichment analyses, should primarily be viewed as hypothesis-generating, as these findings alone do not demonstrate the role of EV-associated miRNAs in inducing a neurotoxic phenotype. METH (10 mg/kg/d for 7 days, twice daily, i.p.) has been shown to activate the TLR4/TRIF/Peli1 signaling axis, promoting inflammatory signaling in the mouse brain [158]. Another interesting study demonstrated that chronic METH exposure (5 mg/kg/day for 30 days, i.p.) in mice, followed by intravenous administration of mesenchymal stem cell-derived (MSC) exosomes from bone marrow, decreases inflammatory mediators including Iba-1, TNF-α, and NF-κB, as well as improves spatial learning and memory, together with a significant increase in the expression of neuronal NeuN and Doublecortin, thus promoting hippocampal neurogenesis [159]. This supports neuroprotective and regenerative effects of MSC-derived exosomes in METH-induced neurological damage, highlighting EV-based therapies as promising interventions [158,159]. Additional reports also show that direct METH exposure (2 mM for 24 h) reduced PTEN/Rab7a signaling and impaired autophagosome–lysosome fusion in SH-SY5Y cells, and exosomes released from these SH-SY5Y cells demonstrate differential miRNA expression, which are potential regulators of PTEN, suggesting that altered exosomal miRNA cargo may contribute to METH-induced impairment of neuronal autophagic flux through the PTEN-Rab7a signaling pathway [155]. While these findings suggest that METH-induced neuronal exosomes may participate in the regulation of autophagy, altered miRNA expression alone does not establish that individual EV-associated miRNAs are responsible for the observed PTEN/Rab7a-dependent effects, and the contribution of specific miRNA cargo requires further experimental validation. METH exposure has subsequently been described to confer α-synuclein pathology through exosome mediated communication between neurons and astrocytes. Specifically, METH exposure at a 2 mM dose for 24 h on the SH-SY5Y neuroblastoma cell line and U87MG human primary glioblastoma cell line (astrocytes) co-cultured in a Transwell setup showed pathologic α-synuclein generation from astrocytes. Further, this study demonstrated a decrease in nuclear receptor-related protein (Nurr1) abundance in the astrocytes and subsequent release of pro-inflammatory cytokines, causing an inflammatory state [156]. Collectively, these findings indicate that METH exposure can alter EV abundance and molecular cargo, and METH-EVs can influence several METH-related cellular and neurobehavioral effects. However, the strength of mechanistic evidence varies across studies. Changes in miRNA expression and findings from pathway-enrichment analyses should therefore be viewed as hypothesis-generating rather than direct evidence that a specific EV-associated miRNA causes neurotoxicity. Although some studies have provided direct functional or target-validation evidence, the direct role of individual EV cargo molecules remains largely unresolved. Further EV-transfer, gain- and loss-of-function, and rescue experiments are needed to establish their specific contributions to METH-induced neurobiological changes.

Although the molecular pathways discussed above are well-established contributors to METH-induced reinforcement, behavioral sensitization, and drug-seeking behavior, the extent to which they are influenced by EV-mediated communication remains unclear. Recent studies suggest that METH exposure alters EV-associated cargo, including miRNAs and inflammatory proteins, which may affect synaptic function, neuroimmune signaling, and neuronal homeostasis [53,55,162,166]. While these changes are relevant to the neurobiological adaptations associated with chronic METH exposure, they do not necessarily indicate that EVs directly contribute to addiction. For example, changes in EV-associated miRNA profiles have been observed alongside METH-induced conditioned place preference and alterations in synaptic signaling pathways [166]. Other studies have shown that EV-associated cargo can contribute to neuroinflammation and synaptodendritic injury following METH exposure [55]. Furthermore, it has also been demonstrated that circulating EVs from individuals undergoing acute METH withdrawal induced anxiety- and depression-like behaviors, hippocampal synaptic abnormalities, and microglial activation in mice. Importantly, EV-associated SAA1 contributed to these effects, as SAA1-enriched EVs reproduced withdrawal-associated behavioral abnormalities, whereas SAA1 neutralization attenuated these responses [71]. However, these studies also provide different types of evidence. The first one suggests a relationship between EV-associated molecular changes and addiction-related behavior, whereas the second one demonstrates the involvement of EVs in neurotoxic processes without establishing a direct role in drug reinforcement or relapse. Importantly, EV-mediated anxiety- and depression-like behaviors during METH withdrawal should be distinguished from drug-seeking and reinstatement behaviors, as these represent different behavioral outcomes. Taken together, these findings suggest that EV signaling may contribute to the lasting neurobiological effects of METH exposure. However, further studies using EV-specific interventions in established addiction models are needed to determine whether EVs directly influence the development, persistence, or recurrence of addictive behaviors.

5. EVs as Mediators of METH Withdrawal and Abstinence

METH addiction, followed by symptoms of withdrawal and abstinence, leads to psychological disorders along with intellectual and cognitive impairments [137,167,168]. There are two types of METH withdrawal: (1) acute (occurs during the first 1–2 weeks after cessation and is characterized by prominent affective, sleep, and craving-related symptoms) and (2) protracted (persists for several weeks to months after cessation with residual mood, cognitive, and craving-related disturbances which increases vulnerability to relapse) [169,170]. Enrichment of plasma-derived exosomal miRNAs- hsa-mia-451a and hsa-mir-21a was observed in patients with acute METH withdrawal (7-day and 3- and 12-month withdrawal periods); further, psychological comorbidities in these subjects correlated with a significant decrease in neurotransmitters like GABA and choline, and an increase in serotonin, compared to healthy controls [70]. In a study on Korean subjects, miR-137 in circulating EVs (cEVs) was reported to serve as a blood biomarker of METH abstinence. In total, 37 METH-abstinent subjects were divided into two groups comprising: (1) abstinence within 3 months, and (2) abstinence for 3 months or more. Patients abstinent for ≤3 months and those abstinent for >3 months both showed significantly reduced cEV miR-137 in comparison to age-matched healthy controls, thus demonstrating that miR-137 reduction was independent of duration of the METH use or abstinence. However, miRNA-137 was shown to be increased with age in METH abstinent subjects, whereas the healthy participant group showed an age-dependent decline of EV miRNA-137 levels, suggesting reduced cEV miR-137 as a potential stable biomarker of METH abstinence and indicating the functional role of aging in driving differential abstinence-associated behavior [171]. It is important to

mention that further validation of EV-associated biomarkers in larger, independent clinical cohorts is necessary to establish their diagnostic or prognostic utility in METH use disorder. To delineate the immunomodulatory phenomenon associated with withdrawal symptoms in METH use disorders, investigators profiled peripheral cytokines, immune-cell status, and plasma exosomal RNAs like lncRNA, mRNA, and miRNA in patients undergoing acute (7–14 days) and protracted (~1 year) withdrawal. Exosomal RNA sequencing identified 327 differentially expressed mRNAs (203 upregulated and 124 downregulated) and 411 differentially expressed lncRNAs during acute withdrawal; furthermore, these mRNAs targeted pathways related to chemokine signaling, leukocyte migration, platelet activation, and neurodegeneration. On the other hand, during protracted withdrawal, 518 mRNAs showed differential expression (227 upregulated and 291 downregulated), while out of 525 lncRNAs, 188 were upregulated and 337 were downregulated. The differentially expressed mRNAs targeted proteasome, protein export, oxidative phosphorylation, and Parkinson's disease-associated pathways, suggesting the circulating exosome mediated regulatory landscape changes substantially across withdrawal stages. Notably, exosomal CXCL5 and CXCL4/PF4 were specifically elevated during acute withdrawal but remained unchanged during protracted withdrawal, suggesting their potential as biomarkers of acute METH withdrawal. Overall, prolonged abstinence was associated with substantial improvement in immune-related abnormalities, while exosome derived transcriptomic changes were associated with neurological disease-associated pathways [172]. Studies on 2–3-year-old female cynomolgus macaques showed important findings on EV-derived microRNAs and their role during METH abstinence. METH was administered subcutaneously (s.c.) using a 28-day ramp-up protocol, with doses ranging from 0.1 to 0.6 mg/kg per injection and administration frequencies increasing from one to three times daily (s.i.d., b.i.d., or t.i.d.). Clinical parameters were monitored throughout the study, with the clinical signs of the animals observed at least once daily for any potential complications, followed by an 8-week drug-free abstinence period, followed by assessment of circulatory EVs in the serum. A subsequent mouse study was conducted following a ramp-up protocol with variable METH doses (0.5–4.0 mg/5.0 mL/kg) and was also used to assess METH abstinence. In brief, mice were injected with i.p. administration of METH 1–3 times daily (s.i.d., b.i.d., or t.i.d.) for a total of 14 days, following abstinence for 1, 3, and 6 weeks. Further, circulatory serum EVs were extracted and characterized for miRNAs. In this study, both models have demonstrated that circulating EV miR-137 was downregulated due to reduced SYNCRIP-dependent miRNA sorting into the exosomes of the dorsal striatum during METH abstinence [173]. These findings suggest that circulating EV miR-137 may serve as a potential biomarker of persistent molecular changes in the striatum during METH abstinence. Another interesting study investigated circulating EV alterations during METH abstinence in 97 acute-withdrawal (≤14 days), 93 protracted-withdrawal (15–365 days), and 94 healthy male participants. Proteomic analysis identified 998 EV-associated proteins, 121 of which were differentially expressed in acute withdrawal (101 upregulated, 20 downregulated). Notably, SAA1 and serum amyloid A2 (SAA2) were elevated during acute withdrawal and normalized during protracted withdrawal [71]. Specifically, EV-associated SAA1 was markedly elevated during acute withdrawal, while returning to control levels during protracted withdrawal, supporting its potential as a withdrawal-stage biomarker. Intravenous administration of MA-AW EVs (EVs from METH abstinence acute withdrawal patients) to C57BL/6 mice induced persistent anxiety- and depression-like behaviors and produced hippocampal dendritic and synaptic abnormalities without overt neuronal loss. MA-AW EVs also increased glial reactivity, particularly microglial activation, while minocycline attenuated behavioral abnormalities. Moreover, SAA1-enriched EVs generated from SAA1-overexpressing 293T cells, when administered to C57BL/6 mice, resulted in behav-

ioral abnormalities like increased anxiety-like behavior in the open-field test (OFT) and elevated plus maze (EPM), depression-like behavior in the forced swim test (FST), and reduced sucrose preference indicative of anhedonia in the sucrose preference test (SPT), whereas neutralization of SAA1 with a specific antibody attenuated these effects [71]. Next, primary hippocampal neurons and microglia derived from neonatal Sprague-Dawley rats were also administered MA-AW EVs. MA-AW EVs impaired neuronal dendritic complexity and synaptic organization and induced pro-inflammatory microglial responses and neuronal dendritic abnormalities. EV-SAA1 similarly promoted microglial inflammation and directly impaired neuronal dendritic and synaptic organization, suggesting both microglia-dependent and direct neuronal effects [71]. Collectively, these findings identify circulating EVs, particularly EV-associated SAA1, as potential mediators of acute METH withdrawal by regulating microglial inflammatory responses, dendritic and synaptic dysfunction, and withdrawal behaviors.

Overall, these studies identify EVs as potential biomarkers and mediators of METH withdrawal and abstinence, with altered EV cargo contributing to neuroinflammation, synaptic dysfunction, and withdrawal-associated neurobehavioral abnormalities (summarized in Table 2). Interpretation of these findings should also consider substantial clinical and experimental heterogeneity across METH studies. EV profiles and associated neurobiological outcomes may vary with METH dose, route, duration, pattern of use, age, sex, and comorbidities. Importantly, the withdrawal stage is a major source of variability, as acute withdrawal, protracted withdrawal, and prolonged abstinence may exhibit distinct EV molecular profiles. Polysubstance use is another important confounder, particularly in clinical studies, because concurrent or prior exposure to opioids, cocaine, cannabis, alcohol, nicotine, or other substances may independently influence neuroinflammation and EV cargo, complicating attribution of observed changes specifically to METH alone. Therefore, future studies should be undertaken to characterize EVs in association with METH exposure characteristics, polysubstance use, and demographic variables.

Table 2. METH dependence and abstinence-based changes in the CNS and EVs.

METH-Dependence/ Abstinence	Molecular Pathways	CNS and Behavioral Changes	EV Cargoes	Nature of Evidence
METH dependence	-	Anxiogenic and Depressive behavior	Reduced exosomal miRNAs: hsa-miR-143-3p, hsa-miR-200a-3p, hsa-miR-363-3p, and hsa-miR-125b-5p, in the plasma of human subjects [163]	Associative
METH dependence	-	Reduced synaptic plasticity along with drug-craving behavior	Reduced exosomal miRNAs: miR-301a-3p, miR-382-5p, and miR-628-5p in the plasma of human subjects [53]	Associative & bioinformatic
METH dependence	-	Reduced synaptic plasticity and enhanced memory impairment	Enhanced miR-9-3p levels in the serum-derived exosomes from human subjects [164]	Associative

Table 2. *Cont.*

METH-Dependence/Abstinence	Molecular Pathways	CNS and Behavioral Changes	EV Cargoes	Nature of Evidence
METH dependence	Activates dopaminergic and GABAergic pathways	Increased conditioned place preference (CPP), indicating enhanced drug-associated reward or preference	Enhanced serum-exosomal levels of miR-218b, miR-194-5p, and miR-152-3p in rats; predicted targets implicated dopaminergic/GABAergic signaling and neuronal plasticity [165]	Associative & bioinformatic
METH dependence	Suppresses neuregulin-1 or NRG1/ErbB4 signaling	Hippocampal dysfunction in the CA1 region	Increased miR-183-5p in the mouse hippocampus [174]	Functional
METH dependence	Alters glutamatergic signaling and TNF/MAPK pathways, resulting in apoptosis	Addictive neuroplasticity	Enhanced levels of miR-1b, miR-181a-5p, miR-200a-3p, and miR-375-3p in hippocampus and serum exosomes of rats; bioinformatic analyses show target pathways related to synaptic transmission [166]	Associative & bioinformatic
METH abstinence	Inhibits CRTC1/CREB signaling pathway	Reduced synaptic plasticity and neuronal growth associated with mild anxiety and depressive-like behaviors	Reduced plasma-exosomal miR-184-3p and miR-4433a-5p levels in human subjects [162]	Associative evidence with molecular target validation
METH abstinence	Significant decrease in neurotransmitters like GABA, along with an increase in serotonin	Decline in neuronal development and function and enhanced withdrawal behavior	Enhanced plasma-derived exosomal miRNAs-hsa-mia-451a and hsa-mir-21a in human subjects [70]	Associative
METH abstinence	-		Reduced miR-137 expression in circulating EVs in METH-abstinent individuals [171]	Associative
METH abstinence	-	Overactivation of the immune system, persistent increases in inflammatory cytokines and chemokines, and activation of Parkinson's disease-associated pathways	Associated with elevated exosomal CXCL5 and CXCL4/PF4 levels in plasma during acute withdrawal while remaining unchanged during protracted withdrawal in humans [172]	Associative & bioinformatic

Table 2. *Cont.*

METH-Dependence/ Abstinence	Molecular Pathways	CNS and Behavioral Changes	EV Cargoes	Nature of Evidence
METH abstinence	-	Causes neurodegeneration and promotes a behavioral shift toward egocentric spatial learning	Associated with decreased sera and striatal tissue levels of miR-137 in both non-human primate model and mice [173]	Functional molecular evidence; EV-specific function not established
METH abstinence	Neuronal and synaptic dysregulation pathway dependent on microglial activation and release of pro-inflammatory cytokines	Triggers hippocampal, dendritic, and synaptic abnormalities without overt neuronal loss in the acute withdrawal phase, and showed depression-like behavior, while no changes were observed in the protracted-withdrawal phase.	Elevated plasma EV-derived SAA1 levels in the acute withdrawal phase, while reverting during protracted withdrawal in mice [71]	Functional

Abbreviations: miRNA: micro-RNA; GABA: gamma-aminobutyric acid; NRG1: Neuregulin 1; ErbB4: Erb-B2 Receptor Tyrosine Kinase 4; CA1: Cornu Ammonis 1; TNF: Tumor Necrosis Factor; MAPK: Mitogen-Activated Protein Kinase; CRTC1: CREB-regulated transcription coactivator 1; CREB: cAMP response element-binding protein; cEV: circulating EV; CXCL5: chemokine (C-X-C motif) ligand 5; CXCL4: chemokine (C-X-C motif) ligand 4; PF4: platelet factor 4; SAA1: serum amyloid A1; Nature of evidence: Functional EV evidence includes studies in which EV administration or changes in EV-associated cargo produce measurable biological effects; Associative evidence describes studies reporting changes in EV levels or their molecular cargo in relation to METH exposure, withdrawal, or behavioral outcomes; Bioinformatic evidence refers to studies that use computational analyses to identify potential molecular targets or signaling pathways without experimentally confirming their functions. Studies that report both EV-associated changes and computational predictions are classified as associative and bioinformatic evidence.

6. Effect of METH in HIV Comorbidity and Related Neurological Disorders

METH has been proven to enhance the urge for sexual behavior associated with a high risk of HIV transmission from infected individuals to drug-seeking individuals, resulting in worsening immune suppression and increased viral load [65]. Further, substance use has a direct correlation with an increase in non-adherence to antiretroviral therapy in HIV patients, thus worsening the disease pathogenesis [12,175–179]. Incidentally, METH users with HIV infection showed higher viral load in the brain than corresponding non-METH users [65,180–182]. Furthermore, HIV and METH dependence have synergistic effects involving changes in frontostriatal circuits and neurotoxicity [183]. Studies in rats identified molecular synergy in exacerbating HIV pathogenesis-related neurocognitive defects through microglia-mediated NLRP3 inflammasome activation [184]. Combined METH and HIV-1 exposure altered TAAR1-mediated signaling in astrocytes, with co-exposure increasing TAAR1 expression and intracellular cAMP signaling. These changes impaired EAAT-2-mediated glutamate clearance, potentially contributing to excessive extracellular glutamate and excitotoxicity [185]. Additionally, METH exposure on promonocytic U1 HIV-infected cells showed the release of EVs facilitating syncytia formation and enhanced HIV-induced neuropathogenesis, involving the enhanced expression of ICAM-1 and viral Nef protein. Additionally, in cultured BV2 microglial cells, combined 0.1 mM METH and

100 nM HIV-1 Tat exposure synergistically activated the AIM2 inflammasome, caspase-1 activation, IL-1β and IL-18 production, and GSDMD cleavage, thereby promoting microglial pyroptosis. Suppression of AIM2 attenuated these responses, supporting AIM2-dependent inflammasome activation as a mechanism underlying METH-Tat-induced microglial inflammation and pyroptosis. An additional study in the same line demonstrated that co-exposure of METH (0.1 mM) and HIV-1 Tat (100 nM) (12 h exposure) on BV2 cells resulted in synergistic microglial activation along with AIM2-dependent pyroptosis and reduced cell viability, thus indicating that METH potentiates HIV-Tat-mediated microglial inflammation [149]. Intriguingly, METH was also shown to potentiate HIV-1 gp120-induced neurotoxicity by enhancing NLRP3 inflammasome assembly, GSDMD cleavage, and pro-inflammatory cytokine production in rat primary microglial cultures [186], while aggravating gp120-mediated synaptic dysfunction through increased expression of NLRP3, ASC, and procaspase-1 in hippocampal slices [184]. These studies demonstrate the role of METH on enhancement of HIV/HIV-viral proteins mediated neurotoxicity, warranting a broader assessment of how EVs released from METH and HIV co-exposed cells contribute to viral propagation, neuroinflammation, and neuronal dysfunction.

EVs released during HIV/SIV infection consistently package viral proteins (e.g., Env/gp120, Gag, Nef, Tat), viral RNAs (genomic RNA, TAR, miRNA) that can enhance viral spread, immune activation, and neurotoxicity along with host inflammatory mediators (cytokines, chemokines), adhesion molecules, kinases, and restriction factors, as well as raft-like lipids (cholesterol, sphingomyelin, ceramide, phosphatidylserine) and numerous miRNAs/mRNAs that together modulate immune responses, neuronal integrity, and reservoir dynamics [187–191]. Plasma-EVs from people living with HIV (PLWH) on cART are enriched in inflammatory cytokines and chemokines (e.g., IL-1α, TNF-α, CXCL10), supporting a role in chronic immune activation. EVs from HIV+ patients carry viral proteins such as Nef, gp120, Tat, and Gag, which can induce CD4+ T-cell apoptosis, activate latent reservoirs, and promote neurotoxicity and neuroinflammation [191–193]. Neuronal/glial-derived EVs from HIV-infected cells have been reported to alter glucose metabolism and glutamate homeostasis, promote neuroinflammation, and transfer neurotoxic viral proteins even under conditions of replicative latency, thereby sustaining chronic CNS injury [28,191,194]. In longitudinal SIV + macaque studies, plasma EV numbers increase ~50% from baseline in response to acute infection, return toward baseline under ART, and show a modest rise with ART-treatment interruption, paralleling systemic viral and inflammatory dynamics [195]. Proteomic analysis of serum exosomes from SIV-infected rhesus macaques revealed differential enrichment of ~236 proteins linked to latent viral reactivation (e.g., heat shock transcription factor 1), complement and inflammatory pathways, unfolded protein response, mitochondrial dysfunction, defective autophagy, and neuropathology [196]. EVs from cervicovaginal lavage and blood from SIV-infected macaques carry both host and viral proteins in EV-enriched fractions, suggesting roles in mucosal transmission, local inflammation, and CNS neuropathogenesis [196–198]. In an in vitro study, human endothelial cells were exposed to EVs isolated from HIV-1 Nef-transfected HEK-293 cells, resulting in transfer of Nef to the recipient endothelial cells. In the same study, SIV-Nef was detected in EVs isolated from the plasma of SIV-infected macaques, while further experimentation demonstrated the uptake of SIV-Nef-carrying EVs in naïve CD4+ T cells from uninfected macaques. These findings imply that EVs may facilitate Nef delivery to uninfected cells and thereby contribute to HIV/SIV pathogenesis [199].

Previous reports from our lab have also shown that HIV-1 viral protein Tat drives astrocytic EV biogenesis and pathological cargo loading in the context of HAND-associated Alzheimer's-like pathology. In a foundational study, Sil et al. (2020) [200] demonstrated that HIV-1 Tat induces astrocytic amyloidosis via the HIF-1α/lncRNA BACE1-AS axis,

wherein Tat stabilizes HIF-1α, enabling nuclear translocation and formation of a HIF-1α/BACE1-AS complex that transcriptionally activates BACE1 and stabilizes BACE1 mRNA, resulting in increased APP cleavage and Aβ42 production; this pathway was validated in SIV-infected rhesus macaques and postmortem brain tissue from HIV+ individuals with cognitive impairment [200]. Extending these findings to opiate comorbidity, chronic morphine exposure in rhesus macaques induced amyloid accumulation in the frontal cortex and basal ganglia through the HIF-1α/BACE1 axis and increased astrocyte-derived EV (ADEV) release enriched in amyloid cargoes (AβmOC64 and N-terminally cleaved APP) and IL-1β; HIF-1α silencing reduced both EV release and amyloid cargo loading, implicating HIF-1α in EV biogenesis and pathological cargo loading [51]. Building on these, our subsequent study demonstrated that HIV-1 Tat exposure of human primary astrocytes significantly increased release of exomere-enriched ADEVs carrying toxic amyloid cargoes (APP, Aβ); these Tat-ADEVs were taken up by hippocampal neurons and caused significant loss of dendritic arborization and impaired synaptic integrity, while ADEVs from HIF-1α-silenced astrocytes showed restoration of the dendritic parameters [201]. Finally, we translated these mechanistic findings to a therapeutic proof-of-concept using CD34+ NSG humanized mice infected with HIV-1, in which intranasal delivery of HIF-1α siRNA-loaded ADEVs over a ~2-month period suppressed HIF-1α expression, reduced APP, AβmOC64, Aβ fibrils, and hyperphosphorylated tau, attenuated glial activation, partially restored synaptic proteins, and showed modest improvement in memory impairments and startle response, thus establishing ADEV-mediated HIF-1α knockdown as a promising non-invasive therapeutic strategy for HAND-associated Alzheimer's-like pathology [202]. In addition to ADEVs, our subsequent study demonstrated that HIV-1 Tat exposure activated the microglial NLRP3 inflammasome, resulting in packaging of NLRP3 and mature IL-1β into microglia-derived EVs (MDEVs) that were taken up by neurons; these Tat-MDEVs caused synaptodendritic injury manifested by reduced PSD95, synaptophysin, and vGLUT1 expression, increased inhibitory neuronal markers GAD65 and gephyrin, and significant loss of dendritic spines. In addition to Tat-MDEV-NLRP3 cargoes, one of our most recent studies demonstrated that HIV Tat induces ferroptosis [203] and dysregulated ferritinophagy in microglia [204], resulting in enrichment of ferroptosis cargoes in the MDEVs [204] and transfer of these MDEV-ferroptotic cargoes to neurons, resulting in iron accumulation, oxidative stress, mitochondrial dysfunction, altered excitatory/inhibitory synaptic signaling, and synaptodendritic injury [205]. These effects were abrogated when neurons were treated with EVs derived from NLRP3-silenced microglia [206]. Taken together, these studies establish that EVs released from HIV-1 Tat-exposed astrocytes and microglia serve as critical intercellular conduits for transferring pathological cargoes, including toxic amyloids, NLRP3 inflammasome components, and ferroptotic mediators, to neurons, thereby linking glial-mediated synaptodendritic injury associated with HIV-associated neurocognitive disorders. METH increases EV release from multiple CNS-relevant cell types in the presence of HIV, carrying pathological cargoes (HIV-Nef, ICAM-1, α-synuclein, miR-29a) that promote neuroinflammation, syncytia formation, synaptodendritic injury, and potentially facilitate HIV persistence in the brain.

METH use disorder is consistently associated with increased EV release in plasma, from brain tissue, and macrophage cultures, indicating that psychostimulant exposure is a strong stimulus for vesicular shedding. In chronically HIV-infected promonocytic U1 cells and uninfected U937 macrophages, METH increases secretion of both large (10 K) and small (100 K) EVs and upregulates EV markers (e.g., HSC70), confirming enhanced EV biogenesis [28,54]. EVs from METH-treated, HIV-infected promonocytic U1 cells show increased ICAM-1 and VCAM expression, which promotes cellular clustering and syncytia formation in naïve macrophages, which can facilitate cell–cell viral spread. METH also selec-

tively enriches Nef in small EVs (100 K fraction) isolated from HIV-infected promonocytic U1 cells in a dose-dependent manner, and combined METH + cART further enhances Nef in some EV fractions, potentially amplifying EV-mediated CD4+ T-cell apoptosis, immune activation, and reservoir activation [54]. Additionally, METH robustly alters EV-miRNA cargo (e.g., upregulated miR-29a-3p in brain-derived EVs, and hundreds of differentially expressed miRNAs in rat plasma EVs) that drive inflammatory and synaptodendritic injury, yet how these METH-EV RNA signatures intersect with HIV replication, tropism, and reservoir dynamics in vivo remains largely unresolved [55]. Importantly, while METH increases brain viral load in SIV-infected macaques, detailed characterization of EVs specifically from HIV + METH-dependent macaques/subjects is still lacking, so the precise cargo changes that mediate METH-associated enhancement of SIV/HIV infectivity remain to be elucidated. Current reports strongly suggest that METH increases EV biogenesis, selectively enriches pro-adhesive proteins (ICAM-1, VCAM) and Nef, and alters EV-miRNA cargo in ways that promote inflammation and neurodegeneration. However, comprehensive multi-omic profiling of EVs from HIV+ humans and SIV+ macaques with METH exposure, especially integrating lipidomics, proteomics, and small and long RNA sequencing in cell-type-specific EVs (e.g., brain-derived EVs, macrophage EVs), is still needed to mechanistically link specific cargo changes to increased HIV infectivity, reservoir dynamics, and HAND progression. Addressing these gaps will be important for understanding whether METH enhances EV-mediated transfer of HIV co-receptors and viral proteins, alters EV lipid rafts in ways that facilitate viral docking or fusion, or modifies EV-associated miRNA networks involved in viral entry, replication, and latency. These mechanisms remain scantily studied and warrant further investigation.

7. Conclusions & Future Perspectives

METH is a highly addictive psychostimulant that can produce complex and potentially long-lasting changes at the molecular, synaptic, cellular, and neural-circuit levels. Evidence from both human studies and pre-clinical models shows that METH-associated pathology extends well beyond its acute effects on monoaminergic neurotransmission. It involves maladaptive dopaminergic and glutamatergic plasticity, oxidative and mitochondrial stress, impaired autophagy, BBB dysfunction, and neuroimmune activation. In particular, microglial and astrocytic activation, together with inflammasome signaling involving NLRP3, AIM2, NLRP6, and NLRP1, provides important links between cellular stress, inflammation, synaptic dysfunction, and behavioral abnormalities. Collectively, these interconnected mechanisms may contribute to the persistence of cognitive and affective disturbances during chronic METH use and withdrawal, as well as the continued vulnerability to relapse discussed throughout this review.

An increasingly important aspect of METH neuropathology is its interaction with EV-mediated intercellular communication. METH addiction and abstinence can alter EV biogenesis or release, as well as EV molecular cargo, including microRNAs and proteins. Importantly, EVs appear to be more than biomarkers of cellular injury. By transferring biologically active molecules between cells, they can influence inflammatory signaling, oxidative stress, autophagic processes, synaptic function, and neuronal viability. Evidence is particularly strong for changes in circulating and microglia-derived EVs, although neuronal, endothelial, and peripheral immune-cell compartments have also been implicated. For example, METH-associated EV miR-29a can promote inflammatory and synaptodendritic responses, whereas EV-associated SAA1 during acute withdrawal can induce microglial activation and synaptic abnormalities. However, the evidence linking EVs to METH-associated neurological and behavioral changes is still emerging. Some studies have shown that EV-associated cargo can contribute to neuroinflammation, synaptic injury, and behavioral dis-

turbances during withdrawal, while others have reported changes in EV molecular profiles following METH exposure or identified possible signaling pathways through bioinformatic analyses. Although these findings suggest that EVs may play a role in METH-related neurobiological changes, they do not yet demonstrate a direct involvement in drug reinforcement, drug-seeking behavior, or relapse. Further studies using EV-specific interventions in established addiction models are therefore needed to better understand their contribution to these processes. The potential relevance of EV signaling may extend beyond active METH use to withdrawal, abstinence, and relapse, although its direct involvement in relapse remains to be established. Dynamic changes in circulating EV-associated miRNAs and proteins across different stages of METH withdrawal suggest that EV cargo may reflect evolving neurobiological states rather than simply cumulative drug exposure. These molecular signatures could eventually serve as minimally invasive biomarkers for identifying METH exposure, characterizing withdrawal-associated neuroinflammation and neuropsychiatric symptoms, monitoring recovery, and potentially assessing biological changes associated with relapse. However, this translational potential remains early-stage. Longitudinal studies involving larger and more diverse cohorts will be necessary to establish the specificity, reproducibility, and predictive value of individual EV-associated molecules or multimolecular EV signatures. Together, these observations suggest that EV cargo differs with different stages of METH use, abstinence, and relapse, and can modulate recipient cells to induce sustained pathological signaling beyond the period of active drug exposure (summarized in Figure 1).

However, these findings should be interpreted with some caution, as methodological differences across studies may contribute to the observed variation. In blood-based studies, factors such as the use of plasma or serum, the choice of anticoagulant, the extent of platelet removal, and differences in sample processing, centrifugation, storage, and freeze–thaw conditions can all affect the abundance and molecular composition of the EV populations recovered [207,208]. Further variability may arise from differences in EV separation methods. For example, ultracentrifugation protocols can vary in centrifugal force, run time, and washing steps. These differences can affect EV recovery and may also lead to the co-isolation of lipoproteins, protein complexes, and other non-EV particles [209,210]. The interpretation of EV-associated miRNA findings may also be influenced by several technical factors, including differences in RNA extraction efficiency, the naturally low abundance of RNA, hemolysis or cellular contamination, the analytical platform used, and the choice of normalization method [211,212]. Furthermore, biological effects observed with EV-enriched preparations cannot always be attributed directly to EV cargo, as co-isolated soluble or non-vesicular components may also contribute to these responses. Appropriate controls are therefore important for distinguishing EV-specific effects from those caused by other components in the preparation. Overall, differences in EV isolation techniques, cargo analysis, and functional assays should be considered when comparing findings across METH-related studies and drawing conclusions about underlying mechanisms or potential biomarkers. EV cargoes, especially miRNAs, can be a potential biomarker candidate in METH addiction and abstinence, thus warranting further investigation. However, further validation in larger, independent clinical cohorts is indispensable before establishing their diagnostic or prognostic utility.

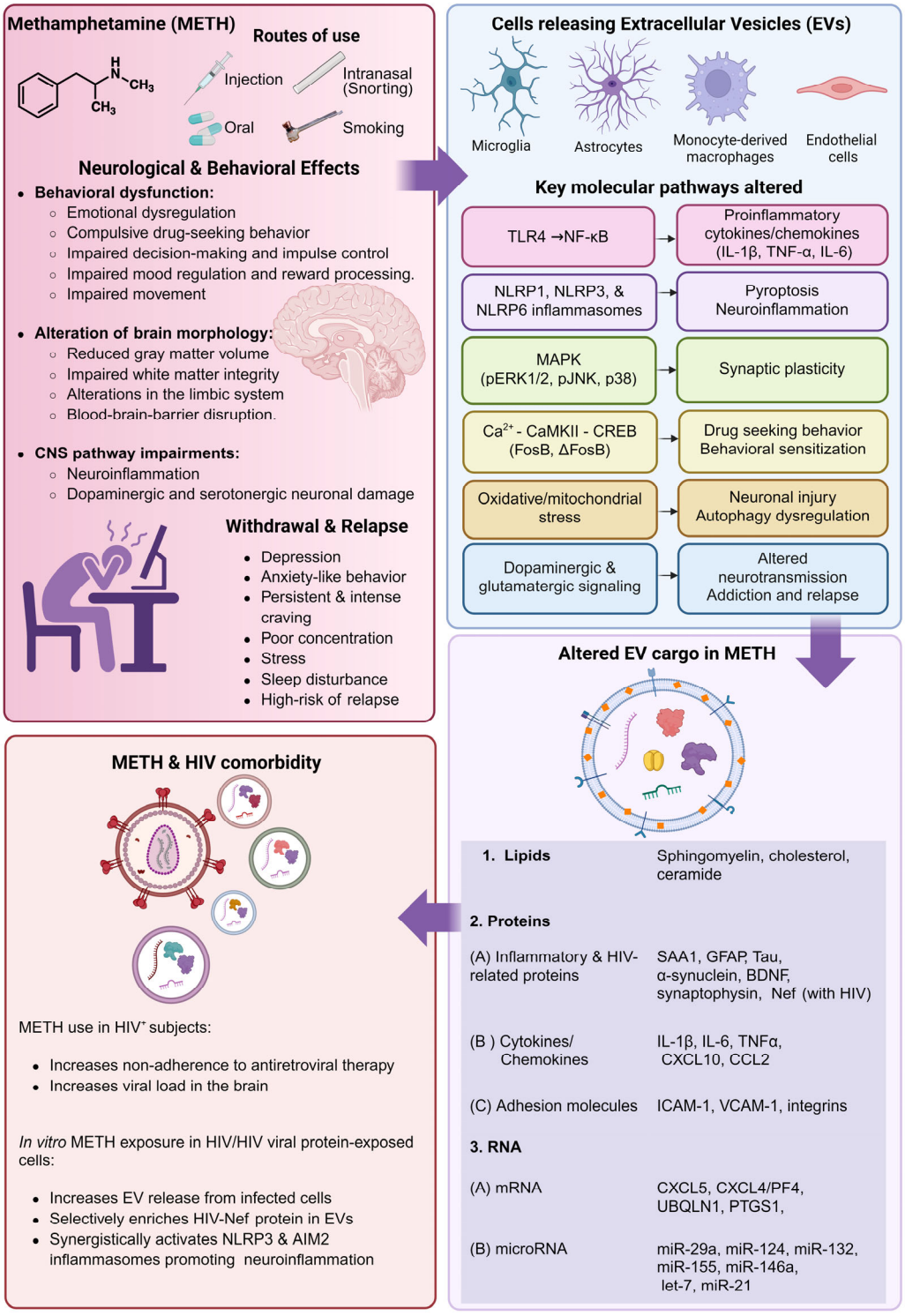

Figure 1. Role of EVs in Methamphetamine-mediated neurological disorders: Methamphetamine (METH) exposure induces neurological and behavioral dysfunction and induces extracellular vesicle (EV) release from microglia, astrocytes, macrophages, and endothelial cells. METH-EVs modulate inflammatory, oxidative stress, and neurotransmitter pathways while altering EV lipid, protein, mRNA, and microRNA cargoes. These METH and METH-EV-mediated altered molecular pathways contribute to neuroinflammation, neuronal injury, synaptic dysfunction, drug-seeking behavior, and relapse. Further, METH enhances HIV infectivity, EV release, and HIV-associated cargoes, thereby amplifying inflammasome activation and neuroinflammation.

An important limitation of the existing literature is the considerable variability in METH doses and exposure conditions across experimental models. In vitro studies have employed METH concentrations ranging from 10–150 μM to as high as 2–2.5 mM, while in vivo rodent studies have used doses of 2–10 mg/kg, with some studies employing doses of 30 mg/kg or higher. In contrast, METH concentrations commonly reported in human blood are approximately 0.1–0.5 mg/L (0.67–3.35 μM), although higher concentrations may occur during severe intoxication [213,214]. Melega et al. reported a blood range of 0.1–11.1 μM METH concentrations in METH users [214], while a large Swedish forensic study documented blood concentrations of as high as 5.6 mg/L (~37.5 μM) in METH users [215]. Post-mortem toxicological studies have confirmed the presence of METH in human brain tissue, with evidence of tissue-specific distribution and variability in brain-to-blood concentration ratios [215–217]. Additionally, human PET data were used to estimate a total brain concentration of ~14 μM following a 30-mg acute METH dosing (scanning performed for 90 min after injection) [218]. The total postmortem tissue concentrations of METH are not equivalent to free extracellular concentrations found in the living human brain. Thus, particularly the concentrations used in in vitro studies substantially exceed typical human systemic exposure. Moreover, differences in pharmacokinetics, metabolism, and tissue distribution limit direct comparisons between animal dosing regimens and human exposure. This distinction is especially important for METH because its plasma half-life is approximately 60–70 min in rats versus 10–12 h in humans. Consequently, high-exposure studies provide mechanistic evidence under the tested conditions but do not establish that the same effects occur across human cohorts. These limitations underscore the need for clinically relevant exposure models, appropriate cell viability assessments, and cautious interpretation when extrapolating experimental findings to human METH-associated neurological disorders.

Beyond these dose-related and translational limitations, several important knowledge gaps remain regarding the factors governing METH-induced EV biogenesis, cargo composition, and their functional significance across different stages of METH use disorder. The cellular origins of many circulating EV populations are still incompletely defined, and it remains unclear how factors such as METH dose, duration, and route of exposure, sex, age, polysubstance use, HIV status, and stage of addiction or withdrawal influence EV biogenesis and cargo selection. Among these variables, withdrawal stage and polysubstance use warrant particular consideration, as differences between acute withdrawal, protracted abstinence, and concurrent exposure to other substances may substantially influence EV-associated molecular profiles and complicate attribution of observed changes specifically to METH. Future studies should therefore integrate longitudinal human cohort studies with mechanistically controlled experimental models and standardized approaches for EV isolation and characterization. Moreover, studies linking METH use to altered EV size distributions remain limited, warranting robust, systemically generated studies on EV size and METH use. Furthermore, investigations should determine whether EV signatures can reliably distinguish active METH exposure, acute withdrawal, prolonged abstinence, and relapse, and whether manipulating specific EV cargo or EV-mediated signaling pathways can alter disease-related phenotypes rather than correlate with them. The relevance of EV signaling may extend beyond active METH use to withdrawal, abstinence, and relapse. Ultimately, a more complete understanding of METH use disorder will require integrating neurotransmitter dysregulation, maladaptive neuroplasticity, neuroinflammation, and EV-mediated intercellular communication rather than viewing these processes in isolation. Determining how METH reshapes EV biogenesis, cargo sorting, trafficking, and recipient-cell responses may uncover previously unrecognized pathways connecting peripheral immune alterations with neuronal and glial dysfunction in the brain. At the

same time, systematic characterization of EV-associated proteins and RNAs may support the development of minimally invasive biomarkers for disease staging, treatment monitoring, and potentially relapse-associated states. In the longer term, establishing whether pathogenic EV signaling can be selectively disrupted, or whether beneficial EVs can be engineered for therapeutic delivery, could provide new avenues for intervention.

EV-mediated mechanisms may be particularly important in HIV, where METH can exacerbate neuroinflammation, BBB dysfunction, and viral neuropathogenesis. Understanding these interactions may help to clarify the increased risk of reactivation of viral latency and worsening of neurocognitive impairments in virally suppressed HIV-infected individuals with METH use disorder.

Thus, future EV research provides a promising framework for connecting molecular mechanisms underlying METH addiction, withdrawal, neurological dysfunction, and comorbid HIV-neuropathological conditions with promising biomarker development and therapeutic strategies.

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