Neurotoxicity of Methamphetamine

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Contents

Abstract

Recreational consumption of the highly addictive psychostimulant methamphetamine is becoming a serious public health problem worldwide. Recent estimates indicate that methamphetamine abuse has increased in the last decade and that only cannabis is used by a greater number of consumers. Despite its popularity, methamphetamine is a known neurotoxin that damages dopaminergic terminals

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in the striatum, as indicated by reductions in striatal levels of dopamine and its metabolites and a sustained decrease in the expression of markers for dopaminergic terminals such as TH and DAT. In addition, methamphetamine affects the cell bodies of these same dopaminergic neurons in the substantia nigra, resulting in cell loss. The mechanisms underlying dopaminergic neurotoxicity are the focus of intense research, and knowledge in this area has expanded in recent decades. Evidence from previous studies points to dysregulation of dopamine, oxidative stress, DNA damage, and mitochondrial dysfunction as the main causes of methamphetamine neurotoxicity. The dopamine receptors D1 and D2 also play an important role in methamphetamine-induced neurotoxicity since inactivation of either receptor is neuroprotective against methamphetamine. Recent results from clinical research indicate that methamphetamine abusers have a higher risk of developing Parkinson's disease; this is in keeping with results in laboratory animals and confirms the persistence of methamphetamine-induced dopaminergic injury. These findings suggest that neuroprotective strategies that are effective against methamphetamine-induced toxicity are also promising candidates for preventive therapy for Parkinson's disease and other persistent dopaminergic injuries.

Keywords

Amphetamine derivatives • Designer drugs • Dopamine • Drug addiction • Methamphetamine • Neurotoxicity • Parkinson's disease • Psychomotor stimulants

List of Abbreviations

ATS	Amphetamine-type stimulants
$D1R^{-/-}$	D1R knockout mice
D1R	Dopamine D1 receptor
$D2R^{-/-}$	D2R knockout mice
D2R	Dopamine D2 receptor
DAT	Dopamine transporter
DOPAC	3,4-Dihydroxyphenylacetic acid
Gclm	γ-Cysteine ligase modulatory subunit
Gclc	γ-Cysteine ligase catalytic subunit
GPx	Glutathione peroxidase
HVA	Homovanillic acid
MDMA	3,4-Methylendioxymethamphetamine also called "ecstasy"
Nrf2	Nuclear factor-erythroid 2-related factor 2
PD	Parkinson's disease
ROS	Reactive oxygen species
SNpc	Substantia nigra pars compacta
TH	Tyrosine hydroxylase
VMAT2	Vesicular monoamine transporter

1 Introduction

1.1 Background, Medical Use, and Epidemiology

Methamphetamine (*N*-methyl-1-phenylpropan-2-amine) is a synthetic drug first used clinically at the beginning of the twentieth century. Therapeutic use of methamphetamine was based on its sympathicomimetic properties, but its current illicit use as a recreational drug in several countries around the world is due to its psychostimulant effects. It is also used for weight loss and for enhancing alertness, focus, motivation, and mental clarity for extended periods of time. Methamphetamine is structurally related to the neurotransmitters dopamine and phenylethylamine, and to other psychostimulant drugs like amphetamines (Fig. 1).

Methamphetamine has proven to be highly addictive and its abuse can result in severe psychological and physical dependence. It is therefore classified as a Schedule II drug under the Convention on Psychotropic Substances. Methamphetamine abuse is increasingly recognized as a major global health problem (Degenhardt et al. 2008). The 2012 World Drug Report (UNODC 2012) suggests that the global use of amphetamine-type stimulants (ATS; methamphetamine,

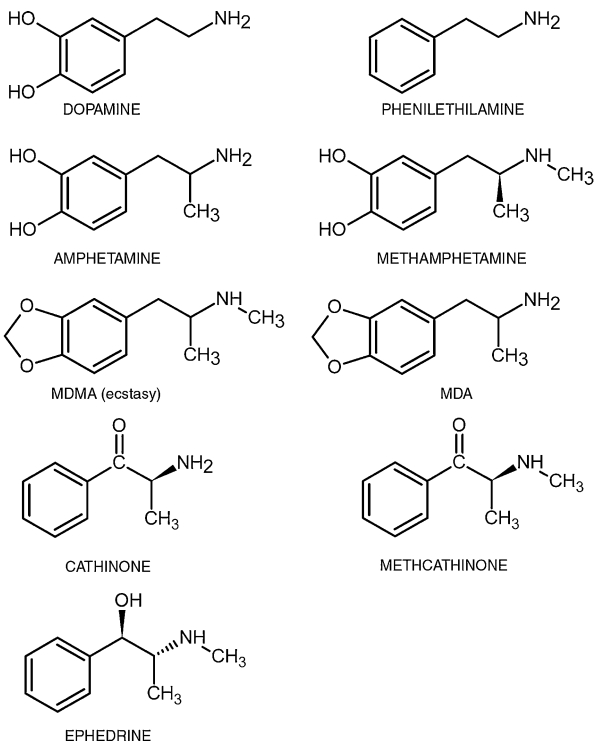

Fig. 1 Chemical structures of methamphetamine and related amphetamine compounds. All compounds share chemical structure with dopamine and therefore have strong effects on the dopamine system

amphetamine, and methcathionine) (excluding "ecstasy") is second only to cannabis. They were used by 14–53 million people in 2010 equivalent to 0.3–1.2 % of the global population aged 15–64 years.

1.2 Administration Routes and Patterns of Methamphetamine Use

Methamphetamine comes in several forms. The hydrochloride salt of methamphetamine is a white, crystalline, bitter, odorless powder. It is water-soluble and strongly hygroscopic (absorbs water quickly). The common street names "speed," "Meth," or "chalk" refer to the salt, while "crystal," "crystal Meth," and "ice" refer to crystalline methamphetamine, a form purer than the powder. Methamphetamine is also known by a variety of other names, including shabu, batu, D-meth, tina, and glass. Methamphetamine freebase is oily and is uncommon on the street.

Methamphetamine can be taken orally (in pill form), by intravenous injection, smoking, snorting (in powder form), or by anal or vaginal insertion of a suppository. The effects experienced by the user last 6–8 h, depending on the rate at which methamphetamine reaches the blood, which depends on the route of administration. The faster the drug reaches the blood, the greater the "high" and other effects experienced by the user.

Following administration by any route, methamphetamine is distributed through most organs, including the lungs, liver, and stomach. Moderate levels reach the brain by crossing the blood–brain barrier. It also crosses the placenta and is secreted into breast milk. Methamphetamine is metabolized in the liver, with the main metabolites including the active compounds amphetamine, 4-hydrooxyamphetamine, and norephedrine. The concentration of amphetamine metabolite peaks at 10–24 h post-administration.

Methamphetamine abuse has two distinct use patterns. The first, characterized by low intensity use, does not confer psychological dependence. The second, known as "binge" use, consists of repeated redosing, usually by inhalation or injection, for several days in a row; generally, withdrawal symptoms occur when drug delivery is stopped abruptly.

1.3 Methamphetamine: Mechanism of Action and Effects

Methamphetamine's effects on the body are due to its structural resemblance to the neurotransmitter dopamine (Fig. 1). It easily crosses the blood–brain barrier, reaches the brain, and enters the axons of dopaminergic neurons through the dopamine transporter DAT and by passive diffusion (Granado et al. 2011a; Krasnova and Cadet 2009). Once inside the axon, methamphetamine triggers the release of dopamine from synaptic storage vesicles, resulting in an unusually high concentration of dopamine in the cytoplasm (Krasnova and Cadet 2009). Methamphetamine cannot directly activate dopamine receptors; rather, it acts as an indirect

dopamine agonist that increases dopamine concentration in the synapse by increasing dopamine release and blocking dopamine uptake. Methamphetamine also releases norepinephrine and serotonin by a similar mechanism; however, in the brain, methamphetamine is selectively concentrated in norepinephrine and dopamine nerve terminals because it is a substrate for the molecular transporters present in these terminals.

As a consequence of this increased release of dopamine in several areas of the brain, methamphetamine produces a number of acute psychological effects including euphoria (also known as "flash" or "rush", and lasting only several minutes). After this first short period, other feelings and behaviors may appear, including a false sense of self-confidence and power (delusions of grandeur), loquacity, moodiness, irritability, anxiousness, nervousness, aggressiveness, and violent behavior. Methamphetamine consumption has many acute adverse physical effects, including hyperthermia, increase in blood pressure and heart rate, mydriasis (pupil dilatation), logorrhoea, grinding teeth (trismus and bruxia), gastrointestinal irritation, appetite loss, itching, welts on skin, hyperactivity, involuntary body movements, irreversible damage to blood vessels in the brain resulting in cerebrovascular accidents, arrhythmia, tachycardia, cardiovascular collapse, and death. The most common symptoms of chronic methamphetamine abuse include temporomandibular joint syndrome, tooth erosion, and myofacial pain, all manifestations of acute trismus and bruxia. Other long-term symptoms are loss of appetite, weight loss, accelerated aging, nose bleeding, and "Meth mouth," an oral disease characterized by tooth erosion, extensive caries, decayed surfaces, missing teeth, tooth wear, plaque, and calculus (Fig. 2). Methamphetamine is highly addictive, and its use can result in tolerance: The effects decrease gradually with chronic use; thus, increased dosages are required to achieve the desired effects.

2 Methamphetamine Induces Neurotoxicity

Repeated methamphetamine administration results in neurotoxicity, primarily affecting dopaminergic neurons in the nigrostriatal system as reflected by long-lasting reductions in levels of dopamine and its metabolites (DOPAC and HVA), dopaminergic markers such as tyrosine hydroxylase (TH) (the rate-limiting enzyme for dopamine synthesis), and DAT (Krasnova and Cadet 2009) (Fig. 3).

Several studies have demonstrated dopaminergic axon loss in the striatum after repeated methamphetamine use, indicated by loss of TH and DAT immunoreactivity. Although there is partial recovery of axonal TH and DAT immunoreactivity, some loss persists for long periods. Other amphetamine compounds such as ecstasy (MDMA) also produce this persistent axonal loss, which correlates with dopaminergic cell body loss in the substantia nigra pars compacta (SNpc), as demonstrated by rigorous stereology/cell counts with TH and Nissl staining and by use of cell death markers such as Fluorojade (Granado et al. 2008a). Apoptotic cell bodies, an irrefutable marker of cell death, have also been observed in the SNpc of methamphetamine-treated mice (Ares-Santos et al. 2012, 2013; Granado et al. 2011a).

Fig. 2 Methamphetamine abuse can produce accelerated aging, "METH mouth," and "METH mites." (**a**). Physical aspect of a woman at several time points during 4 years of methamphetamine abuse. (**b**). Case of "Meth Mouth". (**c**). Sores on the skin known as "meth mites," which result from scratching to relieve the feeling of having small bugs under the skin that methamphetamine abusers may experience (Taken from drug enforcement administration www.dea.gov)

Interestingly, the compartments of the mouse striatum – the striosomes and matrix – differ in their vulnerabilities to methamphetamine, (Granado et al. 2010) (see Fig. 4). Striosomes, which are connected to the limbic system and are functionally associated with reward-related behaviors and emotional events (White and Hiroi 1998), are more vulnerable to methamphetamine-induced dopaminergic terminal loss than the matrix, which is connected to sensorimotor regions of the brain and is more closely associated with normal motor functions.

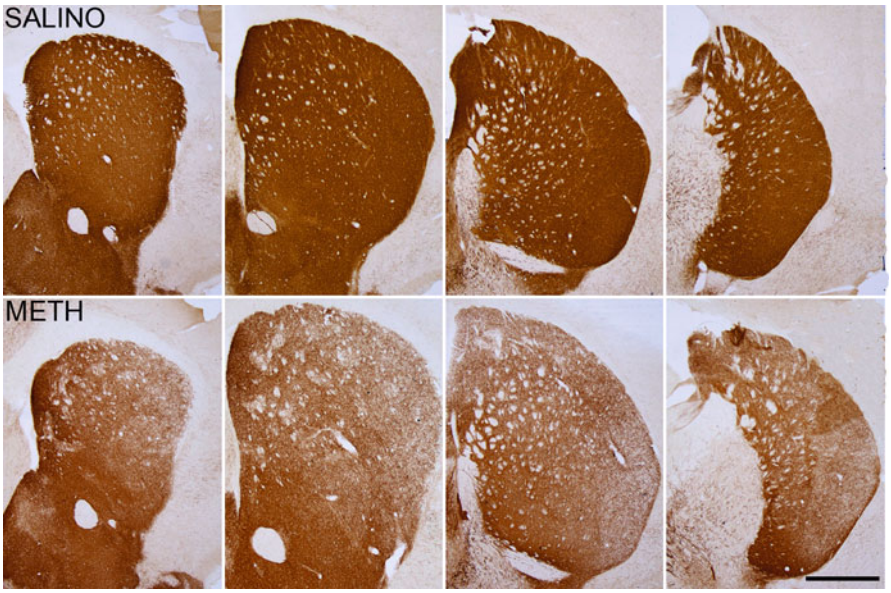

Fig. 3 Methamphetamine induces a loss of striatal TH-ir, indicative of dopamine fiber loss. Photomicrographs of striatal sections from mice treated with saline or methamphetamine stained for TH. METH produced a marked loss in striatal TH-ir that persists 7 days after treatment. Scale bar indicates 500 μm

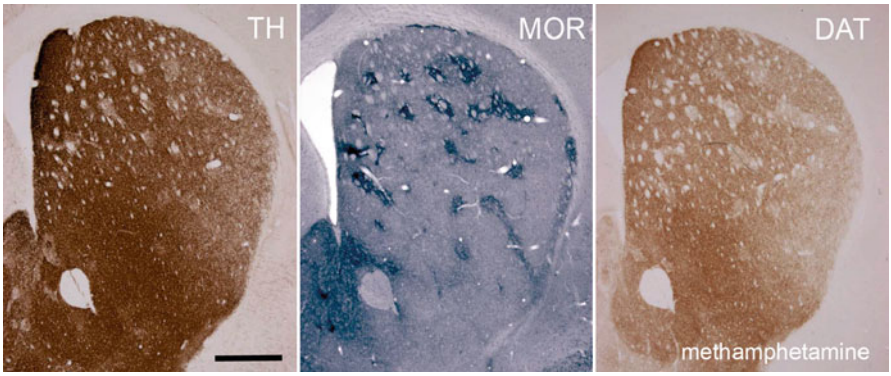

Fig. 4 Striatal vulnerability to methamphetamine. Methamphetamine toxicity occurs primarily in the striatum. Methamphetamine produces a preferential loss of TH and DAT in the striosomal compartment compared to the matrix. Photomicrographs of mouse brain sections 7 days after saline or methamphetamine treatment were stained for TH, MOR-1, and DAT. MOR-1 is a marker for striosomes. Scale bar indicates 500 μm (Modified from Granado et al. (2010))

The pattern of dopamine degeneration in the striatum is similar to that observed in the early stages of other neurodegenerative diseases such as Huntington's disease, hypoxic ischemic injury, and treatment with MPTP, a selective neurotoxin for dopaminergic neurons that is frequently used as a model of Parkinson's disease.

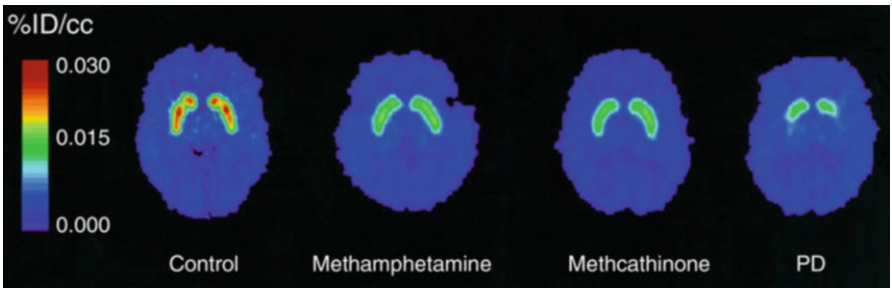

Fig. 5 Reduced DAT function in methamphetamine users. PET images showing accumulation of [11C] WIN-35,428 in the striatum of a control subject, an abstinent methamphetamine subject, an abstinent methcathinone subject, and a PD patient 70–90 min after injection of [11C] WIN-35,428 (Taken from McCann et al. (1998))

The nucleus accumbens is resistant to methamphetamine-induced dopaminergic axon loss (Granado et al. 2010), paralleling the effects of Parkinson's disease (Hurtig et al. 2000).

Methamphetamine neurotoxicity has also been demonstrated in humans (Fig. 5). PET studies in methamphetamine abusers found a reduction in DAT density in the caudate nucleus (26 % loss) and the putamen (21 % loss) after a short period of abstinence (Volkow et al. 2001a). Other authors reported similar DAT loss even 3 years after methamphetamine withdrawal (McCann et al. 1998). Studies in postmortem striatal tissue from chronic methamphetamine abusers showed a significant dopamine reduction concomitant with loss of DAT and TH immunoreactivity, indicative of dopamine nerve fiber loss. These effects have been related to loss of neurological function, including memory loss and motor and verbal learning impairments (Volkow et al. 2001b). Methamphetamine also causes neurotoxicity in other brain areas including somatosensory parietal, frontal, and piriform cortex, olfactory bulb, and hippocampus, where apoptotic neurons have been found following exposure to the drug.

These studies in animals and in human abusers suggest that methamphetamine consumers may be more susceptible to neurodegenerative diseases like Parkinson's disease (PD), raising important concerns about the use and abuse of amphetamines clinically and recreationally. A very recent clinical study shows that methamphetamine users have a 76 % greater risk of developing PD than normal subjects. Although these studies have not yet confirmed neuronal damage in the substantia nigra of human methamphetamine abusers, results in animals support this loss and are consistent with the idea that repeated methamphetamine abuse predisposes the abuser to PD. Methamphetamine also causes neuronal loss in other brain areas such as the olfactory bulb, cortex, hippocampus, and striatum, as indicated by increased apoptosis, increased numbers of TUNEL-positive cells, and decreased numbers of neurons in the brains of methamphetamine-treated laboratory animals (Krasnova and Cadet 2009).

3 Mechanisms of Methamphetamine-Induced Neurotoxicity

3.1 Role of Dopamine

Methamphetamine consumption greatly increases the dopamine concentration in brain synapses. Excessive dopamine in the synaptic cleft is responsible for most of the physical and psychological effects of the drug, including addiction and psychomotor stimulant effects. An imbalance in the distribution of dopamine in the brain also seems to give rise to neurotoxicity, a fact that explains the localization of drug-induced degeneration to dopaminergic terminals (Fig. 6).

Following synthesis in dopaminergic neurons, dopamine is first released to the cytosol before being stored in vesicles where it is protected from metabolism and auto-oxidation. Methamphetamine induces a redistribution of dopamine inside the terminal, releasing dopamine from the vesicles into the cytosol where it is a substrate for metabolic and oxidative reactions, resulting in the production of dopamine quinones, superoxide anions, and hydrogen peroxide and hydroxyl radical species. This can further promote the oxidation of cytosolic dopamine, generating oxidative stress and leading to mitochondrial dysfunction and damage in the dopaminergic terminal (Cadet and Krasnova 2009; Thomas et al. 2008). The detrimental role of excessive cytosolic dopamine and its implication in the neurotoxic effects of methamphetamine is supported by the fact that when dopamine synthesis is inhibited by αMPT, protection against methamphetamine toxicity is observed (Albers and Sonsalla 1995; Ares-Santos et al. 2012). Moreover, pretreatment with L-DOPA, a precursor of dopamine, and treatment with reserpine, which releases dopamine from vesicles to the cytoplasm, both potentiate methamphetamine toxicity (Albers and Sonsalla 1995; Granado et al. 2011a).

3.2 Implications of Oxidative Stress

Reactive nitrogen species and reactive oxygen species (ROS) are by-products of normal physiological metabolism in the brain, but excessive production of these reactive species can damage cell components, including lipids by lipid peroxidation, proteins by formation of protein carbonyls, and mitochondrial and nuclear DNA by peroxidation of these macromolecules. These reactive species impair mitochondrial respiratory chain enzymes and inhibit sodium-potassium ATPase, generating oxidative and nitrosative stress that leads to metabolic collapse and necrotic or apoptotic cell death. These oxidative stress cascades occur in several neurodegenerative disorders including Parkinson's disease.

Methamphetamine administration increases levels of extra-vesicular dopamine in the cytosol, where it can be metabolized by MAO or auto-oxidized in a process that generates toxic dopamine quinones. These dopamine quinones can damage cell proteins by binding to cysteine residues or by generating hydrogen peroxide (H_2O_2) and superoxide anions ($O_2\bullet-$), considered a major culprit in methamphetamine toxicity. The methamphetamine-induced increase in

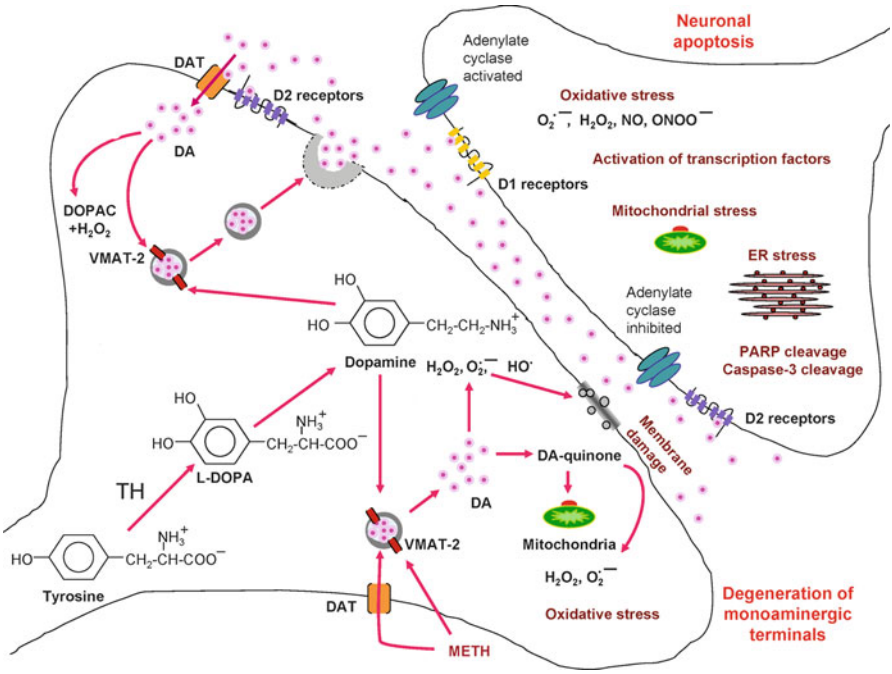

Fig. 6 Schematic representation of cellular and molecular events involved in methamphetamine-induced dopamine terminal degeneration and neuronal apoptosis within the striatum. The figure summarizes findings of various studies that have addressed the role of dopamine, oxidative stress, and other mechanisms in methamphetamine toxicity. Methamphetamine enters dopaminergic neurons via DAT and passive diffusion. Within these neurons, methamphetamine enters synaptic vesicles through VMAT2 and causes dopamine release into the cytoplasm via changes in pH balance. In the cytoplasm, dopamine auto-oxidizes to form toxic dopamine quinones, generating superoxide radicals and hydrogen peroxides via quinone cycling. Subsequent formation of hydroxyl radicals through interactions of superoxides and hydrogen peroxide with transition metals leads to oxidative stress, mitochondrial dysfunctions, and peroxidative damage to presynaptic membranes. The toxic effects of released dopamine might occur through activation of dopamine receptors, as dopamine receptor antagonists block degeneration of dopamine terminals (Modified from Krasnova and Cadet (2009))

interaction of superoxides and hydrogen peroxide with transition metals like iron can lead to the formation of hydroxyl radicals (•OH) that cause oxidative stress, mitochondrial dysfunction, and peroxidative damage to dopaminergic terminal membranes (Krasnova and Cadet 2009).

The methamphetamine-induced increase in oxidative stress likely results from an imbalance between ROS production and the capacity of antioxidant enzyme systems to scavenge ROS: Methamphetamine both increases ROS production and reduces levels of the ROS scavengers CuZnSOD, catalase, glutathionine, and peroxiredoxins in the brain (Jayanthi et al. 1998; Li et al. 2008). Oxidative stress increases the susceptibility of the striosomal compartment to methamphetamine-induced dopaminergic toxicity (Granado et al. 2010). SOD is less abundant in

striosomes than in the matrix (Medina et al. 1996), which may explain the greater vulnerability of the striosomes to methamphetamine neurotoxicity. This is supported by the finding that transgenic mice overexpressing CuZnSOD are resistant to methamphetamine-induced striatal neuronal damage (Hirata et al. 1996). Furthermore, antioxidants like ascorbic acid (vitamin C), vitamin E, bromocriptine (a hydroxyl radical scavenger), and coenzyme Q10 (antioxidant and mitochondrial energy enhancer) attenuate methamphetamine toxicity (Wagner et al. 1986). Free radical scavengers like PBN (α-phenyl-*N*-terbutil nitrone) also reduce neurotoxic damage (Yamamoto and Zhu 1998), without altering the hyperthermic response that follows methamphetamine administration and that contributes to methamphetamine neurotoxicity (see Sect. 3.3 below).

3.3 Role of Hyperthermia

Methamphetamine usually produces a hyperthermic response in experimental animals and human meth abusers directly proportional to the dosage of the drug and to the ambient temperature in the place of treatment. This hyperthermia can be lethal (it is the first cause of methamphetamine-induced deaths) and can promote long-term neurotoxicity, as a relationship has been observed between the hyperthermic response and the neurotoxicity induced by the drug (Ares-Santos et al. 2012; Bowyer et al. 1994; Granado et al. 2010, 2011a). Strategies that reduce or avoid this hyperthermic response after methamphetamine treatment, like administering the drug at low ambient temperatures (4 °C) or pretreatment with pharmacologic agents like diclocipine or haloperidol, prevent or attenuate drug-induced neurotoxicity (Albers and Sonsalla 1995; O'Callaghan and Miller 1994). Furthermore, methamphetamine administration at high ambient temperatures promotes the hyperthermic response and increases neurotoxicity (Ares-Santos et al. 2012; Bowyer et al. 1994; Granado et al. 2011a; Miller and O'Callaghan 2003).

The correlation between hyperthermia and neurotoxicity is believed to be due to the fact that hyperthermia can potentiate DAT function (Xie et al. 2000), increasing free radicals and dopamine oxidation in the brain (Krasnova and Cadet 2009; LaVoie and Hastings 1999). Conversely, hypothermia has inhibitory effects on oxidative stress, as it reduces dopamine oxidation (LaVoie and Hastings 1999) and the formation of hydroxyl radicals (Fleckenstein et al. 1997; Krasnova and Cadet 2009). Moreover, neurons in animals maintained at low body temperature have reduced energetic demand, which may be protective since administration of methamphetamine produces striatal loss of ATP, possibly as a consequence of metabolic stress in dopaminergic neurons (Chan et al. 1994).

In contrast to these results, other pharmacological and genetic studies indicate that while hyperthermia contributes to methamphetamine-induced dopaminergic neurotoxicity, it is not required. For example, reserpine, a pharmacologic agent that dramatically lowers body temperature, strongly potentiates methamphetamine-induced neurotoxicity while blocking the hyperthermic response (Albers and Sonsalla 1995; Ares-Santos et al. 2012; Granado et al. 2011a). These results

indicate that blocking the hyperthermic response is not sufficient to protect against neuronal damage. Moreover, total or partial inactivation of DAT, nNOS, IL-6, or c-jun protects against methamphetamine-induced toxicity without altering the hyperthermic response. Thus, methamphetamine-induced hyperthermia contributes to, but is not required for, the neurotoxic effects of the drug.

3.4 Role of Dopamine Receptors and Dopaminergic System

As evidence points to dysregulation of dopamine as the primary cause of methamphetamine-induced neurotoxicity, the role of the dopaminergic system in this process has also been evaluated. In particular, as methamphetamine acts as an indirect dopamine agonist, several studies have focused on elucidating the role of dopamine receptors. There are five different dopamine receptors (D1–D5), which fall into two families based on pharmacologic classification: the D1-like receptors (D1 and D5) and the D2-like receptors (D2, D3, and D4). Both families are involved in behavior and cognition, voluntary movement, motivation, punishment and reward, attention, working memory and learning, and in several neurodegenerative diseases like PD (Darmopil et al. 2009; Granado et al. 2008b; Ortiz et al. 2010).

Pharmacologic studies with the D2 receptor antagonists sulpiride, eticlopride, and raclopride have shown a dose-dependent prevention of methamphetamine toxicity in mice (Albers and Sonsalla 1995; Eisch and Marshall 1998). However, these compounds do not differentiate between members of the D2 receptor family, so it was not clear which receptor(s) mediate the protective effect. Recent studies using genetically modified mice lacking dopamine receptor D2 demonstrated that it is specifically the D2 receptor that is involved in methamphetamine toxicity as its genetic inactivation prevented the loss of dopaminergic striatal markers and inhibited the loss of dopaminergic neurons in the substantia nigra (Granado et al. 2011a).

D2 receptors are localized pre- and postsynaptically. At presynaptic locations, D2 receptors control dopamine release and thereby regulate extra-synaptic dopamine levels, which are involved in non-dopaminergic toxicity, such as in striatal medium spiny neurons, cortical and hippocampal neurons and neuropil. In addition, D2 receptors form heteromeric protein-protein complexes with DAT localized in the dopaminergic terminals that potentiate DAT activity. Blockade or inactivation of the D2R decreases striatal DAT activity. Since DAT knockout mice exhibit full protection against methamphetamine-induced dopaminergic toxicity (Fumagalli et al. 1998), indicating that active DAT is required for this neurotoxicity, the decrease in DAT is likely a major factor in the reduction of methamphetamine-induced dopaminergic toxicity induced by blockade or inactivation of the D2R. Moreover, fast scan cyclic voltammetry indicates that dopamine D2R$^{-/-}$ mice have lower vesicular dopamine content, resulting in lower cytosolic dopamine levels. This also contributes to the reduction in methamphetamine-induced toxicity because cytosolic dopamine levels determine the severity of the toxicity (Granado et al. 2011a).

Receptors from the D1 family are also involved in methamphetamine-induced neurotoxicity as their pharmacologic inactivation with antagonists like SCH23390 also confers protection (Sonsalla et al. 1986). Genetic inactivation of the dopamine D1 receptor (D1R) also protected against reductions in striatal TH and DAT expression and against loss of dopaminergic neurons in the substantia nigra following methamphetamine administration, indicating that the D1R in particular is involved in methamphetamine neurotoxicity (Ares-Santos et al. 2012). Neuroprotection afforded by D1R inactivation is due in part to inhibition of hyperthermia, but also to the redistribution of dopamine inside the terminal. Animals lacking the D1R store more dopamine in vesicles and therefore have a reduced cytosolic dopamine pool compared to WT mice (Ares-Santos et al. 2012). Blockade of D1/D5R also suppresses activation of caspases 3 and 8, mediators of the calcineurin/NFAT/FasL-dependent apoptotic cell death pathway (Jayanthi et al. 2005; Krasnova and Cadet 2009); this may also contribute to the neuroprotective effects of D1/D5 blockade or inactivation. Finally, SCH23390 decreases dopamine-induced oxidation and cytotoxicity mediated by ERK and JNK activation (Chen et al. 2004).

Other components of the dopaminergic system are also involved in methamphetamine-induced toxicity. Vesicular monoamine transporter (VMAT2) takes up dopamine from the cytosol to store it in synaptic vesicles, decreasing dopamine oxidation. Methamphetamine interacts with VMAT2 to cause a possible association of vesicles inside the dopaminergic terminal, increasing the release of dopamine to the cytosol and thereby increasing oxidative stress (Sulzer et al. 2005). VMAT2 knockout mice, with higher levels of cytosolic dopamine, are more sensitive to methamphetamine dopaminergic toxicity and show greater expression of oxidative stress markers than WT animals (Larsen et al. 2002). Other results are in line with these findings, showing that VMAT2 becomes nitrated 1 h after methamphetamine administration, which may reduce its activity (Eyerman and Yamamoto 2005), and that methamphetamine also reduces VMAT2 expression in the striatum (Krasnova and Cadet 2009).

3.5 Role of Glutamate and Nitric Oxide

Methamphetamine produces excitotoxicity by increasing glutamate release in the striatum (Nash and Yamamoto 1992), activating *N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. Stimulation of these receptors increases intracellular levels of Ca^{2+}, causing activation of kinases, lipases, and proteases that damage the cytoskeleton, generating free radicals and DNA damage (Sattler and Tymianski 2000). Pharmacological studies using MK801, a noncompetitive NMDA receptor antagonist, prevented the long-term dopamine loss induced by methamphetamine (Sonsalla et al. 1989).

Moreover, NMDA receptor overactivation results in the production of superoxide radicals (O_2•−) and nitric oxide (NO). When these two species react with each other, peroxynitrite ($ONOO^-$), a more potent oxidative species, is formed, further

potentiating neurotoxicity. To examine the contribution of this strong oxidizing agent to methamphetamine toxicity, a previous study measured the formation of neural 3-nitrotyrosine (3-NT), a product of tyrosine nitration that indicates irreversible structural modification of proteins (Butterfield et al. 2011) that can lead to loss of physiological cell functions, appearance of abnormal functions, and eventually cell death. A single injection of methamphetamine produced a significant rise in 3-NT concentrations in the striatum, signifying the involvement of $ONOO^-$ in the destructive effects methamphetamine abuse. Genetic or pharmacological inactivation of nNOS, the enzyme that produces nitric oxide in the brain, considerably reduced methamphetamine neurotoxicity without affecting the hyperthermic response (Itzhak et al. 1998, 2002), likely due to loss of peroxynitrite production.

3.6 Role of Astroglial and Microglial Activation

The central nervous system (CNS), consisting of the brain and the spinal cord, is an "immune privileged" area with an immune system distinct from that in the rest of the body. Microglia, a type of glial cell, are the resident macrophages of the central nervous system, and so act as its first and main form of active immune defense. These cells are normally in a resting state, but become activated after certain types of CNS damage, as a part of the innate immune response. Activated microglia migrate rapidly to the damage sites and secrete reactive species including proinflammatory cytokines (IL-1β, IL-6, TNF-α) chemokines, prostaglandins, reactive oxygen species (ROS), nitric oxide, and superoxide to protect the brain. However, overactivation can be detrimental, producing cell death and astroglial dysfunction.

Amphetamines increase microglia activation in the striatum, hippocampus, cortex, and SN, with populations peaking 1 day after administration. Following administration of methamphetamine or other amphetamine derivatives, activated microglia are found in the areas in which dopaminergic neurotoxicity occurs, and the intensity of activation seems to be correlated with the level of dopaminergic damage. The highest levels of microglial activation occur in the dorsal striatum, an area highly affected by methamphetamine treatment, while the nucleus accumbens, more resistant than the striatum to methamphetamine-induced dopaminergic toxicity, has relatively few reactive microglia. Methamphetamine administration also results in increased levels of the three principal proinflammatory cytokines: IL-1β, IL-6, TNF-α, that in large part result from microglial activation (Clark et al. 2013). Thus, microglial activation represents a direct response to damage by amphetamines and is part of the cascade leading to neuronal damage (Thomas et al. 2008). Although many of the molecules secreted by activated microglia have been implicated in methamphetamine-induced neurotoxicity, and some anti-inflammatory drugs like ketoprofen, indometacin, tetracycline, and minocycline can protect against methamphetamine-induced microglial activation and neurotoxicity, attenuating microglial activation is not sufficient to protect against methamphetamine neurotoxicity (Sriram et al. 2006).

CNS damage is also accompanied by reactive gliosis, the injury-induced activation of astrocytes. The most abundant cells in the human brain, astrocytes, provide nutrients to nervous tissue, maintain extracellular ion balance, support other brain cells, and play an important role in repair and scar formation in the CNS after injury.

Recently, the immunomodulatory role of astrocytes has begun to emerge. Astrocytes can be protective, increasing levels of glutathionine (an antioxidant), facilitating sprouting, and providing growth factors, guidance molecules, and scaffolding for axonal regeneration, but they can also initiate several neuroinflammatory pathways and release inflammatory cytokines, some of which are neurotoxic. However, it seems likely that astrocytes play a positive role in limiting neuroinflammation and the balance between the activation of microglia and astrocytes leads to a detrimental or beneficial outcome (Clark et al. 2013).

Reactive gliosis is considered a universal reaction to CNS damage and is used as a sensitive marker of neuronal damage. Methamphetamine increases astrocyte activation in the striatum, as seen by increased expression of the marker glial fibrillary acidic protein (GFAP), which reaches maximal levels between 3 and 7 days after drug administration (Fig. 7). However, there are much earlier indications of reactive gliosis: Within a few minutes of methamphetamine delivery, there is already a 20 % increase in the magnitude of the Ca^{2+} fluorescence signal in striatal astrocytes, and a 50–60 % increase in the number of responding astrocytes, indicating a primary astrocytic response (Granado et al. 2011b). As with microglia, activation of astrocytes takes place in the areas the most affected by methamphetamine, while the astrocyte population does not increase in the nucleus accumbens, where dopaminergic damage is normally not significant. Briefly, neuroinflammatory mechanism could in part contribute to the gradually escalating deleterious effects of methamphetamine.

3.7 Nrf2 and Inflammation Play a Role in Methamphetamine-Induced Neurotoxicity

The transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2) has been recently shown to have a protective role against methamphetamine-induced dopaminergic neurotoxicity (Granado et al. 2011b). Nrf2 is considered a master regulator of redox homeostasis, as it regulates the expression of a group of genes that encode the phase 2 detoxification enzymes, including heme oxygenase-1 (HO-1), NADPH quinone oxidoreductase (NQO1), and the catalytic and modulatory subunits of γ-glutamyl synthase (GCLM, GCLC) (Clark and Simon 2009; Johnson et al. 2008). In normal conditions, Nrf2 has a very short half-life because of its interaction with the BTB-Kelch protein Keap1, which promotes Nrf2 degradation by the proteasome (Lo et al. 2006). However, oxidant molecules are able to disrupt the Keap1/Nrf2 complex, rescuing Nrf2 from proteasomal degradation and allowing its entry into the nucleus and transcriptional activity.

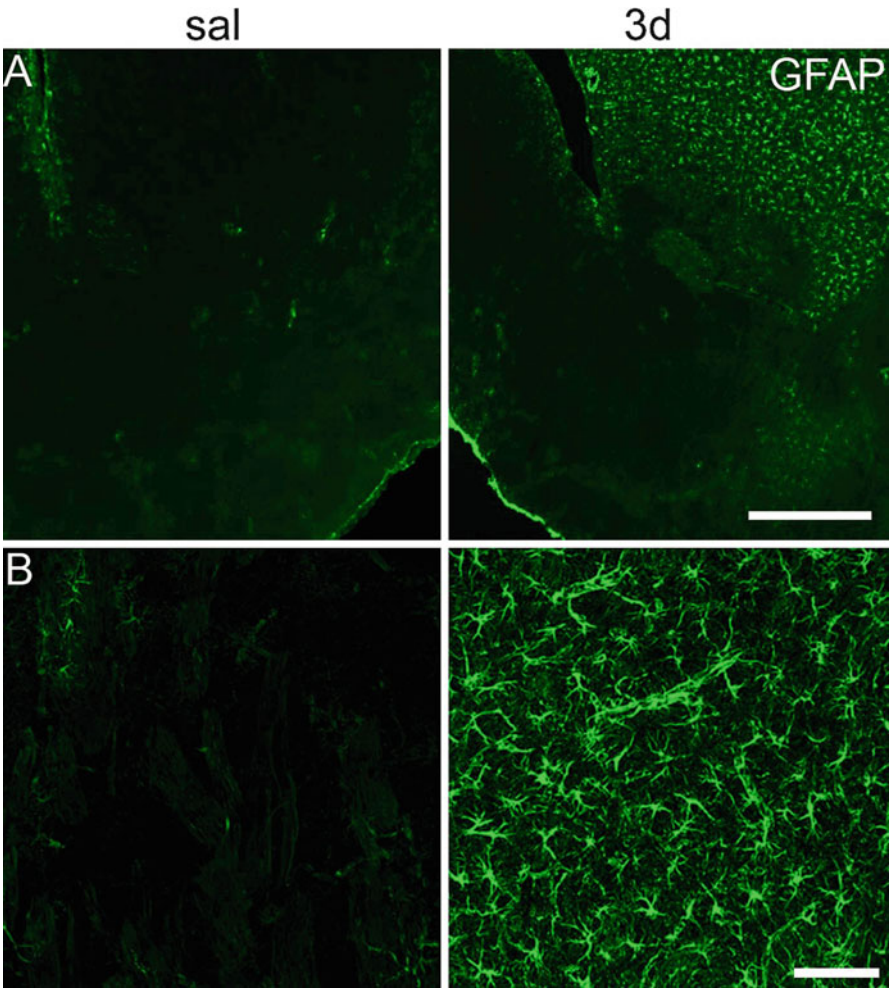

Fig. 7 Methamphetamine induces astrogliosis in mouse striatum but not in nucleus accumbens. (**a**) Photomicrographs of striatal sections from methamphetamine-treated mice stained for GFAP. (**b**) Enlargements of striatum sections shown in (**a**). Animals were killed 3 days after methamphetamine treatment. Methamphetamine increased GFAP staining in the striatum but not in nucleus accumbens. Bar indicates 500 μm for (**a**) and 100 μm for (**b**) (Modified from Granado et al. (2010))

Recent studies have shown that Nrf2 is activated by methamphetamine administration (Jayanthi et al. 2009) and that it plays a crucial role in the protection of dopaminergic neurons against oxidative stress by detoxifying mitochondrial complex I inhibitors and downregulating genes involved in the brain innate immune response. Mice lacking Nrf2 are more susceptible than WT mice to methamphetamine toxicity (Granado et al. 2011b), showing exacerbated hyperthermia, enhanced striatal TH and DAT-fiber loss, and greater decrease in dopamine levels,

and increased dopamine and nigrostriatal dopaminergic alterations and gliosis following administration of methamphetamine (Granado et al. 2011b). This is due to the fact that in the absence of Nrf2, ARE-regulated genes in the striatum, including HO-1 and other antioxidant genes, are not induced by methamphetamine as they are in WT mice, leading to increased oxidative stress, accumulation of ROS (Chen et al. 2009), and ultimately to dopamine fiber loss. In addition, cytokine mRNA levels (TNF-α, IL-1β), gliosis, and astrocytosis in the striatum were elevated to a greater extent in methamphetamine-treated $Nrf2^{-/-}$ mice than WT mice, indicating that the proinflammatory effects of methamphetamine treatment are potentiated in the absence of Nrf2.

Moreover, $Nrf2^{-/-}$ mice treated with methamphetamine presented significantly lower levels of modulatory subunits of γ-cysteine ligase (Gclm) and glutathione peroxidase (GPx) than WT animals, meaning that the detoxificating response is reduced in absence of Nrf2, a fact that could contribute to the increased detrimental effects induced by the drug.

Our finding that Nrf2-deficient mice were more sensitive than WT mice to methamphetamine-induced striatal damage further demonstrates that Nrf2 activation is part of a defensive response to methamphetamine neurotoxicity that involves modulation of methamphetamine-induced inflammation and oxidative stress (Granado et al. 2011b). This defensive modulation of inflammation and oxidative stress by Nrf2 is also seen following administration of other ROS-generating toxins like MPTP (Chen et al. 2009) and lipopolysaccharide (Rangasamy et al. 2004). Intriguingly, Nrf2 deficiency potentiates methamphetamine-induced neurotoxicity in the striatum but not in the SN. It is possible that methamphetamine differentially activates Nrf2-ARE transcription pathways in the striatum and the SN. Thus, differential regulation of Nrf2 by methamphetamine in SN and striatum might explain the lack of effect of $Nrf2^{-/-}$ on neurotoxicity in the SN in our study. These results strongly support the hypothesis that methamphetamine produces dopaminergic neurotoxicity through a process involving inflammation and oxidative stress (Granado et al. 2011b).

3.8 Role of Mitochondrial Dysfunction and DNA Damage

Methamphetamine-induced neurotoxicity also causes mitochondrial dysfunction and DNA damage. Mitochondria are the main source of cellular energy through activation of the ATP-producing mitochondrial respiratory chain, or electron transport chain, composed of a series of four complexes (I–IV). Methamphetamine is a cationic and lipophilic molecule that can diffuse into the mitochondria, where it is able to inhibit ATP synthesis (Asanuma et al. 2000; Krasnova and Cadet 2009). Administration of high doses of methamphetamine in rats decreases the activity of complexes II and IV of the respiratory chain in the striatum and prefrontal cortex, even in the absence of hyperthermia (Brown et al. 2005). Moreover, methamphetamine reduces ATP accumulation, resulting

in mitochondrial dysfunction. The increase in reactive oxygen and nitrogen species may also contribute to the observed mitochondrial dysfunction.

Elevated production of oxygen- and nitrogen-based radicals and related non-radical products leads to the oxidation of essential macromolecules, including DNA. DNA damage plays a role in the pathogenic mechanism of methamphetamine, as the drug increases DNA oxidation in the striatum, hippocampus, substantia nigra, and olfactory bulb (Jeng et al. 2006), causing apoptotic cell death in experimental animals (Deng and Cadet 2000). In particular, the 8-oxoguanine (8-oxoG) produced in DNA lesion may mispair with adenine, causing transversions or mutations, altering the DNA binding of nuclear transcription factors or blocking RNA polymerase, resulting in altered or delayed transcription of proteins. DNA repair genes, including members of the BER pathway, are upregulated in adult mice after methamphetamine administration, suggesting that increased repair activity is induced to counteract the oxidative DNA damage induced by the drug.

4 Neuroprotective Strategies Against Methamphetamine-Induced Neurotoxicity

As basic and clinical research findings begin to elucidate mechanisms of methamphetamine-induced neurotoxicity, potential neuroprotective strategies are being proposed. Since oxidative stress and mitochondrial dysfunction are important factors in methamphetamine-induced neurodegeneration, pretreatment with antioxidants like *N*-acetyl-L-cysteine, ascorbic acid, vitamin E, or coenzyme Q_{10} was evaluated and shown to have protective effects against depletion of monoaminergic axons (Krasnova and Cadet 2009). Melatonin, another antioxidant compound that also has antiapoptotic effects, also reduces methamphetamine-induced depletion of dopaminergic markers in the striatum (Hirata et al. 1998).

Other strategies for avoiding an increase in oxidative stress, such as preventing methamphetamine-induced hyperthermia by pharmacologic treatment or by maintaining animals at cool ambient temperatures during drug administration, also reduce mortality and neurotoxicity.

Controlling the amount of dopamine in the cytosol, where it is susceptible to oxidation, causing oxidative stress, is another neuroprotective strategy. Increasing VMAT2, the vesicular monoamine transporter that sequesters dopamine into vesicles, and administering dopamine uptake inhibitors or dopamine receptor antagonist, all provide protection against methamphetamine -induced degeneration of striatal dopamine terminals. In addition, some trophic factors provide protection against the toxic effects of methamphetamine. Administration of glial cell line-derived neurotrophic factor (GDNF) or brain-derived neurotrophic factor (BDNF) can prevent methamphetamine-mediated reduction in dopamine in the striatum and caspase activation (Dluzen 2004; Matsuzaki et al. 2004; Melega et al. 2000).

Since neuroinflammation may contribute to methamphetamine neurotoxicity, some cytokines, such as interferon gamma, TNF-alpha among others, have been

evaluated and were shown to protect against methamphetamine toxicity. Estrogen can also protect against methamphetamine-induced damage to the nigrostriatal dopamine system (D'Astous et al. 2005). Pretreatment with tamoxifen, a selective estrogen receptor modulator, showed neuroprotective effect against methamphetamine-induced toxicity and attenuated inflammatory response in the striatum when used alone but abolishes estrogen's positive effects when combined with this hormone. While both treatments prevented dopamine decrease, estrogen protected more efficiently other dopaminergic parameters, suggesting that overall estrogen is more effective than tamoxifen as a neuroprotectant of the nigrostriatal dopaminergic system (Bourque et al. 2007; D'Astous et al. 2005).

5 Conclusion

Methamphetamine is a synthetic drug used worldwide, mostly for recreational purposes, due to its powerful psychostimulant effects. It has addictive effects due to its structural analogy with dopamine, but also has neurotoxic effects on the dopaminergic system. The drug causes a reduction in dopamine markers in the striatum similar to that seen in the early stages of neurodegenerative diseases like PD, Huntington's disease, and hypoxic/ischemic injury. In experimental animals, methamphetamine also causes neuron loss in the substantia nigra. This may explain why patients who abuse methamphetamine have a predisposition to future development of PD. Among the mechanisms responsible for methamphetamine's neurotoxic effects are oxidative stress, hyperthermia, microglia and astroglia activation, mitochondrial dysfunction, DNA damage, and elevated levels of glutamate and nitric oxide. The recent implication of the D1 and D2 receptors in methamphetamine-induced neurotoxicity suggests that targeting these receptors may be a promising strategy for development of new approaches to prevention and treatment of methamphetamine addiction and its neurotoxic effects. In addition, the neuropathological similarities between methamphetamine neurotoxicity and PD and the demonstrated predisposition of methamphetamine abusers to developing PD indicate that similar therapeutic approaches may be useful in the early stages of PD and related neurodegenerative diseases.

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