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Escalating Methamphetamine Regimen Induces Compensatory Mechanisms, Mitochondrial Biogenesis, and GDNF Expression, in Substantia Nigra

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ABSTRACT

Methamphetamine (MA) produces long-lasting deficits in dopaminergic neurons in the long-term use via several neurotoxic mechanisms. The effects of MA on mitochondrial biogenesis is less studied currently. So, we evaluated the effects of repeated escalating MA regimen on transcriptional factors involved in mitochondrial biogenesis and glial-derived neurotrophic factor (GDNF) expression in substantia nigra (SN) and striatum of rat. In male Wistar rats, increasing doses of MA (1–14 mg/kg) were administrated twice a day for 14 days. At the 1st, 14th, 28th, and 60th days after MA discontinuation, we measured the PGC1α, TFAM and NRF1 mRNA levels, indicator of mitochondrial biogenesis, and GDNF expression in SN and striatum. Furthermore, we evaluated the glial fibrillary acidic protein (GFAP) and Iba1 mRNA levels, and the levels of tyrosine hydroxylase (TH) and α-synuclein (α-syn) using immunohistochemistry and real-time polymerase chain reaction (PCR). We detected increments in PGC1α and TFAM mRNA levels in SN, but not striatum, and elevations in GDNF levels in SN immediately after MA discontinuation. We also observed increases in GFAP and Iba1 mRNA levels in SN on day 1 and increases in Iba1 mRNA on days 1 and 14 in striatum. Data analysis revealed that the number of TH^+ cells in the SN did not reduce in any time points, though TH mRNA levels was increased on day 1 after MA discontinuation in SN. These data show that repeated escalating MA induces several compensatory mechanisms, such as mitochondrial biogenesis and elevation in GDNF in SN. These mechanisms can reverse MA-induced neuroinflammation and prevent TH-immunoreactivity reduction in nigrostriatal pathway. J. Cell. Biochem. 118: 1369–1378, 2017.

KEY WORDS: METHAMPHETAMINE; MITOCHONDRIAL BIOGENESIS; NIGROSTRIATAL PATHWAY; GDNF

Methamphetamine (MA) is an illicit psychostimulant that is widely abused. The immediate effects of MA are associated with an increase in the release of monoamines, specially dopamine (DA), concentrations in the synaptic cleft. MA can produce long-lasting deficits in dopaminergic cell bodies and terminals in the long-term use [Krasnova and Cadet, 2009; Marshall and O'dell, 2012]. Among the different dopaminergic pathways in the brain, nigrostriatal neurons are more sensitive to MA-induced neurotoxicity, whereas mesolimbic and mesocortical pathways are less affected [Granado et al., 2010; Yamamoto et al., 2010]. There is evidence that MA reduces the striatal markers of dopaminergic nerve terminals including DA level, its metabolites, biosynthetic enzymes, receptors, and transporters in rodents [Kousik et al., 2014; O'callaghan et al., 2014; Shin et al., 2014; Lohr et al., 2015], nonhuman primates [Morrow et al., 2011], and human [Volkow et al., 2001]. Furthermore, MA induces neuronal apoptotic death and reduction in dopaminergic neuron cell bodies of substantia nigra (SN) [Sonsalla et al., 1996; Hirata and Cadet, 1997, Ares-Santos et al., 2014; Kousik et al., 2014]. The mechanisms underlying this neurodegeneration are not fully understood currently, but a considerable body of data indicates that oxidative stress, neuroinflammation, excitotoxicity, as well as mitochondrial dysfunction

Abbreviations: α-syn, alpha synuclein; BDNF, brain-derived neurotrophic factor; DA, dopamine; DAB, 3, 3′-diamino benzidine tetrahydrochloride; GDNF, glial-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; HRP, horse radish peroxidase; Iba1, ionized calcium-binding adapter molecule 1; MA, methamphetamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NRF, nuclear respiratory factor; PBS, phosphate buffer saline; PGC1α, PPARγ coactivator 1α (PGC1α); PPARγ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; SN, substantia nigra; SNpc, substantia nigra pars compacta; TFAM, mitochondrial transcription factor A; TH, tyrosine hydroxylase.

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play an essential role [Barr et al., 2006; Krasnova and Cadet, 2009; Ares-Santos et al., 2013; Shin et al., 2014]. DA quinones, produced by non-enzymatic degradation of DA following MA exposure, are powerful activators of microglia. Activated microglia releases neurotoxic and pro-inflammatory cytokines, and can exacerbate MA neurotoxicity [Yamamoto et al., 2010]. Microglia and astroglia activation have been shown in the midbrain and striatum of rodents [Robson et al., 2013, 2014; O'callaghan et al., 2014; Shin et al., 2014; Zhang et al., 2015] and human MA abusers [Sekine et al., 2008] after acute MA administration and precedes degeneration of dopaminergic terminals.

MA can diffuse through the cell membranes of the intracellular organelles, including mitochondria due to the lipophilic properties [Yamamoto et al., 2010], and induces mitochondrial dysfunction by decreases the respiratory chain complexes activity [Brown et al., 2005]. Mitochondrial dynamics properties such as mitochondrial fission/fusion and biogenesis are critical for normal neuronal function and survival [Chan, 2012; Chaturvedi and Beal, 2013]. Peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1α (PGC1α), a key regulator of mitochondrial biogenesis, is an activator of several transcription factors including nuclear respiratory factors (NRF1 and NRF2) and nuclear encoded mitochondrial genes including mitochondrial transcription factor A (TFAM) and cytochrome c [Finck and Kelly, 2006; Chaturvedi and Beal, 2008]. Impairment of PGC1α function, and down regulation of its mitochondrial target genes, leads to abnormalities in mitochondrial function and energy metabolism, and ultimately neuronal death [Chaturvedi and Beal, 2008]. There is evidence that MA-mediated reactive oxygen species (ROS) generation results in increase the levels of the mitochondrial fission protein, which leads to mitochondrial fragmentation and subsequent apoptosis [Tian et al., 2009; Parameyong et al., 2013]. However, little is known about the effects of repeated MA exposure on mitochondrial biogenesis in SN and striatum. The present study evaluated the gene expression of factors involved in mitochondrial biogenesis and GDNF, a critical growth factor for dopaminergic neurons survival, in the brains of rats that received repeated injections of MA immediately after and over a long period of drug discontinuation. Since, in our previous study, we did not observe any MA-induced DA-related behavioral motor deficits, here, to elucidate the molecular and cellular mechanisms of MA-induced toxicity, we measured neuroinflammation, TH, and α-syn expression in SN and striatum of rat.

MATERIALS AND METHODS

ANIMALS

The subjects were adult male Wistar rats, weighting 220–260 g, obtained from our breeding colony (Neuroscience Research Center). Animals were housed as five in a cage with food and water available ad libitum, under a standard 12 h-light/12 h-dark cycle and temperature of 23 ± 2°C. Rats were allowed 5–6 days of habituation to the animal colony. All the experiments followed the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1996) and was approved by the ethics committee for animal research of the Shahid Beheshti University of Medical Sciences. All experiments were performed at the same time during the day to avoid circadian variations.

SYSTEMIC DRUG INJECTION

MA) hydrochloride (synthesized and analyzed by Laboratory of Medicinal Chemistry, School of Pharmacy, Tehran University of Medical Sciences, Iran) was freshly dissolved in 0.9% saline solution before each administration. Rats received repeated escalating doses (1–14 mg/kg, ip, twice a day, for 14 consecutive days) at a volume of 1 ml/kg. Control group received injections of saline, ip, at the same volume twice a day, for 14 consecutive days. Escalating regimens were used in order to mimic human MA abuse. In MA group injections began with 1 mg/kg in the first day, and gradually increased, 1 mg/kg per day. Injections were performed at 9:00 in the morning and 3:00 in the afternoon.

TISSUE PREPARATION

Rats were decapitated and SN and striatum were immediately dissected on ice, and frozen in liquid nitrogen. After snap freezing in liquid nitrogen, the tissues were transferred to the −80°C freezer until RNA extraction. Another set of animals were used for histological analysis. For this reason, rats were deeply anaesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and then transcardially perfused with phosphate buffer saline (PBS), pH 7.4, followed by 4% paraformaldehyde. The brains were removed from the skull and kept in the same fixative for the next 48 h. A standard paraffin tissue preparation was used for immunohistochemistry. Coronal sections from the striatum and SN (5 μm) were cut on a microtome and used for immunohistochemical staining.

IMMUNOHISTOCHEMISTRY

Paraffin sections through matched coronal levels of the SN (anterior–posterior from bregma: −4.92 to −5.16 mm) and striatum (anterior–posterior from bregma: 0.48 to 0.72 mm) were stained with rabbit anti-TH and anti α-syn antibodies using standard immunoperoxidase techniques. Briefly, paraffin sections of rat midbrain were deparaffinized and hydrated in distilled water. Antigenic sites were exposed by incubating sections in citra antigen retrieval buffer (pH = 6) for 20 min at 96°C using a microwave. Following antigen retrieval, slides were cooled for an additional 20 min. Endogenous peroxidase activity was quenched by treating sections with a 0.3% solution of hydrogen peroxide (aq). Non-specific binding was blocked by a 30 min incubation in 10% normal goat serum. The sections were then incubated overnight at 4°C with rabbit polyclonal anti-TH (1:1000, Abcam, ab112) and, rabbit polyclonal anti a-synuclein (1:1000, Thermo Scientific, PA1-18264) antibodies. TH- and α-syn- immunoreactivities were detected by horse radish peroxidase (HRP) conjugated secondary antibody, and visualized by incubation in liquid 3,3′-Diamino benzidine tetrahydrochloride (DAB) followed by counterstaining with Mayer's hematoxylin. The outline of SN-pars compacta (SNpc) were drawn at low power (×4) using defined anatomic landmarks [Stephenson et al., 2007] and the number of immunoreactive neurons was counted at higher power (×20). Four animals were used per group, and values from the four sections were averaged for each animal.

RNA ISOLATION AND qPCR PROTOCOL

Total RNA was extracted from brain using YTzol (Yektatajhiz azma, Tehran, Iran) according to the manufacturer's instructions. The RNA quality was assessed by determining 28S and 18S ribosomal RNA bands using electrophoresis. Two hundred and fifty nano grams of total RNA was then reverse transcribed to cDNA using TransScript First-Strand cDNA Synthesis Kit (Pars toos, Tehran, Iran) according to manufacturer's protocol. In short, RNA template, primer, and nuclease free H_2O were mixed and incubated at 65°C for 5 min and chilled on ice. Then, the mixture was mixed with RT-Premix2X and incubated 10 min at 65°C, 60 min at 50°C, 10 min at 70°C and held at 4°C until further use. The resulting cDNA was then used to quantitatively measure the expression of genes using PCR Master Mix (Ampliqon) reagents by the following cycling conditions; activation 5 min 95°C, denaturation 30 s 95°C, annealing 30 s, extension 30 s 72°C. Real-time PCR was performed with SYBR Green Real-Time PCR Master Mix (Ampliqon) reagents using ABI System. The threshold cycles (Ct) were used to quantify the mRNA levels of the target genes. Relative gene expressions were calculated by the $2^{-\Delta\Delta Ct}$ method [Butler et al., 2014, Xu et al., 2014]. Primers for RT-qPCR used here are shown in Table I.

STATISTICAL ANALYSIS

Data are presented as mean ± standard error of the mean (SEM). Data were analyzed using one-way ANOVA. Relevant differences were analyzed pair-wise by post hoc comparisons with Tukey's test, to determine specific group differences. Statistical analyses were performed using 16th version of statistical package for the social sciences (SPSS) and the threshold for statistical significance was set at $P<0.05$.

RESULTS

TH mRNA LEVEL WAS INCREASED IN THE SN IMMEDIATELY AFTER MA DISCONTINUATION

The number of TH^+ neurons of SN were evaluated quantitatively after MA-treatment using immunohistochemistry (Fig. 1a). Although, there was partial reduction in the relative number of TH^+ in the SN on day 14 following MA treatment, one-way ANOVA analysis revealed that the number of TH immuno-positive neurons of SN were not different ($F_{(4,\ 15)}=0.667$, $P=0.629$) between the MA- and vehicle-treated groups in all time points (Fig. 1b). However, in the case of TH mRNA level, statistical analysis indicated significant difference between groups ($F_{(4,\ 15)}=30.575$, $P<0.001$). The Tukey post hoc test demonstrated an increase in the TH gene expression at 1 day after MA discontinuation ($P<0.001$) (Fig. 1c), but this increase was reversible. We also observed no significant differences in TH immunoreactivity density of dopaminergic terminals qualitatively in striatum (Fig. 1d).

MA DECREASED THE LEVELS OF α-syn mRNA, NOT PROTEIN, IN SN

MA administration had no effects on nigral α-syn protein content at either time post-MA using immunohistochemistry ($F_{(4,\ 15)}=0.342$, $P=0.844$) (Fig. 2a, b). At the mRNA level, data analysis, unexpectedly, indicated significant differences ($F_{(4,\ 15)}=21.199$, $P<0.001$) between groups, with decreases in α-syn gene expression at day 14 ($P<0.05$), 28 ($P<0.01$), and 60 ($P<0.05$) following last injection (Fig. 2c). However, MA administration were without any effect on striatal α-syn protein level at either time post-MA qualitatively in immunohistochemistry (Fig. 2d), as well as in qPCR ($F_{(4,\ 15)}=0.994$, $P=0.454$) (Fig. 2e).

REPEATED MA EXPOSURE INCREASED GFAP AND Iba mRNA LEVELS REVERSIBLY IN THE SN AND STRIATUM

One-way ANOVA analysis demonstrated that repeated MA administration increased nigral GFAP, marker of astroglial activation ($F_{(4,\ 15)}=15.414$, $P<0.01$), and Iba-1 mRNA, marker of microglial activation ($F_{(4,\ 15)}=47.274$, $P<0.001$). These increases were seen on day 1 post-MA treatment (GFAP, $P<0.05$; Iba-1, $P<0.001$) and reached the normal level on day 14 (Fig. 3a, b). In striatum, there was no change in GFAP mRNA level ($F_{(4,\ 15)}=0.497$, $P=0.739$), but elevated Iba-1 mRNA was seen on days 1 and 14 post-MA treatment ($P<0.01$and $P<0.01$, respectively) (Fig. 3c, d).

THE EFFECTS OF REPEATED MA EXPOSURE ON GENES INVOLVED IN MITOCHONDRIAL BIOGENESIS

Escalating MA regimen changed the expression of genes involved in mitochondrial biogenesis in SN and striatum (Fig. 4). In SN, ANOVA analysis indicated increases in PGC1α ($F_{(4,\ 15)}=49.906$, $P<0.001$) and TFAM ($F_{(4,\ 15)}=21.276$, $P<0.001$), but not in NRF1 ($F_{(4,\ 15)}=2.576$, $P=0.119$) on day 1 post-MA treatment. At the other time points we did not observe any significant changes on gene expression. (Fig. 4a–c). In the case of striatum, significant differences in PGC1α mRNA was seen between groups ($F_{(4,\ 15)}=4.748$, $P<0.05$), with decrease at 1 day after MA discontinuation ($P<0.05$) (Fig. 4d). TFAM and NRF1 expression was not changed at any time

TABLE I. Primer Sequences Used (5′ 3′) For qPCR

Gene	Forward primer	Reverse primer
Tyrosine hydroxylase	AGTACAAGCACGGTGAACCA	GATGCTGTCCTCTCGGTAGC
a-Synuclein	ACCAAGACTATGAGCCTGAAGC	ACTGAGCACTTGTACGCCAT
GFAP	AACCGCATCACCATTCCTGT	TCCTTAATGACCTCGCCATCC
Iba1	TCGTCATCTCCCCACCTAAG	ATCAAACTCCATGTACTTCGTCTTG
GDNF	CACCAGATAAACAAGCGGCG	TCGTAGCCCAAACCCAAGTC
PGC1*a*	GTGCAGCCAAGACTCTGTATGG	GTCCAGGTCATTCACATCAAGTTC
TFAM	AGAGTTGTCATTGGGATTGG	CATTCAGTGGGCAGAAGTC
NRF1	AAATTGGGCCACATTACAGGG	GTTGCATCTCCTGAGAAGCG
β-Actin	TCTATCCTGGCCTCACTGTC	AACGCAGCTCAGTAACACTCC

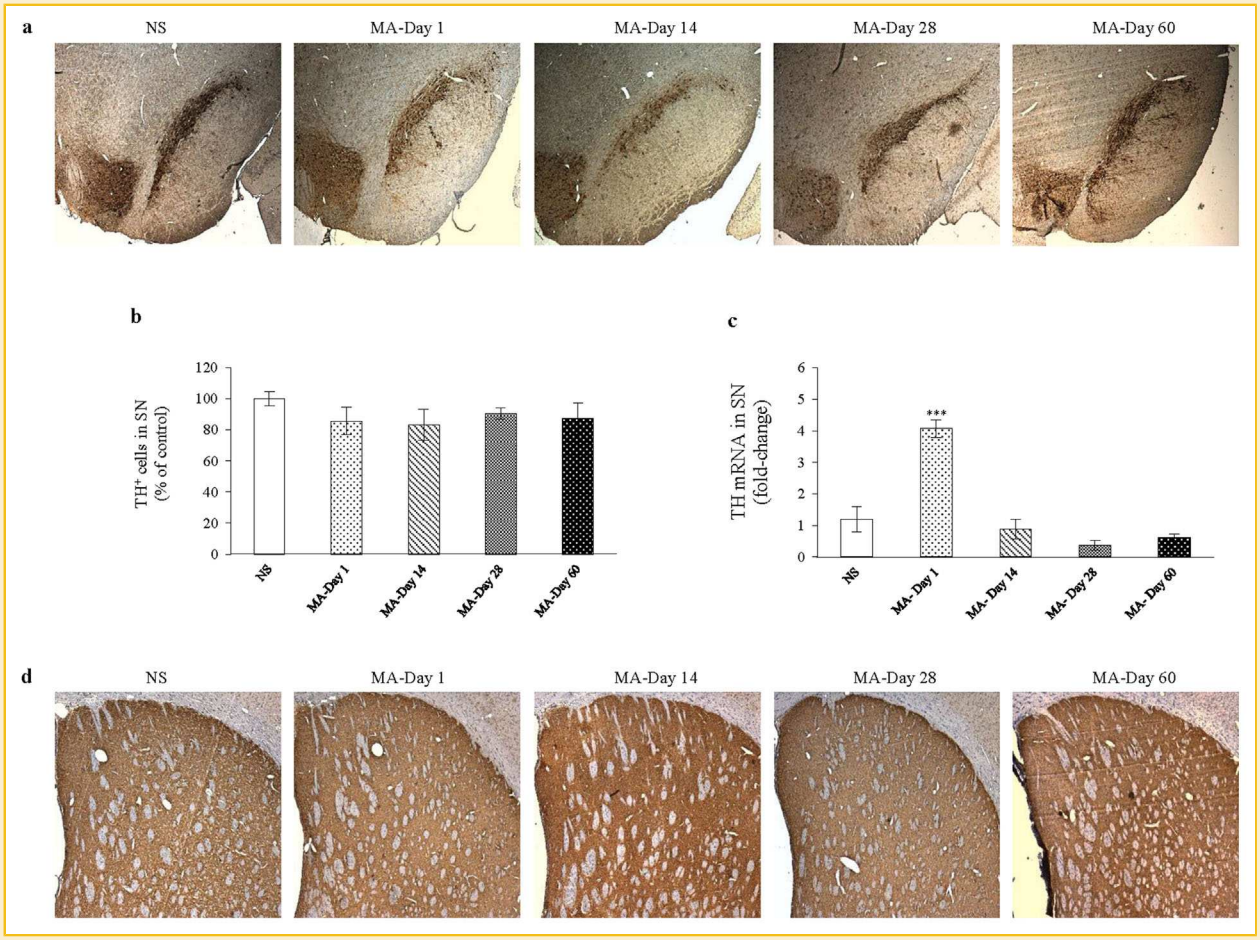

Fig. 1. The effect of escalating MA administration on TH protein and mRNA levels in SN and striatum. Rats received repeated escalating injections of MA (1–14 mg/kg; n = 4 for each time point) or normal saline (NS, n = 4). MA did not decrease the number of TH^+ dopaminergic neurons in histological analysis (a, b), but unexpectedly it increased TH mRNA on day 1 in SN. Relative values were normalized based on β-actin (c). No change was observed in TH-immunoreactivity in striatum at any time points post-treatment (d). $^{***}P < 0.001$ versus NS.

point in the striatum of MA-treated animals [$F_{(4,\ 15)} = 0.555$, $P = 0.701$; $F_{(4,\ 15)} = 1.249$, $P = 0.357$, respectively] (Fig. 4e, f).

MA EXPOSURE ELEVATED GDNF mRNA LEVELS IN THE SN BUT NOT IN THE STRIATUM

Repeated MA administration induced GDNF in the dopaminergic neurons of SN ($F_{(4,\ 15)} = 14.579$, $P < 0.001$) on day 1 post-MA treatment, and this increase returned to normal level on day 14 (Fig. 5a). GDNF mRNA was not changed in striatum at any time points following MA injections ($F_{(4,\ 15)} = 2.119$, $P = 0.161$) (Fig. 5b).

DISCUSSION

TH

Although there was partial qualitative reduction in the relative number of TH^+ in the SN on day 14 following MA treatment, but quantitative analysis revealed no decreases in the number of TH^+ neurons by MA injections. However, we observed increase in TH mRNA at day 1 after last injection with no differences noted at the other times. In line with our finding, it has been shown that MA increased TH expression immediately after MA administration in rat [Braun et al., 2011], and TH activity in neuronal cultures exposed to long-term MA [Larsen et al., 2002]. These findings, consistent with our results, reveals that escalating MA doses increase TH expression immediately after MA abstinence. Furthermore, one in vivo study demonstrated that, MA administration increased TH phosphorylation at ser40 in SN 48 h after escalating, not binge, MA [Keller et al., 2011]. About the striatum, while several studies demonstrated long-lasting reductions in TH protein following MA [Ares-Santos et al., 2014], we did not observe any reduction in TH immunoreactivity. Since no decreases was seen in the number of dopaminergic neurons in the SN, it was expected that no change would be observed in the striatal TH protein expression.

α-SYN

It was previously reported that exposure to high MA doses increased the expression of α-syn in dopaminergic neurons in SN [Fornai et al., 2005; Jiang et al., 2014] and striatum [Butler et al., 2014], of rodents

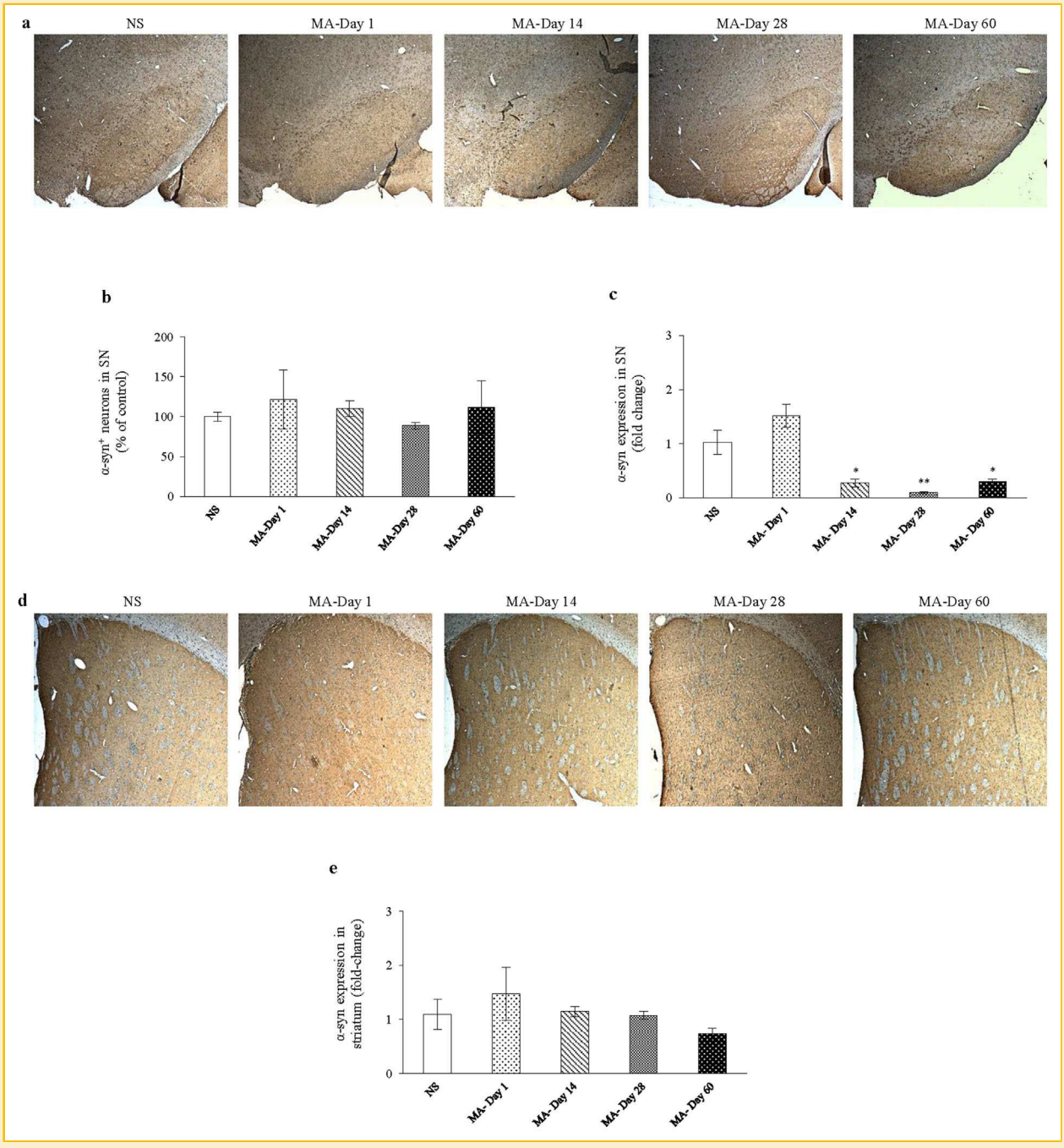

Fig. 2. The effect of escalating MA administration on **α**-syn protein and mRNA levels in SN and striatum. Rats received repeated escalating injections of MA (1–14 mg/kg; n = 4 for each time point) or normal saline (NS, n = 4). MA did not change the number of **α**-syn^{+} nigral neurons in histological analysis (a, b), but at mRNA level, it decreased **α**-syn expression on day 14, 28, and 60 after final injections. Relative values were normalized based on **β**-actin (c). In striatum, we observed no change in **α**-syn protein (d) and mRNA (e) levels following MA treatment. $^{*}P < 0.05$, $^{**}P < 0.01$ versus NS.

which is associated with the neuronal loss and motor dysfunction [Jiang et al., 2014]. Despite the elevation of high molecular weight α-syn species (>19 kDa) following MA repeated high doses in mice, there was no change in the α-syn mRNA levels in the striatum [Butler et al., 2014]. Consistent with this finding, we also observed no change in striatal α-syn mRNA level, but there was reduction in its mRNA in SN. Although, the cause of decrease in α-syn mRNA levels is unclear, this MA-induced response might be one of the compensatory consequences of MA-mediated neurotoxicity. In support of this idea it has been indicated that autophagy is rapidly upregulated in response to repeated low doses of MA [Castino et al., 2008; Cadet et al., 2011; Ma et al., 2014], suggesting the adaptive

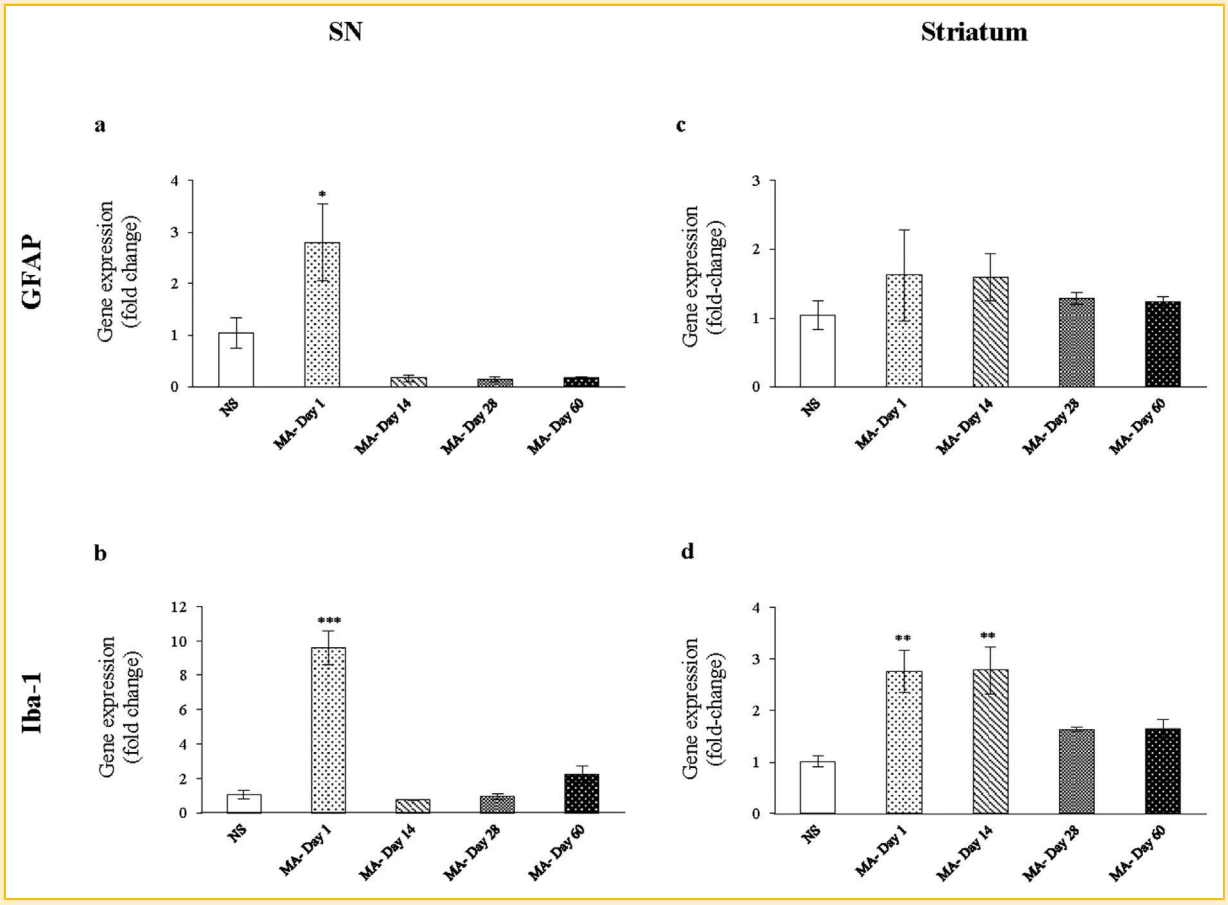

Fig. 3. MA-induced neuroinflammation in SN and striatum. Rats received repeated escalating injections of MA (1–14 mg/kg; n = 4 for each time point) or normal saline (NS, n = 4). Relative values were normalized based on β-actin. One-way ANOVA analysis revealed that MA increased GFAP and Iba-1 expression 1 day following last injection in SN (a, b). In striatum, we observed no change in GFAP mRNA, but increased Iba-1 mRNA at day 1 and 14 after MA discontinuation (c, d). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus NS.

defensive responses against the MA-induced neurotoxic effects. Presence of α-syn aggregates in autophagy lysosomal structures was reported in cells exposed to MA, a condition compatible with cell survival [Castino et al., 2008]. It has been documented that MA binds to the N-terminus of α-syn and causes a conformational change in the protein that can lead to stabilization of the oligomeric form of α-syn implicated in neurotoxicity induction [Butler et al., 2014]. Here, we measured the level of normal form of α-syn protein, but not fibrils or oligomers forms. The effect of MA repeated escalating doses on these pathogenic forms of α-syn should be studied. This MA-induced adaptive response may be the reason for unchanged α-syn protein expression observed in the brain tissues after repeated MA administration in our study.

GFAP, Iba-1

Various aspects of neuroinflammation, characterized by the activation of microglia and astroglia, are believed to be contributory factors in MA neurotoxicity. It has been reported that Iba1 [Robson et al., 2013] and GFAP [Robson et al., 2014; Zhang et al., 2015] mRNA levels were increased in parallel to their immunoreactivity after MA treatment. Consistent with these findings we revealed that escalating MA regimen also induced glial activation via increased GFAP and Iba1 mRNA in SN and striatum. The later decreases in GFAP mRNA to less than normal levels can be attributed to negative feedbacks and/or late pathological responses to repeated MA exposure. However, the importance of this decrease at the protein and functional levels need to be studied.

GDNF

We indicated that GDNF mRNA expression was increased in SN on day 1 following MA treatment. Since GDNF is a critical factor for dopaminergic neurons survival, this response may act as a compensatory mechanism to protect dopaminergic neurons against MA-induced neurotoxicity. GDNF also increases TH protein [Salvatore et al., 2009] and TH phosphorylation at Ser31 in striatum and SN of rat [Salvatore et al., 2004] and results in elevated DA release in striatum. It has been shown that exposure to low concentrations of MA causes transcriptional changes, such as pro-survival proteins, involved in rendering the nigrostriatal dopaminergic system refractory to subsequent oxidative stress [El Ayadi and Zigmond, 2011]. In this regard, upregulation of GDNF and brain-derived neurotrophic factor (BDNF) is observed by MA low doses

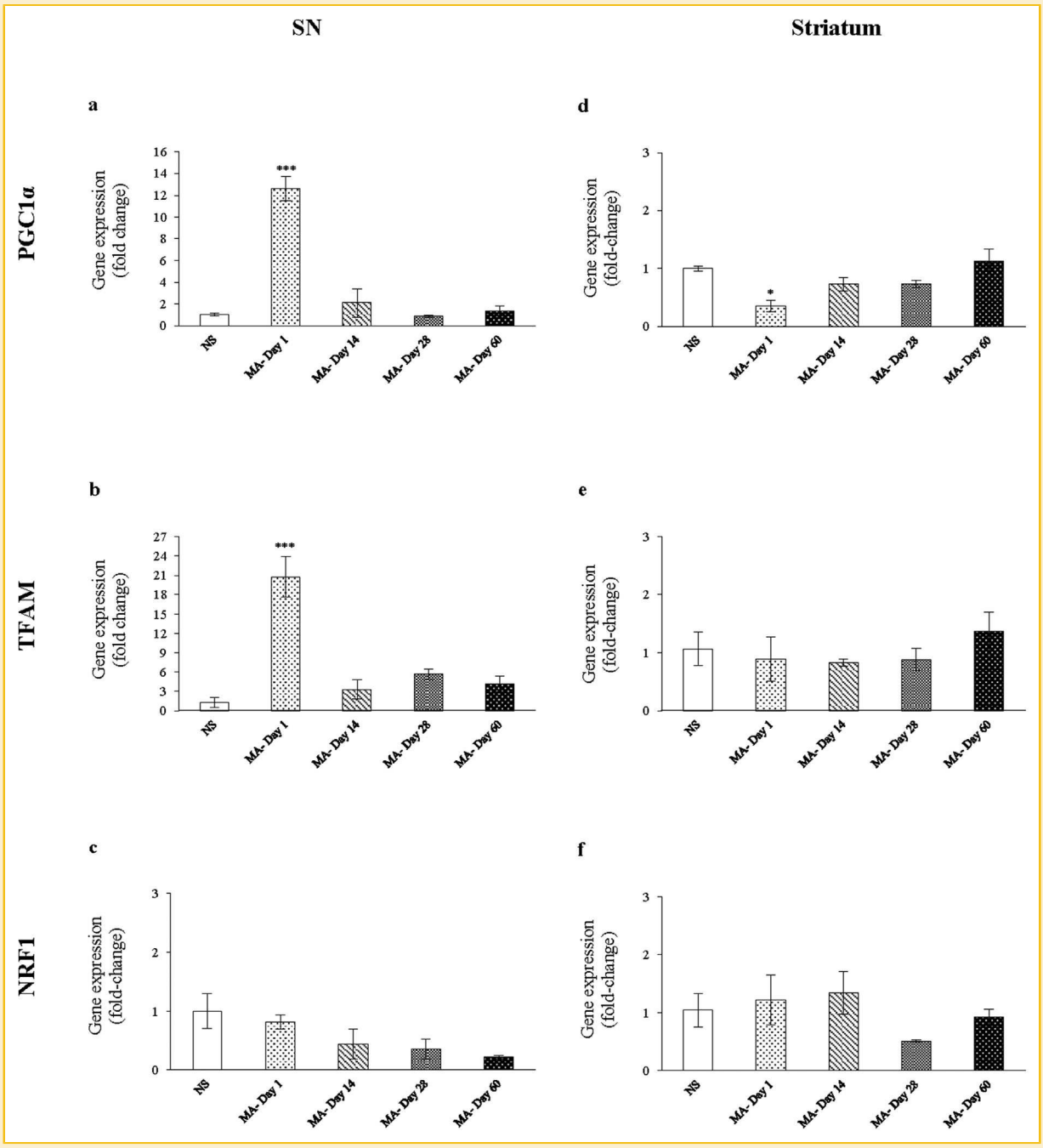

Fig. 4. MA effects on genes involved in mitochondrial biogenesis in SN and striatum. Rats received repeated escalating injections of MA (1–14 mg/kg; n = 4 for each time point) or normal saline (NS, n = 4). Data analysis revealed that MA increased PGC1α and TFAM expression 1 day following the last injection, but it could not induce changes in NRF1 in SN (a–c). In striatum, we observed reversible reduction just in the case of PGC1α mRNA at day 1 after MA discontinuation (d). TFAM and NRF1 were not changed at any time points (e, f). Relative values were normalized based on β-actin. $^{*}P < 0.05$, $^{***}P < 0.001$ versus NS.

exposure to protect the nigrostriatal dopaminergic pathway against the deleterious effects of the toxic psychostimulant [Cadet et al., 2009; Braun et al., 2011]. It can be concluded that elevation in GDNF mRNA observed in this study prevented MA-induced detrimental effects in nigrostriatal dopaminergic pathway.

MITOCHONDRIAL BIOGENESIS

PGC1α, in addition to its role in mitochondrial biogenesis, is also required for the induction of many ROS-detoxifying enzymes [Spiegelman, 2007; Li et al., 2011]. It has been documented that MA induces oxidative stress in human MA

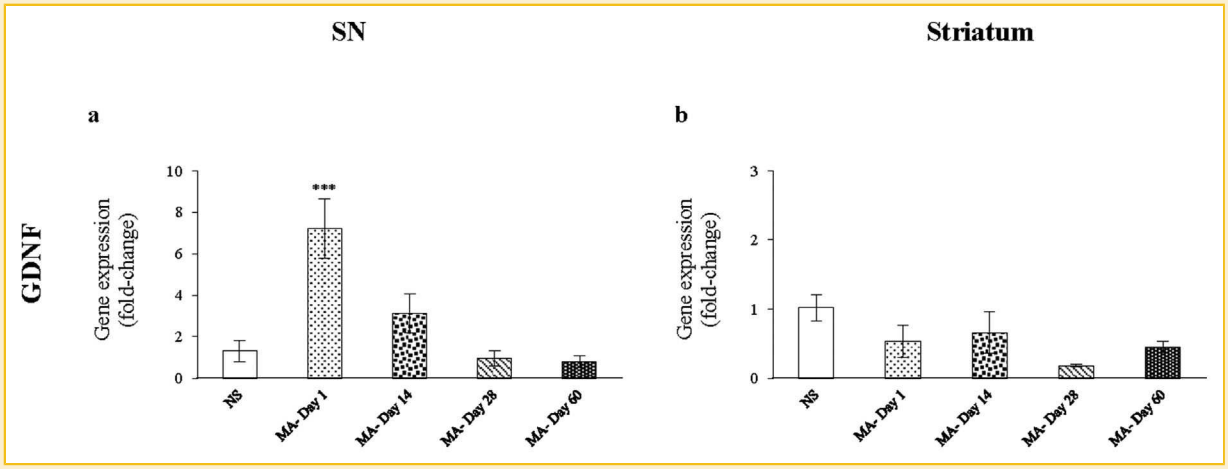

Fig. 5. MA-induced changes in GDNF expression. Rats received repeated escalating injections of MA (1–14 mg/kg; n = 4 for each time points) or normal saline (NS, n = 4). Relative values were normalized based on β-actin. Data analysis revealed that MA increased GDNF expression 1 day following the last injection in SN (a), but about the striatum, there was no change in GDNF mRNA level (b). $^{***}P < 0.001$ versus NS.

abusers [Fitzmaurice et al., 2006] and animal models [Yamamoto et al., 2010; Vaghef and Babri, 2014] by disrupting the balance between ROS production and the capacity of antioxidant systems to scavenge ROS [Kobeissy et al., 2008]. Neurotoxic effects of amphetamines can be attenuated by free radical scavengers and antioxidants or overexpression of antioxidant enzymes [Yamamoto et al., 2010]. Elevated PGC1α levels dramatically protected neural cells from oxidative stress, induced by H_2O_2, and cell death [St-Pierre et al., 2006; Irrcher et al., 2009]. Furthermore, increased vulnerability to MPTP-induced degeneration of nigral dopaminergic neurons was observed in PGC1α knockout mice, suggesting a critical role of PGC1α in neuroprotection [Corona and Duchen, 2015]. In the context of damage and oxidative stress, neurons initiate adaptive responses such as activation of mitochondrial biogenesis. Increased protein and mRNA levels of PGC1α, TFAM, and NRF1 in the early hours of recirculation have been reported in animal ischemia and hypoxia models. These increases were reversible and reached the normal levels later [Gutsaeva et al., 2008; Yin et al., 2008; Mehta et al., 2012]. Furthermore, it has been shown that the expression of PGC1α and downstream mitochondrial antioxidant enzymes are markedly increased in astrocytes in active multiple sclerosis (MS) lesions, to protect against oxidative stress and inflammation [Nijland et al., 2014] . Activation of PGC1α increased the expression of nuclear-encoded subunits of the mitochondrial respiratory chain and prevented the dopaminergic neuron loss in Parkinson's disease models [Corona and Duchen, 2015]. In general, it could be concluded that PGC1α activation could result in decreased oxidative challenge, by upregulation of genes involved in mitochondrial biogenesis, increased the number of mitochondria, and antioxidant enzymes activation [Radak et al., 2013; Wang et al., 2016]. Consistent with these findings, we observed increased PGC1α and TFAM mRNA levels in SN on day 1 post-MA abstinence. Although, the changes in gene transcription and mRNA levels may not always reflect the changes in protein levels, however, in several studies changes in mRNA level are along with changes in protein level. In case of factors involved in mitochondrial biogenesis it has been shown that increases in mRNA levels of PGC1α [Gutsaeva et al., 2008] and NRF1 [Yin et al., 2008] can result in elevation in their protein levels. This response may act as a component of the endogenous adaptive mechanisms to prevent MA-induced neurotoxicity in nigrostriatal pathway at behavioral (our not published previous study) and molecular levels. In support of this idea, we observed no increases in α-syn protein and mRNA, as well as not persistent neuoinflammation in SN and striatum.

It is important to point out that the compensatory responses in terms of MA-induced gene expression might be regionally specific. These regional differences can be secondary to the fact that SN is the site of origin for dopaminergic neurons whereas striatum is mainly composed of dopaminergic terminals [Cadet et al., 2009]. Therefore, obtained results from intrinsic striatal non-dopaminergic neurons were different from those observed in SN.

CONCLUSION

We demonstrated that exposure to escalating doses of MA not only did not destruct mitochondrial biogenesis but also induced overexpression of genes involved in this process. Activated mitochondrial biogenesis prevented long-lasting inflammation and aggregation of α-syn in SN and striatum. In addition, we also observed increases in GDNF mRNA immediately after MA discontinuation. Survival of dopaminergic neurons may be attributed to the GDNF upregulation. These findings indicate that increasing MA regimen induced compensatory and protective mechanisms in dopaminergic neurons to prevent MA-induced neurotoxicity.

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