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Disruption of striatal glutamatergic/GABAergic homeostasis following acute methamphetamine in mice

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ABSTRACT

Methamphetamine leads to functional changes in basal ganglia that are linked to impairment in motor activity. Previous studies from our group and others have shown that a single high-methamphetamine injection induces striatal dopaminergic changes in rodents. However, striatal glutamatergic, GABAergic and serotoninergic changes remain elusive under this methamphetamine regimen. Moreover, nothing is known about the participation of the receptor for advanced glycation end-products (RAGE), which is overexpressed upon synaptic dysfunction and glial response, on methamphetamine-induced striatal dysfunction. The aim of this work was to provide an integrative characterization of the striatal changes in amino acids, monoamines and astroglia, as well as in the RAGE levels, and the associated motor activity profile of C57BL/6 adult mice, 72 h after a single-high dose of methamphetamine (30 mg/kg, i.p.). Our findings indicate, for the first time, that methamphetamine decreases striatal glutamine, glutamate and GABA levels, as well as glutamine/glutamate and GABA/glutamate ratios, while serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels remain unchanged. This methamphetamine regimen also produced dopaminergic terminal degeneration in the striatum, as evidenced by dopamine and tyrosine hydroxylase depletion. Consistently, methamphetamine decreased the locomotor activity of mice, in the open field test. In addition, increased levels of glutamine synthase and glial fibrillary acidic protein were observed. Nevertheless, methamphetamine failed to change RAGE levels. Our results show that acute methamphetamine intoxication induces pronounced changes in the striatal glutamatergic/GABAergic and dopaminergic homeostasis, along with astrocyte activation. These neurochemical and glial alterations are accompanied by impairment in locomotor activity.

1. Introduction

Striatum, one of the major brain areas impacted by methamphetamine (METH), represents the main input into the basal ganglia (BG), relaying neural information to the BG output structures. Striatal neurons, mainly GABAergic projection medium spiny neurons (MS), receive convergent synaptic inputs from dopaminergic and serotonergic afferences coming from the substantia nigra pars compacta (SNc) and Raphe nuclei, together with glutamatergic afferents from the entire area of the cortex. Less than 5% of striatal neuronal cells are cholinergic and GABAergic interneurons (Tisch et al., 2004). Decreased levels of tyrosine hydroxylase (TH) (EC 1.14.16.2) and dopamine (DA) have been associated with motor and cognitive impairments in METH addicts (Wilson et al., 1996; Volkow et al., 2001; Sekine et al., 2006). Detrimental effects on striatal dopaminergic, but not on serotonergic markers, were consistently reproduced in rodent models using acute high-dose METH treatments (Krasnova and Cadet, 2009). Glutamate (GLU) was also suggested to play a role in METH-induced striatal neurotoxicity (Simoes et al., 2008; Yamamoto et al., 2010). METH appears to increase striatal GLU via a polysynaptic pathway, involving DA and GABA (Mark et al., 2004). However, the impact of METH on the striatal GLU homeostasis is unknown, particularly the role of astrocytes on this parameter. The impact of toxic doses of METH on the GABAergic system in the brain is still not clear. While Haughey et al. (2000) reported that METH did not affect the GABAergic system, the stimulant drug was suggested to cause degeneration of

Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; DA, dopamine; DOPAC, dihydroxyphenyl acetic acid; HVA, homovanilic acid; 5-HT, 5-hydroxytriptamine; 5-HIAA, 5-hydroxyindoleacetic acid; GAD, glutamic acid decarboxylase; GFAP, glial fibrillary acidic protein; GLU, glutamate; GLN, glutamine; GS, glutamine synthase; PAG, phosphate-activated glutaminase; METH, methamphetamine; OPA, *o*-phthalaldehyde; MCE, β-mercaptoethanol; RAGE, receptor for advanced glycation end-products; TH, tyrosine hydroxylase.

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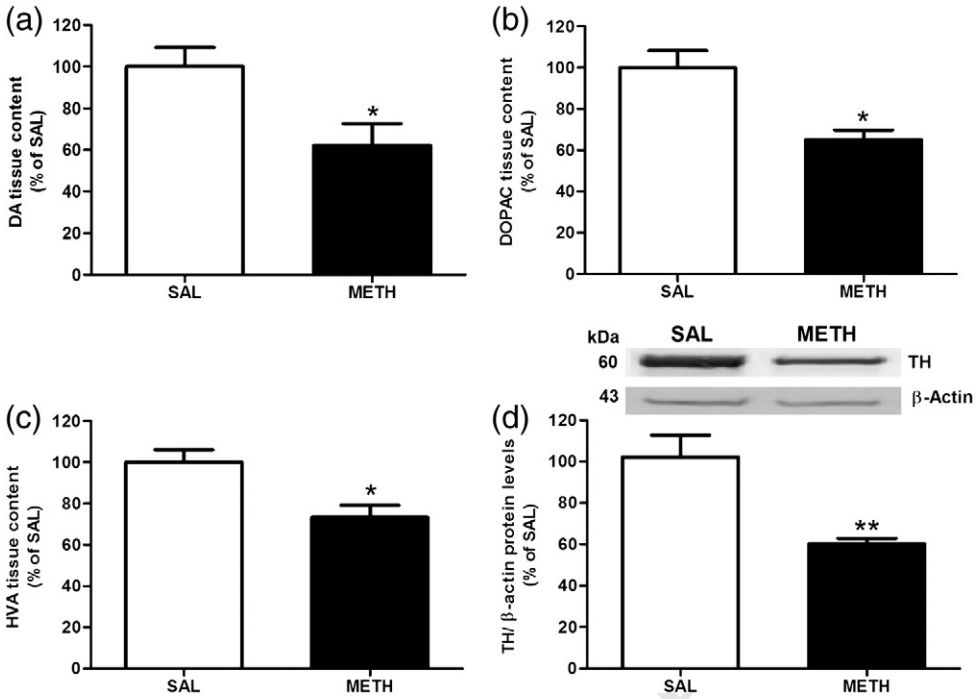

Fig. 1. METH disrupts dopamine homeostasis in the striatum. (a) DA, (b) DOPAC and (c) HVA striatal tissue contents were significantly decreased 72 h following a single dose of METH (30 mg/kg, i.p.). Saline (SAL), DA, DOPAC and HVA levels were: 494.3 ± 23.6, 115.6 ± 13.4 and 51.4 ± 16.4 ng/mg protein, respectively. (d) TH protein levels were also significantly decreased at this end-point. $^{*}P<0.05$, $^{**}P<0.01$, versus SAL-treated animals. The results are expressed as mean percentage of SAL ± S.E.M. of 6 animals per group. Statistics were performed by using unpaired Student's *t*-test.

striatal neurons expressing glutamic acid decarboxylase (GAD, a GABA-synthesizing enzyme) (E.C. 4.1.1.15) (Jayanthi et al., 2004; Zhu et al., 2006a). In contrast, Zhang et al. (2006) showed that METH induced a long-term increase in striatal GAD67 expression. The synthesis of brain GLU and GABA are both linked to a substrate cycle involving glutamine (GLN) metabolism in neurons and astrocytes (Bak et al., 2006). This has led to the concept of the GLU–GLN cycle, which predicts that GLU leaving the neuronal compartment is transferred to the astrocytic compartment, where GLU is metabolized, at least partly, to GLN, via a highly active GLN synthase (GS) (EC 6.3.1.2) pathway. GLN subsequently returns to neurons to be hydrolyzed to GLU by phosphate-activated glutaminase (PAG) (EC 3.5.1.2). GLN released by astrocytes serves additionally as a precursor for GABA, via GLU, through GAD. Evidence of a neuronal glial GLU cycle dysfunction in METH abusers has been recently provided (Sailasuta et al., 2010). A depletion of the glutamatergic system was also reported in recently abstinent METH users (Ernst and Chang, 2008). Additionally, astrocytic striatal activation was demonstrated to be a hallmark of METH neurotoxicity in rodents (O'Callaghan and Miller, 1984; Pubill et al., 2003; Zhu et al., 2006b; O'Callaghan et al., 2008). However, the effect of METH on this neuron-astrocyte cross-talk is not completely understood. The up-regulation of receptor for advanced glycation end-products (RAGE) has been described as playing a pivotal role on synaptic function, glial response and oxidative stress homeostasis (Ding and Keller, 2005). On the other hand, oxidative stress and glial-modulated inflammatory processes are envisaged as playing a role on striatal METH neurotoxicity (Krasnova and Cadet, 2009). Thus, the study of RAGE on a METH-neurotoxic setting has been intriguingly overlooked.

The aim of this work was to provide an integrative characterization of striatal changes in amino acids (GLU and GABA pathways), monoamines (DA, 5-HT pathways), astroglial markers and RAGE levels, 72 h after administration of a single-high dose (30 mg/kg, i.p.) of METH in adult C57BL/6 mice. Spontaneous locomotor activity of mice was also assessed 24 and 48 h following this METH regimen, through the open field test. Our results show, for the first time, that a single injection of a high dose of METH (30 mg/kg, i.p.) induces GLU and GABA depletion, concurrently with dopaminergic neurodegeneration in the striatum, at 72 h post-METH injection. This study also shows that marked changes in the striatal neurochemical homeostasis, along with astrocyte activation, coexist with unchanged RAGE expression 72 h post-METH injection. Importantly, this striatal toxicity accompanies mice sustained hypoactivity.

2. Materials and methods

2.1. Animals

Male adult C57BL/6J mice (3–4 months old; 20–28 g; Charles River Laboratories, Barcelona, Spain) were housed 3 per cage, under controlled environmental conditions (12-h light/dark schedule, at room temperature of 21 ± 1 °C, with food and water supplied ad libitum). All experiments were approved by the Institutional Animal Care and Use Committee from Faculty of Medicine, Coimbra University, and were

Table 1
METH does not change serotonin homeostasis in the striatum.

	Indolamine tissue content (% of SAL; mean ± S.E.M.)	
	5-HT	5-HIAA
SAL (n = 6)	100.0 ± 10.5	100.0 ± 7.6
METH (n = 6)	90.6 ± 10.4	80.8 ± 7.8

A single dose of METH (30 mg/kg, i.p.) does not affect serotonin homeostasis in striatum. Saline 5-HT and 5-HIAA striatal levels were 26.9 ± 4.1 and 19.1 ± 4.8 ng/mg protein, respectively. The results are expressed as mean percentage of saline (SAL) ± S.E.M. of 6 animals per group. Statistics were performed by using unpaired Student's *t*-test.

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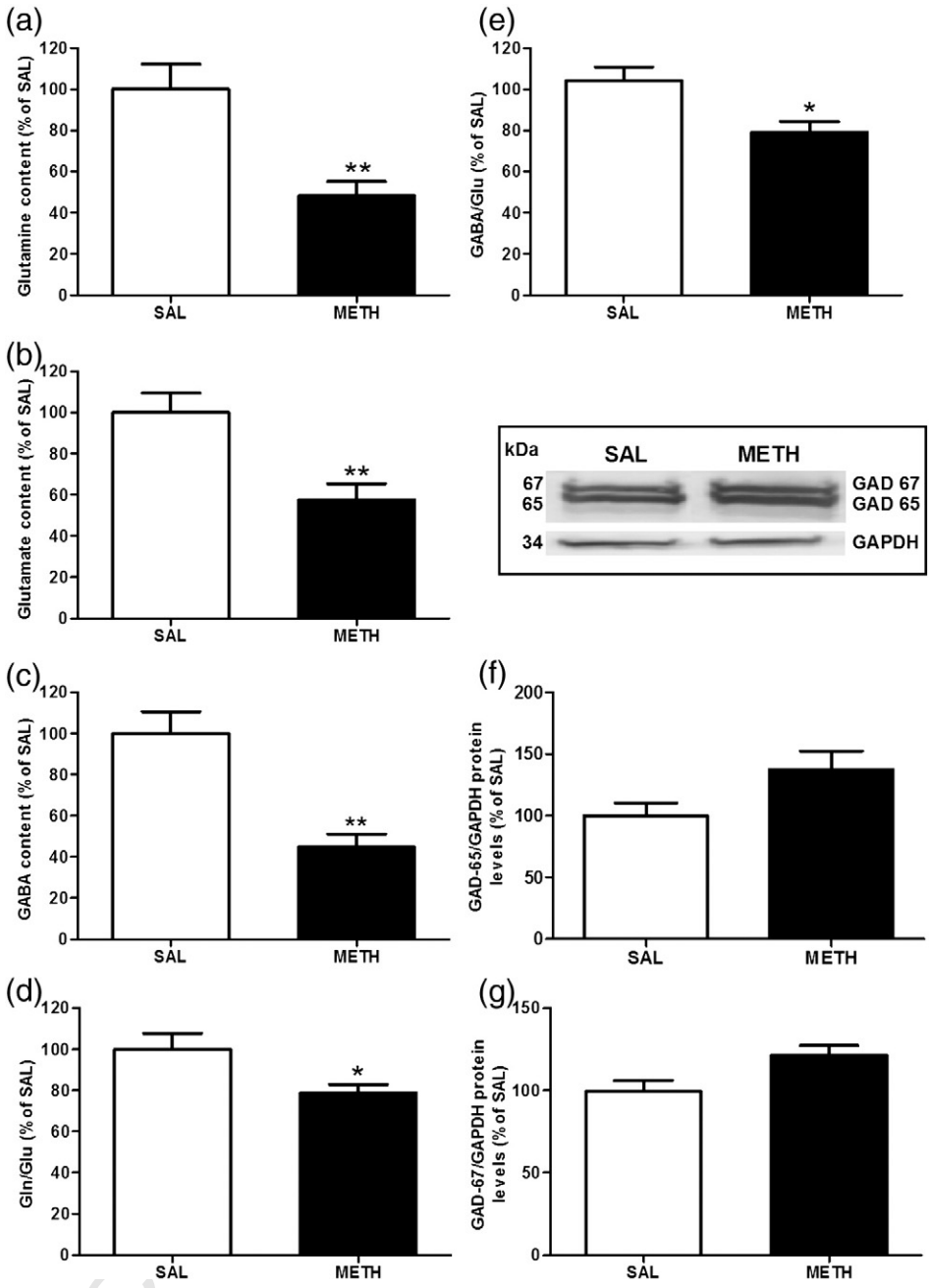

Fig. 2. METH decreases amino acid homeostasis in the striatum. (a) GLN, (b) GLU and (c) GABA striatal tissue contents were significantly decreased 72 h following a single dose of METH (30 mg/kg, i.p.). (d,e) METH also significantly decreased GLN/GLU and GABA/GLU ratios at this end-point. Saline (SAL) GLN, GLU and GABA levels were: 29.4 ± 4.6, 34.7 ± 7.22 and 13.3 ± 2.3 μg/mg protein, respectively. (f,g) METH did not change GAD65/67 protein levels in striatum at 72 h. $^{*}P<0.05$, $^{**}P<0.01$, versus SAL-treated animals. The results are expressed as mean percentage of SAL ± S.E.M. of 6 animals per group. Statistics were performed by using unpaired Student's *t*-test.

performed in accordance with the European Community directive (2010/63/EU). All efforts were made to minimize animal suffering and to reduce the number of animals used. The ARRIVE guidelines have been followed.

2.2. Drugs and chemicals

We were issued permission to import methamphetamine. HCl was purchased from Sigma-Aldrich (St. Louis, MO, USA) by INFARMED, Portugal (National Authority of Medicines and Health Products). Standards for DA, DOPAC, HVA, 5-HT, 5-HIAA, L-GLN, GLU, GABA, *o*-phthaldehyde (OPA) and β-mercaptoethanol (MCE) were purchased from Sigma-Aldrich. The other used chemicals (*ultrapure* and *pro analysis* quality) were purchased from Sigma-Aldrich and Merck AG (Darmstadt, Germany).

2.3. Drug administration

Six animals were injected intraperitoneally with a single-dose of METH (30 mg/kg, i.p.) or with saline solution (0.9% NaCl). Body temperature was assessed with a rectal probe every 30 min following injection, up to 3 h. Twenty-four and 48 h following METH injection, animals were tested for locomotor activity in the open field test. Animals were then sacrificed by decapitation 72 h post-treatment and striata were dissected on ice and stored at −80 °C until further analyses. This end-point was chosen based on previous literature

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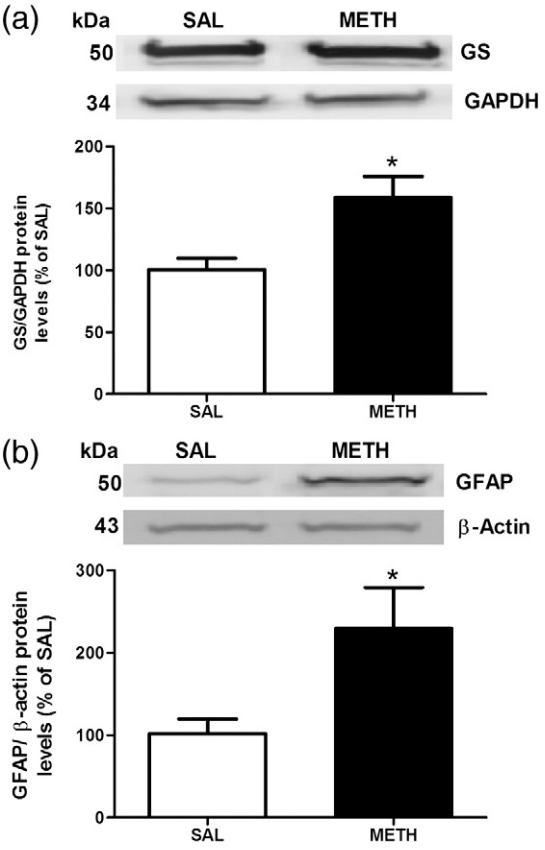

Fig. 3. METH increases GS and GFAP protein levels in the striatum. (a) GS and (b) GFAP protein levels were significantly increased 72 h following a single dose of METH (30 mg/kg, i.p.). *$P<0.05$ versus saline (SAL)-treated animals. The results are expressed as mean percentage of SAL ± S.E.M. of 6 animals per group. Statistics were performed by using unpaired Student's *t*-test.

demonstrating dopaminergic neurotoxicity at 72 h after the administration of METH (Krasnova and Cadet, 2009). Left brain areas were used for the determination of monoamine (DA, DOPAC, HVA, 5-HT and 5-HIAA) and amino acid (GLN, GLU and GABA) contents by HPLC-ED and HPLC-fluorescence detection, and right areas were used for the quantification of protein expression by Western-blot. In the present study, all animals survived this dosing regimen and none showed convulsions or weight reductions. Regarding body temperature, animals treated with METH displayed hyperthermia starting at 30 min, peaking at 2 h (36.5 ± 0.2 °C vs 38.8 ± 0.2 °C, $P<0.001$; peak temperature for controls and METH respectively) and returning to normal values at 3 h. This transient hyperthermia is consistent with that shown by Xu et al. (2005), using the same METH dose.

2.4. HPLC

2.4.1. Monoamine assessment by HPLC-ED

Left striata were sonicated in ice-cold 0.2 M perchloric acid (250 µL) and centrifuged (15,500×*g*, 7 min, 4 °C). Supernatants were filtered (9000×*g*, 10 min, 4 °C) using 0.2 µm Nylon microfilters (Spin-X® Centrifuge Tube Filter) and stored at −25 °C until further analyses. Fifty microliters of each supernatant was neutralized with 10 M KOH/5 M Tris and used for amino acid analysis (see below). The pellet was resuspended in 1 M NaOH and stored at −80 °C for total protein quantification by the bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific, MA, USA). A Gilson HPLC system was used to determine DA, DOPAC, HVA, 5-HT and 5-HIAA concentrations in striatum as described previously (Pereira et al., 2006). These compounds were separated on a reversed-phase Waters Spherisorb ODS2 column (24.6 mm×250 mm; 5 µm) with a mobile phase (pH = 3.8) consisting of 0.1 M sodium acetate trihydrate, 0.1 M citric acid monohydrate, 0.5 mM sodium octane sulphonate, 0.15 mM EDTA, 1 mM dibutylamine and 10% methanol (vol/vol). A flow rate of 1.0 mL/min was maintained for 60 min, and detection of the chromatographed compounds was achieved using a glassy carbon working electrode set at 0.75 V. Sensitivity was set at 2 nA/V. Monoamine concentration was determined by comparison with peak areas of standards, and expressed in nanogram per mg of protein.

2.4.2. Amino acid assessment by HPLC-fluorescence detection

The levels of the amino acids GLN, GLU and GABA were analyzed in a Gilson-ASTED HPLC system (Rego et al., 1996). Samples were separated on a Hichrom ACE type column (150×4.6 mm, 5 µM C18) at a flow rate of 2.5 mL/min for 45 min, using a ternary solvent system consisting of solvent A [37.5 mM sodium phosphate, 50 mM propionic acid, 7% acetonitrile and 3% dimethyl sulfoxide (pH 6.2)], solvent B (40% acetonitrile, 33% methanol and 7% dimethyl sulfoxide) and solvent C [62.5 mM sodium phosphate, 50 mM propionic acid, 7% acetonitrile and 3% dimethyl sulfoxide (pH 5.5)]. The amino acids were detected as fluorescent derivatives after precolumn derivatization with OPA/MCE, using a Gilson fluorescent detector model 121, with excitation at 340 nm and emission at 410 nm. Amino acid concentration was determined by comparison with peak areas of amino acid standards, and expressed in microgram per mg of protein.

2.5. Western blotting analysis

For measuring TH, GAD 65/67, GFAP, GS and RAGE levels, total extracts were obtained as previously described by Simoes et al. (2008). Right striata were homogenized in lysis buffer (50 mM Tris–HCl pH 7.4/0.5% Triton X-100, 4 °C), supplemented with a protease inhibitor cocktail (1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 1 µg/mL chymostatin, 1 µg/mL leupeptin, 1 µg/mL antipain and 5 µg/mL pepstatin A; Sigma-Aldrich) and centrifuged (15,500×*g*, 15 min, 4 °C) to discard insoluble material. Total protein concentration was determined using the BCA method (Smith et al., 1985), and supernatants were stored at − 80 °C until further use. Equal amounts of protein (10 µg — TH, GAD 65/67, GFAP, GS and 40 µg — RAGE) were loaded and separated by electrophoresis on sodium dodecyl sulfate polyacrylamide gel electrophoresis (12%), transferred to a polyvinylidene difluoride membrane (Millipore, Madrid, Spain), and blocked with 5% nonfat dry milk in phosphate buffer saline for 1 h at room temperature. The membranes were probed with mouse anti-TH (1:5000; Millipore, MA, USA), rabbit anti-GAD65/67 (1:1000; Chemicon, Millipore), mouse anti-GFAP (1:5000; Millipore), goat anti-GS (1:500; Santa Cruz Biotechnology, CA, USA), and rabbit anti-RAGE (1:5000; Millipore), overnight at 4 °C. Membranes were then incubated with alkaline phosphatase-conjugated secondary antibodies (1:10,000 anti-mouse, anti-goat – Sigma-Aldrich – or anti-rabbit — GE Healthcare, USA). Finally, membranes were visualized on a Storm 860 Gel and Blot Imaging System (GE Healthcare, Buckinghamshire, UK), using an enhanced chemifluorescence detection reagent (ECF, GE Healthcare). To confirm equal protein loading and sample transfer, membranes were reprobed with β-actin (1:10,000; Sigma-Aldrich) or GAPDH (1:5000; Abcam, Cambrigde, UK) antibodies. Densitometric analyses were performed using the Image Quant software. Results were normalized against β-actin or GAPDH, and then expressed as percentage of control.

2.6. Open-field test

The locomotor activity was evaluated in the open field arena at 24 and 48 h after METH injection. The open field was made of wood covered with impermeable Formica (50 cm×50 cm×40 cm) and the experiments were performed in a sound-attenuated room under low-intensity light (12 lx). Each mouse was placed in the center of the apparatus and the exploratory activity was registered for 60 min. Boxes were cleaned before and after each trial with a 10% ethanol solution. Locomotor activity

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data were recorded and analyzed in 5-min intervals using the ANY-maze ™ video tracking system (Stoelting Co., Wood Dale IL, USA).

2.7. Statistical analysis

The data are expressed as means ± S.E.M. Open-field data were analyzed by one-way ANOVA followed by Newman–Keuls Multiple Comparison Test. For other experiments, statistics were performed by using unpaired Student's *t*-test. Significant differences were defined at $P<0.05$. All analyses were performed using GraphPad Prism 5.0 for Windows.

3. Results

3.1. METH-induced changes in dopamine homeostasis in the striatum

The effect of a single injection of METH (30 mg/kg, i.p.) on DA and its metabolite content in striatum is seen in Fig. 1(a,b,c). As expected, METH produced a significant depletion of DA and its metabolites, DOPAC and HVA, in the striatum at 24 h (data not shown; $P<0.05$) and 72 h post-injection (DA, 40%; DOPAC, 35%; HVA, 22%; $P<0.05$). Concordantly, METH also produced a marked reduction in TH levels (40%; $P<0.01$) at 72 h, as shown in Fig. 1(d).

We also analyzed the impact of METH on striatal levels of 5-HT and its metabolite 5-HIAA, Nevertheless, METH did not significantly change 5-HT and 5-HIAA levels in striatum (Table 1) ($P>0.05$). Saline 5-HT and 5-HIAA striatal levels were 26.9 ± 4.1 and 19.1 ± 4.8 ng/mg protein, respectively.

3.2. METH-induced changes in amino acid levels and metabolic enzymes in the striatum

Striatal GLN, GLU and GABA contents were also measured in METH-treated mice. A single dose of METH (30 mg/kg, i.p.) significantly reduced GLN, GLU and GABA levels (52, 42 and 55%, respectively; $P<0.01$) 72 h post-injection, as shown in Fig. 2(a,b,c). METH also decreased both GLN/GLU and GABA/GLU ratios at this endpoint ($P<0.05$) (Fig. 2(d,e)).

Glutamic acid decarboxylase (GAD 65/67) is the rate-limiting enzyme for the biosynthesis of GABA. GAD65 is abundant in the terminals of MS neurons, whereas GAD67 is strongly expressed in the GABAergic interneurons, with the exception of somatostatin-containing GABAergic interneurons (Li et al., 2010). METH failed to change both GAD65 and GAD67 levels in the striatum, 72 h after administration ($P>0.05$) (Fig. 2(f,g)). We also determined the levels of GS, the enzyme responsible for GLU–GLN conversion. Interestingly, METH evoked an increment in the expression of GS at 72 h (159% of SAL; $P<0.05$) (Fig. 3(a)). Because increased levels of GS could not reflect its activity, we hypothesized the occurrence of increased astrogliosis in METH-treated animals. Indeed, striatal astrogliosis (examined by GFAP labeling) was significantly increased 72 h post-METH, as compared to SAL controls (230% of SAL; $P<0.05$) (Fig. 3(b)).

3.3. METH impact in RAGE expression in striatum

Taking into account that RAGE appears to play a role in glial response and oxidative stress, and that there is no literature about the effects of METH treatment on RAGE levels, we determined the striatal levels of this receptor. However, METH did not change RAGE expression in the striatum 72 h after administration, as shown in Fig. 4(a,b,c) ($P>0.05$).

3.4. METH impact on locomotor activity

Little is known about locomotor effects in mice following a single high-METH dose. Therefore, the effects of METH administration on spontaneous locomotor activity of mice were evaluated using the open-field test. METH induced a significant decrease in total distance (cm) traveled by the animals during 1 h sessions, at 24 and 48 h after administration (Fig. 5(a),(b),(c)).

4. Discussion

Our results show, for the first time, a pronounced striatal GLU and GABA depletion, concurrent with dopaminergic neurodegeneration, at 72 h after a single injection of a high dose of METH (30 mg/kg, i.p.), in mice. This METH impact on striatal neurochemistry could be influenced

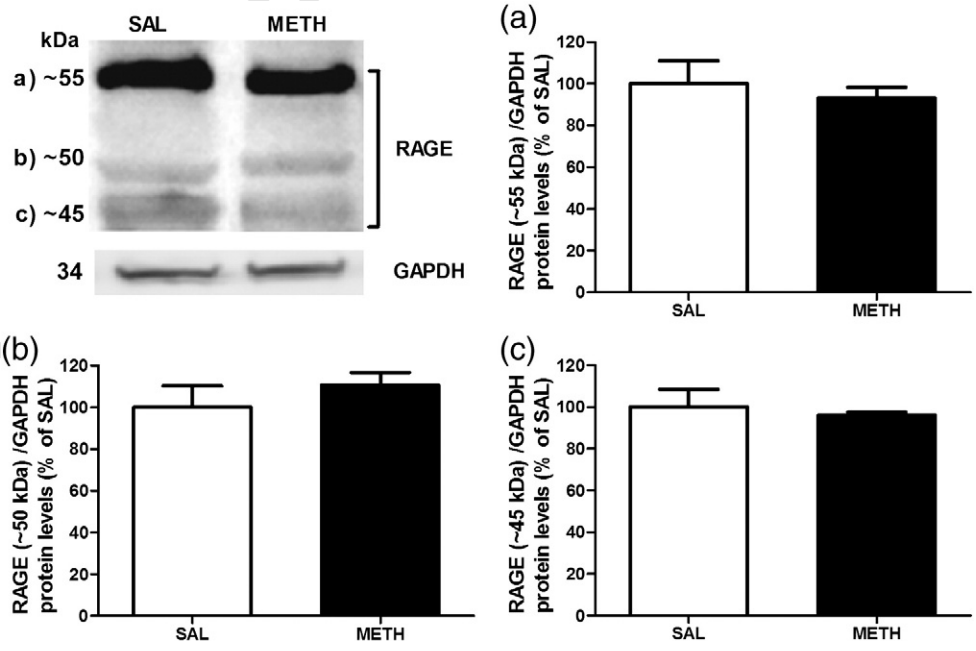

Fig. 4. METH does not change RAGE protein levels in the striatum. (a) ~55 kDa, (b) ~50 kDa and (c) ~45 kDa RAGE levels remained unchanged 72 h following a single dose of METH (30 mg/kg, i.p.). The results are expressed as mean percentage of saline (SAL) ± S.E.M. of 6 animals per group. Statistics were performed by using unpaired Student's *t*-test.

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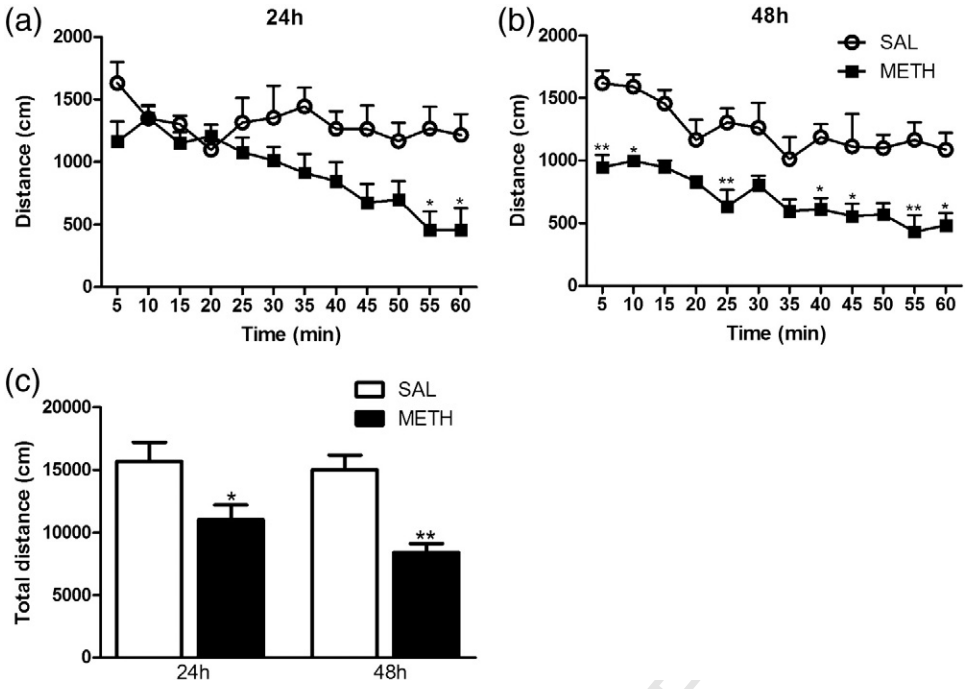

Fig. 5. METH impairs spontaneous locomotor activity of mice in the open field test. The figure represents the distances traveled by the animals in the open-field arena during 60 min in 5 minute intervals (a) 24 h and (b) 48 h following a single dose of METH (30 mg/kg, i.p.). Total distances traveled by the animals are represented in (c). $^{*}P<0.05$, $^{**}P<0.01$, versus saline (SAL)-treated animals. The results are expressed as mean distances in cm ± S.E.M. of 6 animals per group. Statistics were performed by using one-way ANOVA followed by the Newman–Keuls Multiple Comparison Test.

by the observed transient hyperthermia. Indeed, the influence of body temperature on METH-induced striatal neurotoxicity was previously highlighted (Ali et al., 1994). Typically, rodent METH research uses a single-day regimen of multiple 5–10 mg/kg i.p. injections (neurotoxic/binge dosing paradigm) which results in loss of DA content, as well as TH levels, in the early withdrawal period within 72 h post-treatment (Miller et al., 2000; Pereira et al., 2006). In the present study, we used a single METH injection (30 mg/kg, i.p.) to reduce the inherent complexity present in repeated dosage regimens and, thus, to better uncover the biochemical and molecular profile of METH striatal neurotoxicity (Pereira et al., 2006; Simoes et al., 2008). As expected, this METH paradigm imposed striatal dopaminergic neurodegeneration, as seen by the degree of DA and TH depletion, as well as DOPAC and HVA depletion. These results further confirm that the paradigm used in this work disrupts DA homeostasis, as suggested by Zhu et al. (2005). Since dopaminergic synapses constitute hardly 9% of all synapses in the striatum (Arbuthnott and Wickens, 2007), we further analyzed other striatal neurotransmitters. Although METH is primarily seen as DA neurotoxin, this psychostimulant may also damage other monoaminergic profiles, namely serotonin (Krasnova and Cadet, 2009). However, rodent studies are not consistent regarding METH toxicity against 5-HT systems (Krasnova and Cadet, 2009). For example, Fumagalli et al. (1998) showed 5-HT depletion in C57BL/6J, which was not replicated by Grace et al. (2010). In the present study, we failed to detect significant changes in both striatal 5-HT and its metabolite 5-HIAA, in METH (30 mg/kg, i.p.)-treated mice. This intriguing selectivity of METH toxicity towards DA terminals, sparing 5-HT terminals within the striatum, deserves further investigation.

Although the vast majority of striatal synapses (about 80%) are glutamatergic (mainly cortical and thalamic inputs) (Wilson, 2007), there is no information on both total striatal GLU and GLN levels following METH administration. GLU and GLN contents of brain homogenates mainly reflect neuronal and astrocytic pools, respectively (Waagepetersen et al., 2003). GLN concentration also reflects the amount of GLU uptaken by astrocytes (Bak et al., 2006). One potential explanation for the GLU depletion observed in the present study is an impaired GLU/GLN cycling. This GLU/GLN cycle between astrocytes and neurons is the major source of neuronal GLU, and therefore a critical step in excitatory neurotransmission, as well as in excitotoxicity (Benjamin and Quastel, 1974; Hertz et al., 1999; Broer and Brookes, 2001). Herein we report a METH-induced decrease of GLN/GLU ratio, which suggests an incomplete GLU recycling, in spite of an increase in GS levels. This could be accounted for by METH-induced astrogliosis, as demonstrated by an increase in GFAP levels. Moreover, upregulation of GS is also seen as a hallmark of reactive astrocytosis (Cammer and Downing, 1991). Indeed, this astrogliosis might contribute to diminish GLN synthesis, as suggested by reduced GLN levels. For example, inflammation associated with reactive astrocytes was previously shown to decrease GS activity (Muscoli et al., 2005). Additionally, previous findings showed that astrogliosis is inversely correlated with a reduction in GLN levels (Pekny et al., 1999). However, the GLU/GLN cycle is not a stoichiometric cycle, but rather an open pathway that interfaces with many other metabolic pathways, including oxidation for energy, and incorporation of GLU into proteins. For example, the decrease in GLU + GLN could also mean that they were consumed to replenish a compromised Krebs cycle, consistently with a disruption of striatal metabolism and oxidative stress induced by METH injection (Yamamoto and Raudensky, 2008). Moreover, we are the first showing striatal RAGE levels following METH injection. However, although astrocytes are reactive, we failed to observe changes in RAGE levels.

Both dopaminergic and glutamatergic terminals impinge into GABAergic striatal neurons. Although it was previously reported that K^{+}-depolarization increased GLU and GABA striatal extracellular levels following repeated administration of METH (Bustamante et al., 2002), the impact of METH on total striatal GABA levels is unknown. Herein, we also showed that METH decreased total GABA content in the striatum. Nuclear magnetic resonance studies have shown that GLN is the major precursor for GABA synthesis in rat in vivo (Patel et al., 2001). Recently, it was also suggested that GABA synthesis in rat striatum may be regulated in part by GLN (Wang et al., 2007). Furthermore, inhibition of GLN transport can deplete GLU and GABA in brain slices (Rae et al., 2003). In accordance, our data suggest that GLN deficits could lead to

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reductions in GLU content and, thus, also in GABA levels. Additionally, this METH dosing could have also decreased GAD activity, as suggested by the decrement in GABA/GLU ratio. Moreover, these neurochemical results (GABA depletion) are in line with those reported by other authors, using 30–40 mg/kg of METH, and showing GABAergic striatal cell death by immunohistochemical staining and TUNEL histochemistry (Jayanthi et al., 2004; Zhu et al., 2006a). Finally, herein, both GAD65 and GAD67 levels showed a tendency to increase. This may represent a compensatory mechanism to correct for the impaired GABA transmission.

Clinical studies showed deficits in motor skills, including motor slowing, in abstinent METH abusers (Volkow et al., 2001; Toomey et al., 2003). However, the influence of METH-induced neurochemical and structural striatal changes on the motor behavior of animals has not yet been systematically studied, especially under neurotoxic single METH doses. In the present study, the open-field analysis showed that METH induced a sustained impairment in locomotor activity, at 24 and 48 h after exposure, suggesting that METH-exposed mice were less active than controls. This locomotor impairment could be a functional readout of the severe striatal neurochemical alterations and astrogliosis, displayed by these animals. The early onset of hypolocomotion accompanies the DA or TH depletion, starting as early as 6–24 h, as observed by us (data not shown), and by others (O'Callaghan and Miller 1994; O'Callaghan et al., 2008). Increases in GFAP were also described within the time frame of the locomotor deficits seen here (O'Callaghan and Miller 1994; O'Callaghan et al., 2008). Finally, this behavioral result is consistent with recent studies showing that multiple METH injections evoked hypolocomotion in mice (Grace et al., 2010; Shen et al., 2011).

5. Conclusion

Our results clearly show that an acute single METH exposure is a good model for the study of METH intoxication, evoking profound changes in striatal DA levels and locomotor impairment, along with newly described changes in GLU and GABA homeostasis, underlying astrogliosis. Considering that the present dose of METH may mimic METH overdose incidents in humans, these findings might offer new avenues for a comprehensive understanding of METH striatal dysfunction, which may help identifying new therapeutic strategies.

Authors' contribution

FCP and CFR designed the research. FCP injected the animals and collected brain samples. FCP, TCO and SN performed HPLC analysis. SV and AST performed Western blot analysis. CS and RDP performed open-field experiments. FCP, TCO and SV analyzed the data. FCP and TCO wrote the paper. CR, SA, RDP and CFR contributed with scientific expertise and revisions of the paper.

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Conflict of interest statement

None.

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