Effect of a binge-like dosing regimen of methamphetamine on dopamine levels and tyrosine hydroxylase expressing neurons in the rat brain

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Effect of a binge-like dosing regimen of methamphetamine on dopamine levels and tyrosine hydroxylase expressing neurons in the rat brain

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Abstract:
Methamphetamine, an amphetamine derivative, is a powerful psychomotor stimulant and commonly used drug of abuse. This study examined the effect of binge-like methamphetamine (MA) dosing (4 x 4mg/kg, s.c., 2 hours apart) on regional dopamine and dopaminergic metabolite levels in rat brain at a range of early time points after final dose (2 – 48 hours). Body temperature was elevated when measured 2 hours after the last dose. MA increased dopamine levels in the frontal cortex 2 and 24 hours after the last dose. The dopamine level was also increased in the amygdala at 24 hours. No change was observed in the striatum at any time point, but levels of the dopamine metabolite DOPAC were markedly reduced at 24 and 48 hours. Tyrosine hydroxylase expression is induced downstream of dopamine activity, and it is the rate limiting enzyme in dopamine synthesis. The effect of MA binge-like dosing on the volume of tyrosine hydroxylase containing cell bodies and the area fraction of tyrosine hydroxylase containing fibres was also assessed. MA increased the area fraction of tyrosine hydroxylase fibres in the frontal cortex and reduced the volume of tyrosine hydroxylase containing cell bodies 2 hours after last dose in the ventral tegmental area and the substantia nigra. An increase in cell body volume in the substantia nigra was observed 48 hours after treatment. These findings collectively highlight the importance of the dopaminergic system in methamphetamine induced effects, identify the frontal cortex, amygdala and striatum as key regions that undergo early changes in response to binge-like methamphetamine dosing and provide evidence of time-dependent effects on the cell bodies and fibres of tyrosine hydroxylase expressing neurons.

Keywords: Methamphetamine; Dopamine; Dopamine metabolites; Tyrosine hydroxylase; Hyperthermia

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Introduction

Methamphetamine (MA), a synthetic derivative of amphetamine, is a very potent psychomotor stimulant and is well known for its high potential for abuse. MA intake by an array of routes of administration results in wide distribution in the periphery and the brain, where it interacts and reverses the function of mono-amine transporters, enhancing synaptic levels of dopamine, noradrenaline and serotonin\(^1\). Evidence suggests that the mechanism underlying the psychomotor and neurotoxic properties of amphetamine and MA are similar and they also have similar potency\(^2,3\). Using in vivo microdialysis, it has been demonstrated that amphetamine and other amphetamine-like stimulants including MA rapidly increase extracellular levels of dopamine in dopamine-rich brain regions such as the striatum\(^4,6\). Depletion of dopamine days after drug exposure is another well-established feature of MA and amphetamine-like drugs of abuse\(^7-11\), however dopamine tissue levels in the hours immediately following the rapid increase in extracellular levels, within the first 48 hours after drug exposure is less widely investigated, and is the subject of the present study.

It has been suggested that low doses of MA affect the CNS by activation of D2 receptors, while high doses additionally activate D1 receptors\(^12,13\). Following stimulation of D1-like receptors, the subsequent increase in cAMP and influx of calcium activates protein kinase A, which can phosphorylate cAMP response element binding protein (CREB). It is known that drugs such as amphetamine and cocaine activate D1 receptors and induce phosphorylation of CREB\(^14\). Phosphorylated CREB acts as a transcriptional factor for many downstream genes such as tyrosine hydroxylase\(^15\).

Tyrosine hydroxylase is an enzyme that catalyses the conversion of L-tyrosine to L-DOPA. It is the rate-limiting enzyme in the synthesis of dopamine and noradrenaline. Many studies have shown an alteration in tyrosine hydroxylase following MA exposure. For example, a transient increase in tyrosine hydroxylase mRNA has been demonstrated in the rat locus coeruleus following acute MA treatment\(^16\) and an increase in tyrosine hydroxylase protein was observed in the mouse cerebellar cortex 3 days after a binge MA dosing regimen that correlated with the motor dysfunction caused by the drug\(^17\). In contrast, chronic use of MA has been shown to cause a decrease in tyrosine hydroxylase levels\(^18,19\) that may be persistent\(^20\) and post-mortem studies of chronic MA users have shown reduced dopamine levels and tyrosine hydroxylase expression in the striatum\(^21\).

Animal models have shown that single or repeated doses of methamphetamine promotes behavior and biochemical changes. Single large doses of methamphetamine up to 15mg/kg have been used\(^22\), although recent studies\(^23,24\) have demonstrated that repeated lower doses reduce risk of mortality and more closely mimics that used by humans. A regimen using 4 injections of methamphetamine at 2 hour intervals is the most common approach\(^25-27\).
Dopaminergic fibres project to the striatum (nigrostriatal pathway), the cerebral cortex (mesocortical pathway) and the limbic system (mesolimbic pathway). There is considerable literature on the effects of MA in the striatum, but less information on the mesocortical and mesolimbic target sites. This study investigated the early effect of binge-like MA dosing on dopamine neurotransmitter levels in the frontal cortex and the amygdala, in addition to the striatum, at time points from 2 hours after final binge dose, up to 48 hours. The effect of MA binge dosing on neurons expressing tyrosine hydroxylase over the same time period was also assessed, specifically the cell body volume of neurons in the substantia nigra and ventral tegmental area, and the area fraction of tyrosine hydroxylase containing fibres in the frontal cortex. The acute hyperthermia triggered by MA has been associated with deficits in the dopaminergic system, oxidative stress and ultimately with neurodegeneration, so it was of interest to correlate the changes in dopaminergic neurons with changes in body temperature to advance understanding of the effect of methamphetamine on dopaminergic neurons.
Materials and Methods

Sprague Dawley rats (250 – 350 g) were used in this study. Procedures were carried out under the guidelines of the Animal Welfare Committee of the National University of Ireland, Galway and in accordance with the EU Directive (2010/63/EU).

Rats (n = 6 or 7 per time point) were housed singly and maintained on a 12 hour light/dark cycle (lights on at 08:00h and off at 20:00h). The housing facility was temperature (20°C ± 2°C) and humidity (40-60%) controlled. Food and water were available ad libitum.

(+/-) Methamphetamine-HCl was purchased under license from Sigma-Aldrich, USA. Rats were administered four doses of MA, 4mg/kg s.c. (calculated as free base), 2 hours apart or saline for control. Repeated dosing of MA at 2 hour intervals is commonly used to mimic binge-like drug taking behavior 24, 26, 27. The 4mg/kg dose used in this study was previously shown to be the highest safe level in our hands (data not shown).

Body temperature was measured in animals treated with methamphetamine and the saline treated controls by rectal thermometer at 2, 24 or 48 hours after the last dose, immediately prior to sacrifice and brain dissection for analysis.

Concentrations of dopamine, its metabolites and 1-3-4-dihydroxyphenylamine (L-DOPA) were measured by HPLC with electrochemical detection using the method of 29. Briefly, each brain was dissected on ice into regions of interest (frontal cortex, amygdala and striatum) and stored at -80°C until further processing. The fresh frozen brain tissue (n=7 per group) was sonicated in ice cold homogenizing buffer, and the supernatant separated on a Merck Licrosorb RP-C18 column. N-Methyl-5-HT (2ng/20µl) was used as the internal standard in all samples. An external standard mix containing 200ng/20µl of dopamine, L-DOPA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was run between every 5 samples to recalibrate the system and minimize any drift in amine retention times as sampling proceeded. Peak height data from chromatograms together with data obtained from brain tissue weights and internal and external standard mixtures were used to calculate neurotransmitter concentration in each brain sample in terms of ng/g of fresh tissue.

For immunohistochemistry, animals (n = 6 per group) were deeply anaesthetized with pentobarbital and transcardial perfusion was performed with 4% v/v paraformaldehyde. The brains were stored in 20% w/v sucrose in PBS with 0.1% w/v sodium azide at 4°C. Coronal brain sections of 40µM thickness were cut on a microtome and floated in Trizma Buffered Saline (TBS) with 1% w/v azide solution. Sections were washed with TBS (3 x 5 minutes), placed in 0.75% v/v hydrogen peroxide/methanol in distilled water for 5 minutes in order to quench the endogenous peroxides in the tissue, followed by further washes in TBS (3 x 5 minutes). Sections were then blocked with 3% v/v normal horse serum in Triton-X TBS (TXTBS) for 60 minutes, to avoid unspecific binding. Then sections were incubated for 24 hours at room temperature and constant agitation in TXTBS containing
tyrosine hydroxylase antibody (mouse, anti-rat; 1:1000 dilution, Vector Laboratories) and 1% v/v normal horse serum. After incubation, sections were washed in TBS (3 x 10 minutes) and incubated for 3 hours in TBS containing biotinylated horse anti-mouse secondary antibody (1:200 dilution; Vector Laboratories) in 1% v/v normal horse serum. After incubation, sections were rinsed in TBS (3 x 10 minutes) and incubated in avidin-biotin-peroxidase complex (ABC) in TBS 1% v/v normal horse serum for 2 hours. Sections were then rinsed in TBS (3 x 10 minutes) and incubated for 5 minutes in 0.02% 3,3-diaminobenzidine-4HCl (DAB) and 0.01% v/v H2O2 in TBS to reveal immunoreactivity. The reaction was stopped by washing the section in TBS (3 x 5 minutes). The sections were then mounted on glass-gelatinised slides and air-dried overnight. Sections were then dehydrated in a series of ethyl alcohol (5 minutes in 50% alcohol, two times 5 minutes in 70% alcohol and 5 minutes in 100% alcohol), cleared with xylene (2 x 5 minutes) and coverslipped with DePex mounting medium.

Quantification of tyrosine hydroxylase fibres in the frontal cortex:
Photomicrographs (20x magnification; five images per slice, six per animal) of the prefrontal cortex (region Fr2, interaural 13.7mm, bregma +4.7mm, \(^{30}\)) were analysed. Tyrosine hydroxylase fibre area was determined using Image J software. For this, images were first converted to grey scale 8-bit images, threshold defined and the area fraction of stained fibres was assessed by dividing by the area of the field of view.

Estimation of nuclear volume of tyrosine hydroxylase containing neurons in the Substantia Nigra (SN) and Ventral Tegmental Area (VTA):
Photomicrographs (20x; two images from each hemisphere) of the substantia nigra (pars reticulata and pars compacta) and ventral tegmental area were analysed. Volume-weighted mean volume (\(\bar{V}_{v Nuc}\)), was estimated as described by \(^{31}\), and is an estimate of cell body volume. Briefly, this was determined by the point sampled intercept method \(^{32}\). A lattice of test points on lines was superimposed randomly onto the cell bodies in each particular field. When a point hit a cell body, a line was drawn through the point from one cell body margin to the other. These lines produced point-sampled intercepts whose length were measured in cm, changed to \(\mu m\) and divided by the magnification of the picture (in this case 500), to give the real size (\(l_o\)). The number was then cubed and the mean multiplied by \(\pi/3\). The average overall intercepts gave an unbiased estimate of the cell body volume volume (\(\bar{V}_{v Nuc}\); \(\bar{V}_{v Nuc} = \pi/3.l_o^3\)). All photomicrographs were taken at level Interaural 4.2mm; bregma -4.8mm, \(^{30}\)).

Statistical comparisons were made by performing analysis of variance (one-way ANOVA). Inter-group comparisons were assessed using Dunnett’s multiple comparisons post-hoc test. Results were expressed as mean ± standard error of the mean (S.E.M) on graphs and mean ± standard deviation (SD) on tables.
Results:

Results showed a significant main effect on dopamine in the frontal cortex (F (3, 24) = 13.76; p<0.0001; Fig. 1a), with a significant increase in dopamine evident at 2 hours (p < 0.001) and 24 hours (p < 0.01), returning to normal by 48 hours following MA binge-dosing. No change in dopamine expression was observed in the striatum (F (3, 24) = 2.30; p>0.05, Fig. 1b). There was a significant main effect on dopamine levels in the amygdala (F (3, 24) = 6.87, p < 0.01; Fig. 1c), with a significant increase in dopamine 24 hours after final MA dose, but not any other time point.

No changes in the levels of the dopamine precursor L-DOPA, or the dopamine metabolite HVA was observed in the frontal cortex (L-DOPA: F (3, 24) = 1.78, p>0.05; HVA: F (3, 24) = 0.42, p>0.05), striatum (L-DOPA: F (3, 24) = 5.35, p>0.05; HVA: F (3, 24) = 0.37, p>0.05), or amygdala (L-DOPA: F (3, 24) = 1.14, p>0.05; HVA: F (3, 24) = 1.10, p>0.05) at any time point following MA treatment (Table 1). However, there was a significant main effect on DOPAC levels in the striatum (F (3, 24) = 226.9, p<0.001). DOPAC levels were significantly reduced in the striatum 24 hours (p < 0.001) and 48 hours (p < 0.05) following binge-like MA treatment (Table 1), but no significant change was observed in the frontal cortex (F (3, 24) = 0.93, p>0.05) or amygdala (F (3, 24) = 0.37, p>0.05).

Table 1 L-DOPA, DOPAC and HVA concentrations in brain tissue following a binge-like regimen of MA

<table>
<thead>
<tr>
<th>Time</th>
<th>L-DOPA ng/g fresh tissue</th>
<th>DOPAC ng/g fresh tissue</th>
<th>HVA ng/g fresh tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frontal Cortex</td>
<td>Striatum</td>
<td>Amygdala</td>
</tr>
<tr>
<td>Control</td>
<td>177±49</td>
<td>198±90</td>
<td>1518±356</td>
</tr>
<tr>
<td>2h</td>
<td>222±36</td>
<td>210±53</td>
<td>1899±763</td>
</tr>
<tr>
<td>24h</td>
<td>161±71</td>
<td>233±71</td>
<td>1876±524</td>
</tr>
<tr>
<td>48h</td>
<td>213±67</td>
<td>179±104</td>
<td>1471±542</td>
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Results were analyzed by One Way ANOVA followed by Dunnett’s post hoc analysis and are expressed as mean ± SD (n=7). *p<0.05, **p<0.001 vs control.

The effect of the changes in dopamine and its metabolite levels on dopamine turnover rate were assessed using the formula [DOPAC] + 33/ [Dopamine]. The most pronounced changes in dopamine turnover were observed in the frontal cortex and striatum. In the frontal cortex, dopamine turnover was markedly reduced (approx. 4 - fold reduction) in MA treated animals, 2 hours and 24 hours after MA treatment (Table 2), but had recovered by 48 hours. This change in turnover was due to the higher levels of dopamine in the frontal cortex at those time points after MA dosing (Fig. 1a), while DOPAC and HVA levels were relatively unaffected (Table 1). MA treatment also markedly affected dopamine turnover in the striatum, 24 hours after MA treatment (also approaching 4 – fold reduction; Table 2),
which had recovered by 48 hours. This change in turnover was attributable to the substantially reduced DOPAC levels in the striatum observed at that time point (Table 1). Dopamine turnover was also reduced in the amygdala 24 hours after MA treatment, but the change was less pronounced (Table 2) and was largely caused by increased dopamine levels (Fig. 1c) at that time point.

Table 2 Dopamine turnover rate following a binge-like regimen of MA

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<th>Striatum</th>
<th>Amygdala</th>
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<tr>
<td><strong>Control</strong></td>
<td>3.78</td>
<td>1.20</td>
<td>2.51</td>
</tr>
<tr>
<td><strong>2h</strong></td>
<td>0.94</td>
<td>1.25</td>
<td>2.40</td>
</tr>
<tr>
<td><strong>24h</strong></td>
<td>1.07</td>
<td>0.35</td>
<td>1.42</td>
</tr>
<tr>
<td><strong>48h</strong></td>
<td>4.53</td>
<td>1.27</td>
<td>1.68</td>
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As expected, a significant main effect on body temperature was observed (F (3, 24) = 49.98; p < 0.001; Fig. 2), with a significant increase 2 hours after the last dose that returned back to normal levels by the 24h time point (Fig. 2).

There was a significant main effect on the volume ($V_{Nuc}$) of tyrosine hydroxylase cell bodies both in the substantia nigra (F (3, 20) = 18.15, p < 0.01; Fig. 3a) and the ventral tegmental area (F (3, 20) = 5.60, p < 0.05; Fig. 3b). In both regions, cell body volume was significantly decreased 2 hours after the MA regimen (4 doses 2 hours apart), (Fig. 3a and 3b). In the ventral tegmental area, levels of $V_{Nuc}$ were back to normal by 24 hours and no change was observed at 48 hours (Fig. 3b). In the substantia nigra levels were also back to normal by 24 hours but an increase was observed in the $V_{Nuc}$ 48 hours after the last dose of MA regimen (p < 0.01; Fig. 3a).

MA had a significant main effect on the area fraction of tyrosine hydroxylase fibres in the frontal cortex, (F (3, 20) = 4.25, p < 0.05; Fig. 4). The area fraction increased 2 hours after MA treatment but returned back to normal level by 24 hours and no change was seen at 48 hours (Fig. 4).
Discussion:

MA rapidly increases extracellular levels of dopamine by reverse transport through the dopamine transporter (DAT)\(^3^4\). Additionally, animal models have shown that MA causes a conformational change in the Vesicular Monoamine Transporter 2 (VMAT-2), resulting in the redistribution of neurotransmitter from synaptic vesicles to the cytosol\(^3^5\). The literature also contains many studies which have shown a subsequent decrease in dopamine following MA administration when measured many days after the last dose\(^2^0\),\(^2^6\),\(^3^6\),\(^3^7\).

The results presented here could reflect a short-term increase in dopamine expression in order to support the effect on release of dopamine by MA in the initial hours after drug use. The short-lived increase observed was particularly evident in the frontal cortex, which is an area not well studied following MA dosing. Dopamine levels measured in control animals in this study are in line with those previously reported in the literature\(^3^8\). Previous studies using microdialysis have shown that MA increases extracellular DA levels\(^6\),\(^3^9\),\(^4^0\), and the literature also demonstrates that MA has been shown to produce long-lasting depletion of striatal dopamine (DA) resulting from damage to mesostriatal terminals\(^3^6\),\(^4^1\),\(^4^2\). Most of these studies have focused on timepoints later than our study, normally 7 days after injection. The present study was not designed to observed dopamine levels later than 48h which may explain the difference from the literature.

Concerns about the neurotoxicity of methamphetamine following repeated administration are very well documented\(^2^6\),\(^4^3\),\(^4^4\). Methamphetamine has been shown to induce toxicity in vivo and in vitro\(^2^6\), affecting in particular, but not only, dopaminergic neurons\(^4^5\). Many studies have shown that the psychological and neurotoxic effects are related to dopamine release\(^9\),\(^4^6\),\(^4^7\), although the molecular and cellular mechanisms involved in this process remain to be clarified.

Dopamine turnover was reduced to the most pronounced extent in the frontal cortex and the striatum, although there was a reduction in turnover in all three regions at different time points. The change in dopamine turnover in the frontal cortex and amygdala was associated with the raised dopamine levels in brain tissue, but reduced DOPAC levels were the main contributory factor to the reduced dopamine turnover observed in the striatum. DOPAC and HVA are main metabolites of dopamine following the combined action of monoamine oxidase and catechol-O-methyl transferase (COM-T). DOPAC levels were reduced in the striatum at 24 and to a lesser extent, 48 hours after dosing. Ordinarily, DOPAC is metabolised to HVA by COM-T, but can also be oxidised by hydrogen peroxide to produce toxic metabolites such as peroxynitrite\(^4^8\). There was no significant change in HVA in any brain region, including the striatum, at any time point in this study. Understanding of how MA induces neurotoxicity is still incomplete, but it is likely that the formation of reactive oxygen species is a contributory factor\(^1^0\),\(^4^9\),\(^5^0\). In the present study, MA treatment may have triggered increased catabolism of DOPAC via hydrogen peroxide oxidation in the striatum, which would promote neurotoxicity. A reduction of
dopamine metabolites and dopamine have previously been demonstrated in mouse striatum 3 days after MA binge-like treatment with a substantially higher dose (10mg x 4 doses, 2 hours apart) than used in our study \(^48\). Another study by the same group showed a dose-response effect of MA on dopamine and its metabolites in rat striatum. A single dose of 10mg/kg (and up to 40 mg/kg) reduced dopamine, DOPAC and HVA in the striatum, but 5mg/kg reduced DOPAC alone \(^51\), which is consistent with our observation. Many studies have previously shown long term damage to dopaminergic nerve terminals in the striatum \(^52,\) \(^53\). It would be of interest to investigate further the link between DOPAC levels and neurotoxicity in future work.

Tyrosine hydroxylase gene expression is closely controlled by transcriptional and post-transcriptional mechanisms \(^54\)-\(^56\). MA induced dopamine release is thought to increase cAMP activity, resulting in CREB phosphorylation, which can increase tyrosine hydroxylase expression \(^15\). In this study, there was no change observed in the level of the dopamine precursor L-DOPA in any brain region assessed, but the expression of tyrosine hydroxylase in fibres in the frontal cortex (area fraction of tyrosine hydroxylase containing fibres in the frontal cortex) was increased 2 hours after the binge-like MA dosing, suggesting an upregulation of TH activity in the frontal cortex. The frontal cortex was chosen because the most pronounced changes in dopamine turnover were observed in that area in the current study, but it would be of great interest to assess the impact of MA on the expression of tyrosine hydroxylase in fibres in other brain regions, particularly the amygdala and striatum in future work.

The frontal cortex is a region known to influence motor function, decision-making and emotion, important features to be considered in drug addiction. Methamphetamine-impairs cognition and changes in behaviour following methamphetamine administration to nonhuman primates is produced in part by neuronal changes in the frontal lobes \(^57\). The Frontal cortex is also important in reward via the mesocortical pathway. Likewise, the amygdala is a very important brain region involved with the expression and perception of emotion, and has been implicated in the pathophysiology of affective disorders and drug addiction. The prefrontal cortex is involved in higher-order cognition and emotion and taking into account that drug addiction involves maladaptive behaviour, the prefrontal cortex is a logical structure in this context \(^58\). There are some lines of evidence that implicate prefrontal and limbic dysfunction with addiction. Polysubstance abusers show a smaller prefrontal gray matter than controls \(^59\) and psychostimulant abusers have decreased gray matter in brain regions including the ventromedial orbitofrontal cortex, anterior cingulate cortex and superior temporal cortex relative to controls \(^60\). It would be of great interest to study the longer - term changes in dopaminergic neurons in a model of MA addiction.

Interestingly, our observations at the 2 hour time point showing a significant reduction in the volume of tyrosine hydroxylase cell bodies in the substantia nigra and the ventral tegmental area correlates with the time point when the rats exhibited significant hyperthermia. The decrease in the size of cell bodies in the VTA and SN could be directly caused by the increase in body temperature seen 2 hours after the last dose of MA - as the temperature increases cells can lose water and shrink. Hyperthermia is a well
recognized feature of neurotoxicity induced by amphetamine and its derivates such as MA. Changes in body temperature following MA administration can be a good indicator of neurotoxicity, as severe hyperthermia has been correlated with neurodegeneration. Hyperthermia produced by MA may cause induction of heat shock protein (HSP)-72 and trigger the ultimate depletion of dopamine concentration. Furthermore, studies have shown that when hyperthermia is prevented by placing the rats in a cold environment, dopaminergic deficits are significantly attenuated. The mechanism by which MA and amphetamine like substances increase body temperature is not fully clarified, but studies have shown that hyperthermia can be prevented by a dopamine synthesis inhibitor (α-methyl-p-tyrosine), a dopamine D1 receptor antagonist (SCH23390) or a dopamine D2 receptor antagonist (eticlopride), indicating that dopamine and dopamine receptors play an important role in MA-induced hyperthermia. Further work directly correlating body temperature to cell volume changes in dopaminergic neurons would be very valuable, as would further study into the mechanism involved in the effect.

It is also of interest to note that cell bodies of tyrosine hydroxylase expressing neurons in the SN were significantly increased in size 48h following binge drug taking regimen, which potentially may trigger long lasting change in the striatum. As previously mentioned, MA has been demonstrated to alter nerve terminal endings in the striatum in rats, to cause enlargement of striatal volume in adult abusers of the drug and a reduction in striatal volume in children exposed to MA prenatally. The findings in this study suggest that increased catabolism of DOPAC leading to reactive oxygen species generation may be involved, although this needs to be confirmed directly. Further study investigating if the early changes observed in the current study are the initial steps in the development of neurotoxic changes in response to MA would be very valuable.

Binge-like MA dosing had a time- and region-specific effect on the dopaminergic system in the rat brain. It also exerted time-dependent effects on the expression of tyrosine hydroxylase in fibres in the frontal cortex, and also on the cell bodies of tyrosine hydroxylase containing neurons in the substantia nigra and ventral tegmentum. These findings collectively highlight the importance of the dopaminergic system in MA induced effects and toxicity, and also identify the frontal cortex, amygdala and striatum as important regions that undergo early changes in response to binge-like MA dosing. Further study will provide valuable insights into the role of these brain regions in MA addiction and also the extent and mechanism of neurotoxicity caused by the drug.

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Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflict of interest: The authors declare that they have no conflict of interest.
Fig. 1 Dopamine in (a) frontal cortex, (b) striatum and (c) amygdala following a binge-like regimen of methamphetamine (MA). Results were analyzed by one-way ANOVA followed by Dunnett’s post hoc analysis and are expressed as mean ± S.E.M. (n=7), **p<0.01, ***p<0.001 vs control.

Fig. 2 Body temperature following a binge-like regimen of methamphetamine (MA). Results were analyzed by one-way ANOVA followed by Dunnett’s post hoc analysis and are expressed as mean ± S.E.M. (n = 7), ***p<0.001 vs control.

Fig. 3. Effect of methamphetamine (MA) on cell body volume (\(\bar{V}_{\text{Nuc}}\)) of tyrosine hydroxylase containing cell bodies in (a) substantia nigra (SN) and (b) ventral tegmental area (VTA) over time. Results were analysed by one-way ANOVA followed by Dunnett’s post hoc analysis and expressed as mean ± S.E.M. (n = 6), *p<0.05, **p<0.01 vs control. Inset shows representative images (x20 magnification) of tyrosine hydroxylase containing cell bodies in the two areas respectively.

Fig. 4 Effect of methamphetamine (MA) on area fraction of tyrosine hydroxylase containing fibres in the frontal cortex over time. Results were analysed by one-way ANOVA followed by Dunnett’s post hoc analysis and are expressed as mean ± S.E.M. (n = 6), *p<0.05 vs control. Inset shows representative images (x20 magnification) of tyrosine hydroxylase containing fibres in frontal cortex.
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a. Frontal Cortex

![Graph showing dopamine levels in the Frontal Cortex over different time periods (control, 2h, 24h, 48h).](image)

b. Striatum

![Graph showing dopamine levels in the Striatum over different time periods (control, 2h, 24h, 48h).](image)

c. Amygdala

![Graph showing dopamine levels in the Amygdala over different time periods (control, 2h, 24h, 48h).](image)