



Provided by the author(s) and University of Galway in accordance with publisher policies. Please cite the published version when available.

Title	Time and region-dependent manner of increased brain derived neurotrophic factor and TrkB in rat brain after binge-like methamphetamine exposure
Author(s)	Moreira da Silva Santos, Andreia; Gorman, Adrienne M.; Kelly, John P.; Doyle, Karen M.
Publication Date	2019-11-03
Publication Information	Moreira da Silva Santos, Andreia, Gorman, Adrienne M., Kelly, John P., & Doyle, Karen M. (2020). Time and region-dependent manner of increased brain derived neurotrophic factor and TrkB in rat brain after binge-like methamphetamine exposure. <i>Neuroscience Letters</i> , 715, 134606. doi: <a href="https://doi.org/10.1016/j.neulet.2019.134606">https://doi.org/10.1016/j.neulet.2019.134606</a>
Publisher	Elsevier
Link to publisher's version	<a href="https://doi.org/10.1016/j.neulet.2019.134606">https://doi.org/10.1016/j.neulet.2019.134606</a>
Item record	<a href="http://hdl.handle.net/10379/15732">http://hdl.handle.net/10379/15732</a>
DOI	<a href="http://dx.doi.org/10.1016/j.neulet.2019.134606">http://dx.doi.org/10.1016/j.neulet.2019.134606</a>

Downloaded 2024-04-26T11:18:07Z

Some rights reserved. For more information, please see the item record link above.



# **Time and region-dependent manner of increased Brain Derived Neurotrophic Factor and TrkB in rat brain after binge-like methamphetamine exposure**

Andreia Moreira da Silva Santos<sup>1,2</sup>, Adrienne M. Gorman<sup>3</sup>, John P. Kelly<sup>1</sup>, Karen M. Doyle<sup>1</sup>

<sup>1</sup>School of Medicine and Galway Neuroscience Centre, National University of Ireland, Galway, Ireland

<sup>2</sup>Centro Universitário Unievangélica. Anápolis - GO CEP: 75083-515, Brasil

<sup>3</sup>School of Natural Sciences and Apoptosis Research Centre, National University of Ireland, Galway, Ireland

Corresponding Author details: Karen Doyle: karen.doyle@nuigalway.ie

## **Abstract**

Methamphetamine (MA), a synthetic derivate of amphetamine, has become a major drug of abuse worldwide. This study investigated the effect of binge-like MA dosing (4 x 4 mg/kg, s.c., 2 hours (h) apart) at a range of different time points (from 2 h to 7 days after treatment) on brain-derived neurotrophic factor (BDNF) levels and its receptors, TrkB and p75<sup>NTR</sup>. BDNF levels were significantly increased in the frontal cortex from 2 to 36 h after treatment, returning to normal within 48 h after treatment. In the striatum, BDNF expression was increased at 12 and 24 h after binge-like MA treatment and had returned to normal at 36 h. Increased expression of the TrkB receptor was observed in the frontal cortex at 2, 24 and 48 h after MA treatment and in the striatum at 24 and 48 h after the MA regimen. A significant increase in the p75<sup>NTR</sup> receptor was also noted in the striatum but not the frontal cortex, and it was less pronounced than the effect on TrkB receptor expression. These findings show that the binge-like regimen of MA affects expression of BDNF and its receptors, particularly the TrkB receptor, in a time and region dependent manner, and highlights the importance of the frontal cortex and the striatum in the response following MA binge-like dosing.

## **Key-words**

Methamphetamine, Brain derived neurotrophic factor, TrkB receptor, p75<sup>NTR</sup>

## **Acknowledgements:**

This research was funded by a College of Science Scholarship, National University of Ireland, Galway. The authors acknowledge the contribution of Prof. Afshin Samali, School of Natural Sciences, NUI Galway, to this study. The authors acknowledge the facilities and scientific and technical assistance of the Centre for Microscopy & Imaging at the National University of Ireland Galway ([www.imaging.nuigalway.ie](http://www.imaging.nuigalway.ie)).

## **Introduction**

Methamphetamine (MA), a synthetic derivative of amphetamine, has become a major drug of abuse worldwide. It is a very potent psychomotor stimulant (1) Although MA and other amphetamines can have similar effects, differences also exist. Goodwin and colleagues (2) have shown that MA induces the release of 5 times more dopamine than amphetamine, which is responsible for the euphoric and addictive properties of MA. The effects of MA last longer and it causes more toxic effects than amphetamine. The N-methyl group that differentiates MA from amphetamine decreases the polarity of the molecule, enabling it to better penetrate the blood brain barrier (3).

Drug abuse, such as that associated with MA, leads to long-lasting alterations in neurons in the brain reward circuitry, that may be responsible for the development of addiction and the vulnerability to relapse (4). Furthermore, there is evidence that, when ingested repetitively at high doses, such as that which occurs with binge-like drug-taking behaviour, MA produces measurable neurotoxicity in animal models and humans (5-7). Some of the well-established effects of high dose and chronic MA in the brain include increased dopaminergic activity (8-10), formation of reactive oxygen species (11, 12) and induction of endoplasmic reticulum stress (13, 14).

Many studies have shown that dopamine has an important role in MA induced neuronal damage (15-18). In normal physiological conditions dopamine is stable in the nerve terminal but excessive levels of dopamine in the cytosol can damage dopamine neurons (15, 19). The toxicity is thought to be mediated by the generation of reactive oxygen species (ROS) and cytotoxic quinones (20, 21). Additionally, damage and degeneration of serotonergic terminals has been observed (17).

Dopamine receptors are positively or negatively coupled to adenylyl cyclase. D1- like ( $G_s$ ) and D2- like ( $G_i$ ) receptors have opposing effects, whereby they stimulate or inhibit adenylyl cyclase respectively (22). Activation of adenylyl cyclase by D1 receptors can lead to cAMP-dependent activation of protein kinase (PKA) which is responsible for phosphorylation of the transcription factor cAMP response element-binding protein (CREB) (23). pCREB is the major transcription factor involved in the expression of

neurotrophins, including Brain derived neurotrophic factor (BDNF) (24, 25). Neurotrophin functions pre- and post- synaptically are well established (26-29) but how they mediate their diverse responses in neurons is yet to be fully clarified.

BDNF mediates its pro-survival effects through the tropomyosin receptor kinase B (TrkB) receptor (30, 31). Pro-BDNF mediates mainly apoptotic signaling through p75<sup>NTR</sup> (32). BDNF can influence synaptic plasticity (26) and has been reported to be involved in drug cravings and addiction (33, 34). In this context, BDNF has been suggested to potentiate the dopaminergic activity promoted by psychostimulants and sensitization of dopamine neurons after withdrawal (35, 36). BDNF has also been suggested to serve a neuroprotective function in response to chronic cocaine exposure (33).

In humans, studies have shown that the concentration of BDNF in plasma of MA users is raised (37). Prior studies have shown that MA increased brain BDNF mRNA in a time-dependent manner in adult rats (38). Increased BDNF protein levels have also been previously reported in the hippocampus and striatum of rat pups following MA exposure (39). Additionally, TrkB mRNA has previously been shown to be transiently increased in the brain following MA exposure (38). Furthermore, BDNF signaling through TrkB receptors in striatal areas has been linked to withdrawal symptoms (40) and cravings (41).

Previously in our laboratory, we demonstrated that MA treatment leads to a dose- and region- specific increase in dopamine and BDNF expression in rat brain 2 h after a binge-dose regimen of MA (42, 43). To further increase our understanding of how MA affects BDNF expression and activity, in this study we investigated the longer-term effect of MA on BDNF expression over time, using a range of time points from 2h to 7 days. To investigate the functional implications of changes in BDNF expression, we also investigated the changes in expression of TrkB and p75<sup>NTR</sup>.

## **Material and methods**

### **Animals**

Sprague Dawley rats (250 – 350 g) were used in this study and were purchased from Charles River laboratories, UK. 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 = 6-8 per time point) were housed singly and maintained on a 12 h light/dark cycle (lights on at 08:00h and off at 20:00h). The housing facility was temperature ( $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) and humidity (40-60%) controlled. Food and water were available *ad libitum*.

### **Drugs**

(+) Methamphetamine-HCl was purchased under license from Sigma-Aldrich, USA. Repeated dosing of MA at 2 h intervals is commonly used to mimic binge-like drug taking behaviour (8, 38, 44). Rats were administered four doses of MA, 4 mg/kg as freebase or saline (control), 2 h apart, according to our protocol previously described (42, 43). Room temperature was controlled throughout the experimental procedure and body temperature was measured by insertion of a rectal thermometer (42). Rats were euthanized by decapitation at 2 h, 24 h, 72 h or 7 days after the last dose and BDNF expression was measured. To further expand the results and the time points, another experiment was carried out and rats were euthanized at 2, 12, 24, 36 or 48 h after MA treatment. All drug treatments were administered during daytime hours. The wide array of timepoints in the second experiment meant that some tissue was harvested during the day (2, 24, 48 h after the last MA dose) and some at night (12, 36 h after the last MA dose). It has been shown previously (45, 46) that the levels of neurotrophins vary during the 24 h circadian cycle. To account for diurnal variation, two controls were employed – one control for tissue harvested during the day and another for tissue harvested at night. TrkB and p75<sup>NTR</sup> receptor expression was also assessed in this study.

### **Protein analysis**

Expression of BDNF was measured by ELISA (R&D, DY248). Expression of TrkB and p75<sup>NTR</sup> receptors was assessed by Western blotting. Briefly, brain regions were dissected on ice into regions of interest (frontal cortex, striatum, amygdala, hippocampus, cerebellum and olfactory bulb). Brain tissue was homogenized in lysis buffer (137 nM

NaCl, 20 mM Trizma HCl, 10% v/v Glycerol and 1% v/v Igepal) on ice during the procedure. Following centrifugation at 4<sup>0</sup>C, the supernatant was stored at -80<sup>0</sup>C until further analysis.

For Western blot analysis, protein samples were diluted (1:5) in Laemmli's SDS-PAGE sample buffer (62.5 mM Tris-HCl pH 6.8, 2% w/v SDS, 5% v/v β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.05% v/v bromophenol blue) and heated to 95<sup>0</sup>C for 5 minutes. Approximately 40 μg protein was then resolved on 12% SDS-PAGE gel. A broad-range (6.5-175 kDa) pre-stained protein ladder was run alongside the protein samples. Gels were electrophoresed at 70 V for 30 minutes, followed by 100 V for 1-1.5 h in gel running buffer (25 mM Trizma base, 2M glycine, 3 mM SDS). SDS-PAGE gels were electrophoretically transferred onto nitrocellulose membrane for 1.5 h at 110V in transfer buffer (10 mM CAPS, pH 11, 20% v/v methanol). Membranes were blocked for 1 h in blocking buffer (phosphate buffered saline (PBS) containing 0.05% v/v Tween 20 and 5% v/v non-fat milk). The membrane was then incubated overnight at 4<sup>0</sup>C with the anti-TrkB antibody (1:500, Abcam ab18987, glycosylated) or anti-p75<sup>NTR</sup> antibody (1:1000, Abcam ab8874), diluted in blocking buffer containing 0.1% v/v sodium azide. Unbound antibody was removed by 3 x 10 minute washes with PBS, containing 0.05% v/v Tween<sub>20</sub>, at room temperature. After the washings, the membrane was incubated with secondary antibody (1:10000) for 1.5 h at room temperature, Goat Anti-Rabbit IgG H&L (ab205718) for TrkB and Goat Anti-Rabbit IgG H&L (ab205718) for p75<sup>NTR</sup>. This was followed by 3 x 10 minute washings to remove unbound antibody. Membranes were incubated in 2 ml supersignal West Pico Western blot detection solution for 5 minutes at room temperature. Blots were stripped and re-probed for TrkB or p75<sup>NTR</sup> and protein bands were then visualized using X-ray film in the dark and quantified using the ImageJ analysis tool. Values were normalised against actin expression.

### **Statistical Analysis**

Statistical comparisons were made by performing analysis of variance (one-way or two-way ANOVA, as appropriate) and Dunnett's multiple-comparisons *post hoc* test. For one-way ANOVA analysis, the independent variable was time, and for two-way ANOVA, the independent variables were time and treatment. All results were expressed as mean ± standard error of the mean (SEM). Results were considered significant when  $p < 0.05$ .

## Results

In the first experiment, tissue was harvested 2, 24, 72 h and 7 days after the last dose of MA. BDNF levels were significantly increased by MA in the frontal cortex ( $F_{1,40}=19.30$ ,  $p<0.0001$ ). There was a significant effect of time, with levels raised at 2 and 24 h and reverting to normal levels at 72 h ( $F_{3,40}=18.35$ ,  $p<0.0001$ ) (Fig.1). In the striatum BDNF expression was also significantly increased by MA ( $F_{1,40}=12.66$ ,  $p<0.001$ ). There was a significant effect of time ( $F_{3,40}=51.73$ ,  $p<0.0001$ ) (Fig.1) with raised BDNF expression at 24 h and reverting to normal levels at 72 h. In the other brain regions, no significant changes were observed (Table 1).

**Figure 1. MA administration induces elevation of BDNF levels in the frontal cortex and striatum.** BDNF expression measured by ELISA in (A) frontal cortex and (B) striatum following a binge-like regimen of MA at 4 mg/kg or saline (control). Results (n=8) were analysed by two-way ANOVA analysis and are expressed as mean  $\pm$  S.E.M., \* $p<0.05$

**Table 1. Effect of MA treatment on BDNF levels (pg/mg of protein) in brain regions at different time points**

BDNF expression in brain regions following binge like regimen of MA 4 mg/kg or saline (control). Results (n=8) were analysed by Two Way ANOVA followed by Dunnett's *post hoc* analysis and are expressed as mean  $\pm$  S.E.M.; \* $p<0.05$ . Results in bold represent control values.

To confirm our findings and to further elucidate the time response relationship, in a further experiment we introduced more early time points (2, 12, 24, 36 and 48 h). A significant increase in BDNF in frontal cortex at 2, 12, 24 and 36 h was found ( $F_{6,42} = 11.92$ ,  $p<0.0001$ ) (Fig. 2) reverting to normal levels at 48 h in comparison to the control day. Similarly, a significant increase in BDNF in the striatum was observed at 12 and 24

h ( $F_{6,42} = 37.41$ ,  $p < 0.0001$ ) (Fig. 2), reverting to normal levels at 36 h in comparison to the control night.

**Figure 2. Expanded time course showing elevation of BDNF levels in response to MA in the frontal cortex and striatum.** BDNF level in the (A) Frontal cortex \* $p < 0.05$  vs control day, # $p < 0.05$  vs control night and (B) Striatum \* $p < 0.05$  vs control day, # $p < 0.05$  vs control night following a binge-like regimen of MA 4mg/kg or saline (control). Results were analyzed by one-way ANOVA (vs control day or control night as appropriate) followed by Dunnett post hoc analysis and are expressed as mean  $\pm$  S.E.M.

Changes in the BDNF receptors, TrkB and p75<sup>NTR</sup>, were evaluated in the frontal cortex and striatum and results show that expression of TrkB receptor in the frontal cortex was increased at 2, 24 and 48h ( $F_{3,8} = 51.87$ ,  $p < 0.0001$  vs control). Expression of TrkB receptors in the striatum was also increased at 24 and 48h ( $F_{3,8} = 44.41$ , \* $p < 0.0001$  vs control) (Figure 3) after the MA regimen (4mg/kg, 4 doses, 2 h apart). No difference was noted in the levels of p75<sup>NTR</sup> receptor in the frontal cortex ( $F_{3,8} = 2.74$ ,  $p = 0.113$ ), but in the striatum, there was a significant main effect ( $F_{3,8} = 4.86$ ,  $p = 0.033$ ), but post-hoc analysis did not identify significance at any particular timepoint (Figure 4).

**Figure 3.** TrkB receptor in (A) the frontal cortex and (B) striatum following a binge-like regimen of MA or saline (control). The figure presented here is representative of independent Western Blot (n=6). Actin was used as loading control. Values are normalised levels of TrkB/actin ratios. Results were analysed by One Way ANOVA followed by Dunnett's post hoc analysis and are expressed as mean  $\pm$  S.E.M.;  $p < 0.05$ .

**Figure 4.** p75<sup>NTR</sup> receptor in (A) the frontal cortex and (B) striatum, following a binge-like regimen of MA 4mg/kg or saline (control). The figure presented here is representative of independent Western Blot (n=6). Actin was used as loading control. Values are normalised levels of TrkB/actin ratios. Results were analysed by One Way ANOVA followed by Dunnett's post hoc analysis and are expressed as mean  $\pm$  S.E.M.

## Discussion

In this study, we show a region-dependent increase in the expression of BDNF over time, most prominent in the frontal cortex and striatum. These findings build on a previous study by our group in which we observed a region- and dose-dependent elevation of BDNF expression 2 h after MA treatment (42). Other studies, have reported that BDNF protein levels in brain (39) and BDNF mRNA levels in brain (38) can be increased by binge-like regimens of MA administration. A transient increase in serum BDNF after MA withdrawal in human addicts, which decreases over time has also previously been reported (47).

BDNF is not only important in the development of dopaminergic neurons (48) but it also has a role in the function and plasticity in the mature brain (26). We have previously shown that raised BDNF expression correlates with enhanced dopamine levels in the frontal cortex of rats treated with MA (42). Deficiency in the dopamine transporter (DAT) in rats has been shown to reduce BDNF mRNA expression in the frontal cortex (49). It has been suggested that changes in expression levels of BDNF in the frontal cortex could also influence striatal levels through anterograde transportation mechanisms (49).

The data shown here shows MA regulation of BDNF expression in the frontal cortex and striatum at different time points. With the advent of neuroimaging studies additional brain areas, especially the frontal cortex (50), have been implicated in drug addiction while the role of striatum is much more studied in the literature (51-53).

Previous work has shown that BDNF mRNA is increased by MA in an array of brain regions, including hippocampal, brain stem and cortical regions (38). MA has also been shown to increase BDNF levels in the hippocampus 24 hours post-binge administration (54). In the present study, we also observed a slight increase in the hippocampus at 24h, but the effect did not reach significance. Our data highlights a more pronounced effect of MA on the striatum and the frontal cortex. Interestingly, cocaine (55) and MDMA (56) have also been shown to increase BDNF mRNA expression in the prefrontal cortex after drug administration.

TrkB, the high affinity receptor for mature BDNF, exhibited substantial increases in expression following MA exposure in the brain regions with the greatest increases in BDNF expression, i.e., the frontal cortex and striatum. Through the TrkB receptor, BDNF activates signal transduction cascades leading to neural plasticity, neurogenesis, stress resistance, cell survival (57) and intercellular signalling (58). BDNF-TrkB signalling also supports differentiation, maturation and survival of neurons in the nervous system (59, 60). Unlike TrkB signalling, p75<sup>NTR</sup> activation is able to induce either survival or apoptosis depending on the ligand that binds and downstream signalling activated (61). Prior studies have linked BDNF with a neuroprotective effect following a neurotoxic insult (62-65). The functional effect of the increases we have observed in BDNF and TrkB expression following MA exposure is not fully understood. We suggest that raised BDNF and TrkB expression may be a response to protect neurons within the first 48 h following MA binge-like drug treatment.

Previous studies have demonstrated differences in regional TrkB receptor expression in brain (66). The results in the present study suggest that control rats may express more TrkB and p75<sup>NTR</sup> in the striatum than the frontal cortex, but further work would be required to confirm this finding.

The ELISA kit used in this study contained a primary antibody that was raised against the mature form of BDNF, however, it is likely that pro-BDNF expression was also measured in this study. It is known that the precursor neurotrophin proteins, including proBDNF, bind with high affinity to the receptor p75<sup>NTR</sup>, while the mature form of BDNF binds with weak affinity to p75<sup>NTR</sup> (57). Activation of the p75<sup>NTR</sup> receptor in neurons is linked with adverse outcomes, such as apoptosis (32). Here we observed a slight, but significant increase in p75<sup>NTR</sup> receptor expression in the striatum, but no significant change in expression in the frontal cortex, where the most pronounced change in BDNF expression occurred. This finding suggests that mature BDNF probably accounts for the majority of the raised BDNF expression we observed, although it would be very interesting to confirm this using more specific ELISAs for mature- and pro-forms of BDNF in the future.

Studies have shown that plasma BDNF is raised in chronic MA abusers (37) and serum BDNF levels decrease during MA withdrawal (67). We have shown here that that the increase in BDNF expression in the brain is also transient. We suggest that raised BDNF and TrkB receptor expression may likely influence the plastic changes underlying drug craving and addiction, in addition to a neuroprotective response to drugs of abuse.

## **Conclusion**

Taken together, our findings show that a binge-like regimen of MA affects BDNF and its receptor TrkB in a time- and region-dependent manner and highlights the importance of MA-induced effects in the frontal cortex and striatum in particular. More studies are needed to clarify the functional effects of the raised BDNF levels and what this elevation means in terms of MA neurotoxicity and drug addiction.

## **Funding**

This study was funded by a Scholarship from the College of Science, National University of Ireland, Galway.

## **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.

## **Ethical statement**

All animal experiments were carried out in accordance with EU Directive 2010/63/EU. Formal approval to conduct the experiments described was obtained from the Animal Research Ethics Committee in NUI, Galway. I attest that all efforts were made to minimize the number of animals used and their suffering.

Conflict of interest: The authors declare that they have no conflict of interest.

## References

1. Gonzales R, Mooney L, Rawson RA. The methamphetamine problem in the United States. *Annu Rev Public Health*. 2010;31:385-98.
2. Goodwin JS, Larson GA, Swant J, Sen N, Javitch JA, Zahniser NR, et al. Amphetamine and methamphetamine differentially affect dopamine transporters in vitro and in vivo. *J Biol Chem*. 2009;284(5):2978-89.
3. MacKenzie RG, Heischouer B. Methamphetamine. *Pediatr Rev*. 1997;18(9):305-9.
4. Weiss F, Koob GF. Drug addiction: functional neurotoxicity of the brain reward systems. *Neurotox Res*. 2001;3(1):145-56.
5. Krasnova IN, Cadet JL. Methamphetamine toxicity and messengers of death. *Brain Res Rev*. 2009;60(2):379-407.
6. Ares-Santos S, Granado N, Espadas I, Martinez-Murillo R, Moratalla R. Methamphetamine causes degeneration of dopamine cell bodies and terminals of the nigrostriatal pathway evidenced by silver staining. *Neuropsychopharmacology*. 2014;39(5):1066-80.
7. Moszczynska A, Callan SP. Molecular, Behavioral, and Physiological Consequences of Methamphetamine Neurotoxicity: Implications for Treatment. *J Pharmacol Exp Ther*. 2017;362(3):474-88.
8. Wallace TL, Gudelsky GA, Vorhees CV. Methamphetamine-induced neurotoxicity alters locomotor activity, stereotypic behavior, and stimulated dopamine release in the rat. *J Neurosci*. 1999;19(20):9141-8.
9. Metzger RR, Haughey HM, Wilkins DG, Gibb JW, Hanson GR, Fleckenstein AE. Methamphetamine-induced rapid decrease in dopamine transporter function: role of dopamine and hyperthermia. *J Pharmacol Exp Ther*. 2000;295(3):1077-85.
10. Brown JM, Riddle EL, Sandoval V, Weston RK, Hanson JE, Crosby MJ, et al. A single methamphetamine administration rapidly decreases vesicular dopamine uptake. *J Pharmacol Exp Ther*. 2002;302(2):497-501.
11. Yamamoto BK, Zhu W. The effects of methamphetamine on the production of free radicals and oxidative stress. *J Pharmacol Exp Ther*. 1998;287(1):107-14.
12. Quinton MS, Yamamoto BK. Causes and consequences of methamphetamine and MDMA toxicity. *AAPS J*. 2006;8(2):E337-47.
13. Irie Y, Saeki M, Tanaka H, Kanemura Y, Otake S, Ozono Y, et al. Methamphetamine induces endoplasmic reticulum stress related gene CHOP/Gadd153/ddit3 in dopaminergic cells. *Cell Tissue Res*. 2011;345(2):231-41.
14. Shah A, Kumar A. Methamphetamine-mediated endoplasmic reticulum (ER) stress induces type-1 programmed cell death in astrocytes via ATF6, IRE1alpha and PERK pathways. *Oncotarget*. 2016;7(29):46100-19.
15. Cubells JF, Rayport S, Rajendran G, Sulzer D. Methamphetamine neurotoxicity involves vacuolation of endocytic organelles and dopamine-dependent intracellular oxidative stress. *J Neurosci*. 1994;14(4):2260-71.
16. Liu X, Matochik JA, Cadet JL, London ED. Smaller volume of prefrontal lobe in polysubstance abusers: a magnetic resonance imaging study. *Neuropsychopharmacology*. 1998;18(4):243-52.
17. Wrona MZ, Yang Z, Zhang F, Dryhurst G. Potential new insights into the

molecular mechanisms of methamphetamine-induced neurodegeneration. *NIDA Res Monogr.* 1997;173:146-74.

18. Uhl GR. Hypothesis: the role of dopaminergic transporters in selective vulnerability of cells in Parkinson's disease. *Ann Neurol.* 1998;43(5):555-60.

19. Miyazaki I, Asanuma M, Diaz-Corrales FJ, Fukuda M, Kitaichi K, Miyoshi K, et al. Methamphetamine-induced dopaminergic neurotoxicity is regulated by quinone-formation-related molecules. *FASEB J.* 2006;20(3):571-3.

20. Tse DC, McCreery RL, Adams RN. Potential oxidative pathways of brain catecholamines. *J Med Chem.* 1976;19(1):37-40.

21. Graham DG. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol Pharmacol.* 1978;14(4):633-43.

22. Simon MI, Strathmann MP, Gautam N. Diversity of G proteins in signal transduction. *Science.* 1991;252(5007):802-8.

23. Delghandi MP, Johannessen M, Moens U. The cAMP signalling pathway activates CREB through PKA, p38 and MSK1 in NIH 3T3 cells. *Cell Signal.* 2005;17(11):1343-51.

24. Finkbeiner S, Tavazoie SF, Maloratsky A, Jacobs KM, Harris KM, Greenberg ME. CREB: a major mediator of neuronal neurotrophin responses. *Neuron.* 1997;19(5):1031-47.

25. Xue W, Wang W, Gong T, Zhang H, Tao W, Xue L, et al. PKA-CREB-BDNF signaling regulated long lasting antidepressant activities of Yueju but not ketamine. *Sci Rep.* 2016;6:26331.

26. Schinder AF, Poo M. The neurotrophin hypothesis for synaptic plasticity. *Trends Neurosci.* 2000;23(12):639-45.

27. Poser S, Impey S, Xia Z, Storm DR. Brain-derived neurotrophic factor protection of cortical neurons from serum withdrawal-induced apoptosis is inhibited by cAMP. *J Neurosci.* 2003;23(11):4420-7.

28. Segal RA. Selectivity in neurotrophin signaling: theme and variations. *Annu Rev Neurosci.* 2003;26:299-330.

29. Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, et al. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75<sup>NTR</sup> and sortilin. *J Neurosci.* 2005;25(22):5455-63.

30. Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem.* 2003;72:609-42.

31. Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci.* 2003;4(4):299-309.

32. Dechant G, Barde YA. The neurotrophin receptor p75<sup>(NTR)</sup>: novel functions and implications for diseases of the nervous system. *Nat Neurosci.* 2002;5(11):1131-6.

33. Corominas M, Roncero C, Ribases M, Castells X, Casas M. Brain-derived neurotrophic factor and its intracellular signaling pathways in cocaine addiction. *Neuropsychobiology.* 2007;55(1):2-13.

34. Davis MI. Ethanol-BDNF interactions: still more questions than answers. *Pharmacol Ther.* 2008;118(1):36-57.

35. Pu L, Liu QS, Poo MM. BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal. *Nat Neurosci.* 2006;9(5):605-7.

36. Kalivas PW, O'Brien C. Drug addiction as a pathology of staged neuroplasticity. *Neuropsychopharmacology.* 2008;33(1):166-80.

37. Kim DJ, Roh S, Kim Y, Yoon SJ, Lee HK, Han CS, et al. High concentrations of plasma brain-derived neurotrophic factor in methamphetamine users. *Neurosci Lett.* 2005;388(2):112-5.

38. Braun AA, Herring NR, Schaefer TL, Hemmerle AM, Dickerson JW, Seroogy KB, et al. Neurotoxic (+)-methamphetamine treatment in rats increases brain-derived neurotrophic factor and tropomyosin receptor kinase B expression in multiple brain regions. *Neuroscience*. 2011;184:164-71.
39. Grace CE, Schaefer TL, Herring NR, Skelton MR, McCrea AE, Vorhees CV, et al. (+)-Methamphetamine increases corticosterone in plasma and BDNF in brain more than forced swim or isolation in neonatal rats. *Synapse*. 2008;62(2):110-21.
40. Ren Q, Ma M, Yang C, Zhang JC, Yao W, Hashimoto K. BDNF-TrkB signaling in the nucleus accumbens shell of mice has key role in methamphetamine withdrawal symptoms. *Transl Psychiatry*. 2015;5:e666.
41. Li X, Rubio FJ, Zeric T, Bossert JM, Kambhampati S, Cates HM, et al. Incubation of methamphetamine craving is associated with selective increases in expression of Bdnf and trkb, glutamate receptors, and epigenetic enzymes in cue-activated fos-expressing dorsal striatal neurons. *J Neurosci*. 2015;35(21):8232-44.
42. Moreira da Silva Santos A, Kelly JP, Doyle KM. Dose-Dependent Effects of Binge-Like Methamphetamine Dosing on Dopamine and Neurotrophin Levels in Rat Brain. *Neuropsychobiology*. 2017;75(2):63-71.
43. Moreira da Silva Santos A, Kelly JP, Dockery P, Doyle KM. Effect of a binge-like dosing regimen of methamphetamine on dopamine levels and tyrosine hydroxylase expressing neurons in the rat brain. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;89:303-9.
44. Pereira FC, Lourenco ES, Borges F, Morgadinho T, Ribeiro CF, Macedo TR, et al. Single or multiple injections of methamphetamine increased dopamine turnover but did not decrease tyrosine hydroxylase levels or cleave caspase-3 in caudate-putamen. *Synapse*. 2006;60(3):185-93.
45. Bova R, Micheli MR, Qualadrucci P, Zucconi GG. BDNF and trkB mRNAs oscillate in rat brain during the light-dark cycle. *Brain Res Mol Brain Res*. 1998;57(2):321-4.
46. Dolci C, Montaruli A, Roveda E, Barajon I, Vizzotto L, Grassi Zucconi G, et al. Circadian variations in expression of the trkB receptor in adult rat hippocampus. *Brain Res*. 2003;994(1):67-72.
47. Ren W, Tao J, Wei Y, Su H, Zhang J, Xie Y, et al. Time-Dependent Serum Brain-Derived Neurotrophic Factor Decline During Methamphetamine Withdrawal. *Medicine (Baltimore)*. 2016;95(5):e2604.
48. Hyman C, Hofer M, Barde YA, Juhasz M, Yancopoulos GD, Squinto SP, et al. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature*. 1991;350(6315):230-2.
49. Fumagalli F, Racagni G, Colombo E, Riva MA. BDNF gene expression is reduced in the frontal cortex of dopamine transporter knockout mice. *Mol Psychiatry*. 2003;8(11):898-9.
50. Goldstein RZ, Volkow ND. Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. *Am J Psychiatry*. 2002;159(10):1642-52.
51. Lobo MK, Nestler EJ. The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. *Front Neuroanat*. 2011;5:41.
52. Everitt BJ, Robbins TW. From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. *Neurosci Biobehav Rev*. 2013;37(9 Pt A):1946-54.
53. Yager LM, Garcia AF, Wunsch AM, Ferguson SM. The ins and outs of the striatum: role in drug addiction. *Neuroscience*. 2015;301:529-41.
54. Garcia-Cabrerizo R, Garcia-Fuster MJ. Comparative effects of amphetamine-like

psychostimulants on rat hippocampal cell genesis at different developmental ages. *Neurotoxicology*. 2016;56:29-39.

55. Le Foll B, Diaz J, Sokoloff P. A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning. *Neuroreport*. 2005;16(2):175-8.

56. Hemmerle AM, Dickerson JW, Herring NR, Schaefer TL, Vorhees CV, Williams MT, et al. (+/-)3,4-methylenedioxymethamphetamine ("ecstasy") treatment modulates expression of neurotrophins and their receptors in multiple regions of adult rat brain. *J Comp Neurol*. 2012;520(11):2459-74.

57. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci*. 2015;11(6):1164-78.

58. Sasi M, Vignoli B, Canossa M, Blum R. Neurobiology of local and intercellular BDNF signaling. *Pflugers Arch*. 2017;469(5-6):593-610.

59. Acheson A, Conover JC, Fandl JP, DeChiara TM, Russell M, Thadani A, et al. A BDNF autocrine loop in adult sensory neurons prevents cell death. *Nature*. 1995;374(6521):450-3.

60. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci*. 2001;24:677-736.

61. Wehner AB, Milen AM, Albin RL, Pierchala BA. The p75 neurotrophin receptor augments survival signaling in the striatum of pre-symptomatic Q175(WT/HD) mice. *Neuroscience*. 2016;324:297-306.

62. Singh S, Ahmad R, Mathur D, Sagar RK, Krishana B. Neuroprotective effect of BDNF in young and aged 6-OHDA treated rat model of Parkinson disease. *Indian J Exp Biol*. 2006;44(9):699-704.

63. Wu CL, Chen SD, Hwang CS, Yang DI. Sonic hedgehog mediates BDNF-induced neuroprotection against mitochondrial inhibitor 3-nitropropionic acid. *Biochem Biophys Res Commun*. 2009;385(1):112-7.

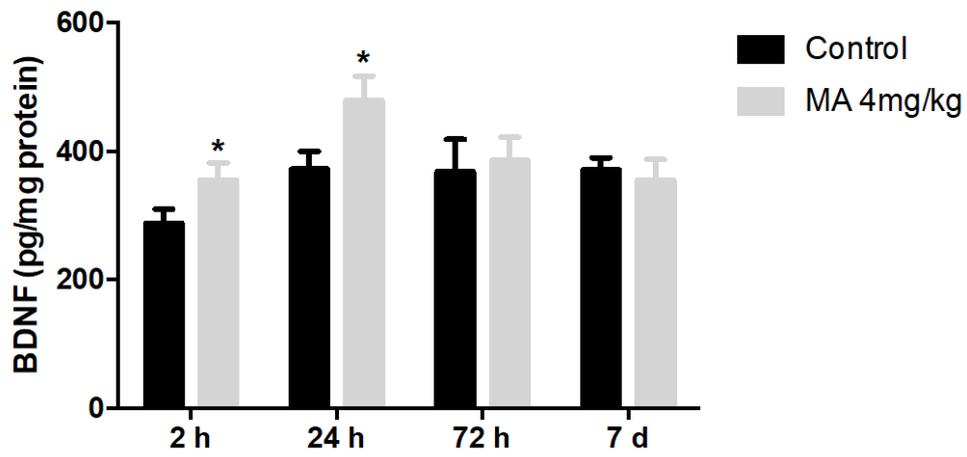
64. Motaghinejad M, Motevalian M, Babalouei F, Abdollahi M, Heidari M, Madjd Z. Possible involvement of CREB/BDNF signaling pathway in neuroprotective effects of topiramate against methylphenidate induced apoptosis, oxidative stress and inflammation in isolated hippocampus of rats: Molecular, biochemical and histological evidences. *Brain Res Bull*. 2017;132:82-98.

65. Dluzen DE. The effect of gender and the neurotrophin, BDNF, upon methamphetamine-induced neurotoxicity of the nigrostriatal dopaminergic system in mice. *Neurosci Lett*. 2004;359(3):135-8.

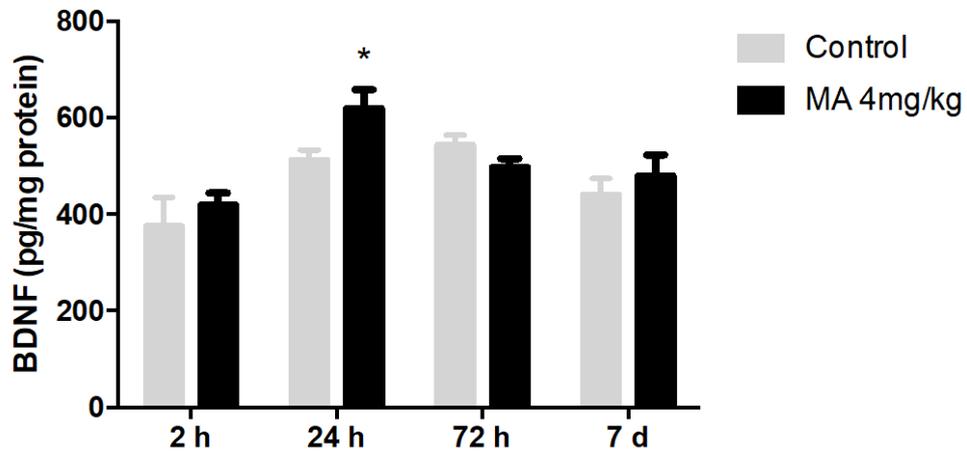
66. Kotloski R, McNamara JO. Reduction of TrkB expression de novo in the adult mouse impairs epileptogenesis in the kindling model. *Hippocampus*. 2010;20(6):713-23.

67. Chen PH, Huang MC, Lai YC, Chen PY, Liu HC. Serum brain-derived neurotrophic factor levels were reduced during methamphetamine early withdrawal. *Addict Biol*. 2014;19(3):482-5.

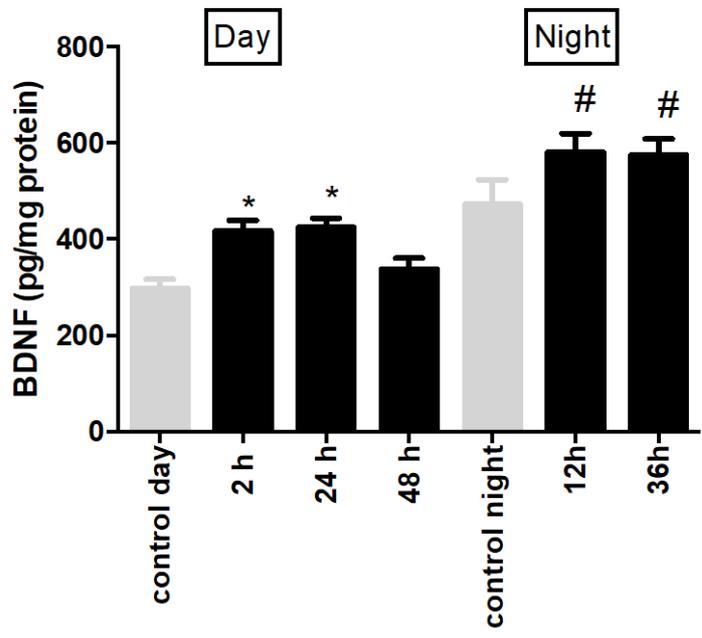
A. BDNF expression in frontal cortex



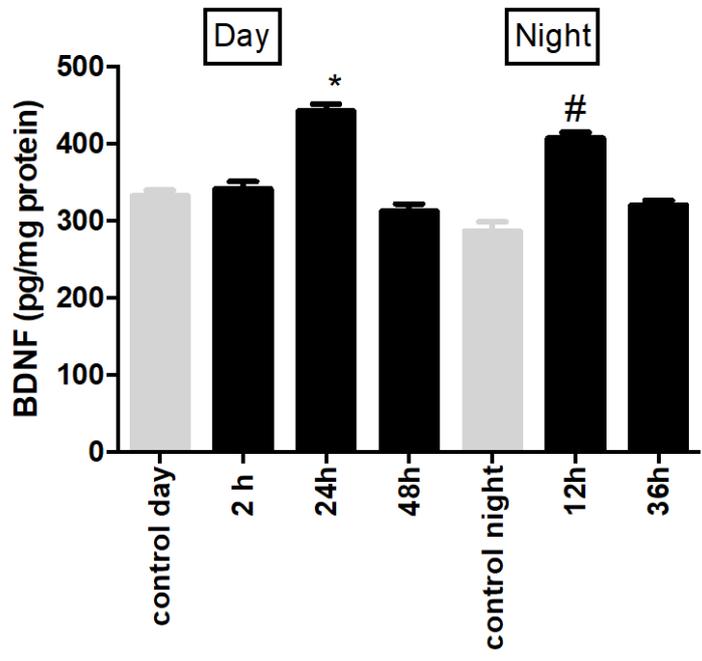
B. BDNF expression in striatum



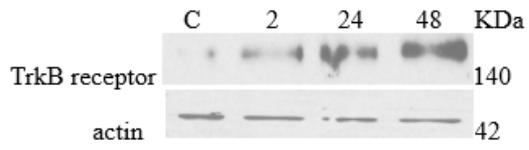
A. BDNF expression in frontal cortex



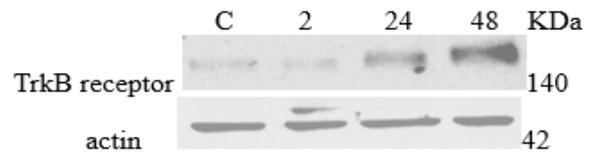
B. BDNF expression in striatum



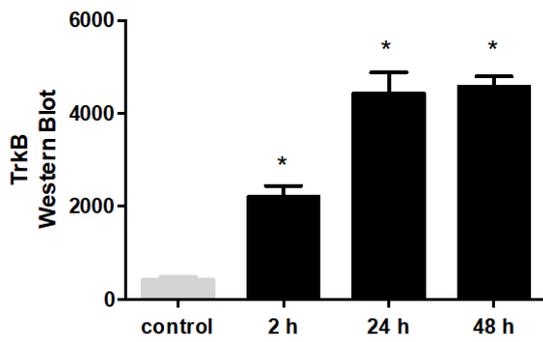
A. TrkB receptor in frontal cortex



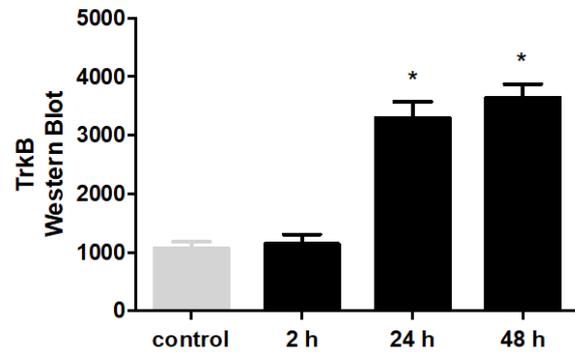
B. TrkB receptor in striatum



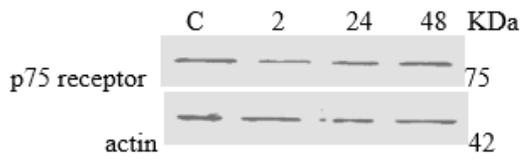
A. TrkB receptor expression in frontal cortex



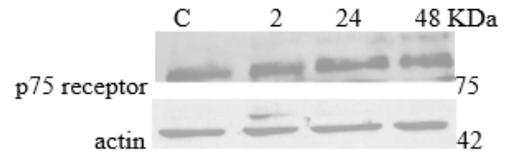
B. TrkB receptor expression in striatum



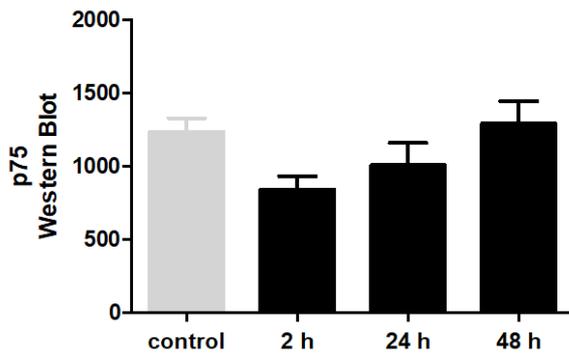
A. p75<sup>NTR</sup> receptor in frontal cortex



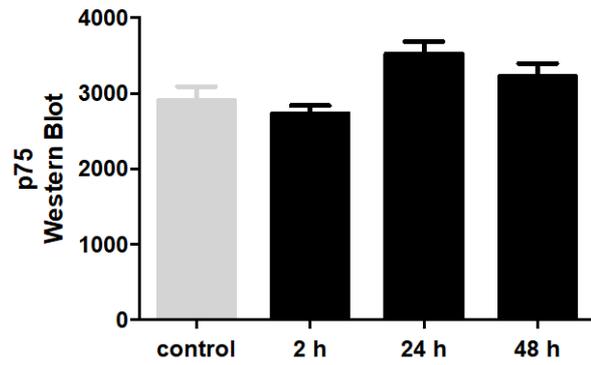
B. p75<sup>NTR</sup> receptor in striatum



A. p75 receptor expression in frontal cortex



B. p75 receptor expression in striatum



**Table 1. Effect of MA treatment on BDNF levels (pg/mg of protein) in brain regions at different time points**

Group	2 h	24h	72h	7 days	
Cerebellum	<b>633±30</b>	<b>570±47</b>	<b>631±98</b>	<b>559±94</b>	$F_{1,40} = 2.65$ $p=0.11$
	474±18	522±90	599±50	630±143	
Amygdala	<b>398±33</b>	<b>307±80</b>	<b>549±38</b>	<b>344±13</b>	$F_{1,40} = 0.72$ $p=0.40$
	432±80	336±74	450±194	279±16	
Hippocampus	<b>505±104</b>	<b>558±91</b>	<b>637±31</b>	<b>684±11</b>	$F_{1,40} = 1.32$ $p=0.26$
	545±35	621±42	553±61	590±30	
Olfactory Bulb	<b>617±78</b>	<b>663±58</b>	<b>659±54</b>	<b>501±27</b>	$F_{1,40} = 1.45$ $p=0.24$
	639±53	627±34	665±50	581±39	

BDNF expression in brain regions following binge like regimen of MA 4 mg/kg or saline (control). Results (n=8) were analyzed by Two Way ANOVA followed by Dunnett's *post hoc* analysis and are expressed as mean±S.E.M.; \* $p<0.05$ . Results in bold represent control values.