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Research report

# Chronic treatment with supraphysiological levels of corticosterone enhances D-MDMA-induced dopaminergic neurotoxicity in the C57BL/6J female mouse

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**Abstract**

Chronic stress and extended periods of elevated circulating glucocorticoids have been reported to exacerbate excitotoxicity-induced hippocampal neuronal injury in rat. Despite continued interest in the effects of protracted exposure to stress or glucocorticoids, there has been little examination of how other types of neurotoxicity may be exacerbated or blocked, by stress. Here we examined the effects of chronic supraphysiologic levels of corticosterone on D-3,4-methylenedioxymethamphetamine (D-MDMA)-induced striatal dopaminergic neurotoxicity in the female C57BL/6J mouse. Corticosterone (5 mg, 15 mg or placebo) pellets were implanted to continuously elevate circulating glucocorticoids and create a model of the ultimate effect of chronic activation of the hypothalamic–pituitary–adrenal axis. After 7 days, a neurotoxic regimen of D-MDMA was administered (20 mg/kg s.c. every 2 h×4); thymus, spleen, striatum and hippocampus were collected 72 h later. Significant involution of thymus and spleen confirmed the bioavailability of the corticosterone at both dosages. D-MDMA increased the striatal levels of the astrocyte-localized protein glial fibrillary acidic protein (GFAP, a marker of gliosis); both dosages of corticosterone exacerbated this increase but only the 15 mg pellet exacerbated the decrease in tyrosine hydroxylase protein. Corticosterone alone or in combination with D-MDMA produced no neural injury in hippocampus, as measured by GFAP. Our work indicates corticosterone was able to increase the vulnerability of the striatum, but not the hippocampus to D-MDMA. An examination of other mouse strains and models of neurotoxic injury would be useful in determining the general validity of the glucocorticoid neuroendangerment hypothesis. Published by Elsevier Science B.V.

*Theme:* Neural basis of behaviour

*Topic:* Stress, neuronal plasticity, drugs of abuse: amphetamines and other stimulants

*Keywords:* HPA axis; Hippocampus; Striatum; Dopamine; Serotonin; D-MDMA

## 1. Introduction

The hypothalamic–pituitary–adrenal (HPA) axis is one of the major body systems activated in response to stress or homeostatic disturbance. Consequently, chronic stress could be viewed as a state in which the HPA axis is activated for extended periods and, as such, it would engender a prolonged elevation in levels of circulating glucocorticoids. Such extended exposure to elevated glucocorticoids levels is suspected to have detrimental effects on a number of body systems including the nervous system [15–17]. For example, repeated exposure to certain stressors or to exogenous corticosterone exacerbates the excitotoxic neuronal death induced in hippocampus by the neurotoxicant kainic acid [27–29]. Despite the continued interest in chronic stress and its consequences for brain integrity and function, there has been little examination of whether or how prolonged stress can exacerbate the neurotoxic response of brain areas other than the hippocampus.

The striatum and prefrontal cortex are among the non-hippocampal brain regions considered to be vulnerable to the effects of chronic stress, however; this vulnerability has been infrequently considered in the context of the

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toxicological actions of exogenous agents [1,7,9]. Many investigations have shown that acute stress procedures can affect various aspects of dopamine neurotransmission. Consequently, chronic stress has been examined most often for the role it may play in disease states believed to affect dopamine neurotransmission. For example, aberrant function of the HPA axis has been hypothesized to underlie the pathophysiology of disorders such as depression and drug abuse [6]. There has been, as well, great interest in how chronic stress or persistent exposure to glucocorticoids may alter the pharmacological actions of agents targeting dopamine neurotransmission. The paucity of data related to chronic stress and neurotoxicity in striatum may be related to the lack of convenient or reliable models of dopaminergic neurotoxicity. For example, 6-hydroxydopamine reliably produces striatal damage but it cannot be given systemically; the requirement for intracerebral injection of this compound may introduce variables such as altered blood–brain barrier function, etc., that can make interpretation of the data difficult. In other models (e.g., 3-nitroproprionic acid) the compound can be administered systemically but the degree of striatal damage often is inconsistent [26].

The discovery that several substituted amphetamines cause striatal dopaminergic neurotoxicity in the mouse may now provide an in vivo model for examination of the interaction between chronic stress and neurotoxicity in this brain area [25,14]. Thus, we elected to study the impact of chronic stress, as mimicked by the implantation of sustained release corticosterone pellets, on dopaminergic neurotoxicity induced by D-3,4-methylenedioxymethamphetamine (MDMA). MDMA produces marked damage to striatal dopaminergic nerve terminals as evidenced by decreases in dopamine, its metabolites, and tyrosine hydroxylase (TH) protein as well as by an increase in glial fibrillary acidic protein (GFAP), an astrocyte protein that serves as a marker of injury-induced gliosis. Our data indicate that supraphysiological levels of corticosterone increase MDMA-induced striatal dopaminergic neurotoxicity. Although hippocampus is not a brain area targeted by MDMA it is considered to be vulnerable to insult by high levels of corticosterone and thus was also chosen for evaluation. No hippocampal gliosis or alterations in serotonin or norepinephrine content occurred in response to either corticosterone alone or in combination with MDMA. These results suggest that high circulating levels of corticosterone, such as those experienced during chronic stress, may enhance the neurotoxicity of agents targeting the striatum.

## 2. Materials and methods

### 2.1. Materials

The following drugs and chemicals were kindly provided by or obtained from the sources indicated: high-performance liquid chromatography (HPLC) standards (Sigma, St. Louis, MO, USA); D-MDMA (Research Technology Branch, National Institute on Drug Abuse, Rockville, MD, USA). Reagents used for HPLC were of HPLC-grade (ESA, Chelmsford, MA, USA). Twenty-one-day release pellets containing placebo, 5 mg or 15 mg of corticosterone were obtained from Innovative Research of America (Sarasota, FL, USA).

### 2.2. Animals

All procedures were carried out according to protocols approved by the institutional Animal Care and Use Committee and in accordance with the NRC *Guide for the Care and Use of Laboratory Animals* (National Academy Press, 1996). Female C57BL/6J mice (Jackson Labs, Bar Harbor, ME, USA) 5 to 10 weeks of age were maintained in a colony certified by the American Association for Accreditation of Laboratory Animal Care. Our previous experiments demonstrated that this strain and sex of mice were susceptible to D-MDMA-induced neurotoxicity [20,25]. Upon receipt, the mice were housed in groups of 6–8 in a temperature controlled ($21\pm1$ °C) and humidity controlled ($50\pm10$%) colony room maintained under filtered positive pressure ventilation on a 12-h light–12-h dark cycle beginning at 06.00 EDT. The plastic tub cages (30.5 cm×30.5 cm×15 cm) were bedded with approximately 4 cm of heat-treated pine shavings. Food (ProLab ISOPRO RMH 3000, irradiated food containing 22% crude protein, 5% crude fat, 5% crude fiber, 6% ash and 2.5% added minerals) and water were available ad libitum.

### 2.3. Corticosterone and placebo pellet implantation

Twenty-one-day-release pellets containing either, placebo, 5 mg corticosterone or 15 mg corticosterone were implanted under halothane anaesthesia. A small (5 mm) incision was made at the nape of the neck using a trocar after local disinfectant had been applied. The pellet was inserted and the incision closed with a stainless steel surgical staple (Roboz Surgical, Rockville, MD, USA). Animals were allowed to recover under a heat lamp. Pellets were left in place for 7 days before injection with either saline or D-MDMA.

### 2.4. Group assignment

On the day of dosing, mice (29 weeks of age) were weighed and their number within the group marked on their tails with a laboratory marker (Sharpie non-toxic permanent marker) so that individual mice could be followed throughout the experiment.

### 2.5. Drug or vehicle administration

D-MDMA (20 mg/kg of mouse body weight, calculated as base) or saline vehicle (0.9%) was administered subcutaneously (s.c.) in a volume of 1 ml/100 g body weight, every 2 h, for a total of four injections. To minimize circadian influences on toxicity, the first injection was always given between 09.00 and 10.00 h. This dosage regimen causes reproducible decrements in striatal dopamine (DA) and other markers of neural damage in C57BL/6J mice [12,20,25].

### 2.6. Temperature measurement

As body temperature is known to play a role in the neurotoxic actions of MDMA [11,12,20,33,3] rectal temperature was recorded with a Bat-10 thermometer coupled to a RET-3 mouse rectal probe (Physitemp, Clifton, NJ, USA) lubricated with mineral oil to determine if any observed effects were due to treatment-induced alterations in this variable. To facilitate temperature measurements, mice were placed under a 'Quonset hut'-shaped piece of foam that was approximately the length of the mouse and that was blocked at the front end. Mice were held by the base of the tail while the temperature probe was inserted to a pre-marked depth of 1.8 cm. This method minimized handling, and in conjunction with the use of a fast-rise time of the rectal probe made it possible to obtain reliable measurements of rectal temperature in less than 30 s per mouse. Temperature sampling times were immediately before each injection, 2 h after last injection and 24 h after first injection. Room temperature averaged 22 °C during pellet implantation surgery and recovery and during D-MDMA or saline dose administration.

### 2.7. Brain dissection and tissue preparation

All tissue was obtained 72 h after the initial D-MDMA injection, a time point at which striatal dopaminergic neurotoxicity is evident as indicated by markers of neural injury and decreases in dopamine content (see Ref. [25] for a complete time course). Immediately after decapitation, whole brains were removed from the skull with the aid of blunt curved forceps. Striatum was dissected free-hand on a thermoelectric cold plate (Model TCP-2, Aldrich, Milwaukee, WI, USA) using a pair of fine curved forceps (Roboz, Washington, DC, USA). Striatum from the left side of the brain was weighed, frozen on dry ice and stored at −70 °C for subsequent analysis of DA, serotonin (5-HT), norepinephrine (NE) and metabolites [homovanillic acid (HVA); 3,4-dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA)]. Striatum and hippocampus from the right side of each brain was weighed, frozen and stored as above and later processed for determination of GFAP and TH by sandwich enzyme-linked immunosorbent assay (ELISA) as described below.

### 2.8. DA analysis

DA, DOPAC, HVA and other neurotransmitter substances were analysed by HPLC with electrochemical detection using the following system: tissue homogenates were prepared by sonication (Kontes Micro ultrasonicator/cell disruptor) on ice using a 30-s pulse in 0.2 M perchloric acid, containing 3,4-dihydroxybenzylamine 1 μM as internal standard. The homogenate was centrifuged at 10,000 *g* for 15 min, and the resulting supernatant immediately injected using the autosampler described below. Each brain area was prepared in a standard volume (striatum 0.3 ml, hippocampus 0.2 ml, cortex 0.5 ml) then results were expressed as μg/g original tissue weight. Sample (10 μl) was injected using a temperature controlled (4 °C) Waters 717 Plus Autosampler (Waters, Milford, MA, USA) connected to a Waters 515 HPLC pump. The sample was passed over a reversed-phase $C_{18}$ column (Waters Symmetry, 250×4.6 mm, 5 μm, 100 Å). Analytes were detected using the Waters 464 pulsed electrochemical detector (range 10 nA, potential 700 mV) connected by means of the Waters bus SAT/IN module to a computer using Millenium Software 32. The mobile phase consisted of 75 mM sodium dihydrogenphosphate, 1.7 mM 1-octanesulfonic acid, 25 μmol ethylendiaminetetraacetic acid and 10% (v/v) acetonitrile. All components were adjusted to a pH of 3.0 with phosphoric acid, pumped at a flow rate of 1 ml/min. Under these conditions the average run time is 30 min with representative retention times (in min) for NE (5.99), 4-dihydroxybenzylamine (DHBA, internal standard, 8.24), DOPAC (8.93), DA (11.28), 5-HIAA (13.57), HVA (19.77), 5-HT (26.1). Quantitation was achieved by the use of the internal standard (10 pmol DHBA per injection) method using daily standard curves of each analyte (0.5 to 25 pmol per injection). The limit of detection is 0.5 pmol per injection, interassay variation is ±3%.

### 2.9. GFAP immunoassay

Frozen striatum and hippocampus (right side) were homogenized by sonification in 10 volumes of hot (90–95 °C) 1% sodium dodecyl sulfate (SDS). The resulting homogenate was frozen at −80 °C until the day of assay. GFAP was assayed according to modifications [24,25] of a previously described sandwich ELISA [23]. Total protein concentration of the SDS homogenate used for the GFAP immunoassay was determined by the method of Smith et al. [35].

### 2.10. TH immunoassay

TH was assayed from the same SDS homogenates using a fluorescence-based ELISA adapted from the GFAP assay [37]. Briefly, a mouse anti-TH monoclonal antibody

(Calbiochem catalogue No. 657010) was used to capture TH from dilutions of the SDS homogenates. The detection antibody was a rabbit anti-TH polyclonal antibody (Calbiochem catalogue No. 657012) and quantification was achieved using anti-rabbit antibody conjugated with horseradish peroxidase (Amersham catalogue No. NA934) using Quantablu Substrate (Pierce catalogue No. 31402) as the peroxidase substrate. Values were generated with an Fmax Plate Reader (Molecular Devices, Sunnyvale, CA, USA) set at 305/405 nm.

### *2.11. Statistical analysis*

The JMP (version 4.04) statistical analysis software package (SAS Institute, Cary, NC, USA) was used for all data analysis. Individual variables (DA, GFAP, etc.) were evaluated by analysis of variance (ANOVA) followed by the Fisher's protected least significant difference test ($\alpha$ level set at 0.05).

The TAUC, a composite measure of temperature, was calculated for each mouse by the application of Simpson's rule to temperatures measured at times 0, 2, 4, 6, and 8 h. This measure represents the area under the curve of a plot of temperature (°C) versus time (h), and has units of 0C-h. The TAUC reflects the impact of experimental conditions and the interaction of mouse characteristics with those conditions in the generation of heat. See Ref. [12] for a more complete discussion of this variable.

## 3. Results

### *3.1. Effect of corticosterone pellets and D-MDMA treatment on immune organs*

Thymus and spleen weights were chosen as endpoints to evaluate the bioeffectiveness of the sustained release corticosterone. This strategy served to avoid the repeated blood collection necessary to obtain corticosterone levels during the experiment. Thymus weight and/or size especially serves as an established biomarker of the bioactivity of glucocorticoids (see Ref. [2] for a discussion). As expected, implantation of the corticosterone pellets caused a marked involution of the thymus as indicated by a reduction in thymic weight, expressed as thymus weight in mg/100 m body weight (Fig. 1A). Compared to the placebo pellet, the 5 and 15 mg corticosterone pellets resulted in a decrease in relative thymus weight of 25 and 50%, respectively. MDMA treatment, itself also caused a significant reduction of thymus weight (15% in the placebo group) and exacerbated the decrease in the corticosterone pellet groups, an additional 33 and 15% decrease in the 5 and 15 mg groups, respectively. Spleen weights also were significantly decreased by at least 30% in the 15 mg corticosterone pellet implanted animals (expressed as mg spleen per 100 g body weight, Fig. 1B). Adrenal weights

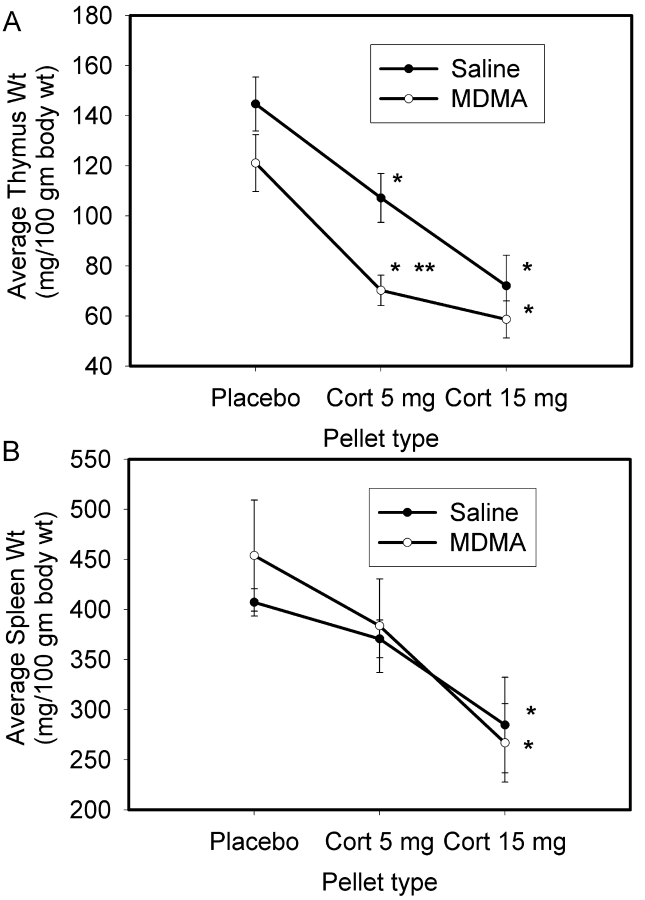


Fig. 1. Effects of pellet type and D-MDMA treatment on thymus and spleen weight. Closed symbols indicate saline-treated animals, open symbols indicate D-MDMA treated, error bars indicate S.E.M. for each group. Each group consisted of 6–8 animals. Animals were implanted with pellets under halothane anaesthesia 7 days prior to drug treatment. D-MDMA treatment consisted of four injections of D-MDMA 20 mg/kg, given subcutaneously every 2 h. Saline treatment consisted of four injections of saline (200 μl), given subcutaneously every 2 h. Thymus and spleen weights were determined 72 h after the first dose of D-MDMA or saline. Weights are expressed as mg tissue weight per 100 g body weight. (A) Effects on relative thymus weight: while both the main effects of pellet ($F_{2,40}=22.4105$, $P<0.0001$) and treatment ($F_{1,39}=8.8308$, $P=0.005$) were significant, the interaction, treatment×pellet was not significant ($F_{1,39}=0.6384$, $P=0.5177$). A single asterisk indicates a significant difference in relative thymus weight compared to placebo implanted group $P<0.05$, a double asterisk indicates a significant difference in relative thymus weight compared to saline-treated group with corresponding pellet, $P<0.05$. (B) Effects on relative spleen weight: only the main effect of pellet was significant in reduction of spleen relative weight ($F_{2,40}=7.9383$, $P=0.0015$), while the effect of treatment with MDMA ($F_{1,40}=0.1854$, $P=0.6695$) and the interaction treatment×pellet ($F_{1,40}=0.3323$, $P=0.7196$) were not significant.

were not altered by either corticosterone or D-MDMA (data not shown). Although the thymus and spleen involution caused by corticosterone appears to be dose-dependent, the daily dosage able to effect these changes was not measured, however, it can be estimated. At the time of tissue harvest, the pellets had been in place for 10 days. The pellets were designed to release a stable level over a 21

day period, such that a 5 mg pellet should release 0.24 mg/day and a 15 mg pellet should release 0.71 mg/day. Assuming the pellets released at stable daily levels and given that the mice weighed approximately 26 g, daily dosages for the 5 and 15 mg pellets can be estimated at 9.2 and 27.3 mg/kg/day, respectively.

### *3.2. Effect of corticosterone on D-MDMA-induced depletion of striatal TH, DA and its metabolites*

Evidence of the neurotoxic effect of D-MDMA includes the loss of the DA, the terminal marker TH, as well as a decrease of DA metabolites [25]. Relative to saline-treated animals TH protein levels were decreased in all mice receiving D-MDMA but the protein loss was greater in those receiving the highest dose of corticosterone (49, 41 and 73% for the placebo, 5 and 15 mg pellet, respectively). Corticosterone alone did not affect TH levels (Table 1). Although D-MDMA appeared to produce a greater DA depletion in mice receiving the highest dosage of corticosterone (63, 66 and 80% for the placebo, 5 and 15 mg pellet groups, respectively) this numerical deficit was not significant. The 15 mg dosage of corticosterone, however, did significantly exacerbate the decrement caused by D-MDMA in DA metabolites DOPAC (39, 60 and 74% in the placebo, 5 and 15 mg pellet groups, respectively) and HVA (27, 21 and 52% in the placebo, 5 and 15 mg pellet groups, respectively) (see Fig. 2 legend for statistics). The corticosterone pellet alone, in the absence of D-MDMA had no significant effect on the striatal concentration of DA or its metabolites (Table 1).

### *3.3. Effect of corticosterone and D-MDMA on striatal 5-HT and 5-HIAA*

Treatment with D-MDMA did not induce significant changes in striatal 5-HT or its metabolite 5-HIAA (Fig. 3). In addition, corticosterone, either alone or in combination with D-MDMA, did not cause an alteration in striatal 5-HT or its metabolite (Table 1).

### *3.4. Effect of corticosterone on D-MDMA-induced elevation of striatal GFAP*

Commensurate with our previous findings, D-MDMA induced a very large elevation in the striatal concentration of GFAP, a marker of astrocytic hypertrophy in response to neuronal damage (Fig. 4). Treatment with corticosterone significantly enhanced this gliotic response (4.8, 6.0 and 6.3 fold in the placebo, 5 and 15 mg pellet groups, respectively). Corticosterone, alone, had no significant effect on striatal GFAP (Table 1).

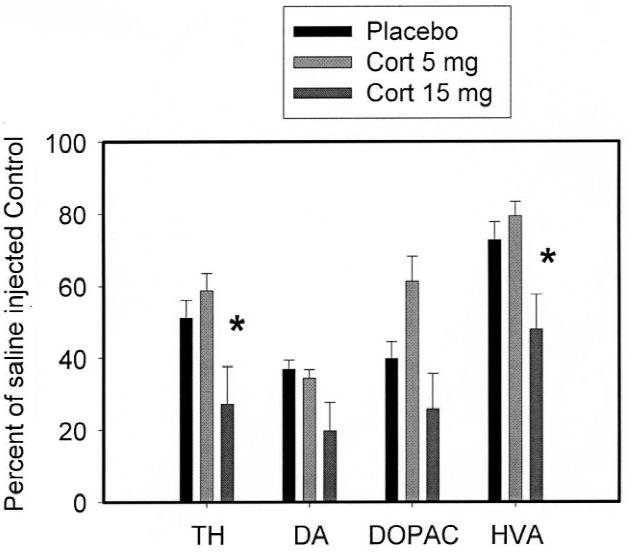


Fig. 2. Effects of corticosterone on D-MDMA-induced depletion of striatal TH, DA and DA metabolites DOPAC and HVA. DA, metabolites and TH were determined in brain tissue at a time point that was 72 h after the first dose of saline or D-MDMA and 10 days after implantation of the pellet. Each group consisted of 6–8 animals. Data are expressed as percent of the appropriate saline-treated control. Control values (saline-injected animals implanted with each pellet type) for striatal TH, DA, DOPAC, and HVA are listed in Table 1. Striatal TH was significantly depleted by MDMA in all pellet groups ($F_{5,40}=13.38$, $P<0.0001$) and was depleted to a greater extent in the 15 mg corticosterone pellet group than in the 5 mg corticosterone pellet group (see asterisk sign, $P<0.05$). D-MDMA treatment caused a significant depletion of striatal DA in all pellet implanted animals ($F_{5,40}=28.77$, $P<0.0001$) however there was no statistically significant difference in the extent of depletion in corticosterone versus placebo pellet groups. Striatal DOPAC concentrations were significantly depleted by D-MDMA in all pellet groups ($F_{5,40}=51.64$, $P<0.0001$) but there was no significant difference in the extent of depletion between placebo and corticosterone pellet groups. MDMA treatment caused a significant depletion of striatal HVA ($F_{5,40}=8.59$, $P<0.0001$) and the extent of depletion in the 15 mg corticosterone pellet implanted group was significantly greater than in the placebo and 5 mg corticosterone groups, (see asterisk, $P<0.05$).

Table 1
Absolute values for TH, neurotransmitters and GFAP obtained from brain sections of saline-injected, pellet-implanted (control) animals

| Pellet type | Striatal TH (μg/mg total protein±S.E.M.) | Striatal DA (μg/g brain tissue±S.E.M.) | Striatal DOPAC (μg/g brain tissue±S.E.M.) | Striatal HVA (μg/g brain tissue±S.E.M.) | Striatal 5-HT (μg/g brain tissue±S.E.M.) | Striatal 5-HIAA (μg/g brain tissue±S.E.M.) | Striatal GFAP (μg/mg total protein±S.E.M.) | Hippocampal 5-HT (μg/g brain tissue±S.E.M.) | Hippocampal 5-HIAA (μg/g brain tissue±S.E.M.) | Hippocampal GFAP (μg/g total protein±S.E.M.) |
|---|---|---|---|---|---|---|---|---|---|---|
| Placebo | 0.63±0.07 | 19.08±1.64 | 4.51±1.01 | 1.67±0.13 | 1.58±0.13 | 0.67±0.05 | 0.158±0.006 | 1.71±0.10 | 0.57±0.03 | 1.03±0.06 |
| 5 mg Corticosterone | 0.64±0.07 | 21.99±1.00 | 3.35±0.43 | 1.68±0.11 | 1.86±0.30 | 0.70±0.06 | 0.163±0.010 | 1.78±0.09 | 0.57±0.03 | 0.99±0.04 |
| 15 mg Corticosterone | 0.71±0.03 | 19.32±1.93 | 4.24±0.75 | 1.83±0.14 | 1.79±0.16 | 0.68±0.05 | 0.160±0.013 | 1.51±0.04* | 0.51±0.03 | 0.99±0.08 |

* Indicates significant difference from placebo pellet implanted animals, $P<0.05$.

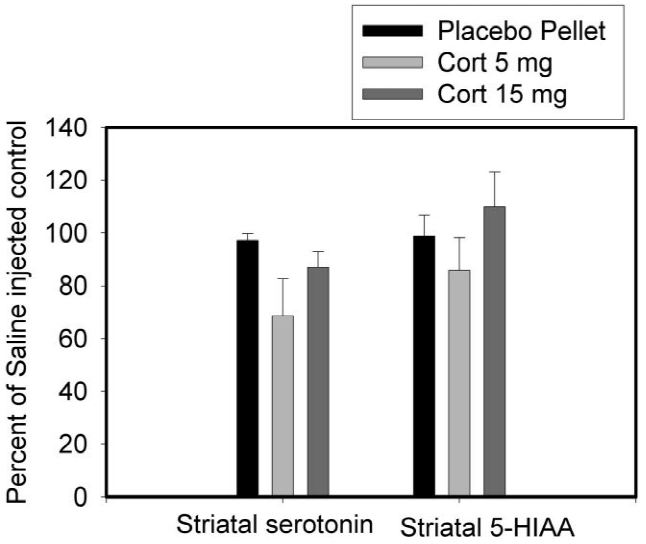


Fig. 3. Effect of corticosterone and D-MDMA on striatal 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA). Black bars indicate placebo pellet implanted animals, light grey bars indicate corticosterone 5 mg pellet implanted animals and dark grey bars indicate corticosterone 15 mg pellet implanted animals treated with D-MDMA 20 mg/kg every 2 h×4. Each group consisted of 6–8 animals. Data is expressed as percent of the saline-treated control. Saline-injected-control values (μg/g brain tissue) are listed in Table 1 D-MDMA 20 mg/kg s.c. every 2 h for four doses had no effect on striatal 5-HT or 5-HIAA levels when analysed 72 h after the first injection. Pellet type also had no impact on striatal 5-HT or 5-HIAA levels.

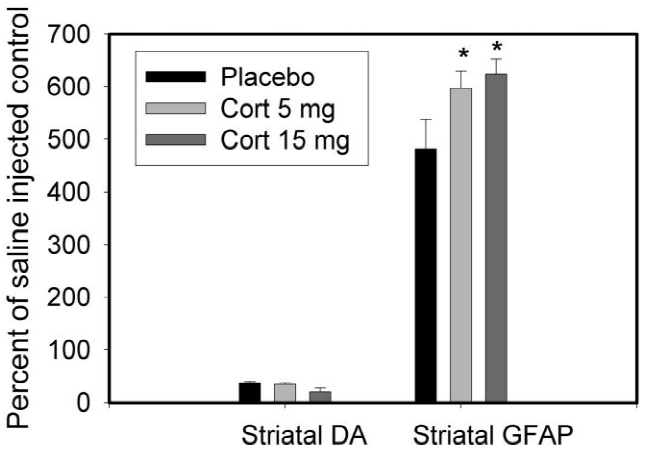


Fig. 4. Effect of corticosterone, placebo pellets and D-MDMA on striatal concentrations of DA and GFAP at 72 h after the first dose. Black bars indicate placebo pellet implanted animals, light grey bars indicate corticosterone 5 mg pellet implanted animals and dark grey bars indicate corticosterone 15 mg pellet implanted animals treated with D-MDMA 20 mg/kg every 2 h×4. Each group consisted of 6–8 animals. Data is expressed as percent of the saline-treated control. Saline-injected-control values for striatal DA and GFAP are listed in Table 1. D-MDMA treatment caused a significant depletion of striatal DA as shown in Fig. 2. D-MDMA treatment also caused a significant elevation of GFAP in all pellet groups ($F_{5,40}=103.87$, $P<0.0001$). Furthermore, the elevation of GFAP was significantly greater in both the corticosterone pellet implanted groups than in placebo ($P<0.05$). A single asterisk indicates a significant difference from the placebo pellet-implanted D-MDMA-treated group ($P<0.05$).

### *3.5. Effect of corticosterone and D-MDMA on hippocampal neurotransmitters and GFAP*

In contrast to the pronounced striatal dopaminergic neurotoxic effect induced by D-MDMA administration, this neurotoxicant did not alter hippocampal 5-HT, 5-HIAA, NE or GFAP (Fig. 5). In addition, there was no significant effect by corticosterone on hippocampal 5-HIAA, NE or GFAP (Table 1). The higher dosage of corticosterone did, however, lower hippocampal 5-HT levels by approximately 12% whether the mice received saline or D-MDMA (Table 1).

### *3.6. Effect of pellet corticosterone concentration on temperature modulation caused by D-MDMA*

Treatment with D-MDMA elevates mouse body temperatures and a correlation exists between striatal damage and body temperature elevations [12]. As expected, treatment with D-MDMA (20 mg/kg s.c. every 2 h for four doses) caused an elevation of body temperature over the 8-h dose period (Fig. 6A). The greatest temperature elevation was observed in the D-MDMA-treated group implanted with 15 mg corticosterone pellets. At the 4, 6, and 8 h time points, average rectal temperatures were significantly higher in

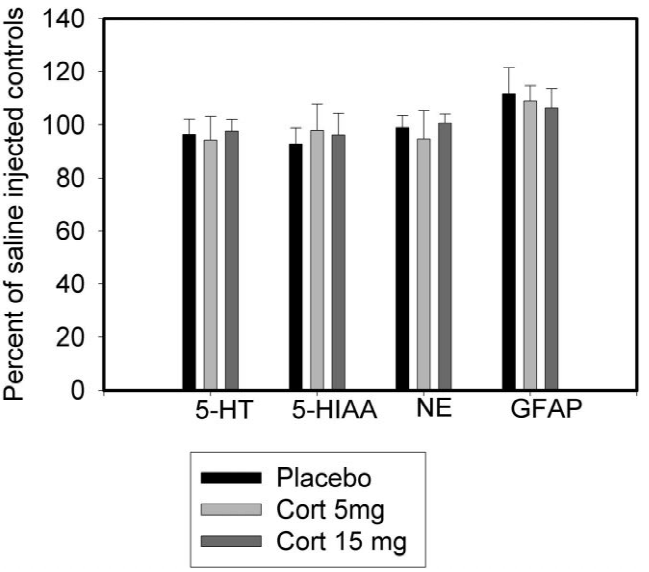


Fig. 5. Effects of pellet type and D-MDMA on hippocampal neurotransmitter concentrations and on hippocampal GFAP. Black bars indicate placebo pellet implanted animals, light grey bars indicate corticosterone 5 mg pellet implanted animals and dark grey bars indicate corticosterone 15 mg pellet implanted animals treated with D-MDMA 20 mg/kg every 2 h×4. Each group consisted of 6–8 animals. Data is expressed as percent of the saline-treated control. Saline-injected-control values for hippocampal 5-HT and NE (μg/g brain tissue) are listed in Table 1. Treatment with D-MDMA caused no significant depletions of hippocampal 5-HT, 5-HIAA or NE and caused no significant elevation of hippocampal GFAP. The 15 mg corticosterone pellet caused a slight but significant reduction of hippocampal 5-HT in both saline and D-MDMA-treated groups (see Table 1, $P<0.05$).

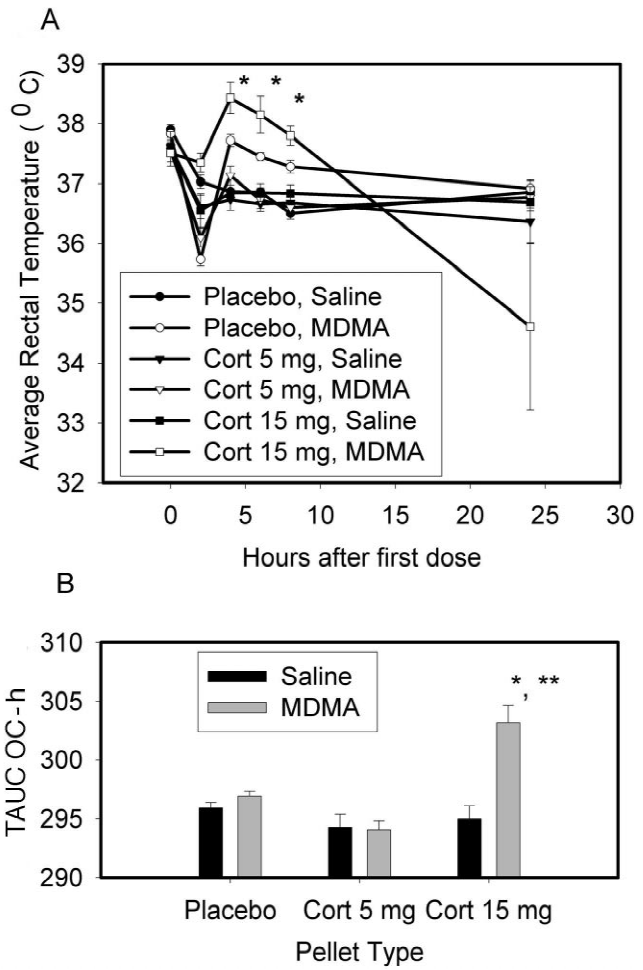


Fig. 6. Effects of pellet type on the thermomodulation caused by D-MDMA. Each group consisted of 6–8 animals. (A) Time course of body temperature elevations caused by D-MDMA (20 mg/kg s.c. every 2 h×4 doses) versus saline (200 μl every 2 h×4 doses). Symbols indicate the average rectal temperature for each group, error bars indicate the S.E.M. of rectal temperature, as follows: placebo pellet implanted, saline-injected animals (solid circles); placebo pellet implanted, D-MDMA-injected animals (open circles), corticosterone 5 mg implanted, saline-injected (solid triangles), corticosterone 5 mg implanted, D-MDMA-injected animals (open triangles), corticosterone 15 mg implanted, saline-treated animals (solid squares), corticosterone 15 mg implanted, D-MDMA-treated animals (open squares). At the 4, 6 and 8 h timepoints, combined corticosterone 15 mg pellets with MDMA treatment resulted in significantly higher rectal temperature, compared to other MDMA-treated animals. Asterisk indicates a significantly elevated temperature compared to the placebo pellet implanted-MDMA-treated group $P<0.05$. (B) Saline-treated animals (solid black bar) are compared to D-MDMA-treated animals (light grey bar) in each pellet content category. Each group consisted of 6–8 animals. Temperature AUC is calculated as described in Materials and methods. A single asterisk indicates a significant effect of D-MDMA to cause elevated AUC compared to saline ($P<0.05$). A double asterisk indicates that the D-MDMA in Cort15 mg pellet implanted animals gives a higher temperature elevation compared to D-MDMA-treated animals in any other pellet category ($P<0.05$).

this group than in any other D-MDMA-treated group. Since the time course of rectal temperature alteration by MDMA is biphasic, we have found a convenient way to compare thermal effects of drugs is to construct a composite measure, which integrates the temperature curve over 8 h (TAUC, Fig. 6B). We have previously found that the TAUC is positively correlated with striatal damage caused by D-MDMA [12]. The 15 mg corticosterone pellet plus D-MDMA resulted in a significantly higher TAUC (303.16) compared to D-MDMA treatment of the other pellet groups (296.92 in placebo, and 294.08 in corticosterone 5 mg pellet groups).

## 4. Discussion

Our data indicate that supraphysiological levels of corticosterone can enhance the striatal dopaminergic neurotoxicity engendered by treatment with D-MDMA. In agreement with our previous findings [25], D-MDMA reduced the striatal content of TH protein, DA and its metabolites. These decreases in markers of DA terminal integrity were accompanied by marked astrogliosis, as indicated by a robust elevation in GFAP. Treatment with corticosterone enhanced this striatal neurotoxicity. These findings stand in contrast to our previous work in which we examined the ability of orally administered corticosterone to exacerbate the striatal damage induced by D-amphetamine [21]. In this prior study corticosterone was administered in the drinking water at a dosage that did not involute the thymus and did not alter the striatal neurotoxicity of D-amphetamine. Given the similarity in the pattern of striatal damage for these amphetamines it is unlikely that the different effects of corticosterone observed in these two studies were due to different mechanisms of action of the two amphetamines. Rather, it seems likely that the exacerbation of neurotoxicity occurs only with exposure to a supraphysiological dosage (i.e., capable of causing thymic involution) of the steroid. In the present work, both dosages of corticosterone were capable of involuting the thymus and both were able to enhance D-MDMA neurotoxicity. As the exacerbated neurotoxicity observed here was moderate it should be determined if a longer exposure to supraphysiological dosages would result in a greater enhancement of neurotoxicity. Our current results do, however, suggest that chronic stress or other conditions producing prolonged exposure to glucocorticoids may make the nigral–striatal pathway more vulnerable to neurotoxic injury.

The detection and characterization of the molecular substrates underlying amphetamine-induced damage to the striatum is an area of intense study but as yet there is no consensus regarding the mechanism of neurotoxicity. Because of the presumed linkage between certain reactive chemical events and neurodegeneration many investigators have proposed oxidative stress as a common link in the neurotoxicity effected by the various amphetamines [5,34,22,4,10,13,8]. Hydroxyl radicals, peroxynitrite, superoxide radicals, sulfhydryl oxidants, and reactive

quinones have all been proposed as possible mediators of the damage because the amphetamines have actions (e.g., DA release, etc.) that may promote conditions suitable for the intra- or extracellular oxidation of DA with the resultant generation of reactive products. Glucocorticoids also are believed to cause damage through reactive pathways primarily through metabolic compromise of neurons, which leaves them vulnerable to oxygen radicals. For example, high levels of corticosterone can compromise antioxidant enzyme capacity making a region vulnerable to neurotoxicants that effect their actions through generation of free radicals [18,19].

Regardless of the mechanism responsible for striatal damage caused by amphetamines it is quite clear that manipulation of ambient temperature or of the core temperature of the animal can alter substituted amphetamine neurotoxicity [3,20]. We, and others have shown that exposure to conditions or agents (e.g., restraint, ethanol, etc.) able to reduce body temperature during the amphetamine dosage period or dosing at a lowered ambient temperature are able to reduce neurotoxicity of these agents. Conversely, elevations in ambient or body temperature can enhance their dopaminergic neurotoxicity. The data from this study would suggest that corticosterone can increase D-MDMA striatal neurotoxicity through an increase in body temperature. The mice implanted with the 15 mg corticosterone pellet receiving D-MDMA had a pronounced elevation in body temperatures over those given just D-MDMA. This group also showed increased striatal gliosis and increased DA terminal damage. In the 5 mg pellet group D-MDMA provoked an increase in striatal gliosis but no increase in body temperature and no evidence of damage to striatal DA elements. As body temperature has been clearly linked to the dopaminergic neurotoxicity of D-MDMA in the mouse it is likely that the increased striatal damage in the high dosage corticosterone group is due to the ability of this steroid to increase body temperature during the period of dosing with D-MDMA. Conversely, the observed increase in striatal gliosis observed in the lower dosage corticosterone group most likely represents damage to nondopaminergic elements of the striatum caused by D-MDMA. This damage is likely not due to the ability of corticosterone itself to damage striatum as neither dosage of corticosterone changed the basal levels of GFAP.

Reports of cognitive impairment, neuronal loss and atrophy as well as neuritic degeneration support the idea that continuous exposures to high dosages of glucocorticoids compromise the function and integrity of the hippocampus [30,36]. Thus, we were surprised that the supraphyiological dosages of corticosterone utilized here produced no evidence of hippocampal injury as indicated by the lack of an astrocytic response. Although our exposure time was not as long as that in most of the published reports it is unlikely this is the cause of our failure to observe hippocampal damage as we have not observed injury in this structure with much longer exposure times (Benkovic and Miller, unpublished data). Because most of the published reports of hippocampal endangerment by glucocorticoids have utilized rat, our results may represent a difference in the susceptibility of the mouse in general, or of this strain of mouse in particular, to the hippocampal neurotoxicity of glucocorticoids. Although the C57BL/6J strain is highly susceptible to striatal neurotoxicants such as MPTP and the substituted amphetamines, it is resistant to the hippocampal damage caused by kainic acid [31,32]. Strain dependent differences in expression of death-signal transduction proteins or tissue-specific differences in concentration of antioxidant enzymes or other protective proteins may explain the increased resistance of this strain to hippocampal insult. Treatment with corticosterone also failed to make the hippocampus vulnerable to insult by D-MDMA but was able to increase the vulnerability of striatum a brain area targeted by this neurotoxicant. Our data suggest glucocorticoids can enhance neurotoxic damage but only in areas already subject to injury by the compound and do not support the idea that glucocorticoids will promote neuroendangerment in non-target areas.

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## References

[1] R.S. Ahima, R.E. Harlan, Differential corticosteroid regulation of type II glucocorticoid receptor-like immunoreactivity in the rat central nervous system: topography and implications, Endocrinology 129 (1991) 226–236.

[2] S.F. Akana, C.S. Cascio, J. Shinsako, M.F. Dallman, Corticosterone: narrow range required for normal body and thymus weight and ACTH, Am. J. Physiol. 249 (5 Pt. 2) (1985) R527–532.

[3] J.F. Bowyer, B. Gough, W. Slikker Jr., G.W. Lipe, G.D. Newport, R.R. Holson, Effects of a cold environment or age on methamphetamine-induced dopamine release in the caudate putamen of female rats, Pharmacol. Biochem. Behav. 44 (1993) 87–98.

[4] K.B. Burrows, G. Gudelsky, B.K. Yamamoto, Rapid and transient inhibition of mitochondrial function following methamphetamine or 3,4-methylenedioxymethamphetamine administration, Eur. J. Pharmacol. 398 (2000) 11–18.

[5] J.L. Cadet, B. Ladenheim, J. Hirata, R.B. Rothman, J. Ali, E. Carlson, C. Epstein, T. Moran, Superoxide radicals mediate the biochemical effects of methylenedioxymethamphetamine (MDMA): evidence from using CuZn-superoxide dismutase transgenic mice, Synapse 21 (1995) 169–176.

[6] M. Cyr, M. Morissett, N. Barden, S. Beaulieu, J. Rochford, T. Di Paolo, Dopaminergic activity in transgenic mice underexpressing glucocorticoid receptors: effect of anitidepressants, Neuroscience 102 (2001) 151–158.

[7] C.H. Defiore, B.B. Turner, [$^3$H]Corticosterone binding in the caudate-putamen, Brain Res. 278 (1983) 93–101.

[8] M.E. Gotz, G. Kunig, P. Riederer, M.B.H. Youdim, Oxidative stress: free radical production in neural degeneration, Pharmacol. Ther. 63 (1994) 37–122.
[9] L.E. Haynes, M.R. Griffiths, R.E. Hyde, D.J. Barber, I.J. Mitchell, Dexamethasone induces limited apoptosis and extensive sublethal damage to specific subregions of the striatum and hippocampus: implications for mood disorders, Neuroscience 104 (2001) 57–69.
[10] S.Z. Imam, J. el-Yazal, G.D. Newport, Y. Itzhak, J.L. Cadet, W. Slikker Jr., S.F. Ali, Methamphetamine-induced dopaminergic neurotoxicity: role of peroxynitrite and neuroprotective role of antioxidants and peroxynitrite decomposition catalysts, Ann. N.Y. Acad. Sci. 939 (2001) 366–380.
[11] E.A. Johnson, J.P. O'Callaghan, D.B. Miller, Repeated exposure results in the loss of the neuroprotective properties of restraint-induced hypothermia in the substituted amphetamine model of striatal dopaminergic neurotoxicity, Soc. Neurosci. Abstr. 24 (855.4) (1998) 2154.
[12] E.A. Johnson, D. S Sharp, D.B. Miller, Restraint as a stressor in mice: against the dopaminergic neurotoxicity of D-MDMA, low body weight mitigates restraint-induced hypothermia and consequent neuroprotection, Brain Res. 875 (2000) 107–118.
[13] M.J. LaVoie, T.G. Hastings, Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: evidence against a role for extracellular dopamine, J. Neurosci. 19 (1999) 1484–1491.
[14] B.J. Logan, R. Laverty, W.D. Sanderson, Y.B. Yee, Differences between rats and mice in MDMA(methylenedioxymethamphetamine) neurotoxicity, Eur. J. Pharmacol. 152 (1988) 227–234.
[15] B.S. McEwen, Protective and damaging effects of stress mediators, New Engl. J. Med. 338 (1998) 171–179.
[16] B.S. McEwen, The neurobiology of stress: from serendipity to clinical relevance, Brain Res. 886 (2000) 172–189.
[17] B.S. McEwen, Effects of adverse experiences for brain structure and function, Biol. Psychiatry 48 (2000) 721–731.
[18] L.J. McIntosh, K.E. Hong, R.M. Sapolsky, Glucocorticoids may alter antioxidant enzyme capacity in the brain: baseline studies, Brain Res. 791 (1998) 209–214.
[19] L.J. McIntosh, K.M. Cortopassi, R.M. Sapolsky, Glucocorticoids may alter antioxidant enzyme capacity in the brain: kainic acid studies, Brain Res. 791 (1998) 215–222.
[20] D.B. Miller, J.P. O'Callaghan, Environment-, drug- and stress-induced alterations in body temperature affect the neurotoxicity of substituted amphetamines in the C57Bl/6J mouse, J. Pharmacol Exp. Ther. 270 (1994) 752–760.
[21] D.B. Miller, J.P. O'Callaghan, Neurotoxicity of D-amphetamine in the C57BL/6J and CD-1 mouse: interactions with stress and the adrenal system, Ann. N.Y. Acad. Sci. 801 (1996) 148–167.
[22] W.L. Nixdorf, K.B. Burrows, G.A. Gudelsky, B.K. Yamamoto, Enhancement of 3,4-methylenedioxymethamphetamine neurotoxicity by the energy inhibitor malonate, J. Neurochem. 77 (2001) 647–654.
[23] J.P. O'Callaghan, Quantification of glial fibrillary acidic protein: comparison of slot-immunobinding assays with a novel sandwich ELISA, Neurotoxicol. Teratol. 13 (1991) 275–281.
[24] J.P. O'Callaghan, Measurement of glial fibrillary acidic protein, in: L.G. Costa (Ed.), Current Protocols in Toxicology, Wiley, New York, in press.
[25] J.P. O'Callaghan, D.B. Miller, Neurotoxicity, profiles of substituted amphetamines in the C57BL/6J mouse, J. Pharmacol. Exp. Ther. 270 (1994) 741–751.
[26] S. Ouary, N. Bizat, S. Altairac, H. Menetrat, V. Mittoux, F. Conde, P. Hantraye, E. Brouillet, Major strain differences in response to chronic systemic administration of the mitochondrial toxin 3-nitroproprionic acid in rats: implications for neuroprotection studies, Neuroscience 97 (2000) 521–530.
[27] R.M. Sapolsky, A mechanism for glucocorticoid toxicity in the hippocampus: increased neuronal vulnerability to metabolic insults, J. Neurosci. 5 (1985) 1228–1232.
[28] R.M. Sapolski, L.C. Krey, B.S. McEwen, Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging, J. Neurosci. 5 (1985) 1222–1227.
[29] R.M. Sapolsky, Glucocorticoids, stress and exacerbation of excitotoxic neuron death, Semin. Neurosci. 6 (1994) 323–331.
[30] R.M. Sapolsky, Stress, glucocorticoids, and damage to the nervous system: the current state of confusion, Stress 1 (1996) 1–19.
[31] P.E. Schauwecker, O. Steward, Genetic determinants of susceptibility to excitotoxic cell death: Implications for gene targeting approaches, Proc. Natl. Acad. Sci. USA 94 (1997) 4103–4108.
[32] P.E. Schauwecker, Seizure-induced neuronal death is associated with induction of c-Jun N-terminal kinase and is dependent on genetic background, Brain Res. 884 (2000) 116–128.
[33] C.J. Schmidt, C.K. Black, G.M. Abbate, V.L. Taylor, Methylenedioxymethamphetamine-induced hyperthermia and neurotoxicity are independently mediated by 5-HT2 receptors, Brain Res. 529 (1990) 85–90.
[34] M. Shankaran, B. K Yamamoto, G.A. Gudelsky, Ascorbic acid prevents 3,4-methylenedioxymethamphetamine (MDMA)-induced hydroxyl radical formation and the behavioral and neurochemical consequences of the depletion of brain 5-HT, Synapse 40 (2001) 55–64.
[35] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, R.H. Gartner, M.D. Provenzano, E.K. Fujimoto, B.J. Olson, D.C. Klenk, Measurement of protein using bicinchoninic acid, Anal. Biochem. 150 (1985) 76–85.
[36] N. Sousa, N.V. Lukoyanov, M.D. Madeira, O.F.X. Almeida, M.M. Paula-Barbosa, Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement, Neuroscience 97 (2000) 253–266.
[37] G.R. Schmidt, R.S. Yemm, K.D. Childs, J.P. O'Callaghan, K.L. Hossner, The detection of central nervous system tissue on beef carcasses and in comminuted beef, J. Food Protect. 64 (2001) 2047–2052.