7,8-dihydroxyflavone blocks the development of behavioral sensitization to MDPV, but not to cocaine: differential role of the BDNF-TrkB pathway

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Role of BDNF in behavioral sensitization to MDPV.

ABSTRACT

3,4-Methylenedioxypyrovalerone (MDPV) acts as a dopamine transporter blocker and exerts powerful psychostimulant effects. In this study we aimed to investigate the bidirectional cross-sensitization between MDPV and cocaine, as well as to evaluate the role of the BDNF-TrkB signaling pathway in the development of locomotor sensitization to both drugs.

Mice were treated with MDPV (1.5 mg/kg) or cocaine (10 or 15 mg/kg) once daily for 5 days. After withdrawal (10 days), animals were challenged with cocaine (8 mg/kg) or MDPV (1 mg/kg). For biochemical determinations, MDPV (1.5 mg/kg) or cocaine (15 mg/kg) were administered acutely or repeatedly, and BDNF, D3R and G9a transcription levels as well as pro- and mature BDNF protein levels were determined.

Our results demonstrate that repeated administration of MDPV or cocaine sensitizes to cocaine and MDPV locomotor effects. After an acute or a repeated exposure to MDPV, cortical mRNA BDNF levels were increased, while a decrease in mBDNF protein levels in the nucleus accumbens 2h after repeated exposure was evidenced. Interestingly, such decline was involved in the development of locomotor sensitization, thus the pretreatment with 7,8-dihydroxyflavone (10 mg/kg), a TrkB agonist, blocked the development of sensitization to MDPV but not to cocaine, for which no changes in the BDNF-TrkB signaling pathway were observed at early withdrawal.

In conclusion, a bidirectional cross-sensitization between MDPV and cocaine was evidenced. Our findings suggest that decreased BDNF-TrkB signaling has an important role in the behavioral sensitization to MDPV, pointing TrkB modulation as a target to prevent MDPV sensitization.

KEYWORDS:

MDPV; Cocaine; Sensitization; BDNF; 7,8-dihydroxyflavone

1. INTRODUCTION

During the last decade the illicit drug market has changed considerably due to the emergence of New Psychoactive Substances which include synthetic cathinones. The popularity of cathinones as recreational drugs has been increasing since they broke into the drug market, so that they are used as substitutes for other classical psychostimulants such as cocaine or ecstasy.

3,4-methylenedioxipyrovalerone (MDPV) is one of the most popular synthetic cathinones and one of the main ingredients of the so-called “bath salts” [1,2]. As many psychostimulants, MDPV produces some of its neurochemical effects by interacting with the transporters of monoamine neurotransmitters. In the same way as cocaine, MDPV increases dopamine (DA) levels in the synaptic cleft by inhibiting its uptake. Animal studies indicate that MDPV is 10-50-fold more potent as a dopamine transporter (DAT) blocker than cocaine [3,4]. Moreover, it has also been demonstrated that MDPV exerts powerful psychostimulant, rewarding and reinforcing effects related to cocaine at one tenth-doses [5], pointing to a high abuse liability and thus a presumable upward consumption of this substance in the next years, probably favored by its affordable cost. Therefore, new findings about MDPV and its relationship with addiction are of special interest. The research on this topic becomes important

considering the potential health and social consequences associated with newly emerging molecular variants of this drug.

Behavioral sensitization to psychostimulants is the process whereby repeated intermittent exposure to drugs results in a progressive and enduring increase in the motor stimulant response to the drug [6–10]. Furthermore, behavioral sensitization is a long-lasting phenomenon, shown to persist for at least one year after cessation of drug administration [11]. Hence, repeated drug exposure may induce dynamic changes in neural processes, which may influence susceptibility to drug abuse and relapse by increasing the reinforcing value of acute drug administration [9,12]. It is also known that the ventral tegmental area (VTA) is essential for the development of behavioral sensitization, whereas the nucleus accumbens (NAcc) seems to be necessary for its expression [13,14]

There is convincing evidence that the brain-derived neurotrophic factor (BDNF) along with its specific receptor, tropomyosin receptor kinase B (TrkB), have a key role in the behavioral abnormalities observed in rodents after psychostimulant administration [15–17]. BDNF present in NAcc and dorsal striatum ischiefly , but not exclusively, supplied by anterograde axonal transport from cortical pyramidal neurons in frontal cortex [18–20]

Research done over the last two decades, has reported BDNF to be involved in the long-term neuronal adaptations leading to functional modifications in the synapses associated with cocaine abuse and related behaviors [17,21,22]. Moreover, it is also involved in the behavioral abnormalities and neurotoxicity induced by methamphetamine consumption [23]. Hence, the BDNF-TrkB signaling may be a potential therapeutic target for treating drug addiction.

Transcription of the BDNF gene is under control of not only transcriptional factors but also of epigenetic mechanisms including chromatin remodeling and DNA methylation [24]. G9a is a histone 3 lysine 9 (H3K9me2)-specific dimethyltransferase that acts as a negative regulator of BDNF signaling through TrkB [25]. It is also known that repeated exposure to cocaine induces long-lasting epigenetic changes in the brain, modifying the chromatin structure of DNA via histone acetylation, phosphorylation, and/or methylation [26,27]. For instance, Maze et al. [28] demonstrated that H3K9me2 is substantially reduced in the NAcc after chronic cocaine exposure due to decreased levels of G9a. In this sense, it has been identified an essential role for H3K9m3 and G9a in cocaine-induced structural and behavioral plasticity [28].

At the same time BDNF, synthesized in either VTA neurons or neurons originating from the cortex, controls dopamine 3 receptor (D3R) expression [29]. Interestingly, hyperresponsiveness to drug-associated cues and context-dependent behavioral sensitization might be related to hypersensitive postsynaptic dopaminergic receptors. Among them, D3R seems to be a key target since it is highly expressed in the shell of NAcc, in which DA release is mainly triggered by drugs [30]. Furthermore, D3R expression controls behavioral sensitization and BDNF [29,31], which at the same time controls D3R expression and enhances the conditioned reward and locomotor activity induced by cocaine [32].

Drug-induced locomotor sensitization has been described for both cocaine and MDPV [33–36]. The fact that different addictive drugs produce the same effect implies that they may exert some of their effects through the same neural mechanisms. Furthermore, cross-sensitization between other addictive drugs has also been described [37,38]. For this reason, the present study aims to investigate for the first

time the bidirectional cross-sensitization between MDPV and cocaine, as well as to determine and compare the changes induced by either acute or chronic exposure to MDPV or cocaine on the transcription of plasticity genes in early abstinence. Thus, this work is mainly focused on the role of BDNF-TrkB signaling pathway in the development of behavioral sensitization to MDPV and cocaine, and also its target gene, *d3r,* and the regulator histone methyltransferase G9a.

2. MATERIALS AND METHODS

2.1. Animals

Male adolescent (PND 41-44) Swiss CD-1 mice (Charles River, Spain) were used for all experiments due to its optimal sensitivity to the reinforcing and psychostimulant effects of cocaine (McKerchar et al., 2015). Animals were randomly assigned to an experimental group and housed six per cage in temperature-controlled conditions (22 ± 1 ºC) under a 12 h light/dark cycle and had *ad libitum* access to standard food and water. During the behavioral manipulations, researchers were not aware of the treatment that each animal had received. All the procedures adhered to the guidelines of the European Community Council (2010/62/EU) and ARRIVE and were approved by the Animal Ethics Committee of the University of Barcelona. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Materials

Pure racemic MDPV · HCl was synthesized and characterized in our laboratory as described (Novellas et al., 2015). Cocaine was provided by the Spanish National Institute of Toxicology. MDPV and cocaine solutions for injection were prepared in 0.9% NaCl (saline, pH=7.4) immediately before administration.

Both TrkB ligands 7,8-Dihydroxyflavone (7,8-DHF) and ANA-12 (N-[2-[[(Hexahydro-2-oxo-1H-azepin-3-yl)amino]carbonyl]phenyl]-benzo[b]thiophene-2-carboxamide) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were prepared as a micro-suspension in phosphate-buffered saline containing 0.5% (w/v) carboxymethyl cellulose and 0.1% (v/v) Tween-80.

Specific ELISA mBDNF and proBDNF Kits were purchased from Biosensis (Thebarton SA, Australia), the protease and phosphatase inhibitor cocktail from Abcam (Cambridge, UK) and the organic solvents ethanol, chloroform and isopropanol, from Scharlab (Barcelona, Spain) The rest of reagents were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Drug administration protocol and experimental design

For cross-sensitization experiments animals followed the administration regime depicted in Figure 1.

A moderate dose of MDPV (1.5 mg/kg, s.c.) eliciting hyperlocomotion was chosen for this study. This dose, in mice, is equivalent to a dose of approximately 8 mg in humans [40,41] which is in the middle range of the doses most commonly used by consumers [42]. The doses of cocaine (10 and 15 mg/kg, i.p.) have been chosen on the basis that MDPV is at least 10-fold more potent than cocaine *in vivo* and *in vitro* [3–5,43]. No higher doses were used since those we selected predominantly induce locomotor activity (e.g., horizontal forward locomotion, even though some occasional licking were observed), whereas higher doses could produce non-ambulatory stereotypies, such as

head bobbing, sniffing or licking that could have interfered the analysis because of the competition between non-ambulatory behaviors and locomotor activity.

The sensitization procedure consisted of three phases over 17 days: habituation, treatment and challenge. In the habituation phase (the two days prior treatment, days -1 and 0), mice were handled for 10 min, injected with saline (5 ml/kg) and immediately placed for 45 min in a black Plexiglass open field arena. After the habituation phase, mice were injected with saline (5 ml/kg), MDPV (1.5 mg/kg s.c.) or cocaine (10 mg/kg or 15 mg/kg i.p.) once daily for five consecutive days (days 1-5). Animals were placed in the open field arena immediately after each saline or drug injection and their horizontal locomotor activity (HLA) was recorded. Ten days after the final administration (day 15), all mice were challenged with cocaine (8 mg/kg i.p.) or MDPV (1 mg/kg s.c.) and their HLA was registered again.

For biochemical determinations, acute and chronic exposure regimes were designed. In acute experiments, a single dose of saline (5 ml/kg), MDPV (1.5 mg/kg s.c.) or cocaine (15 mg/kg i.p.) were injected and animals were sacrificed 1h or 2h post-administration for the subsequent study of different markers. The chronic administration protocol was the same as the one used for the cross-sensitization experiments (Figure 1) but sacrificing the animals 2h, 24h or 10 days post-treatment (day 15).

2.4. Horizontal locomotor activity (HLA) measurement

The animals were given their treatment (saline, MDPV or cocaine solutions) and immediately placed in a black Plexiglass open field arena (25 x 25 x 40 cm) under low-

light conditions and white noise. HLA was video-monitored for 30 min using a specific tracking software (Smart 3.0 Panlab, Barcelona, Spain). All HLA experiments were performed during the resting-phase of the animals (between 8:00 am and 2:00 pm).

2.5. Effects of 7,8-DHF on the development of behavioral sensitization after repeated exposure to cocaine or MDPV

To evaluate the sensitizing effects of MDPV and cocaine under a previous treatment with 7,8-DHF, mice were assigned to one of the following four groups: vehicle + saline; vehicle + MDPV or cocaine; 7,8-DHF + saline and 7,8-DHF + MDPV or cocaine. 7,8-DHF (10 mg/kg, i.p.) was administered 30 min prior to the saline or drug injection, to ensure constant levels of the agonist throughout all the procedure. The treatment schedule used was the same as in the cross-sensitization experiments, but the HLA was only recorded after the challenge with MDPV or cocaine, on day 15. The 7,8-DHF dose as well as the administration schedule were selected according to those used in multiple studies in the literature [23,44].

2.6. Tissue sample preparations

Mice treated according to the administration protocols described above were sacrificed by cervical dislocation, 1h or 2h after an acute dose and 2h, 24h or 10 days after the repeated regime, for the analysis of different factors including: G9a, BDNF and D3R. NAcc, ventral striatum (VS) containing NAcc, or medium prefrontal cortex (mPFC), when appropriate, were quickly dissected out and stored at -80ºC until use. Particularly, for the dissection of the NAcc and the mPFC, brains were rapidly removed

and placed in an ice-cold mouse brain acrylic matrix (Alto, Agnthos, Sweden). Two double edge blades were properly inserted to obtain a 2 mm (2 mm anterior to bregma) or 1 mm (from 2 to 3 mm anterior to bregma) thick slices, respectively, which contained the regions of interest [45]. The NAcc was micropunched away from the rest of the brain tissue.

Tissue samples for ELISA analysis were processed following the instructions provided by the kit manufacturer. Briefly, NAcc samples were thawed and homogenized through sonication at 4°C in 100 volumes of RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% (v/v) NP-40 and 0.5% (w/v) sodium deoxycholate, pH=7,5-8) containing a protease and phosphatase inhibitor cocktail. Once homogenized, samples were kept on ice for 30 min. Sample sonication and cooling with ice were performed twice. Homogenates were centrifuged at 14,000 x *g* for 30 min at 4ºC and the resulting supernatants (total lysates) were collected and kept at -80ºC until use. Protein content was determined using the Bio-Rad Protein Reagent (Bio Rad, Inc., Madrid, Spain).

2.7. Quantification of mBDNF and proBDNF protein levels

The determination of proBDNF and mBDNF was performed following the instructions of the corresponding ELISA kit. In brief, the kit consisted of a microplate pre-coated with mouse monoclonal anti-mature or polyclonal anti-proBDNF capture antibodies. Then, samples containing unknown amount of the target protein were added and bound to the capture antibody. After washing steps to rid the microplate of unbound substances, a biotinylated anti-mature or anti-proBDNF detection antibody, respectively, and horseradish peroxidase (HRP)-conjugated streptavidin were added for detection. The addition of a substrate (3,3',5,5'-tetramethylbenzidine,

TMB) yielded a colored reaction product that was directly proportional to the concentration of mature or proBDNF present in samples and protein standards.

2.8. Total RNA extraction and gene expression determination

Total RNA isolation from ventral striatum or mPFC was carried out by means of a TRIsure™ reagent-Chloroform based extraction protocol. TRIsure™ (Bioline, Meridian Bioscience Inc., UK), monophasic solution of phenol and guanidinium isothiocyanate, facilitates disruption of cells during homogenization and effectively inhibits DNase and RNase activity. Simultaneously, solubilizes biological material and denatures protein. After solubilization, the addition of chloroform causes phase separation, where protein is extracted to the organic phase, DNA resolves at the interface, and RNA remains in the aqueous phase [46]. RNA content in the samples was measured at 260 nm, and sample purity was determined by the A260/280 ratio in a NanoDrop™ ND-1000 (Thermo Scientific).

Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and the Veriti® thermal cycler (Applied Biosystems, Foster, CA, USA). Briefly, complementary DNA (cDNA) was synthesized in a total volume of 20 μL by mixing 1 μg of total RNA and the appropriate volumes of each kit reagent.

Quantitative real-time polymerase chain reaction (qPCR) was performed using the StepOnePlus™ Real-Time PCR System and the Taqman one-step PCR Master Mix (Applied Biosystems, Foster, CA, USA). In brief, 25 ng of total cDNA was added per 20-μl reaction mixture containing sequence-specific primers and Taqman probes

from Applied Biosystems Mm0113261_m1 for G9a, Mm04230607_s1 for BDNF, Mm00432887_m1 for D3R and Mm00607939_s1 for β-actin. The β-actin transcript level was used to normalize differences in sample loading and preparation. The reaction conditions were as follows: the holding stage was initiated by a 2-min incubation at 50ºC and 10 min of polymerase activation at 95ºC, followed by a cycling stage of 40 cycles at 95ºC for 15 s and 60ºC for 60 s. All reactions were performed in triplicate. Fold-changes in gene expression were determined using the $2^{-\Delta\Delta Ct}$ method (comparative Ct method) for each experimental sample [47] . All primer-probe combinations were optimized and validated for relative quantification of gene expression. All experiments included a no-template control, and real-time PCR reactions also included a no-reverse transcriptase (no-RT) control for which no amplification products were seen, indicating that cDNA was amplified rather than residual genomic DNA.

2.9. Conditioned place preference (CPP) test

The place conditioning protocol used was non-biased as described previously [48] CPP was performed in three phases: preconditioning, conditioning and test. During the preconditioning phase (day 1), mice had free access and roam among the three compartments of the apparatus for 15 min. The time spent in each compartment was recorded by computerized monitoring software (Smart 3.0 Panlab, Barcelona, Spain). During the conditioning phase (days 2-5; sessions 1-8), mice were given 7,8-DHF (10 mg/kg i.p.), 30 min before the s.c. administration of MDPV (1.5 mg/kg) or saline. After the MDPV injection, the animals were immediately placed into one of the two conditioning compartments for 20 min (sessions 1, 3, 5 and 7). On the alternate

sessions (2,4,6 and 8), mice were placed in the other compartment for 20 min after being given a saline injection. Two conditioning sessions per day were performed, separated by a 5-hours period. Control animals received saline every session. The preference test (day 6) was conducted as the preconditioning phase. A preference score was expressed in seconds and calculated for each animal as the difference between the time spent in the drug-paired compartment in the test minus the time spent in the same compartment in the preconditioning phase.

2.10. Data acquisition and statistical analysis

Data were expressed as mean ± SEM. Differences between groups were compared using one- or two-way ANOVA where appropriate. One-way ANOVA was used when analyzing differences due to the factor “treatment” in the experiment, whereas two-way ANOVA was performed when two different factors were analyzed: “treatment x time”. The α error probability was set at 0.05. Significant differences ($P<0.05$) were analyzed using the Tukey’s post hoc test for multiple comparisons measures where appropriate only if F achieved the necessary level of statistical significance ($P<0.05$) and no significant variance in homogeneity was observed. Every set of results was tested in the calculator QuickCalcs of GraphPad software, which performs Grubbs' test (extreme studentized deviate method), to determine whether any of the values in the set was a significant outlier from the rest. Statistic calculations were performed using GraphPAD Prism 6.0 software.

3. RESULTS

3.1. Cross-sensitization between MDPV and cocaine

To evaluate the ability of cocaine to sensitize to the motor stimulant effects of MDPV and vice versa, a bidirectional cross-sensitization experiment was performed following the administration regime displayed in Figure 1, in which two different doses of cocaine were tested (10 mg/kg and 15 mg/kg, i.p.).

During the treatment phase, HLA was measured daily and immediately after each saline or drug injection. MDPV induced an acute hyperlocomotion that, unlike cocaine, significantly increased with repeated daily exposure (Figure 2). Accordingly, two-way ANOVA revealed an effect of the day ($F_{4,368}$=8.681, P<0.001), treatment ($F_{3,92}$=131.3, P<0.001) and the interaction between both factors ($F_{12,368}$=1.931, P<0.05) (n=24/group). Moreover, MDPV-treated mice showed a significantly higher locomotor activity at a dose of 1.5 mg/kg during the treatment compared to cocaine at both doses tested (10 and 15 mg/kg).

When analyzing the HLA after a challenge with MDPV (1 mg/kg, s.c.) or cocaine (8 mg/kg i.p.) on day 15 after withdrawal (Figure 3A and 3B), a higher locomotor response was evidenced in both MDPV and cocaine-treated mice compared to the control group. Accordingly, one-way ANOVA reported an effect of treatment for both challenge drugs (MDPV challenge: $F_{3,44}$=8.697, P<0.001, n=12/group; cocaine challenge: $F_{3,43}$=14.15, P<0.001, saline, cocaine 15 mg/kg and MDPV-treated groups, n=12/group; cocaine 10 mg/kg group, n=11). On the other hand, no differences were found between MDPV and cocaine-pretreated mice after the challenge with MDPV. However, it is noteworthy that, after the challenge with cocaine, MDPV-pretreated mice showed a higher locomotor response compared to cocaine 10 mg/kg-pretreated mice (P<0.05) but not

to those pretreated with cocaine 15 mg/kg. Therefore, repeated administration of cocaine 15 mg/kg or MDPV 1.5 mg/kg developed equivalent locomotor sensitization. Thus, the dose of 15 mg/kg of cocaine was chosen for the subsequent experiments.

3.2. Effect of MDPV and cocaine on G9a methyltransferase, BDNF and D3R expression

To further investigate the role of the BDNF-TrkB pathway in the development of behavioral sensitization, we assessed and compared the changes occurred in related genes after both an acute and a repeated exposure to MDPV and cocaine.

3.2.1. After an acute dose of MDPV or cocaine

G9a methyltransferase mRNA in VS was determined 1h and 2h post-injection. Two-way ANOVA revealed an effect of treatment ($F_{2,29}$=8.166, P<0.01) and time ($F_{1,29}$=14.02, P<0.001) with interaction between both factors ($F_{2,29}$=8,090, P<0.01) (Figure 4A). 1 hour after an acute dose of MDPV (1.5 mg/kg) or cocaine (15 mg/kg), the levels of G9a mRNA were similarly increased in cocaine (P<0.05) and MDPV (P<0.01)-treated mice compared to the saline group. However, this overexpression had rapidly declined at 2 hours. Nevertheless, this decline was more marked for the MPDV-treated mice than for those treated with cocaine, so significant differences were found between both groups at 2 hours (P<0.01) as well as among the MDPV-treated mice when comparing between the two time-points (P<0.001) (saline 1h and MDPV 1h groups, n=5/group, cocaine 1h, saline 2h and MDPV 2h groups, n=6/group; cocaine 2h group, n=7).

In addition, when assessing the expression of the transferase in the mPFC 2h post-injection, no changes were observed neither by MDPV nor by cocaine administration (saline group, n=5; cocaine and MDPV groups, n=7/group) (Figure 4B).

In the same brain area, one-way ANOVA of *bdnf* mRNA levels 2h post-administration yielded a significant effect of treatment ($F_{2,16}$=23.73, P<.001) (Figure 4C). Particularly, an acute MDPV injection induced a significant overexpression of this factor (P<0.001), whereas no changes by cocaine were observed (saline group, n=5; cocaine and MDPV groups, n=7/group).

Since cortical BDNF controls D3R expression in the NAcc, the mRNA levels encoding this receptor were also assessed 2h post-administration in VS (containing NAcc). Nevertheless, no changes in D3R mRNA levels were evidenced in this area (Figure 4D) (saline group, n=6; cocaine and MDPV groups, n=7/group).

3.2.2. After a repeated exposure to MDPV or cocaine

G9a gene expression was determined 2h and 24h after the last drug injection. Maze et al [28] described the effect of repeated cocaine exposure on G9a expression in NAcc at 24h but, due that we did not find changes at this time point, we assessed its levels also at 2h after drug administration.

As shown in Figure 5A, two-way ANOVA (n=6/group) reported a significant effect of treatment ($F_{2,30}$=3.811, P<0.05), time ($F_{1,30}$=28.60, P<0.001) and the interaction between both factors ($F_{2,30}$=9.743, P<0.001). Particularly, repeated cocaine exposure upregulated G9a in VS 2h post-treatment (P<0.01), whereas MDPV did not alter significantly its expression. However, when measuring G9a mRNA 24h post-treatment,

no changes were observed for cocaine, pointing to a transient overexpression of the methyltransferase, which had returned to control values at 24h of withdrawal. Regarding MDPV, despite no effect was observed after two hours, a significant reduction of G9a expression over time was evidenced when comparing between both time-points ($P<0.05$).

We did not detect any effect induced by MDPV or cocaine on G9a transcript in mPFC 2h post-treatment (n=6/group) (Figure 5B).

In parallel, BDNF and D3R gene expression were determined 2h post-treatment in mPFC and VS, respectively. One-way ANOVA (n=6/group) revealed a significant effect of treatment ($F_{2,15}=7.590$, $P<0.01$). Repeated exposure to MDPV, but not to cocaine, increased BDNF expression ($P<0.05$) (Figure 5C). At the same time, a similar pattern of expression was observed for D3R, although the differences observed did not reach statistical significance (n=6/group) (Figure 5D).

Given the fact that those fluctuations in the level of BDNF transcription after drug exposure may not necessarily affect overall protein expression, pro- and mBDNF protein levels were assessed in dissected tissue from NAcc. Such protein levels were determined 2h after-treatment, but also 24h and 10 days after the last administration of both substances.

Despite the observed increment in *bdnf* gene transcription induced by MDPV in mPFC, neither MDPV nor cocaine treatment altered proBDNF levels in NAcc (n=6/group) (Figure 6A).

Surprisingly, two-way ANOVA revealed a significant effect of time ($F_{2,44}=4.782$, $P<0.05$) and treatment ($F_{2,44}=7.650$, $P<0.01$) in mBDNF expression, although no

interaction between both factors was evidenced. Therefore, despite no differences were observed in propeptide levels, mBDNF was decreased shortly after a chronic MDPV exposure ($P<0.05$), even though its levels were restored after 10 days of withdrawal. In cocaine-treated-mice, no changes were observed at any of the time-points assessed until 10 days of withdrawal (saline 2h and day 10, cocaine 2h and 24h, MDPV 2h and day 10 groups, n=6/group; saline 24h and MDPV 24h groups, n=5/group; cocaine day 10 group, n=7) (Figure 6B).

3.3. Effect of 7,8-DHF on the development of locomotor sensitization after repeated administration of MDPV or cocaine

Given the decrease of mBDNF protein levels observed 2h after a chronic MDPV exposure, we sought to determine if this change was involved in the development of locomotor sensitization to the drug. To investigate this participation, 7,8-DHF, a TrkB receptor agonist, was administered 30 min prior to every drug injection (pretreatment).

From the experiments with MDPV (Figure 7A), one-way ANOVA revealed a significant effect of treatment ($F_{3,32} = 19,64$, $P<0.001$) (n=8/group). As we expected, post-hoc analysis showed that exposure to MDPV (1.5 mg/kg) significantly increased ($P<0.0001$) acute MDPV (1 mg/kg)-induced locomotion 10 days after treatment, compared to its control group (vehicle + saline treated). Interestingly, pretreatment with 7,8-DHF significantly blocked ($P<0.001$) such development of sensitization. Moreover, control (saline) animals pretreated with 7,8-DHF or the vehicle showed a similar locomotor response, which evidences the null effect of this agonist on the locomotor activity by itself.

To prove that the effect of 7,8-DHF in the development of locomotor sensitization to MDPV was mainly due to its ability to bind and selectively activate TrkB, ANA-12, an antagonist of this receptor, was administered previously to the flavone. One way-ANOVA, ($F_{3,31}$ = 20.15, p<0.001) revealed that both groups exposed to MDPV (vehicle + MDPV and ANA-12+7,8-DHF+MDPV) developed behavioral sensitization (25592 ± 716, P<0.001 and 21343 ± 1192, P<0.01, respectively, vs saline-treated groups). This result suggested that ANA-12 prevented the binding of 7,8-DHF to TrkB and thereby its further activation, which seems to be involved in the effects induced by the agonist. No changes in the locomotor activity of control (saline) animals pretreated with ANA-12 + 7,8-DHF were observed.

On the other hand, in the experiments with cocaine, no effect of 7,8-DHF was evidenced in the development of behavioral sensitization (Figure 7B). One-way ANOVA ($F_{3,42}$=16.33, P<0.001) evidenced that locomotor sensitization was similarly developed in both cocaine-treated groups, regardless they were pretreated or not with 7,8-DHF. (“Vehicle + saline”, “7,8-DHF + saline”, “vehicle + cocaine” groups, n=12/group; “7,8-DHF + MDPV” group, n=10).

3.4. Effect of 7,8-DHF on the place conditioning induced by MDPV

The CPP paradigm was used to study whether 7,8-DHF could modify the conditioning properties of MDPV, aside from the development of locomotor sensitization.

The percentages of time spent in both compartments during the preconditioning phase were 51.48 ± 2.45 % and 48.57 ± 2.45 % (p>0.05), indicating a total lack of preference for either side. 4 mice from the saline and MDPV groups were withdrawn from the

experiment due to an initial preference for one of the compartments (>65% of the total session time). One more animal from the MDPV group was withdrawn after the test day because it was a significant outlier ("Saline" and "7,8-DHF" groups, n=12/group; "MDPV" group, n=11 and "7,8-DHF + MDPV" group, n=13).

On the test day (day 6, post-conditioning), one-way ANOVA revealed a significant effect of treatment ($F_{3,44}$=16.50, P<0.001). Accordingly, the repeated administration of MDPV (1.5 mg/kg, s.c.) produced a preference for the MDPV-paired compartment (P<0.001) which was not modified by the previous administration of 7,8-DHF. Furthermore, 7,8-DHF did not exert any conditioning or aversive effect by itself (Figure 8).

4. **DISCUSSION**

In a previous work, we reported that exposure of adolescent mice to MDPV sensitizes to cocaine effects and induces a higher vulnerability to cocaine abuse in adulthood [48]. Cocaine abuse represents a heavy burden of disease in many countries, becoming a global problem. Any factor that increases the vulnerability to cocaine abuse must be carefully evaluated. In this sense, the present study examines more closely the common pathways involved in the effects of MDPV and cocaine, using the same dose of MDPV previously described, and also mice at the beginning of periadolescence. Despite the risks associated with new cathinones use, little is known about their consequences, especially among adolescents, although the exposure pattern in this age group is very similar to that observed among adults [49].

Based on their pharmacological mechanism, we tested the possible cross-sensitization between cocaine and MDPV. Following our schedule, during the induction of

sensitization, only MDPV elicited a hyperlocomotion that significantly increased with repeated daily exposure, whereas cocaine did not. Even so, regarding to the expression of sensitization (challenge day, 10 days after withdrawal), we observed that the induction of sensitization by cocaine 15 mg/kg was equivalent to that induced by MDPV 1.5 mg/kg. Additionally, our results demonstrate that repeated administration of MDPV or cocaine sensitizes to cocaine or MDPV locomotor effects, respectively.

Moreover, this is the first time that it is evidenced that the challenge with MDPV triggers an enhanced locomotor activity in animals pretreated with cocaine. This is of chief significance since sensitization-like phenomena has been frequently proposed to account for relapses in drug consumption. In this line, here we demonstrate that previous consumption of one of these psychostimulants may favor relapse when a new dose is taken after a withdrawal period an also may favor the onset of an addiction to the other drug when taken for the first time.

In previous studies [34,48] we found that, although repeated exposure to MDPV resulted in an increase in ΔFosB expression, as it occurs after cocaine exposure, other factors were not affected by this cathinone derivative. This suggested that additional pathways are involved in the MDPV effects. To gain more in-depth knowledge of these mechanisms, this study aimed to investigate the role of BDNF in the locomotor sensitization induced by MDPV and cocaine, as well as to assess the effects of a single administration of both psychostimulants in such signaling pathway.

Dynamic alteration of BDNF expression and the consequent adjustments in brain functioning and neuronal plasticity are subjected to environmental changes (i.e., drug exposure) by means of altered epigenetic programming. Differential cocaine-induced effects at specific *bdnf* promoters are mediated by distinct epigenetic mechanisms. In

mammals, the trimethylation of histone H3 on lysine 9, which is catalyzed by the methylytransferase G9a, is commonly associated with gene silencing [50]. It has been described that there is an increase of G9a expression in NAcc after an acute dose of cocaine, but a decrease after repeated doses, which favors the expression of genes epigenetically controlled by this methyltransferase such as ΔFosB, BDNF or Arc [28].

In the present study we found an early significant increase in G9a expression induced after an acute dose of cocaine or MDPV, which rapidly reverted. It is noteworthy that this decline was more marked for MDPV. However, when a schedule of repeated administrations was used, we also obtained an early very significant increase of G9a mRNA induced by cocaine, which disappeared 24 h after treatment. Again, the signal was more transient in the case of MDPV and, in fact, the early increase did not reach statistical significance. Taken together, we hypothesize that the initial increase might have taken place much earlier than when we performed the determination.

Nevertheless, in a previous work from our lab [34], an overexpression of this transferase was observed in the VS 24h after a higher and longer MDPV exposure (1.5 mg/kg, 2 doses in a day x 7 days). All over, our findings suggest that G9a expression after drug intake may be time and dose-dependent and, moreover, its regulation runs temporarily different for cocaine than for MDPV.

BDNF gene is differentially regulated in regions of the mesolimbic pathway during the withdrawal or abstinence period following repeated cocaine administration [51]. Therefore, although the repressive role of G9 in the NAcc, no effect of this methyltransferase has been described in mPFC after cocaine exposure, but an increased histone acetylation [52]. In this sense, we considered it was worth to investigate if the changes in the methyltransferase transcription induced by these

psychostimulants were region-specific. G9a expression in mPFC was not altered neither by acute nor by repeated doses of both drugs, pointing that the changes in this histone methyltransferase produced by MDPV or cocaine administration occur only in the striatum, where probably play a relevant role in the regulation of behavioral responses to these DAT blockers [53].

Considering the discrepancy observed in the epigenetic regulation according to the brain area (VS or mPFC), we determined the effect of cocaine and MDPV on *bdnf* transcription. Given the fact that Le Foll [54] described that a single exposure to cocaine (20 mg/kg) increases *bdnf* mRNA expression throughout the cortex in the adult rat, but not in the NAcc, we only determined *bdnf* expression in mPFC 2h after the injection of the psychostimulants. In our study, using adolescent mice and a lower dose of cocaine (15 mg/kg), we did not observe such increase in *bdnf* transcription. However, a clear difference between cocaine and MDPV equivalent treatments must be highlighted. A single dose of MDPV, but not of cocaine, was enough to upregulate BDNF mRNA levels, similarly to what had been reported after a semichronic treatment. Nevertheless, the G9a methyltransferase expression in the same brain area remained unaffected. Hence, we can conclude that, shortly after an acute or repeated MDPV administration, BDNF mRNA expression in mPFC increases without being apparently under the control of G9a-mediated epigenetic mark.

In adult mice, it has also been described a close relationship between increased BDNF mRNA expression in the cortex and an early increase of D3R mRNA in the NAcc [55]. In our study, using adolescent mice, even though MDPV generated an increase of the neurotrophin transcript, it did not cause any change in accumbal D3R mRNA, after acute or repeated exposure.

Based on the changes observed in mPFC, we decided to investigate BDNF protein in the NAcc. BDNF is initially synthesized as its precursor (proBDNF) and subsequently cleaved into mature BDNF (mBDNF), which can be anterogradely transported to its target neurons [56] In this sense, levels of both the pro (2h post-administration) and the mature isoforms (2h, 24h and 10 days after administration) of this protein (proBDNF and mBDNF, respectively) were determined. Despite the early increment observed in *bdnf* transcription induced by MDPV, neither MDPV nor cocaine treatment altered proBDNF protein levels in NAcc.

We would like to emphasize once again the fact that alterations of G9a after repeated doses did not translate into any modification of the immature protein levels, so even though G9a is an important factor involved in cocaine-induced plasticity, it does not seem to exert a direct influence on the *de novo* synthesis of BDNF.

Regarding cocaine, no effect on *bdnf* mRNA transcription, proBDNF or mBDNF protein levels were evidenced at any time until 10 days of withdrawal. These findings agree with the lack of changes in BDNF expression observed during early cocaine withdrawal [57,58]. In fact, changes only appear after 3 weeks- 30 days of withdrawal. On the other hand, the effects of cocaine in BDNF protein synthesis generate controversy. Li and Wolf [15] summarized these effects in a long table in which only one of the 30 cites referred to a study performed in mice [59]. Zhang and co-workers exposed C57BL/6J mice to 20 mg/kg cocaine for seven consecutive days. Under these conditions, BDNF expression was slightly induced in the NAcc and caudate putamen (CPu). The rest of the existing literature report the effects of cocaine on BDNF especially in rats, at doses greater than 15 mg/kg, and mainly after a long withdrawal. In contrast, the effects that the authors observed shortly after repeated cocaine doses

are controversial when examining BDNF mRNA and protein levels [20,57]. Overall, although cocaine exposure generally leads to increases in BDNF levels in reward-related brain regions, exceptions have also been observed. So, the regional selectivity and timing of cocaine effects can vary widely depending on the experimental conditions [15].

In the same way, we demonstrated that repeated MDPV administrations induced temporal and regional-specific changes in BDNF expression, with a decoupling between transcriptional and translational processes. Conversely to the discrete up-regulation of mRNA levels in mPFC, mBDNF protein levels were reduced in the NAcc just after treatment, although they were restored after 10 days of withdrawal. Fumagalli et al. [60] also described a decoupling between transcriptional and translational processes shortly after repeated cocaine exposure (five daily injections, 5mg/kg), which differently modulates BDNF mRNA in PFC and protein levels in rat striatum. Concretely, cocaine increased BDNF mRNA levels 2h after the last injection with no changes in mBDNF protein levels in the striatum. Moreover, it has been described that BDNF protein levels in the NAcc progressively increase after withdrawal from cocaine self-administration, but this gradual increase does not derive from local protein synthesis in NAcc neurons [20,58].

Considering these unexpected results, a new *in vivo* experiment was carried out in sought to determine if such decline in mBDNF levels induced by the cathinone was related to locomotor sensitization. Therefore, a new sensitization experiment was carried out, but in this case the animals were pretreated with 7,8-DHF, a BDNF receptor (TrkB) agonist. Daily administration of 7,8-DHF 30 minutes prior to the injection of MDPV during the induction period completely blocked the development of

sensitization to the cathinone. In addition, to prove that the effect of the flavone was exclusively mediated via TrkB activation, the same experiment was carried out in the presence of ANA-12, a potent and selective TrkB antagonist. ANA-12 prevented the effect of the flavone in such a way that the group ANA-12 + 7,8-DHF + MDPV developed locomotor sensitization after an MDPV challenge.

Taken together, these results suggest that the decrease in mBDNF levels, and hence of its receptor stimulation, is involved in the sensitization developed to MDPV. In fact, the same effect of 7,8-DHF on the development of behavioral sensitization after repeated administration of methamphetamine (METH) was described by Ren et al. [23]. So, even though MDPV and methamphetamine have substantial differences regarding their mechanism of action, a decrease in BDNF protein levels has been found during early withdrawal of both substances [61]. Therefore, this suggests that METH and MDPV abusers may suffer from a severe dysfunction on BDNF-TrkB signaling, which is importantly involved in the development of behavioral sensitization. In fact, mouse models with reduced BDNF expression exhibit a variety of alterations in the DA system [62], which indicates that BDNF has some direct influence on this system.

It is noteworthy that, regarding mBDNF levels, the differences between cocaine and MDPV observed in the *in vitro* determinations agree with the results of the *in vivo* experiments. So, the expression of cocaine-induced locomotor sensitization, for which no changes in mBDNF levels were observed, was not altered by the previous administration of the flavone. Therefore, other signaling pathways may be involved in the development of cocaine-induced sensitization despite the great similarity of its mechanism of action with that of MDPV.

Nevertheless, when assessing the rewarding properties of MDPV in the CPP paradigm, 7,8-DHF did not modify CPP acquisition, suggesting that the BDNF/BDNF-TrkB modulation has a specific effect on the motor sensitization to the cathinone. In fact, behavioral sensitization and CPP have been proven to be modulated by different mechanisms. Associative memory develops correlation between drug-induced affective states and contextual cues, triggering craving and some goal-directed behavior toward drugs, like that seen in the CPP paradigm. However, the transition from recreational to pathological and compulsive drug seeking may involve associative (conditioned to the open field arena) and non-associative processes, such as sensitization, whereby the neural substrate mediating the response to the drug is directly augmented [62,63].

In summary, repeated administration of MDPV or cocaine cross-sensitizes to their locomotor effects. When using equivalent sensitization doses of both psychostimulants, only MDPV induces early changes in BDNF transcription, and this ability to activate the mPFC and increase *bdnf* gene expression is preserved after a repeated treatment. However, although the cathinone increases cortical BDNF mRNA, the accumbal levels of the mature protein significantly decrease. Therefore, exposure to psychostimulants does not always affect *bdnf* mRNA and protein expression in the same direction, possibly due to the complex regulation of BDNF synthesis and transport. Alternatively, upregulation of BDNF mRNA levels might be a compensatory response to the primary loss of mBDNF protein. Our findings demonstrate that BDNF, but not D3R plays a role in the development of MDPV-induced locomotor sensitization, which may influence susceptibility to drug abuse and drug relapse. Given the protective role of BDNF, we cannot rule out the possibility that neurons expressing lower levels

of BDNF after repeated MDPV injections could be more vulnerable because of reduced trophic support [64] .

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AUTHOR CONTRIBUTIONS

E.E. conceived and designed the research study. LDC performed the main part of research study and acquired data. SV conducted parts of experiments. RLA and DP contributed to analysis and interpretation of data. JC and DP were involved in revising the manuscript critically for important intellectual content. LDC and EE wrote the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

LEGENDS FOR FIGURES

Figure 1

Drug administration protocol and experimental design. After two days of habituation (days -1 and 0), mice were treated with saline (5ml/kg), MDPV (1.5 mg/kg s.c.) or cocaine (10 mg/kg or 15 mg/kg i.p.), once daily for five consecutive days (days 1-5) and their horizontal locomotor activity was recorded. 10 days later (day 15), all mice were challenged with MDPV (1 mg/kg s.c.) or cocaine (8 mg/kg i.p.) and their HLA recorded.

Figure 2

Horizontal locomotor activity (30 min) induced by saline, cocaine (10 mg/kg or 15 mg/kg, i.p.) and MDPV (1.5 mg/kg, s.c.) administration during the five days of treatment. Bars represent mean ± SEM of the distance travelled after each injection. ***P< 0.001 vs the saline-matched day. $P<0.05 and $$$P<0.001 vs the cocaine 10 mg/kg-matched day. @@@P<0.001 vs the cocaine 15 mg/kg-matched day. ##P<0.01 and ###P<0.001 vs MDPV day 1.

Figure 3

Effect of cocaine (10 mg/kg (Coc 10) or 15 mg/kg (Coc 15), i.p.) and MDPV (1.5 mg/kg (MDPV 1.5), s.c.) treatment on the horizontal locomotor activity (30 min) induced by MDPV (A) or cocaine (B). Bars represent mean ± SEM of the distance travelled after a single MDPV (1 mg/kg, s.c.) or cocaine (8 mg/kg, i.p.) injection 10 days after treatment. **P<0.01 and ***P<0.001 vs saline group. $ P<0.05 vs cocaine 10 mg/kg.

Figure 4

Effect of a single dose of MDPV (1.5 mg/kg, s.c.) or cocaine (15 mg/kg, i.p.) on the mRNA expression encoding G9a in VS 1h and 2h post-injection (A), G9a in mPFC 2h post-injection (B), BDNF in mPFC 2h post-injection (C), and D3R in VS 2h post-injection (D). Results are expressed as mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 vs saline group. #P<0.05 vs cocaine 2h. $$$ P<0.001 vs MDPV 1h.

Figure 5

Effect of MDPV (1.5 mg/kg, s.c.) and cocaine (15 mg/kg, i.p.) repeated exposure on the mRNA expression encoding G9a in VS 2h and 24h post-treatment (A), G9a in mPFC 2h post-treatment (B), BDNF in mPFC 2h post-treatment (C), and D3R in VS 2h post-treatment (D). Results are expressed as mean ± SEM. *P<0.05 and **P<0.01 vs saline group. $P<0.05 and $$$P<0.001 vs 2h-matched drug.

Figure 6

Effect of MDPV (1.5 mg/kg, s.c.) and cocaine (15 mg/kg, i.p.) repeated exposure on protein levels in NAcc of proBDNF 2h after exposure (A), and mBDNF 2h, 24h and 10 days after the exposure (B). Results are expressed as mean ± SEM. *P<0.05 vs saline group. #P<0.05 vs MDPV.

Figure 7

Effect of 7,8-DHF (10 mg/kg, i.p.) pre-treatment on the development of locomotor sensitization to MDPV 1.5 mg/kg (A) or cocaine 15 mg/kg (B). Bars represent mean ± SEM of the distance travelled after an MDPV (1 mg/kg s.c.) or cocaine (8 mg/kg, i.p.) challenge 10 days after treatment. ***P<0.001 vs saline-treated groups. $$$P<0.001 vs vehicle + MDPV group.

Figure 8

Effect of 7,8-DHF on MDPV (1.5 mg/kg s.c.)-induced conditioned place preference. The x-axis represents the treatment group, and the y-axis represents the preference score (difference between the time (s) spent in the drug-paired compartment on the test and pre-conditioning day). ***P<0.001 vs saline group.

REFERENCES

[1] M.E. John, C. Thomas-Rozea, D. Hahn, Bath Salts Abuse leading to New Onset Psychosis and Potential for Violence, Clin. Schizophr. Relat. Psychoses. (2014) 1–14. doi:10.3371/CSRP.JORO.061314.

[2] D. Zuba, P. Adamowicz, B. Byrska, Detection of buphedrone in biological and non-biological material--two case reports, Forensic Sci. Int. 227 (2013) 15–20. doi:10.1016/j.forsciint.2012.08.034.

[3] M.H. Baumann, J.S. Partilla, K.R. Lehner, E.B. Thorndike, A.F. Hoffman, M. Holy, R.B. Rothman, S.R. Goldberg, C.R. Lupica, H.H. Sitte, S.D. Brandt, S.R. Tella, N.V. Cozzi, C.W. Schindler, Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive “bath salts” products, Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 38 (2013) 552–562. doi:10.1038/npp.2012.204.

[4] L.D. Simmler, T.A. Buser, M. Donzelli, Y. Schramm, L.-H. Dieu, J. Huwyler, S. Chaboz, M.C. Hoener, M.E. Liechti, Pharmacological characterization of designer cathinones in vitro, Br. J. Pharmacol. 168 (2013) 458–470. doi:10.1111/j.1476-5381.2012.02145.x.

[5] S.J. Simmons, R.A. Gregg, F.H. Tran, L. Mo, E. von Weltin, D.J. Barker, T.A. Gentile, L.R. Watterson, S.M. Rawls, J.W. Muschamp, Comparing rewarding and reinforcing properties between “bath salt” 3,4-methylenedioxypyrovalerone (MDPV) and cocaine using ultrasonic vocalizations in rats, Addict. Biol. (2016). doi:10.1111/adb.12479.

[6] P.W. Kalivas, B.A. Sorg, M.S. Hooks, The pharmacology and neural circuitry of sensitization to psychostimulants, Behav. Pharmacol. 4 (1993) 315–334.

[7] R. Kuczenski, D. Segal, Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis, J. Neurosci. 9 (1989) 2051–2065.

[8] R.C. Pierce, P.W. Kalivas, A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants, Brain Res. Brain Res. Rev. 25 (1997) 192–216.

[9] T.E. Robinson, K.C. Berridge, The neural basis of drug craving: an incentive-sensitization theory of addiction, Brain Res. Brain Res. Rev. 18 (1993) 247–291.

[10] J.D. Steketee, P.W. Kalivas, Drug wanting: behavioral sensitization and relapse to drug-seeking behavior, Pharmacol. Rev. 63 (2011) 348–365. doi:10.1124/pr.109.001933.
[11] P.E. Paulson, D.M. Camp, T.E. Robinson, Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats, Psychopharmacology (Berl.). 103 (1991) 480–492.
[12] T.J. De Vries, A.N.M. Schoffelmeer, R. Binnekade, H. Raasø, L.J.M.J. Vanderschuren, Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization, Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 26 (2002) 18–26. doi:10.1016/S0893-133X(01)00293-7.
[13] Y. Bjijou, L. Stinus, M. Le Moal, M. Cador, Evidence for selective involvement of dopamine D1 receptors of the ventral tegmental area in the behavioral sensitization induced by intra-ventral tegmental area injections of D-amphetamine, J. Pharmacol. Exp. Ther. 277 (1996) 1177–1187.
[14] M. Cador, Y. Bjijou, L. Stinus, Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine, Neuroscience. 65 (1995) 385–395.
[15] X. Li, M.E. Wolf, Multiple faces of BDNF in cocaine addiction, Behav. Brain Res. 279 (2015) 240–254. doi:10.1016/j.bbr.2014.11.018.
[16] C.L. Pickens, M. Airavaara, F. Theberge, S. Fanous, B.T. Hope, Y. Shaham, Neurobiology of the incubation of drug craving, Trends Neurosci. 34 (2011) 411–420. doi:10.1016/j.tins.2011.06.001.
[17] S.J. Russo, M.S. Mazei-Robison, J.L. Ables, E.J. Nestler, Neurotrophic factors and structural plasticity in addiction, Neuropharmacology. 56 Suppl 1 (2009) 73–82. doi:10.1016/j.neuropharm.2008.06.059.
[18] V. Lessmann, K. Gottmann, M. Malcangio, Neurotrophin secretion: current facts and future prospects, Prog. Neurobiol. 69 (2003) 341–374. doi:10.1016/S0301-0082(03)00019-4.
[19] J.M. Conner, J.C. Lauterborn, Q. Yan, C.M. Gall, S. Varon, Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport, J. Neurosci. Off. J. Soc. Neurosci. 17 (1997) 2295–2313.
[20] C.A. Altar, N. Cai, T. Bliven, M. Juhasz, J.M. Conner, A.L. Acheson, R.M. Lindsay, S.J. Wiegand, Anterograde transport of brain-derived neurotrophic factor and its role in the brain, Nature. 389 (1997) 856–860. doi:10.1038/39885.
[21] D.L. Graham, S. Edwards, R.K. Bachtell, R.J. DiLeone, M. Rios, D.W. Self, Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse, Nat. Neurosci. 10 (2007) 1029–1037. doi:10.1038/nn1929.
[22] U.E. Ghitza, H. Zhai, P. Wu, M. Airavaara, Y. Shaham, L. Lu, Role of BDNF and GDNF in drug reward and relapse: a review, Neurosci. Biobehav. Rev. 35 (2010) 157–171. doi:10.1016/j.neubiorev.2009.11.009.
[23] J.F. McGinty, T.W. Whitfield, W.J. Berglind, Brain-derived neurotrophic factor and cocaine addiction, Brain Res. 1314 (2010) 183–193. doi:10.1016/j.brainres.2009.08.078.
[24] Q. Ren, J.-C. Zhang, M. Ma, Y. Fujita, J. Wu, K. Hashimoto, 7,8-Dihydroxyflavone, a TrkB agonist, attenuates behavioral abnormalities and

neurotoxicity in mice after administration of methamphetamine, Psychopharmacology (Berl.). 231 (2014) 159–166. doi:10.1007/s00213-013-3221-7.
[25] F. Zheng, X. Zhou, C. Moon, H. Wang, Regulation of brain-derived neurotrophic factor expression in neurons, Int. J. Physiol. Pathophysiol. Pharmacol. 4 (2012) 188–200.
[26] H.E. Covington, I. Maze, H. Sun, H.M. Bomze, K.D. DeMaio, E.Y. Wu, D.M. Dietz, M.K. Lobo, S. Ghose, E. Mouzon, R.L. Neve, C.A. Tamminga, E.J. Nestler, A Role for Repressive Histone Methylation in Cocaine-Induced Vulnerability to Stress, Neuron. 71 (2011) 656–670. doi:10.1016/j.neuron.2011.06.007.
[27] W. Renthal, I. Maze, V. Krishnan, H.E. Covington, G. Xiao, A. Kumar, S.J. Russo, A. Graham, N. Tsankova, T.E. Kippin, K.A. Kerstetter, R.L. Neve, S.J. Haggarty, T.A. McKinsey, R. Bassel-Duby, E.N. Olson, E.J. Nestler, Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli, Neuron. 56 (2007) 517–529. doi:10.1016/j.neuron.2007.09.032.
[28] W. Renthal, E.J. Nestler, Epigenetic mechanisms in drug addiction, Trends Mol. Med. 14 (2008) 341–350. doi:10.1016/j.molmed.2008.06.004.
[29] I. Maze, H.E. Covington, D.M. Dietz, Q. LaPlant, W. Renthal, S.J. Russo, M. Mechanic, E. Mouzon, R.L. Neve, S.J. Haggarty, Y. Ren, S.C. Sampath, Y.L. Hurd, P. Greengard, A. Tarakhovsky, A. Schaefer, E.J. Nestler, Essential Role of the Histone Methyltransferase G9a in Cocaine-Induced Plasticity, Science. 327 (2010) 213–216. doi:10.1126/science.1179438.
[30] O. Guillin, J. Diaz, P. Carroll, N. Griffon, J.-C. Schwartz, P. Sokoloff, BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization, Nature. 411 (2001) 86–89. doi:10.1038/35075076.
[31] G. Di Chiara, A. Imperato, Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats., Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 5274–5278.
[32] R. Bordet, S. Ridray, S. Carboni, J. Diaz, P. Sokoloff, J.C. Schwartz, Induction of dopamine D3 receptor expression as a mechanism of behavioral sensitization to levodopa, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 3363–3367.
[33] B.A. Horger, C.A. Iyasere, M.T. Berhow, C.J. Messer, E.J. Nestler, J.R. Taylor, Enhancement of Locomotor Activity and Conditioned Reward to Cocaine by Brain-Derived Neurotrophic Factor, J. Neurosci. 19 (1999) 4110–4122.
[34] M. Buenrostro-Jáuregui, A. Ciudad-Roberts, J. Moreno, P. Muñoz-Villegas, R. López-Arnau, D. Pubill, E. Escubedo, J. Camarasa, Changes in CREB and deltaFosB are associated with the behavioural sensitization induced by methylenedioxypyrovalerone, J. Psychopharmacol. Oxf. Engl. (2016). doi:10.1177/0269881116645300.
[35] L. Duart-Castells, R. López-Arnau, M. Buenrostro-Jáuregui, P. Muñoz-Villegas, O. Valverde, J. Camarasa, D. Pubill, E. Escubedo, Neuroadaptive changes and behavioral effects after a sensitization regime of MDPV, Neuropharmacology. (2018). doi:10.1016/j.neuropharm.2018.10.005.
[36] M. Hirabayashi, S. Okada, S. Tadokoro, Comparison of sensitization to ambulation-increasing effects of cocaine and methamphetamine after repeated administration in mice, J. Pharm. Pharmacol. 43 (1991) 827–830.
[37] L. Shuster, G. Yu, A. Bates, Sensitization to cocaine stimulation in mice, Psychopharmacology (Berl.). 52 (1977) 185–190.

[38] C.N. Lessov, T.J. Phillips, Cross-sensitization between the locomotor stimulant effects of ethanol and those of morphine and cocaine in mice, Alcohol. Clin. Exp. Res. 27 (2003) 616–627. doi:10.1097/01.ALC.0000062760.17530.74.
[39] P. Vezina, J. Stewart, The effect of dopamine receptor blockade on the development of sensitization to the locomotor activating effects of amphetamine and morphine, Brain Res. 499 (1989) 108–120.
[40] J. Novellas, R. López-Arnau, M.L. Carbó, D. Pubill, J. Camarasa, E. Escubedo, Concentrations of MDPV in rat striatum correlate with the psychostimulant effect, J. Psychopharmacol. Oxf. Engl. (2015). doi:10.1177/0269881115598415.
[41] S. Reagan-Shaw, M. Nihal, N. Ahmad, Dose translation from animal to human studies revisited, FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 22 (2008) 659–661. doi:10.1096/fj.07-9574LSF.
[42] European Monitoring Centre for Drug and Drug Addiction, EMCDDA–Europol Joint Report on a new psychoactive substance: MDPV (3,4-methylenedioxypyrovalerone), Httpwwwemcddaeuropaeupublicationsjoint-ReportMDPV. (2014). about:blank (accessed September 4, 2014).
[43] R. Kolanos, E. Solis, F. Sakloth, L.J. De Felice, R.A. Glennon, “Deconstruction” of the Abused Synthetic Cathinone Methylenedioxypyrovalerone (MDPV) and an Examination of Effects at the Human Dopamine Transporter, ACS Chem. Neurosci. 4 (2013) 1524–1529. doi:10.1021/cn4001236.
[44] Q. Ren, J.-C. Zhang, Y. Fujita, M. Ma, J. Wu, K. Hashimoto, Effects of TrkB agonist 7,8-dihydroxyflavone on sensory gating deficits in mice after administration of methamphetamine, Pharmacol. Biochem. Behav. 106 (2013) 124–127. doi:10.1016/j.pbb.2013.03.016.
[45] G. Paxinos, K.B.J. Franklin, The Mouse Brain in Stereotaxic Coordinates, Gulf Professional Publishing, 2004.
[46] D.C. Rio, M. Ares, G.J. Hannon, T.W. Nilsen, Purification of RNA Using TRIzol (TRI Reagent), Cold Spring Harb. Protoc. 2010 (2010) pdb.prot5439. doi:10.1101/pdb.prot5439.
[47] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods San Diego Calif. 25 (2001) 402–408. doi:10.1006/meth.2001.1262.
[48] R. López-Arnau, M.A. Luján, L. Duart-Castells, D. Pubill, J. Camarasa, O. Valverde, E. Escubedo, Exposure of adolescent mice to 3,4-methylenedioxypyrovalerone increases the psychostimulant, rewarding and reinforcing effects of cocaine in adulthood, Br. J. Pharmacol. 174 (2017) 1161–1173. doi:10.1111/bph.13771.
[49] M.B. Forrester, Adolescent synthetic cathinone exposures reported to Texas poison centers, Pediatr. Emerg. Care. 29 (2013) 151–155. doi:10.1097/PEC.0b013e3182808ae2.
[50] C. Martin, Y. Zhang, The diverse functions of histone lysine methylation, Nat. Rev. Mol. Cell Biol. 6 (2005) 838–849. doi:10.1038/nrm1761.
[51] D.M. McCarthy, A.N. Brown, P.G. Bhide, Regulation of BDNF expression by cocaine, Yale J. Biol. Med. 85 (2012) 437–446.
[52] F.M. Vassoler, S.L. White, H.D. Schmidt, G. Sadri-Vakili, R.C. Pierce, Epigenetic Inheritance of a Cocaine Resistance Phenotype, Nat. Neurosci. 16 (2013) 42–47. doi:10.1038/nn.3280.
[53] I. Maze, D. Chaudhury, D.M. Dietz, M. Von Schimmelmann, P.J. Kennedy, M.K. Lobo, S.E. Sillivan, M.L. Miller, R.C. Bagot, H. Sun, G. Turecki, R.L. Neve, Y.L. Hurd, L. Shen, M.-H. Han, A. Schaefer, E.J. Nestler, G9a influences neuronal

subtype specification in striatum, Nat. Neurosci. 17 (2014) 533–539. doi:10.1038/nn.3670.
[54] B. Le Foll, J. Diaz, P. Sokoloff, A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning, Neuroreport. 16 (2005) 175–178.
[55] B. Le Foll, J. Diaz, P. Sokoloff, A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning, Neuroreport. 16 (2005) 175–178.
[56] F. Fumagalli, F. Moro, L. Caffino, A. Orrù, C. Cassina, G. Giannotti, A. Di Clemente, G. Racagni, M.A. Riva, L. Cervo, Region-specific effects on BDNF expression after contingent or non-contingent cocaine i.v. self-administration in rats, Int. J. Neuropsychopharmacol. Off. Sci. J. Coll. Int. Neuropsychopharmacol. CINP. 16 (2013) 913–918. doi:10.1017/S146114571200096X.
[57] J.W. Grimm, L. Lu, T. Hayashi, B.T. Hope, T.-P. Su, Y. Shaham, Time-Dependent Increases in Brain-Derived Neurotrophic Factor Protein Levels within the Mesolimbic Dopamine System after Withdrawal from Cocaine: Implications for Incubation of Cocaine Craving, J. Neurosci. 23 (2003) 742–747.
[58] D. Zhang, L. Zhang, D.W. Lou, Y. Nakabeppu, J. Zhang, M. Xu, The dopamine D1 receptor is a critical mediator for cocaine-induced gene expression, J. Neurochem. 82 (2002) 1453–1464.
[59] F. Fumagalli, L. Di Pasquale, L. Caffino, G. Racagni, M.A. Riva, Repeated exposure to cocaine differently modulates BDNF mRNA and protein levels in rat striatum and prefrontal cortex, Eur. J. Neurosci. 26 (2007) 2756–2763. doi:10.1111/j.1460-9568.2007.05918.x.
[60] C. Chen, C. Kostakis, R.J. Irvine, J.M. White, Increases in use of novel synthetic stimulant are not directly linked to decreased use of 3,4-methylenedioxy-N-methylamphetamine (MDMA), Forensic Sci. Int. 231 (2013) 278–283. doi:10.1016/j.forsciint.2013.06.007.
[61] A.K. Apawu, F.K. Maina, J.R. Taylor, T.A. Mathews, Probing the ability of presynaptic tyrosine kinase receptors to regulate striatal dopamine dynamics, ACS Chem. Neurosci. 4 (2013) 895–904. doi:10.1021/cn4000742.
[62] S.A. Carmack, J.S. Kim, J.R. Sage, A.W. Thomas, K.N. Skillicorn, S.G. Anagnostaras, The competitive NMDA receptor antagonist CPP disrupts cocaine-induced conditioned place preference, but spares behavioral sensitization, Behav. Brain Res. 239 (2013) 155–163. doi:10.1016/j.bbr.2012.10.042.
[63] T.E. Robinson, K.C. Berridge, Review. The incentive sensitization theory of addiction: some current issues, Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363 (2008) 3137–3146. doi:10.1098/rstb.2008.0093.
[64] Q.-S. Yan, M.-J. Feng, S.-E. Yan, RNA interference-mediated inhibition of brain-derived neurotrophic factor expression increases cocaine's cytotoxicity in cultured cells, Neurosci. Lett. 414 (2007) 165–169. doi:10.1016/j.neulet.2006.12.016.

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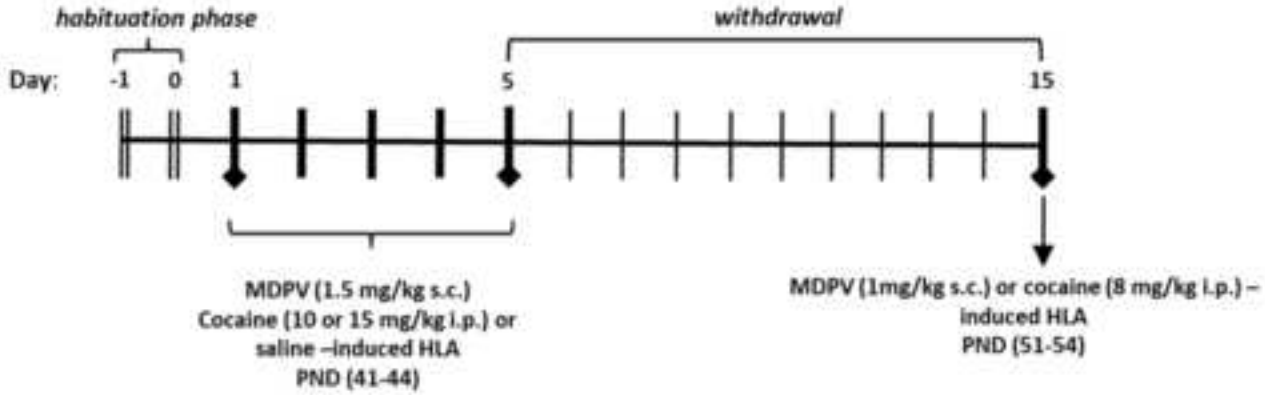

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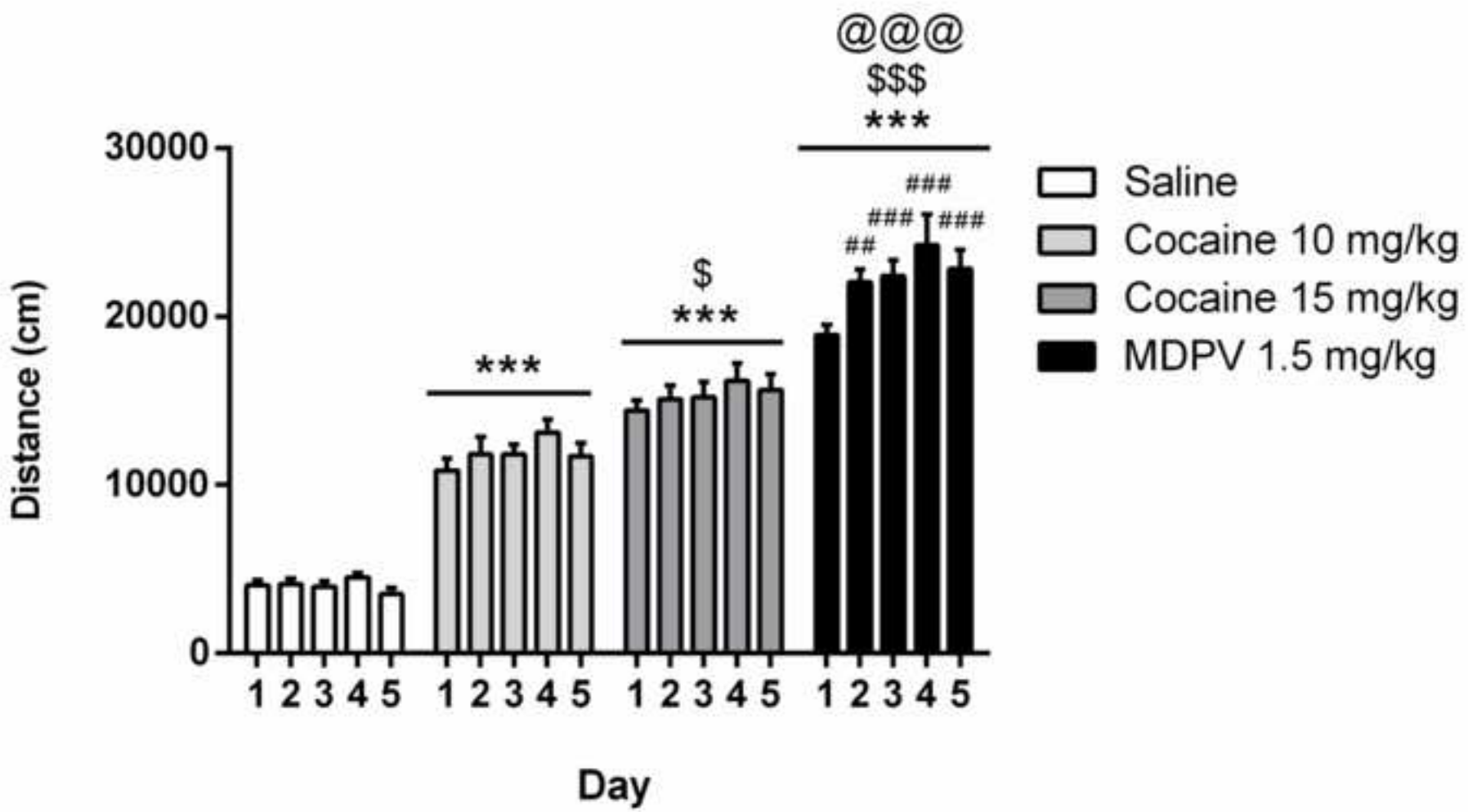

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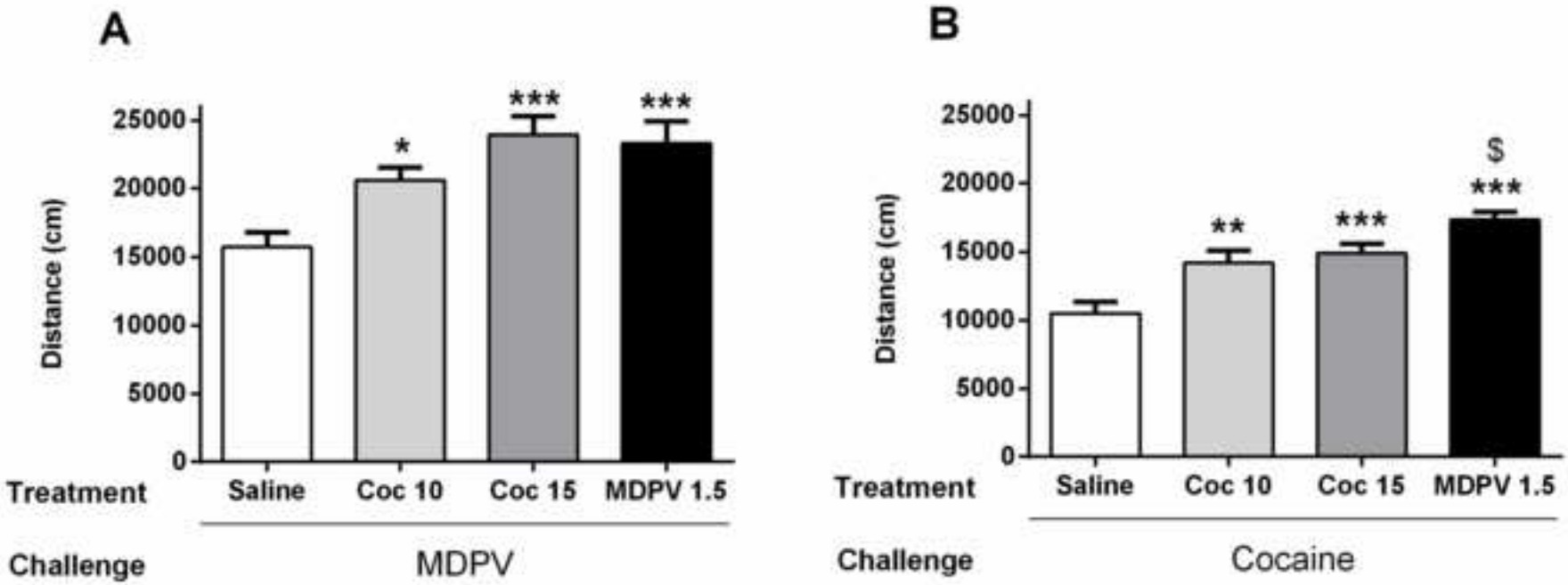

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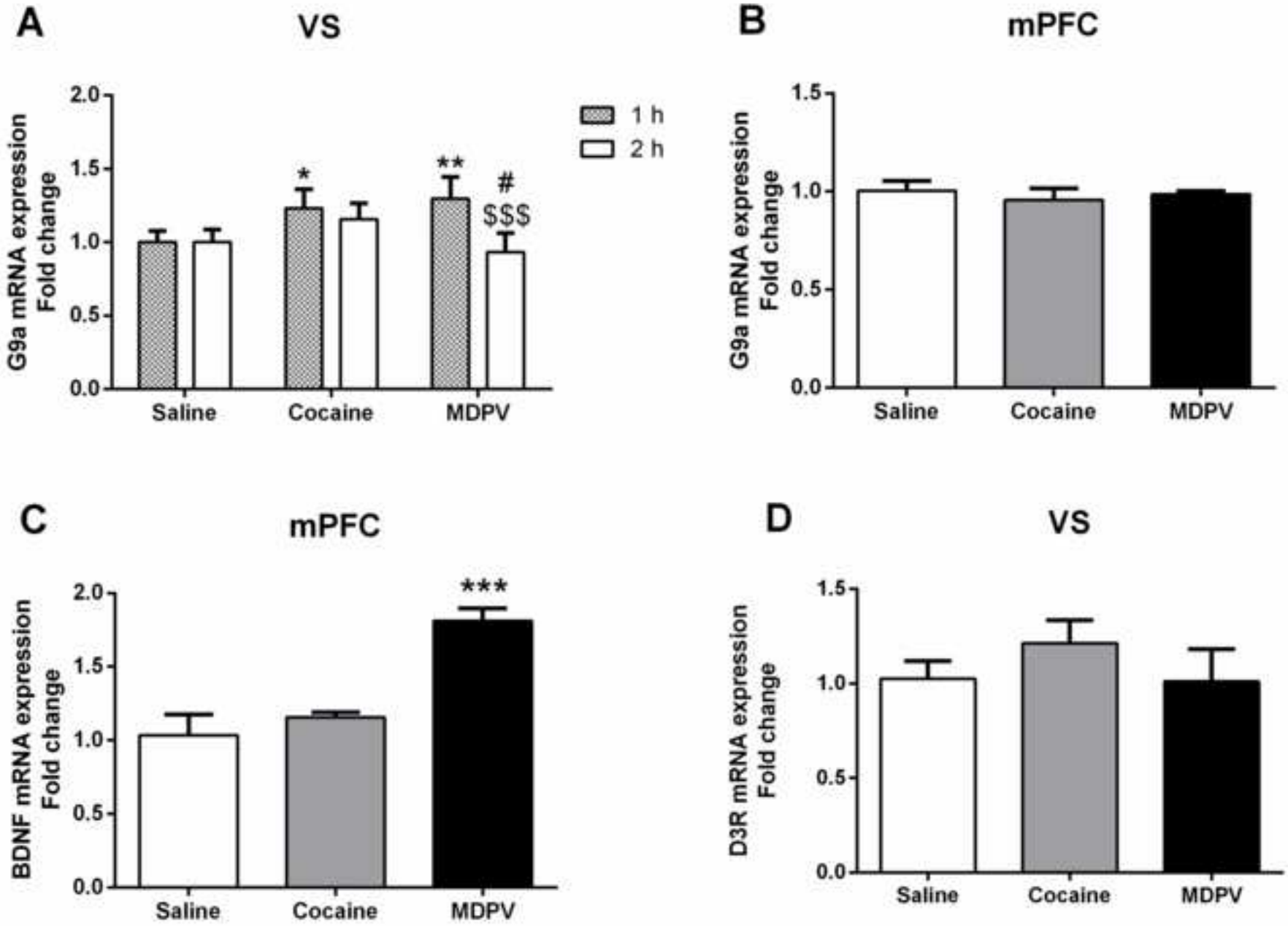

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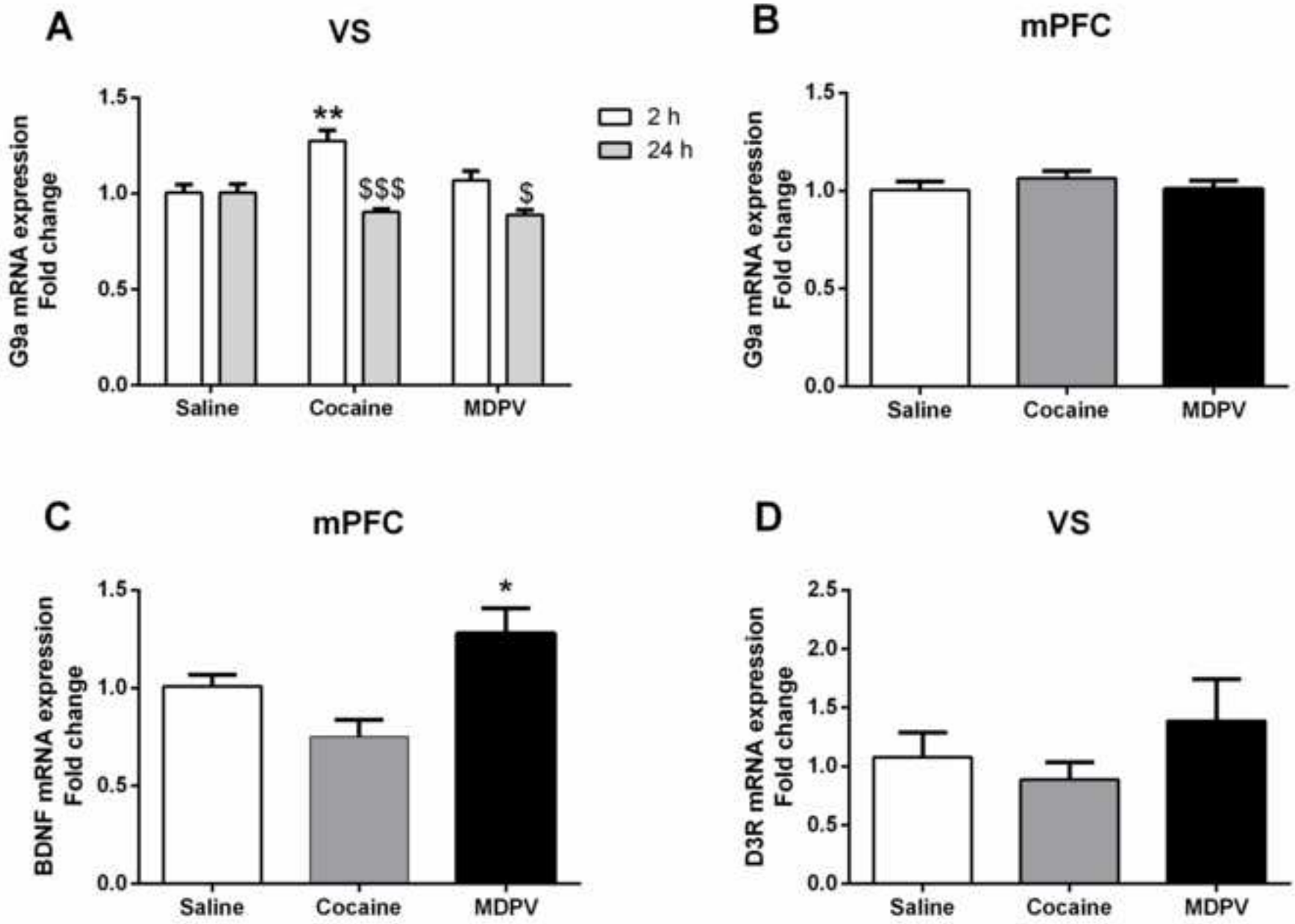

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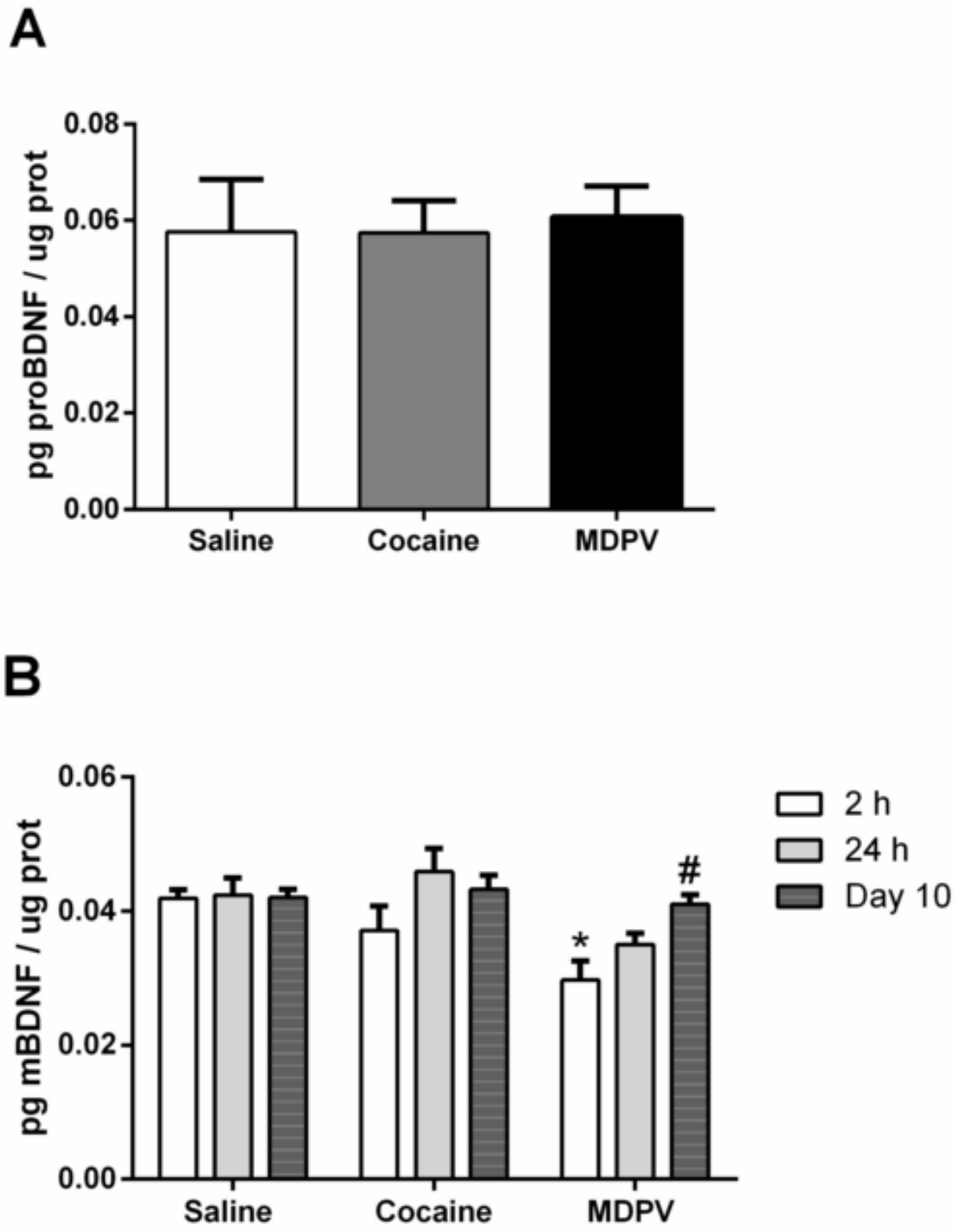

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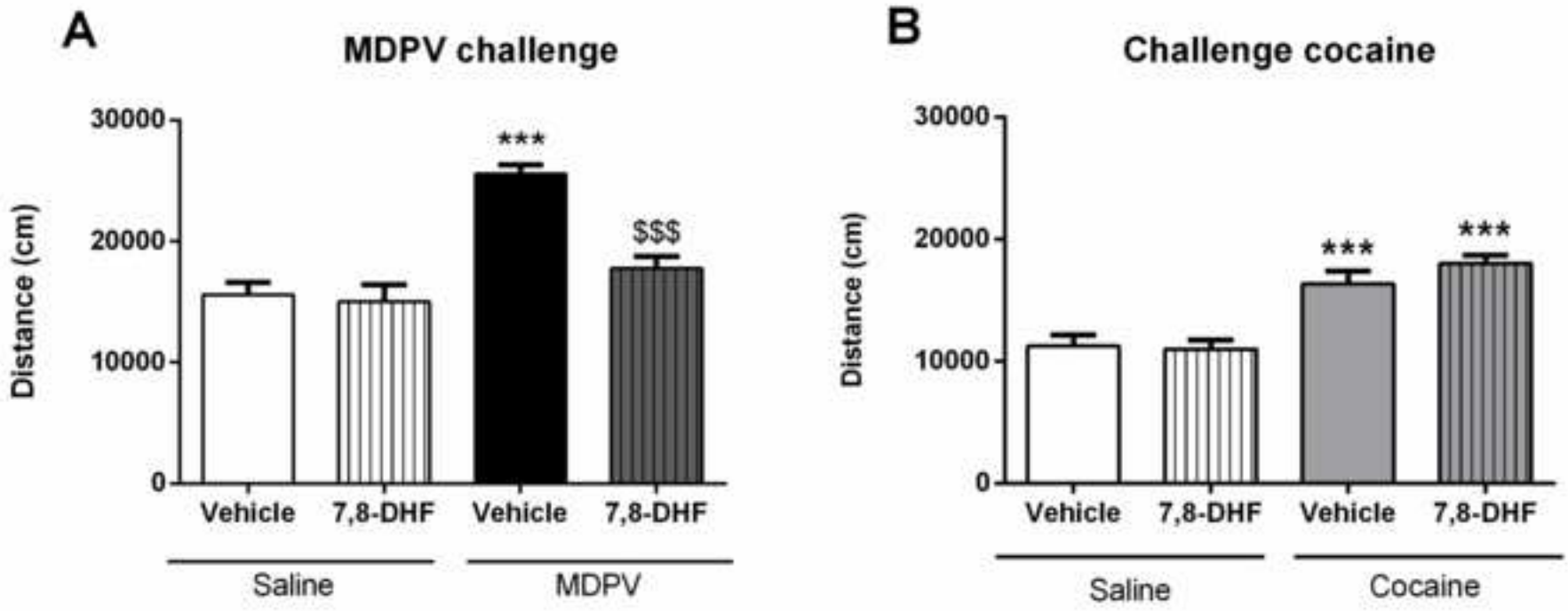

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