

**Modulation of MDMA-induced behavioral and transcriptional effects by
the delta opioid antagonist naltrindole in mice**

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Abstract

The delta opioid system is involved in the behavioral effects of various drugs of abuse. However, only few studies have focused on the possible interactions between the opioid system and the effects of MDMA. In order to examine the possible role of the delta opioid system in MDMA-induced behaviors in mice, locomotor activity and conditioned place preference were investigated in the presence of naltrindole, a selective delta opioid antagonist. Moreover, the consequences of acute and chronic MDMA administration on *Penk* (pro-enkephalin) and *Pomc* (pro-opiomelanocortin) gene expression were assessed by quantitative real-time PCR. The results showed that, after acute MDMA administration (9mg/kg; i.p.), naltrindole (5mg/kg, s.c.) was able to totally block MDMA-induced hyperlocomotion. *Penk* expression gene was not modulated by acute MDMA but a decrease of *Pomc* gene expression was observed that was not antagonized by naltrindole. Administration of the antagonist prevented the acquisition of MDMA-induced conditioned place preference, suggesting an implication of the delta opioid receptors in this behavior. Following chronic MDMA treatment only the level of *Pomc* was modulated. The observed increase was totally blocked by naltrindole pretreatment. All these results confirm the interactions between the delta opioid system (receptors and peptides) and the effects of MDMA.

Introduction

MDMA (3, 4-methylenedioxymethamphetamine, “ecstasy”) is a phenylethylamine derivative, with psychostimulant properties, widely used by young adults during night parties. MDMA has various acute effects in humans: hyperactivity, hyperthermia, cognitive disturbances, elevated anxiety, serotonin syndrome, etc. (Morgan, 2000, Morton, 2005, Parrott, 2002). Some of these effects can be found in animals (Callaway and Geyer, 1992, Gold and Koob, 1989, Green et al., 2003, O'Shea et al., 2006). Surprisingly, while the rewarding effects of MDMA have been well established in several species (monkeys, rats, mice) by the use of various paradigms, including intravenous self-administration or conditioned place preference (CPP) (Lile et al., 2005, Robledo et al., 2004, Salzman et al., 2003, Schenk et al., 2003), the mechanisms leading to these effects are still unclear. MDMA is a complex drug that may act on several neurochemical systems. This substituted amphetamine increases the extracellular concentrations of monoamines, including dopamine (DA) and serotonin (5-HT), primarily by acting as a substrate for reuptake transporters (Bankson and Cunningham, 2001, Di Chiara, 1995, Gough et al., 1991, Simantov, 2004).

The endogenous opioid system is known to be involved in the processes of reward and reinforcement. The effects induced by various psychostimulants can be modulated by the opioid system (Bilsky et al., 1992, Kuzmin et al., 1997, Suzuki et al., 1994). Naltrindole (NTI) is a selective delta-opioid receptor antagonist. The delta opioid system is notably localized in the ventral and dorsal striatum, the hippocampus and the amygdala. The implication of the delta opioid system in the effects of various drugs has been highlighted by studies showing that NTI can block or attenuate cocaine (Menkens et al., 1992; Suzuki et al., 1994a), morphine (Suzuki et al., 1994b) and methamphetamine (Suzuki et al., 1994a)-induced conditioned place preference. NTI has also been shown to block cocaine-induced facilitation

of pressing for rewarding brain stimulation (Reid et al., 1993). In the case of MDMA, previous studies have shown that the delta opioid system is involved in the effects of this drug. The delta opioid receptor antagonist NTI blocks MDMA's enhancement of pressing for reinforcing brain stimulation (Reid et al., 1996). Moreover, the delta opioid receptor interacting peptides, enkephalins have been shown to mediate at least in part the MDMA-induced hyperlocomotion in mice (Compan et al., 2003).

In order to better understand the implication of the delta opioid system in both acute and chronic MDMA effects, we investigated the effects of the delta opioid antagonist naltrindole. First, we demonstrated the total selectivity of naltrindole on delta receptor using the locomotor activity and the hot plate test. Then, we investigated the effect of naltrindole on MDMA-induced hyperlocomotion and conditioned place preference (CPP). Finally, we evaluated the consequences of acute and chronic MDMA administration on the mRNA levels of two delta-opioid receptor interacting peptides: pro-enkephalin (*Penk*) and pro-opiomelanocortin (*Pomc*) using quantitative real-time PCR (Q-PCR).

Materials and methods

Animals:

Male CD1 mice (20-22 g at the beginning of the experiments) (Charles River, L'arbresle, France) were housed in groups of 12 in a room with a 12 h light/dark cycle and temperature controlled environment (20-21°C). Food and water were provided *ad libitum*. Animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) as well as French law, with the standard ethical guidelines and under the control of the Ethical Committee of the Faculty. Every effort was made to minimize the number of animals used and their discomfort.

Chemicals and pharmacological treatments:

MDMA hydrochloride (3,4-methylenedioxymethamphetamine) was synthesized in the laboratory of Pr H. Galons (Université Paris Descartes, Paris, France). SNC80 ((+)-4-[(α R)- α -((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide) and U-50,488 (*trans*-(+)-3,4-dichloro-N-methyl-N-(2-(1 pyrrolidinyl) cyclohexyl) benzeneacetamide methanesulfonate) were purchased from TOCRIS (Missouri, USA). Methadone and naltrindole (NTI) were purchased from Sigma (Saint-Quentin Fallavier, France). MDMA, methadone, U-50,488, and naltrindole were dissolved in saline solution (0.9% NaCl). Naltrindole (5 mg/kg) was administered subcutaneously (s.c.) immediately before testing. This dose was chosen on the basis of the literature, with higher doses we would have lost the selectivity of the antagonist. It has been shown that there is no dose-effect in the CPP paradigm (Grobowski et al, 2008), the use of a higher dose of naltrindole would therefore alter the selectivity of this compound without changing its effect in that behavioural test. SNC80 (5 mg/kg, i.p.), methadone (3 mg/kg, i.p.) and U-50,488 (10 mg/kg, s.c.) were administered immediately before the test. These doses were chosen to ensure selectivity for

their respective opioids receptors respectively. The dose of MDMA (9 mg/kg, i.p.) was chosen based on previous behavioral and transcriptional studies in the same mouse strain (Salzmann et al., 2006, Salzmann et al., 2003). In all cases the injection volume was 0.1 ml / 10 g.

Locomotor activity:

The locomotor activity of mice was measured just after the drug injections during 60 min in an actimeter (Immetronic, Bordeaux, France) composed of eight cages of transparent plastic of equal size (19 x 11 x 14 cm) under low illumination (< 5 lux). One mouse was placed in each box to record its movements. Displacements were measured by photocell beams located across the long axis and above the floor. Locomotor activity was recorded for 60 minutes. Horizontal locomotor activities were expressed in scores (mean \pm s.e.m) as the number of interruptions of the photocell beams recorded on total activity (60 min).

Conditioned Place Preference (CPP) paradigm:

As previously described an unbiased place preference conditioning procedure was used (Salzmann et al., 2003). The place preference apparatus consisted of two conditioning compartments (15x15x15 cm) separated by a neutral area. The conditioning compartments were differentiated by a distinctive sensory cue: the wall-coloring pattern (black or strips). Movements and location of mice were recorded by computerized monitoring software (Video track, Viewpoint, Lyon, France). The protocol consisted of three phases. Preconditioning phase: drug-naïve mice had free access to all compartments for 20 min, and the time spent in each compartment was recorded. Conditioning phase: this phase consisted of 6 days where each conditioning chamber was closed. On the first conditioning day, mice were treated with NTI (5 mg/kg s.c.) or saline just before MDMA (9 mg/kg, i.p) or saline injection according to their group and placed immediately after the last injection in one of the conditioning

environments individually for 20 min. The following conditioning day, all mice were given saline in the opposite compartment, and this sequence was repeated during the next 4 days. The designation of drug-paired chamber was random and resulted in an approximately equal representation of the two conditioning chambers as the drug-paired chamber across groups for all experiments. Post-conditioning phase: this phase took place 24h after the final conditioning session (day 7). This phase was carried out exactly as the preconditioning phase. Results were expressed in scores (mean \pm s.e.m.) calculated as the difference between the time spent in the drug-paired compartment during the post-conditioning phase minus the time spent in the same compartment in the preconditioning phase.

Hot plate test:

The test was based on that described by Eddy and Leimbach (Eddy and Leimbach, 1953). A glass cylinder (16 cm high, 16 cm diameter) was used to keep the mouse on the heated surface (52 \pm 0.5 °C) of the plate. The latency period until the mouse jumped was registered by means of a stop-watch (cut-off time = 240 sec). Results were expressed as means of jump latency \pm s.e.m.

Dissection, RNA extraction and reverse transcription for quantitative real-time PCR (Q-PCR):

For acute treatment of MDMA, mice were killed 2h after injection of the drugs, (a different group of mice had been used for behavioural study) whereas for CPP experience mice were killed the day of the test just after behavioural study, by cervical dislocation. The brain was quickly removed, frozen in isopentane at -50°C, and placed in an acrylic matrice (David Kopf Instruments, Phymep, France) allowing the reproducible slicing of 1mm coronal sections. A section of 2 mm was cut, corresponding approximately to bregma +0.26 mm to -0.46mm according to The Mouse Brain Paxinos and Franklin Atlas (Academic Press, 2nd

edition, 2001). Dorsal striatum was then dissected free-hand on ice within the slice, frozen in isopentane and stored at -80 °C until processing.

Total RNA was extracted from bilateral striata with RNeasy Qiagen mini kit according to the lipid tissue protocol of the manufacturer (Qiagen, Courtaboeuf, France). Quantification of total RNA was assessed using a NanoDrop[®] ND-1000 spectrophotometer. Reverse transcription of RNA was performed in a final volume of 20µl containing 1X RT-PCR buffer (3mM MgCl₂, 75mM KCl and 50mM tris-HCl), 500µM each deoxynucleotide triphosphate, 20U RNasin ribonuclease inhibitor (Promega, France), 10mM DTT, 100U of superscript II RNase H⁻ reverse transcriptase (Invitrogen, France), 1.5µM random hexamers (GE Healthcare, France) and 500ng of total RNA. Samples were incubated at 25°C for 10 min, then 42°C for 30 min and at 99°C for 5 min. cDNAs were kept at -20°C.

Real-time quantitative PCR (Q-PCR):

The primer nucleotide sequences were chosen with the assistance of Oligo 6.42 software (MedProbe, Oslo, Norway). Sequences of the primers used for amplification were as followed: *Pomc* upper 5'-GCAGGGGTCTTCTCATTCC-3' and lower 5'-AGAGCCGACTGTGAAATCTG-3'; *Penk* upper 5'-ACGCCCCGAGTGGTGGAT-3' and lower 5'-GCCCCCGTATCTTTTCTC-3'. The primer nucleotide sequences used for *Hprt* (hypoxanthine guanine phosphoribosyl transferase) have been previously described (Salzmann et al, 2006). Gene expression was assessed by real-time quantitative RT-PCR. Amplification was detected by SYBR[®] Green fluorescence on 7900HT sequence detection system (Applied Biosystems, France). Thermocycling was carried out in a final volume of 20µl containing 0.5 µM of each upper and lower primer and 10µl of Power SYBR[®] Green PCR Master Mix 2X. 8µl of diluted cDNA were added to this mix. The thermal cycling conditions were 2 min at 50°C then 10 min at 95°C followed by 40 amplification cycles at

95°C for 15 sec, 65°C or 60°C for 45 sec and 95°C for 15 sec. Quantification was made on the basis of a calibration curve using cDNA from an untreated mouse striata. In addition to the genes of interest, the *Hprt* transcript was also quantified and each sample was normalized on the basis of its *Hprt* content. Results were expressed as gene of interest transcript/*Hprt* transcript.

Statistical analysis:

All series of data were analyzed with Statview 5.0 software (SAS Institute Inc., NC, USA). For locomotor activity, conditioned place preference and real time quantitative RT-PCR results, data were analyzed using one-way ANOVA between subjects, followed by a Bonferroni test for *post hoc* comparisons. The level of significance was set at $P < 0.05$.

Results:

Selectivity of the delta opioid antagonist: naltrindole

In a first series of experiments, selectivity of the delta opioid antagonist, naltrindole (5mg/kg, s.c) on opioid system was investigated. We used opioid agonists and appropriate behavioral tests.

1) Delta response

The delta response was assessed with the selective delta opioid receptor agonist SNC80 using locomotor activity test. One-way ANOVA showed a significant treatment effect ($F(3,36) = 5.567, p < 0.0033$). *Post-hoc* analysis revealed that NTI totally blocked the SNC80-induced locomotor activity. (Fig1a)

2) Mu response

The mu response was assessed with the mu opioid receptor agonist methadone using the hot plate test. One-way ANOVA showed a significant treatment effect ($F(3,39) = 77.10, p < 0.0001$). *Post-hoc* analysis revealed that NTI had no effect on methadone-induced antinociception response. (Fig1b).

3) Kappa response

The kappa response was assessed with the kappa opioid receptor agonist U-50,488 using the locomotor activity test. One-way ANOVA showed a significant treatment effect ($F(3,36) = 7.06, p = 0.0009$). *Post-hoc* analysis revealed that NTI had no effect on U50, 488-induced hypolocomotion (Fig1c).

Effect of naltrindole on MDMA-induced hyperlocomotion

One-way ANOVA showed a significant treatment effect ($F(3, 33) = 8.345, p = 0.0003$). Administration of MDMA increased locomotor activity as compared to saline-treated animals (saline/MDMA vs saline/saline). *Post-hoc* analysis revealed that NTI (5 mg/kg s.c.) was able to block this hyperactivity (saline/MDMA vs naltrindole/MDMA). No significant difference was found between saline/saline and naltrindole/saline treated animals (Fig. 2).

Effect of acute MDMA and NTI administrations on Pomc and Penk mRNA levels in the striatum:

The effects of naltrindole pretreatment on MDMA-induced *Pomc* and *Penk* gene expression were analyzed by real-time quantitative RT-PCR (Fig. 3a, b). In the case of *Pomc*, one-way ANOVA revealed significant differences in transcript levels in the striatum (Fig. 3a) ($F(3,41) = 4.745, p = 0.0062$). *Post hoc* comparisons showed a significant decrease of *Pomc* transcription 2h after MDMA administration (9 mg/kg). The administration of NTI did not alter MDMA-induced decrease of *Pomc* (saline-saline vs NTI-MDMA $p=0.05$). Thus NTI had no effect on this MDMA-induced decrease of *Pomc* mRNA level. No significant effects were observed on *Penk* gene expression following MDMA and MDMA + naltrindole ($F(3, 43) = 1.622, p = 0.1981$ (Fig. 3b).

Role of delta opioid receptors activation on the rewarding properties of MDMA:

NTI was used in order to assess the role of delta opioid receptors in MDMA-induced conditioned place preference. One-way ANOVA ($F(3, 84) = 5.682, p = 0.0014$) revealed a significant treatment effect between the four groups of animals. *Post hoc* comparisons showed a significant effect of MDMA as compared to control group (MDMA/saline vs saline/saline: P

= 0.0002) and a significant effect of naltrindole pretreatment on the acquisition of MDMA-induced conditioned place preference (saline/MDMA vs naltrindole/MDMA: $P = 0.0068$) (Fig. 4).

Effect of chronic MDMA and NTI administrations on Pomc and Penk gene expression in the striatum:

Mice were killed the day of the test just after behavioral study. One-way ANOVA showed no significant effect of MDMA chronic treatment on *Penk* transcript level (Fig 5b) ($F(3,40) = 0.669$, $p = 0.5763$), while a significant treatment effect on the *Pomc* transcript (Fig.5a) ($F(3,34) = 6.293$, $p = 0.0016$) was found. *Post hoc* comparisons showed a significant increase of *Pomc* in MDMA-treated animals as compared to control mice (MDMA/saline vs saline/saline). This increase was blocked by co-administration of the delta opioid antagonist, naltrindole. No significant difference was found between saline/saline and naltrindole/saline treated animals

Discussion

Behavioral effects of MDMA have been studied in rodents for many years (Ball and Rebec, 2005, Daza-Losada et al., 2007, Gold and Koob, 1989, Green et al., 2003), but the mechanisms underlying these effects are still poorly understood. Few studies have focused on the possible interaction between the delta opioid system and the effects of this substituted amphetamine (Compan et al., 2003; Reid et al., 1996). Indeed this system is known to be involved in the behavioral effects of various drugs of abuse such as cocaine (Menkens et al., 1992; Reid et al. 1993), morphine (Suzuki et al., 1994b) and methamphetamine (Suzuki et al., 1994a). However, only partial approaches have been used until now, studying either the peptides or the opioid receptors. These observations led us to study the role of the delta opioid receptor and the regulation of its interacting peptides to have an overview of the role that endogenous delta opioid system may have in MDMA-induced behaviors in mice. [Since Robledo and coworkers have already shown that the rewarding properties of MDMA are preserved in mice lacking \$\mu\$ -opioid receptor, we focused on the delta system because \(Robledo et al., 2004\).](#)

We first verified that, in our experimental conditions, NTI selectively blocked a delta opioid behavioral response and did not interfere with mu or kappa responses. We then studied the effect of NTI on MDMA-induced behavioral responses. Acute administration of naltrindole blocked the MDMA-induced hyperlocomotion in mice suggesting a role of the delta receptors in this behavior. Two endogenous opioid peptides may bind to these receptors: enkephalins and beta-endorphin (β -endorphin). A contribution of enkephalins in acute locomotor effects of MDMA has already been proposed by Compan et al. (Compan et al., 2003). MDMA could facilitate a release of these peptides, and their interaction with delta opioid receptor activation might increase the dopamine release (Svingos et al., 1999) leading to D1 dopamine receptor activation known to be involved in the effect of MDMA on

locomotion (Benturquia et al., 2008). Another possible mechanism would be that MDMA-induced increase of the extracellular levels of 5-HT and dopamine could increase enkephalins release (Compan et al., 2003, Gobaille et al., 1994).

In order to determine whether MDMA could modulate the genomic expression of delta opioid peptides, we studied the transcript levels of the precursors of the two peptides: *Pomc* and *Penk*. We chose to work on the dorsal striatum, this dopaminergic structure is implicated in the two studied behaviours: locomotion (Dankova et al., 1975, Hruska and Silbergeld, 1979) and reward (Hurd and Herkenham, 1993, Robbins et al., 1990). We observed that acute MDMA administration had no significant effects on the transcription level of the *Penk* gene suggesting that these peptides are not involved in the observed behaviour. However, we cannot exclude the hypothesis that the synthesis of new enkephalins might be needless, the pre-existing pool being sufficient to mediate the action of this peptide. In the case of *Pomc* gene expression, data showed that MDMA-induced a decrease that was not antagonised by naltrindole. These results suggest that MDMA exerts an inhibition of *Pomc* gene expression in the striatum that is independent of delta opioid receptors.

In order to determine whether the delta opioid system was implicated in MDMA rewarding properties we studied the effect of naltrindole on MDMA-induced CPP. In agreement with previous studies, MDMA (9mg/kg, i.p.) induced a conditioned place preference (Salzmann et al., 2003). Naltrindole significantly blocked the rewarding properties of the substituted amphetamine. These data suggest that activation of delta opioid receptors, subsequent to MDMA administration, plays an important role in the acquisition of MDMA-induced conditioned place preference consistent with an involvement of the delta opioid system in the reinforcing properties of MDMA as previously suggested by the study of Reid and coworkers (Reid et al., 1996).

In order to assess the expression levels of the opioid peptides during the post-conditioning CPP test, we studied their mRNA levels. We found that, as observed after the acute treatment, the mRNA level of *Penk* was not affected by chronic MDMA administration. In the case of *Pomc*, an increase of the mRNA level was found, that could be blocked by naltrindole pretreatment. The *Pomc* gene encode various peptides among which β -endorphin. It has been shown that β -endorphin peptide plays a key role in reinforcing properties of drugs of abuse as cocaine (Marquez et al., 2008, Roth-Deri et al., 2003), ethanol (Marinelli et al., 2003), amphetamine (Olive et al., 2001), heroin (Amalric et al., 1987) and THC (Solinas et al., 2004). It has been shown that these drugs of abuse produced elevated level of β -endorphin in mesolimbic structures. Our results suggest that the rewarding effects of MDMA may involve a strong release of β -endorphin during the CPP that would subsequently necessitate *de novo* synthesis of the peptide. Indeed, further studies are needed to assess if the observed increase in *Pomc* mRNA level, after MDMA-induced CPP, is associated or not with an increase of β -endorphin peptide level. Interestingly, an increase in 5-HT levels has been shown to induce an increase in *Pomc* expression and β -endorphin level in various brain structures (Winberg and Lepage, 1998, Zangen et al., 1999). It is therefore possible that chronic MDMA treatment increases *Pomc* expression through its action on 5-HT transporter.

Increase in *Pomc* mRNA could have been due to either the effects of repeated MDMA administration or to the effects of learning the CPP procedure. In our experimental conditions, we cannot differentiate the contributions of these two effects. The observed modulation is likely due to a combination of both effects since the learning procedure is important for the establishment of CPP.

The results of the present study demonstrate that the blockade of delta opioid receptors is able to antagonize MDMA-induced hyperlocomotion and CPP. Moreover, our molecular

results suggest that chronic MDMA administration may increase the release of endogenous opioid peptides, which activate delta opioid receptors and participate in the MDMA-induced hyperlocomotion and reward. Although, the peptide precursor affected in acute and chronic MDMA treatment is the same, the mechanisms involved seem different. Further studies are needed to determine the implication of the β -endorphin peptides in the observed modulation of the behavioral effects of MDMA by the delta opioid system.

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Titles and legend to figures

Figure 1: Selectivity of naltrindole (5 mg/kg) on delta, kappa and mu opioid receptors-induced behaviors.

Mice were injected with the delta selective agonist, SNC80 (5 mg/kg) (a) or the kappa selective agonist, U50, 488 (10 mg/kg) (c) in presence or in absence of naltrindole and were immediately placed in the actimeter to record horizontal locomotor activity for 60 min. For the mu receptor (b) mice were injected with the mu agonist, methadone (3 mg/kg, i.p.) in presence or in absence of naltrindole and were immediately placed on the hot plate. The latency period until the mouse jumped was registered. **p<0.01, ***p<0.001 as compared to saline-saline treated group, ##p<0.01, ###p<0.001 as compared to naltrindole-saline treated group, ^^ p<0.01 as compared to saline-MDMA treated group. (n = 9-10 per group).

Figure 2: Effects of naltrindole treatment on MDMA-induced horizontal locomotor activity.

Mice were injected with MDMA (9 mg/kg) ± naltrindole (5 mg/kg) and were immediately placed in the actimeter to record horizontal locomotor activity for 60 min. ***p<0.001 as compared to saline-saline treated group, ###p<0.001 as compared to naltrindole-saline treated group, ^^ p<0.01 as compared to Saline-MDMA treated group. (n = 9-10 per group).

Figure 3: Real-time quantitative PCR of *Pomc* and *Penk* gene expression on MDMA-induced hyperlocomotion.

Mice were injected with MDMA (9mg/kg) ± naltrindole (5 mg/kg) and sacrificed 2h after treatments. Expression of *Pomc* (Fig.5a) and *Penk* (Fig.5b) were analyzed by real-time quantitative RT-PCR. Results are expressed as mean ± s.e.m of gene interest/Hprt ratio (n =

12 per group). * $p < 0.05$ ** $p < 0.01$ as compared to saline-saline treated group, ### $p < 0.01$ as compared to naltrindole-saline treated group.

Figure 4: Effect of naltrindole on the acquisition of MDMA-induced CPP.

Mice (n=9-10 per group) received naltrindole (5 mg/kg) or saline immediately before MDMA (9 mg/kg) or saline during the conditioning phase. Data are expressed as mean \pm s.e.m in score values calculated as difference between post-conditioning and preconditioning time spent in the compartment associated with the drug (n = 10-12 per group). *** $p < 0.001$ as compared to saline-saline treated group, ### $p < 0.01$ as compared to naltrindole-saline treated group, ^^ $p < 0.01$ as compared to saline-MDMA treated group. (n = 9 -10 per group).

Figure 5: Real-time quantitative PCR of *Pomc* and *Penk* gene expression on MDMA-induced conditioned place preference.

Mice were conditioned with MDMA (9 mg/kg) \pm naltrindole (5 mg/kg.). Animals were sacrificed the day of the test just after behavioral study. Results are expressed as mean \pm s.e.m of gene of interest/Hprt ratio (n=10-12 per group). ** $p < 0.01$ as compared to saline-saline treated group, ### $p < 0.001$ as compared to naltrindole-saline treated group treated, ^^ $p < 0.01$ as compared to saline-MDMA treated group.