



**HAL**  
open science

## **Acute and chronic effects of methamphetamine on tele-methylhistamine levels in mouse brain: selective involvement of the D(2) and not D(3) receptor.**

Séverine Morisset-Lopez, Catherine Pilon, Joël Tardivel-Lacombe, D Weinstein, William Rostene, C Betancur, Pierre Sokoloff, Jean Charles Schwartz, Jean-Michel Arrang

### ► To cite this version:

Séverine Morisset-Lopez, Catherine Pilon, Joël Tardivel-Lacombe, D Weinstein, William Rostene, et al.. Acute and chronic effects of methamphetamine on tele-methylhistamine levels in mouse brain: selective involvement of the D(2) and not D(3) receptor.. *Journal of Pharmacology and Experimental Therapeutics*, 2002, 300 (2), pp.621-8. 10.1124/jpet.300.2.621 . hal-02082246

**HAL Id: hal-02082246**

**<https://hal.science/hal-02082246v1>**

Submitted on 15 Feb 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# **Acute and Chronic Effects of Methamphetamine on *Tele*-Methylhistamine Levels in Mouse Brain: Selective Involvement of the D<sub>2</sub> and not D<sub>3</sub> Receptor**

S. MORISSET, C. PILON, J. TARDIVEL-LACOMBE, D. WEINSTEIN, W. ROSTENE, C. BETANCUR, P. SOKOLOFF, J.-C. SCHWARTZ, and J.-M. ARRANG

*Unité de Neurobiologie et Pharmacologie Moléculaire (U.109) de l'INSERM, Centre Paul Broca, 2 ter rue d'Alésia, 75014 Paris, France (S.M., C.P., J.T.-L., D.W., P.S., J.-C.S., J.-M.A.); and Imagerie Cellulaire des Neurorécepteurs et Physiopathologie Neuroendocrinienne, Hôpital Saint-Antoine, 184 rue du Faubourg Saint-Antoine, Paris, France (W.R. C.B.)*

Running title: Methamphetamine and histaminergic neurons

Corresponding author: Jean-Michel Arrang, Unité de Neurobiologie et Pharmacologie Moléculaire (U109), Centre Paul Broca de l'INSERM, 2 ter rue d'Alésia, 75014 Paris, France. e-mail: [arrang@broca.inserm.fr](mailto:arrang@broca.inserm.fr)

List of abbreviations: t-MeHA, *tele*-methylhistamine; HA, histamine; 5-HT, 5-hydroxytryptamine; bp, base pair; (R) $\alpha$ -MeHA, (R) $\alpha$ -methylhistamine; HAL, haloperidol; KET, ketanserin; CPX, ciproxifan; MET, methamphetamine; SAL, saline solution.

Section: Neuropharmacology

**Footnotes:** This work was supported by a grant from the Fondation pour la Recherche Médicale to S.M.

## ABSTRACT

We have explored the role of endogenous dopamine in the control of histaminergic neuron activity in mouse brain regions evaluated by changes in *trans*-methylhistamine (t-MeHA) levels. *In vitro*, methamphetamine released [<sup>3</sup>H]noradrenaline but failed to release [<sup>3</sup>H]histamine from synaptosomes. *In vivo*, methamphetamine enhanced t-MeHA levels by about two-fold with ED<sub>50</sub>'s of ~1 mg/kg in caudate putamen, nucleus accumbens, cerebral cortex and hypothalamus. This response selectively involved the D<sub>2</sub> and not the D<sub>3</sub> receptor as indicated by its blockade by haloperidol and by its persistence following administration of nafadotride, a D<sub>3</sub>-receptor preferential ligand, or in (-/-) D<sub>3</sub>-receptor deficient mice. The t-MeHA response to methamphetamine was delayed as compared to the locomotor activating effect of this drug, suggesting that it is of compensatory nature. In agreement, ciproxifan, an inverse agonist known to enhance histamine neuron activity, decreased the hyperlocomotion induced by methamphetamine.

Repeated methamphetamine administration resulted in the expected sensitization to the hyperlocomotor effect of the drug but did not modify either the ED<sub>50</sub> or the E<sub>max</sub> regarding t-MeHA levels. However, it resulted in an enhanced basal t-MeHA level (+30-40%) which was sustained for at least 11 days after withdrawal in hypothalamus, striatum and cerebral cortex and suppressed by haloperidol. Hence both the acute and chronic administration of methamphetamine enhances histamine neuron activity, presumably in a compensatory manner.

Repeated methamphetamine administration also resulted in a modified balance in the opposite influences of dopamine and serotonin on histaminergic neurons as revealed by the enhanced response to haloperidol and abolished response to ketanserin, respectively.

Histamine neurons originate from the tuberomammillary nucleus in the hypothalamus and project in a highly divergent manner to many brain regions namely in the limbic system where the highest density of terminals is encountered (Schwartz et al., 1991, 1995; Onodera et al., 1994). Whereas the function of these neurons in physiological processes such as arousal, attention or hormonal controls is well documented, very little was known about their possible involvement in brain disorders.

Recently, however, several pieces of evidence have started to suggest a possible involvement of brain histaminergic neurons in the pathophysiology of schizophrenia and/or the action of typical and atypical antipsychotic agents. Overdose of various H<sub>1</sub>-receptor antagonists of the first generation was repeatedly reported to result in toxic psychoses with hallucinations resembling schizophrenia and the hallucinogenic potential of these drugs has even led to abuse (Sangalli, 1997). In several open studies famotidine, a H<sub>2</sub>-receptor antagonist, was found to improve schizophrenic patients, a finding that remains to be confirmed in control studies.

Methamphetamine, a psychotogenic drug that induces an enhanced dopamine release in schizophrenic patients (Laruelle et al., 1996), was shown to enhance histamine release in dialysates of rat striatum. This response was completely blocked by haloperidol, an antagonist at dopamine D<sub>2</sub>-like receptors (Ito et al., 1996a). Consistent with a positive influence of endogenous dopamine in the control of histamine neuron activity, we recently showed that typical antipsychotic agents, such as haloperidol, reduce the level of *te/e*-methylhistamine in mouse brain (Morisset et al., 1999).

Furthermore, repeated administration of methamphetamine, which results in behavioral sensitization to dopamine agonists, a cardinal feature of schizophrenia, was accompanied by an enhanced histamine release in rat striatum. This effect was blocked by haloperidol, presumably reflecting an increased tonic dopaminergic influence on histaminergic neurons (Ito et al., 1996a). Consistent with this proposal, Prell et al. (1995) showed that the level of *te/e*-methylhistamine, a major histamine metabolite, was significantly elevated in the cerebrospinal fluid of patients with chronic schizophrenia, either under neuroleptic treatment or not, suggesting that hyperactivity of dopaminergic transmission was associated with an enhanced activity of histaminergic neurons.

In contrast to typical neuroleptics, we recently reported that atypical antipsychotics, such as clozapine and olanzapine, enhance histamine neuron activity. This effect was attributable to blockade of 5-HT<sub>2</sub> receptors *in vivo* and suggested the existence of a balance between a positive and negative tonic influence of dopamine and serotonin, respectively, on histamine neuron activity (Morisset et al., 1999).

In the present work, we have further explored the influence of endogenous dopamine on histamine neuron activity. To this purpose we have i) assessed a possible direct histamine-releasing activity of methamphetamine *in vitro*, ii) evaluated the effects of acute and repeated treatment with methamphetamine on *tele*-methylhistamine levels in several brain areas, iii) assessed the role of D<sub>2</sub> and D<sub>3</sub> receptors in the response to methamphetamine. In addition, we have assessed the changes in the respective tonic influences of endogenous dopamine and serotonin in mice displaying behavioral sensitization to methamphetamine.

## MATERIALS AND METHODS

**Animals.** Male Swiss mice (18-20 g) (Iffa-Credo, L'Arbresle, France) were housed with free access to food and water in a room maintained at 21-22°C under a 12 h/12 h light-dark cycle with lights on from 7 a.m. The D<sub>3</sub>-receptor mutant mice were obtained as previously described (Accili et al. 1996). Subsequent generations were obtained by crossing heterozygote mice. DNA was prepared from a piece of their tail (3-5 mm), using the DNAeasy kit (Qiagen) and amplified with the mixture of primers 5'-GCA GTG GTC ATG CCA GTT CAC TAT CAG-3' and 5'-CCT GTT GTG TTG AAA CCA AAG AGG AGA GG-3', amplifying exon 3 of the wild-type D<sub>3</sub>-receptor, and 5'-TGG ATG TGG AAT GTG TGC TGC GAG-3' and 5'-GAA ACC AAA GAG AGG GCA GGA C-3', amplifying the PGK cassette of the mutated gene. Agarose gel electrophoresis allowed us to detect homozygote wild-type mice (a single band at 137 bp), homozygote mutated mice (a single band at 200 pb) and heterozygote mice (two bands at 137 and 200 bp). D<sub>3</sub> receptor mutant mice (-/-) and their wild-type (+/+) littermates were used for the experiments.

**Determination of *tele*-methylhistamine levels in brain.** Drugs dissolved in saline solution (0.9% NaCl w/v) were administered intraperitoneally. After treatment, animals were sacrificed by decapitation. The brain was dissected out and the cerebral cortex, caudate putamen, nucleus accumbens or hypothalamus were homogenized in 10 volumes (w/v) of ice-cold perchloric acid (0.4 N). The clear supernatant was stored at -20°C immediately after centrifugation (4,000 x g for 20 min). *Tele*-methylhistamine (t-MeHA) levels were determined using an enzyme-immunoassay derived from a radio-immunoassay described previously (Garbarg et al., 1989, 1992). Briefly, t-MeHA of the sample was derivatized with p-benzoquinone (BZQ) (2.8 mg/ml). The reaction was allowed to proceed at pH 7.9 for 3 h, then 2 M glycine was added to eliminate the excess of BZQ. The derivatized extract was mixed with t-MeHA-BZQ-Leu-Tyr-Acetylcholinesterase as a tracer and an antiserum raised in rabbits against t-MeHA conjugated with bovine serum albumin via p-

benzoquinone in a plate (Nunc Immuno-Plate Maxi-Sorp Surface) pretreated with swine antirabbit IgG (Cayman Chem. Co.) After incubation for 16 h at 15°C, plates were washed and the substrate for acetylcholinesterase, the Ellmann's reagent, was added. After 5 h, the optical density was measured with a Dynatech Mr 5000 at 405 nm. The limit of the detection was 5 pg of t-MeHA.

**Locomotor activity.** Mice were introduced into a IMétronic actimeter (Pessac, France), consisting in individuals boxes (length = 20 cm, width = 10 cm and height = 10 cm) placed in a quiet room. After a 30-min habituation period, locomotor activity was evaluated by numbering infra-red crossed beams during the next 90-min period. Mice received saline or methamphetamine 30 min after placement into the actimeter. When required, ciproxifan was administered 2 h before methamphetamine treatment. When locomotor sensitization elicited by repeated treatment with methamphetamine would be evaluated, animals were trained to the actimeter by being placed 1 h daily for three consecutive days before the beginning of methamphetamine-treatment.

**[<sup>3</sup>H]histamine and [<sup>3</sup>H]noradrenaline release from synaptosomes.** Release experiments with synaptosomes were performed according to Arrang et al., (1985) with slight modifications (Garbarg et al, 1992). A crude synaptosomal preparation from mouse cerebral cortex, striatum or hypothalamus, was preincubated for 30 min with [<sup>3</sup>H]L-histidine (0.4 μM) or [<sup>3</sup>H]noradrenaline ([<sup>3</sup>H]NA) (30 nM) at 37°C. Synaptosomes were then washed and resuspended in fresh 2 mM K<sup>+</sup>-Krebs-Ringer's medium. Synaptosomes were then incubated for 3 min with or without methamphetamine at various concentrations in 2 mM Krebs-Ringer's medium. Incubations were ended by a rapid centrifugation and [<sup>3</sup>H]amine levels in the supernatant were determined, either directly ([<sup>3</sup>H]NA) or after isolation by ion-exchange chromatography ([<sup>3</sup>H]HA)(Garbarg et al., 1983). As a control, synaptosomes were also depolarized for 3 min with 30 mM K<sup>+</sup> (final concentration) before measurement of the [<sup>3</sup>H]HA released in the supernatant (Garbarg et al, 1992).

**Analysis of data.** Dose-response curves were fitted with Prism program (Graph-Pad Software, San Diego, CA.). This program was also used to draw curves. Statistical evaluation of the results was performed using one-way or two-way ANOVA where appropriate. Comparisons between individual groups were conducted using the Student-Newman-Keuls procedure or the Mann-Whitney test.

**Radiochemicals and drugs.** The drugs and their sources were as follows: ciproxifan and nafadotride (Laboratoire Bioprojet, Paris, France), haloperidol (Janssen Pharmaceutica, Beerse, Belgium), ketanserin (Research Biochemicals International,

Natick, MA) and methamphetamine (Sigma, St Louis, MO, USA). L-[2,5-<sup>3</sup>H]histidine (50 Ci/mmol) and [<sup>3</sup>H]noradrenaline (48 Ci/mmol) were purchased from Amersham, UK. All drug weights are expressed as free base.

## RESULTS

**Effect of methamphetamine on spontaneous efflux of [<sup>3</sup>H]HA from synaptosomes.** Synaptosomes of mouse cerebral cortex were used to study the effect of methamphetamine on the release of newly synthesized [<sup>3</sup>H]-histamine. The spontaneous efflux of [<sup>3</sup>H]HA (at 2 mM K<sup>+</sup>) represented 15 ± 2% of the total [<sup>3</sup>H]HA in cerebral cortex synaptosomes and was not significantly modified after incubation for 3 min with methamphetamine at concentrations up to 30 µM (Fig 1). In contrast, the spontaneous efflux of [<sup>3</sup>H]NA was enhanced by methamphetamine in a concentration-dependent manner (EC<sub>50</sub> = 0.3 µM), with a maximal increase of ~80 % (Fig. 1). Whereas a highly significant [<sup>3</sup>H]HA release was evoked over spontaneous efflux by 30 mM K<sup>+</sup>, methamphetamine (1-10 µM) did not significantly modify the spontaneous efflux of [<sup>3</sup>H]HA from synaptosomes of cerebral cortex, striatum or hypothalamus (Table 1).

**Effect of a single administration of methamphetamine on t-MeHA levels and locomotor activity.** Methamphetamine (MET) enhanced t-MeHA levels in striatum (Fig.2, Table 2), cerebral cortex and hypothalamus (Table 2) in a dose-dependent manner. In the first two regions, the increase was of similar amplitude (about +150%), whereas it was of lower amplitude (about + 80%) in the hypothalamus. MET displayed a similar potency at increasing t-MeHA levels in the three brain regions, with ED<sub>50</sub> values of ~1mg/kg (Table 2). In striatum, the increase in t-MeHA levels induced by MET (2 mg/kg) was similar in the caudate putamen and nucleus accumbens (~+20% and ~+80% at 30 min and 3h following administration, respectively) (Table 3).

Saline administration did not produce any change, whereas MET 0.5- 4 mg/kg increased locomotor activity in a dose-dependent manner. However, following a higher dose of MET (8 mg/kg), the activity decreased as compared with lower doses. This effect was associated with an increase in stereotyped behaviours observed in forms of rearing, continuous sniffing, head twitching or circular movement, which competed with locomotor activity (Fig. 2).

The acute administration of MET (2 mg/kg, i.p.) increased significantly striatal t-MeHA levels as compared with controls, with a maximal change (~100%) observed 3h after administration, and increased locomotor activity showing a peak 30 min after administration (Fig.3). Whereas the hyperlocomotor activity declined rapidly, t-MeHA

levels progressively increased to reach a maximum after 3 h and then gradually decreased, returning to control values only 12 h after administration (Fig. 3). In addition, 15 h after administration, t-MeHA levels were significantly decreased (-22%), with a maximal change (-40%) observed 18 h after injection and then returned to control values 48 h after administration (Fig. 3). The MET-induced hyperlocomotor activity was significantly reduced following administration of the H<sub>3</sub>-receptor antagonist / inverse agonist ciproxifan (3 mg/kg, i.p.) (Fig. 4).

Haloperidol, a dual D<sub>2</sub>/D<sub>3</sub>-receptor antagonist used alone induced a slight but significant decrease of basal t-MeHA levels (-27%) and reversed the t-MeHA accumulation induced by methamphetamine (-86%) (Fig. 5). Nafadotride, a preferential D<sub>3</sub>-receptor antagonist, did not either modify basal t-MeHA levels, nor affect the t-MeHA accumulation induced by methamphetamine (Fig. 5). Moreover a significant increase in t-MeHA level was also observed 3 h after administration of MET (2 mg/kg, i.p.) to D<sub>3</sub>-receptor deficient mice (-/-), in the cerebral cortex (+83%), striatum (+106%) and hypothalamus (+50%) and was in a similar amplitude as that observed in control mice (+/+) (Fig. 6).

**Effect of repeated administration of methamphetamine on t-MeHA levels and locomotor activity.** In mice pretreated with two daily injections of saline or MET (2 mg/kg), the hyperlocomotor effect of MET was progressively enhanced after the 5<sup>th</sup> and 9<sup>th</sup> injection as compared with the first injection whereas the responsiveness was not modified after repeated administration of saline (Fig. 7A).

Following the same MET-pretreatment (2 mg/kg, b.i.d for 4 days), the basal t-MeHA level measured in striatum at day 5 was significantly increased (+36%), as compared with control mice (having received saline) (Fig. 7B). In the cerebral cortex or hypothalamus, the same type of change was observed but did not reach statistical significance (data not shown). In the MET-pretreated mice, striatal t-MeHA levels were increased 3h following MET (2 mg/kg) to an extent (+109%) similar to that observed in saline-pretreated mice (+130%). The response to a lower dose of MET (0.5 mg/kg) was also of the same amplitude (+60%) as in saline-pretreated mice (+56%) (Fig.7B).

Changes were also evaluated after withdrawal, i.e. in mice pretreated for 5 days with MET (2 mg/kg, b.i.d.), and studied after either a 2-day or 11-day interruption of treatment. At the 11<sup>th</sup> day of withdrawal, basal t-MeHA levels were significantly enhanced in the three brain regions analyzed, the change being the highest (+44%) in striatum where it had remained at a level similar to that already reached on day 5 of treatment (Tables 4 and 5). On the other hand, changes in striatal t-MeHA levels induced by MET did not show any modification in responsiveness to the drug: neither the ED<sub>50</sub> nor the E<sub>max</sub> were significantly modified



at any time during the treatment or withdrawal periods. In contrast mice still displayed increased locomotor responsiveness to MET during the withdrawal period when it was even more pronounced than at day 5 of the treatment period (Table 5).

At the 11<sup>th</sup> day of withdrawal, the decrease in striatal t-MeHA levels elicited by haloperidol was enhanced in MET-sensitized mice whereas the opposite response to ketanserin was abolished and the response to ciproxifan was not significantly modified (Fig.8).

## DISCUSSION

Our results show that methamphetamine affects histaminergic neuron activity in brain both acutely and in a long-term fashion. A single administration of methamphetamine enhances t-MeHA levels markedly in all brain regions analyzed. This response occurs with ED<sub>50</sub>'s of about 1 mg/kg, in the same range as those required to elicit the hyperlocomotor response in the same animals, whereas at doses above 4 mg/kg the locomotion is reduced in relationship with the appearance of stereotypies (Hirabayashi and Alam, 1981). The enhanced t-MeHA level very likely reflects an enhanced activity of all histaminergic neurons which is consistent with increased histamine levels in microdialysates of rat striatum (Ito et al., 1996a). There is no evidence for selective projections of histaminergic subpopulations to particular brain regions (Köhler et al., 1985). However, the maximal effect of methamphetamine on t-MeHA levels was higher in the cerebral cortex and striatum than in the hypothalamus. Its influence on histamine levels was also stronger in the cortex and striatum than in the diencephalon (Ito et al., 1996b). These findings may reveal some functional heterogeneity among histaminergic neurons, as suggested from the regulation of histamine release by presynaptic galanin receptors (Arrang et al., 1991).

This response to methamphetamine does not reflect a direct effect of the drug upon histaminergic neurons since it did not release [<sup>3</sup>H]histamine from synaptosomes. In contrast, its expected effect on [<sup>3</sup>H]noradrenaline release was observed in the same preparations. Releasing effects of amphetamines on catecholamines and indolamines result mainly from their interaction with the plasma membrane transporters rather than with the vesicular monoamine transporter VMAT2 (Giros et al., 1996). VMAT2 transports histamine and is expressed in histaminergic neurons (Peter et al., 1995), whereas no active uptake of HA could be evidenced at the plasma membrane (Schwartz et al., 1991). This difference between histamine and other monoamine neurons accounts for the lack of direct histamine-releasing effect of methamphetamine that we evidence here.

Our data show that the enhancing effect of methamphetamine on t-MeHA levels results from the stimulation of histaminergic neurons by endogenous dopamine

activating selectively D<sub>2</sub> receptors. In agreement, this effect was completely blocked by haloperidol, a D<sub>2</sub>/D<sub>3</sub>-receptor antagonist, but remained unchanged either after administration of nafadotride used at a dose inducing a selective blockade of the D<sub>3</sub> receptor (Sautel et al., 1995), or in the brain of mice lacking functional D<sub>3</sub> receptors. In agreement, endogenous dopamine released by methamphetamine enhances striatal histamine release by interacting with D<sub>2</sub>-like receptors (Ito et al., 1996a). Moreover, we have shown that haloperidol as well as other "typical" neuroleptics (Morisset et al., 1999) decrease basal t-MeHA levels by ~20%, thereby revealing a tonic activation of histaminergic neurons by endogenous dopamine under basal conditions. Using [<sup>125</sup>I]iodosulpride as a ligand, D<sub>2</sub>-like receptor binding sites have been evidenced by autoradiography at the level of the tuberomammillary nucleus (Bouthenet et al., 1987), an area in which D<sub>3</sub> receptors could not be detected (J. Diaz, personal communication). Therefore, endogenous dopamine may directly activate histamine neurons by interacting with D<sub>2</sub> receptors located upon their perikarya or dendrites, as also supported by retrograde transport studies showing that some of the dopamine-containing axons emanating from the ventral tegmental area or substantia nigra project to the tuberomammillary nucleus (Ericson et al., 1989). D<sub>2</sub> receptors located on histaminergic nerve endings do not seem to be involved since apomorphine fails to significantly affect histamine release from slices of rat cerebral cortex (Schwartz et al., 1990). Although it cannot be entirely ruled out, some involvement of endogenous serotonin in the enhancing effect of methamphetamine on t-MeHA levels seems unlikely. Serotonin was reported to induce histamine release following local perfusion in the rat hypothalamus (Laitinen et al., 1995) and to increase the firing rate of tuberomammillary neurons *in vitro* (Eriksson et al., 2001). However, these local stimulations may play a minor part in the overall *in vivo* regulation of histamine neurons by endogenous serotonin since we recently showed that histamine neuron activity is under tonic inhibition by endogenous serotonin via 5-HT<sub>2</sub> receptors (Morisset et al., 1999).

Although our findings leave little doubt that the changes in t-MeHA levels induced by methamphetamine are due to an increase in dopamine release, microdialysis studies have shown that maximal extracellular dopamine concentrations are generally achieved within 30 min after drug administration, then declining to basal levels within 3h (Kuczenski and Segal, 1999a). In contrast the changes in t-MeHA levels were maximal at 3h and still highly significant 9h after drug administration, demonstrating a dissociation in the time-course of the two methamphetamine-induced responses. A dissociation was also evidenced in the temporal profiles of dopamine release and stereotypies induced by a single administration of amphetamine and has been explained by the rapid development of a hypersensitivity of dopamine receptors, allowing their activation by low

concentrations of dopamine (Kuczenski and Segal, 1999b). The activation of supersensitive D<sub>2</sub> receptors by endogenous dopamine might also result in the strong and long-lasting changes in t-MeHA levels induced by methamphetamine.

The hyperlocomotor effect observed after drug administration did not parallel either the increase in striatal t-MeHA levels suggesting that histaminergic neurons are not directly involved in the locomotor response to methamphetamine. Even more, the comparison of the two temporal profiles suggests that histaminergic neurons are involved in a compensatory manner in the modulation of this response, inasmuch as t-MeHA levels were also increased in the nucleus accumbens, known to be associated with the regulation of locomotor function (Svensson et al., 1995). Consistent with this proposal, enhanced release of endogenous histamine attenuates stimulant-induced locomotor activity (Itoh et al., 1984; Clapham and Kilpatrick, 1994; Ito et al., 1997), and ciproxifan, a H<sub>3</sub>-receptor inverse agonist known to enhance histamine neuron activity (Morisset et al., 2000a), strongly decrease methamphetamine-induced hyperlocomotion.

Histamine neuron activity exhibits a circadian rhythm and is the highest during arousal, that is during the dark phase in rodents (Schwartz et al., 1991; 1995). In agreement, t-MeHA levels are maximal in the brain during this dark phase (Morisset et al., 2000b). In the present study, methamphetamine was administered during the light phase, that is, when histamine neuron activity is the lowest. The long-lasting decrease in basal t-MeHA levels observed 15h after drug administration might reflect a disruption in the normal circadian pattern of histaminergic neuron activity by the psychomotor stimulant drug.

Repeated exposure to amphetamines is well known to produce behavioral sensitization, characterized by an augmented locomotor response to a subsequent challenge injection. In agreement with previous studies (Hirabayashi and Alam, 1981; Kolta et al., 1985), the intensity of the sensitized behavioral response in our mice was higher at extended withdrawal periods than shortly after the cessation of drug treatment. The effect of a challenge injection of methamphetamine on striatal t-MeHA levels remained unchanged in sensitized animals after the treatment and at any withdrawal times suggesting that histaminergic neurons are not directly involved in the initiation or expression of the locomotor response. However, basal t-MeHA levels were enhanced in various brain regions of sensitized mice showing that repeated administration of methamphetamine induced a long-lasting enhancement of histaminergic neuron activity in the whole brain, which is consistent with the increase in histamine release observed in the striatum of sensitized rats (Ito et al., 1996a). Like the response to acute administration, this effect of chronic treatment with methamphetamine on t-MeHA levels was blocked by haloperidol, strongly suggesting that it resulted from a sensitized release of dopamine from dopaminergic afferents,

leading to a higher degree of activation of D<sub>2</sub> receptors. Although no single neuronal system is likely to be responsible for behavioral sensitization, the increased releasability of dopamine in the nucleus accumbens and striatum of animals sensitized to amphetamine is well documented (Kalivas and Stewart, 1991; Pierce and Kalivas, 1997). Moreover, the role of an enhanced dopamine tone in the enhanced histamine neuron activity observed during sensitization is confirmed by the time-course of changes in basal t-MeHA levels: levels were clearly enhanced immediately following the cessation of the drug treatment and after a long withdrawal period (11 days), but not significantly modified after a shorter withdrawal (2 days), a pattern that parallels sensitized release of dopamine (Paulson and Robinson, 1996; Pierce and Kalivas, 1997).

Our results also indicated that the balance between tonic dopamine and serotonin influences on histamine neuron activity was modified in sensitized animals. As previously described (Morisset et al., 1999), endogenous dopamine (via D<sub>2</sub> receptors) and serotonin (via 5-HT<sub>2</sub> receptors) exerted opposite tonic influences in control mice. However the serotonin influence, of a much larger amplitude than the dopamine influence in controls, was abolished in sensitized animals, as revealed by the disappearance of t-MeHA-enhancing response to ketanserin. Amphetamines also interact with the 5-HT transporter (Hoffman et al., 1991) but the involvement of 5-HT neurons in behavioral sensitization seems unclear (Pierce and Kalivas, 1997).

Behavioral sensitization to psychostimulants may represent an animal model of psychostimulant-induced and idiopathic psychosis (Pierce and Kalivas, 1997). It is interesting that enhanced t-MeHA levels in CSF of schizophrenic patients were reported in one study (Prell et al., 1995), indicating that histamine neuron hyperactivity might be present in both the human disease and the rodent model. The apparent compensatory nature of this hyperactivity, its reduction by antipsychotics and the high density of histaminergic terminals in limbic brain areas, suggests that brain histamine receptors may represent novel targets for the therapeutics of psychotic disorders, an hypothesis which remains to be explored in the case of H<sub>2</sub> and H<sub>3</sub> receptors.

### **Acknowledgements**

We are grateful to A. Galtier for processing this manuscript.

### **REFERENCES**

- Accili D, Fishburn CS, Drago J, Steiner H, Lachowicz JE, Park BH, Gauda EB, Lee EJ, Cool MH, Sibley DR, Gerfen CR, Westphal H and Fuchs S (1996) A target mutation of the D<sub>3</sub> dopamine receptor gene is associated with hyperactivity in mice. *Proc Natl Acad Sci USA* **93**: 1945-1949.

- Arrang JM, Garbarg M and Schwartz JC (1985) Autoregulation of histamine release in brain by presynaptic H<sub>3</sub>- receptors, *Neuroscience* **15**: 553-562.
- Arrang JM, Gulat-Marnay C, Defontaine N and Schwartz JC (1991) Regulation of histamine release in rat hypothalamus and hippocampus by presynaptic galanin receptors, *Peptides* **12**: 1113-1117.
- Bouthenet ML, Martres MP, Sales N and Schwartz JC (1987) A detailed mapping of dopamine D-2 receptors in rat central nervous system by autoradiography with [<sup>125</sup>I]iodosulpride, *Neuroscience* **20**: 117-155.
- Clapham J and Kilpatrick GJ (1994) Thioperamide, the selective histamine H<sub>3</sub> receptor antagonist, attenuates stimulant-induced locomotor activity in the mouse, *Eur J Pharmacol* **259**: 107-114.
- Ericson H, Blomqvist A and Kohler C (1989) Brainstem afferents to the tuberomammillary nucleus in the rat brain with special reference to monoaminergic innervation, *J Comp Neurol* **281**: 169-192.
- Eriksson KS, Stevens DR and Haas HL (2001) Serotonin excites tuberomammillary neurons by activation of Na<sup>+</sup>/Ca<sup>2+</sup>-exchange, *Neuropharmacology* **40**: 345-351.
- Garbarg M, Arrang JM, Rouleau A, Ligneau X, Tuong MD, Schwartz JC and Ganellin CR (1992) S-[2-(4-imidazolyl)ethyl]isothiourea, a highly specific and potent histamine H<sub>3</sub> receptor agonist, *J Pharmacol Exp Ther* **263**: 304-310.
- Garbarg M, Pollard H, Dam Trung Tuong M, Schwartz JC and Gros C (1989) Sensitive radioimmunoassays for histamine and *tele*-methylhistamine in the brain, *J Neurochem* **53**: 1724-1730.
- Garbarg M., Pollard H., Quach T.T. and Schwartz JC. (1983) Methods in brain histamine research, in *Methods in Biogenic Amines Research* (Parvez S., Nagatsu T., Nagatsu I. and Parvez H., eds), pp.623-662. Elsevier Science Publishers, Amsterdam.
- Giros B, Jaber M, Jones SR, Wightman RM and Caron MG (1996) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter, *Nature* **379**: 606-612.
- Hirabayashi M and Alam MR (1981) Enhancing effect of methamphetamine on ambulatory activity produced by repeated administration in mice, *Pharmacol Biochem Behav* **15**: 925-932.
- Hoffman BJ, Mezey E and Brownstein MJ (1991) Cloning of a serotonin transporter affected by antidepressants, *Science* **254**: 579-580.
- Ito C, Onodera K, Sakurai E, Sato M and Watanabe T (1996a) Effects of dopamine antagonists on neuronal histamine release in the striatum of rats subjected to acute and chronic treatments with methamphetamine, *J Pharmacol Exp Ther* **279**: 271-276.
- Ito C, Onodera K, Sakurai E, Sato M and Watanabe T (1996b) The effect of methamphetamine on histamine level and histidine decarboxylase activity in the rat brain, *Brain Res* **734**: 98-102.
- Ito C, Onodera K, Watanabe T and Sato M (1997), Effects of histamine agents on methamphetamine-induced stereotyped behavior and behavioral sensitization in rats, *Psychopharmacology (Berl)* **130**: 362-367.
- Itoh Y, Nishibori M, Oishi R and Saeki K (1984) Neuronal histamine inhibits methamphetamine-induced locomotor hyperactivity in mice, *Neurosci Lett* **48**: 305-309.
- Kalivas PW and Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity, *Brain Res Brain Res Rev* **16**: 223-244.
- Köhler CLW, Swanson L, Haglund L and Wu JW (1985) The cytoarchitecture, histochemistry, and projections of the tuberomammillary nucleus in the rat, *Neuroscience* **16**: 85-110.
- Kolta MG, Shreve P, De Souza V and Uretsky NJ (1985) Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pretreatment with amphetamine, *Neuropharmacology* **24**: 823-829.
- Kuczenski R and Segal DS (1999a) Sensitization of amphetamine-induced stereotyped behaviors during the acute response, *J Pharmacol Exp Ther* **288**: 699-709.

- Kuczenski R and Segal DS (1999b) Sensitization of amphetamine-induced stereotyped behaviors during the acute response: role of D<sub>1</sub> and D<sub>2</sub> dopamine receptors, *Brain Res* **822**: 164-174.
- Laitinen KSM, Tuomisto L and Laitinen JT (1995) Endogenous serotonin modulates histamine release in the rat hypothalamus as measured by in vivo microdialysis, *Eur J Pharmacol* **285**: 159-164.
- Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, D'Souza CD, Erdos J, McCance E, Rosenblatt W, Fingado C, Zoghbi SS, Baldwin RM, Seibyl JP, Krystal JH, Charney DS and Innis RB (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects, *Proc Natl Acad Sci USA*, **93**: 9235-9240.
- Morisset S, Sahm UG, Traiffort E, Tardivel-Lacombe J, Arrang JM and Schwartz JC (1999) Atypical neuroleptics enhance histamine turnover in brain via 5-hydroxytryptamine<sub>2A</sub> receptor blockade, *J Pharmacol Exp Ther* **288**: 590-596.
- Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-lacombe, J, Stark, H, Schunack W, Ganellin CR, Schwartz JC and Arrang JM (2000a) High constitutive activity of native H<sub>3</sub> receptors regulates histamine neurons in brain, *Nature* **408**: 860-864.
- Morisset S, Traiffort E, Arrang JM and Schwartz JC (2000b) Changes in histamine H<sub>3</sub>-receptor responsiveness in mouse brain. *J Neurochem* **74**: 339-346.
- Onodera K, Yamatodani A, Watanabe T and Wada H (1994) Neuropharmacology of the histaminergic neuron system in the brain and its relationship with behavioral disorders, *Prog Neurobiol* **42**: 685-702.
- Paulson PE and Robinson TE (1996) Regional differences in the effects of amphetamine withdrawal on dopamine dynamics in the striatum. Analysis of circadian patterns using automated on-line microdialysis, *Neuropsychopharmacology* **14**: 325-337.
- Peter D, Liu Y, Sternini C, de Giorgio R, Brecha N and Edwards, RH (1995) Differential expression of two vesicular monoamine transporters. *J Neurosci*, **15**: 6179-6188.
- Pierce RC and Kalivas PW (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants, *Brain Res Rev* **25**: 192-216.
- Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz JC, Everitt BJ and Sokoloff P (1999) Selective inhibition of cocaine-seeking behaviour by a partial dopamine D<sub>3</sub> receptor agonist, *Nature* **400**: 371-375.
- Prell GD, Green JP, Kaufmann CA, Khandelwal JK, Morrishow AM, Kirch DG, Linnoila M and Wyatt RJ (1995) Histamine metabolites in cerebrospinal fluid of patients with chronic schizophrenia: their relationships to levels of other aminergic transmitters and ratings of symptoms, *Schizophr Res* **14**: 93-104.
- Sangalli BC (1997) Role of the central histaminergic neuronal system in the CNS toxicity of the first generation H<sub>1</sub>-antagonists, *Prog Neurobiol* **52**: 145-157.
- Sautel F, Griffon N, Sokoloff P, Schwartz JC, Launay C, Simon P, Costentin J, Schoenfelder A, Garrido F et Mann A. (1995) Nafadotride, a potent preferential dopamine D<sub>3</sub> receptor antagonist, activates locomotion in rodents, *J Pharmacol Exp Ther* **275**: 1239-1246.
- Schwartz, JC, Arrang, JM, Garbarg, M, Gulat-Marnay, C and Pollard, H. (1990). Modulation of histamine synthesis and release in brain via presynaptic autoreceptors and heteroreceptors. *Ann N Y Acad Sci* **604**:40-54.
- Schwartz JC, Arrang JM, Garbarg M, Pollard H and Ruat M (1991) Histaminergic transmission in the mammalian brain, *Physiol Rev* **71**: 1-51.
- Schwartz JC, Arrang JM, Garbarg M and Traiffort E, (1995) Histamine, in *Psychopharmacology: The Fourth Generation of Progress*, (Bloom FE and Kupfer eds), pp . 397-405, Raven Press, New York.
- Svensson A, Carlsson ML and Carlsson A, (1995) Crucial role of the accumbens nucleus in the neurotransmitter interactions regulating motor control in mice, *J Neural Transm Gen Sect* **101**:127-148.

**TABLE 1. Effect of methamphetamine on [<sup>3</sup>H]HA release from synaptosomes of mouse cerebral cortex, striatum or hypothalamus**

Synaptosomes allowed to synthesise [<sup>3</sup>H]HA from [<sup>3</sup>H]L-histidine, were suspended in fresh 2mM K<sup>+</sup>-Krebs-Ringer's medium and incubated with methamphetamine (1 or 10 μM) for 3 min. [<sup>3</sup>H]HA in the supernatant was evaluated after isolation by ion-exchange chromatography. Values are means ± S.E.M. of 6 determinations obtained in 2 experiments.

	[ <sup>3</sup> H]HA release (cpm/incubation)		
	Cortex	Striatum	Hypothalamus
Control	271 ± 6	854 ± 4	1500 ± 17
30 mM K <sup>+</sup>	456 ± 7 *	1490 ± 18 *	2763 ± 26 *
1 μM MET	277 ± 3	849 ± 17	1625 ± 10
10 μM MET	286 ± 2	906 ± 7	1602 ± 15

\**p* < 0.01 vs corresponding controls in Mann-Whitney test.

**TABLE 2. Effect of methamphetamine on t-MeHA levels in various mouse brain regions**

Mice received methamphetamine in increasing dosages and were sacrificed 3h after administration of the drug. Values were calculated from dose-response curves similar to that shown in Figure 1. The maximal responses corresponded to levels of 534 ± 27, 312 ± 14 and 225 ± 12 ng/g in the hypothalamus, striatum and cerebral cortex respectively. Values are means ± S.E.M. of data from 12 to 15 animals.

	Hypothalamus	Striatum	Cortex
ED <sub>50</sub> (mg/kg)	0.8 ± 0.3	1.1 ± 0.2	0.7 ± 0.1
Maximal Effect (% of control)	177 ± 5	255 ± 11	256 ± 7

**TABLE 3. Effect of methamphetamine on t-MeHA levels in caudate putamen and nucleus accumbens**

Mice received methamphetamine (2 mg/kg; i.p.) and were sacrificed 30 min or 3h after administration of the drug. Percent changes as compared with controls are indicated between parentheses. Values are means  $\pm$  S.E.M. of data from 5-8 animals.

Time after treatment	t-MeHA levels (ng/g)	
	Caudate putamen	Nucleus accumbens
Controls	86 $\pm$ 17	182 $\pm$ 11
<i>t</i> = 30 min	108 $\pm$ 10 (+ 25%)	219 $\pm$ 10 (+ 21%)
<i>t</i> = 3 h	151 $\pm$ 21 * (+ 75%)	329 $\pm$ 29 * (+ 81%)

\**p* < 0.01 vs corresponding controls in Mann-Whitney test.

**TABLE 4. Changes in basal t-MeHA levels in three brain regions of mice sensitized to methamphetamine**

Mice were treated twice a day for 5 days with 2mg/kg of methamphetamine (sensitized) or saline (controls) and were sacrificed 11days after the last administration. Means  $\pm$  SEM of values from 30 animals.

Treatment	t-MeHA levels (ng/g)		
	Hypothalamus	Striatum	Cortex
Controls	233 $\pm$ 14	101 $\pm$ 16	70 $\pm$ 5
Sensitized	287 $\pm$ 18* (+24%)	145 $\pm$ 8** (+44%)	87 $\pm$ 5* (+23%)

\**p* < 0.05; \*\**p* < 0.001 vs corresponding controls in Mann-Whitney test.



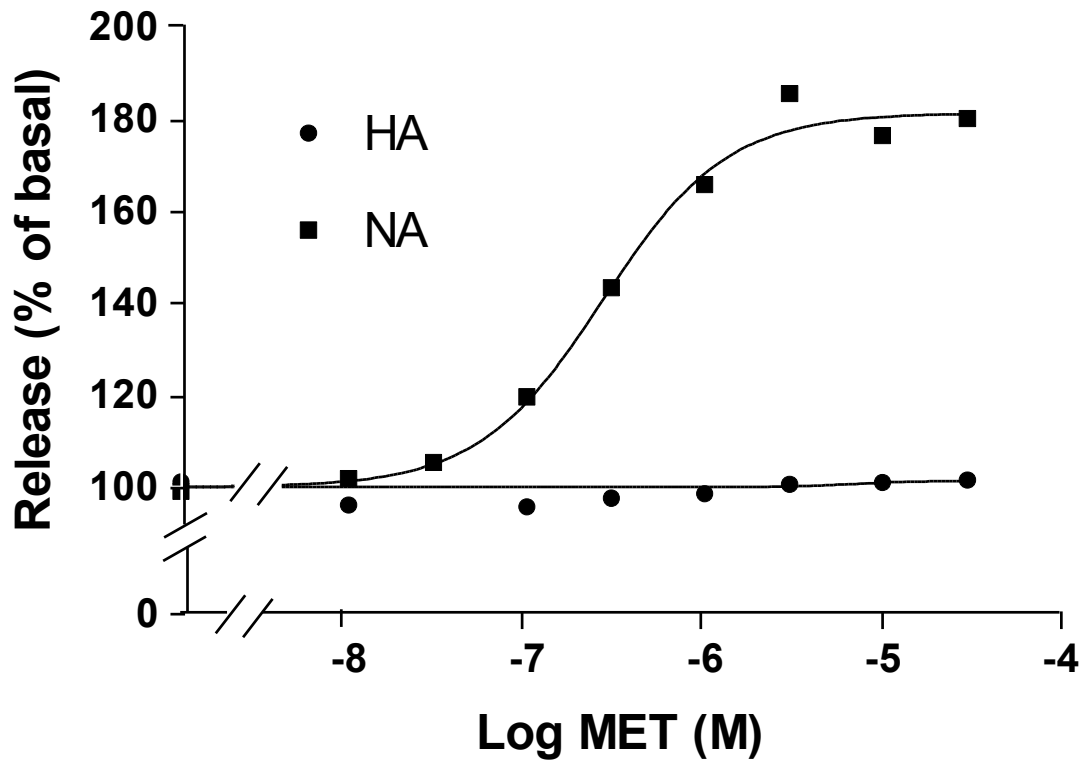
**TABLE 5. Effects of methamphetamine on striatal t-MeHA levels and locomotor activity**

Mice received methamphetamine (2mg/kg b.i.d.) (sensitized) or saline (controls) for 5 days. At the end of the treatment (day 5) and after either a 2-day or a 11-day interruption (days 8 and 17 respectively), changes in t-MeHA levels were evaluated 3h following the administration of saline (basal levels) or methamphetamine in increasing dosages. Values were derived from the dose-response curves. The maximal responses i.e. corresponding to striatal levels of  $260 \pm 14$ ,  $247 \pm 23$ ,  $290 \pm 33$ ,  $290 \pm 33$  ng/g for days 1, 5, 8 and 17 respectively, were not significantly modified in sensitized mice.

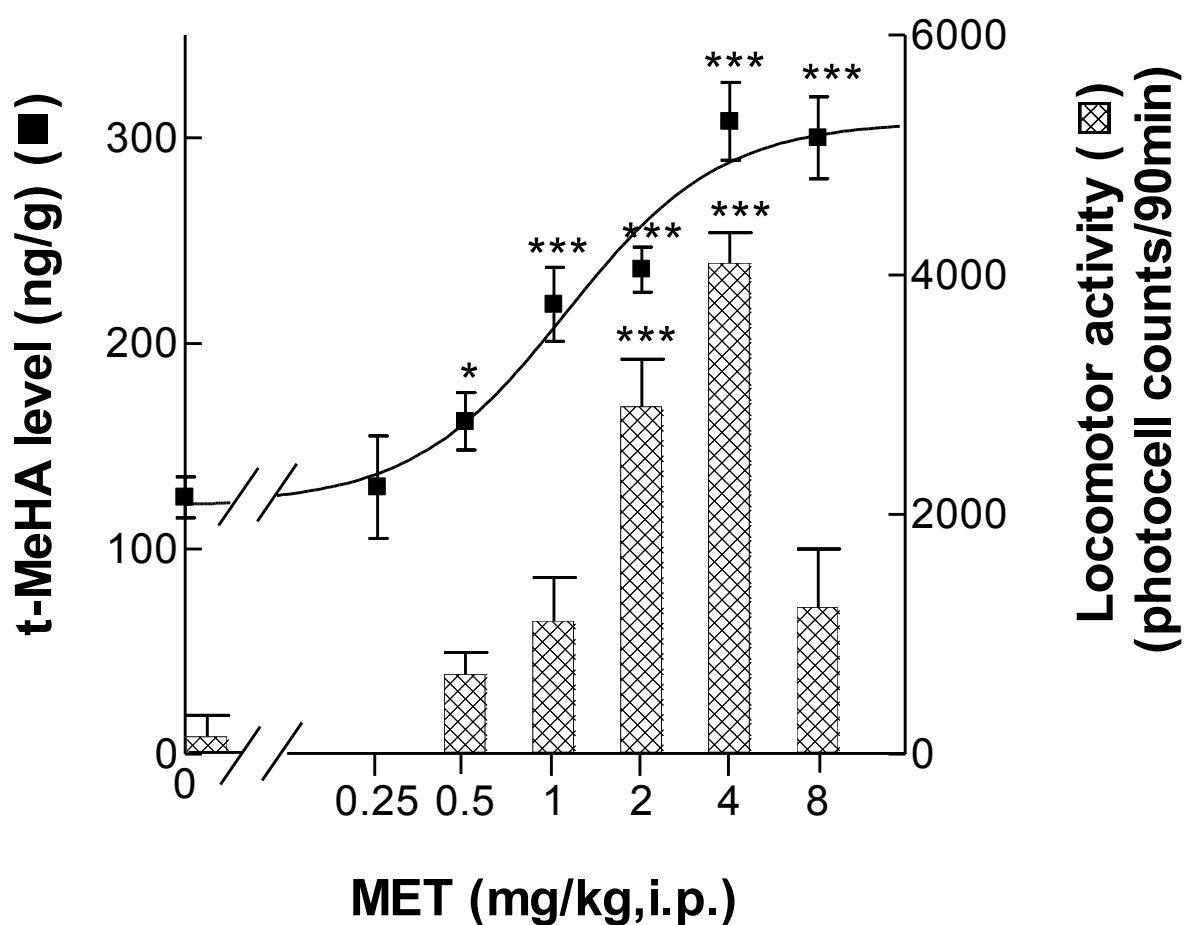
	Treatment Period			Withdrawal Period			
	Day 1	Day 5		Day 8		Day 17	
		Controls	Sensitized	Controls	Sensitized	Controls	Sensitized
Basal t-MeHA levels (ng/g)	$110 \pm 5$	$97 \pm 4$	$132 \pm 11^{\S}$	$124 \pm 7$	$138 \pm 18$	$101 \pm 6$	$145 \pm 8^{\S\S}$
Enhancement of t-MeHA levels							
ED <sub>50</sub> (mg/kg)	$0.7 \pm 0.3$	$0.6 \pm 0.3$	$0.7 \pm 0.3$	$1.1 \pm 0.6$	$2.1 \pm 0.6$	$0.6 \pm 0.3$	$1.6 \pm 0.5$
Emax (% of control)	$252 \pm 18$	$249 \pm 18$	$225 \pm 16$	$234 \pm 27$	$248 \pm 18$	$251 \pm 21$	$231 \pm 17$
Locomotor activity (photocell counts/90min)							
MET 2 mg/kg, i.p.	$2390 \pm 312$		$4472 \pm 493^{**}$		$6177 \pm 1283^*$		$5626 \pm 172^{**}$
MET 0.5 mg/kg, i.p.	$1158 \pm 392$						$3529 \pm 549^{**}$

\*  $p < 0.05$ , \*\*  $p < 0.01$  compared with a single injection (day 1).

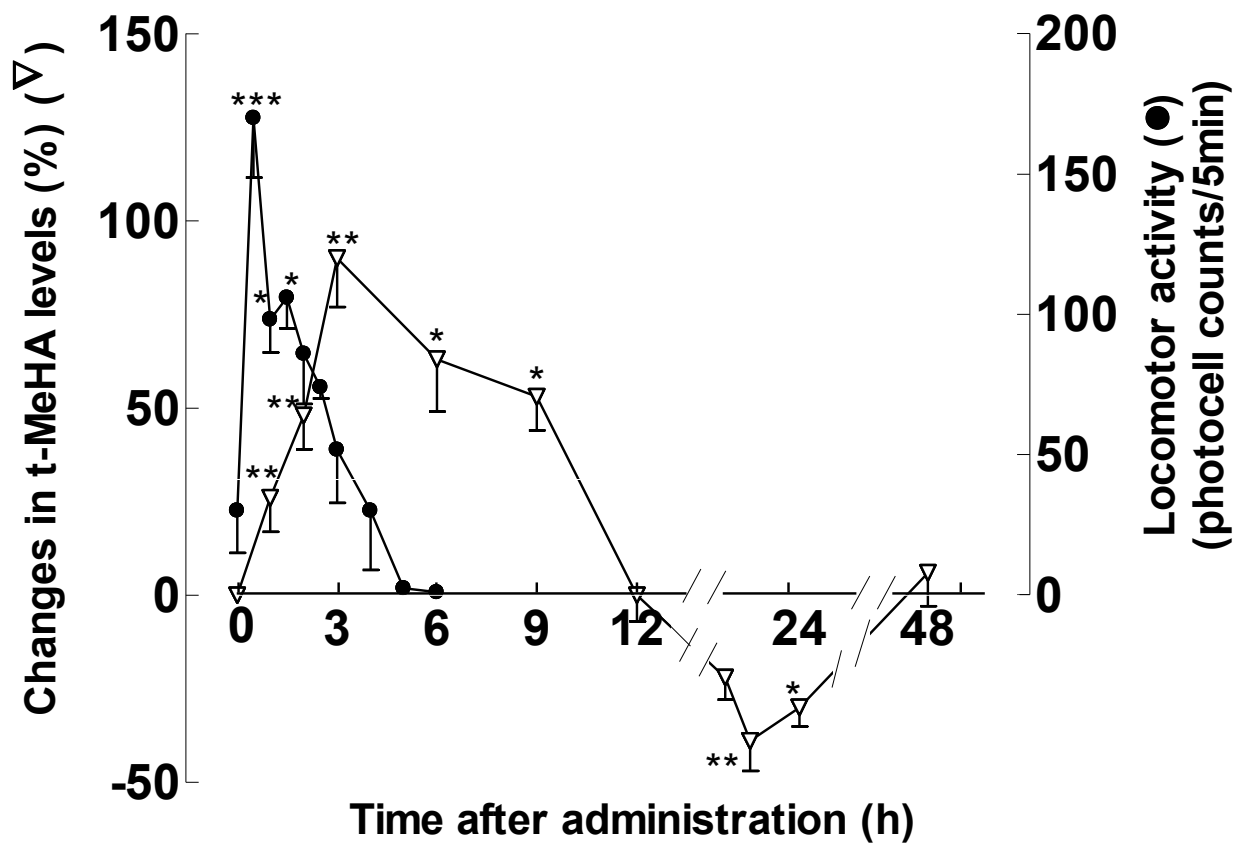
$\S$   $p < 0.05$ ;  $\S\S$   $p < 0.001$  vs respective controls in Mann-Whitney test.



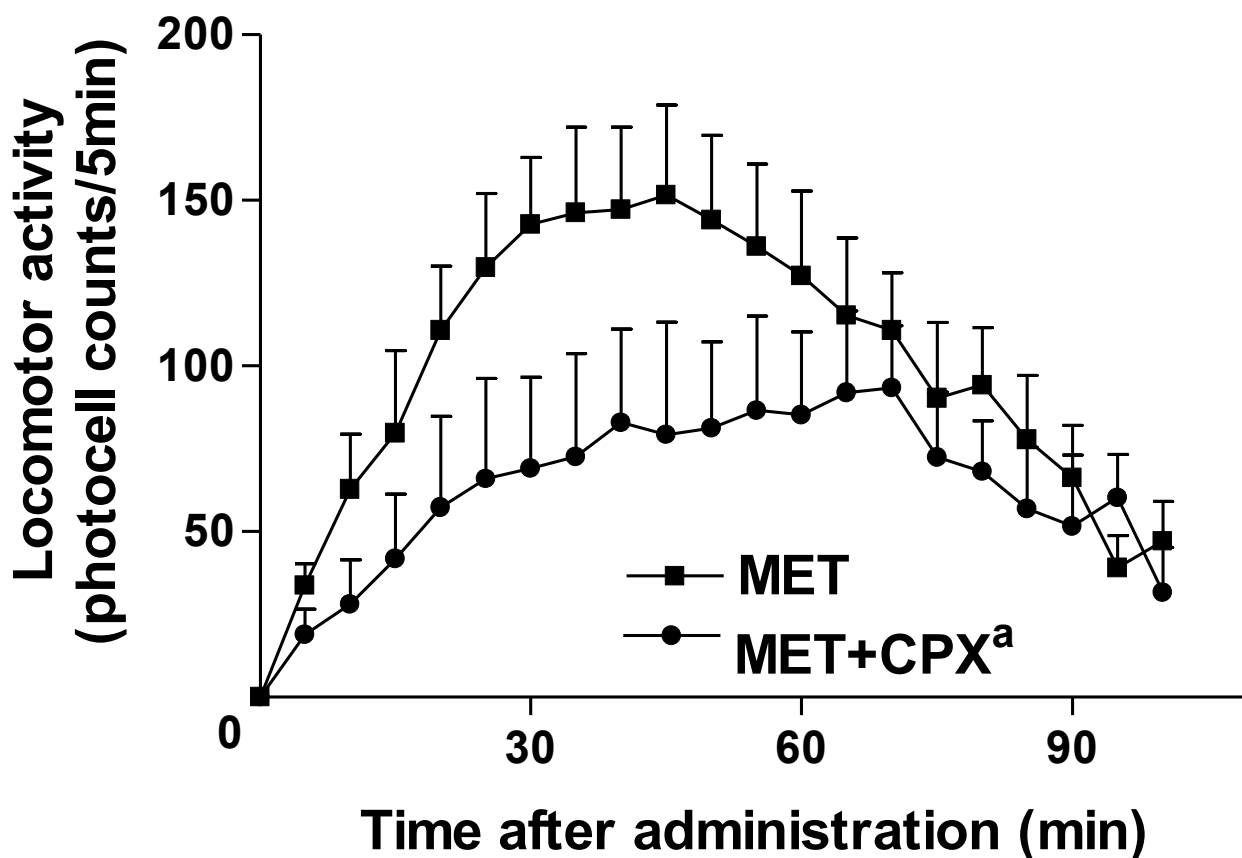
**Fig 1.** Effect of methamphetamine on [<sup>3</sup>H]histamine (HA) or [<sup>3</sup>H]noradrenaline (NA) release from synaptosomes of mouse cerebral cortex. The spontaneous efflux represented  $205 \pm 2$  and  $9927 \pm 97$  cpm/incubation for [<sup>3</sup>H]HA and [<sup>3</sup>H]NA release, respectively. The data are expressed as percent of these values and are means of four determinations from two separate experiments.



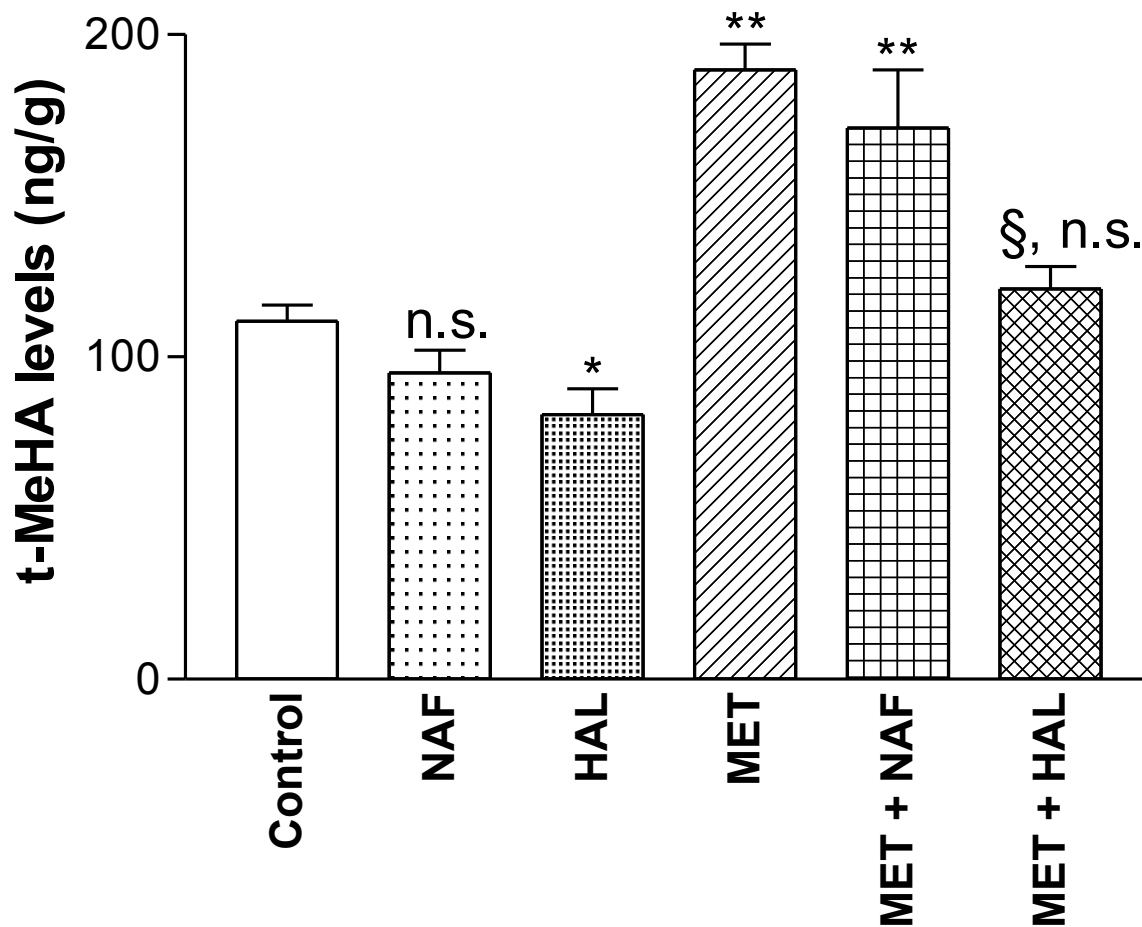
**Fig 2.** Changes in striatal t-MeHA levels and locomotor activity in mice receiving methamphetamine (MET) in increasing dosages. Following a 30min-habituaton period in the actimeter, mice received MET at the indicated dosage and locomotor activity was recorded for 90 min. Another group of mice received MET in increasing dosage and were sacrificed 3h later for measurement of t-MeHA levels. Values are means  $\pm$  SEM from 6 to 8 animals. \* $p < 0.05$ , \*\* $p < 0.001$  vs saline controls in Student-Newman-Keuls test.



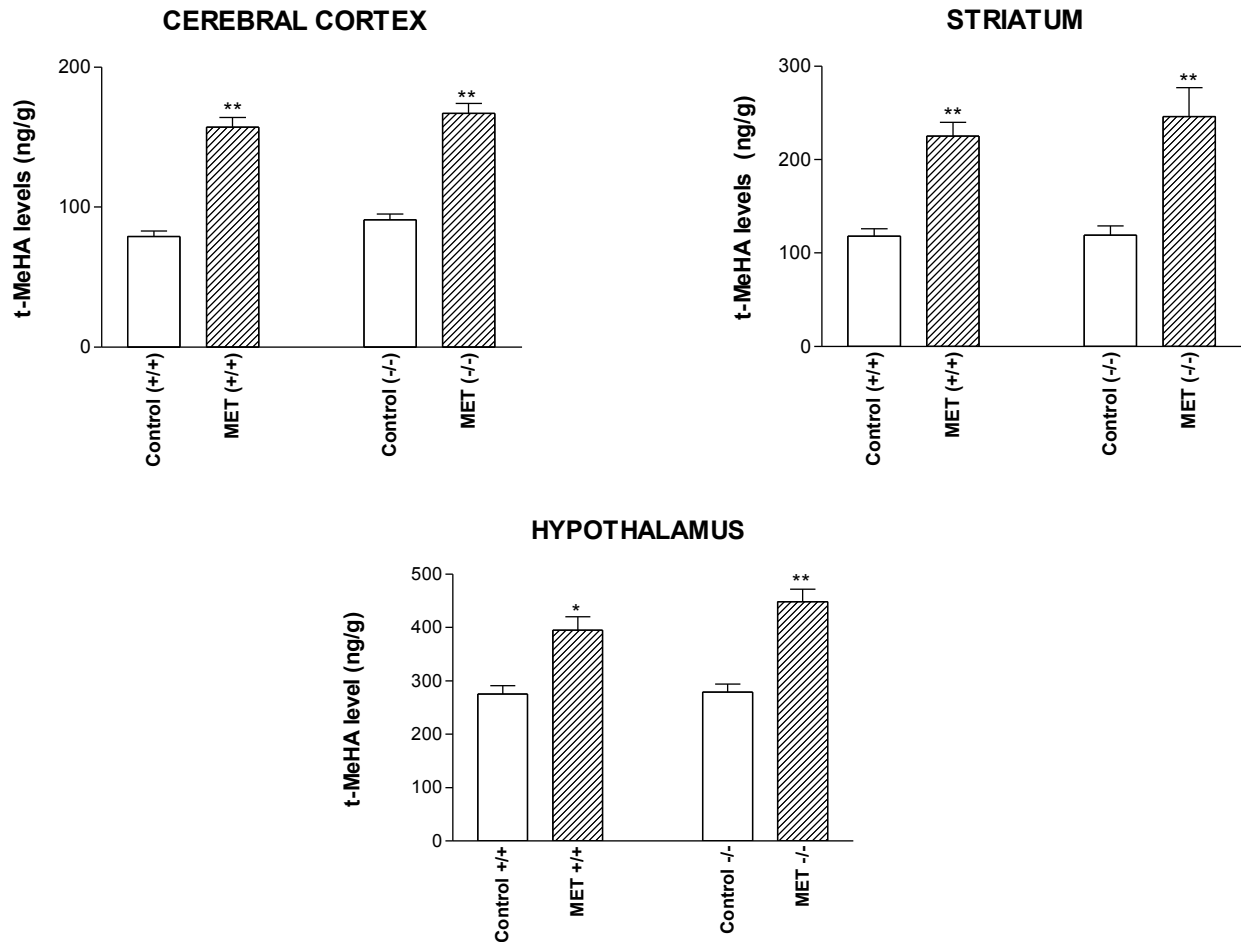
**Fig 3.** Temporal pattern of locomotor activity and concomitant changes in t-MeHA levels after a single injection of methamphetamine (MET, 2mg/kg, i.p.). Mice received methamphetamine (MET, 2mg/kg, i.p) at various times and were all sacrificed between 11 am and 16 pm. t-MeHA levels are expressed in percent increase as compared to levels in corresponding controls ( $139 \pm 18$  ng/g). Values are means  $\pm$  SEM from 6 to 18 animals. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs saline controls in Student-Newman-Keuls test.



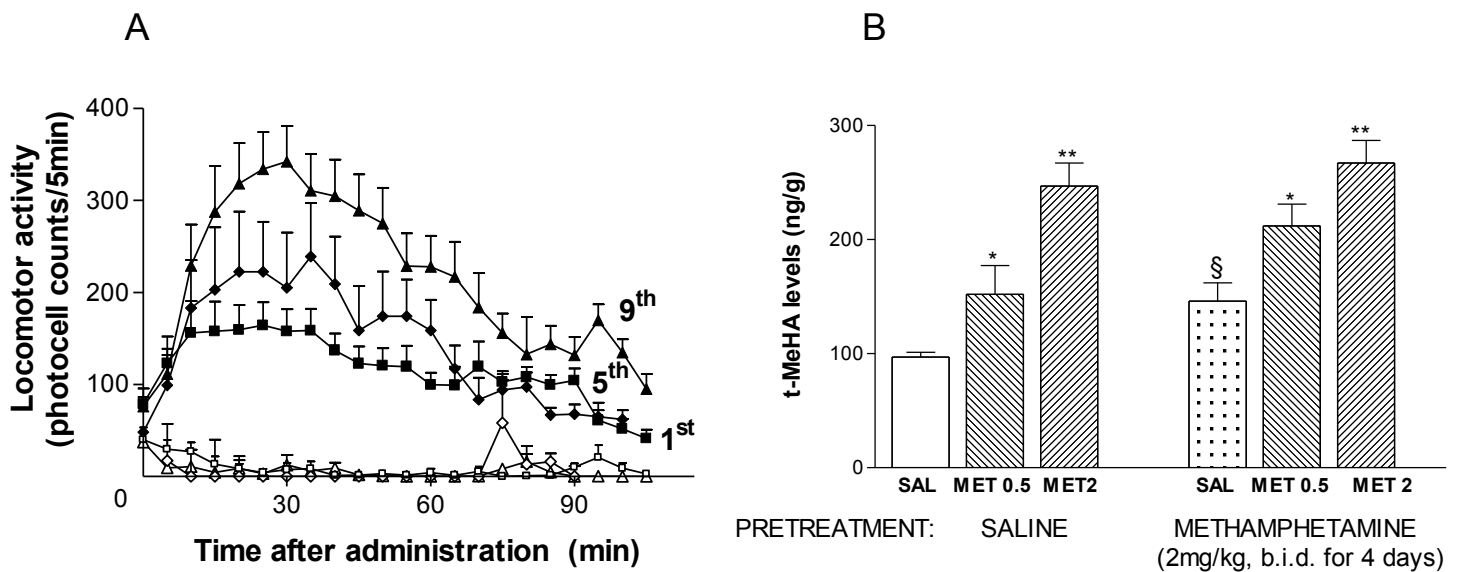
**Fig 4.** Effect of ciproxifan, a H<sub>3</sub>-receptor antagonist/inverse agonist, on the hyperlocomotor activity of methamphetamine. Ciproxifan (CPX) (3 mg/kg; i.p.) was administered 2 h before methamphetamine (MET, 2 mg/kg; i.p.). Each point represents the mean  $\pm$  SEM of the values from 6-8 animals. <sup>a</sup> Two-way ANOVA revealed a significant difference between the two groups of mice (pretreated or not with ciproxifan) [treatment:  $F_{1, 252} = 27.25$ ,  $p < 0.0001$ ; time:  $F_{1, 252} = 4.42$ ,  $p < 0.0001$ ; interaction:  $F_{1, 252} = 0.7$ , n.s.].



**Fig 5.** Effect of haloperidol (HAL, 1 mg/kg,i.p.) and nafadotride (NAF, 2 mg/kg, i.p.) alone or with methamphetamine (MET, 2 mg/kg, i.p.) on striatal t-MeHA levels. Mice were sacrificed 3h after intraperitoneal administration of vehicle or drugs. Values are means  $\pm$  SEM from 12 animals. n.s., non significant, \* $p$ <0.05, \*\*  $p$ <0.001 vs saline control; §  $p$ <0.001 vs methamphetamine alone in Student-Newman-Keuls test.

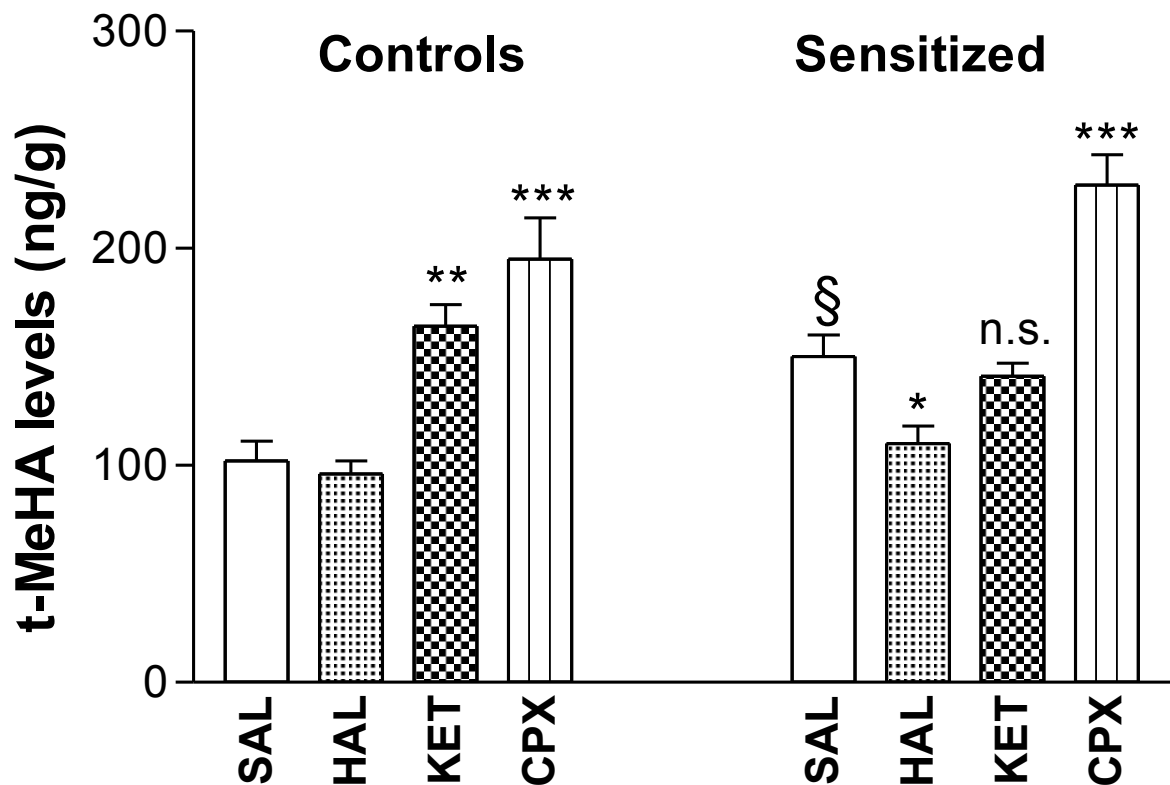


**Fig 6.** Effect of methamphetamine on t-MeHA levels of control mice (+/+) and D<sub>3</sub>-receptor deficient mice (-/-). Mice were sacrificed 3h after the injection of vehicle or methamphetamine (MET 2mg/kg, i.p.). t-MeHA levels are expressed in ng/g of tissue. Means  $\pm$  SEM of values from 12 animals. Two-way ANOVA revealed a significant effect of methamphetamine in control (+/+) and D<sub>3</sub>-receptor deficient mice (-/-) [cerebral cortex:  $F_{1, 28} = 170.6$ ,  $p < 0.0001$ ], [striatum:  $F_{1, 28} = 40.56$ ,  $p < 0.0001$ ] and [hypothalamus:  $F_{1, 28} = 49.66$ ,  $p < 0.0001$ ]. There were no main effect of genotype and no genotype  $\times$  treatment interaction in the three regions. \* $p < 0.01$ ; \*\*  $p < 0.001$  vs the corresponding control in Student-Newman-Keuls test.



**Fig 7.** (A) Effects of repeated methamphetamine pretreatments on locomotor activity. Mice received methamphetamine (2mg/kg, i.p.) twice daily for 5 days. Locomotor activity was evaluated after the 1<sup>st</sup> (■, □), 5<sup>th</sup> (◆, ◇) or 9<sup>th</sup> (▲, Δ) injection of methamphetamine (2mg/kg, filled symbols) or saline solution (open symbols). Means ± SEM of values from 8-10 animals. (B) Comparison of the effect of a challenge dose of methamphetamine (0.5 or 2 mg/kg, i.p.) on striatal t-MeHA levels in mice pretreated twice daily for 4 days with saline solution or methamphetamine (2mg/kg). Means ± SEM of values from 5-10 animals. \* $p < 0.01$ , \*\*  $p < 0.001$  vs corresponding saline controls; §  $p < 0.05$  vs saline control of mice pretreated with saline in Student-Newman-Keuls test.





PRETREATMENT: SALINE

METHAMPHETAMINE  
(2mg/kg b.i.d. , 5 days )

**Fig 8.** Comparison of the effects of ketanserin (KET, 8 mg/kg, i.p.), haloperidol (HAL, 1 mg/kg, i.p.) and ciproxifan (CPX, 3mg/kg, i.p.) on striatal t-MeHA levels in mice pretreated twice daily for 5 days with saline solution (controls) or methamphetamine (2mg/kg) (sensitized) as evaluated after an 11-day interruption of treatment. Means  $\pm$  SEM of values from 12 mice. n.s: non significant, \* $p$ <0.05, \*\* $p$ <0.01; \*\*\* $p$ <0.001 vs corresponding saline controls; §  $p$ <0.01 vs saline control of mice pretreated with saline in Student-Newman-Keuls test.