

Preprotachykinin A gene expression after administration of 3,4-methylene dioxymethamphetamine (Ecstasy)

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Abstract

This study tested the effects of 8 days of subchronic administration of 3,4-methylene dioxymethamphetamine (MDMA) (5 mg/kg b.w.) on preprotachykinin A mRNA levels in discrete rat brain regions. In situ hybridization examined preprotachykinin A mRNA levels in the core and shell of the nucleus accumbens, the islands of Calleja, the olfactory tubercle, the dorsal and ventral caudate–putamen, the bed nucleus of the stria terminalis, the medial preoptic area, the medial habenular nucleus and in the postero-dorsal part of the medial amygdala. Higher levels of preprotachykinin A mRNA were found in the core and shell of the nucleus accumbens, in the islands of calleja, in the olfactory tubercle, in the bed nucleus of the stria terminalis, in the medial habenular nucleus and the postero-dorsal part of the medial amygdala, compared to control animals. Conversely, increased preprotachykinin A mRNA levels were observed in the dorsal and ventral caudate–putamen in MDMA treated when compared to control rats. In the social memory test, MDMA significantly impaired rats' short-term working memory. These results show that chronic exposure to MDMA strongly affects preprotachykinin A mRNA levels in discrete rat brain regions. These changes occur in experimental conditions in which working memory is markedly reduced, suggesting that changes in gene expression of tachykinin mechanisms may contribute to the effects of MDMA on memory function.

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1. Introduction

The amphetamine derivative 3,4-methylene dioxymethamphetamine (MDMA or Ecstasy) stands out as a recreational drug, which has become increasingly abused in Europe and North America over the last 10 years. MDMA abusers have been reported to suffer from cognitive deficits that may be related to the effects exerted on serotonergic and dopaminergic systems and possibly on other neurochemical systems involved in cognitive functions (Ricaurte et al., 1988; Green et al., 1995; Frederick and Paule, 1997).

It possesses both stimulant-like (dopaminergic) and hallucinogen-like (serotonergic) properties (McKenna and Peroutka, 1990; Frederick and Paule, 1997) as a result of its

serotonin (5-HT) and dopamine releasing effects, its ability to inhibit 5-HT and dopamine reuptake and its property to inhibit monoamine oxidase activity (McKenna and Peroutka, 1990; Leonardi and Azmitia, 1994; Gudelsky et al., 1994; Bradberry, 1994; White et al., 1996).

MDMA has been often described in the lay press as being “safe” and is clearly believed to be so by those who misuse the drug, but acute toxic reactions including malignant hyperthermia, convulsions, hepatitis (Dykhuizen et al., 1995; Khakoo et al., 1995) and death have been attributed to MDMA use by young people (Randall, 1992; O'Connor, 1994; Green et al., 1995). Repeated injections of low doses of MDMA were shown to produce long-lasting damage to populations of serotonin-containing axons that project to the forebrain in laboratory rats and monkeys (Ricaurte et al., 1985, 1988; Bannon et al., 1987; O'Hearn et al., 1988; Battaglia et al., 1991; Mamounas et al., 1991).

Dopaminergic neurons can be at risk in humans who repeatedly ingest MDMA, as high doses of MDMA were

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shown to damage forebrain dopamine-containing axons in laboratory rats and appear to selectively damage only dopamine-containing axons in mice (Logan et al., 1988; Stone et al., 1988; Yamamoto and Spanos, 1988; Yaksh et al., 1994; Cadet et al., 1995). MDMA has been shown to affect memory processes, most likely by influencing the serotonergic and dopaminergic system, which have been implicated in learning and memory (Ricaurte et al., 1988).

A number of studies have also implicated the tachykinin substance P, in learning and memory (Schlesinger et al., 1983a,b; Huston and Hasenohrl, 1995).

Its effect appears to be dependent on the brain region in which substance P is injected. Posttrial injection into the substantia nigra or amygdala disrupted, whereas injections into the lateral hypothalamus or septum facilitated, performance of avoidance tasks (Huston et al., 1977). Facilitation of an inhibitory avoidance task was also demonstrated following injection of substance P into the nucleus basalis magnocellularis (Kafetzopoulos et al., 1986; Gerhardt et al., 1992), which is very rich in substance P-ergic terminals (Ljungdahl et al., 1978; Haber and Watson, 1985), and its lesioning is associated with deficits in avoidance tasks in rats (Miyamoto et al., 1985). An effect on memory can also be observed following intracerebroventricular injection of substance P (Kameyama et al., 1998).

In addition, peripheral administration of substance P was shown to improve the performance in a variety of learning tasks, facilitating retention of inhibitory avoidance tasks in mice and rats (Schlesinger et al., 1983a,b), facilitating habituation (Tomaz et al., 1990), positively reinforcing discriminative learning (Schlesinger et al., 1986) and maze performance in aged rats (Hasenohrl et al., 1990, 1994). In this regard, it is noteworthy that aged rats show reduced brain levels of substance P (Dupont et al., 1981) as well as a decrease in preprotachykinin A mRNA levels (Pompei et al., 1999). Substance P has also been shown to possess memory-promoting (in addition to reinforcing and anxiolytic-like) effects when administered systemically or into the nucleus basalis of the ventral pallidum (Hasenohrl et al., 2000).

Interestingly, the gene expression of tachykinin peptides (TK) in the forebrain is strongly influenced by the dopaminergic and the serotonergic systems, raising the possibility that the three systems may strongly interact in the control of memory function.

Three distinct G protein-coupled tachykinin receptors, namely NK₁, NK₂ and NK₃ have been cloned and pharmacologically characterized (Regoli et al., 1987, 1994; Guard and Watson, 1991; Otsuka and Yoshioka, 1993). Autoradiographic studies indicate a wide distribution of NK₁ and NK₃ receptors in the central nervous system, while NK₂ receptors appear to be selectively expressed only in specific brain nuclei (Regoli et al., 1994).

More recently, an orphan receptor resembling the NK₃ receptor, initially claimed to be an atypical opioid receptor, was shown to respond potently to neurokinin B and

was proposed as the NK₄ receptor (Donaldson et al., 1996).

However, it still awaits a detailed pharmacological characterization. Mammalian tachykinins are the products of two distinct genes: substance P/neurokinin A gene, or preprotachykinin gene I or A and the neurokinin B gene or preprotachykinin gene II or B. The neuropeptides substance P, neurokinin A, neuropeptide K and neuropeptide γ are encoded by mRNAs resulting from preprotachykinin A gene transcription, whereas neurokinin B is the only tachykinin derived from preprotachykinin B gene. Alternate RNA splicing of primary transcripts of the preprotachykinin A gene results in the production of three substance P-encoding mRNAs, called α -, β - and γ -preprotachykinin A mRNA (Krause et al., 1987). Previous studies have reported lower striatal preprotachykinin A mRNA amounts in response to the selective dopaminergic neurotoxin 6-hydroxydopamine (Sivam et al., 1987). In contrast, dopamine receptor stimulation increased striatal preprotachykinin A mRNA amounts (Sivam et al., 1987). Attention has also been paid to serotonin as an important trans-synaptic regulator of striatal neuropeptide gene expression (Walker et al., 1991). Previous experiments have demonstrated that prolonged inhibition of serotonin biosynthesis lowered striatal preprotachykinin A mRNA levels, whereas several days of enhanced transmission raised preprotachykinin A message amounts (Walker et al., 1991).

Based upon these findings, the aim of this study was to evaluate gene expression of preprotachykinin A mRNA levels in discrete rat brain regions after a subchronic treatment of MDMA, which results in a pronounced impairment of short-term working memory in rats.

2. Materials and methods

2.1. Subjects

Twenty-four male Sprague–Dawley rats, weighing 230–250 g were employed (Harlan, Correzzana, Milan, Italy). Prior to testing, animals were housed in groups of six in a temperature- and humidity-controlled colony room for 1 week. Lighting was maintained on a 12-h photocycle, with lights on at 7:00 a.m. Water and Purina Rat chow were available ad libitum. At the time of the experiment, rats were placed in individual cages. The present animal study was conducted in accordance with the highest standards of human animal care (EEC council 86/609 authorization; D.L. 27/01/1992, no. 116).

2.2. Experimental procedures

Experimental animals included two groups (12 animals in each group). The first group (control) was injected at 10:00 a.m. with intraperitoneal saline solution, whereas

the second group received an intraperitoneal administration of MDMA (5 mg/kg b.w.). Each animal was injected once a day, and the subchronic treatment lasted for eight consecutive days. Behavioural tests began 30 min after the daily treatment. Lastly, on day 9, rats were sacrificed by decapitation at 10:00 a.m., and brains removed and stored for the *in situ* hybridization procedures.

2.3. *In situ* hybridization

Rats from each group were decapitated, brains were rapidly removed from the skull and immediately frozen on powdered dry ice and were stored at -70°C . Twenty-micrometer-thick sections were cut in the coronal plane on a cryostat microtome, according to the Swanson rat brain atlas (Swanson, 1992), collected on gelatin-coated diethylpyrocarbonate-treated slides and stored at -70°C until hybridization. Prior to hybridization, sections were fixed for 30 min at room temperature, 2% paraformaldehyde/0.1 M sodium phosphate buffer for 1–2 min (pH 7.2), and were then washed in ice-cold $0.5\times$ sodium chloride sodium citrate (SSC) buffer for 1–2 min ($1\times$ sodium chloride sodium citrate = 0.15 M sodium chloride/0.015 M sodium citrate buffer, pH 7.0). Sections were air-dried at room temperature and were hybridized at 37°C in a humidified environment with a solution consisting of 0.2% (w/v) bovine serum albumin, 0.1% (w/v) polyvinylpyrrolidone, $4\times$ SSC buffer, 50% formamide, 100 $\mu\text{g}/\text{ml}$ of salmon sperm DNA and 6×10^6 cpm/ml of ^{33}P -labeled synthetic oligonucleotide probe using terminal transferase. The hybridization mix was added at 100 μl per slide. Glass coverslips were applied and sections were incubated overnight (16 h) at 37°C in humidified covered plastic trays. Following an overnight hybridization, coverslips were removed, and slides were first rinsed in $1\times$ SSC buffer at room temperature for 60 min and then for 60 min at 40°C .

The synthetic oligodeoxyribonucleotides used to detect preprotachykinin A mRNA (5'dCATTAATCCAAAGAA-TGCTGAGGCTTGGGTC and 5'dGCCCATAGTC-CAACAAAGGAATCTGTTTTATG) correspond to the nucleotide sequence coding for amino acids 58–68 and 98–108, respectively, of the rat preprotachykinin A.

A computerized literature search indicated that the oligonucleotide sequence chosen for the preprotachykinin A mRNA probe did not crosshybridize with the mRNA for any other published nucleic acid sequence. To further test the specificity of labeling, test hybridizations were performed in the presence or absence of a 30-fold molar excess of unlabelled preprotachykinin A probe.

Sections were initially apposed to Kodak XAR film for 8 days at room temperature. They were then dipped in Kodak NTB-2 emulsion and permitted to air-dry before they were stored in light-tight slides boxes containing Drierite (Acros Organics, USA). The slides were exposed

for 6 weeks at 4°C . Slides were finally developed by using standard procedure: Slides were developed in Kodak Dektol, rinsed in water, fixed in Kodak fixer and washed. The sections were counterstained with 0.1% Cresyl violet and were coverslipped with Clarion mounting medium (Bio-medex, USA).

2.4. Social memory test

The social memory test was an adaptation of the procedure described by Dantzer et al. (1987) for measuring retroactive facilitation. Experiments were carried out in the animal room under the dark–light cycle. The living cage of the adult rat was positioned under the beam of an infrared spotlight. A juvenile rat was put into this living cage for 5 min (first encounter). The time spent by the adult rat in investigating the juvenile was recorded. Nosing, sniffing, grooming, pawing or close following of the juvenile by the adult were considered indiscriminately as an item of social investigatory behaviour. Thereafter, experimental rats received the last MDMA injection (Day 8), and 120 min later, the same juvenile rat was introduced into the cage for a second encounter (5 min). During each encounter, the duration of the investigatory behaviour of the resident rat was recorded, as social investigation time.

2.5. Statistical analysis

Data are presented as means \pm S.E.M. values. For *in situ* hybridization experiments, relative optical density and reduced silver grain counts were analyzed by a two-way analysis of variance (ANOVA), with treatment group (control, MDMA) as between-subjects and brain regions (core and shell of the nucleus accumbens, islands of Calleja, olfactory tubercle, dorsal and ventral caudate–putamen, bed nucleus of the stria terminalis, medial preoptic area, medial habenular nucleus, postero-dorsal part of the medial amygdala) as within-subjects factors. Post hoc follow-up tests of main effects (Student–Newman–Keuls) and interaction effects were done as required.

For the social memory test, ΔSIT (difference between the second and the first investigation time) was analyzed by means of the Student's *t* test.

Statistical significance was set at $P < 0.05$.

3. Results

3.1. Preprotachykinin A mRNA expression

Silver grains from brain sections were primarily concentrated over the perikarya of neurons. The labeling pattern was consistent from rat to rat in all the brain areas analyzed.

The overall ANOVA revealed a significant treatment effect [$F(1,12)=46.5$, $P<0.001$].

Fig. 1 shows preprotachykinin A mRNA levels in the dopamine terminal regions in the forebrain, such as the core and shell of the nucleus accumbens, the islands of Calleja, the olfactory tubercle, the dorsal and ventral caudate–putamen, the bed nucleus of the stria terminalis, the medial preoptic area, the postero-dorsal part of the medial amygdala and the medial habenular nucleus.

MDMA treatment induced a 21.8% reduction ($P<0.01$) in basal hybridizable preprotachykinin A mRNA in the core of the nucleus accumbens as well as a 20.5% ($P<0.01$) in the shell of the nucleus accumbens compared to control rats. A 25.2% statistically significant lower level ($P<0.01$) of preprotachykinin A mRNA in MDMA-treated rats was observed in the islands of calleja opposite to control rats, whereas the same figure shows a 9.4% statistically significant lower level ($P<0.01$) of preprotachykinin A mRNA in the olfactory tubercle. Preprotachykinin A mRNA levels were shown to be significantly lower by 36% ($P<0.01$) in the bed nucleus of the stria terminalis in MDMA-treated animals compared to control rats. Also, levels of preprotachykinin A mRNA were significantly reduced ($P<0.01$) in the medial preoptic area of MDMA treated with respect to control animals. Finally, a 26.6% and a 24.8% statistically significant lower level of preprotachykinin A mRNA was found in the postero-dorsal part of the medial amygdala ($P<0.01$) and in the medial habenular nucleus ($P<0.01$) of MDMA-treated rats when compared to control animals, respectively.

On the other hand, MDMA treatment induced an 18.5% and a 23.4% statistically significant increase ($P<0.01$) in preprotachykinin A mRNA levels in the dorsal and ventral part of the caudate–putamen respectively, opposite to control rats.

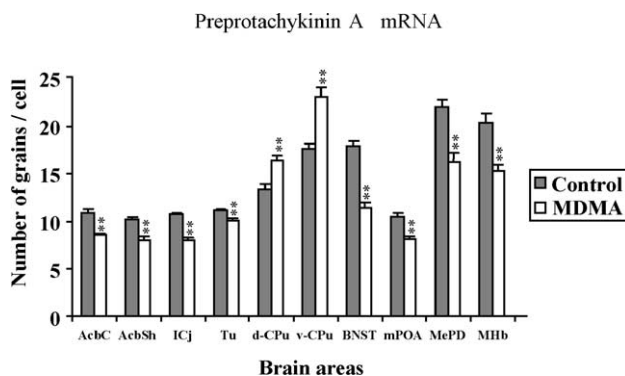


Fig. 1. Preprotachykinin A mRNA levels in the caudate–putamen, dorsal and ventral, respectively (d-CPu) (v-CPu), in the bed nucleus of the stria terminalis (BNST), in the medial preoptic area (mPOA), in the postero-dorsal part of the medial amygdala (MePD) and in the medial habenular nucleus (MHb). Values represent means \pm S.E.M. of 12 animals. One set of sections was collected from each animal. ** $P<0.01$.

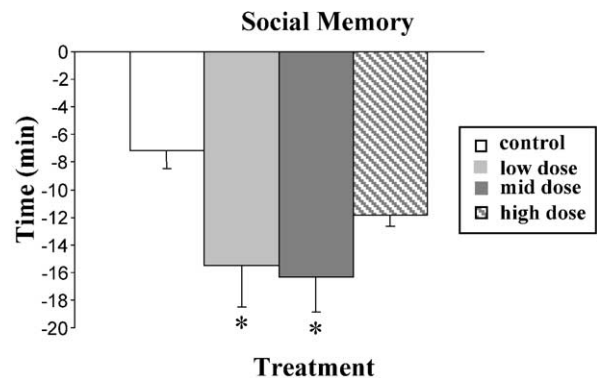


Fig. 2. Social memory test, expressed as Δ SIT (difference between the second and the first investigation time) was analyzed by means of the Student's t test. Values represent means \pm S.E.M. of 12 animals. * $P<0.05$. Animal groups were control (saline solution), low dose (1 mg/kg b.w.), mid dose (5 mg/kg b.w.) and high dose (10 mg/kg b.w.).

3.2. Behavioural results

As shown in Fig. 2, the same chronic treatment with MDMA impaired the short-term working memory, as revealed in the social memory test.

4. Discussion

The findings of the present study show that preprotachykinin A mRNA expression is strongly affected by MDMA administration. Brain areas expressing preprotachykinin A mRNA seem to respond differently to MDMA treatment, either increasing or decreasing its gene expression. Most brain areas decrease expression of preprotachykinin A mRNA after MDMA treatment. Preprotachykinin A mRNA increase was detected in the caudate–putamen.

Previous studies have reported that dopamine regulates neuropeptide gene expression (Robertson et al., 1992) and that dopaminergic drugs also induce expression of the immediate early gene *c-fos* in basal ganglia (Robertson et al., 1991). It is well known that both dopamine or 5-HT influence preprotachykinin A gene expression: Increased synaptic availability of dopamine or 5-HT increases preprotachykinin A gene expression, while reduced extracellular levels of both neurotransmitter reduce preprotachykinin A mRNA expression (Sivam et al., 1987; Robertson et al., 1992).

The dopaminergic system can undergo counterregulation in response to a subchronic psychostimulant treatment regimen, as reported in the literature (Unterwald et al., 1994; Maisonneuve et al., 1995).

Since there are decreased levels of preprotachykinin A mRNA in medial forebrain examined regions, and these regions are terminally innervated by dopaminergic projections from the midbrain, it seems probable that decreased dopamine tone may be reducing preprotachykinin A mRNA levels. Indeed, while activation of 5-HT_{2A} or 5-HT₄ recep-

tors has been shown to increase dopamine release (De Deurwaerdere et al., 1997; Yan et al., 2000) our data suggest that, in response to a subchronic administration of MDMA, there is a compensatory attenuation of dopamine release in forebrain structures leading to decreased tachykinin mRNA levels. This counterregulation of the dopaminergic system in response to subchronic as opposed to acute psychostimulant treatment is well known (Unterwald et al., 1994). Thus, in response to the subchronic administration of MDMA in the present study, there may have been a compensatory attenuation of dopamine release in forebrain structures leading to a decrease in tachykinin mRNA levels.

Neostriatal levels of preprotachykinin A mRNA are also sensitive to changes in 5-HT neurotransmission. In this regard, acute activation of 5-HT_{2A}, 5-HT₃ or 5-HT₄ receptors has been shown to increase dopamine release (Yan et al., 2000; De Deurwaerdere et al., 1997) and preprotachykinin A gene expression. Decreases in extracellular 5-HT, which may be evoked by MDMA, may cause neostriatal preprotachykinin A mRNA levels to fall (Walker et al., 1991).

More difficult is to speculate about the increase in preprotachykinin A mRNA observed in the caudate–putamen. A previous study correlating the effect of MDMA on dopamine release in the caudate–putamen and in the nucleus accumbens (Yamamoto and Spanos, 1988) showed that low doses of this drug of abuse determined a similar release of dopamine in both areas, whereas high doses of the same drug were able to induce by far a more robust increase in dopamine release after acute administration in the caudate. This difference, supposedly related to the diverse dopaminergic innervation in these two areas, might, if only in part, account for the increase in preprotachykinin A mRNA in the caudate–putamen after MDMA treatment.

Interestingly, several studies have highlighted memory impairment in MDMA users, which has been suggested to be related to changes elicited by MDMA on 5-HT and dopamine mechanisms in the forebrain (Ricaurte et al., 1993). Accordingly, the chronic MDMA treatment adopted in the present study induced impairment of working memory in our rats.

Indeed, substance P also has been implicated in learning and memory processes (Ricaurte and McCann, 1992).

Intraperitoneal injection of substance P not only disrupts learning of aversive stimuli when preceded by an acoustic stimuli (Huston and Staubli, 1981), but it also facilitates short- and long-term memory in the radial-maze. To this purpose, it is noteworthy that old rats show lower levels of substance P (Dupont et al., 1981) as well as a decrease in tachykinin mRNA (Pompei et al., 1999).

Also, peripheral administration of substance P is shown to reinforce learning capacity in the spatial-maze memory test (Banks and Kastin, 1985; Hasenhorl et al., 1994). Nevertheless, since substance P does not seem to promptly cross the blood–brain barrier, its action might be partly related to the vasodilatory properties. TK are known to be

present in several brain areas involved in learning and memory processes. Neurons synthesizing TK are strictly associated or colocalized with neurons containing acetylcholine in basal ganglia or dopamine in the striatum (Bannon et al., 1987). In fact, acetylcholine and tachykinins are found in the same brain nuclei. Since substance P content is decreased in patients affected by Alzheimer disease, a contribution of the tachykinergic system might be of some importance in memory and learning processes associated to senile dementia (Cristal and Davies, 1982; Decker and McGaugh, 1991).

More recently, it has been shown that substance P mRNA is also decreased in an animal model of Alzheimer disease (Pompei et al., 1999), further supporting the hypothesis of a contribution of the tachykinergic system to memory and learning.

Taken together, these findings highlight that the tachykinergic system, beyond the well-characterized dopamine and serotonergic systems, is strongly affected after MDMA subchronic exposure and might be of importance to our further understanding of the neurobiological basis of brain areas expressing preprotachykinin A after subchronic exposure to MDMA.

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References

- Banks, W.A., Kastin, A.J., 1985. Peptides and the blood–brain barrier: lipophilicity as a predictor of permeability. *Brain Res. Bull.* 15, 287–292.
- Bannon, M.J., Peter, P.J., Bunney, E.B., 1987. Striatal tachykinin biosynthesis: regulation of mRNA and peptide levels by dopamine agonists and antagonists. *Mol. Brain Res.* 3, 31–37.
- Battaglia, G., Sharkey, J., Kuhar, M.J., De Souza, E.B., 1991. Neuroanatomic specificity and time course of alterations in rat brain serotonergic pathways induced by MDMA (3,4 methylene dioxymethamphetamine) assessment using quantitative autoradiography. *Synapse* 8, 249–260.
- Bradberry, C.W., 1994. Microdialysis assessment of the impact of (+)3,4 methylene dioxymethamphetamine, cocaine, and cocaethylene on serotonergic neurons. *Drug. Dev. Res.* 33, 1–9.
- Cadet, L., Landenheim, B.L., Hirata, H., Rothman, R.B., Ali, S., Carlson, E., Epstein, C., Moran, T.H., 1995. Superoxide radicals mediate the biochemical effects of methylene dioxymethamphetamine (MDMA): evidence from using CuZn-superoxide dismutase transgenic mice. *Synapse* 21, 169–176.
- Cristal, H.A., Davies, P., 1982. Cortical Substance P-like immunoreactivity in cases of Alzheimer's disease and senile, dementia of the Alzheimer type. *J. Neurochem.* 38, 1781–1784.
- Dantzer, R., Bluthé, R.M., Koob, G.F., Le Moal, M., 1987. Modulation of social memory in male rats by neurohypophyseal peptides. *Psychopharmacology* 91, 363–368.

- Decker, M.W., McGaugh, J.L., 1991. The role of interaction between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* 7, 151–168.
- De Deurwaerdere, P., Lhironde, M., Bonhomme, N., Lucas, G., Cheramy, A., Spampinato, U., 1997. Serotonin stimulation of 5-HT₄ receptors indirectly enhances in vivo dopamine release in the rat striatum. *J. Neurochem.* 68, 195–203.
- Donaldson, L.F., Haskell, C.A., Hanley, M.R., 1996. Functional characterization by heterologous expression of a novel cloned tachykinin peptide receptor. *J. Biochem.* 320, 1–5.
- Dupont, A., Savard, P., Merand, Y., Labrie, F., Boissier, J.R., 1981. Age-related changes in central nervous system enkephalins and substance P. *Life Sci.* 29, 2317–2322.
- Dykhuizen, R.S., Brunt, P.W., Atkinson, P., Simpson, J.G., Smith, C.C., 1995. Ecstasy induced hepatitis mimicking viral hepatitis. *Gut* 36, 939–941.
- Frederick, D.L., Paule, M.G., 1997. Effects of MDMA on complex brain function in laboratory animals. *Neurosci. Biobehav. Rev.* 21, 67–78.
- Gerhardt, P., Hasenhorl, R.U., Huston, J.P., 1992. Enhanced learning produced by injection of neurokinin substance P by the N-terminal sequence. *Exp. Neurol.* 118, 302–308.
- Green, A.R., Cross, A.J., Goodwin, G.M., 1995. Review of the pharmacology and clinical pharmacology of 3,4-methylene dioxymethamphetamine (MDMA or “Ecstasy”). *Psychopharmacology* 119, 247–260.
- Guard, S., Watson, S.P., 1991. Tachykinin receptor types: classification and membrane-signalling mechanisms. *Neurochem. Int.* 18, 149–165.
- Gudelsky, G.A., Yamamoto, B.K., Nash, J.F., 1994. Potentiation of 3,4-methylene dioxymethamphetamine-induced dopamine release and serotonin neurotoxicity by 5-HT₂ receptors agonists. *Eur. J. Pharmacol.* 264, 325–330.
- Haber, S.N., Watson, S.J., 1985. The comparative distribution of enkephalin, dynorphin, and substance P in the human globus pallidus and basal forebrain. *Neuroscience* 14, 1011–1024.
- Hasenhorl, R.U., Huston, J.P., Schurman, T., 1990. Neuropeptide substance P improves water maze performance in aged rats. *Psychopharmacology* 101, 23–26.
- Hasenhorl, R.U., Frisch, C., Nikolaus, S., Huston, J.P., 1994. Chronic administration of neurokinin substance P improves maze performance in aged *Rattus norvegicus*. *Behav. Neural Biol.* 62, 110–120.
- Hasenhorl, R.U., Souza-Silva, M.A., Nikolaus, S., Tomaz, C., Brandao, M.L., Schwarting, R.K., Huston, J.P., 2000. Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. *Neuropeptides* 34 (5), 272–280.
- Huston, J.P., Hasenhorl, R.U., 1995. The role of neuropeptides in learning: focus on the neurokinin substance P. *Behav. Brain Res.* 66, 117–127.
- Huston, J.P., Staubli, U., 1981. Substance P and its effects on learning and memory. In: Martinez, J.L., Jensen, R.A., Messing, R.B. (Eds.), *Endogenous peptides and learning and memory processor*. Academic Press, New York, pp. 521–540.
- Huston, J.P., Mueller, C.C., Mondadori, C., 1977. Memory facilitation by post-trial hypothalamic stimulation and other reinforcement. *Biobehav. Rev.* 1, 143–150.
- Khakoo, S.I., Coles, C.J., Armstrong, J.S., Barry, R.E., 1995. Hepatotoxicity and accelerated fibrosis following 3,4 methylene dioxymethamphetamine (“Ecstasy”) usage. *J. Clin. Gastroenterol.* 20, 244–247.
- Kafetzopoulos, E., Holzhauser, M.S., Huston, J.P., 1986. Substance P injected into the region of the nucleus basalis magnocellularis facilitates performance of an inhibitory avoidance task. *Psychopharmacology* 90, 281–283.
- Kameyama, T., Ukai, M., Shinkai, N., 1998. Ameliorative effects of tachykinins on scopolamine-induced impairment of spontaneous alternation performance in mice. *Methods Find. Exp. Clin. Pharmacol.* 20 (7), 555–560. Sep.
- Krause, J., Chirgwin, J., Carter, M., Xu, Z., Hershey, A., 1987. Three rat preprotachykinin mRNAs encode the neuropeptides Substance P and Neurokinin A. *Proc. Natl. Acad. Sci. U. S. A.* 84, 881–885.
- Leonardi, E.T.K., Azmitia, E.C., 1994. MDMA (ecstasy) inhibition of MAO type A and type B: comparisons with fenfluramine and fluoxetine (Prozac). *Neuropsychopharmacology* 10, 231–238.
- Ljungdahl, A., Hokfel, T., Nilsson, G., 1978. Distribution of substance P-like immunoreactivity in the central nervous system of the rat, cell bodies and nerve terminals. *Neuroscience* 3, 861–943.
- Logan, B.J., Lavery, R., Sanderson, W.D., Yee, Y.B., 1988. Differences between rats and mice in MDMA (methylene dioxymethamphetamine) neurotoxicity. *Eur. J. Pharmacol.* 152, 227–234.
- Maisonneuve, I.M., Ho, A., Kreek, M.J., 1995. Chronic administration of a cocaine “binge” alters basal extracellular levels in male rats: an in vivo microdialysis study. *J. Pharmacol. Exp. Ther.* 272, 652–657.
- Mamounas, L.A., Mullen, C.A., O’Hearn, E., Moliver, M.E., 1991. Dual serotonergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. *J. Comp. Neurol.* 314, 558–586.
- McKenna, D.J., Peroutka, S.J., 1990. Neurochemistry and neurotoxicity of 3,4-methylene dioxymethamphetamine (MDMA, Ecstasy). *J. Neurochem.* 54, 14–22.
- Miyamoto, M., Shintani, M., Nagaoka, A., Nagawa, Y., 1985. Lesioning of the rat basal forebrain leads to memory impairments in passive and active avoidance tasks. *Brain Res.* 328, 97–104.
- O’Connor, B., 1994. Hazards associated with the recreational drug “Ecstasy”. *Br. J. Hosp. Med.* 52, 507–514.
- O’Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J., Moliver, M.E., 1988. Methylene dioxymethamphetamine (MDA) and 3,4 methylene dioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. *J. Neurosci.* 8, 2788–2803.
- Otsuka, M., Yoshioka, K., 1993. Neurotransmitter functions of mammalian tachykinins. *Physiol. Rev.* 73, 229–308.
- Pompei, P., Severini, R., Costa, G., Massi, M., Fattoretti, P., Bertoni-Fredari, C., 1999. In situ hybridization analysis of preprotachykinin-A mRNA levels in young and old rats. *Mol. Brain Res.* 64 (1), 132–136.
- Randall, J., 1992. Ecstasy-fueled “rave” parties become dances of death for English youth. *JAMA* 268, 1505–1506.
- Regoli, D., Drapeau, G., Dion, S., D’Orleans-Juste, P., 1987. Pharmacological receptors for substance P and neurokinins. *Life Sci.* 40, 109–117.
- Regoli, D., Boudon, A., Fauchere, J.L., 1994. Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.* 46, 551–599.
- Ricaurte, G.A., McCann, U.D., 1992. Neurotoxic amphetamine analogues: effects in monkeys and implications for humans. *Ann. N.Y. Acad. Sci.* 648, 371–382.
- Ricaurte, G.A., Bryan, G., Strauss, L., Seiden, L., Schuster, C., 1985. Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. *Science* 229, 986–988.
- Ricaurte, G.A., Forno, L.S., Wilson, M.A., DeLanney, L.E., Irwin, I., Moliver, M.E., Langston, J.W., 1988. 3,4 Methylene dioxymethamphetamine selectively damages serotonergic neurons in nonhuman primates. *JAMA* 260, 51–55.
- Ricaurte, G.A., Markowska, A.L., Wenk, G.L., Hatzidimitriou, G., Wlos, J., Olton, D.S., 1993. 3,4 Methylene dioxymethamphetamine, serotonin and memory. *J. Pharmacol. Exp. Ther.* 266, 1097–1104.
- Robertson, H.A., Paul, M.L., Moratalla, R., Graybiel, A.M., 1991. Expression of the immediate early-gene c-fos in basal ganglia: induction by dopaminergic drugs. *Can. J. Neurol. Sci.* 18, 380–383. Supplement.
- Robertson, G.S., Vincent, S.R., Fibiger, H.C., 1992. D1 D2 dopamine receptors differentially regulate c-fos expression in striatonigral and striato-pallidal neurons. *Neuroscience* 49, 285–296.
- Schlesinger, K., Lipsitz, D.U., Peck, P.L., Pellemounter, M.A., Stewart, T.N., Chase, T.N., 1983a. Substance P enhancement of passive and active avoidance conditioning in mice. *Pharmacol. Biochem. Behav.* 19, 661–665.
- Schlesinger, K., Lipsitz, D.U., Peck, P.L., Pellemounter, M.A., 1983b. Substance P reversal of electroconvulsive shock and cycloheximide-induced retrograde amnesia. *Behav. Neural Biol.* 39, 30–39.
- Schlesinger, K., Pellemounter, M.A., Van de Kamp, J., Bader, D.L., Stewart,

- J.M., Chase, T.N., 1986. Substance P facilitation of memory: effects in an appetitively motivated learning tasks. *Behav. Neural Biol.* 45, 230–239.
- Sivam, P.S., Breese, G.R., Krause, J.E., Napier, C.T., Mueller, R.A., Hong, J.-S., 1987. Neonatal and adult 6-hydroxydopamine-induced lesions differentially alter tachykinin and enkephalin gene expression. *J. Neurochem.* 49 (5), 1623–1633.
- Stone, D.M., Johnson, M., Hanson, G.R., Gibbs, J.W., 1988. Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylene dioxymethamphetamine. *J. Pharmacol. Exp. Ther.* 247, 79–87.
- Swanson, L.W., 1992. *Brain Maps: Structure of the Rat Brain* Elsevier, Amsterdam, NL.
- Tomaz, C., Aguiar, M.S., Nogueira, P.C.J., 1990. Facilitation of memory by peripheral administration of substance P and naloxone using avoidance and habituation learning tasks. *Neurosci. Biobehav. Rev.* 14, 447–453.
- Unterwald, E.M., Ho, A., Rubinfeld, J.M., Kreek, M.J., 1994. Time course of the development of behavioral sensitization and dopamine receptor up-regulation during binge cocaine administration. *J. Pharmacol. Exp. Ther.* 270, 1387–1396.
- Walker, P.D., Riley, L.A., Hart, R.P., Miller Jonakait, G., 1991. Serotonin regulation of tachykinin biosynthesis in the rat neostriatum. *Brain Res.* 546, 33–39.
- White, S.R., Obradovic, T., Imel, K.M., Wheaton, M.J., 1996. The effects of methylene dioxymethamphetamine (MDMA, “Ecstasy”) on monoaminergic neurotransmission in the central nervous system. *Prog. Neurobiol.* 49, 445–449.
- Yaksh, T.L., Jessel, T.M., Gamse, R., Kalivas, P.W., 1994. Methylene dioxymethamphetamine depresses glutamate-evoked neuronal firing and increases extracellular levels of dopamine and serotonin in the nucleus accumbens in vivo. *Neuroscience* 62, 155–157.
- Yamamoto, B.K., Spanos, L.J., 1988. The acute effects of methylene dioxymethamphetamine on dopamine release in the awake-behaving rat. *Eur. J. Pharmacol.* 148, 195–203.
- Yan, Q., Reith, M.E., Yan, S., 2000. Enhanced accumbal dopamine release following 5-HT(2A) receptor stimulation in rats pretreated with intermittent cocaine. *Brain Res.* 863, 254–258.