The Journal of Applied Pharmacology, **12**, 101-107(2004)

Behavioral Sensitization and M1 Muscarinic Acetylcholine Receptor mRNA Expression in Methamphetamine-Administered Mice

Kyung In KIM, Jae Han CHO, Hyun Jung PARK, Seok-Yong LEE and Choon-Gon JANG*

Department of Pharmacology, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea

(Received June 5, 2004; Accepted June 24, 2004)

Abstract – Repeated administration of psychostimulants such as amphetamines increases locomotor activity in rodents. These drugs, including methamphetamine, enhance dopaminergic neurotransmission and result in hyper-locomotion and behavioral sensitization. It is well known that the existence of a complex balance between the cholinergic and dopaminergic systems in the central nervous system. Thus, behavioral sensitization by methamphetamine may be related to the expression of the M1 muscarinic acetylcholine receptors gene. The present study investigated the changes of M1R mRNA in hyperlocomotor activity and behavioral sensitization by methamphetamine (2 mg/kg) in mice. Our results showed that M1R mRNA expression was increased in the frontal cortex and the hippocampus region (the CA2 region) in the acute methamphetamine administered group compared to the saline administered group. In the chronic group, M1R mRNA expression was increased in the frontal cortex and the hippocampus regions (CA2 and DG regions) in methamphetamine administered group compared to saline control group. These results indicate that acute or chronic treatment of mathamphetamine leads to the region-specific changes in mRNA expression levels of M1R. Therefore, the present result suggests that M1R may play a role in modulating of methamphetamine-induced behavioral sensitization in mice.

Keywords ☐ Muscarinic receptors, behavioral sensitization, methamphetamine

INTRODUCTION

Psychostimulants are widely abused throughout the world, causing severe medical and social problems. Acute treatment with psychostimulants increases locomotor activity. Repeated administration of low doses of psychostimulants results in progressive increases in the locomotor activities produced by a subsequent dose of the drug; the phenomenon is referred to as behavioral sensitization or reverse tolerance. Animals repeatedly treated with amphetamines showed increased behavioral sensitization (Piazza *et al.* 1990; Gerber *et al.* 2001; Kitanaka *et al.*, 2003). The increased sensitivity to the locomotor stimulating effect of these drugs (behavioral sensitization) is believed to be relevant to the psychopathology, drug addiction and craving that develop in humans abusing psychostimulants (Robinson and Berridge, 1993; Post *et al.*, 1988).

It has been proposed that locomotor stimulation of psychostimulants is associated with the activation of the mesolimbic dopaminergic mechanism. The behavioral effects of the psychostimulants such as methamphetamine and amphetamine increase synaptic levels of the neurotransmitter dopamine (DA). Amphetamines cause the release of DA, and bind to the dopamine transporters (DAT) and inhibit the reuptake of DA into the presynaptic nerve endings (Seiden *et al.*, 1993; Kitayama and Dohi, 1996; Fleckenstein *et al.*, 2000).

Recently, dopaminergic neuron signaling at the striatum was reported to synchronize with hippocampal neurons, and other researchers have suggested that the administration of d-amphetamine causes an increase of extracellular noradrenaline in rat frontal cortex (Wortley *et al.*, 1999; Goto and O'Donnell, 2001). DA release in the striatum is believed to be the main mediator of the locomotor activating properties of psychostimulants. Although these evidences provide that enhanced DA transmission in the nucleus accumbens and striatum is associated with behavioral sensitization to amphetamine (Robinson and Becker, 1986; Robinson *et al.*, 1998), the role of other transmission is also apparent. Recently, a role for acetylcholine (ACh) receptors in modulating psychostimulant induced locomotor activity has been suggested.

Identification of the muscarinic acetylcholine receptors

*Corresponding author
Tel: 031-290-7780, Fax: 031-292-8800
E-mail: jang@skku.ac.kr

(mAChR) subtypes involved in these diverse central muscarinic functions has proven a difficult task, primarily because of the lack of ligands with pronounced subtype selectivity (Buckley *et al.*, 1989; Dörje *et al.*, 1991; Caulfield, 1993; Wess, 1996). The mAChR proteins, typifying G-protein-coupled receptors, have seven putative transmembrane regions joined by alternating intracellular and extracellular loops (Wang *et al.*, 1989). At present, five different genes have been cloned, m1m5, that encode the corresponding mAChR subtypes, termed M1 through M5 (Bonner, 1987). The mRNA transcripts for all of the muscarinic subtypes are expressed in the mammalian brain (Bonner, 1987; Peralta *et al.*, 1987; Buckley *et al.*, 1988) and in peripheral tissues (Maeda *et al.*, 1988). The distinct distribution of the five mRNA suggests functional differences among the receptor subtypes. In general, M1, M3, and M5 receptors preferentially activate the phospholipase C pathway through selective coupling to $G_{q/11}$, whereas M2 and M4 receptor subtypes inhibit adenylyl cyclase by coupling to G_i (Caulfield, 1993). In particular, $G_{q/11}$ coupled M1 muscarinic receptor (M1R) is important in learning and memory, and may play an important role in the regulation of extrapyramidal locomotor function (Fornari *et al.*, 2000; Gerber; *et al.*, 2001). We, therefore, anticipate that M1R may play a role of methamphetamine-induced locomotor activities and/or behavioral sensitization.

Psychostimulants-induced alterations of gene expression have been reported. Alterations in the neural gene expressions and subsequent protein expressions following chronic treatment with psychostimulants are important subcellular events towards behavioral sensitization. The previous study of receptor gene-specific mRNA, or receptor specific immunoreactivity can provide an evidence supporting the pharmacological demonstration of a functional receptor subtype (Caulfield and Birdsall 1998), and *in situ* mRNA hybridization studies have shown that all five mAChRs are expressed in areas of the CNS known to contain cholinergic cell bodies, raising the possibility that multiple mAChRs participate in presynaptic modulation of ACh release (Vilaro *et al.*, 1994). To estimate the role of M1 muscarinic receptor in methamphetamine-induced behavioral sensitization, we measured locomotor activity and behavioral sensitization in methamphetamine administered mice, and examined expression of M1R mRNA for *in situ* hybridization in the several cerebrum regions; the frontal cortex, striatum, and the hippocampus regions (the CA1, CA2, CA3, and the DG regions), of the methamphetamine administered mice.

MATERIALS AND METHODS

Animals and drugs

Male ICR mice (MJ Ltd. Co., Seoul, Korea) weighing 20~25 g were used. They were housed in a group of 9 and in an acrylic fiber cage in temperature and humidity controlled room (25 ± 1°C, 55 ± 5%) with a 12h light/dark cycle (lights on at 7:00 A.M.). The animals had free access to food and drinking water. The drug used was methamphetamine hydrochloride (The USP convention, Inc., Rockville, USA), which was dissolved in physiological saline. The drug solution was administered by intraperitoneal (i.p.) injection in a volume of 10 mg/kg of body weight.

Measurement of locomotor activity in methamphetamine administered mice

To test methamphetamine-induced behavioral locomotor activity, 2 mg/kg of methamphetamine administered by i.p. to mice once every other day for 7 days (day 1, 3, 5, and 7; 4 injections). Each mouse was placed in an activity cage (locomotor box; 30×30×30 cm) and then habituated for 30 min. After the habituation phase, the mice were administered methamphetamine and were returned to the same activity cage and then the horizontal activity was recorded using by videotracking system (NeuroVision, Pusan, Korea). Locomotor activity was immediately measured for 1 h on day 1 served as acute locomotor data and on day 7 served as a behavioral sensitization data.

Sample preparation

The mice were sacrificed by decapitation 2 h after methamphetamine administration (acute session) and the locomotor activity tested mice at day 7 were sacrifice by decapitation 2 h after the administration of the drug (chronic session). Their brains were removed and rapidly frozen in dry ice for 5 min and then stored at -70°C. Coronal brain sections (20 μm) were cut on a cryomicrotome (LEICA CM 1850) at -20°C and than mounted on gelatinized slides. Sections were stored at -70°C until used.

In situ hybridization for M1R mRNA

Oligonucleotide probes, M1R (sequence; 5'-TGG TGC CAA GAC AGT GAT GTT GGG ACT GAC AGC AGG GGG CAC TGA GGT-3', position; 188~235, accession number; NM 007698) were synthesized (Bioneer, Daejeon, Korea). The oligonucleotides were 3'-end labeled with [^{35}S]dATP

(Dupont NEN Life Science Products, MA, USA) using terminal transferase. Slide-mounted brain sections were fixed in 4% paraformaldehyde in 0.1M PBS (pH7.4), washed three times with 0.1M PBS for each 3 min and washed in 2× SSC for 3 min and then rinsed with deionized water. The sections were dried with air stream.

Brain sections were hybridized with 1.6×10^5 cpm/μl of radiorabeled oligonucleotide in hybridization buffer containing 100% formamide, 20× SSC, 50× Denhart's solution, dextran sulfate, 10 mg/ml salmon sperm DNA, 10 mg/ml yeast tRNAs and DTT. The sections were covered with cover slips and incubated at 40 in the oven for over night. After hybridization the cover slips were removed in 1× SSC and the sections were washed 3 times in 1× SSC for each 3 min, washed twice in 1× SSC at 60°C for each 15 min, washed twice in 1× SSC at 35°C for each 15 min and rapidly rinsed in deionized water. The sections were dried under air stream and exposed to Kodak Biomax autoradiographic film for 2 weeks at 4°C. The slide containing a scale of [^{14}C]micro-scale (Amersham Bio-Science, NJ, USA) were co-exposed with the samples. Film development protocol induced a 3 min dip in the developer solution and 10 min in the fixer.

The quantification of the autoradiogram for measurement of mRNA expression levels was performed using a computerized image analysis program (Molecular Dynamics Image Quant software). Coronal brain sections were analyzed in the following brain regions: the frontal cortex, striatum, and the hippocampus subregions (the CA1, CA2, CA3, and the DG regions).

Statistical analysis

All data were presented as the means ± SEM. Locomotor activity data were analyzed by one-way ANOVA followed by Newman-Keuls Multiple Comparison Test. *In situ* hybridization data were analyzed by Students t-test (unpaired). The analyzed data were used to determine the statistical significances of differences between the experimental groups. Criteria for significance were set as $p<0.05$.

RESULTS

Methamphetamine-induced locomotor activity and behavioral sensitization

The mice that received 2mg/kg of methamphetamine on each day 1, 3, 5, and 7 exhibited more movements than mice that received saline to confirm previous study that methamphetamine cause increasing locomotor activity and behavioral sensitization (Piazza *et al.* 1990; Gerber *et al.* 2001; Kitanaka *et al.*, 2003). Data were showed in table I. On day 1, acute methamphetamine administered mice significantly increased locomotor activity compared to saline injected mice ($P<0.001$). On day 7, repeated methamphetamine administered mice also significantly increased movements compared to saline administered mice ($P<0.001$). In addition the mice that repeated administered methamphetamine on day 7 significantly increased behavioral sensitization than acute methamphetamine administered mice on day 1 ($P<0.001$).

Table I. Effect of methamphetamine on locomotor activity and behavioral sensitization in mice.

	Traveled Distance (cm)	
	Acute (day 1)	Chronic (day 7)
Saline	8966 ± 1445	10219 ± 1395
Methamphetamine	36243 ± 1787[a]	54635 ± 1809[a,b]

The mice were administered once every other day for 7 days (1, 3, 5, 7 day) with either saline or methamphetamine (2 mg/kg i.p.). Locomotor activity was evaluated after the first or fourth injection of methamphetamine or saline. Data were presented as the means ± SEM of values. a, $p<0.001$, comparing methamphetamine group (n=9) vs. saline control group (n=9) on day 1 and 7, respectively; b, $p<0.001$, comparing day 1 vs. 7 in methamphetamine administered groups (one-way ANOVA followed by Newman-Keuls Multiple Comparison Test).

Effect of acute administration of methamphetamine on M1R mRNA expression in the brain

It is well known that muscarinic receptors innervate the frontal cortex, striatum, and the hippocampus. We, therefore, examined intensity of M1R mRNA expression in these brain areas. Fig. 1 shows methamphetamine or saline administered brain slices, coronal sections with representative brain areas using by *in situ* hybridization. At acute session, once methamphetamine administration increased M1R mRNA expression in the frontal cortex (161.7± 18.4 %, $p<0.05$). There was no change in M1R mRNA expression in the striatum of acute methamphetamine administered mice (81.74± 13.0%).

In the hippocampus regions, there was no change in M1R mRNA expression levels in the CA1, CA3 and DG regions in acute methamphetamine administered mice compared to saline administered mice (104.9 ± 4.3%, 107.8 ± 9.1%, and 109.4 ± 4.9%, respectively). On the other hand, M1R mRNA expression in the CA2 region significantly increased (120.7 ± 6.4%, $p<0.05$) in acute methamphetamine administered mice compared to saline group (Fig. 2). These results show that acute methamphetamine administration significantly increases M1R mRNA expression in the frontal cortex and in the CA2 regions

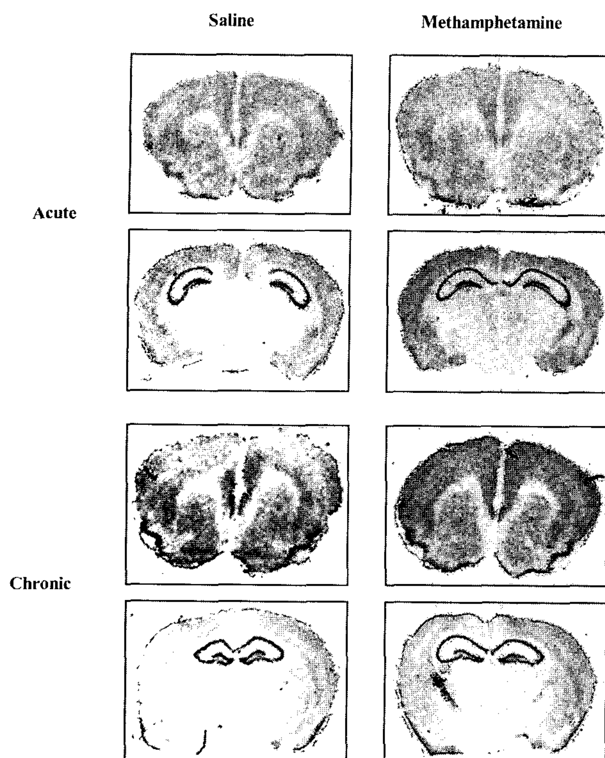

Fig. 1. Autoradiographic film images of M1R mRNA detected by means of in situ hybridization in coronal brain sections of methamphetamine administered mouse. Methamphetamine (2 mg/kg, i.p.) was administered one time (acute) or once every other day for 7 days (chronic; 4 injections) according to the locomotor test paradigm. The brains were sacrificed at 2 h after methamphetamine administration.

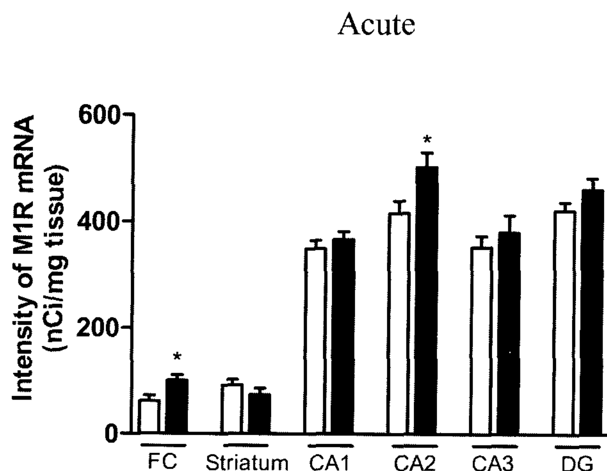

Fig. 2. *In situ* hybridization for M1R mRNA in coronal brain section of acute methamphetamine administered mouse by oligonucleotides labeled with [^{35}S]dATP. Expression levels of M1R mRNA were evaluated 2h after the first administration of methamphetamine (filled bar) or saline (open bar). FC: Frontal cortex, and DG: Dentate gyrus. Data were presented as the means ± SEM of values and were determined using Students t-test for unpaired comparison. *$p<0.05$ comparing saline vs. methamphetamine group.

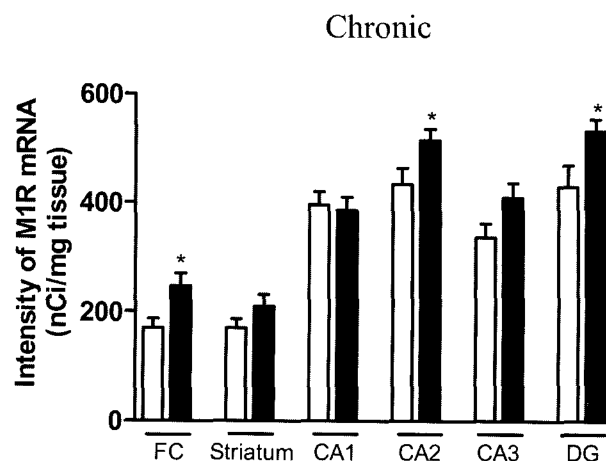

Fig. 3. In situ hybridization for M1R mRNA in coronal brain section of repeated methamphetamine administered mouse by oligonucleotides labeled with [^{35}S]dATP. Expression levels of M1R mRNA were evaluated 2h after the fourth administration of methamphetamine (filled bar) or saline (open bar). FC: Frontal cortex, and DG: Dentate gyrus. Data were presented as the means ± SEM of values and were determined using Students t-test for unpaired comparison. *$p<0.05$, comparing saline vs. methamphetamine group.

in the mice brain.

Effect of repeated administration of methamphetamine on M1R mRNA expression in the brain

Repeated methamphetamine administration significantly increased M1R mRNA expression in the frontal cortex (145.5 ± 13.6%, $p<0.05$). There was no change in M1R mRNA expression in the striatum of repeated methamphetamine administered mice compared to saline administered control mice (123.4± 12.9%).

In the hippocampus regions, M1R mRNA expression had no change in the CA1 and CA 3 regions in the repeated administration of methamphetamine group compared to saline administered group (97.63± 6.2% and 121. 9± 8.0%, respectively).

In the CA2 region, M1R mRNA expression levels significantly increased in repeated administration with methamphetamine compared to saline administered group (118.7 ± 4.9%, $p<0.05$). M1R mRNA expression significantly increased in repeated administration of methamphetamine (123.7 ± 5.1%, $p<0.05$) compared to saline control group in the DG region (Fig. 1 and 3). These results show that repeated methamphetamine administration significantly increases M1R mRNA expression in the frontal cortex and in the hippocampus regions: the CA2 and DG regions compared to saline administration control in the mice brains.

DISCUSSION

In the present study, acute or repeated administration of methamphetamine increased locomotor activity and behavioral sensitization. These data are similar to the previous studies that the acute administration of psychostimulants such as amphetamines enhances locomotor activity in response to treatment of the same or related drugs in rodents (Piazza *et al.* 1990; Gerber *et al.* 2001; Kitanaka *et al.*, 2003). The drugs of abuse that induce behavioral sensitization enhance dopaminergic neurotransmission, which mediates hyperlocomotion, reinforcing effects and rewarding properties by increasing extracellular DA levels in the terminal areas of the nigrostriatal and mesolimbic systems (Kalivas *et al.*, 1993; Wise, 1996; Uhl *et al.*, 2002). Amphetamines cause the release of DA through the DAT via reverse transport mechanisms (Seiden *et al.*, 1993). On the other hand, ACh released by neurons tends to oppose the effects of DA. A large body of evidence suggests the existence of a complex balance between the cholinergic and dopaminergic systems in the basal ganglia. The disruption of this balance could contribute to movement disorders such as parkinsonism (Graybiel, 1990; Di Chiara *et al.*, 1994). Cholinergic and dopaminergic balance is required for normal motor functions (Sousa *et al.*, 2001). M1 muscarinic receptors mediate many cholinergic effects described in the central nervous system, including the cortex, striatum and the hippocampus. It has been implicated in a variety of central processes, including learning and memory, depression, generation of seizures and locomotion (Nathanson, 1987; Wess, 1996). Especially, M1R may play an important role in the regulation of extrapyramidal locomotor function and in certain aspects of schizophrenia (Levey *et al.*, 1991; Bymaster *et al.*, 1999). In addition, dysfunction of the prefrontal cortex has commonly been associated with a deficit state in schizophrenia (Buchnan *et al.*, 1990). In rodents, lesions of the medial prefrontal cortex block the sensitization of hyperlocomotion, but not stereotypy, following a sensitizing amphetamine regimen. The medial parts of the hippocampus appear to be critical sites of dysfunction in schizophrenic patients (Wolf *et al.*, 1995). Our data show that expression of M1R mRNA by *in situ* hybridization was increased in the frontal cortex and the hippocampus region (including the CA2 region) in acute methamphetamine administered mice compared to control group. These results suggest that acute treatment of methamphetamine leads to the region-specific changes in M1R mRNA expression levels.

In methamphetamine-induced behavioral sensitization, the expression levels of M1R mRNA were similar to that of methamphetamine-induced locomotor activity. The M1R mRNA expression in the repeated methamphetamine administration indicated the region-specific increases in the frontal cortex and the hippocampus regions: the CA2 and DG regions, on day 7. These results suggest that the development of behavioral sensitization to methamphetamine is involved in the increased expression of mRNA for M1R in the frontal cortex, the CA2 and DG regions. Previous study suggested that DG region of the hippocampus regions was the most important neural substrate of the locomotor activity of corticotropin-releasing factor (Lee *et al.*, 1989). It is also well known that DA level was increased in the frontal cortex and hippocampus as well as the striatum in methamphetamine-induced behavioral sensitization (Wortley *et al.*, 1999; Goto and O'Donnell, 2001). Thus, although our results had no change in M1R mRNA expression in the striatum, M1R mRNA expression was significantly increased in the frontal cortex and hippocampus regions (including the CA2 and DG regions) in methamphetamine treated mice. These results suggest that the frontal cortex and hippocampus regions (including the CA2 and DG regions) are important target regions in the expression of M1R mRNA in methamphetamine-induced behavioral sensitization.

In several previous studies, the neural correlation of amphetamine-induced behavioral sensitization has been reported to mediate via a reciprocal balance between dopaminergic and inhibitory cholinergic mechanisms. Cholinergic neurons as well as dopaminergic neurons are highly responsive to repeated forced administration of several drugs of abuse (Nestby *et al.*, 1997; Mark *et al.*, 1999). Other study validated that scopolamine treated group more increased methamphetamine-induced behavioral sensitization compared to only methamphetamine treated group. Behavioral sensitization by methamphetamine administration after scopolamine administration might be mediated via a reciprocal balance between the dopaminergic and cholinergic systems in favor of dopaminergic dominance, and that such a balance might be involved in the conditioning to the drug-associated tone conditioned stimulus (Yui *et al.*, 1996). In addition, M1 knockout mice were found to have a significantly elevated level of extracellular DA in the striatum with correspondingly increased locomotor activity (Gerber *et al.*, 2001). These findings demonstrate that M1R function is required for regulation of dopaminergic transmission and for maintaining normal control of locomotor behavior.

In conclusion, the present study revealed a role of the M1R in mediating the locomotor activity of acute and repeated meth-

amphetamine administration. The major findings of the present study are: (a) expression of M1R mRNA is increased mainly in the frontal cortex and the CA2 region in acute methamphetamine administered mice, (b) expression of M1R mRNA is increased mainly in the frontal cortex, the CA2 and DG regions in repeated methamphetamine administered mice. Therefore, our results indicate that methamphetamine increases expression of M1R mRNA, locomotor activity and behavioral sensitization in mice. In further study, we need to examine whether the M1R protein levels are correlated with the mRNA levels in methamphetamine-induced behavioral sensitization.

ACKNOWLEDGEMENT

This research was supported by a grant (M103KV010008 04K2201 00830) from Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology of Republic of Korea.

REFERENCE

Bonner T. I., Buckley N. J., Young A. C. and Brann M. R. (1987) Identification of a family of muscarinic acetylcholine receptor genes. *Science.* **237**, 527-532.

Buchanan R. W., Kirkpatrick B., Heinrichs D. W. and Carpenter W. T. (1990) Clinical correlates of the deficit syndrome of schizophrenia. *Am J Psychiatry.* **147**, 290-294.

Buckley N. J., Bonner T. I. and Brann M. R. (1988) Localization of a family of muscarinic receptor mRNA in rat brain. *J Neurosci.* **8**, 4646-4652.

Buckley N. J., Bonner T. I., Buckley C. M. and Brann M. R. (1989) Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. *Mol Pharmacol.* **35**, 469-476.

Bymaster F. P., Shannon H. E., Rasmussen K., DeLapp N. W., Ward J. S., Calligaro D. O., Mitch C. H., Whitesitt C., Ludvigsen T. S., Sheardown M., Swedberg M., Rasmussen T., Olesen P. H., Jeppesen L., Sauerberg P. and Fink-Jensen A. (1999) Potential role of muscarinic receptors in schizophrenia. *Life Sci.* **64,** 527-534.

Caulfield M. P. (1993) Muscarinic receptors-characterization, coupling and function. *Pharmacol Ther.* **58**, 319-379.

Caulfield M. P. and Birdsall N. J. (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol Rev.* **50**(2), 279-290.

Di Chiara G., Morelli M. and Consolo S. (1994) Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/ NMDA interactions. *Trends Neurosci.* **17**, 228-233.

Dörje F., Wess J., Lambrecht G., Tacke R., Mutschler E. and Brann M. (1991) Antagonist binding profiles of five cloned human muscarinic receptor subtypes. *J. Pharmacol Exp Ther.* **256**, 727-733.

Fleckenstein A. E., Gibb J. W. and Hanson G. R. (2000) Differential effects of stimulants on monoaminergic transporters:pharmacological conse-quences and implications for neurotoxicity. *Eur J. Pharmacol.* **406**, 1-13.

Fornari R. V., Moreira K. M. and Oliveira M. G. (2000) Effects of the selective M1 muscarinic receptor antagonist dicyclomine on emotional memory. *Learn Mem.* **7**(5), 287-292.

Gerber D. J., Sotnikova T. D., Gainetdinov R. R., Huang S. Y., Caron M. G. and Tonegawa S. (2001) Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in M1 muscarinic acetylcholine receptor-deficient mice. *Proc Natl Acad Sci U.S.A* **98**(26), 15312-15317.

Goto Y. and O'Donnell P. (2001) Network synchrony in the nucleus accumbens in vivo. *J. Neurosci.* **21**, 4498-4504.

Graybiel A. M. (1990) Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci.* **13**, 244-253.

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

Kitanaka J., Kitanaka N. and Takemura M. (2003) Chronic methamphetamine administration reduces histamine-stimulated phosphoinositide hydrolysis in mouse frontal cortex. *Biochem Biophys Res Commun.* **300**(4), 932-937.

Kitayama S. and Dohi T. (1996) Cellular and molecular aspects of monoamine neurotransmitter transporters. *Jpn J. Pharmacol.* **72**, 195-208.

Lee E. H. and Tsai M. J. (1989) The hippocampus and amygdala mediate the locomotor stimulating effects of corticotropin-releasing factor in mice. *Behav Neural Biol.* **51**(3), 412-423.

Levey A. I., Kitt C. A., Simonds W. F., Price D. L. and Brann M. R. (1991) Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J. Neurosci.* **11**, 3218-3226.

Maeda A., Kubo T., Mishina M. and Numa S. (1988) Tissue distribution of mRNA encoding muscarinic acetylcholine receptor subtypes. *FEBS Lett.* **239**, 339342.

Mark G. P., Hajnal A., Kinney A. E. and Keys A. S. (1999) Self-administration of cocaine increases the release of acetylcholine to a greater extent than response-independent cocaine in the nucleus accumbens of rats. *Psychopharmacology (Berl).* **143**(1), 47-53.

Nathanson N. M. (1987) Molecular properties of the muscarinic acetylcholine receptor. *Annu Rev Neurosci.* **10**, 195-236.

Nestby P., Vanderschuren L. J., De Vries T. J., Hogenboom F., Wardeh G., Mulder A. H. and Schoffelmeer A. N. (1997) Ethanol, like psychostimulants and morphine, causes long-lasting hyperreactivity of dopamine and acetylcholine neurons of rat nucleus accumbens: possible role in behavioural sensitization. *Psychopharmacology (Berl).* **133**(1), 69-76.

Peralta E. G., Ashkenazi A., Winslow J. A. W., Smith D. H., Ramachandran J. and Capon D. J. (1987) Distinct primary structures, ligand-binding properties and tissue-specific expression of four human muscarinic acetylcholine receptors. *EMBO J.* **6**, 3923-3929.

Piazza P. V., Deminiere J. M., le Moal M. and Simon H. (1990) Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. *Brain Res.* **514**(1), 22-6.

Post R. M. and Weiss S. R. B. (1988) Sensitization and kindling: implications for the evolution of psychiatric symptomatology. In: Kalivas, P. W., Barnes, C. D. (Eds.), Sensitization in the Nervous System. *Telford Press, Caldwell,* 257-292.

Robinson T. E. and Becker J. B. (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration; A review and evaluation of animal models of amphetamine psychosis. *Brain Res Rev.* **11**, 157-198.

Robinson T. E. and Berridge K. C. (1993) The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Res Rev.* **18**, 247-291.

Robinson T. E., Browman K. E., Crombag H. S. and Badiani A. (1998) Modulation of the induction or expression of psychostimulant sensitization by the circumstances surrounding drug administration. *Neurosci Biobehav Rev.* **22**, 347-354.

Seiden L. S., Sabol K. E. and Ricaurte G. A. (1993) Amphetamine: effects on catecholamine systems and behavior. *Annu Rev Pharmacol Toxicol.* **32**, 639-677.

Sousa F. C., Gomes P. B., Noronha E. C., Macedo D. S., Vasconcelos S. M., Fonteles M. M. and Viana G. S. (2001) Effects of dopaminergic and cholinergic interactions on rat behavior. *Life Sci.* **69**(20), 2419-2428.

Uhl G., Lin Z., Metzger T. and Dar D. E. (1998) Dopamine transporter mutants, small molecules, and approaches to cocaine antagonist/dopamine transporter disinhibitor development. In : Amara SG,editor. *Methods in enzymology. Academic Press* **296**, 456-465.

Vilaro M. T., Palacios J. M. and Mengod G. (1994) Multiplicity of muscarinic autoreceptor subtypes? Comparison of the distribution of cholinergic cells and cells containing mRNA for five subtypes of muscarinic receptors in the rat brain. *Mol. Brain Res.* **21**, 30-46.

Wang H., Lipfert L., Malbon C. C. and Bahouth S. (1989) Site-directed anti-peptide antibodies define the topography of the beta-adrenergic receptor. *J Biol Chem.* **264**, 14424-14431.

Wess J. (1996) Molecular biology of muscarinic acetylcholine receptors. *Crit Rev Neurobiol.* **10**, 69-99.

Wise R. A. (1996) Addictive drugs and brain stimulation reward. *Annu Rev Neurosci.* **19**, 319-340.

Wolf M. E., Dahlin S. L., Hu X. T., Xue C. J. and White K. (1995) Effects of lesions of prefrontal cortex, amygdale, or fornix on behavioral sensitization to amphetamine: Comparison with N-methyl-D-aspartate antagonists. *Neurosci.* **69**, 417-439.

Wortley K. E., Hughes Z. A., Heal D. J. and Stanford S. C. (1999) Comparison of changes in the extracellular concentration of noradrenaline in rat frontal cortex induced by sibutramine or d-amphetamine: modulation by a2-adrenoceptors. *Br J Pharm.* **127**, 1860-1866.

Yui K. and Miura T. (1996) Behavioral responses induced by repeated treatment with methamphetamine alone and in combination with scopolamine in rats. *Neuropsychobiology.* **33**(1), 21-7.