Korean J Physiol Pharmacol
Vol 11: 139-144, August, 2007

Effects of Repeated Nicotine Treatment on the Changes in Glutamate Receptor Subunits Levels in Mesocorticolimbic Dopamine Areas

Kuem-Ju Lee[2], Dong-Hoon Kim[1], Song-Hyen Choi[2], You-Chan Shin[1], Sang-Ha Park[1], Bo-Hyun Moon[2], Seung Woo Kang[1], Eujin Cho[1], Sang-Hyun Choi[1], Boe-Gwun Chun[1], Min-Soo Lee[3], and Kyung-Ho Shin[1]

[1]Department of Pharmacology, [2]Graduate School of Biomedical Sciences, [3]Department of Psychiatry, Korea University College of Medicine, Seoul 136-705, Korea

Recent studies suggest that alterations in glutamate receptor subunit levels in mesocorticolimbic dopamine areas could account for neural adaptations in response to psychostimulant drugs. Although many drugs of abuse induce changes in ionotropic glutamate receptor subunits in mesocorticolimbic dopamine areas, the changes of ionotropic glutamate receptor subunits by repeated nicotine treatment in these areas are not known. To answer this question, we injected male Sprague-Dawley rats twice daily with nicotine (0.4 mg/kg) or saline (1 ml/kg) for 10 days. The immunoreactivity of NR1, GluR1, and GluR2 glutamate receptor subunits was examined 16~18 h after the last injection of saline or nicotine. Repeated nicotine treatment significantly increased NR1 levels in the ventral tegmental area (VTA). In addition, repeated nicotine treatment showed a tendency towards an increase in GluR1 levels in the VTA as well as in striatum. However, there was no significant change in glutamate receptor subunits in other areas including nucleus accumbens (NAc). These results demonstrate that repeated nicotine treatment increases NR1 levels in VTA similarly to other drugs of abuse, suggesting that elevated glutamate receptor subunits in the VTA, but not NAc may be involved in the excitation of mesocorticolimbic dopamine neurons by nicotine.

Key Words: Addiction, Dopamine, Glutamate receptor, Nicotine, Synaptic plasticity, Ventral tegmental area

INTRODUCTION

Tobacco smoking is addictive and its use is the single most important preventable health risk in the developed world (Fagerstrom, 2002). Nicotine is known as a component of tobacco that induces habitual smoking (Nisell et al, 1995). Considerable evidence suggests that mesocorticolimbic dopamine (DA) system, which originates in the ventral tegmental area (VTA) and innervates the nucleus accumbens (NAc) and prefrontal cortex (Fallon & Moore, 1978), has been attributed to the positive reinforcement of many drugs of abuse, including nicotine. Nicotine increases the firing rate and burst firing of DA neurons in VTA (Grenhoff et al, 1986; Johnson et al, 1992; Nisell et al, 1996; Fisher et al, 1998), which, in turn, leads to increased release of DA in NAc (Johnson et al, 1992; Chergui et al, 1993). This increase in extracellular DA in the NAc is thought to mediate the positive reinforcement (Corrigall et al, 1992; Corrigall et al, 1994) and rewarding action of nicotine (Schilstrom et al, 1998b). Moreover, nicotine also acts at presynaptic $\alpha 7$ nicotinic acetylcholine receptors (nAChRs) located on glutamatergic afferents to VTA to increase glutamate release (Schilstrom et al, 2000), thereby increasing DA release in NAc through NMDA receptors on dopamine neurons in VTA (Schilstrom et al, 1998a; Svensson et al, 1998; Fu et al, 2000).

Recently, in addition to changes in glutamate release in VTA, it has been suggested that many drugs of abuse induce changes in ionotropic glutamate receptor subunits in mesocorticolimbic dopamine areas. For example, repeated treatment of cocaine (Churchill et al, 1999), morphine (Fitzgerald et al, 1996) or ethanol (Ortiz et al, 1996) increases GluR1 levels, one of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits, in the VTA, which could contribute to increased sensitivity to the locomotor stimulating and rewarding actions of drugs of abuse (Carlezon et al, 1997). Although a single systemic injection does not change GluR1 expression in VTA (Ferrari et al, 2002), changes of ionotropic glutamate receptor subunits by repeated nicotine treatment in mesocorticolimbic dopamine areas have not been studied.

Corresponding to: Kyung-Ho Shin, Department of Pharmacology, Korea University College of Medicine, 1, Anam-dong 5-ga, Sungbuk-gu, Seoul 136-705, Korea. (Tel) 82-2-920-6195, (Fax) 82-2-927-0824, (E-mail) kyungho@korea.ac.kr

ABBREVIATIONS: VTA, ventral tegmental area; DA, dopamine; NAC, nucleus accumbens; nAChR, nicotinic acetylcholine receptor; NMDA, N-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; mPFC, medial prefrontal cortex; SN, substantia nigra.

To answer this question, we injected rats with nicotine for 10 days, and observed the changes of ionotropic glutamate receptor subunits in mesocorticolimbic dopamine areas.

METHODS

Animals and nicotine administration

Male Sprague-Dawley rats (initial weight 240~260 g, Samtaco, Osan, Korea) were received 4 days before experiment and were group housed 3 per cage under a 12 h light-dark cycle (light on at 6:00 A.M.). Food and water were available *ad libitum*. All experimental procedures performed on the animals were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). All rats received injections twice daily (at 10:00 A.M. and 16:00 P.M.) for 10 days with either nicotine [0.4 mg/kg, s.c., (−)-nicotine hydrogen tartate salt, Sigma, St. Louis, MO] or 0.9 % saline (1 ml/kg, s.c.). (−)-Nicotine hydrogen tartrate salt was dissolved in 0.9% saline solution, and adjusted to pH 7.2~7.4 with 1 N NaOH. This dose of nicotine has been shown to enhance levels of extracellular dopamine in the nucleus accumbens and increase locomotor activity in response to subsequent injection of nicotine (Benwell & Balfour, 1992; Benwell et al, 1995).

Western blot analyses of glutamate receptor subunits

Rats were killed by decapitation 16~18 h after the last injection of nicotine or saline, as reported previously for cocaine (Fitzgerald et al, 1996; Boundy et al, 1998) or morphine (Lane-Ladd et al, 1997). Brains were placed in ice-cold artificial cerebrospinal fluid and 1.0 mm-thick coronal slices of brain were obtained with McIlwain tissue chopper (Brinkmann Instruments, Westbury, USA). The appropriate brain regions were dissected on an ice-cooled plate using a 12 or 15-gauge tissue punch according to the method of Wolf et al (1999). Bilateral tissue samples were homogenized in 1% sodium dodecyl sulfate (SDS) (Berhow et al, 1996; Fitzgerald et al, 1996) and frozen in aliquots at −80°C. Protein content was determined using Bio-Rad protein assay (Bio-Rad Laboratories Inc., Hercules, USA). Samples (15~30 μg of protein) were subjected to SDS-polyacrylamide gel electrophoresis with resolving gels containing 8% acrylamide and transferred electrophoretically to PVDF membranes (Bio-Rad Laboratories Inc., Hercules, USA). Proteins were probed for glutamate receptor subunit GluR1 (1 : 2000; Chemicon, Temecula, USA), GluR2 (1 : 2000; Chemicon, Temecula, USA) or NR1 (1:2000; Chemicon, Temecula, USA) separately for 48 h at 4°C. The specificity of these antibodies has been established (Manabe et al, 2000; Terashima et al, 2004; Priel et al, 2005) and these antibodies recognized specific bands at their predicted molecular weights. Proteins were detected using horseradish peroxidase-conjugated IgG (1 : 2000; Vector, Burlingame, USA) followed by chemiluminescence (Amersham Biosciences, Arlington Heights, USA) and exposed to Hyperfilm (Amersham Biosciences, Arlington Heights, USA). Equal loading and transfer of proteins were confirmed in every experiment by analyzing blots with amido black staining (Lane-Ladd et al, 1997).

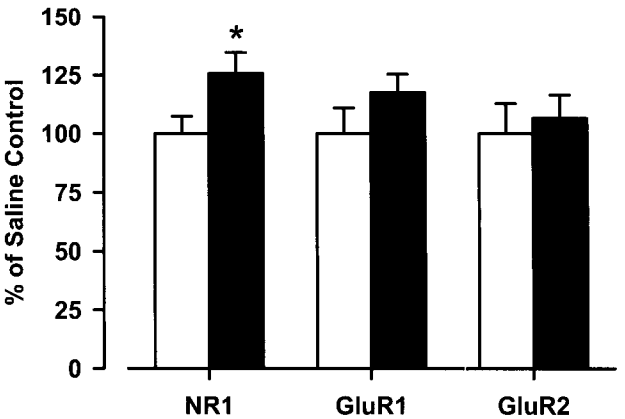

Fig. 1. Densitometric analysis of glutamate receptor subunit levels in ventral tegmental area (VTA) 10 days after repeated injection with saline (white bar) or nicotine (black bar). Data are mean±SEM percent change from values for repeated saline-treated control (abbreviated as saline control) for each immunoblot. Repeated nicotine-treated rats demonstrated a significant increase of NR1 ($p<0.05$) compared to saline control (n=9 per group). *$p<0.05$ compared to saline control.

Fig. 2. Effect of repeated nicotine treatment on the levels of glutamate receptor subunit immunoreactivity in rat ventral tegmental area (VTA) and nucleus accumbens (NAc) as determined by Western blotting. −: repeated saline-treated control, +: repeated nicotine-treated group.

Data analysis

Levels of immunoreactivity were quantified by measuring the optical density of each band using computer-assisted densitometry (NIH Image analysis program, version 1.61). Values of each bands obtained in repeated saline-treated control rats (abbreviated as saline control) were averaged, and the remaining data were normalized as percentage of saline control. Data were combined across blots for statistical analysis. The percent values in the Western blots between saline control and repeated nicotine-treated rats were compared using a two-tailed unpaired Student's *t*-test with a significance set at $p<0.05$.

RESULTS

Effects of repeated nicotine treatment on glutamate receptor subunit changes in the VTA

Repeated nicotine treatment caused a significant increase in NR1 levels (125.8±9.1% of saline control) in the VTA, whereas the same treatment did not alter GluR2

Table 1. Effects of repeated nicotine treatment on percent change of glutamate receptor subunits in nucleus accumbens (NAc), medial prefrontal cortex (mPFC), and substantia nigra (SN). Data are mean±SEM percent change in optical density from saline control

	NR1		GluR1		GluR2	
	Saline	Nicotine	Saline	Nicotine	Saline	Nicotine
NAc	100.0±8.3	94.7±8.8	100.0±10.1	100.0±6.7	100.0±8.3	94.7±8.8
mPFC	100.0±6.3	96.4±6.6	100.0±10.6	106.1±9.8	100.0±9.5	106.4±5.7
SN	100.0±15.4	99.7±17.0	100.0±9.1	103.4±9.0	100.0±6.3	108.9±8.3

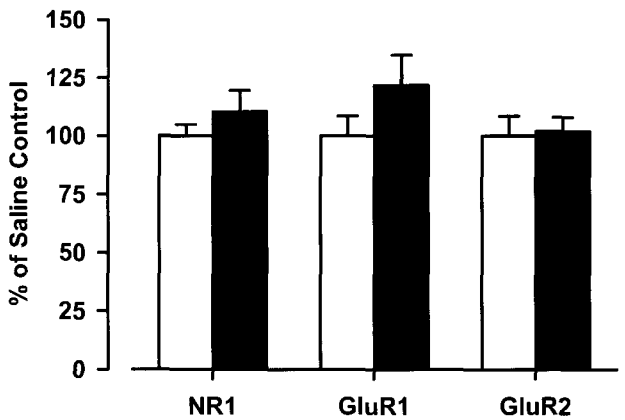

Fig. 3. Densitometric analysis of glutamate receptor subunit levels in striatum 10 days after repeated injection with saline (white bar) or nicotine (black bar). Data are mean±SEM percent change from values for repeated saline-treated control. There was a trend for an increase in GluR1 levels of striatum in repeated nicotine-treated rats (n=9 per group).

levels in the VTA. In addition, repeated nicotine treatment increased GluR1 levels in the VTA (117.6±7.9% of saline control), but this difference did not reach a statistical significance (Fig. 1, 2).

Effects of repeated nicotine treatment on glutamate receptor subunit changes in the NAc

Repeated nicotine treatment slightly decreased NR1 and GluR2 levels compared to saline control, but this effect was not statistically significant ($p > 0.05$). There was no significant change in GluR1 levels following repeated nicotine treatment compared with saline control (Fig. 2, Table 1).

Effects of repeated nicotine treatment on glutamate receptor subunit changes in the striatum

Repeated nicotine treatment caused an increase in GluR1 levels (121.6±13.2% of saline control) and NR1 levels (110.2±9.3% of saline control), respectively, but these effects were not statistically significant ($p > 0.05$). There was no change in GluR2 levels following repeated nicotine treatment compared with saline control (Fig. 3).

Effects of repeated nicotine treatment on glutamate receptor subunit changes in the medial prefrontal cortex (mPFC) and substantia nigra (SN)

Although there was a trend toward an increase in GluR1 levels (106.1±9.8% of saline control) and GluR2 levels (106.4±5.7% of saline control) in mPFC as well as GluR2 levels (108.9±8.3% of saline control) in the substantia nigra, this effect was not significant. Moreover, there was no significant effect of nicotine on NR1 levels in mPFC or SN (Table 1).

DISCUSSION

The present study demonstrates that repeated nicotine treatment significantly increased NR1 levels in VTA, which is similar to that reported after long-term treatments with ethanol (Ortiz et al, 1995) or cocaine (Fitzgerald et al, 1996; Churchill et al, 1999). Extensive pharmacological and physiological evidence shows the presence of NMDA and AMPA receptors in midbrain dopaminergic neurons (Overton & Clark, 1992; Chergui et al, 1993; Wang & French, 1993). The electrical activity of VTA DA neurons and the release of DA in the NAc are strongly regulated by the activation of these glutamate receptors on VTA DA neurons (Suaud-Chagny et al, 1992). The activation of NMDA or AMPA receptors increases firing of VTA DA neurons, but there is a difference in firing nature: NMDA receptor activation increases burst firing while AMPA receptor activation increases sustained firing. Apart from the sustained firing, the burst firing of VTA DA neurons are known to enhance the DA release in the NAc (Suaud-Chagny et al, 1992; Chergui et al, 1993). Furthermore, repeatitive nicotine treatment increases burst firing of VTA neurons in a manner that is antagonized by NMDA receptor antagonists (Nisell et al, 1996). Interestingly, $\alpha 7$ nAChRs are located on presynaptic glutamatergic terminal and, when these receptors are activated by nicotine, glutamate release from presynaptic glutamatergic terminal in VTA is increased (Schilstrom et al, 1998a). Thus, activation of presynaptic $\alpha 7$ nAChR by nicotine enhances glutamate release, which in turn leads to an increased activation of NMDA and AMPA receptors on VTA DA neurons. This hypothesis is supported by the finding that decreased function of NMDA receptor by NR1 antisense oligonucleotide reduces the number of bursts and percentage of DA neurons exhibiting a bursting pattern (Tajiri et al, 2001). Considering these together, increased NR1 levels by repeated nicotine treatment in the present experiment may be one of the

mechanisms by which nicotine increases the excitatory synaptic transmission in the VTA. It would be likely that repeated nicotine treatments increase the sensitivity of the NMDA receptors to subsequent stimulation by nicotine, which may be accompanied by burst firing of DA neuron in VTA (Nisell et al, 1996) as well as an increase in DA release in NAc (Benwell & Balfour, 1992).

Although GluR1 increase in VTA following repeated nicotine treatment was not significant, a tendency toward an increase of GluR1 in VTA may further contribute to the excitation of mesocorticolimbic dopamine neurons (Grillner & Svensson, 2000). Similar to our findings, repeated treatment with ethanol, morphine or cocaine increases GluR1 levels in the VTA (Ortiz et al, 1995; Fitzgerald et al, 1996; Churchill et al, 1999). Elevated GluR1 levels in the VTA may increase the formation of GluR1-homomeric AMPA receptors which can enhance Ca^{2+} influx to glutamate stimulation (Carlezon et al, 1997; Neve et al, 1997). Thus, VTA DA neurons may be more sensitive to AMPA-receptor initiated Ca^{2+} influx by nicotine after repeated nicotine treatment. As with increased NR1 levels in VTA, AMPA receptor activation may contribute further to the increase in the excitability and function of VTA dopaminergic neurons. However, it was reported that chronic administration of nicotine, using osmotic minipump, lead to a decreased firing rate of dopamine neuron in VTA (Rasmussen & Czachura, 1995). The reasons for this different result are not clear, but may be related to the treatment dose, regimen, duration and the time after the last drug dose (Carlezon & Nestler, 2002). For example, sensitization of dopamine release to subsequent challenge dose of nicotine is only observed after daily nicotine injection, but not after constant subcutaneous infusion of nicotine via Alzet minipumps (Benwell et al, 1995). Interestingly, repeated intermittent, but not constant, administration of morphine induces an increase of GluR1 subunit in VTA (Fitzgerald et al, 1996), suggesting that intermittent injection of nicotine causes a different result compared with osmotic minipump injection. In addition to increase in the excitability of VTA DA neurons, elevated GluR1 in the VTA may contribute, in part, to the increased sensitivity to the locomotor stimulating and rewarding actions of nicotine, as suggested by previous studies (Carlezon et al, 1997; Carlezon & Nestler, 2002).

In contrast with the VTA, repeated nicotine treatment did not affect the glutamate receptor subunit levels in the NAc. Similarly, repeated treatment with cocaine (Churchill et al, 1999) or amphetamine (Lu et al, 1999) does not change glutamate receptor subunits levels in the NAc. Given the importance of NAc in expression of drug sensitization, it is surprising that repeated nicotine treatment did not change the subunits of glutamate receptor. However, accumulating evidence shows that nicotinic receptors within VTA, rather than the NAc, are of major importance for nicotine's stimulatory actions on behavior and dopamine release in NAc (Corrigall et al, 1994; Nisell et al, 1994a, b). Although previous studies have focused on the role of the mesocorticolimbic system as a mediator for nicotine addiction, relatively less is known about the role of the nigrostriatal DA systems. Recent study shows that stimulation of nigrostriatal DA neurons may be involved in the expression of behavioral sensitization by repeated nicotine treatment (Shim et al, 2001). For example, the magnitude of increase in dopamine release in striatum by local infusion of nicotine is very similar to that observed in NAc (Shim et al, 2001). In the present study, repeated nicotine treatment showed only a tendency towards an increase in GluR1 levels in striatum, but not other glutamate receptor subunits. In line with this result, no change of MK-801 binding in striatum is observed in chronically nicotine-treated animals (Shoaib et al, 1997). It has been suggested that nicotine acts at $\alpha 7$ nAChRs on striatal glutamate terminals, and then it stimulates dopamine release in vitro (Kaiser & Wonnacott, 2000) and in vivo (Marchi et al, 2002). Moreover, perfusion of AMPA into the striatum causes a dose-dependent increase of dopamine release, which is prevented by AMPA antagonist, indicating that AMPA receptor may exert an excitatory influence on the release of dopamine in the striatum (Hernandez et al, 2003). These results raise the possibility that striatum may be involved in long-term changes following repeated nicotine treatment (Shim et al, 2001). However, there was no significant change in glutamate receptor subunits in medial prefrontal cortex and substantia nigra by repeated nicotine treatments. This result suggests that glutamate receptor subunits in PFC or SN may not be involved with long-term changes following nicotine treatment. However, we cannot exclude possible changes in glutamate receptor subunits after a different paradigm of nicotine injection.

Taken together, the present results demonstrate that repeated nicotine treatment increases NR1 levels in VTA similarly to other drugs of abuse, suggesting that elevated glutamate receptor subunits in the VTA, but not NAc may be involved the excitation of mesocorticolimbic dopamine neurons by nicotine. In addition, these results raise the possibility, although speculative, that elevated glutamatergic transmission in the VTA may be implicated in the increased sensitivity to the locomotor stimulating and rewarding actions of nicotine.

ACKNOWLEDGEMENT

This work was supported by grants from the MRC Research Center for Environmental Toxicogenomics and Proteomics (Korea Science and Engineering Foundations and Ministry of Science & Technology; R13-2003-016-02004-0) and the Korea Health 21 R&D Project (Ministry of Health & Welfare; 03-PJ10-PG13-GD01-0002).

REFERENCES

Benwell ME, Balfour DJ. The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. *Br J Pharmacol* 105: 849–856, 1992

Benwell ME, Balfour DJ, Birrell CE. Desensitization of the nicotine-induced mesolimbic dopamine responses during constant infusion with nicotine. *Br J Pharmacol* 114: 454–460, 1995

Berhow MT, Hiroi N, Nestler EJ. Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. *J Neurosci* 16: 4707–4715, 1996

Boundy VA, Gold SJ, Messer CJ, Chen J, Son JH, Joh TH, Nestler EJ. Regulation of tyrosine hydroxylase promoter activity by chronic morphine in TH9.0-LacZ transgenic mice. *J Neurosci* 18: 9989–9995, 1998

Carlezon WA Jr, Boundy VA, Haile CN, Lane SB, Kalb RG, Neve RL, Nestler EJ. Sensitization to morphine induced by viral-mediated gene transfer. *Science* 277: 812–814, 1997

Carlezon WA Jr, Nestler EJ. Elevated levels of GluR1 in the

midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci* 25: 610–615, 2002

Chergui K, Charlety PJ, Akaoka H, Saunier CF, Brunet JL, Buda M, Svensson TH, Chovet G. Tonic activation of NMDA receptors causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo. *Eur J Neurosci* 5: 137–144, 1993

Churchill L, Swanson CJ, Urbina M, Kalivas PW. Repeated cocaine alters glutamate receptor subunit levels in the nucleus accumbens and ventral tegmental area of rats that develop behavioral sensitization. *J Neurochem* 72: 2397–2403, 1999

Corrigall WA, Coen KM, Adamson KL. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Res* 653: 278–284, 1994

Corrigall WA, Franklin KB, Coen KM, Clarke PB. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology (Berl)* 107: 285–289, 1992

Fagerstrom K. The epidemiology of smoking: health consequences and benefits of cessation. *Drugs* 62(Suppl 2): 1–9, 2002

Fallon JH, Moore RY. Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *J Comp Neurol* 180: 545–580, 1978

Ferrari R, Le Novere N, Picciotto MR, Changeux JP, Zoli M. Acute and long-term changes in the mesolimbic dopamine pathway after systemic or local single nicotine injections. *Eur J Neurosci 15:* 1810–1818, 2002

Fisher JL, Pidoplichko VI, Dani JA. Nicotine modifies the activity of ventral tegmental area dopaminergic neurons and hippocampal GABAergic neurons. *J Physiol Paris* 92: 209–213, 1998

Fitzgerald LW, Ortiz J, Hamedani AG, Nestler EJ. Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. *J Neurosci* 16: 274–282, 1996

Grenhoff J, Aston-Jones G, Svensson TH. Nicotinic effects on the firing pattern of midbrain dopamine neurons. *Acta Physiol Scand* 128: 351–358, 1986

Grillner P, Svensson TH. Nicotine-induced excitation of midbrain dopamine neurons in vitro involves ionotropic glutamate receptor activation. *Synapse* 38: 1–9, 2000

Hernandez LF, Segovia G, Mora F. Effects of activation of NMDA and AMPA glutamate receptors on the extracellular concentrations of dopamine, acetylcholine, and GABA in striatum of the awake rat: a microdialysis study. *Neurochem Res* 28: 1819–1827, 2003

Johnson SW, Seutin V, North RA. Burst firing in dopamine neurons induced by N-methyl-D-aspartate: role of electrogenic sodium pump. *Science* 258: 665–667, 1992

Kaiser S, Wonnacott S. alpha-bungarotoxin-sensitive nicotinic receptors indirectly modulate [(3)H]dopamine release in rat striatal slices via glutamate release. *Mol Pharmacol* 58: 312–318, 2000

Lane-Ladd SB, Pineda J, Boundy VA, Pfeuffer T, Krupinski J, Aghajanian GK, Nestler EJ. CREB (cAMP response element-binding protein) in the locus coeruleus: biochemical, physiological, and behavioral evidence for a role in opiate dependence. *J Neurosci* 17: 7890–7901, 1997

Lu W, Monteggia LM, Wolf ME. Withdrawal from repeated amphetamine administration reduces NMDAR1 expression in the rat substantia nigra, nucleus accumbens and medial prefrontal cortex. *Eur J Neurosci* 11: 3167–3177, 1999

Manabe T, Aiba A, Yamada A, Ichise T, Sakagami H, Kondo H, Katsuki M. Regulation of long-term potentiation by H-Ras through NMDA receptor phosphorylation. *J Neurosci* 20: 2504–2511, 2000

Marchi M, Risso F, Viola C, Cavazzani P, Raiteri M. Direct evidence that release-stimulating alpha7* nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. *J Neurochem* 80: 1071–1078, 2002

Neve RL, Howe JR, Hong S, Kalb RG. Introduction of the glutamate receptor subunit 1 into motor neurons in vitro and in vivo using a recombinant herpes simplex virus. *Neuroscience* 79: 435–447, 1997

Nisell M, Nomikos GG, Hertel P, Panagis G, Svensson TH. Condition-independent sensitization of locomotor stimulation and mesocortical dopamine release following chronic nicotine treatment in the rat. *Synapse* 22: 369–381, 1996

Nisell M, Nomikos GG, Svensson TH. Infusion of nicotine in the ventral tegmental area or the nucleus accumbens of the rat differentially affects accumbal dopamine release. Pharmacol Toxicol 75: 348–352, 1994a

Nisell M, Nomikos GG, Svensson TH. Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. *Synapse* 16: 36–44, 1994b

Nisell M, Nomikos GG, Svensson TH. Nicotine dependence, midbrain dopamine systems and psychiatric disorders. *Pharmacol Toxicol* 76: 157–162, 1995

Ortiz J, Fitzgerald LW, Charlton M, Lane S, Trevisan L, Guitart X, Shoemaker W, Duman RS, Nestler EJ. Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. *Synapse* 21: 289–298, 1995

Ortiz J, Fitzgerald LW, Lane S, Terwilliger R, Nestler EJ. Biochemical adaptations in the mesolimbic dopamine system in response to repeated stress. *Neuropsychopharmacology* 14: 443–452, 1996

Overton P, Clark D. Iontophoretically administered drugs acting at the N-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. *Synapse* 10: 131–140, 1992

Priel A, Kolleker A, Ayalon G, Gillor M, Osten P, Stern-Bach Y. Stargazin reduces desensitization and slows deactivation of the AMPA-type glutamate receptors. *J Neurosci* 25: 2682–2686, 2005

Rasmussen K, Czachura JF. Nicotine withdrawal leads to increased firing rates of midbrain dopamine neurons. *Neuroreport* 7: 329–332, 1995

Schilstrom B, Fagerquist MV, Zhang X, Hertel P, Panagis G, Nomikos GG, Svensson TH. Putative role of presynaptic alpha7* nicotinic receptors in nicotine stimulated increases of extracellular levels of glutamate and aspartate in the ventral tegmental area. *Synapse* 38: 375–383, 2000

Schilstrom B, Nomikos GG, Nisell M, Hertel P, Svensson TH. N-methyl-D-aspartate receptor antagonism in the ventral tegmental area diminishes the systemic nicotine-induced dopamine release in the nucleus accumbens. *Neuroscience* 82: 781–789, 1998a

Schilstrom B, Svensson HM, Svensson TH, Nomikos GG. Nicotine and food induced dopamine release in the nucleus accumbens of the rat: putative role of alpha7 nicotinic receptors in the ventral tegmental area. *Neuroscience* 85: 1005–1009, 1998b

Shim I, Javaid JI, Wirtshafter D, Jang SY, Shin KH, Lee HJ, Chung YC, Chun BG. Nicotine-induced behavioral sensitization is associated with extracellular dopamine release and expression of c-Fos in the striatum and nucleus accumbens of the rat. *Behav Brain Res* 121: 137–147, 2001

Shoaib M, Schindler CW, Goldberg SR, Pauly JR. Behavioural and biochemical adaptations to nicotine in rats: influence of MK801, an NMDA receptor antagonist. *Psychopharmacology (Berl)* 134: 121–130, 1997

Suaud-Chagny MF, Chergui K, Chouvet G, Gonon F. Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. *Neuroscience* 49: 63–72, 1992

Svensson TH, Mathe JM, Nomikos GG, Schilstrom B. Role of excitatory amino acids in the ventral tegmental area for central actions of non-competitive NMDA-receptor antagonists and nicotine. *Amino Acids* 14: 51–56, 1998

Tajiri K, Emori K, Murata M, Tanaka K, Suzuki M, Uehara T, Sumiyoshi T, Ashby CR Jr, Kurachi M. Intracerebroventricular administration of NMDA-R1 antisense oligodeoxynucleotide significantly alters the activity of ventral tegmental area dopamine neurons: an electrophysiological study. *Synapse* 40: 275–281, 2001

Terashima A, Cotton L, Dev KK, Meyer G, Zaman S, Duprat F, Henley JM, Collingridge GL, Isaac JT. Regulation of synaptic strength and AMPA receptor subunit composition by PICK1. *J Neurosci* 24: 5381–5390, 2004

Wang T, French ED. Electrophysiological evidence for the existence of NMDA and non-NMDA receptors on rat ventral tegmental dopamine neurons. *Synapse* 13: 270−277, 1993

Wolf DH, Numan S, Nestler EJ, Russell DS. Regulation of phospholipase Cgamma in the mesolimbic dopamine system by chronic morphine administration. *J Neurochem* 73: 1520−1528, 1999