*Experimental Neurobiology*
Vol. 16, pages 27~33, June 2007

# Characteristics of Cholinergic Receptors in Nigrostriatal Neurons

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## ABSTRACT

Brain dopaminergic system has various important functions such as attention, locomotion, and sensory-motor integration. Although nicotine has a medical problem like addiction, it can activate brain dopaminergic system. The present study was conducted to determine the characteristics of nigrostriatal dopaminergic neurons by focusing on the relative contribution of nicotinic and muscarinic receptors and on sensitivity to alpha-bungarotoxin in nicotinic receptors. Under urethane anesthesia, the responses of nigrostriatal neurons of the male Sprague-Dawley rats to iontophoretically applied cholinergic chemicals were recorded. Nigrostriatal neurons showed high basal firing frequency. These neurons were more activated following the ejection of nicotine than muscarine. The distribution of receptors sensitive to alpha-bungarotoxin was relatively high compared to receptors insensitive to alpha-bungarotoxin. These results suggest that midbrain SNpc neurons reveal the distinct characteristics in terms of nicotinic receptors.

**Key words:** nigrostriatal neurons, nicotine, alpha-bungarotoxin, cholinergic receptors

## INTRODUCTION

Dopamine-containing neurons in the rat substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) receive moderate to dense innervations from mesopontine cholinergic cells (Bolam et el., 1991). The substantia nigra (SN) is located lateral to the A10 dopaminergic cell groups and contains A9 dopaminergic neurons. Dopaminergic cell bodies are located along dorsal part of the SNpc.

Acetylcholine (ACh) (Jacobowitz and Goldberg, 1977) and acetylcholine esterase (AChE) activities (Kobayashi et al., 1975) are high in both the VTA

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and SNpc. Localization of acetylcholine in the SNpc suggests cholinergic innervation to dopaminergic cell bodies.

Nicotinic receptors (nAChR), which are pentameric ligand-gated ion channels, have been shown to be present in the striatum or SN (Zhou et al., 2002; Dajas-Bailador and Wonnacott, 2004; Quik, 2004; Wonnacott et al., 2005). These nicotinic receptors can be activated by striatal dopamine, suggesting a close interaction between the nicotinic and dopaminergic systems (Salminen et al., 2004; McCallum et al., 2005). The cholinergic projection to the SN is originated in the pedunculopontine and lateral dorsal tegmental nuclei (Grace and Bunney, 1983; Clarke et al., 1987; Beninato and Spencer, 1988; Lee et al., 1988; Mitchell et al., 1989). Nicotinic cholinoreceptors are located on dopaminergic cells of the SNpc. Binding site of M1 and M2 muscarinic receptor subtypes are present in the SN. The muscarinic (M1, M2) and nicotinic receptors exist in both the VTA and SN (Clarke and Pert, 1985; London et al., 1985; Cortès et al., 1986; Boksa and Ouirion, 1987; Nastuk and Graybiel, 1991).

In electrophysiological studies, iontophoretically ejected ACh increases the firing frequence at dopaminergic and non-dopaminergic cells in the VTA and SNpc (Lichtensteiger et al., 1976; Lichtensteiger et al., 1982; Waszczak, 1990; Greenfield, 1991). Systemically and locally injected nicotine increases the neuronal firing frequence (Lichtensteiger et al., 1982; Clarke et al., 1985; Grenhoff et al., 1986; Carlson and Foote, 1992). However, the specific responsiveness of the SNpc neurons to cholinergic agents are still unclear. Therefore, the present study was conducted to determine the characteristics of nigrostriatal dopaminergic neurons by focusing on the relative contribution of nicotine and muscarinic receptors and on sensitivity to alpha-bungarotoxin in nicotinic receptors.

## MATERIALS AND METHODS

### *Subjects*

Fifty adult male Sprague-Dawley rats weighing 300±50 g were subjected to the microiontophoretic study. Anesthesia was induced by intraperitoneal administration of urethane (1.25 g/kg) and mounted on a stereotaxic apparatus. Rectal temperature was monitored by a thermistor and maintained between 36.5 and 37.5°C by means of an electrically heated blanket.

### *Electrophysiological recording from the SNpc*

Microelectrophoretic applications of chemicals with calibrated currents were performed using seven-barreled glass capillary pipettes (120F, WPI, Sarasota, Florida, USA) pulled in two stages with a glass micro electrode puller (PE-2, Narishige, Setagaya-ku, Tokyo, Japan). Extracellular single unit recordings were made with 7-barrel microiontophoretic pipettes with a central recording barrel (Lee et al., 1991). The tips of the recording electrodes were broken back under microscope control to a 8～10 mm diameter. The recording barrel was filled with a 2 M NaCl solution. Six surrounding barrels were used for drug ejections and a current balance for current neutralization. Action potentials were screened via a differential amplifier (AM502, Tektronix, Carrollton, TX, USA) and window discriminator (121, WPI, Sarasota, FL, USA), which generated square pulses. These pulses were fed to AD/DA converter (1401 plus, CED, Cambridge, UK) and a personal computer which generated firing rate histograms with a software for electrophysiology (Spike II, Cambridge Electronic Design, Cambridge, U.K.). The side barrels used for drug ejection were filled with the following solutions: 0.5 M nicotine, in 120 mM NaCl, pH 3.5; 5 mM muscarin, in 200 mM NaCl, pH 4; 1 mM dihydro-beta-erythroidine, in 165 mM Nacl, pH 4.5; 5 nM methyllycaconitine, in 165 mM NaCl, pH 4.5. To distinguish the nicotinic acetylcholine receptor subtype, we used dihydro-beta-erythroidine, a nicotinic ACh receptor antagonist (competitive antagonist) and methyllycaconitine (noncompetitive antagonist). Thus, to determine whether the neurons activated by nicotine is sensitive or insensitive to alpha-bungarotoxin, dihydro-beta-erythroidine (insensitive: Bachem, Torrance, CA, USA) and methyllycaconitine (sensitive: Bachem, Torrance, CA, USA) were used.

Presumed dopaminergic neurones within the SNpc are well established electrophysiological criterial (Grace and Bunney, 1983) including; 1) spontanteous firing rate between 5 and 90 spikes 10 s-1 (occurring sometimes in bursts); 2) triphasic

or biphasic waveforms, with an initial positive deflection followed with a prominent negative phase; 3) long action potential (duration 2～4 ms); and, 4) low pitch sound when monitored by an audio-amplifier.

The caudate-putamen (2.2 mm anterior and 1.6 mm lateral to the bregma, 5.4 mm below the cortical surface) was electrically stimulated and the evoked potentials were recorded by the electrode position area in the SNpc (−4.8～−6.2 posterior and 1.6～2.0 mm lateral to the bregma, 7.6～8.2 mm below the cortical surface).

### *Statistics*

Conduction velocity was expressed as the mean± SD. The number of SNpc neurons responsive to chemicals was counted and analyzed by $\chi^2$-test. Probability values smaller than 0.05 were considered significant.

## RESULTS

### *Identification of nigrostriatal neurons*

To observe the response of SNpc neurons to iontophoretically ejected drugs, it is important to determine that each neuron projects or not to the caudate-putamen. In order classify the SNpc neurons, we stimulate the caudate-putamen while recording the responses of SNpc neurons to electrical stimulation. Fig. 1 shows the location of SNpc neurons recorded. A total of 50 cells were found as nigrostriatal neurons. The nigrostriatal SNpc neurons were concentrated in 4.8～5.6 mm posterior to bregma and 1.6～2.0 mm lateral to the midline. Spontaneous firing rate of SNpc neurons was 41.53±4.85 impulse/sec.

### *Conduction velocity of nigrostriatal neurons*

In order to determine the conduction velocity of

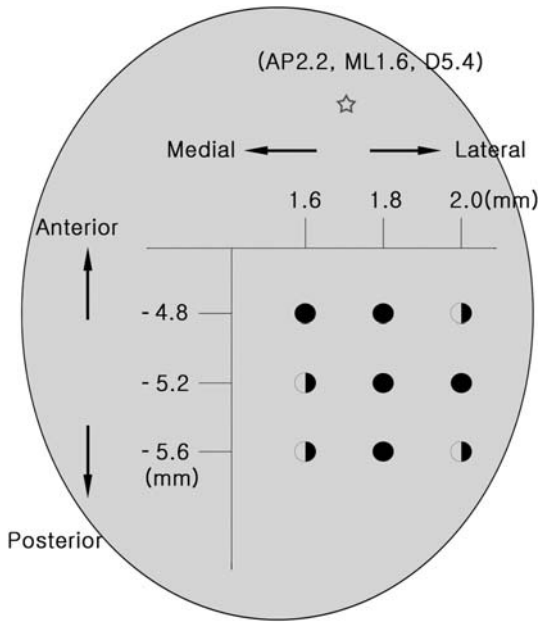


**Fig. 1.** The location of nigrostriatal neurons in horizon plane. (☆: stimulation site, ○: no response, ●: response, ◑: unclear).

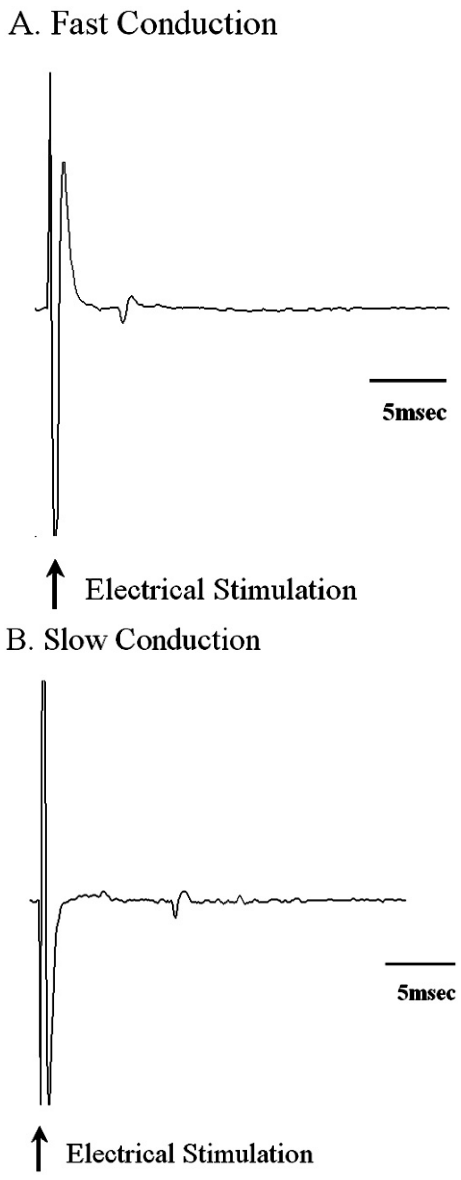


**Fig. 2.** Comparison of two SNpc neurons in terms of different conduction velocities.

nigrostriatal neurons, the latency of responses at SNpc to electrical stimulation of the caudate-putamen was measured and divided by the distance between stimulating and recording electrodes. The conduction velocity of nigrostriatal neurons which projects from the SNpc to the caudate-putamen could be readily classified into two categories showing fast and slow velocities (Fig. 2). Of 50 cells recorded in the SNpc, the fast conduction velocity was 3.30±0.17 m/sec and the slow neuronal conduction velocity was 1.10±0.08 m/sec.

### *Responses of SNpc neurons to iontophoretically ejected drugs*

After the classification of identified nigrostriatal neurons, the effect of iontophoretically injected drugs was observed. Fig. 3 shows the responses of SNpc neurons to microiontophoretically ejected chemicals. SNpc neurons which projects to the striatum responded to iontophoretically ejected nicotine (Fig. 3A). Nicotine was increased the responses of SNpc neurons. The responses of SNpc neurons to iontophoretically ejected nicotine were current-dependent. The increased responses of SNpc neurons to nicotine were current-dependently inhibited by methyllycaconitine. Fig. 3B shows the neuronal responses to the iontophoretically ejected nicotine and dihydro-beta-erythroidine. Dihydro-beta-erythroidine also reduced the neuronal responses to nicotine current-dependently.

Table 1 summarizes the responsiveness of SNpc neurons to iontophoretically administered chemicals. The identified nigrostriatal neurons were responded to iontophoretically ejected nicotine with increased responses. The neurones were more activated by

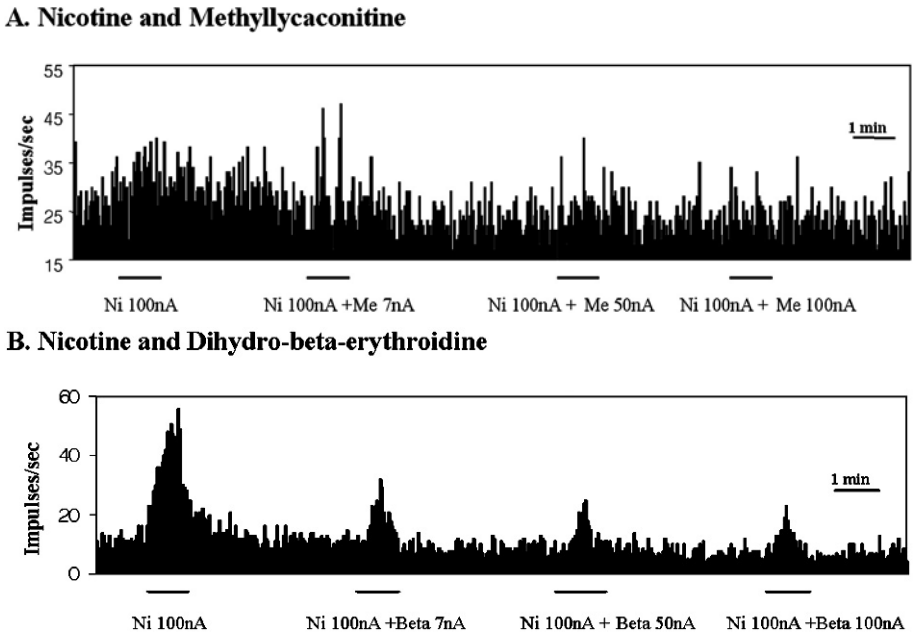


**Fig. 3.** The responses of SNpc neurons to iontophoretically ejected chemicals. Ni: Nicotine, Me: Methyllycaconitine, Beta: Dihydro-beta-erythroidine.

**Table 1.** Responses of SNpc neurons to iontophoretically administered chemicals

| Chemicals | Excitatory | Inhibitory | Biphasic | No effect | Total |
|---|---|---|---|---|---|
| Nicotine (NI) | 25 | 3 | 2 | 20 | 50 |
| Muscarine | 4 | 2 | 2 | 7 | 15 |
| Dihydro-beta-erythroidine (DI) | 0 | 1 | 0 | 9 | 10 |
| Methyllycarconitine (MC) | 0 | 1 | 9 | 0 | 10 |
| NI+DI | 7 | 11 | 0 | 6 | 24 |
| NI+MC | 8 | 17 | 0 | 6 | 31 |

nicotine than muscarine. Thus nigrostriatal neurons were much more sensitive to nicotine than muscarine.

To determine whether the neurons activated by nicotine is sensitive or insensitive to alpha-bungarotoxin, we observated the neuronal response to iontophoretically ejected methyllycaconitine and dihydro-beta-erythroidine. The results suggest that the neurons showed biphagical effects to methyllycaconitine alone but no effects to dihydro-beta-erythroidine alone ($\chi^2$=18, $p<.001$). When nicotine and its antagonist were concurrently ejected, the excitatory effect of nicotine was inhibited. In 11 of 24 nigrostriatal neurons (46%), the nicotinic excitatory effect was reduced by concurrently ejected dihydro-beta-erythroidine. In 17 of 31 neurons (55%), methyllycaconitine inhibited the excitatory effect of nicotine. However, the difference between inhibitory effects of both dihydro-beta-erythroidine and methyllycaconitine was not statistically different ($\chi^2$= 0.469, $p>.05$).

## DISCUSSION

There have been many studies on the effect of nicotine on midbrain dopaminergic neurons in rats. When the nicotine is locally injected in the VTA or SNpc, the efflux of dopamine is increased in the dopaminergic projection areas such as the nucleus accumbens and striatum (Blaha and Winn, 1993; Nisell et al., 1994). Our research observed the relative involvement of the nicotinic and muscarinic receptors in mesoaccumbens dopaminergic and nigrostriatal dopaminergic system neurons. Nicotine produces more excitatory responses than muscarin. Therefore, nigrostriatal neurons more sensitively respond to nicotine than muscarin. It has been shown that the dopaminergic neurons in the SNpc have muscarinic receptor mRNA (Vilaro et al.,1990) but the concentration of the muscarinic receptors is very low (Reisine et al., 1979; Cross and Waddington, 1980; Mash and Potter, 1986). The SNpc neurons show high firing frequency, and also excitatory responses to nicotine. Therefore, SNpc neurons are composed of the relatively high frequency and nicotine-sensitive neurons. Our results showed the current-dependent response to iontophoreticaly ejected nicotine. Similarly, the excitatory response to i.v. injected nicotine was dose-dependent (Armitage et al., 1968; Engberg and Svensson, 1980; Svensson and Engberg, 1980).

To investigate whether the nicotinic receptors of SNpc neurons are sensitive or insensitive to alpha-bungarotoxin, the neuronal response was observed during iontophoretical ejection of the methyllycaconitine or dihydro-beta-erythroidin. There was no particular response to iontophoretic ejection of dihydro-beta-erythroidine and methyllycaconitine alone in the SNpc. However the nicotine induced excitatory response in nigrostriatal neurons was reduced by iontophoretically ejected nicotinic antagonist, dihydro-beta-erythroidine and methyllycaconitine.

The results of the sensitivity to alpha-bungarotoxin in midbrain dopaminergic neurons show that the distribution of receptors sensitive to alpha-bungarotoxin was relatively high compared to receptors insensitive to alpha-bungarotoxin. Alpha-bungarotoxin inhibits the nicotine activity in cerebellar inhibitory interneurons, but not showed specific selective inhibitory effect to nicotine in Purkinje neurons (Graza et al., 1987). Also alpha-bungarotoxin could not inhibit nicotinic and acetylcholinergic activity to various ganglionic preparation (Brown and Fumagalli, 1977; Bursztajn and Gershon, 1977). These results imply that the nicotinic receptor subtypes are different at different areas of the brain. Therefore, the nicotinic receptors of dopaminergic neurons in the midbrain may have different characteristics compared as the nicotinic receptors of the other brain area neurons.

Smoking is associated with a decreased incidence in some neurological diseases (Checkoway and Nelson 1999; Gorell et al., 1999; Allam et al., 2004; Quik, 2004). Accumulating studies suggest that nicotine may be a candidate that mediates this apparent neuroprotection (O'Neill et al., 2002; Quik, 2004). Particularly, smokers have lower parkinson's disease rate than nonsmokers because of the protective effect of nicotine (Baumann et al., 1980; Baron, 1986; Morens et al., 1995). Parkinsonean patients at early stage showed reduced symptoms to tremor, rigidity, bradykinesia and gait disturbances during about 10 to 30 minute of smoking. therefore, the nicotine may have an positive effect for treatment of parkinson's disease (Ishikawa and Miyatake, 1993), schizophrenia (Alder et al., 1992,

1993) and attention deficit hyperactivity disorder (ADHD: Barkley et al., 1990; Pomerleau et al., 1996).

Therefore, nicotine and nicotinic agents may have a useful effect for various diseases such as Alzheimer's disease, Parkinson's disease, schizophrenia, ADHD. These effects may be mediated by brain dopaminergic system. Thus, the further systematic research is needed for the investigation of the effects at nicotine on brain dopaminergic system. The development of nicotine delivery system such as local injection or skin patch may reduce the misuse of nicotine and the risk of health problem and also could suggest more effective treatment with reduction of the side effect of nicotine by systemic injection.

## ACKNOWLEDGEMENTS

This work was supported by the SRC/ERC program of MOST/KOSEF (R11-2005-014).

## REFERENCES

Alder LE, Hoffer LJ, Griffith J, Waldo MC and Freedman R (1992) Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. *Biol Psychiatry* 32:607-616.

Alder LE, Hoffer LJ, Wiser A and Freedman R (1993) Cigarette smoking normalizes auditory physiology in schizophrenics. *Am J Psychiatry* 150:1856-1861.

Allam MF, Campbell MJ, Hofman A, Del Castillo AS and Fernandez-Crehuet Navajas R (2004) Smoking and parkinson's disease: systematic review of prospective studies. *Mov Disord* 19:614-621.

Armitage AK, Hall GH and Morrison CF (1968) Pharmacological basis for the tobacco smoking habit. *Nature* 217:331-334.

Barkley RA, Fischer M, Edelbrock CS and Smallish L (1990) The adolescent outcome of hyperactive children diagnosed by research criteria: I. An 8-year prospective follow-up study. *J Am Acad Child Adolese Psychiatry* 29:546-557.

Baron JA (1986) Cigarette smoking and Parkinson's disease. *Neurology* 36:1490-1496.

Baumann RJ, Jameson HD, McKean HD, Haack DG and Weisberg LM (1980) Cigarette smoking and Parkinson's disease: I. comparison of cases with matched neighbors. *Neurology* 30:839-843.

Beninato M and Spencer RF (1988) The cholinergic innervation of the rat substantia nigra: a light and electron microscopic immunohistochemical study. *Exp Brain Res* 72:178-184.

Blaha CD and Winn P (1993) Modulation of dopamine efflux in the striatum following cholinergic stimulation of the substantia nigra in intact and pedunculopontine tegmental nucleus-lesioned rats. *J Neurosci* 13:1035-1044.

Boksa P and Quirion R (1987) [3 H]N-Methyl-carbamylcholine, a new radioligand for nicotinic cholinergic receptors in brain. *Eur J Pharmacol* 139:323-333.

Bolam JP, Francis CM and Henderson Z (1991) Cholinergic input to dopaminergic neurons in the substantia nigra: a double immunocytochemical study. *Neuroscience* 41:483-494.

Brown DA and Fumagalli L (1977) Dissociation of alpha-Bungarotozin binding and receptor block in the rat superior cervical ganglion. *Brain Res* 129:165-168.

Bursztajn S and Gershon MD (1977) Discrimination between nicotinic receptors in vertebrate ganglia and skeletal muscle by alpha-bungarotoxin and cobra venoms. *J Physiol* 269:17-31.

Carlson JH and Foote SL (1992) Oscillation of interspike interval length in substantia nigra dopamine neurons: effect of nicotine and the dopamine and the dopaminergic D2 agonist LY 163502 on electrophysiological activity. *Synapse* 11:229-248.

Checkoway H and Nelson LM (1999) Epidemiologic approaches to the study of parkinson's disease etiology. *Epidemiology* 10:327-336.

Clarke PB and Pert A (1985) Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. *Brain Res* 348:355-358.

Clarke PB, Hommer DW, Pert A and Skirboll LR (1987) Innervation of substantia nigra neurons by cholinergic afferents from pedunculopontine nucleus in the rat: neuroanatomical and electrophysiological evidence. *Neuroscience* 23:1011-1019.

Cortés R, Probst A, Tobler HJ and Palacios JM (1986) Muscarinic cholinergic receptor subtypes in the human brain. II. Quantitative autoradiographic studies. *Brain Res* 362: 239-253.

Cross AJ and Waddington JL (1980) [3H] Quinuclidinyl benzcilate and [3H] GABA receptor binding in rat substantia nigra after 6-hydroxy-dopamine lesions. *Neurosci Lett* 17: 271-275.

Dajas-Bailador F and Wonnacott S (2004) Nicotinic acetylcholine receptors and the regulation of neuronal signalling. *Trends Pharmacol Sci* 25:317-324.

de la Garza R, Bickford-wimer PC, Hoffer BJ and Freedman R (1987) Heterogeneity of nicotine actions in the rat cerebellum: an in vivo electrophysiogival study. *J Pharmac Exp Ther* 240:689-695.

Engberg G and Svensson TH (1980) Pharmacological analysis of a cholinergic receptor regulation of brain norepinephrine neurons. *J Neural Transm* 49:137-150.

Gorell JM, Rybicki BA, Johnson CC and Peterson EL (1999) Smoking and parkinson's disease: a dose-response relationship. *Neurology* 52:115-119.

Grace AA and Bunney BS (1983) Intracellular and extracellular electrophysiology of nigral dopaminergic neurons-1. Identification and characterization. *Neuroscience* 10:301-315.

Greenfield SA (1991) A non-cholinergic action or acetylcholinesterase (AchE) in the brain: from neuronal secretion to the generation of movement. *Cell Mol Neurobiol* 11:55-77.

Grenhoff J, Aston-Jones G and Svensson TH (1986) Nicotinic effects on the firing pattern of midbrain dopamicne neu-

rons. *Acta Physiol Scand* 128:351-358.

Ishikawa A and Miyatake T (1993) Effects of smoking in patients with early-on-set Parkinson's disease. *J Neurol Sci* 117:28-32.

Jacobowitz DM and Goldberg AM (1977) Determination of acetylcholine in discrete regions of the rat brain. *Brain Res* 122:575-577.

Kobayashi RM, Brownstein M, Saavedra JM and Palkovits (1975) Choline acetyltransferase content in discrete regions of the rat brain. *J Neurochem* 24:637-640.

Lee BH, Lamour Y and Bassant MH (1991) Iontophoretic study of medial septal neurons in the unanesthetized rat. *Neurosci Lett* 128:29-32.

Lee HJ, Rye DB, Hallanger AE, Levey AI and Wainer BH (1988), Cholinergic vs. non-cholinergic efferents from the mesopontine tegmentum to the extrapyramidal motor svstem nuclei. *J Comp Neurol* 275:469-492.

Lichtensteiger W, Felix D, Lienhart R and Hefti F (1976) A quantitative correlation between single unit activity and fluorescence intensity of dopamine neurons in zona compacta of substantia nigra. as demonstrated under the influence of nicotine and physostigmine. *Brain Res* 117: 85-103.

Lichtensteiger W, Hefti F, Felix D, Huwyler T, Melamed E and Schlumpf M (1982) Stimulation of nigrostriatal dopamine neurons by nicotine. *Neuropharmacology* 21:963-968.

London ED, Waller SB and Wamsley JK (1985) Autoradiographic localization of [3H]nicotine binding sites in the rat brain. *Neurosci Lett* 53:179-184.

Mash DC and Potter LT (1986) Autoradiographic localization of M1 and M2 muscarine receptors in the rat brain. *Neuroscience* 19:551-564.

McCallum SE, Parameswaran N, Bordia T, Mclntosh JM, Grady SR and Quik M (2005) Decrease in $\alpha 3/\alpha 6$ nicotinic receptors but not nicotine-evoked dopamine release in monkey brain after nigrostriatal damage. *Mol Pharmacol* 68:737-746.

Mitchell SN, Brazell MP, Joseph MH, Alavijeh MS and Gray JA (1989) Regionally specific effects of acute and chronic nicotine on rates of catecholamine and 5-hydroxytryptamine synthesis in rat brain. *Eur J Phamarcol* 167:311-322.

Morens DM, Grandinetti A, Reed D, White LR and Ross GM (1995) Cigarette smoking and protections from Parkinson's disease: false association or etiologic clue? *Neurology* 45:1041-1051.

Nastuk MA and Graybiel AM (1991) Pharmacologically defined M1 and M2 muscarinic cholinergic binding sites in the cat's substantia nigra: development and maturity. *Dev Brain Res* 61:1-10.

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

O'Neill MJ, Murray TK, Lakics V, Visanji NP and Duty S (2002) The role of neuronal nicotinic acetylcholine receptors in acute and chronic neurodegeneration. *Curr Drug Target CNS Neurol Disord* 1:339-411.

Pomerleau OF, Downey KK, Stelson FW and Pomerleau CS (1996) Cigarette smoking in adult patients diagnosed with attention deficit hyperactivity disorder. *J Subst Abuse* 7: 373-378.

Quik M (2004) Smoking, nicotine and parkinson's disease. *Trends Neurosci* 27:561-568.

Reisine TD, Nagy JI, Beaumont K, Fibiger HC and Yamaura HI (1979) The localization of receptor binding sites in the substantia nigra and striatum of the rat. *Brain Res* 177: 241-252.

Salminen O, Murphy KL, Mcintosh JM, Drago J, Marks MJ, Collins AC and Grady SR (2004) Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol Pharmacol* 65:1526-1535.

Svensson TH and Engberg G (1980) Effect of nicotine on single cell activity in the noradrenergic nucleus locus coeruleus. *Acta Physiol Scand (Suppl)* 479:31-34.

Vilaro MT, Palacios JM and Mengod G (1990) Localization of m5 muscarinic receptor mRNA in rat brain examined by in situ hybridization histochemistry. *Neurosci Lett* 114: 154-159.

Waszczak BL (1990) Differential effects of D1 and D2 dopamine receptor agonists on substantia nigra pars reticulata neurons. *Brain Res* 513:125-135.

Wonnacott S, Sidhpura N and Balfour DJ (2005) Nicotine: from molecular mechanisms to behaviour. *Curr Opin Pharmacol* 5:53-59.

Zhou FM, Wilson CJ and Dani JA (2002) Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol* 53:590-605.