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Pharmacological elevation of endogenous kynurenic acid levels activates nigral dopamine neurons

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Summary. Inhibitors of kynurenine 3-hydroxylase have previously been used to increase endogenous levels of kynurenic acid, an excitatory amino acid receptor antagonist. In the present electrophysiological study PNU 156561A was utilized to elevate endogenous concentrations of kynurenic acid and subsequent effects on the firing pattern of dopamine (DA) neurons of rat substantia nigra (SN) were analyzed. Pretreatment with PNU 156561A (40 mg/kg, i.v., 5–7 h) caused a five-fold increase in endogenous kynurenic acid levels in whole brain five to seven hours after administration and also evoked a significant increase in firing rate and bursting activity of nigral DA neurons. The results of the present study show that a moderate increase in endogenous kynurenic acid levels produces significant actions on the tonic glutamatergic control of the firing pattern of nigral DA neurons, and implicate kynurenine 3-hydroxylase inhibitors as novel antiparkinsonian agents.

Keywords: Amino acids – Kynurenic acid – Firing rate – Burst activity – PNU 156561A – Substantia nigra – Dopamine

Introduction

Midbrain dopamine (DA) neurons are generally divided into two major groups of cells i.e. those located within the substantia nigra (SN) zona compacta, and those of the ventral tegmental area. These two nuclei comprise the origin of the striatal and the mesocorticolimbic DA systems, respectively. The DA neurons strongly influence motor functions and are involved in many behavioural functions such as regulation of cognition, incentive and reward processes (Schultz, 1998). Midbrain DA neurons typically fire in two distinct patterns of activity; either an irregular and relatively slow single spike firing mode or, alternatively, in relative high frequency bursts at intervals with decreasing spike amplitude and increasing spike width within bursts, frequently followed by a period of quiescence (Grace and Bunney, 1984a, b). Mounting evidence suggests that the firing pattern of DA neurons is of central

importance for transmitter release in dopaminergic terminal regions and, hence, for the overall functioning of the midbrain DA systems. Specifically, a switch to a bursting mode of firing leads to a massive release of DA from the terminals and also to activation of postsynaptic neurons as assessed by the expression of immediate-early genes, e.g. C-fos (Gonon, 1988; Chergui et al., 1996). Furthermore, pharmacologically induced cessation of bursting activity of nigral DA neurons is associated with a decrease in DA terminal efflux (Nissbrandt et al., 1994). Whereas single spikes are suggested to enable initiating movements, phasic nerve impulse activity may rather be specifically related to attentional and motivational components of behaviour (Schultz, 1998).

DA neurons fail to exhibit burst firing activity when recorded in the *in vitro* midbrain slice preparation (Sanghera et al., 1984; Wu et al., 1999) indicating that burst firing is not primarily the result of an intrinsic property of the neurons, but requires active afferent inputs that are largely absent in the slice-preparation. Changes in firing pattern of nigral DA neurons, i.e. a conversion from a single firing mode to a bursting mode is suggested to be a result of activation of glutamatergic projections to the SN from e.g. the prefrontal cortex (Carter, 1982; Naito and Kita, 1994) and the subthalamic nucleus (Kita and Kitai, 1988). Thus, iontophoretic application of N-methyl-D-aspartate (NMDA) induces burst activity in midbrain DA neurons both *in vivo* (Overton and Clark, 1992) and *in vitro* (Wu et al., 1999). In addition, local application of kynurenic acid, a broad spectrum excitatory amino acid antagonist, has been shown to inhibit burst firing and to increase the regularity of firing in nigral DA neurons (Charléty et al., 1991). Taken together, mounting evidence demonstrates that glutamatergic projections to the SN play a prominent role in the physiological regulation of neuronal firing pattern, including both the firing rate and burst activity of DA neurons.

Interestingly, the excitatory amino acid receptor antagonist kynurenic acid is synthetised endogenously in astrocytes and, in addition, displays an uneven distribution in human brain (Moroni et al., 1988; see Stone, 1993; Turski et al., 1988). Relatively low endogenous levels of kynurenic acid are found in the human brain (150 pmol/g tissue in human neocortex (Moroni et al., 1988)), and in rats (Speciale et al., 1996; Erhardt et al., 2000). Recently, inhibitors of kynurenine 3-hydroxylase have been introduced. These inhibitors significantly cause an elevation of endogenous kynurenic acid levels by blocking an alternative metabolic pathway for the precursor of kynurenic acid, kynurenine, and thereby promoting the synthesis of kynurenic acid (Speciale et al., 1996). These novel compounds help to widen our knowledge about kynurenic acid as a potentially important modulator of basic glutamatergic responses in brain. Thus, we have recently shown that pharmacological elevation of endogenous brain kynurenic acid levels effectively antagonises the excitatory effects of nicotine on the firing rate of locus coeruleus noradrenergic neurons, suggesting that brain kynurenic acid may play a significant role in the modulation of excitatory amino acid functions during physiological conditions as well as following pharmacological challenges (Erhardt et al., 2000).

In the present study, we analysed whether pharmacologically induced elevation of endogenous kynurenic acid levels, caused by systemic administration of the novel kynurenine 3-hydroxylase inhibitor PNU 156561A interferes with glutamatergic transmission within the SN and thereby influence the basal activity, i.e. firing rate, burst activity and regularity of firing of nigral DA neurons

Material and methods

The experiments were performed on male Sprague-Dawley rats (B&K Universal AB, Sollentuna, Sweden; weighing between 180–250 g).The animals were housed in groups of five and kept under constant temperature (25°C), regulated light/dark conditions, and had free access to food and drinking water. Before i.v. pretreatment with PNU 156561A (dissolved in β-cyclodextrin) or saline, rats were anaesthetised (chloral hydrate; 400 mg/kg, intraperitoneally) and placed individually in plexiglass cages (under a heating lamp in order to maintain body temperature). About four hours later, rats were again anaesthetised (chloral hydrate; 400 mg/kg, intraperitoneally) and mounted in a conventional stereotaxic frame. Additional anaesthesia was given through a lateral tail vein. Throughout the experiments the body temperature of the animals was maintained at 37°C by means of a heating pad. The skull surface was exposed and a 3 mm burr hole was drilled with its centre located approximately 2 mm anterior to lambda and 2 mm lateral to the midline (Paxinos and Watson, 1998). The dura was carefully removed and a single-barrelled micropipette with a tip diameter of approximately 1–2 μm was lowered by means of a hydraulic microdrive (David Kopf Instr., Tujunga, CA, USA) into the region of SN, according to stereotaxic coordinates from the atlas of Paxinos and Watson. The micropipette was filled with 2 M sodium acetate saturated with pontamine sky blue. The *in vitro* impedance of the electrodes were generally 5–8 MΩ, measured in saline at 135 Hz. Single unit potentials were passed through a high input-impedance amplifier and filters. The impulses were discriminated from background noise and fed into a computer, and simultaneously displayed on a digital storage oscilloscope, monitored on an audiomonitor and on a strip chart recorder (Gould). Two to nine cells were recorded from in each rat. All neurons found fulfilling the neurophysiological characteristics previously described for DA neurons in the SN pars compacta (Grace and Bunney, 1983) were included in the study.

The distribution of spikes was analysed on line utilising a Macintosh computer. The software used for the analysis of firing was written in-house using a high level object oriented programming language called "G" (Lab VIEW, National Instruments, Austin, TX, USA). The software was designed to sample and analyze the intervals of an arbitrary number of TTL pulses (corresponding to spikes passing through the discriminating filter) using a time resolution of 1 μs. An interspike interval was designated as the time (in ms) elapsed between the rising edge of two sequential TTL pulses. The software programme sorted the intervals and divided them into 3 ms bins and displayed the results as an interspike time interval histogram (ISH) with regard to the number of intervals corresponding to each bin. In order to avoid artifacts in the sampling procedure time intervals below 20 ms were ignored by the spike analyser. The onset of a burst was determined as an inter-spike interval shorter than 80 ms and the termination of a burst by the next interval longer than 160 ms (Grace and Bunney, 1984b). Cells were considered to be bursting if at least 1 interspike time interval of 100 recorded spikes was below 80 ms. The intervals were analysed with regard to the number of bursts that occurred during each 100 spikes sampling period along with a calculation of the percentage of spikes in bursts. Firing rate, percentage burst firing, and variation coefficient (which was used as a measure of the regularity of firing; Werner and Mountcastle, 1963) were expressed as the median of at least 3 consecutive ISHes.

After the electrophysiologic experiments the rats were killed by decapitation. The brains were rapidly taken out and immediately stored at −70°C for subsequent analysis of kynurenic acid. The samples were acidified with sonication solution (perchloric acid 0.4 M, $Na_2S_2O_5$ (0.1%)), and EDTA (0.05%) which was added in the same amount as the weight of the brain before sonication. The samples were centrifuged at 4,000 g for 10 minutes and to the supernatant 40 μl perchloric acid (70%) was added. Thereafter, the supernatant was centrifuged twice; the last time through a micropore filter (pore size 0.22 μm).

For analysis of kynurenic acid, an isocratic reversed-phase HPLC system was used, including a dual piston, high liquid delivery pump (CMA/250 LC Pump, CMA, Stockholm, Sweden), an Eclipse XDB-C18 column (4.6 × 150 mm, Rockland Tech. Inc., USA) and a fluorescence detector CMA/280 (CMA/Microdialysis Stockholm, Sweden) with a fixed wavelength (excitation: 315–370 nm emission: 395–545 nm). In the present study a mobile phase of 50 mM sodium acetate pH 6.2 (adjusted with acetic acid) and 7% acetonitrile was pumped through the reversed-phase column at a flow rate of 0.25 ml/min. Samples were injected into a Rheodyne injector with a single sample loop of 50 μl (Cotati, California, USA). 0.5 M zinc acetate (not pH adjusted) was delivered post column by a peristaltic pump, S2-Mini (Alitea, Sweden) at a flow rate of 0.15 ml/min. The signals from the fluorescence detector were passed through a MacLab analogue to a digital converter and transferred to a Macintosh computer. The software used for the analysis was MacLab Chart Software. The retention time of kynurenic acid was about 15 minutes and the sensitivity of the method was 0.125 pmol (signal: noise ratio 5:1). Initially, the sensitivity of the fluorescence method was evaluated by injection of a standard mixture of kynurenic acid with concentrations from 1.25 nM to 60 nM. This resulted in a standard plot, which was used to relate the heights of the peaks in the chromatogram to the correct concentration of kynurenic acid in the samples.

Drugs

The following drugs were used: chloral hydrate (Merck, Darmstadt, FRG), PNU 156561A ([(R,S)-2-amino-4-oxo-4-(3′-f′-dichlorophenyl) butanoic acid], kindly donated from C. Speciale, Pharmacia & Upjohn, Milano, Italy and dissolved in 10% β-cyclodextrin), β-cyclodextrin, zinc acetate (Sigma Chemical Co, St. Louis, MO, USA), sodium acetate (Riedelde Haen, Germany), perchloric acid (Kebo lab, Stockholm, Sweden) and acetonitrile (Labasco, Partille, Sweden).

Results

Pretreatment with PNU 156561A (40 mg/kg, i.v., 5–7 h; n = 13) caused an increase in endogenous kynurenic acid levels in whole brain by about 5 times (191 ± 73 pmol/g tissue) compared to saline controls (37 ± 6 pmol/g tissue; n = 12; Fig. 1). This pretreatment was also associated with an increase in firing rate (Fig. 2a) and an increase in burst firing activity of nigral DA neurons (Fig. 2b; n = 41 neurons from 13 PNU 156561A pretreated rats vs. 41 neurons from 12 control rats). No significant effect on the regularity of firing, as assessed by the variation coefficient was observed (Fig. 2c). According to the conventional criteria (e.g., those of Grace and Bunney, 1984a, b) 40 out of 41 neurons were classified as bursting in the group of rats treated with PNU 156561A and 30 out of 41 neurons in the control group. In the data presented, all neurons, bursting or not, have been included. Burst firing displayed typical spike amplitude decreases within bursts in accordance with the studies of

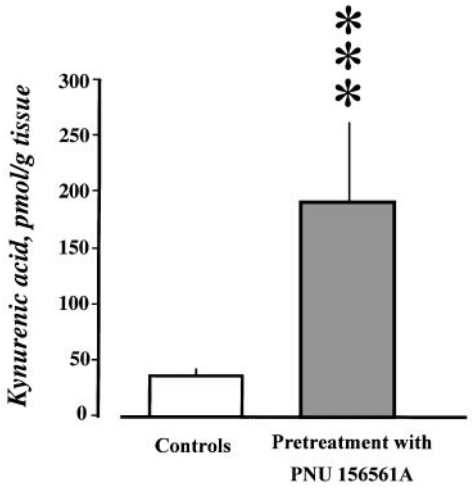

Fig. 1. Kynurenic acid concentrations in whole rat brain, expressed as pmol/g tissue, in controls (n = 12) and in rats pretreated with PNU 156561A (40 mg/kg, i.v., 5–7 h; n = 13). Each value represents means ± SEM. Statistics: ***$P < 0.001$ (Mann-Whitney U-test)

Table 1. Effects of PNU 156561A (40 mg/kg, i.v., 2–4 min before electrophysiological analysis) on the firing rate and spike distribution of dopamine neurons in the substantia nigra[1]

	Firing rate, Hz	Variation coefficient, %	Spikes in bursts, %
Controls	4.17 ± 1.5	71.6 ± 11.5	30.8 ± 14.3
PNU 156561A	4.11 ± 1.6	68.9 ± 10.4	29.2 ± 15.7

[1] Values are means ± SEM from 5 rats.

Grace and Bunney (1984a, b). Following pretreatment with PNU 156561A the number of spikes per burst tended to increase, in some cases up to 20 spikes per burst. Acute administration of PNU 156561A, 2–4 minutes before electrophysiological analysis and 10 minutes prior to decapitation (40 mg/kg, i.v., n = 5), did not change the whole brain levels of kynurenic acid (55.3 ± 14 pmol/g tissue vs. 37 ± 6 pmol/g tissue in saline controls), nor the firing rate, bursting activity or variation coefficient of the nigral DA neurons compared to predrug analyses of the same neuron (Table 1).

Discussion

Mounting evidence shows that excitatory amino acid receptors are involved in the regulation of the firing pattern of midbrain DA neurons (cf. Introduction). The major finding of the present study is that pretreatment with PNU 156561A is associated with a significant increase in the mean firing rate and percentage of spikes fired in bursts of nigral DA neurons. Since acutely administered PNU 156561A did not influence the firing pattern of nigral DA

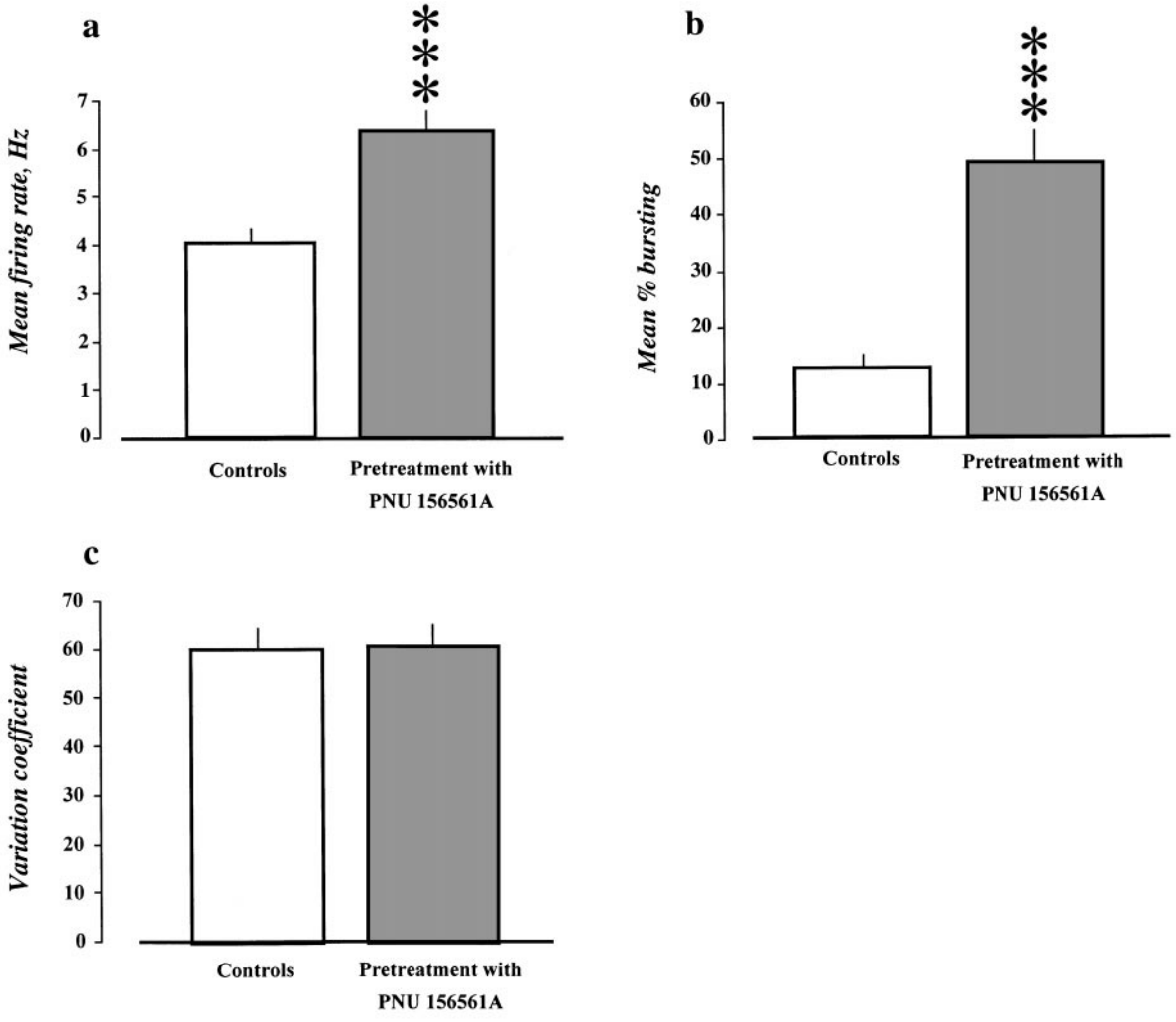

Fig. 2. Effect of PNU 156561A (40 mg/kg, i.v., 5–7 h, n = 41 neurons from 13 rats) on (**a**) firing rate, (**b**) bursting activity and (**c**) regularity of firing, assessed by the variation coefficient, of SN DA neurons compared to controls (n = 41 neurons from 12 rats). Each value represents means ± SEM. Statistics: ***P < 0.001 vs. controls (Mann-Whitney U-test)

neurons these effects are in all probability mediated via an increased level of endogenous kynurenic acid induced by the kynurenine 3-hydroxylase inhibitor.

The activation of nigral DA neurons observed following elevated levels of the endogenous glutamatereceptor antagonist appears paradoxical in view of the prominent role of excitatory amino acids in the control of firing rate and bursting activity of these neurons. In fact, local application by microiontophoresis or pneumatic ejections of kynurenic acid have been shown to cause a cessation of burst firing and to induce pacemaker-like firing pattern in SN DA neurons (Charléty et al., 1991), whereas intra-

cerebroventricular administration of kynurenic acid was without effect in this regard (Tung et al., 1991). This discrepancy may be related to different concentrations of kynurenic acid at sites of action. Thus, a large body of previous electrophysiological data suggests that, at relatively low concentrations, kynurenic acid preferably acts as a non-competitive antagonist of the NMDA receptor ion channel complex (acting on the strychnine-insensitive glycine recognition site), whereas at higher concentrations (millimolar), the compound also acts as a competitive antagonist at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptors (Stone, 1993). In addition, the PNU 156561A-induced elevation of kynurenic acid may be restricted to other compartments than those reached by exogenous kynurenic acid applied by local application or intracerebroventricular administration. Thus, PNU 156561A probably increases endogenous kynurenic acid in microcompartments where endogenous synthesis of kynurenic acid naturally occurs and where the turnover is rapid, e.g. in astrocytes, in close vicinity to and inside synaptic clefts (Schwarcz et al., 1992). At these sites the concentration of kynurenic acid may be high enough at least to antagonise NMDA receptors. In fact, the present results bear a striking similarity to the stimulatory effects of systemic administration of the non-competitive NMDA receptor antagonists MK-801 (Murase et al., 1993), or phencyclidine (French et al., 1993) on the firing of midbrain DA neurons. This activation has been suggested to involve GABAergic and/or non-NMDA receptor mechanisms (Ceci and French, 1989; Mathé et al., 1998). It appears likely that the elevated concentrations of brain kynurenic acid may primarily reduce the activity of GABAergic projections from SN zona reticulata to the nigral DA neurons. In fact, micropressure application of kynurenic acid onto the GABA-containing neurons of the SN zona reticulata have been shown to decrease the spontaneous firing rate of these neurons via antagonism of NMDA receptors (Schmitt et al., 1999). Since somatodendritic $GABA_B$-receptors have been shown to tonically dampen firing rate and burst firing of nigral DA neurons (Engberg et al., 1993; Erhardt et al., 1999), a decreased release of GABA within the SN zona compacta would promote firing rate and bursting activity of these neurons. In such a situation, where somatodendritic NMDA receptors of the nigral DA neurons are blocked and the GABAergic influence of these neurons are attenuated, non-NMDA receptors may play a more pronounced role (Zhang et al., 1994).

In all, our findings suggest a potential role for pharmacological agents that modulate endogenous kynurenic acid in pathophysiological states in brain in which excitatory amino acid neurotransmission is implicated. In addition, the activation of SN DA neurons caused by administration of PNU 156561A also indicates a potential and novel role of this type of compound in states involving DA deficiency in brain, i.e. Parkinson's disease. Previous clinical studies of NMDA receptor antagonists have as yet failed to provide robust support for clinical use of these compounds, and have in some cases revealed a potential of these compounds to cause neuropsychological side-effects, including psychotic symptoms (Javitt and Zukin, 1991). Although the assessment of a tentative psychotomimetic potential of PNU 156561A is well

beyond the scope of this study, increased levels of endogenous kynurenic acid elicit pharmacological actions on central DA neurons that are similar to those evoked by systemically administered NMDA receptor antagonists, e.g. phencyclidine, that possesses a high propensity to cause psychotomimetic effects (Javitt and Zukin, 1991). Nevertheless, treatment with kynurenine 3-hydroxylase inhibitors may represent a novel and potentially less drastic means of ameliorating Parkinsonian symptoms compared to NMDA receptor antagonists per se. Furthermore, given the neuroprotective actions of kynurenine 3-hydroxylase inhibitors (Speciale et al., 1996) such treatment might be of additional therapeutic value when regarding the insidious, neurode-generative aspects of Parkinsons disease.

In conclusion, the present results show that an increase in endogenous kynurenic acid levels effectively stimulate the firing of nigral DA neurons. The very moderate increase in kynurenic acid levels, i.e. within the same order of magnitude as those found in human neocortex under physiological conditions (150 mpol/g tissue; (Moroni et al., 1988)), to produce this effect argues that endogenous kynurenic acid may modulate basic glutamatergic responses in brain. This finding may open up new perspectives regarding the physiological and/or pathophysiological significance of endogenous kynurenic acid. Furthermore, by virtue of their capacity to activate nigral DA neurons, kynurenine 3-hydroxylase inhibitors may represent a conceptually novel class of antiparkinsonian agents.

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References

Carter CJ (1982) Topographical distribution of possible glutamatergic pathways from the frontal cortex to the striatum and substantia nigra in rats. Neuropharmacology 21: 379–383

Ceci A, French ED (1989) Phencyclidine-induced activation of ventral tegmental A10 dopamine neurons is differentially affected by lesions of the nucleus accumbens and medial prefrontal cortex. Life Sci 45: 637–646

Charléty PJ, Grenhoff J, Chergui K, De La Chapelle B, Buda M, Svensson TH, Chouvet G (1991) Burst firing of mesolimbic dopamine neurons is inhibited by somatodendritic application of kynurenate. Acta Physiol Scand 142: 105–112

Chergui K, Nomikos GG, Mathe JM, Gonon F, Svensson TH (1996) Burst stimulation of the medial forebrain bundle selectively increase Fos-like immunoreactivity in the limbic forebrain of the rat. Neuroscience 72: 141–156

Engberg G, Kling-Petersen T, Nissbrandt H (1993) $GABA_B$-receptors activation alters the firing pattern of dopamine neurons in the rat substantia nigra. Synapse 15: 229–238

Erhardt S, Nissbrandt H, Engberg G (1999) Activation of nigral dopamine neurons by the selective GABA(B)-receptor antagonist SCH 50911. J Neural Transm 106: 383–394

Erhardt S, Hajos M, Lindberg A, Engberg G (2000) Nicotine induced excitation of locus coeruleus neurons is blocked by elevated levels of endogenous kynurenic acid. Synapse 37: 104–108

French ED, Mura A, Wang T (1993) MK-801, phencyclidine (PCP), and PCP-like drugs increase burst firing in rat A10 dopamine neurons: comparison to competitive NMDA antagonists. Synapse 13: 108–116

Gonon FG (1988) Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. Neuroscience 24: 19–28

Grace AA, Bunney BS (1983) Intracellular and extracellular electrophysiology of nigral dopamine neurons. Identification and characterization. Neuroscience 10: 301–315

Grace AA, Bunney BS (1984a) The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 4: 2866–2876

Grace AA, Bunney BS (1984b) The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4: 2877–2890

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148: 1301–1308

Kita H, Kitai ST (1987) Efferent projections of the subthalamic nucleus in the rat: light and electron microscopic analysis with the PHA-L method. Comp Neurol 260: 435–452

Mathe JM, Nomikos GG, Schilstrom B, Svensson TH (1998) Non-NMDA excitatory amino acid receptors in the ventral tegmental area mediate systemic dizocilpine (MK-801) induced hyperlocomotion and dopamine release in the nucleus accumbens. J Neurosci Res 51: 583–592

Moroni F, Russi P, Lombardi G, Beni M, Carla V (1988) Presence of kynurenic acid in the mammalian brain. J Neurochem 51: 177–180

Murase S, Mathé JM, Grenhoff J, Svensson TH (1993) Effects of dizocilpine (MK 801) on rat midbrain dopamine cell activity: differential actions on firing pattern related to anatomical localization. J Neural Transm 91: 13–25

Naito A, Kita H (1994) The cortico-nigral projection in the rat: an anterograde tracing study with biotinylated dextran amine. Brain Res 637: 317–322

Nissbrandt H, Elverfors A, Engberg G (1994) Pharmacologically induced cessation of burst activity in nigral dopamine neurons; significance for the terminal dopamine efflux. Synapse 17: 217–224

Overton P, Clark D (1992) 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

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, 4th edn. Academic Press, New York

Sanghera MK, Trulson ME, German DC (1984) Electrophysiological properties of mouse dopamine neurons: in vivo and in vitro studies. Neuroscience 12: 793–801

Schmitt P, Souliere F, Dugast C, Chouvet G (1999) Regulation of substantia nigra pars reticulata neuronal activity by excitatory amino acids. Naunyn Schmiedebergs Arch Pharmacol 360: 402–412

Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80: 1–27

Schwarcz R, Du F, Schmidt W, Turski WA, Gramsbergen JB, Okuno E, Roberts RC (1992) Kynurenic acid, a potential pathogen in brain disorders. Ann NY Acad Sci 648: 140–153

Speciale C, Wu H-Q, Cini M, Marconi M, Varasi M, Schwarcz R (1996) (R,S)-3,4-dichlorobenzoylalanine (FCE 28833A) causes a large and persistent increase in brain kynurenic acid levels in rats. Eur J Pharmacol 315: 263–267

Stone TW (1993) Neuropharmacology of quinolinic and kynurenic acids. Pharmacol Rev 45: 309–379

Tung CS, Grenhoff J, Svensson TH (1991) Kynurenate blocks the acute effect of haloperidol on midbrain dopamine neurons recorded in vivo. J Neural Transm Gen Sect 84: 53–64

Turski WA, Nakamura M, Todd WP, Carpenter BK, Whetsell WO Jr, Schwarcz R (1988) Identification and quantification of kynurenic acid in human brain tissue. Brain Res 454: 164–169

Werner G, Mountcastle VB (1963) The variability of central neural activity in a sensory system and its implification for the central reflection of sensory events. J Neurophysiol 26: 958–977

Wu YN, Shen KZ, Johnson SW (1999) Presynaptic inhibition preferentially reduces in N-methyl-D-aspartate receptor-mediated component of transmission in rat midbrain dopamine neurons. Br J Pharmacol 127: 1422–1430

Zhang J, Chiodo LA, Freeman AS (1994) Influence of excitatory amino acid receptor subtypes on the electrophysiological activity of dopaminergic and nondopaminergic neurons in rat substantia nigra. J Pharmacol Exp Ther 269: 313–321

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