Amino Acids (2002) 23: 105–109
DOI 10.1007/s00726-001-0116-4

Amino
Acids
Printed in Austria

Transcription factors involved in the pathogenesis of L-DOPA-induced dyskinesia in a rat model of Parkinson's disease

M. A. Cenci

Department of Physiological Sciences, Section of Neurobiology, Lund University. Wallenberg Neuroscience Centre, Lund, Sweden

Received July 6, 2001
Accepted August 6, 2001
Published online June 17, 2002

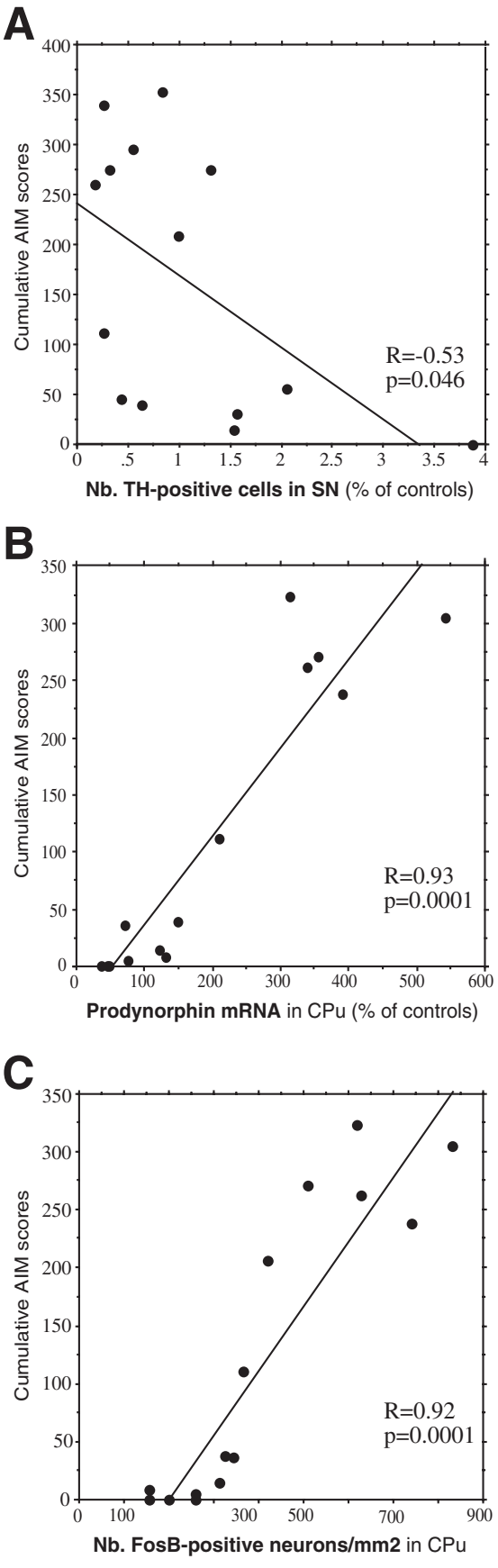
Summary. L-DOPA-induced dyskinesia (abnormal involuntary movements) is one of the most debilitating complications of chronic L-DOPA pharmacotherapy in Parkinson's disease. It is generally agreed that dyskinesia arises as a consequence of pulsatile dopamine-receptor stimulation in the brain, causing downstream changes in genes and proteins. Advance in our understanding of such changes is critically dependent on the availability of suitable animal models. We have introduced a new method to classify and rate L-DOPA-induced abnormal involuntary movements (AIMs) in 6-hydroxydopamine (6-OHDA) lesioned rats. This method allows us to dissect the molecular correlates of a dyskinetic motor response to L-DOPA in this species. One of the most prominent molecular changes underlying the development of dyskinesia in the rat consists in the striatal induction of prodynorphin gene expression by L-DOPA. This effect is mediated by FosB-related transcription factors of 32–37 kDa, which are co-induced with prodynophin in striatal neurons of the "direct pathway". Both AIM development and the associated upregulation of prodynorphin mRNA by L-DOPA are significantly inhibited by the intrastriatal infusion of *fos*B antisense. Antisense-mediated knockdown of CREB (cyclic AMP response-element binding proteins) has however no effect. Our results identify *fos*B as a potential target for adjunctive antiparkinsonian therapies.

Kewords: Immediate-early genes – Movement disorder – Basal ganglia – Dynorphin – Striatonigral

Pharmacological dopamine (DA) replacement with L-DOPA remains the most effective treatment for Parkinson's disease. However, this treatment is associated with the development of debilitating complications. After a variable time (generally a few years), L-DOPA loses the ability to produce a stable improvement of parkinsonian motor symptoms, and elicits abnormal involuntary movements (dyskinesia) in the vast majority of patients (for review see Obeso et al., 2000). It is generally agreed that dyskinesia development depends on complex molecular and neurochemical changes, which are produced by the treatment itself at sites downstream the nigrostriatal DA neuron (for review see Calon et al., 2000). Advance in our understanding of such changes is critically dependent on preclinical studies performed in animal models.

Our group has recently developed a model of L-DOPA-induced dyskinesia in the rat. Unilaterally 6-hydroxydopamine (6-OHDA) lesioned rats, receiving repeated injections of L-DOPA at therapeutical doses (6–8 mg/kg/day) gradually develop abnormal involuntary movements (AIMs) affecting the side of the body contralateral to the lesion (Cenci et al., 1998). These movements appear during the 2–3 hours that follow an injection of the drug, simulating the time course of peak-dose dyskinesia in Parkinson's disease (Lee et al., 2000). Rat AIMs can be classified in 4 subtypes based on their topographical distribution (orolingual, axial, limb and locomotive AIMs), and can be quantified according to a severity scale similar to that used in clinical dyskinesia ratings (Lee et al., 2000; Cenci et al., 1998).

We found that 6-OHDA/-lesioned rats exhibit a large individual variability in their predisposition to L-DOPA-induced AIMs, and that this variability is only partially accounted for by the extent of nigral DA-cell loss (Fig. 1A). On the other hand, there is a very strong, positive correlation between the severity of L-DOPA-induced dyskinesia and the upregulation of prodynorphin mRNA, which is induced by L-DOPA in the DA-denervated striatum (Fig. 1B). Striatal levels of prodynorphin gene expression are increased much above normal control values in dyskinetic animals (Cenci et al., 1998). The prodynorphin



transcript encodes for inhibitory neurotransmitters (dynorphins), which are released in the target structures of striatal efferent neurons upon DA-receptor stimulation (You et al., 1994; Lavin and Garcia-Munoz, 1985). In unilaterally 6-OHDA lesioned rats, upregulation of striatal prodynorphin is causally linked with behavioral sensitization and AIM development (Newman et al., 1996; Andersson et al., 1999; and own unpublished data). These and other findings indicate that the induction of prodynorphin gene expression by L-DOPA is one of the most prominent molecular changes underlying the development of dyskinesia in the rat.

Our group has undertaken a series of experiments in order to clarify the mechanisms by which L-DOPA causes an induction of the prodynorphin gene in striatal neurons. We have used a combination of antisense technology, in vivo gene induction assays, and electrophoretic mobility shift assays (EMSA) in order to identify transcription factors that may mediate this response.

The cyclic AMP response-element binding protein (CREB) is a constitutive transcription factor which becomes activated by phosphorylation on a critical serine residue (for review see Herdegen and Leah, 1998). In cultured striatal neurons, CREB mediates prodynorphin gene induction by DA (Cole et al., 1995). We therefore addressed the question whether CREB may mediate the induction of the same gene by L-DOPA in the 6-OHDA-lesioned striatum. Knockdown of CREB in the sensorimotor striatum was achieved by either single injection or continuous infusion of CREB antisense over several days, obtaining a partial or total suppression of CREB immunoreactivity (Andersson et al., 2001). Surprisingly, CREB

Fig. 1. Simple regression of L-DOPA-induced AIM scores on: (**A**) number of tyrosine hydroxylase (TH)-immunoreactive neurons in the 6-OHDA-lesioned substantia nigra (*SN*); (**B**) levels of prodynorphin (PDyn) mRNA in the DA-denervated caudate-putamen (*CPu*); (**C**) number of FosB/ΔFosB-like immunoreactive neurons in the same structure. *R*, Pearson's correlation coefficient; *p*, probability value. This set of data was collected from a group of unilaterally 6-OHDA-lesioned animals which were treated for 3 weeks with methyl L-DOPA (6 mg/kg/day, i.p.; combined with 15 mg/kg benserazide). Ratings of L-DOPA-induced AIMs were carried out 3 times a week. The cumulative AIM scores recorded from each animal during the treatment period were used for regression analysis. Prodynorphin mRNA levels and number of TH-positive neurons are expressed as percentage of the values measured from the unlesioned side of the brain (controls)

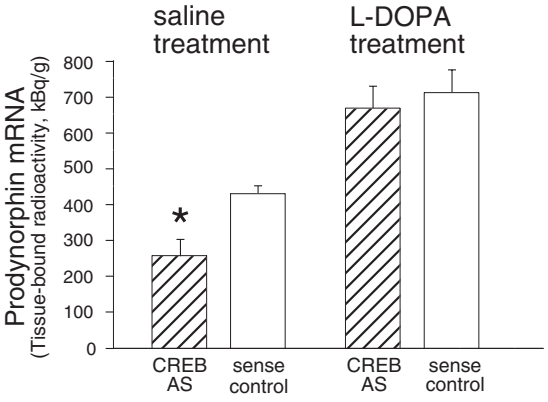


Fig. 2. Antisense-mediated CREB knockdown reduces basal (unstimulated) levels of prodynorphin mRNA, but has no effect on prodynorphin gene induction by L-DOPA in the 6-OHDA lesioned striatum. These data were collected from animals sustaining continuous intrastriatal infusion of CREB antisense or a control oligonucleotide via osmotic minipumps (14-day duration). L-DOPA (8 mg/kg, i.p., combined with 15 mg/kg benserazide) or saline were injected acutely on the 14th day. Levels of prodynorphin mRNA were measured by in situ hybridization in the proximity of the infusion cannula. Values show means + SEM, * $p < 0.05$; n 6 rats per group (from Andersson et al., 2001)

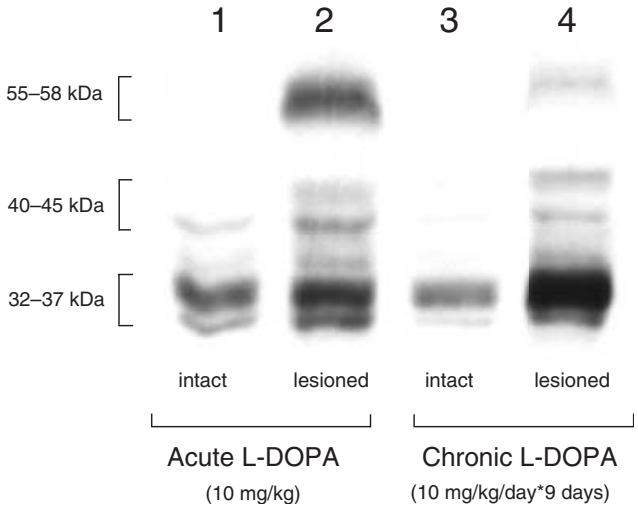


Fig. 3. Western blot analysis of proteins extracts from unilaterally 6-OHDA-lesioned rats sustaining acute or chronic treatment with L-DOPA. Immunoblots were stained with an antibody that recognizes all Fos family proteins. The DA-denervated striatum is shown in lanes *2* and *4*, while the contralateral intact side is shown in lanes *1* and *3*

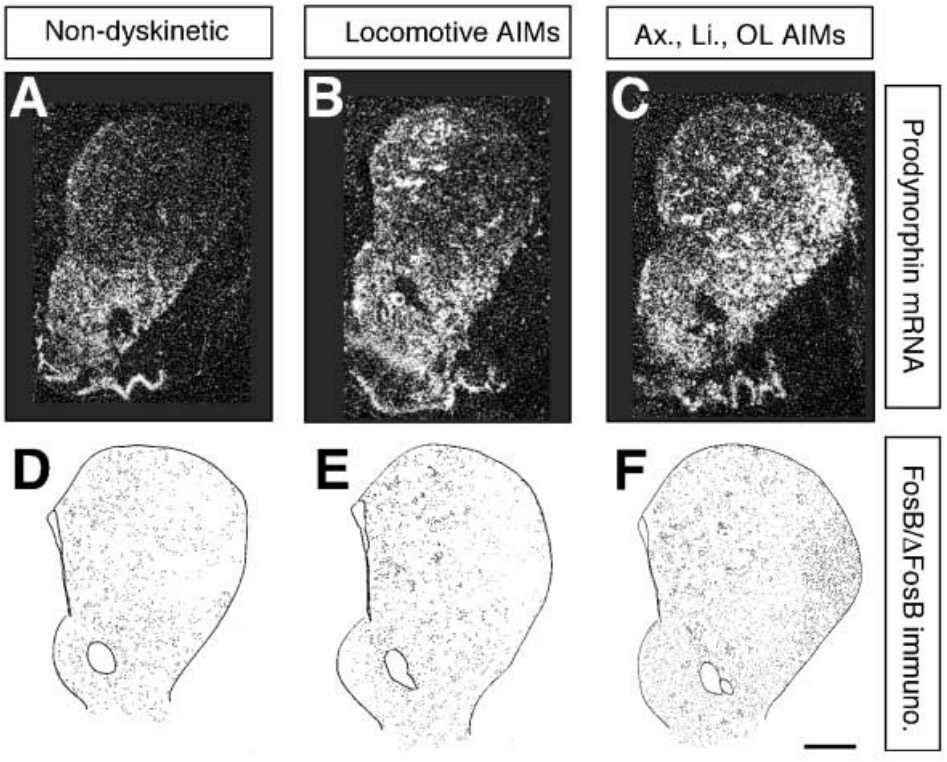


Fig. 4. Somatotopic relationship between L-DOPA-induced AIM subtypes and prodynorphin or *fos*B gene expression in the DA-denervated caudate-putamen. The upper panel (**A–C**) shows in situ hybridization autoradiographs of prodynorphin mRNA from one chronically L-DOPA-treated but non-dyskinetic rat (**A**), one animal which exhibited only locomotive AIMs (**B**), and another one that also manifested severe limb, trunk and orofacial AIMs during the course of the treatment (**C**). The lower panel (**D–F**) shows camera lucida drawings of FosB/ΔFosB-like immunoreactive neurons in the DA-denervated striatum from the same animals. Both of the markers under investigation are expressed above basal levels in striatal subregions that are somatotopically related to the type(s) of AIMs induced by L-DOPA. Scale bar, 1 mm (from Andersson et al., 1999)

knockdown was unable to block or attenuate prodynorphin gene induction by L-DOPA, although it was effective in reducing basal (unstimulated) levels of prodynorphin gene expression in the intact striatum (Fig. 2). We thus turned our attention to the Fos family of transcription factors, which are induced in striatal neurons upon DA agonist treatment (Robertson et al., 1989). Using an antibody against a peptide that is common to all Fos family proteins, we found that L-DOPA induced high levels of Fos-like immunoreactive proteins in the 6-OHDA lesioned striatum, and that the type of proteins induced was conditioned by the regimen of drug administration. High levels of c-Fos (~55 kDa) were found after an acute injection of L-DOPA, whereas repeated administration of the drug caused downregulation of c-Fos, and upregulation of proteins in the 32–37 kDa range (Fig. 3). In agreement with previous studies (Doucet et al., 1996; Bronstein et al., 1994), we established that such proteins belonged to the FosB subfamily of transcription factors (Andersson et al., 1999). The *fos*B gene encodes for full length FosB (~45 kDa) and its truncated splice variant, ΔFosB (~33 kDa). Upon repeated application of an inducing stimulus, ΔFosB can undergo post-translational modifications, and generate highly stable isoforms, the chronic FRAs (35–37 kDa; Chen et al., 1997; for review see Hope et al., 1998). Using an antibody that recognizes all FosB isoforms, we analysed the relationship between striatal FosB-like immunoreactivity, on one hand, and the severity and topographic subtype(s) of L-DOPA-induced AIMs, on the other hand (Andersson et al., 1999). Striatal FosB-like immunoreactivity was increased several fold above control levels in dyskinetic animals, but did not differ from controls in rats that did not develop a dyskinetic motor response to L-DOPA (Andersson et al., 1999; see also Fig. 1C). The induced FosB/ΔFosB proteins were localized to striatal subregions mediating the type(s) of AIM which had been elicited by the treatment in a given animal (Fig. 4D–F). In particular, locomotive AIMs were associated with high FosB/ΔFosB-like immunoreactivity in the medial CPu (Fig. 4E). On the other hand, axial, limb and orolingual AIMs were associated with an induction of *fos*B gene expression in the lateral caudate-putamen (Fig. 4F), which controls limb usage and orolingual movements in the rat (Dickson et al., 1994). The induced FosB-related proteins were colocalized with prodynorphin mRNA on both a regional (Fig. 4A–C) and a single cell level (Andersson et al., 1999). In order to demonstrate

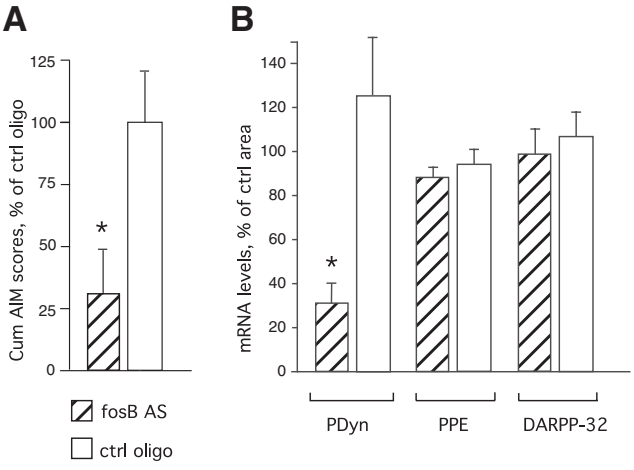


Fig. 5. Antisense-mediated *fos*B knockdown inhibits both the development of AIMs during chronic L-DOPA treatment (**A**), and the associated upregulation of prodynorphin mRNA in the 6-OHDA-lesioned striatum (**B**). In situ hybridization histochemistry was used to measure the levels of different mRNAs which are important to the function of striatal efferent neurons. Measurements were performed in the proximity of the infusion cannula, and expressed as percentage of the values found in a control area, which had not been reached by the antisense. Only the prodynorphin transcript was reduced by *fos*B antisense. Data were collected from animals sustaining continuous intrastriatal infusion of *fos*B antisense or a control oligonucleotide (ctrl oligo) via osmotic minipumps for 23 days. L-DOPA (6 mg/kg/day, i.p., combined with 15 mg/kg benserazide) was given on days 2–23 after pump implantation. AIM ratings were carried out a total of 9 times (3 times a week) by an experimentally blinded investigator. N = 8–9 in each the antisense and ctrl. oligo. group, asterisks indicate statistical significance. *PDyn*, prodynorphin; *PPE*, preproenkephalin; *DARPP-32*, DA- and adenosine 3′,5′-monophosphate-regulated phosphoprotein (from Andersson et al., 1999)

a causal relationship between striatal *fos*B induction and prodynorphin upregulation by L-DOPA, we infused an antisense oligonucleotide against *fos*B in the 6-OHDA-lesioned striatum both before and during a chronic course of treatment with L-DOPA. Antisense-mediated *fos*B knockdown caused a significant attenuation of L-DOPA-induced AIMs (Fig. 5A) and blocked prodynorphin gene induction by L-DOPA (Fig. 5B). EMSA analysis of striatal protein extracts from dyskinetic rats revealed a specific increase in DNA binding activity at both AP-1- and CRE-like enhancers from the prodynorphin promoter, and this binding was displaced by anti-FosB antibodies (Andersson et al., 2001). This demonstrates that FosB/ΔFosB-related proteins, which are induced by L-DOPA in dyskinetic animals, can actually interact with the prodynorphin promoter at both AP-1- and CRE-like sites (for a review on the the biology of Fos

and Jun transcription factors, see Herdegen and Leah, 1998).

In conclusion, the data summarized in this review demonstrate that FosB/ΔFosB-related proteins (32–37 kDa) mediate changes in striatal gene expression that are closely associated with the development of L-DOPA-induced dyskinesia in a rat model of Parkinson's disease. Furthermore, our data indicate that dyskinesia can be prevented by treatment strategies that inhibit striatal *fos*B induction by L-DOPA.

Acknowledgements

This work was supported by grants from the Swedish Association of the Neurologically Disabled, the Craaford Foundation, the Greta and Johan Kock Foundations, the Thorsten and Elsa Segerfalk Foundation, the Åke Wiberg Foundation, Consul Thure Carlssons Foundation, Harald and Greta Jeansson Foundation and the Swedish Medical Research Council (contract nb. K2001-33X-13480-02B to M.A.C.).

References

Andersson M, Hilbertson A, Cenci MA (1999) Striatal *fos*B expression is causally linked with l-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. Neurobiol Dis 6: 461–474

Andersson M, Konradi C, Cenci MA (2001) cAMP response element-binding protein is required for dopamine-dependent gene expression in the intact but not the dopamine-denervated striatum. J Neurosci 21: 9930–9943

Bronstein DM, Ye H, Pennypacker KR, Hudson PM, Hong J-S (1994) Role of a 35 kDa fos-related antigen (FRA) in the long-term induction of striatal dynorphin expression in the 6-hydroxydopamine lesioned rat. Mol Brain Res 23: 191–203

Calon F, Tahar AH, Blanchet PJ, Morissette M, Grondin R, Goulet M, Doucet JP, Robertson GS, Nestler E, Di Paolo T, Bedard PJ (2000) Dopamine-receptor stimulation: biobehavioral and biochemical consequences. Trends Neurosci 23: S92–S100

Cenci MA, Lee CS, Björklund A (1998) L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. Eur J Neurosci 10: 2694–2706

Chen J, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ (1997) Chronic Fos-related antigens: stable variants of ΔFosB induced in brain by chronic treatments. J Neurosci 17: 4933–4941

Cole RL, Konradi C, Douglass J, Hyman SE (1995) Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. Neuron 14: 813–823

Dickson PR, Lang CG, Hinton SC, Kelley AE (1994) Oral stereotypy induced by amphetamine microinjection into striatum: an anatomical mapping study. Neuroscience 61: 81–91

Doucet JP, Nakabeppu Y, Bedard PJ, Hope BT, Nestler EJ, Jasmin BJ, Chen JS, Iadarola MJ, St-Jean M, Wigle N, Blanchet P, Grondin R, Robertson GS (1996) Chronic alterations in dopaminergic neurotransmission produce a persistent elevation of deltaFosB-like protein(s) in both the rodent and primate striatum. Eur J Neurosci 8: 365–381

Herdegen T, Leah JD (1998) Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res Rev 28: 370–490

Hope BT (1998) Cocaine and the AP-1 transcription factor complex. Ann NY Acad Sci 844: 1–6

Lavin A, Garcia-Munoz M (1985) Electrophysiological changes in substantia nigra after dynorphin administration. Brain Res 369: 298–302

Lee CS, Cenci MA, Schulzer M, Bjorklund A (2000) Embryonic ventral mesencephalic grafts improve levodopa-induced dyskinesia in a rat model of Parkinson's disease. Brain 123: 1365–1379

Newman DD, Rajakumar N, Flumerfelt BA, Stoessl AJ (1997) A kappa opioid antagonist blocks sensitization in a rodent model of Parkinson's disease. NeuroReport 8: 669–672

Obeso JA, Olanow CW, Nutt JG (2000) Levodopa motor complications in Parkinson's disease. Trends Neurosci 23: S2–S7

Robertson HA, Peterson MR, Murphy K, Robertson GS (1989) D1-dopamine receptor agonists selectively activate striatal *c*-fos independent of rotational behaviour. Brain Res 503: 346–349

You ZB, Herrera-Marschitz M, Nylander I, Goiny M, O'Connor WT, Ungerstedt U, Terenius L (1994) The striatonigral dynorphin pathway of the rat studied with in vivo microdialysis–II. Effects of dopamine D1 and D2 receptor agonists. Neuroscience 63: 427–434

Authors' address: Angela Cenci-Nilsson, Wallenberg Neuroscience Centre, BMC A11, S-221 84 Lund, Sweden, Fax +46-46-2220561, E-mail: Angela.Cenci-Nilsson@mphy.lu.se