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Effects of prefrontal cortical lesions on neuropeptide and dopamine receptor gene expression in the striatum-accumbens complex

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Accepted 24 March 1998

Abstract

In the rat, neurochemical, behavioral, and anatomical investigations suggest that medial prefrontal cortical input modulates the activity of the basal ganglia. To understand how prefrontal dysfunction might alter striatal-accumbens function, in situ hybridization histochemistry with S35-labeled oligonucleotide probes was used to assess changes in striatal-accumbens gene expression following bilateral excitotoxic ibotenic acid (IA) lesions of the rat medial prefrontal cortex. Quantitative densitometry was used to measure changes in mRNA levels for preproenkephalin A (ENK), D1 dopamine receptor, protachykinin (SubP), glutamic acid decarboxylase (GAD65), and D2 dopamine receptor. No differences were found between sham and lesion groups for ENK, D1, SubP, or GAD65 mRNA levels in the striatum or nucleus accumbens (NAC). D2 receptor mRNA levels were, however, significantly higher in the dorsomedial striatum and in the core area of the NAC of the lesioned rats. Although the functional significance of increased D2 mRNA is unclear, these findings demonstrate that glutamate mPFC projections modulate gene expression in relatively regionally-localized subcortical neuronal populations.

Keywords: mPFC lesions; In situ hybridization histochemistry; Basal ganglia; D2; D1; Enkephalin; GAD65; Substance P

1. Introduction

Comparatively little is known about the changes in gene expression induced by disruptions of cortical/striatal interconnections. Using the rat, the present study explores the role of the medial prefrontal cortex (mPFC) in the modulation of gene expression in the striatum and nucleus accumbens (NAC). The mPFC innervates both the striatum and the NAC in a topographically specific manner [2,3,5,21,41,50,70]. In the rat, the mPFC, defined by its connection to the mediodorsal nucleus of the thalamus, innervates the dorsal aspect of the striatum while the ventral striatum is innervated by prelimbic and infralimbic frontal regions [3,5,10,41,70]. Flouro-gold injections into both the shell and core regions of the NAC produce extensive retrograde neuronal labeling in the mPFC [5]. Thus, alterations in mPFC activity could alter gene expression in the striatum and NAC directly. Additionally, the mPFC directly modulates mesencephalic dopamine neurons in the substantia nigra pars compacta (SN) and ventral tegmental area (VTA), which in turn send projections to both the striatum and NAC [14,33,52,73]. Although direct projections from the mPFC to the SN and VTA are considerably less numerous than mPFC projections to the striatum [52], prefrontal cortex modulates striatal dopamine release through projections directly onto mesencephalic dopamine cell groups, rather than at the terminal level within the striatum [33]. In all cases, the mPFC output comes from glutamatergic pyramidal neurons [25].

Disrupting prefrontal projections to the striatum-accumbens complex may induce both motor and cognitive deficits [26,64]. The modulatory role of the mPFC on motor activity has been examined in a number of behavioral and neurochemical investigations [1,4,7,12,13,15,16,25,27–29,31,35–37,53,57,59–62]. Behaviorally, transient increases in spontaneous and amphetamine-induced locomotion have been observed in rats with excitotoxic lesions of the mPFC [28]. Hyperactivity in lesioned rats also has been observed under novel, open
field testing procedures [4]. Ibogenic acid (IA) lesions of the mPFC elevate dopamine and its metabolites in the striatum and NAC of rats exposed to mild subchronic stress [27], while DA, homovanillic acid, and 3, 4-dihydroxyphenylacetic acid (DOPAC) levels transiently increase in the anterior portion of the striatum in mPFC lesioned animals [28]. Rats with 6-hydroxydopamine lesions of the mPFC showed elevated DOPAC levels in the NAC following footshock [15,16]. These findings suggest that the mPFC inhibits stress-induced motor responses subserved by subcortical motor pathways [7,16].

A number of studies demonstrate that changes in ENK, SubP, and dynorphin mRNA levels often parallel changes in dopamine-mediated activity in cells found in the striatum and NAC [54]. These changes were observed in striatopallidal and striatonigral neurons following chronic haloperidol treatment in a rodent model of tardive dyskinesia [17], and after 6-hydroxydopamine lesions of the nigrostriatal dopamine system in conjunction with apomorphine treatments [22,23]. In both the striatum and NAC, GABA-containing type II spiny neurons can be divided into those that express primarily D1 or D2 mRNA; moreover, D1 expressing neurons co-express substance P and dynorphin while D2 cells co-express enkephalin mRNA [23]. D1 expressing neurons are part of the striatopallidal or direct pathway, while D2 expressing neurons are thought to participate in the striatopallidal or indirect pathway.

Therefore, we hypothesized that the mPFC may modulate subcortical gene expression in the basal ganglia either secondarily through synapses upon dopamine neurons in the midbrain and/or dopamine terminals within the striatum, or possibly directly through synapses upon striatal neuronal elements. It is likely that the mPFC modulates striatal-accumbens gene expression in a regionally specific manner than reflects anatomic connectivity. In so far as alterations in the production of mRNA reflect changes in the functional activity of neurons in a particular structure [17], in situ hybridization histochemistry semi-quantitatively assesses the modulation of neuronal populations by mPFC input into the striatum-accumbens complex. Pycock et al. [60] demonstrated that damage to the mPFC produced an increased density of striatal-accumbens dopamine receptors, while Flores et al. [19] found similar changes in D2 mRNA expression. Accordingly, we hypothesize that mPFC lesions should have their primary effect upon D2 mRNA expression within the striatum-accumbens complex.

2. Materials and methods

2.1. Surgery

Male Sprague–Dawley rats (Zivic Miller Labs.), initially weighing 220–240 g, were maintained under a 12:12-h light/dark cycle (lights on 0700–1900) with food and water freely available. Fourteen rats received vehicle infusions (sham) and 15 rats received IA (lesion). Surgery was performed as previously described [23,33]. Briefly, after induction of anesthesia (Equithesin 3 ml/kg), rats were randomly assigned to receive vehicle (artificial cerebrospinal fluid, pH = 7.4) or IA (Sigma, St. Louis, MO) (10 μg/μl) injections at a rate of 0.2 μl/min bilaterally into the mPFC (5 μg/0.5 μl) at AP +3.2 mm, ML ±0.7 mm, VD −3.9 mm relative to bregma. The cannulae remained in place for 3 min after the infusion. After the operation, the rats were transferred to their home cages and allowed to recover. Eight lesion and 6 sham animals were sacrificed 2 weeks after the operation, while 7 lesion and 8 sham animals were sacrificed 6 weeks after surgery. The extent and location of the lesion was verified in thionin-stained sections obtained from the frontal portions of the brains, and five experimental animals were subsequently excluded from the study because of incomplete or poorly placed lesions. As described previously [27,35], the lesion encompassed the following cortical areas: cingulate cortex 1 (Cg1), cingulate cortex 3 (Cg3), infralimbic cortex (IL), and a medial part of the frontal cortex (Fr2) in the anterior–posterior extension (3.2–2.7 mm relative to bregma), according to Paxinos and Watson [58]. Fig. 1 shows the extent of the lesion along the anterior–posterior dimension. The lesions largely spared the more posterior aspects of the mPFC and did not extend into the underlying white matter.

2.2. Slide preparation

Immediately following decapitation, the rat brains were removed, frozen in dry ice, and refrigerated at −80°C. Tissue was then sectioned on a cryostat at 20-μm intervals and thaw-mounted onto gelatin-coated and twice-subbed glass slides. Slides were collected by placing four consecutive coronal sections on two consecutive slides 2 sec-
m intervals

and immediate following the series were retained for histo-

logical purposes in order to match coronal sections be-

between and within the two groups. The coronal sections from the midstriatum most closely correspond to Plate 12 in the Paxinos and Watson atlas (+AP 1.45 relative to bregma) [58]. After mounting, the slides were dried and stored at −80°C.

2.3. In situ hybridization histochemistry

Briefly, in situ hybridization was performed as follows [81]: Slides were thawed at room temperature for 10 min, fixed in 4% formaldehyde/phosphate-buffered saline for 5 min, treated with 0.25% acetic anhydride in 0.1 M tri-

ethanolamine HCl for 10 min, and delipidated in a series
Fig. 1. Representation of the extent of the lesion in rat mPFC along the anterior–posterior dimension. In general, the lesions were more anterior, sparing some of the more posterior aspects, and did not infringe upon the underlying white matter.
of solutions with increasing concentrations of ethanol and chloroform. Hybridization buffer (25 μl/section) (50% formamide, 600 mM NaCl, 80 mM Tris–HCl (pH 8.0), 4 mM EDTA, 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate, 0.2 mg/ml sodium heparin, 100 mM dithiothreitol, 10% dextran sulfate) containing approximately 5 × 10^5 d.p.m. of an oligonucleotide probe, labeled using terminal deoxynucleotidyl transferase (Boehringer Mannheim, Mannheim, Germany) and alpha S^35^-dATP (New England Nuclear, Boston, MA, 1000 Ci/mM), complementary to the mRNA of interest, was pipetted onto each slide.

Antisense oligonucleotide probes were targeted against bases 829–877 for D1 receptor mRNA [49], 28–75 for D2 receptor mRNA [6,48], 388–435 of rat preproenkephalin A mRNA [79], 1298–1345 for GAD65 mRNA [71], and those encoding the first 16 amino acids of exon 3 of the rat SubP precursor mRNA for Substance P [80].

After covering with parafilm, the slides were incubated overnight at 37°C. The following day, the parafilm was removed, and the slides were washed in a series of 1×SSC at 60°C, followed by washes at room temperature. After rinsing with water and ethanol, the slides were air-dried. After drying, the slides and appropriate 14C standards (American Radiochemicals, St. Louis, MO) were apposed to Kodak X-Omatic film, which was exposed for 14–42 days, developed, and scanned into a Macintosh computer for subsequent quantitative densitometry.

Measurement values for the different brain regions were interpolated along the standard curve generated by the 14C standards. Quantitative densitometry was performed on a Macintosh Quadra 950AV computer using the NIH Image program (developed by Wayne Rasband at the US National Institutes of Health and available from the Internet by anonymous FTP from zippy.nimh.nih.gov or on floppy disk from the National Technical Information Service, Springfield, Virginia, Part No. PB95-500195GEI). As shown in Fig. 2, the striatum was divided into dorsal, dorsomedial, dorsolateral, ventromedial, and ventrolateral regions, and the NAC was divided into core and shell regions in accordance with previous in situ studies [20,22].

2.4. Statistics

Separate MANOVAs were performed for each neuropeptide and dopamine receptor subtype mRNA using lesion status and time after surgery as main effects and anatomical region as a within subjects factor. In order to control for multiple comparisons, post-hoc Bonferroni t-tests were used (Statview 4.0, Abacus Concepts, 1992). Criterion for statistical significance after Bonferroni correction was set at \( P \leq 0.01 \) for ENK, D1 dopamine recep-

![Fig. 2. Histographic representation of the levels of D2 mRNA in sham vs. lesioned rats across the subregions of the striatal complex, expressed as μCi/g fresh tissue ± S.E.M. A lesion effect was seen only for D2 mRNA expression in the dorsomedial striatum and core of the nucleus accumbens.](image)
tor, SubP, and GAD65 mRNA levels. Results were designated at trend level between 0.01 and 0.05. For D2 dopamine receptor mRNA levels, criterion for statistical significance was set at $P \leq 0.05$, as there was an a priori hypothesis that lesion status would increase D2 receptor mRNA levels.

3. Results

The patterns of mRNA expression for the neuropeptides and dopamine receptor subtypes included in this study were similar to previous reports [8,9,20,38,39,43–46]. Fig. 2 shows regional variations of D2 receptor subtype gene expression across the striatum–accumbens complex in sham and lesion animals at two different time-points. D2 receptor mRNA levels showed a dorsal–ventral gradient, highest in the dorsal and dorsolateral striatum and lowest in the NAC. In contrast, D1 receptor mRNA levels were relatively homogenous, highest in the dorsolateral striatum and NAC shell, and lowest in the NAC core. GAD65 mRNA levels were highest in the NAC and lowest in the dorsal regions of the striatum. ENK mRNA levels also showed an inverse gradient, highest in the NAC core and shell and ventrolateral striatum, and lower more dorsally.

As Table 1 indicates, IA lesions of the mPFC produced an elevation in striatal D2 dopamine receptor mRNA, but did not appear to alter striatal or NAC levels of SubP, GAD65, ENK, or D1 dopamine receptor mRNA. A signifi-

| Table 1 |

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<tr>
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<td>Two weeks</td>
<td>Six weeks</td>
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<tr>
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<td>0.0549 ± 0.0037</td>
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<td>0.0719 ± 0.0045</td>
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<td>0.0464 ± 0.0036</td>
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<td></td>
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<td>Six weeks</td>
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<td>0.0378 ± 0.0018</td>
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<td>Dorsostralateral STR</td>
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<td>Ventrolateral STR</td>
<td>0.0933 ± 0.0067</td>
<td>0.0794 ± 0.0063</td>
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<td>NAC core</td>
<td>0.0475 ± 0.0064</td>
<td>0.0443 ± 0.0044</td>
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<tr>
<td>NAC shell</td>
<td>0.0863 ± 0.0045</td>
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<td></td>
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<tr>
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<td>0.0244 ± 0.0024</td>
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<td>Dorsal STR</td>
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<td>Dorsostralateral STR</td>
<td>0.0194 ± 0.0014</td>
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<td>Ventrolateral STR</td>
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<td>NAC core</td>
<td>0.0326 ± 0.0019</td>
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<tr>
<td>NAC shell</td>
<td>0.0390 ± 0.0012</td>
<td>0.0424 ± 0.0016</td>
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Measurements were made in the dorsomedial, dorsal, dorsolateral, and ventrolateral striatum, and core and shell of the nucleus accumbens. A lesion effect was seen only for D2 mRNA expression in the dorsomedial striatum and core of the nucleus accumbens.
cant main effect for lesion status was found for D2 receptor mRNA levels in the striatum-accumbens complex \( (F = 3.29; \ df = 1, 14; \ P \leq 0.03) \). In particular, D2 receptor mRNA alterations were found in both the dorsomedial aspect of the striatum \( (P \leq 0.014) \) as well as in the core of the NAC \( (P \leq 0.0098) \).

4. Discussion

The main finding of this study is a higher level of D2 dopamine receptor mRNA expression in the dorsomedial striatum and NAC core in IA mPFC young adult lesioned rats. This finding in part confirms and extends the report of Flores et al. [19], which noted an increase in D2 receptor mRNA in the dorsomedial striatum at age 60 days following PFC lesions at age 7 days, although their lesion site was more dorsal. These authors also noted a lesion-induced increase in D2 receptor mRNA in the shell of the NAC at age 60 days, whereas we found changes in the core. Differential placement of the lesion might account for this discrepancy between the two studies. The NAC core receives a dense projection from the anterior cingulate cortex [5]. Our lesion damaged cortical regions F2, CG1, CG3, and IL (as per Ref. [58]), whereas the lesion of Flores et al. [19] only damaged F2. In addition, as our lesions were large, they may have had a differential impact upon the function of midbrain dopaminergic input to the striatal complex compared to the lesion of Flores et al. [19]. Our findings are also in agreement with the work of Pycock et al. [60], who found an increased density of D2-like receptors in the striatum and nucleus accumbens of rats that had received medial prefrontal injections of 6-hydroxydopamine.

Despite region specific increases in striatal D2 receptor mRNA, there were no changes in ENK mRNA, which is co-localized with D2 receptor mRNA in a subset of striatal type II spiny neurons [22]. While this finding is counter-intuitive, it does suggest that there may be independent regulation of D2 receptor and ENK mRNA expression at the subcellular level. No changes were found in SubP, GAD65, or D1 receptor mRNA, suggesting that the mPFC exerts its primary modulatory effect on neurons in the striatopallidal (indirect) pathway. Moreover, the disruption appears to affect D2 mRNA levels in regions of the striatum-accumbens complex that are known to receive considerable glutamatergic input from the mPFC.

The increased D2 mRNA expression in the striatum-accumbens complex may be secondary to lesion-induced alterations in mesencephalic dopamine input to the striatum-NAC. Tract-tracing and electrochemical studies have demonstrated that the mPFC projects directly onto mesencephalic dopamine cell groups which, in turn, give rise to projections to the striatum-accumbens complex [52,55,73]. Recent microdialysis studies suggest that dopamine release in the rat basal ganglia is tonically modulated by the mPFC projections to mesencephalic dopamine cell groups [33,73]. Lastly, studies utilizing intrastriatal injections of haloperidol and D(−)AP5 support the notion that there are separate sites of action for the effects of dopamine and glutamate in the basal ganglia [78]. Therefore, the observed changes in D2 mRNA may be the result of: (1) alterations in the function of mesencephalic neurons that innervate the striatum and accumbens and that are directly modulated by the mPFC; (2) a direct loss of mPFC glutamatergic input into the striatum-accumbens complex; (3) a combination of alterations in both the mono- and disynaptic projections from the mPFC to the basal ganglia.

Behavioral and neurochemical studies provide evidence that glutamate input from the mPFC has effects on dopamine activity in the basal ganglia [11,24,47,51,72,74]. Glutamate antagonists block the cataleptic effects of haloperidol, a potent D2 antagonist [51]. Dopamine turnover in the striatum is altered by noncompetitive NMDA antagonists [47,72]. The NAC is another zone of dopamine–glutamate interactions. In the NAC, these interactions appear in part to be mediated by non-NMDA receptors. AMPA injected in the NAC can reduce the prepulse inhibition in rats, which is blocked by haloperidol administration or 6-OHDA lesions of the NAC [74]. Electron microscopy studies suggest that glutamate can directly modulate dopamine release via NMDA glutamate receptors on dopamine axons in the NAC [24]. Since both the striatum and NAC are sites of glutamate–dopamine interactions, disruption of mPFC glutamatergic projections could affect dopamine receptor gene expression in circumscribed subregions of the basal ganglia.

While we did not measure D2 receptor protein density following IA-mediated mPFC lesions, other studies of this issue have yielded variable results. In contrast to the increased D2-like receptor density noted by Flores et al. [19], Jaskiw et al. [30] did not find any changes following adult mPFC lesions almost identical to those in this study. Unlike Flores et al. [19], Jaskiw et al. [30] used whole striatal-accumbens homogenates, which may have obscured regional changes within the basal ganglia. In addition, differences in lesion location and extent might explain the discrepancy in findings between the two studies. Another study found increased striatal dopamine receptor binding following cortical infarctions caused by bilateral ligation of the middle cerebral artery [12], but, due to different lesion boundaries, this study is not comparable to those by Flores et al. [19] or Jaskiw et al. [30]. No mRNA assays were performed in those rats that received these cortical infarctions.

The subcellular localization of striatal D2 receptors might explain how a prefrontal lesion could produce increased D2 receptor mRNA without a corresponding increase in receptor protein. An up-regulation of striatal D2 mRNA levels could lead to an increase in D2 receptor density on cell bodies and dendrites within the striatal complex. However, measurements of actual receptor den-
sity in this region might be unchanged due to a loss of presynaptically located D2 receptors on prefrontal axon terminals. There is evidence that presynaptic D2 receptors are located on the mPFC efferents [18,40,63], which presumably would be destroyed by the lesion. However, there is some dispute about the localization of D2 receptors on cortico-striatal axons [40]. Lastly, protein transport may obscure the relationship between mRNA expression and receptor density in a given brain region [38,39].

A number of methodological issues (e.g., assay sensitivity, binding selectivity, differential signal quenching of isotopes in different areas of the brain) make the receptor density findings difficult to relate directly to in situ hybridization studies. It is also important to note that in situ hybridization studies semi-quantitatively measure relative rather than absolute changes in gene expression. In situ hybridization is also subject to concerns regarding sensitivity and binding selectivity although this study employed probes that have been well-characterized in previous in situ work in the basal ganglia. Finally, regional localization of mRNA expression within adjacent subregions of the striatal-accumbens complex could be contaminated by scatter from the radioactively-labeled cells.

The absence of changes in D1, SubP, ENK, and GAD65 gene expression between lesion and sham groups suggests that the striatum and NAC are selectively and subtly altered by mPFC lesions. Drugs known to alter striatal metabolic activity differentially alter gene expression in the striatum [23]. A behavioral pharmacology study indicates that compounds binding to striatal dopamine receptors differentially affect the expression of mRNA for dopamine receptors and neuropeptides in the striatum-accumbens complex [17]. GAD65 mRNA has been used as an indirect measure of GABA activity in the striatum [9]. GABA is the primary output neurotransmitter of the striatum-accumbens complex 17. GAD65 mRNA has been used as an indirect measure of GABA activity in the striatum [9]. GABA is the primary output neurotransmitter of the striatum-accumbens complex. The present study, D2 mRNA changes were not coexistent with any other gene expression alterations, so it is likely that mPFC lesions produce a subtle effect on neuronal activity in the striatum and NAC. In contrast, chronic haloperidol treatment produces substantial changes in gene expression in the striatum and NAC, reflecting its more dramatic and widespread effects on the function of these structures [17].

The rat striatum and NAC have structural and functional homologies to their namesakes in the human brain that may be compromised in schizophrenia [34,64]. In non-human primates, the NAC is the site of convergence for projections arising from many structures, including the amygdala and hippocampus, the entorhinal and anterior cingulate cortices, and the ventral tegmental area [32]. The striatum is another convergence zone for multiple cortical inputs, and its dorsal aspect is especially susceptible to alterations arising from traditional antipsychotic agents that bind to the D2 receptor. Weinberger [75,76] has hypothesized that schizophrenia is a neurodevelopmental connectivity disorder affecting temporo-limbic-prefrontal cortical (TLPFC) activity. Within this TLPFC circuit, special focus has been placed on both the NAC and striatum as both are major convergence zones [77]. Increases in the D2-like family of dopamine receptors have been observed in the striata of schizophrenic patients in many [65–69] but not in all studies [42,61]. Alterations in the levels of mRNA for the D2-like family of dopamine receptors might be part of the primary or secondary neuropathological changes underlying schizophrenia. D1 receptors appear to be unaffected in this disorder [56,65]. This study suggests that an excitotoxic mPFC lesion may affect D2 mRNA expression in the striatum-accumbens complex. Speculatively, abnormal striatal-accumbens D2 gene expression might be a consequence of a more widely distributed cortical miscommunication primary to the neuropathology of schizophrenia.

5. Conclusion

The present study found a moderate increase in D2 receptor mRNA expression in both the dorsomedial aspect of the striatum and the core of the NAC following bilateral excitotoxic lesions of the rat mPFC. Regional patterns of basal mRNA expression for ENK, SubP, D1 receptor, and GAD65 were consistent with previous studies and did not change in the lesion group. The changes in D2 receptor mRNA demonstrate that the mPFC modulates subcortical D2 receptor gene expression, with some regional specificity. The selective changes in D2 but not D1 receptor mRNA levels suggest that the mPFC exerts a subtle modulatory action on striatopallidal neurons. The absence of changes in ENK mRNA expression suggests that ENK can be regulated independently from D2 receptor mRNA. This study supports the notion that glutamate projections from the mPFC in rats has an impact upon dopamine neurotransmission within the striatum-accumbens complex.

Acknowledgements

The authors would like to thank Dr. Daniel R. Weinberger for his creative input in experimental design and editorial comments in reviewing this manuscript. They also would like to thank Dr. Wu Ma for generously providing the GAD65 probe and Dr. W. Scott Young III for the ENK and SubP probes used for the present study.

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