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Molecular adaptations of striatal spiny projection neurons during levodopa-induced dyskinesia

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Levodopa treatment is the major pharmacotherapy for Parkinson’s disease. However, almost all patients receiving levodopa eventually develop debilitating involuntary movements (dyskinesia). Although it is known that striatal spiny projection neurons (SPNs) are involved in the genesis of this movement disorder, the molecular basis of dyskinesia is not understood. In this study, we identify distinct cell-type-specific gene-expression changes that occur in subclasses of SPNs upon induction of a parkinsonian lesion followed by chronic levodopa treatment. We identify several hundred genes, the expression of which is correlated with levodopa dose, many of which are under the control of activator protein-1 and ERK signaling. Despite homeostatic adaptations involving several signaling modulators, activator protein-1–dependent gene expression remains highly dysregulated in direct pathway SPNs upon chronic levodopa treatment. We also discuss which molecular pathways are most likely to dampen abnormal dopaminergic signaling in spiny projection neurons, hence providing potential targets for antidyskinetic treatments in Parkinson’s disease.

Significance

Parkinson’s disease is characterized by a set of motor features that depend on a loss of dopamine-producing cells in the midbrain. The most common pharmacotherapy for Parkinson’s disease is dopamine replacement with levodopa administration. The majority of patients receiving this treatment develop debilitating abnormal involuntary movements, termed “levodopa-induced dyskinesia.” It is known that striatal projection neurons (SPNs) are involved in the genesis of levodopa-induced dyskinesia, but the genes involved in this process are not fully understood. We reveal the gene-expression profiles of different classes of SPNs during chronic levodopa administration. We correlate gene expression to mouse behavior, predicting which genes are most likely involved in the emergence of levodopa-induced dyskinesia, and which are thus potential targets for new antidyskinetic treatments.

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The authors declare no conflict of interest.

Data deposition: The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE55096).

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tissue, and so cell-type-specific whole-genome gene-expression studies are not attainable from whole-tissue dissections. Knowledge of cell-type-specific molecular changes would reveal important insights in the pathophysiology of levodopa-induced dyskinesia, as well as possibly reveal new therapeutic targets for their treatment. To study these changes, we have used a well-validated mouse model of levodopa-induced dyskinesia (19, 20) in combination with a cell-type-specific mRNA translational profiling approach called translating ribosome affinity purification (TRAP) (21).

Dopamine depletion led to a small number of gene-expression changes in both dSPNs and iSPNs, and in particular, to changes in regulators of MAPK signaling in dSPNs. Chronic levodopa administration induced a small number of changes to iSPN gene expression. In contrast, dSPNs displayed a dramatic increase in cyclic AMP-responsive element binding (CREB), AP-1, and ERK-dependent gene expression. The treatment-induced pattern of gene changes indicates that dSPNs mount homeostatic molecular responses targeting CREB and ERK-mediated signaling, which are, however, inefficient to down-regulate AP-1-dependent gene expression. By comparing chronic low-dose and high-dose levodopa regimens, we identified genes for which expression was correlated with severity of drug-induced dyskinesia. These dose-dependent expression changes were found only in dSPNs, not iSPNs. In dSPNs, several hundred genes were correlated with dose and abnormal involuntary movements, including a large subset predicted to be under the regulation of AP-1 and ERK-activated transcription factors. We also discuss the molecular changes in dSPNs that are most likely to counteract the effects of excessive D1 receptor activation.

Results

An overview of all gene-expression changes reported below is presented graphically in Figs. 1 and 2.

Effects of Striatal Dopamine Depletion upon SPNs. The loss of dopamine innervation primes the striatum for the development of dyskinesia (3). A complete understanding of the pathogenesis of dyskinesia thus requires knowledge of the changes that occur in the dopamine-depleted striatum. To profile the cell-type-specific responses of striatal SPNs to striatal dopamine depletion, we conducted TRAP analysis (21) of the two major classes of these neurons: dSPNs, which express the dopamine receptor 1α (Drd1a), and iSPNs, which express the dopamine receptor 2 (Drd2). To disrupt dopamine innervation to both of these SPN populations that reside in the striatum, we injected the neurotoxin 6-hydroxydopamine (6-OHDA), unilaterally in the medial forebrain bundle in hemizygous Drd1-TRAP and Drd2-TRAP mice (kept on a C57BL/6J genetic background) (21). This lesion procedure causes nigral dopamine (6-OHDA), unilaterally in the medial forebrain bundle in hemizygous Drd1-TRAP and Drd2-TRAP mice (kept on a C57BL/6J genetic background) (21). This lesion procedure causes nigral dopamine cell death within a few days, along with a widespread and near-complete loss of dopaminergic innervation to the entire dorsal striatum on one side of the brain (a hemiparkinsonian model) (20) (Fig. S1). TRAP-purified mRNAs from either Drd1a- or Drd2-expressing SPNs were reverse-transcribed, amplified, and used to interrogate Affymetrix 430_2.0 GeneChip microarrays. Two-hundred twenty-six genes (represented by 291 probe-sets) in dSPNs, and 156 genes (represented by 196 probe-sets) in iSPNs were differentially expressed upon striatal dopamine depletion (the effect of dopamine depletion is defined here by the comparison of saline-treated 6-OHDA-lesioned mice vs. saline-treated sham-lesioned mice) (Datasets S1–S3) (7–10 replicates per group, and differential expression defined by a fold-change of 1.5 or greater and Benjamini–Hochberg adjusted P value less than α = 0.10, Welch’s t test). We performed a simple overlap analysis of gene-associated pathway annotations to reveal functional sets of genes altered in the two SPN types upon dopamine depletion.

In dSPNs, among the most significant pathways altered upon striatal dopamine depletion (Benjamini–Hochberg adjusted P value ≤ 0.05) were the IL-3, EGFR, and MAPK signaling pathways (Dataset S4). Regarding the latter, Dusp dual-specificity phosphatases are capable of dephosphorylating both tyrosine and serine/threonine residues, and as such are negative regulators of MAPK signaling (22). Thus, decreased expression of various Dusp gene products, negative regulators of ERK/MAPK signaling, may contribute to the well-documented supersensitivity of ERK signaling in dopamine-depleted dSPNs (23). In iSPNs, among the most significant pathways altered upon striatal dopamine depletion were the TGF-β signaling and G-protein signaling pathways (Dataset S5); included in the latter was an increase in Gng4, Gng5, and Pde1c expression. An alteration to Pde1c expression may represent a homeostatic mechanism to compensate for a decrease of dopamine-dependent Drd2 receptor signaling: decreased Drd2 receptor signaling as a result of dopamine depletion would be predicted to lead to increased cAMP levels, which could be counteracted by increases in levels of Pde1c, a protein that catalyzes hydrolysis of cAMP.

When comparing the dopamine-depletion–induced gene-expression profiles of dSPNs and iSPNs, there is an overlap of 22 genes represented by 38 probe-sets (for these common genes see Dataset S2). Most of these genes changed in opposite directions in the two cell types. Although the Drd1a and Drd2 receptors are expected to have some opposite signaling effects through coupling to Gαolf and Gi, respectively, this small number of overlapping genes with opposite directional changes suggests that dopamine depletion is not simply producing inverted effects in these two cell types through Gαolf and Gi signaling.

Effects of Levodopa Treatment on Dopamine-Depleted SPNs. We next examined the effects of levodopa treatment upon the molecular profiles of dopamine-depleted dSPNs and iSPNs, as striatal SPNs mediate both the therapeutic response to levodopa and the emergence of levodopa-induced dyskinesia (e.g., ref. 24). In chronically dopamine-depleted hemiparkinsonian mice, the
therapeutic effect of levodopa can be assessed using tests of spontaneous forelimb use, which is reduced on the side contralateral to the lesion and promptly restored by levodopa. At the same time, dyskinesia can be assessed by scoring abnormal involuntary movements (AIMs) that affect axial, orofacial, and limb muscles (19, 25). To define striatal TRAP profiles associated with these behavioral effects, Drd1a- and Drd2-driven TRAP mice with medial forebrain bundle lesions were treated chronically with levodopa using either a high- or a low-dose regimen. Both treatment regimens improved the lesion-induced motor deficit in spontaneous forelimb use (Fig. S2A). However, only the high-dose regimen readily induced AIMs, which reached maximal severity by the end of the treatment (Fig. S2B). The cumulative AIM (dyskinesia) scores recorded during the treatment period were approximately twice as large in the high-dose levodopa group as in the low-dose one (Fig. S2C).

The TRAP analysis of dSPNs revealed that over 3,100 genes (represented by 4,545 probe-sets) displayed altered expression upon dopamine depletion followed by chronic low-dose levodopa treatment; 1,352 genes were up-regulated and 1,758 genes were down-regulated (Dataset S6). [Note that—extremely rarely—different probe-sets for one gene did not directionally agree, potentially because of splicing differences or else poor probe design, and hence summations may appear off (in this case 3,100 instead of 3,110). Any such discrepancies are actually because of this phenomenon.] Among the most significant pathways uniting these changes were the EGFR1 signaling, TGF-β receptor signaling, IL-2 signaling, insulin signaling, and MAPK signaling pathways (Dataset S7), the latter including an up-regulation of the Dusp negative modulators of ERK signaling. This dramatic up-regulation of Dusp genes was a notable reversal of the down-regulation of Dusp genes seen upon dopamine depletion alone. Because Dusp gene expression is known to be up-regulated by the MAPK pathway itself (26), these changes to Dusp gene expression likely represent a homeostatic feedback response to increases in MAPK signaling. Following chronic high-dose levodopa treatment, 4,603 genes (represented by 7,118 probe-sets) changed expression in dopamine-depleted dSPNs; 1,898 genes were up-regulated and 2,733 genes were down-regulated (Dataset S8). Of these genes altered upon chronic high-dose treatment, 3,635 genes (represented by 5,573 probe-sets) also had statistically significant changes (of any magnitude) following chronic low-dose treatment with levodopa (Dataset S9). All of the most significantly enriched pathways among genes changing with chronic low-dose levodopa were also represented in analysis of the high-dose data (Dataset S10), signifying that chronic high-dose levodopa treatment (associated with severe dyskinesia) does not elicit a completely different gene-expression response compared with low-dose treatment (associated with milder dyskinesia). A notable exception, however, was the “regulation of actin cytoskeleton” pathway induced by chronic high-dose treatment. Alterations to expression of genes in this pathway may result in unique
changes to the structure of dSPNs upon induction of severe dyskinesia. To identify regulators of the dSPN response to chronic levodopa, we collected data on the presence of conserved sequence motifs in the promoters of the altered genes [sequence motifs and target prediction from SwissRegulon (27)] and used a hypergeometric test to assign significance to overrepresentation of these motifs among the genes whose expression was altered. Analysis of the regulatory motifs of genes whose expression was up-regulated upon high-dose treatment revealed that CREB, AP-1 (e.g., Fos and Jun), and ERK-dependent (e.g., Elk) motifs were among the most often represented [see “up” tab in Dataset S11; note that near the top of this dataset’s rankings are motifs that are present in the regulatory elements of almost all neuronal-expressed genes (e.g., Sp1), and are thus not considered to reflect enrichment in the high-dose dataset]. This finding is of interest given what appear to be homeostatic changes present in the dSPN high-dose levodopa treatment data (Dataset S8). For example, although ERK2 (Mapk1) levels are slightly increased, many Dusp genes are up-regulated, changes that would be predicted to decrease ERK-dependent signaling. Gene expression of several adenylyl cyclases are decreased, phosphodiesterases increased, regulator of G protein signaling proteins increased, Creb (Crebl) and Crebhp decreasing, and Crem (Icer) increased, suggesting that, as for ERK signaling, a homeostatic response down-regulates cAMP-PKA-CREB signaling as well. In contrast to this homeostatic pattern of both CREB- and ERK-related gene changes, many AP-1 factors were dramatically up-regulated (Fos, Fosb, Fosl1, Fosl2, Jun, and Junb are all up-regulated) (Fig. S3 and Dataset S8), and only slight decreases to facilitators of AP-1 signaling were observed (for example, Mapkbp1, Mapk8ip2). Taken together, these data suggest that CREB, AP-1, and ERK signaling are major drivers of the transcriptional response to chronic high-dose levodopa administration in dSPNs, and although transcriptional changes to dampen CREB and ERK signaling are evident, homeostatic responses to decrease AP-1 signaling are minimal. This lack of decreased AP-1 signaling may be responsible for the persistent elevations in CREB and ERK-dependent gene expression, as we find that many genes that are altered upon chronic high-dose levodopa treatment (e.g., Homer1) contain all three (CREB, AP-1, and ERK-target) binding motifs (Dataset S11). CREB binding increased contrast to the changes above, iSPNs had few significant alterations in mRNA expression upon dopamine depletion followed by chronic levodopa treatment. Only 72 genes (represented by 84 probe-sets) in total had an altered expression profile (48 up, 24 down) upon chronic low-dose administration (Dataset S12). No pathways with statistically significant enrichment were observed, possibly because of the small number of genes involved. Upon dopamine depletion followed by chronic high-dose levodopa treatment, 415 genes (represented by 533 probe-sets) had significant positive dose–responses and were thus associated with more severe AIMS, whereas 192 genes (230 probe-sets) were anticorrelated with dose, and thus AIMS (Dataset S16). No genes had statistically significant excess correlation with AIMS, after accounting for the effect of dose and multiple testing adjustments (SI Materials and Methods and Fig. S4). In iSPNs, no genes had significant correlations with either dose or AIMS after multiple testing adjustments.

The following genes had the most significant correlations between expression and dose in dSPNs (Dataset S16): Gpr39, Fndc9, Cth, Trh, Srrn1, Ier3, Tnf2, Cdk11b, Nrk4a (Nurr1), Itch, Scn2, and Fosl1 (Fra-1). Of these genes, only Trh (positively correlated) has previously been linked to levodopa-induced dyskinesia (28). Fosb has previously been shown to have a causal role in the development of dyskinesia (29), and we now suggest that Fosl1 (positively correlated) (Fig. 3) may have a similar role. Ier3 (positively correlated) (Fig. 3) encodes an inhibitor of protein phosphatase 2A-dependent ERK dephosphorylation (30), and as such may augment ERK signaling. Itch, expression of which was anticorrelated to AIMS (Fig. 3), is an E3 ubiquitin ligase that regulates c-Jun (Jun) levels (31); our data demonstrate that lower levels of Itch expression (presumably resulting in higher levels of c-Jun) correlate with the levodopa response. No genes linked to CREB signaling were among those with the most significant correlations between gene expression and dose.

Analysis of pathway annotations revealed that MAPK Signaling (including changes to Dusp genes) was among the pathways most significantly enriched among genes correlated with dose in dSPNs (hypergeometric test, Benjamin–Hochberg adjusted P value < 0.05) (Dataset S17), whereas genes related to cell-cycle control, DNA replication, and B-cell receptor signaling were among the most anticorrelated in this cell type (Dataset S18). Finally, we searched for overrepresentation of regulatory motifs among the genes whose expression was most correlated to dose in dSPNs: our analysis revealed that CREB, AP-1, and ERK-dependent motifs were often found in the regulatory elements of these genes (Dataset S19).

See Dataset S20 for all statistical results discussed in this study.

Discussion
In this study we have used a combination of a hemiparkinsonian mouse model of PD (19, 20) and a refined TRAP methodology (21) to identify cell-type–specific gene-expression changes in dSPNs and iSPNs as induced by dopamine depletion and pharmacological dopamine replacement. All mice with complete medial forebrain bundle lesions of the nigrostriatal pathway promptly develop dyskinetic behaviors in response to levodopa (20). When studying the effects of levodopa treatment in this animal model, however, comparisons can be made between animals with severe or mild dyskinesia by using different doses of levodopa. This approach is clinically relevant, as levodopa dosage is one of the most important risk factors for dyskinesia in PD, if not the most important one (32). Dopamine depletion followed by chronic low- or high-dose levodopa treatment revealed massive changes to mRNA translation profiles in dSPNs, compared with relatively modest changes in iSPNs (Figs. 1 and 2). These results, combined with the fact that dSPNs (but not iSPNs) demonstrated gene-expression changes that had correlations between dose and AIMS (Dataset S16), strongly suggest that dSPNs are involved in the genesis of levodopa-induced dyskinesia. It is likely that this movement disorder also involves significant adaptations of the iSPN, which, however, do not seem to require pronounced transcriptional and translational changes. Interestingly, although low- and high-dose levodopa treatments both led to more decreases to gene expression than increases to gene expression in dSPNs, the opposite was true for iSPNs (Fig. 2).
Notable Homeostatic Alterations upon Chronic Levodopa Treatment.

See SI Discussion for an extended discussion of MAPK-related pathways. In addition to the pathways analyses discussed in the Results, we systematically examined groups of genes whose expression is known to participate in dopamine signaling in SPNs (33): G protein-coupled receptors (GPCRs) and their regulators, as well as various ionotropic channels (Fig S3). We did not observe large changes in expression of any dopamine receptor genes. However, among many genes whose products regulate dopamine receptor trafficking or G protein coupling, we did observe an up-regulation of Rgs16 expression in iSPNs and Rgs6 expression in dSPNs upon chronic levodopa treatment. Rgs6 protein displays GTPase-activating function specifically toward Gαolf (34), and thus we predict that this change in gene expression reflects a major homeostatic down-regulation of Drd1a (which is Gαolf-coupled) signaling. Rgs16 protein displays GTPase-activating function toward Gαq (35), and as such is poised to be a homeostatic regulator of Drd2 activity. Although we did not observe changes to mGluR5 (Grm5) expression, we noted a dramatic up-regulation of both Homer1 and Tamalin (Grasp) in both dSPNs and iSPNs, changes that are predicted to increase surface expression or functional coupling of mGluR5 in both cell types. Most notable among the metabotropic receptor group, we observed a dramatic up-regulation of the somatostatin receptor genes Sstr2 and Sstr4 in dSPNs. These changes are of great potential relevance to counteracting Drd1a signaling in dSPNs, as both of these somatostatin receptors are Gi4 coupled, dSPNs express a high level of Gi4 protein (21), and aspiny GABAergic interneurons provide an endogenous source of somatostatin in the striatum. Another change of note is the large up-regulation to SK3/KCc2.3(KCnmc3) in dSPNs. Based on the normal function of SK3/KCc2.3(KCnmc3) (36), its up-regulation may serve to dampen dSPN excitability in the face of abnormal Drd1a activation.

Genes That Display Correlations Between Expression Level and Levodopa Dose. Given that our high- and low-dose levodopa treatment group animals had pronounced differences in dyskinesia severity (see AIM scores, Fig S2 B and C), we tested for correlations between gene expression and behavioral response to levodopa dose (Dataset S16). Together with our regulatory motif analysis (Dataset S19), these data indicate that regulators of both AP-1 and ERK signaling are strongly involved in the emergence of dyskinesia, a conclusion that is widely supported by previously published research. Single-gene analysis revealed that the following genes displayed the highest correlations between expression levels and levodopa dose in dSPNs: Gpr39, Fnkdc9, Cstb, Trh, Sstr1, Ier3, Timp2, Cdk11b, Nr4a2 (Nurr1), Icch, Scp2, and Fosl1 (see Dataset S16 for full ranked list). Although Trh has been recently linked to dyskinesia by Graybiel and colleagues (28), the other genes in this list for which we have demonstrated correlation of expression with levodopa dose and AIMS represent compelling novel targets for future studies. For example, the multiple functions of Gpr39 (a receptor of the ghrelin superfamily) in animal models of neuropsychiatric conditions have just started to be addressed (37, 38), and its role in the response to PD treatment is currently unknown. Being a GPCR, Gpr39 may represent an attractive target for therapeutic development. Furthermore, Nr4a2 (Nurr1), a gene known to be involved in the development and maintenance of a dopaminergic neuronal phenotype (39), has been implicated in the etiology of PD (40). Our data now indicate that this factor may also regulate the molecular response of dSPNs to dyskinesiogenic levodopa treatment.

Summary

In this study, we present the full transcriptional profiles of dSPNs and iSPNs in experimental parkinsonism and levodopa-induced dyskinesia, providing a fundamental contribution to this field of research. We find that dSPNs show a large number of gene-expression changes in response to chronic levodopa treatment and that by comparison, iSPNs show few changes. We have determined which genes have the highest correlations between expression level and levodopa dose (AIMs correlated), and are thus most likely to be involved in the generation of levodopa-induced dyskinesia. We have demonstrated that, although there are increases to the expression of various MAPK-related genes in dSPNs, there are also several homeostatic changes induced by the treatment to down-regulate MAPK signaling. There is evidence for down-regulation of expression of CREB family transcription factors in dSPNs, but this is not the case for AP-1 family transcription factors (e.g., Jun, Fos), suggesting that cellular homeostatic mechanisms fail to dampen an AP-1-regulated gene network during chronic levodopa treatment in this cell type. Finally, we observed large increases in expression of Sstr2, Sstr4, and SK3/KCc2.3(KCnmc3) genes in dSPNs during chronic levodopa
support the use of dopaminergic agents with preferential D2-like receptor activity as a first-line therapy to prevent dyskinesia in PD (41).

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The profound molecular adaptations of dSPN and the scarce response of iSPN during dyskinesiogenic treatment with levodopa treatment, all of which were AIMs-correlated. These changes to the ion channel subunit and membrane receptors may serve to counteract an increased D1 receptor-mediated excitability close to the cell surface (as opposed to downstream transcriptional regulators, which are subject to signaling pathway cross-talk). In particular, Stryr2 and Stryr4 (for which specific agonists are currently being developed) represent novel potential targets for the treatment of levodopa-induced dyskinesia. The profound molecular adaptations of dSPN and the scarce response of iSPN during dyskinesiogenic treatment with levodopa.