Gene therapy blockade of dorsal striatal p11 improves motor function and dyskinesia in parkinsonian mice

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Complications of dopamine replacement for Parkinson's disease (PD) can limit therapeutic options, leading to interest in identifying novel pathways that can be exploited to improve treatment. p11 (S100A10) is a cellular scaffold protein that binds to and potentiates the activity of various ion channels and neurotransmitter receptors. We have previously reported that p11 can influence ventral striatal function in models of depression and drug addiction, and thus we hypothesized that dorsal striatal p11 might mediate motor function and drug responses in parkinsonian mice. To focally inhibit p11 expression in the dorsal striatum, we injected an adeno-associated virus (AAV) vector producing a short hairpin RNA (AAV.sh.p11). This intervention reduced the impairment in motor function on forced tasks, such as rotarod and treadmill tests, caused by substantia nigra lesioning in mice. Measures of spontaneous movement and gait in an open-field test declined as expected in control lesioned mice, whereas AAV.sh.p11 mice remained at or near normal baseline. Mice with unilateral lesions were then challenged with L-dopa (levodopa) and various dopamine receptor agonists, and resulting rotational behaviors were significantly reduced after ipsilateral inhibition of dorsal striatal p11 expression. Finally, p11 knockdown in the dorsal striatum dramatically reduced L-dopa–induced abnormal involuntary movements compared with control mice. These data indicate that focal inhibition of p11 action in the dorsal striatum could be a promising PD therapeutic target to improve motor function while reducing L-dopa–induced dyskinesias.

gene therapy | p11 | Parkinson's disease | dyskinesia | striatum

Pharmacologic replacement of depleted dopamine is the primary therapeutic approach to treating Parkinson's disease (PD). Although this usually improves the major motor problems of this disorder, complications of medical therapy can often limit both dosing and effectiveness. Among the most common adverse effects limiting dopamine replacement therapy for PD is the development of abnormal involuntary movements (AIMs), also known as levodopa-induced dyskinesia (LID) (1). Treatment of LID usually requires reducing the dosage of dopaminergic medications to below the threshold for major complications, although certain pharmacotherapies or surgeries can improve LID as well (1). Understanding both the anatomic location and molecular pathways underlying dyskinesia responses to dopamine replacement therapy is necessary to develop improved therapies, which can reduce motor symptoms without this debilitating problem.

Previous studies have identified certain signaling pathways that may influence the development of dyskinesia. The primary site of action of L-dopa (levodopa) on PD motor symptoms after conversion to dopamine is the dorsal striatum, owing to the loss of the normal dopaminergic inputs from the substantia nigra pars compacta (2). This same region has also been shown to be responsible for motor complications of L-dopa therapy, including LID. Specifically, neurons harboring the D1 dopamine receptor appear to be primarily involved in these responses (3–5). Furthermore, other signaling pathways, including the serotonin 5-HT1B receptor, seem to modulate the response of these neurons to dopamine replacement therapy (6, 7). Nonetheless, it has been difficult to identify potential therapeutic targets that both improve motor function and reduce dyskinesia.

Here we demonstrate that dorsal striatal p11 is a key regulator of dopamine responses in PD. We previously reported that p11, a small adaptor protein also known as S100A10, binds to specific serotonin receptor subtypes, including 5-HT1B (8–10). Because activation of the 5-HT1B serotonin receptor (5-HT1BR) reduces dyskinesia, and p11 binds to 5-HT1BR and potentiates 5-HT1B activity, we hypothesized that dorsal striatal p11 may influence the response to dopamine replacement therapy. We found that inhibition of p11 expression in the dorsal striatum improved motor function in parkinsonian mice. Surprisingly, blockade of dorsal striatal p11 expression profoundly inhibited dyskinesias in response to chronic L-dopa treatment, to a greater extent than pharmacologic activation of 5-HT1B in controls. This indicates that inhibition of striatal p11 is a promising potential target to block dyskinesias while improving motor function in PD, and that these effects likely occur through a mechanism other than 5-HT1B.

Results

Reduction of p11 in the Dorsal Striatum Significantly Improves Rotarod and Treadmill Performance in Parkinsonian Mice. To address the influence of striatal p11 on motor function, we generated serotype 2 adeno-associated virus (AAV) vectors encoding for either a short hairpin RNA (shRNA) to block murine p11 expression (AAV.sh.p11) or an shRNA against firefly luciferase (AAV.sh.Luc) as a negative control (Fig. S1 *A* and *B*) (8). At 12 wk after bilateral

Significance

Medications for Parkinson's disease (PD) are designed to replace lost dopamine. Although effective, they often cause abnormal involuntary movements (AIMs), also called dyskinesias, which can be difficult to resolve without worsening PD symptoms. We report that p11, a small protein necessary for neurotransmitter receptor function, is critical to dopamine responses in a mouse PD model. Blocking p11 production in the dorsal striatum, a brain region that responds to dopamine and regulates movement, can improve forced movements and normalize spontaneous movements in parkinsonian mice while dramatically reducing AIMs after dopamine replacement therapy. Our data identify a new target for therapeutic development to both improve symptoms and reduce drug-related side effects in human PD.

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intrastriatal injection of the AAV vectors, the mice demonstrated a significant increase in the time spent on an accelerating rotarod compared with controls ($P < 0.05$), suggesting that inhibition of striatal p11 expression could modestly improve motor function even in normal mice (Fig. 1*C*). Then, 6 wk later, these same animals were subjected to unilateral 6-hydroxydopamine (6-OHDA) lesions, and after another 6 wk, overall rotarod performance declined in all groups, as expected. The better performance of the p11 knockdown group compared with controls was maintained after lesioning, however ($P < 0.05$) (Fig. 1*C*).

Mice were also tested before and after lesioning on an accelerating treadmill, and were assayed for their ability to maintain ambulation at a given speed. Before lesioning, nearly all animals were able to ambulate effectively at on a forced treadmill, with no difference between groups. After lesioning, there was a progressive decline in the ability of mice with either no virus or intrastriatal infusion of control virus to ambulate on the treadmill, with no animal able to walk at a rate of 3 cm/s at 7 wk after lesioning (Fig. 1*D*). In contrast, 60% of the AAV.sh.p11 mice were still able to ambulate effectively on the treadmill at that time point ($P = 0.01$, two-tailed Fisher's exact test). These data indicate that reduction of striatal p11 could improve motor performance after 6-OHDA lesioning and might even improve the ability of normal mice to respond to motor challenges.

Histological assessment on completion of the motor studies indicated comparable expression throughout most of the striatum in both groups, using immunostaining for yellow fluorescent protein (YFP) expressed from a second cassette in both vectors (Fig. 1*B*). There was no evidence of loss of medium spiny neurons, as measured by DARPP-32 staining (Fig. 1*B*), and no evidence of any difference in nigral dopaminergic neuronal loss between groups, as measured by tyrosine hydroxylase (TH) staining (Fig. S1*D*). This indicates that the motor effects after inhibition of dorsal striatal p11 expression were due to altered striatal function rather than to differences in neuronal survival compared with controls.

Reduction of p11 in the Dorsal Striatum Normalizes Spontaneous Motor Behaviors in Parkinsonian Mice. To determine whether blockade of striatal p11 expression influences spontaneous motor function, we analyzed open-field activity in mice both before and after 6-OHDA lesioning. Before the lesion, there was no difference in any parameter between control and AAV.sh.p11 mice. After lesioning, control mice exhibited a substantial decline in several parameters, including the number of ambulatory episodes and average velocity of gait, with a concomitant increase in time spent resting (Fig. 2 *A*–*C*). As with the rotarod and treadmill tests, dorsal striatal p11 knockdown significantly improved these parameters. Unlike the modest improvements in treadmill and rotarod performance, however, the open-field parameters in AAV.sh.p11 mice after lesioning were indistinguishable from the prelesioning baseline behaviors (Fig. 2 *A*–*C*). There was no evidence of pathological hyperactivity in these mice; they were no different from either control group before lesioning, and motor parameters were maintained but not increased after lesioning. This finding further

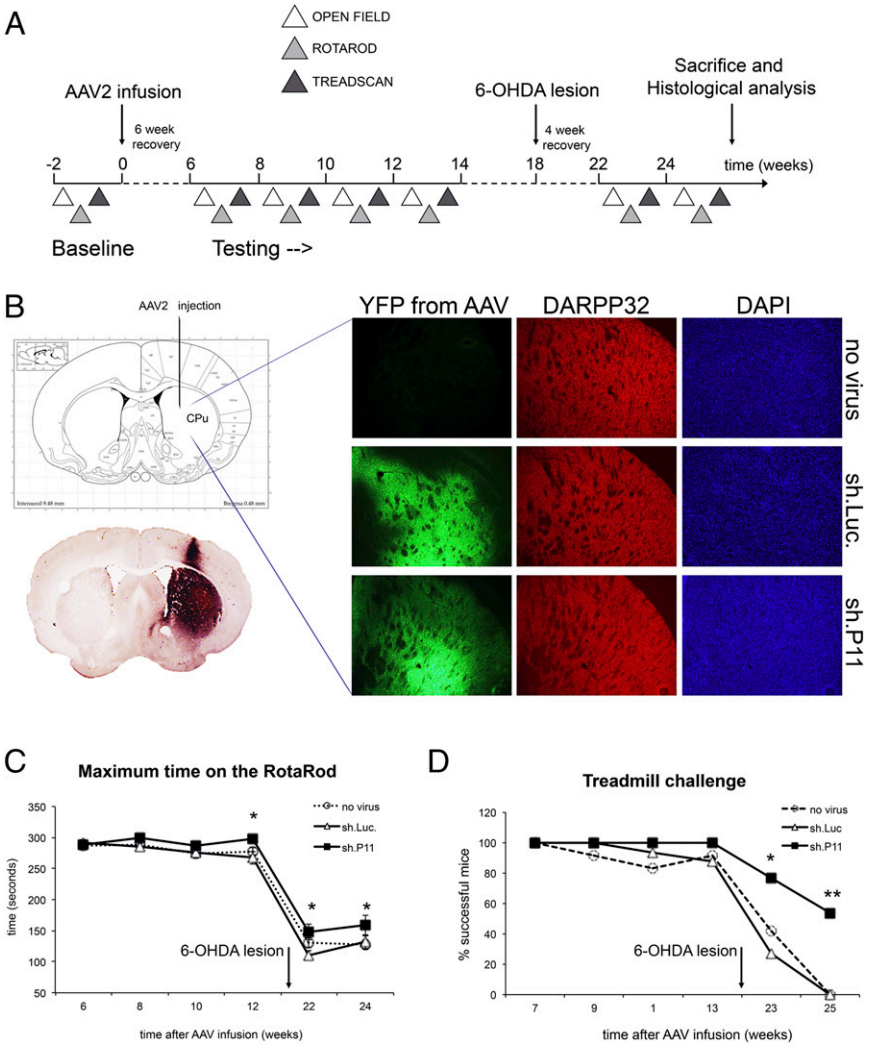

Fig. 1. Reduction of p11 in the dorsal striatum improves evoked motor behavior in adult mice. (*A*) Diagram of the experimental design. (*B*) Immunostaining demonstrating equivalent striatal AAV vector transduction based on YFP expression, with similar overall cell numbers based on staining for DARPP-32 and DAPI. (*C*) Knockdown of p11 in the dorsal striatum of adult mice increased endurance on the rotarod test. A significant increase in rotarod time was observed in AAV.sh.p11 mice just before the 6-OHDA lesion, and this increase was maintained after the lesion. *$P < 0.05$, sh.p11 relative to sh.Luc controls, two-tailed *t* test. (*D*) Striatal p11 reduction improved performance on the treadmill speed challenge after the 6-OHDA lesion compared with controls. *$P < 0.05$, **$P < 0.01$, sh.p11 relative to sh.Luc controls, two-tailed Fisher's exact test. The numbers of mice per experimental group were as follows: no virus, $n = 12$; sh.Luc, $n = 10$; sh.P11, $n = 11$.

supports the conclusion that inhibition of dorsal striatal p11 expression improves motor function in parkinsonian mice, and suggests that spontaneous motor activity may be affected to a greater degree than movement in response to a forced challenge.

Blockade of Striatal p11 Expression Influences the Response to Dopamine in a Mouse Model of PD. Given the effect of p11 on evoked and spontaneous motor behaviors, we next determined whether altered p11 expression after 6-OHDA lesioning could influence motor responses to dopamine. A loss of striatal dopamine after the creation of unilateral 6-OHDA nigral lesions leads to rotational behaviors following administration of dopamine agonists, owing to hypersensitivity of striatal dopamine receptors only in the lesioned hemisphere. To test the effect of p11 loss on striatal responses to dopamine, we randomized mice into equal groups based on their rotational counts in response to the dopamine receptor agonist apomorphine and then administered injections of either AAV.sh.p11 or control AAV.sh.Luc in the ipsilateral striatum. A third group of mice received no viral injection. After a 6-wk period to optimize AAV expression, the mice were challenged again with apomorphine. There was a significant reduction in apomorphine-induced rotations in the AAV.sh.p11 group compared with both the control AAV.sh.Luc and no virus groups ($P < 0.0001$) (Fig. 3*B*).

To evaluate a more clinically relevant condition, we then tested the animals with L-dopa, which is the gold standard therapy for human PD. Again, the AAV.sh.p11 group showed a significant reduction in rotational behaviors compared with controls ($P < 0.001$) (Fig. 3*C*). To determine whether this effect was specific to a single dopamine receptor subtype, we challenged mice with the D1 receptor agonist SKF81297 and the D2 receptor agonist quinpirole. Again, there was a significant reduction in rotations in response to either agonist compared with controls ($P < 0.001$ for the SKF81297 group; $P < 0.0001$ for the quinpirole group), indicating that the effect was not specific to one dopamine receptor subtype (Fig. 3 *D* and *E*). These data are similar to the results seen with focal striatal dopamine replacement therapies, such as gene therapy and cell transplantation (11, 12). Therefore, our results suggest that p11 knockdown normalizes dorsal striatal function ipsilateral to the lesion, with the resultant decrease in up-regulation of dopamine receptor activity leading to reduced striatal asymmetry and decreased rotations.

Down-Regulation of p11 Expression Significantly Reduces L-Dopa–Induced AIMs. Dyskinesia is one of the major complications of dopamine replacement therapy, and, based on our foregoing findings, we hypothesized that p11 might influence the development of AIMs in parkinsonian mice. To test this, mice received unilateral 6-OHDA lesions and 1 mo later were randomized to equal groups based on rotational turns after systemic apomorphine administration. AAV vectors were then injected into the ipsilateral striatum, and starting 8 wk later, the mice received daily therapy with high-dose L-dopa for 3 wk to induce AIMs (Fig. 4*A*). The AIMs induced in mice by chronic L-dopa administration can be classified into four phenotypes: limb, orolingual, axial, and locomotor dyskinesias (13). On the first testing day after L-dopa administration, compared with the mice receiving control AAV.sh.Luc, those with intrastriatal AAV.sh.p11 ipsilateral to the 6-OHDA lesion demonstrated significant reductions in all AIM subscores and in the composite scores of the axial, limb, and orolingual (ALO) and the total composite of ALO plus locomotor scores (Fig. 4 *B* and *C*).

To determine the stability of this effect, animals were scored periodically over 3 wk of daily L-dopa administration, and this profound effect was observed on each testing day (Fig. 4 *D–I*). Because p11 has been shown to potentiate 5-HT1B receptor activity, we examined the effect of inhibiting striatal p11 on AIM scores after coadministration of the 5-HT1B agonist CP94253 with L-dopa. Although modest reductions in AIM subscores and total composite score were observed in control mice, as expected, this effect was not seen in the mice receiving intrastriatal AAV.sh.p11 (Fig. 4 *D–I*). After CP94253 administration, the mice returned to baseline, and the effect of L-dopa continued for another 1 wk. Immunostaining for YFP confirmed equivalent AAV transduction in control and AAV.sh.p11 mice (Fig. S2). These data demonstrate that inhibition of dorsal striatal p11

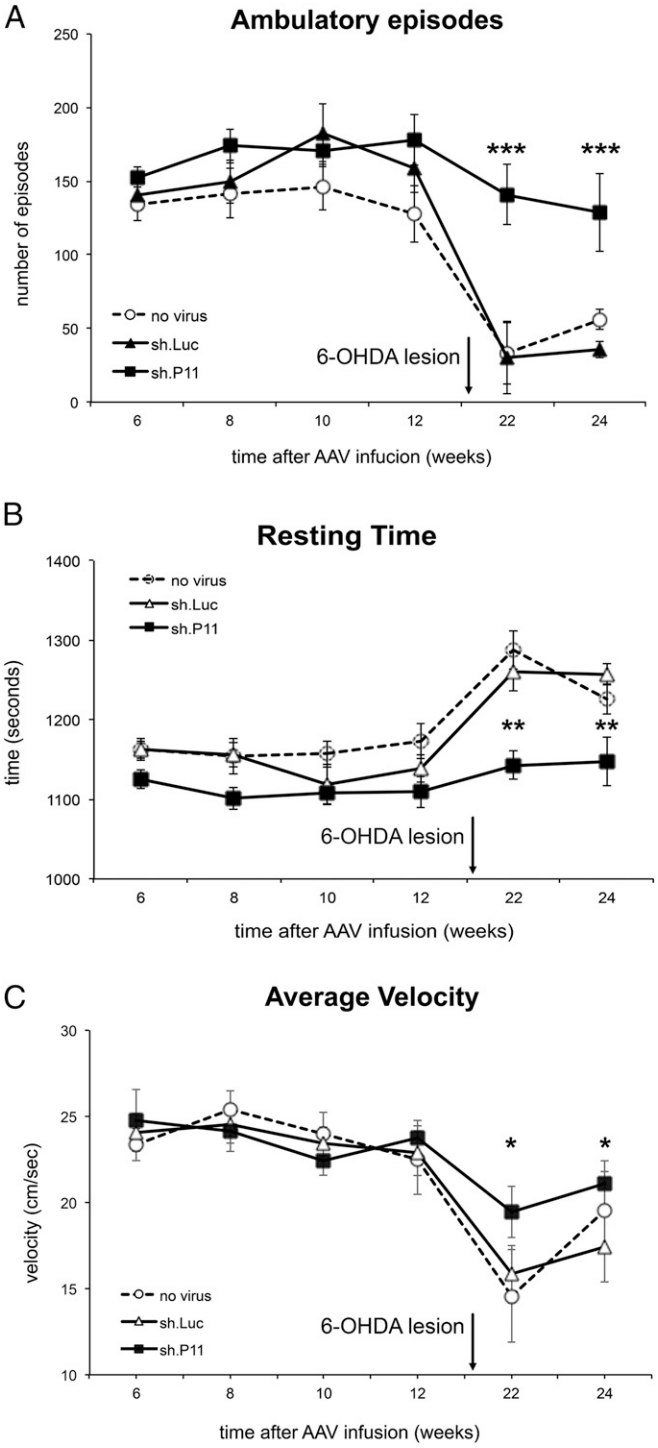

Fig. 2. Reduction of p11 in the dorsal striatum improved spontaneous open-field motor behavior in 6-OHDA lesioned mice. After an initial 5-min acclimation period, open-field activity was measured over 25 min using a photocell-based tracking system in a Plexiglas arena. Compared with control mice receiving AAV.sh.luc, 6-OHDA lesioned mice with intrastriatal AAV.sh.p11 ipsilateral to the lesion showed normalization of ambulatory episodes (*A*), resting time (*B*), and average gait velocity (*C*). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, sh.p11 relative to sh.Luc controls, two-tailed *t* test. The numbers of mice per group were as follows: no virus, $n = 12$; sh.Luc, $n = 10$; sh.P11, $n = 11$.

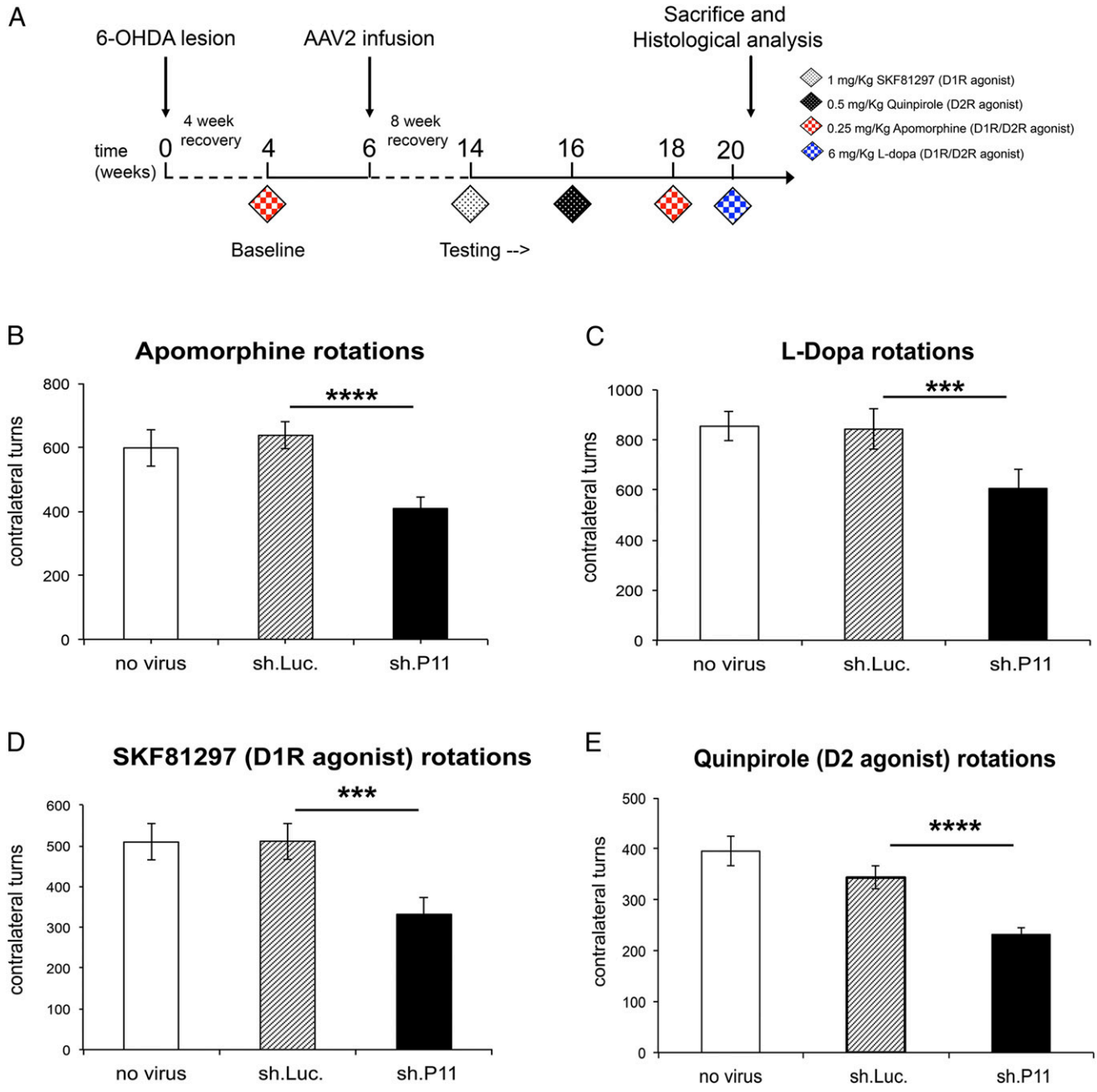

Fig. 3. Reduction of p11 in the dorsal striatum decreases rotational turns in response to dopamine agonists in adult mice. (*A*) Diagram of the experimental design. (*B–E*) Contralateral rotations were decreased in animals with inhibition of striatal p11 ipsilateral to the 6-OHDA lesions compared with lesioned mice receiving a control AAV after administration of apomorphine (*B*), L-dopa (*C*), D1R agonist SKF81297 (*D*), and D2R agonist quinpirole (*E*). ***$P < 0.001$, ****$P < 0.0001$, sh.p11 relative to sh.Luc controls, two-tailed *t* test. The numbers of mice per group were as follows: no virus, $n = 19$; sh.Luc, $n = 18$; sh.P11, $n = 18$. The experiment was repeated three times.

expression is profoundly antidyskinetic, to a greater degree than pharmacologic 5-HT1B receptor activation.

Discussion

Dyskinesia represents one of the most disabling consequences of dopamine replacement therapy for PD. The small adaptor protein p11 has been identified as critical for the function of ventral striatum in models of depression and addiction (8, 14, 15). Here we identify p11 as a protein that profoundly influences the development of abnormal involuntary movements in parkinsonian mice exposed to dopamine replacement therapy. We examined p11 in the dorsal striatum, and found that inhibition of p11 expression resulted in a reduction in AIMs after L-dopa administration. Furthermore, significant improvements in motor behavior were seen, indicating that therapies directed at blocking the action of p11 in the dorsal striatum could improve motor function while reducing abnormal movements. The effect on rotarod and treadmill testing was significant but small, whereas complete normalization of spontaneous motor activity was seen. This suggests that there might be an effect on motivation as well as on movement. Histological analyses confirmed that our transduction was limited to the dorsal striatum, and thus any motivational or behavioral consequences were unlikely to be related to off-target effects of p11. Our data are also consistent with the observation in our companion report (16), which demonstrated improvement in tacrine-induced tremor in transgenic global p11

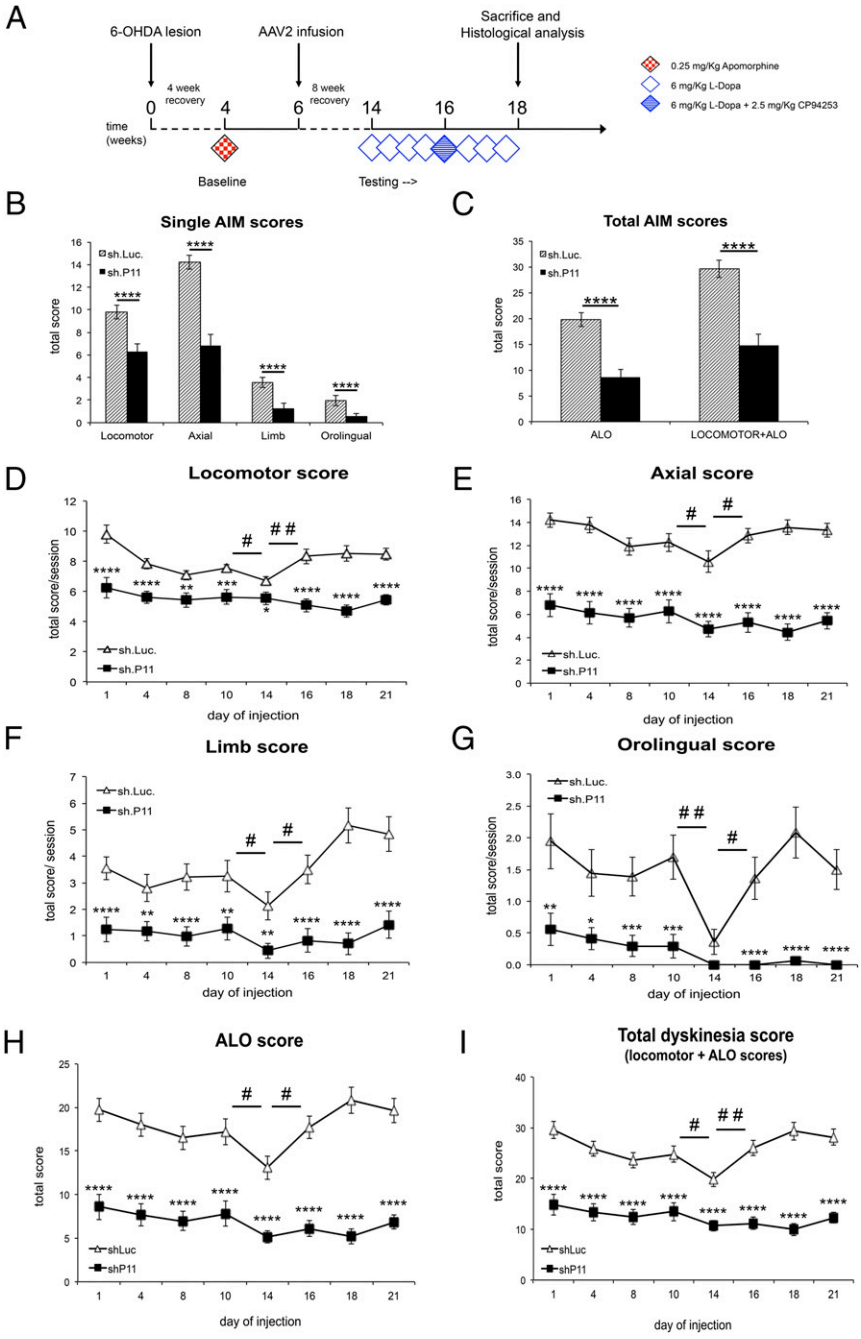

Fig. 4. Reduction of striatal p11 expression significantly reduces L-dopa-induced AIMs. (*A*) Diagram of the experimental design. (*B*) Individual AIM subscores per group in a single testing session. (*C*) Total AIMs composite score in a single testing session. (*D*–*G*) Individual AIM subscores per group across the entire 3 wk of daily L-dopa treatment. On day 14, the mice also received the 5-HT1B agonist CP94253. (*H*) Composite AIM scores of ALO subscores across the entire study. (*I*) Composite total AIM scores (ALO plus locomotor subscores) across the entire study. Administration of CP94253 to control mice modestly reduced AIMs by roughly 20% in all subscores and in total composite AIM scores compared with L-dopa alone, but this effect was not observed in AAV.sh.p11 mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, sh.p11 relative to sh.Luc controls; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ for day 14 sh.Luc compared with either day 10 or day 16 for the same group. $n = 18$ sh.Luc mice; $n = 17$ sh.p11 mice. The experiment was repeated three times.

knockout (KO) mice, an effect replicated in cell type-specific cholinergic p11 KO mice. Taken together, our data suggest that the loss of p11 within cholinergic neurons of the dorsal striatum can improve a variety of motor functions, both evoked and spontaneous. This will be explored more fully in future studies.

Our data raise intriguing questions regarding p11's mechanism of action. The original description of p11 as a potential mediator of neuronal activity identified the 5-HT1B receptor as a key binding partner (17). p11 was found to bind to and increase surface presentation of 5-HT1B receptors, thereby potentiating their activity, and this was confirmed in subsequent studies. Because 5-HT1B agonists are known to reduce dyskinesias (6), we had originally hypothesized that inhibition of p11 expression would block the effect of 5-HT1B agonists, and possibly worsen baseline AIMs. In fact, we found the opposite result. Although focal knockdown of p11 expression in the dorsal striatum did reduce the effect of a 5-HT1B agonist, the overall effect of reduced p11 was profoundly antidyskinetic. Given the dramatic reduction in AIMs, it is difficult to determine whether the effect of reducing p11 in the dorsal striatum on 5-HT1B agonist function is due to decreased 5-HT1B activity or to a floor effect in which a further reduction in AIMs cannot be readily appreciated. Regardless, our data indicate that inhibition of p11 in the dorsal striatum is antidyskinetic, and that this action is not mediated through previously reported potentiation of 5-HT1B activity.

This action also represents, to our knowledge, the first reported potential therapeutic benefit of inhibiting rather than increasing p11 activity, which is again consistent with the finding of reduced AIMs in total p11 KO mice reported in our companion paper (16). The companion study also reported reduced sensitization to rotational behaviors following L-dopa administration in lesioned p11 KO mice, but no difference compared with control mice at baseline. We did observe reduced rotations without sensitization, and we hypothesize

that this reflects improved functioning of neurons in the denervated striatum, leading to reduced receptor hypersensitivity and reduced rotations. This would explain the reduced rotations, reduced AIMs, and improved motor function, which were also reported in the companion paper. This isolated difference between studies likely reflects either variations due to p11 loss during development in KO mice compared with later loss in normal adult mice or potential effects elsewhere in the brain or body in KO mice compared with focal knockdown after viral infusion in otherwise normal mice.

Several other p11 actions that could mediate the effect described here have been reported. In many cases, p11 forms a heterotetramer with annexin A2, and this appears to be necessary for interaction with a variety of receptors and channels, such as the TASK-1 potassium channel (18). p11 also has been reported to interact with SMARCA3, a chromatin remodeling factor, and this interaction appears to be important for some antidepressant effects of p11 (19). Most relevant to our current findings, however, is a recent report of p11 binding to the mGluR5 metabotropic glutamate receptor (20). In prefrontal cortex, p11 was found to potentiate the effects of mGluR5 to regulate depression-like behaviors. mGluR5 also has been shown to play a role in dyskinesia, and unlike 5-HT1B, mGluR5 appears to potentiate abnormal involuntary movements after dopamine replacement therapy (21, 22). Striatal medium spiny projection neurons and cholinergic interneurons express mGluR5 receptors, and pharmacologic inhibition of mGluR5 is antidyskinetic (23, 24); thus, our finding that genetic inhibition of p11 is antidyskinetic could be consistent with this mechanism.

Identification of p11 inhibition as a previously unidentified antidyskinesia pathway raises the potential of exploiting this for therapeutic development. Dyskinesia is a common complication of dopamine replacement therapy, is often dose-limiting, and can be sufficiently difficult to manage such that many patients opt for deep brain stimulation surgery in an attempt to obtain better control without symptomatic decline (1). The complex actions of p11 in different brain regions highlight the difficulties in developing systemic pharmacotherapies for neurologic disease. Although our data suggest that inhibition of p11 throughout the brain might reduce dyskinesia, our previous study in the ventral striatum indicates that this also could cause or worsen depression, which is a major comorbidity of PD (8, 25). Our data suggest that systemic p11 inhibition might be effective in reducing dyskinesia in patients without major depression, whereas for more complex patients, focal therapies, such as gene therapy to specifically inhibit striatal p11, could be optimal for improving dyskinesia and motor function while limiting potential unintended consequences of extrastriatal inhibition.

Materials and Methods

Generation of AAV Vectors Expressing shRNAs. To silence p11 expression, we generated an shRNA against mouse p11 shRNA (sh.p11) and a luciferase shRNA (sh.Luc) as a negative control. These were packaged into AAV vectors using a two-plasmid system in HEK 293 cells. The production and purification procedures are described in detail in *SI Materials and Methods*.

Animals. Wild type C57BL/6 mice, obtained from Charles River Laboratory, were housed two to five per cage and kept at 22 °C on a reverse 12-h light/12-h dark cycle, with standard mouse chow and water provided ad libitum throughout the duration of the study. All animal procedures were approved by the Institutional Animal Care and Use Committee of Weill Cornell Medical College and were in accordance with National Institutes of Health guidelines.

Stereotactic Surgery and Behavioral Assessments. Viral vectors were infused stereotactically into the striata of 8- to 12-wk-old mice, followed by behavioral assessments 6–8 wk later. Details of the surgical infusion and behavioral assessments are provided in *SI Materials and Methods*.

Immunohistochemistry. On completion of all behavioral assessments, mice were deeply anesthetized with sodium pentobarbital (150 mg/kg) and transcardially perfused with 4% paraformaldehyde (PFA). Brains were extracted and post-fixed overnight in 4% PFA, cryoprotected in 30% sucrose, and cut into 40-μm sections using a microtome.

Free-floating sections were treated with various antibodies to visualize proteins of interest, including TH, DARPP-32, and YFP, using immunofluorescence or immunoperoxidase labeling. Detailed information is provided in *SI Materials and Methods*.

Statistical Analysis. The two-tailed *t* test was used for statistical comparisons of all paired animal group data with the exception of the treadmill gait system. All data are expressed as mean ± SEM. The gait system behavior data were evaluated using the two-tailed Fisher's exact test. A P value < 0.05 was considered to indicate statistical significance.

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