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Effects of 5-HT1A receptor stimulation on striatal and cortical M1 pERK induction by L-DOPA and a D1 receptor agonist in a rat model of Parkinson's disease

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Abstract

Motor symptoms of Parkinson's disease are commonly treated using L-DOPA although long-term treatment usually causes debilitating motor side effects including dyskinesias. A putative source of dyskinesia is abnormally high levels of phosphorylated extracellular-regulated kinase (pERK) within the striatum. In animal models, the serotonin 1A receptor agonist ±8-OH-DPAT reduces dyskinesia, suggesting it may exhibit efficacy through the pERK pathway. The present study investigated the effects of ±8-OH-DPAT on pERK density in rats treated with L-DOPA or the D_1 receptor agonist SKF81297. Rats were given a unilateral dopamine lesion with 6-hydroxydopamine and primed with a chronic regimen of L-DOPA, SKF81297 or their vehicles. On the final test day, rats were given two injections: first with ±8-OH-DPAT, the D_1 receptor antagonist SCH23390 or their vehicles, and second with L-DOPA, SKF81297 or their vehicles. Rats were then transcardially perfused for immunohistological analysis of pERK expression in the striatum and primary motor cortex. Rats showed greater dyskinesia in response to L-DOPA and SKF81297 after repeated injections. Although striatal pERK induction was similar between acute and chronic L-DOPA, SKF81297 caused the largest increase in striatal pERK after the first exposure. Neither compound alone affected motor cortex pERK. Surprisingly, in the ventromedial striatum, ±8-OH-DPAT potentiated L-DOPA-induced pERK; in the motor cortex, ±8-OH-DPAT potentiated pERK with L-DOPA or SKF81297. Our results support previous work that the striatal pERK pathway is dysregulated after dopamine depletion, but call into question the utility of pERK as a biomarker of dyskinesia expression.

Keywords

Dopamine; Serotonin; Striatum; Motor cortex; Extracellular-regulated Kinase; Parkinson's disease

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1. Introduction

The most effective symptomatic treatment for Parkinson's disease (PD) is the dopamine (DA) precursor L-DOPA (Cenci et al., 2011). However, most PD patients who take L-DOPA will gradually develop motor side effects including L-DOPA-induced dyskinesia (LID) that increase in severity over time and are due, in part, to super-sensitization of striatal DA receptors (Ahlskog and Muenter, 2001; Feyder et al., 2011). Though the molecular mechanisms of LID are only partially understood, DA loss and subsequent DA replacement therapy result in abnormal striatal plasticity via pathological enhancement of synaptic long-term potentiation and reduced long-term depression (Jenner, 2008; Picconi et al., 2003).

Dysregulation of the striatal extracellular-regulated kinase (ERK) signaling pathway is a candidate mechanism for LID since this molecular cascade promotes synaptic plasticity (Thomas and Huganir, 2004). Phosphorylation of ERK (pERK) stimulates its kinase activity and formation of striatal pERK is promoted by at least two distinct pathways, one mediated by Ras proteins and another by D_1 receptors (D_1Rs; Santini et al., 2008; Shiflett and Balleine, 2011). In rodent and primate models of PD, L-DOPA (through D_1R-mediated mechanisms) and D_1R agonists robustly increase striatal pERK, the magnitude of which often correlates with the severity of dyskinesia (Gerfen et al., 2002; Papadeas et al., 2004; Pavon et al., 2006; Santini et al., 2009, 2010; Westin et al., 2007). Moreover, pharmacological inhibition of pERK formation reduces LID without affecting the efficacy of L-DOPA, suggesting a distinct role for pERK in LID (Lindgren et al., 2009; Santini et al., 2007).

Research into the long-term effects of DA replacement on pERK has yielded conflicting results. Some studies have shown that pERK induction by L-DOPA or D_1R agonists is highest after the first drug exposure, implying that pERK is involved in DA receptor sensitization (Santini et al., 2007, 2010; Papadeas et al., 2004). Others have found that pERK is highest after repeated L-DOPA exposure, suggesting pERK may be a biomarker of dyskinesia expression (Pavon et al., 2006). Thus, the precise relationship between the expression of striatal pERK and dyskinesia remains elusive.

In animal models, serotonin 1A receptor (5-HT_{1A}R) agonists reduce both LID and D_1R-mediated dyskinesia (Bibbiani et al., 2001; Dupre et al., 2008, 2011, 2013). Following DA-depletion, the expression of 5-HT_{1A}Rs within the striatum and primary motor cortex (M1) increases, an effect further potentiated by L-DOPA treatment (Frechilla et al., 2001; Huot et al., 2012). Although 5-HT_{1A}R stimulation enhances pERK levels via activation of the Ras pathway in vitro (Garnovskaya et al., 1996; Raymond et al., 1999) and may do so in vivo (Buritova et al., 2009) it is currently unknown how 5-HT_{1A}Rs impact pERK within the striatum and M1 of a PD brain. Given that pERK is presently thought of as a marker of dyskinesia, 5-HT_{1A}R agonism would be expected to reduce pERK expression in a PD model.

This study investigated how pERK levels in the corticostriatal circuit are affected by compounds that either stimulate or attenuate dyskinesia in a unilateral rat model of PD. Brains were analyzed following a de novo dose of L-DOPA or a D_1R agonist and after repeated exposures to these drugs. It was expected that administration of a 5-HT_{1A}R agonist would reduce pERK induction by both L-DOPA and a D_1R agonist.

2. Results

2.1. Experiment 1: Effects of 5-HT_{1A}R stimulation on L-DOPA-induced striatal and M1 pERK activation

2.1.1. Behavioral Testing—Figure 1A contains a timeline for experiment 1. Rats were assigned to one of six treatment groups based on scores on the forepaw adjusting steps test, a metric of forelimb akinesia (see section 4.5.). The average total intact score was 21% and motor ability did not differ between groups ($F_{5,32}$=0.13, p=.985). LID development during the 10 days of chronic L-DOPA treatment was monitored using the abnormal involuntary movements (AIMs) scale (see section 4.4.). Within-subjects comparisons indicated that treatment day had a significant impact on AIMs (χ^2=20.49, p<.001) such that scores were increased on the 10th day relative to the 1st day (Fig. 2A; Z=3.58, p<.001), but not on the 10th day relative to the 6th day (Z=0.16, p=.877). These results indicate that rats sensitized to L-DOPA in a typical manner (Cenci and Lundblad, 2007), but reached a stable level of responding by the 10th day. A between-subjects analysis using the Kruskal-Wallis test showed that the treatment groups did not differ in their AIMs scores on day 1, 6 or 10 (Fig. 2B). On day 11, rats were injected with two compounds: the 5-HT_{1A} agonist ±8-OH-DPAT (DPAT), the D_1 antagonist SCH23390 (SCH) or vehicle (VEH) and L-DOPA or VEH. A subset of animals (~1/3) was monitored for AIMs at 10 and 20 min after the final injection (prior to sacrifice). Rats given their 1st dose of L-DOPA did not display visible AIMs at these time points. However, rats on their 11th dose of L-DOPA showed an average summed AIMs score of 1.5, while rats pre-treated with DPAT averaged 0.5 AIMs and rats given SCH averaged a score of 1 on the AIMs scale.

2.1.2. Regional changes in striatal pERK expression—A 3-way mixed model 2×4×6 ANOVA was utilized to examine effects of lesion, striatal region, and treatment group on pERK. Findings are depicted in figure 3. A main effect of lesion revealed a 16-fold greater number of pERK-positive cells in the lesioned than intact hemisphere ($F_{1,34}$=37.93, p<.001). A main effect of striatal region was due to the fact that the two medial areas had more pERK than the lateral areas ($F_{2.3,77.3}$=26.93, p<.001). An effect of treatment group was also seen whereby rats given the 5-HT_{1A}R agonist DPAT and/or L-DOPA had greater pERK-positive cell counts ($F_{5,34}$=5.38, p=.001). The 2-way interactions were also significant: lesion*group ($F_{5,34}$=5.98, p<.001); lesion*region ($F_{2.2,75.4}$=25.25, p<.001); region*group ($F_{11.4,77.3}$=3.40, p=.001). Lastly, the 3-way interaction was significant (lesion*region*group: $F_{11.1,75.4}$=3.94, p<.001) and was subject to post-hoc comparisons.

Acute administration of L-DOPA caused a significant increase in pERK that was confined to the dorsomedial striatum, while chronic L-DOPA caused an upregulation in the dorsomedial and ventromedial areas (Fig. 3A, 3C, 3E). No combination of treatments caused a significant upregulation of pERK in the dorsolateral or ventrolateral regions (Fig. 3B, 3D). There were no differences between acutely and chronically L-DOPA-treated rats. Surprisingly, among rats given chronic L-DOPA, pre-treatment with DPAT caused an increase in pERK in the ventromedial striatum (Fig. 3C, 3E). DPAT did not affect pERK levels among rats given acute L-DOPA. As expected, pre-treatment with SCH blocked pERK induction in both the dorsomedial and ventromedial striatum (Fig. 3A, 3C). In the intact striatum, no treatments significantly altered pERK levels in any region.

2.1.3. Regional changes in M1 pERK expression—A 3-way mixed model 2×2×6 ANOVA was used to analyze the effect of lesion, M1 region and treatment group on pERK. There was no effect of lesion indicating that pERK levels did not differ between the M1 hemispheres ($F_{1,34}$=0.95, p=.337). An effect of region reflected increased dorsal M1 pERK labeling relative to ventral M1 ($F_{1,34}$=29.94, p<.001). Drug treatments increased pERK

levels commensurate with a significant main effect of group ($F_{5,34}$=6.69, p<.001). A region*group interaction ($F_{5,34}$=6.47, p<.001) was the only significant 2nd or 3rd order effect. Post-hoc comparisons were performed based on this interaction, by collapsing across hemispheres and analyzing treatment effects at a regional level. Administration of L-DOPA did not change pERK levels in M1. However, when co-administered with acute or chronic L-DOPA, DPAT significantly increased pERK in the dorsal M1 (Fig. 4A, 4C). In the ventral M1, DPAT only significantly increased pERK among the acutely L-DOPA-treated rats (Fig. 4B).

2.2. Experiment 2: Effects of 5-HT_{1A}R stimulation on D_1R agonist-induced striatal and M1 pERK activation

2.2.1. Behavioral Testing—Figure 1B contains a timeline for experiment 2. Rats to be treated with the D_1R agonist SKF81297 (SKF) were assigned to one of six groups based on their scores on the amphetamine-induced rotation test (see section 4.6.). The treatment groups did not differ in rotation scores ($F_{5,26}$=0.13, p=.984) and the average rat rotated ipsilateral to lesion four times per minute. The 3 days of priming with the D_1R agonist SKF significantly impacted AIMs (χ^2=25.94, p<.001). Rats showed higher scores on the 3rd day of priming than on the 1st day (Fig. 2C; Z=3.52, p<.001). Surprisingly, median AIMs were slightly but significantly higher on the 2nd day (Median=56.5) than on the 3rd day (Median=55) of priming (Z=2.26, p=.024). Still, these results indicate that SKF-induced dyskinesia was greater with chronic exposure than acute, and that dyskinesia was relatively stable by the 3rd day of priming (consistent with Dupre et al., 2008, 2011). Among the three groups receiving chronic SKF, there was no significant difference in AIMs expression on the three priming days (Fig. 2D). On the final day of the experiment, rats were given an injection of two compounds: DPAT or VEH and SKF or VEH. All animals were monitored for AIMs at 10 and 20 min after the final injection prior to sacrifice for pERK analysis. During this time period, rats given their 1st dose of SKF averaged a sum of 4.5 on the AIMs scale, which was reduced by DPAT administration to a median score of 0. Rats given their 4th dose of SKF averaged a score of 7 AIMs during this time period, while DPAT co-administration reduced total AIMs to 2.5.

2.2.2. Regional changes in striatal pERK expression—A 3-way mixed model 2×4×6 ANOVA was utilized to examine effects of lesion, striatal region, and treatment group on pERK. Results are depicted in figure 5. A main effect of hemisphere showed more than 8-fold greater pERK-positive cell counts in the lesioned striata than in the intact striata ($F_{1,26}$=61.69, p<.001). A significant effect of region revealed greater pERK upregulation in the medial striatum relative to the lateral striatum ($F_{1.6,42.3}$=9.57, p=.001). SKF increased pERK levels yielding an effect of treatment group ($F_{5,26}$ = 9.86, p<.001). There was a significant lesion*group interaction ($F_{5,26}$=9.78, p<.001), but all other interactions did not reach the threshold for statistical significance.

Post-hoc comparisons in the lesioned striata demonstrated that, on the final test day, acute administration of SKF caused a large increase in pERK in all four striatal regions relative to both VEH and chronic SKF (Fig. 5A–E). By contrast, rats given chronic SKF showed significantly increased pERK only in the dorsomedial and ventromedial striata compared to their controls, which were also primed with SKF for 3 days, but given VEH on the day of sacrifice (Fig. 5A, 5C). Pre-treatment with DPAT did not modify SKF-induced increases in pERK in any region. In the intact striatum, the only statistically significant comparison was an increase in ventromedial striatal pERK amongst rats given chronic SKF compared to those primed with SKF and given acute VEH.

2.2.3. Regional changes in M1 pERK expression—A 3-way mixed model 2×2×6 ANOVA examined the impact of lesion, region and treatment group on pERK. A main effect of lesion revealed 26% greater pERK levels in M1 ipsilateral to DA lesion than in the contralateral M1 ($F_{1,25}$=7.23, p=.013). Levels of pERK were higher in the dorsal region than the ventral region ($F_{1,25}$=18.59, p<.001). Treatment group affected pERK cell counts ($F_{5,25}$=3.65, p=.013), but no interactions reached significance. Results indicated that DPAT, when co-administered with acute SKF, increased pERK in the ventral—but not dorsal—M1 when compared to acute SKF alone (Fig. 6A–C).

3. Discussion

3.1. Principal Findings

Previous research has implicated pERK in dyskinesia pathophysiology and 5-HT_{1A}R agonists in the treatment of dyskinesia (Bibianni et al., 2001; Eskow et al., 2007; Santini et al., 2008) so it was hypothesized that DPAT would reduce pERK induction by L-DOPA and SKF. Unexpectedly, DPAT potentiated pERK induction by L-DOPA in the ventromedial striatum without affecting SKF-induced striatal pERK (Figs. 3, 5). Neither L-DOPA nor SKF alone significantly changed M1 pERK levels, but co-administration of DPAT increased M1 pERK (Figs. 4, 6). Acute SKF induced greater striatal pERK than chronic SKF (Fig. 5), but acute and chronic L-DOPA caused equivalent striatal pERK (Fig. 3).

3.2. 5-HT_{1A}R Stimulation Potentiates pERK Induction in the Striatum and M1

This is the first study to examine modulation of striatal pERK levels by a 5-HT_{1A}R agonist in a PD model. Previous work reported that 5-HT_{1A}R stimulation did not affect striatal pERK in intact rat brains (Chen et al., 2002). This was replicated in the intact striatum of the current study. More importantly, after DA lesion, 5-HT_{1A}R stimulation increased pERK levels in the ventromedial striatum (Fig. 3C). It is not known why 5-HT_{1A}Rs gain control over striatal pERK after DA loss; however, research has shown that DA lesions upregulate striatal and cortical 5-HT_{1A}Rs (Frechilla et al., 2001; Huot et al., 2012), which may enhance the ability of 5-HT_{1A}Rs to alter corticostriatal signaling.

Previous research has shown that the 5-HT transporter inhibitor fluoxetine blocked striatal pERK induction by L-DOPA in a rat model of PD (Inden et al., 2012). The effects of fluoxetine on pERK were attributed to 5-HT_{1A}R activation since pERK suppression was reversed by a 5-HT_{1A}R antagonist. Thus, the work of Inden et al. (2012) suggests that some 5-HT_{1A}Rs negatively regulate striatal pERK. This is surprising in light of the present findings that DPAT potentiated ventromedial striatal pERK.

One explanation may be that two distinct intracellular signaling pathways mediated by post-synaptic striatal 5-HT_{1A}Rs have opposing effects on striatal pERK formation and subsequent dyskinesia. Striatal D_1R stimulation can activate pERK and promote dyskinesia (Feyder et al., 2011; Gerfen et al., 2002; Santini et al., 2010; Papadeas et al., 2004) while 5-HT_{1A}Rs reduce D_1R-mediated dyskinesia (Dupre et al., 2011, 2013), suggesting that striatal 5-HT_{1A}Rs may alter dyskinesia by reducing D_1R supersensitivity. By contrast, some 5-HT_{1A}Rs increase pERK by activating the Ras pathway (Raymond et al., 1999) and overactivity of the Ras pathway promotes dyskinesia (Fasano et al., 2010). This implies a pathway for 5-HT_{1A}Rs to enhance dyskinesia.

These paradoxical effects suggest that topographically distinct 5-HT_{1A}Rs may both promote and inhibit dyskinesia by potentiating and inhibiting pERK activity, respectively. However, dyskinesia suppression appears to be the predominant behavioral effect of DPAT, including at the dose used in the present study (1 mg/kg: Dupre et al., 2007, 2011, 2013). Bidirectional modulation of pERK by 5-HT_{1A}Rs may also explain why investigators see region-specific

modulation of pERK by compounds that activate 5-HT_{1A}Rs (e.g. Buritova et al., 2009; Chen et al., 2002).

We also investigated pERK expression in M1 using a PD model, since corticostriatal inputs are considered to be critically important in LID pathophysiology (Jenner, 2008; Picconi et al., 2003). Alone, neither L-DOPA nor SKF significantly increased M1 pERK (Figs. 4, 6). When DPAT was co-administered with DA compounds, increased pERK formation was found in both the DA-lesioned and intact M1. The largest increase in pERK expression was seen in the dorsal M1, which is primarily involved in intracortical signaling (Geyer et al., 2000). Smaller changes were seen in the ventral M1, which is responsible for cortical outputs to the striatum, thalamus, brain stem and spinal cord (Geyer et al., 2000). While previous work has established that 5-HT_{1A}R agonists increase prefrontal cortex pERK (Buritova et al., 2009), the current study shows that this pattern extends to M1 as well.

There was a large lesion*group interaction influencing pERK expression in the striatum (Exp. 1 η_p^2 = .468; Exp. 2 η_p^2 = .653), but this interaction was not statistically significant in the motor cortex. We interpret this interaction as demonstrating that in the intact striatum, treatment did not influence pERK staining, but in the lesioned striatum, L-DOPA, SKF and DPAT all lead to significant increases in pERK. This suggests that the DA system is a critical regulator of ERK in the striatum and that local depletion of DA renders the striatal ERK cascade hypersensitive to changes in DA and 5-HT transmission. This is not the case in M1: while treatment with DPAT did affect pERK, the change was bilateral and not related to DA lesion. The key regulatory mechanisms governing ERK activity in M1 remain unknown.

3.3. SKF and L-DOPA Differentially Modulate Striatal pERK

Within the striatum, we found that animals given chronic SKF had more than 10-fold greater lesioned-side pERK cell count than animals given chronic L-DOPA (cf. Supplementary Fig. 1A, 1C), despite having relatively equivalent amounts of dyskinesia (cf. Fig. 2B, 2D). Thus, at a given level of dyskinesia, it appears that D_1R agonists increase pERK to a much greater extent than L-DOPA. This is likely due to the fact that L-DOPA-derived DA exhibits opposing effects on pERK via receptor-specific effects: previous work has shown that, among medium spiny neurons expressing DA receptors, D_1R stimulation tends to increase pERK and D_2 receptor activation tends to reduce pERK (Gerfen et al., 2002; Kim et al., 2006; Lindgren et al., 2009). Recent research in the striatum has shown the initial doses of L-DOPA upregulate pERK among medium spiny neurons while chronic administration shifts L-DOPA-induced pERK induction towards cholinergic interneurons (Ding et al., 2011). In the present study, we were unable to verify which cell subtypes were expressing pERK since valid identification requires dual-labeling for pERK and a molecular fingerprint.

Given the association of pERK with dyskinesia, it was surprising that pERK staining was greatest in the medial striatum, which receives primarily prefrontal cortex input. Much less pERK was observed in the lateral striatum, which receives chiefly motor cortex input (Balleine et al., 2007; Shiflett and Balleine, 2011; Figs. 3, 5). While SKF initially caused a large increase in pERK in all striatal areas, by the last treatment, the increase was only significant in the medial striatum (Fig. 5). Interestingly, activity of the ERK cascade in the medial striatum is critical for promoting goal-oriented learning (Balleine et al., 2007; Shiflett and Balleine, 2011). Since impulsive behavior is a common side-effect of DA replacement therapy in PD (Voon et al., 2009), it is possible that abnormal DA-regulated medial striatal pERK activity may account for this type of compulsive behavior.

Alternatively, following anti-PD therapy, the downstream effects of pERK activation may be more rapid and permanent in the lateral vs. medial striatum. Since a key role for ERK is to promote epigenetic modifications of histones and DNA (Thomas and Huganir, 2004), it may be that pERK activity in the lateral striatum causes inexorable modification of striatal cells such that future pERK upregulation is not required to maintain these epigenetic changes. The current findings suggest that future striatal ERK investigations should take care to differentiate striatal regions.

3.4. Acute vs. Chronic Exposure Differences in Striatal pERK

There has been some discord in the literature regarding whether striatal pERK induction by L-DOPA is greatest upon the first exposure. Santini et al. (2007, 2010) and Ding et al (2011) showed that acute L-DOPA (20 and 25 mg/kg, respectively) increased pERK more than chronic L-DOPA, which suggests that pERK is primarily involved in the creation of dyskinesia liability rather than the maintenance of dyskinesia liability. However, other work by Pavon et al. (2006) found that chronic L-DOPA (25 mg/kg) caused greater pERK than acute L-DOPA, which implicates pERK as a biomarker of dyskinesia severity.

The results from the present study suggest that, to some extent, pERK induction by L-DOPA is a marker of both the development and expression of LID. We used a much lower dose of L-DOPA (6 mg/kg) than most previous investigators and found that acute and chronic administration increased striatal pERK by the same amount (Fig. 3). This mirrors the findings of Westin et al. (2007), who used similar methods and a slightly higher dose of L-DOPA (10 mg/kg). Although different species require different doses to achieve commensurate effects, these lower L-DOPA doses are more likely to be physiologically relevant for human PD patients.

In contrast to L-DOPA, all striatal regions showed greater pERK induction by SKF with the acute dose than with the chronic dose (Fig. 5). These results are in accord with Papadeas and colleagues (2004) who reported the highest SKF-induced pERK on the 1st day of drug exposure although higher doses were employed (3 mg/kg vs. 0.8 mg/kg). Therefore, at least for D_1R agonists, it does appear that pERK levels provide information about the process of D_1R sensitization since dyskinesia severity and pERK expression are inversely correlated across test days.

3.5. Potential Utility of pERK as a Dyskinesia Biomarker

A key question for the field is the extent to which changes in striatal pERK can be interpreted as a biomarker of dyskinesia severity. Our results suggest a disconnect between dyskinetic behavior and pERK expression: this was most striking in experiment 2 where acute SKF induced greater striatal pERK than chronic SKF (Fig. 5) even while producing less dyskinesia (Fig. 2C). Moreover, SKF-induced pERK waned in the lateral (motor) striatum compared to VEH over repeated treatments. Additionally, acute L-DOPA induced less dyskinesia than chronic L-DOPA (Fig. 2A), even while producing the same levels of pERK activation (Fig. 3). Comparing between experiments, chronic L-DOPA and SKF caused similar amounts of dyskinesia despite the fact that SKF induced much greater pERK staining than L-DOPA (although at the time of transcardial perfusion, SKF-induced AIMs were likely greater than L-DOPA-induced AIMs; cf. AIMs results in sections 2.1.1 and 2.2.1; also cf. Fig. 2A, 2C). In the present study, we investigated pERK levels at only one time point (20 min after L-DOPA or SKF), which is prior to peak dyskinesia severity. Given this, we cannot rule out the possibility that 5-HT_{1A} agonists may accelerate pERK induction without increasing maximal induction levels or, conversely, may attenuate pERK activation at a later time point, such as during peak dyskinesia.

Finally, despite the well-documented finding that 5-HT_{1A}R agonists reduce dyskinesia evoked by L-DOPA or SKF (e.g. Bibbiani et al., 2001; Dupre et al., 2011, 2013; Eskow et al., 2007) we found that DPAT either had no effect on or increased the expression of striatal pERK. Taken as a whole, our results call into question the utility of pERK as a stable biomarker of dyskinesia expression. Rather, the present data do suggest that DA depletion causes a dysregulation of the striatal pERK pathway, rendering it conditionally hypersensitive to pharmacological compounds acting on the DA and 5-HT systems.

4. Experimental Procedure

4.1. Animals

This study used male Sprague-Dawley rats (Taconic Farms, Hudson, NY, USA) that were 8 weeks old and 225–250 g upon arrival. Rats were kept in plastic cages and given free access to water and standard laboratory rat food. The colony room was maintained at 22–23°C on a 12 h light/dark cycle. Throughout the study, animals were cared for in full accordance with the guidelines of the Institutional Animal Care and Use Committee of Binghamton University and the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academic Press, 2011).

4.2. Drugs

Despiramine hydrochloride (25 mg/ml; Sigma-Aldrich, St. Louis, MO, USA) and D-amphetamine (2.5 mg/ml; Sigma-Aldrich) were dissolved in dH_2O. Buprenorphine hydrochloride (0.03 mg/ml; Hospira Inc., Lake Forest, IL, USA), ±8-OH-DPAT hydrobromide (1 mg/ml; Sigma-Aldrich), and SCH23390 hydrochloride (1 mg/ml; Sigma-Aldrich) were dissolved in dH_20 containing 0.9% NaCl (saline). SKF81297 hydrobromide (0.8 mg/ml; Sigma-Aldrich) was dissolved in a mixture of 20% dimethyl sulfoxide and 80% saline. 6-hydroxydopamine hydrobromide (6-OHDA; 3 mg/ml; Sigma-Aldrich) was dissolved in saline with 0.1% ascorbic acid. L-DOPA methyl ester hydrochloride (6 mg/ml; Sigma-Aldrich) and the peripheral decarboxylase inhibitor benserazide hydrochloride (15 mg/ml; Sigma-Aldrich) were dissolved in the same vehicle, which was saline with 0.1% ascorbic acid. Sodium pentobarbital (260 mg/ml; Fort Dodge Animal Health, Fort Dodge, IA, USA) was suspended in an alcohol/dH_2O mixture by the manufacturer.

4.3. Surgeries

One week after arrival, rats were given a unilateral DA lesion to the left medial forebrain bundle. Prior to surgery, rats were given injections of desipramine (25 mg/kg, ip) to protect norepinephrine neurons, and buprenorphine (0.03 mg/kg, ip) as pre-emptive analgesia. Animals were then anaesthetized with 1–2% isoflurane (Baxter Healthcare Corp., Deerfield, IL, USA) mixed with oxygen (2 L/min). The following coordinates relative to bregma were used to target the medial forebrain bundle according to the rat brain atlas of Paxinos and Watson (1998): AP −1.8 mm; ML +2.0 mm; DV −8.6 mm, with the incisor bar 5 mm below the interaural line. A 10 μl syringe with 26 gauge needle was lowered into the target site. 6-OHDA (12 μg in 4 μl) was injected at a constant flow rate of 2 μl/min for 2 min and timed to begin 30 min after desipramine injection. The needle was withdrawn 5 min later. Stainless steel wound clips were used to close the surgical site and animals received one additional dose of buprenorphine (0.03 mg/kg, ip) the day after surgery.

4.4. Abnormal Involuntary Movements Test

The AIMs test is a metric of dyskinesia. Rats were monitored for AIMs using a procedure modified from Cenci and Lundblad (2007) that is described in detail in Lindenbach et al. (2011). Following treatment with L-DOPA (6 mg/kg, sc) or SKF (0.8 mg/kg, sc), rats were placed in clear, plastic cylinders and rated by trained observers blind to the experimental

condition for 1 min every 10 min over a 120 min period. Individual dyskinesia severity scores ranging from 0 (not present) to 4 (severe and not interruptible) were given for axial, limb and orolingual dyskinesias. The three AIMs subtypes were summed to create a single AIMs score for data analysis.

4.5. Experiment 1: Effects of 5-HT_{1A}R stimulation on L-DOPA-induced striatal and M1 pERK activation

6-OHDA-lesion surgery was performed on 49 rats that were 9 weeks old. Two weeks later, the forepaw adjusting steps test was performed for use as a behavioral correlate of unilateral DA depletion (Chang et al., 1999). Rats were held such that they had only one free forelimb; for each trial, rats were moved laterally across a table at a steady rate of 90 cm/10 sec. Each stepping test consisted of six trials for each forepaw, alternating between directions (for more detail see Eskow et al., 2007). To create a "percent intact" stepping score, the total number of steps with the lesioned forelimb were divided them by total number of steps with the intact forelimb and multiplied by 100. Lower scores indicate a greater Parkinsonian impairment. Rats were excluded from the study if their percent intact score was more than 50%. Percent intact scores were used to rank-order the rats according to degree of impairment and assign them to six equivalently-Parkinsonian treatment groups.

At three weeks post-lesion, rats were started on a daily treatment of either L-DOPA (6 mg/kg, sc) or VEH for a period of 10 days, referred to as the "chronic" treatment. On days 1, 6 and 10 of the chronic treatment phase, rats were monitored for AIMs subsequent to drug injection. Statistical comparisons were used to verify that the pre-sorted groups developed dyskinesias that were stable on the last two test days (a within-subjects analysis) and equivalent between groups on all test days (a between-subjects analysis).

On day 11, each rat received two drug injections, referred to as "acute" treatments. The 1st acute treatment consisted of one of the following: the 5-HT_{1A}R agonist DPAT (1 mg/kg, sc), the D_1R antagonist SCH (1 mg/kg, sc) or VEH. Ten min later, animals received the 2nd acute treatment, which was either L-DOPA (6 mg/kg, sc) or VEH. Twenty min later, rats were injected with a lethal dose of sodium pentobarbital and transcardially perfused, first with ice-cold phosphate buffered saline (PBS) then with 4% formaldehyde suspended in PBS. This 20 min post-L-DOPA time point was chosen for optimal pERK expression based on previously published work (Gerfen et al., 2002; Westin et al., 2007). Our final analysis used data from 42 rats and two were considered to be outliers for both the striatal and M1 analysis (Supplementary Fig. 1A–B). After removing these outliers, each treatment group contained 6–7 rats.

4.6. Experiment 2: Effects of 5-HT_{1A}R stimulation on D_1R agonist-induced striatal and M1 pERK activation

6-OHDA lesion surgery was performed on 36 rats that were 9 weeks old. Two weeks later, rats were assessed for amphetamine-induced rotations, which is a test frequently used to screen for DA lesion severity (Chang et al., 1999). Following injection of D-amphetamine (2.5 mg/kg, ip), rotations ipsilateral to DA lesion were counted for 1 min every 5 min over 90 min. Rats were assigned a rank based their score, and these ranks were used to create six equivalently-lesioned treatment groups.

One week later, three of the six groups received injections of the D_1R agonist SKF (0.8 mg/kg, sc) while the other three groups received VEH on three separate occasions 2–3 days apart, referred to as "chronic" treatments. Immediately following injections, AIMs were observed. Two days later, rats received two treatments, referred to as "acute" treatments: DPAT (1 mg/kg, sc) or VEH followed by SKF (0.8 mg/kg, sc) or VEH. The second

treatment was given immediately following the first treatment injection, and doses were selected based on previously published work (Dupre et al., 2007, 2008). Twenty min after the injections, animals were killed by sodium pentobarbital injection and transcardially perfused with PBS then 4% formaldehyde in PBS. The final analysis included data from 32 rats, of which one rat was considered to be an outlier in the M1 analysis (Supplementary Fig. 1D). After removing the outlier, each treatment group contained 5–6 rats.

4.7. Tissue Processing and Immunohistochemistry

Coronal sections (40 μm thickness) were cut for the entire rostral-caudal axis of the brain using a microtome (Model SM2000R; Leica Microsystems Inc., Bannockburn, IL, USA). Six slices, each containing the striatum and M1, were selected for processing from each rat. The section spanned from 1.60 mm to 0.40 mm anterior to bregma with 240 μm between each section.

All slices for a given rat were placed in the same well of a plastic plate and triple-washed with PBS between incubations. Endogenous peroxidase activity was removed by immersing slices in 0.3% H_2O_2 for 30 min. A rabbit polyclonal biotinylated primary antibody was used (Item 9101, Cell Signaling, Danvers, MA, USA). This antibody binds to pERK1/2 with epitopes that are selective for single or dual phosphorylation at residues Thr202 and Tyr204 for ERK1 and residues Thr187 and Tyr189 for ERK2. The primary antibody (1:50 dilution) was incubated for 40 h in a blocking buffer containing bovine serum albumin, normal goat serum and the detergent Triton X-100 (Sigma-Aldrich). Slices were then bathed in a polyclonal goat anti-rabbit biotinylated secondary antibody (Item AP132B, Millipore, Billerica, MA, USA) at a 1:200 dilution in blocking buffer for 1 h. Subsequently, slices were bathed for 1 h in an avidin-biotin mixture prepared according to manufacturer's protocol (VectaStain Elite ABC Kit, Vector Laboratories, Burlingame, CA, USA). For the chromagen step, SigmaFast kits (Sigma-Aldrich) containing 3,3′-diaminobenzidine were used according to manufacturer's instructions. Slices were incubated in the 3,3′-diaminobenzidine mixture for 90 sec and placed immediately in a PBS bath. Sections were then mounted on glass slides, dehydrated in ethanol and the endogenous lipids were removed before cover slipping.

Light microscopic analysis was performed under brightfield optics with a stereotaxic microscope (Axioscope 2 Plus, Zeiss, Oberkochen, Germany). Striatal cells that were immunoreactive for pERK were visualized at 20x magnification (area = 0.25 mm^2) and photomicrographs were taken in the dorsolateral, dorsomedial, ventrolateral and ventromedial striata. M1 cells were examined at 10x magnification (area = 1 mm^2) using photomicrographs of the dorsal and ventral cortices.

Positively-labeled cells were quantified with the profile count method using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Cell counts were performed by a researcher blind to treatment group. Using ImageJ, full-color photomicrographs were first rendered in 8-bit grayscale. Displayed pixel values were adjusted by increasing minimum pixel value (pure black) while decreasing maximum pixel value (pure white) to conform with values found in each individual photomicrograph. Contrast and threshold were adjusted to separate positively stained cells from background. Next, images were rendered in pure black and white with the pixel intensity threshold modified by the researcher for each photomicrograph. An optimal threshold for detection of cells and exclusion of artifacts was determined using a side-by-side comparison of the modified image to the original full-color photomicrograph. Subsequently, the analyze particles feature was used to count the number of black objects with a circularity index of at least 0.25 and a squared pixel size of 150–2000 (in the striatum) or 100–1500 (in M1).

4.8. Statistical Analysis

Statistical analysis was performed using SPSS v20 (IBM, Chicago, IL, USA) with alpha set at 0.05 for all tests. Since the AIMs scale has ordinal intervals, we used medians as our measure of central tendency with median absolute deviation (M.A.D.) as our variance estimate. The non-parametric Kruskal-Wallis test was used to compare median AIMs scores between groups, while the Friedman test and Wilcoxon sign-rank test were used for within-subjects comparisons. All other data are reported using means and standard error of the mean (S.E.M.). For these data, parametric statistics were performed using ANOVAs and Fisher's LSD planned comparisons where appropriate.

Heteroschedasticity was assessed with Mauchley's test of sphericity or Levene's test for inequality of variance. When Mauchley's test or Levene's test were statistically significant, we used Huyhn-Feldt corrections or Welch's t-test, respectively (in such cases, the degrees of freedom were often fractions). Rats were considered to be outliers if the sum of their lesioned-side pERK cell counts for an entire structure (striatum or M1) were more than two standard deviations from the mean of the treatment group. In such a case, cell counts for that rat in that structure were not used for inferential statistics. Individual subject data (including outliers) for pERK cell counts in the striatum and M1 of the 6-OHDA-lesioned hemisphere are presented in supplementary figure 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

5-HT	Serotonin
5-HT$_{1A}$R	Serotonin lA receptor
6-OHDA	6-hydroxydopamine
AIMs	Abnormal involuntary movements
D$_1$R	D$_1$ receptor
DA	Dopamine
DPAT	±8-OH-DPAT
ERK	Extracellular-regulated kinase
LID	L-DOPA-induced dyskinesia
M1	Primary motor cortex
M.A.D	Median Absolute Deviation
PD	Parkinson's disease

pERK	phosphorylated extracellular-regulated kinase
SCH	SCH23390
SKF	SKF81297
S.E.M	Standard Error of the Mean
VEH	Vehicle

References

Ahlskog JE, Muenter MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. Mov Disord. 2001; 16:448–458. [PubMed: 11391738]

Balleine BW, Delgado MR, Hikosaka O. The role of the dorsal striatum in reward and decision-making. J Neurosci. 2007; 27:8161–8165. [PubMed: 17670959]

Bibbiani F, Oh JD, Chase TN. Serotonin 5-HT1A agonist improves motor complications in rodent and primate parkinsonian models. Neurology. 2001; 57:1829–1834. [PubMed: 11723272]

Buritova J, Berrichon G, Cathala C, Colpaert F, Cussac D. Region-specific changes in 5-HT1A agonist-induced Extracellular signal-Regulated Kinases 1/2 phosphorylation in rat brain: A quantitative ELISA study. Neuropharmacology. 2009; 56:350–361. [PubMed: 18809418]

Cenci MA, Lundblad M. Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice. Curr Protoc Neurosci. 2007; Chapter 9(Unit 25)

Cenci MA, Ohlin KE, Odin P. Current options and future possibilities for the treatment of dyskinesia and motor fluctuations in Parkinson's disease. CNS Neurol Disord Drug Targets. 2011; 10:670–684. [PubMed: 21838677]

Chang JW, Wachtel SR, Young D, Kang UJ. Biochemical and anatomical characterization of forepaw adjusting steps in rat models of Parkinson's disease: studies on medial forebrain bundle and striatal lesions. Neuroscience. 1999; 88:617–628. [PubMed: 10197780]

Chen J, Shen C, Meller E. 5-HT1A receptor-mediated regulation of mitogen-activated protein kinase phosphorylation in rat brain. Eur J Pharmacol. 2002; 452:155–162. [PubMed: 12354565]

Ding Y, Won L, Britt JP, Lim SA, McGehee DS, Kang UJ. Enhanced striatal cholinergic neuronal activity mediates L-DOPA-induced dyskinesia in parkinsonian mice. Proc Natl Acad Sci USA. 2011; 108:840–5. [PubMed: 21187382]

Dupre KB, Eskow KL, Negron G, Bishop C. The differential effects of 5-HT1A receptor stimulation on dopamine receptor-mediated abnormal involuntary movements and rotations in the primed hemiparkinsonian rat. Brain Res. 2007; 1158:135–143. [PubMed: 17553470]

Dupre KB, Eskow KL, Barnum CJ, Bishop C. Striatal 5-HT1A receptor stimulation reduces D1 receptor-induced dyskinesia and improves movement in the hemiparkinsonian rat. Neuropharmacology. 2008; 55:1321–1328. [PubMed: 18824001]

Dupre KB, Ostock CY, Eskow Jaunarajs KL, Button T, Savage LM, Wolf W, Bishop C. Local modulation of striatal glutamate efflux by serotonin 1A receptor stimulation in dyskinetic, hemiparkinsonian rats. Exp Neurol. 2011; 229:288–299. [PubMed: 21352823]

Dupre KB, Ostock CY, George JA, Eskow Jaunarajs KL, Hueston CM, Bishop C. Effects of 5-HT Receptor Stimulation on D1 Receptor Agonist-Induced Striatonigral Activity and Dyskinesia in Hemiparkinsonian Rats. ACS Chem Neurosci. 2013; 4:747–760. [PubMed: 23496922]

Eskow KL, Gupta V, Alam S, Park JY, Bishop C. The partial 5-HT1A agonist buspirone reduces the expression and development of l-DOPA-induced dyskinesia in rats and improves l-DOPA efficacy. Pharmacol Biochem Behav. 2007; 87:306–314. [PubMed: 17553556]

Fasano S, Bezard E, D'Antoni A, Francardo V, Indrigo M, Qin L, Dovero S, Cerovic M, Cenci MA, Brambilla R. Inhibition of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) signaling in the striatum reverts motor symptoms associated with L-dopa-induced dyskinesia. Proc Natl Acad Sci USA. 2010; 107:21824–21829. [PubMed: 21115823]

Feyder M, Bonito-Oliva A, Fisone G. L-DOPA-Induced Dyskinesia and Abnormal Signaling in Striatal Medium Spiny Neurons: Focus on Dopamine D1 Receptor-Mediated Transmission. Front Behav Neurosci. 2011; 5:1–11. [PubMed: 21267359]

Frechilla D, Cobreros A, Saldise L, Moratalla R, Insausti R, Luquin M, Del Rio J. Serotonin 5-HT(1A) receptor expression is selectively enhanced in the striosomal compartment of chronic parkinsonian monkeys. Synapse. 2001; 39:288–296. [PubMed: 11169778]

Garnovskaya MN, van Biesen T, Hawe B, Casanas Ramos S, Lefkowitz RJ, Raymond JR. Ras-dependent activation of fibroblast mitogen-activated protein kinase by 5-HT1A receptor via a G protein beta gamma-subunit-initiated pathway. Biochemistry. 1996; 35:13716–13722. [PubMed: 8901512]

Gerfen CR, Miyachi S, Paletzki R, Brown P. D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J Neurosci. 2002; 22:5042–5054. [PubMed: 12077200]

Geyer S, Matelli M, Luppino G, Zilles K. Functional neuroanatomy of the primate isocortical motor system. Anat Embryol. 2000; 202:443–474. [PubMed: 11131014]

Huot P, Johnston TH, Koprich JB, Winkelmolen L, Fox SH, Brotchie JM. Regulation of cortical and striatal 5-HT1A receptors in the MPTP-lesioned macaque. Neurobiol Aging. 2012; 33:9–19. [PubMed: 20122755]

Inden M, Abe M, Minamino H, Takata K, Yoshimoto K, Tooyama I, Kitamura Y. Effect of Selective Serotonin Reuptake Inhibitors via 5-HT1A Receptors on L-DOPA-Induced Rotational Behavior in a Hemiparkinsonian Rat Model. J Pharmacol Sci. 2012; 119:10–19. [PubMed: 22510520]

Jenner P. Molecular mechanisms of L-DOPA-induced dyskinesia. Nat Rev Neurosci. 2008; 9:665–677. [PubMed: 18714325]

Kim DS, Palmiter RD, Cummins A, Gerfen CR. Reversal of supersensitive striatal dopamine D1 receptor signaling and extracellular signal-regulated kinase activity in dopamine-deficient mice. Neuroscience. 2006; 137:1381–1388. [PubMed: 16388913]

Lindenbach D, Ostock CY, Eskow Jaunarajs KL, Dupre KB, Barnum CJ, Bhide N, Bishop C. Behavioral and Cellular Modulation of L-DOPA-Induced Dyskinesia by β-Adrenoceptor Blockade in the 6-Hydroxydopamine-Lesioned Rat. J Pharmacol Exp Ther. 2011; 337:755–765. [PubMed: 21402691]

Lindgren HS, Ohlin KE, Cenci MA. Differential involvement of D1 and D2 dopamine receptors in L-DOPA-induced angiogenic activity in a rat model of Parkinson's disease. Neuropsychopharmacology. 2009; 34:2477–2488. [PubMed: 19606087]

Papadeas ST, Blake BL, Knapp DJ, Breese GR. Sustained extracellular signal-regulated kinase 1/2 phosphorylation in neonate 6-hydroxydopamine-lesioned rats after repeated D1-dopamine receptor agonist administration: implications for NMDA receptor involvement. J Neurosci. 2004; 24:5863–5876. [PubMed: 15229233]

Pavon N, Martin AB, Mendialdua A, Moratalla R. ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. Biol Psychiatry. 2006; 59:64–74. [PubMed: 16139809]

Paxinos, G.; Watson, W. The rat brain in stereotaxic coordinates. 4. Academic Press; San Diego: 1998.

Picconi B, Centonze D, Håkansson K, Bernardi G, Greengard P, Fisone G, Cenci MA, Calabresi P. Loss of bidirectional striatal synaptic plasticity in L-DOPA–induced dyskinesia. Nat Neurosci. 2003; 6:501–506. [PubMed: 12665799]

Raymond JR, Mukhin YV, Gettys TW, Garnovskaya MN. The recombinant 5-HT1A receptor: G protein coupling and signalling pathways. Br J Pharmacol. 1999; 127:1751–1764. [PubMed: 10482904]

Santini E, Valjent E, Usiello A, Carta M, Borgkvist A, Girault JA, Herve D, Greengard P, Fisone G. Critical Involvement of cAMP/DARPP-32 and Extracellular Signal-Regulated Protein Kinase Signaling in L-DOPA-Induced Dyskinesia. J Neurosci. 2007; 27:6995–7005. [PubMed: 17596448]

Santini E, Valjent E, Fisone G. Parkinson's disease: levodopa-induced dyskinesia and signal transduction. FEBS J. 2008; 275:1392–9. [PubMed: 18279379]

Santini E, Alcacer C, Cacciatore S, Heiman M, Hervé D, Greengard P, Girault JA, Valjent E, Fisone G. l-DOPA activates ERK signaling and phosphorylates histone H3 in the striatonigral medium spiny neurons of hemiparkinsonian mice. J Neurochem. 2009; 108:621–633. [PubMed: 19187092]

Santini E, Sgambato-Faure V, Li Q, Savasta M, Dovero S, Fisone G, Bezard E. Distinct changes in cAMP and extracellular signal-regulated protein kinase signalling in L-DOPA-induced dyskinesia. PLoS One. 2010; 5:e12322. [PubMed: 20808799]

Shiflett MW, Balleine BW. Contributions of ERK signaling in the striatum to instrumental learning and performance. Behav Brain Res. 2011; 218:240–247. [PubMed: 21147168]

Thomas GM, Huganir RL. MAPK cascade signalling and synaptic plasticity. Nat Rev Neurosci. 2004; 5:173–183. [PubMed: 14976517]

Voon V, Fernagut PO, Wickens J, Baunez C, Rodriguez M, Pavon N, Juncos JL, Obeso JA, Bezard E. Chronic dopaminergic stimulation in Parkinson's disease: from dyskinesias to impulse control disorders. Lancet Neurol. 2009; 8:1140–1149. [PubMed: 19909912]

Westin JE, Vercammen L, Strome EM, Konradi C, Cenci MA. Spatiotemporal Pattern of Striatal ERK1/2 Phosphorylation in a Rat Model of L-DOPA–Induced Dyskinesia and the Role of Dopamine D1 Receptors. Biol Psychiatry. 2007; 62:800–810. [PubMed: 17662258]

Highlights

- 5-HT_{1A}R activation potentiated pERK activity in the striatum and M1
- Serotonin and DA drugs had large effects on striatal pERK only if DA was depleted
- Induction of striatal pERK by a D_1R agonist was highest on the first dose
- Increase in striatal pERK by L-DOPA was constant across test days

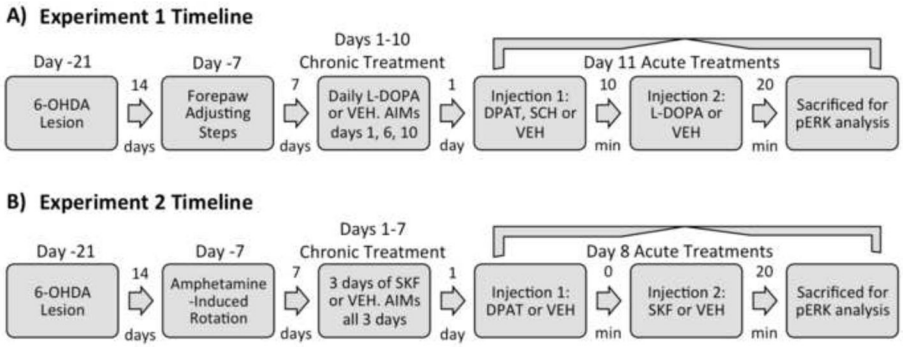

Figure 1.

A) Timeline for experiment 1. Six treatment groups were created based on forepaw adjusting steps scores. Half the rats were given daily L-DOPA (6 mg/kg) for 10 days while half were given VEH. On day 11, DPAT (1 mg/kg), SCH (1 mg/kg) or VEH were given prior to L-DOPA or VEH and sacrificed 20 min later. **B)** Timeline for experiment 2. Six treatment groups were created based on amphetamine-induced rotation scores. Between days 1 and 7, half the rats were given three doses of the D_1 agonist SKF (0.8 mg/kg) while the other half were given VEH. On day 8, rats received DPAT (1 mg/kg) or VEH prior to SKF or VEH and were sacrificed 20 min later.

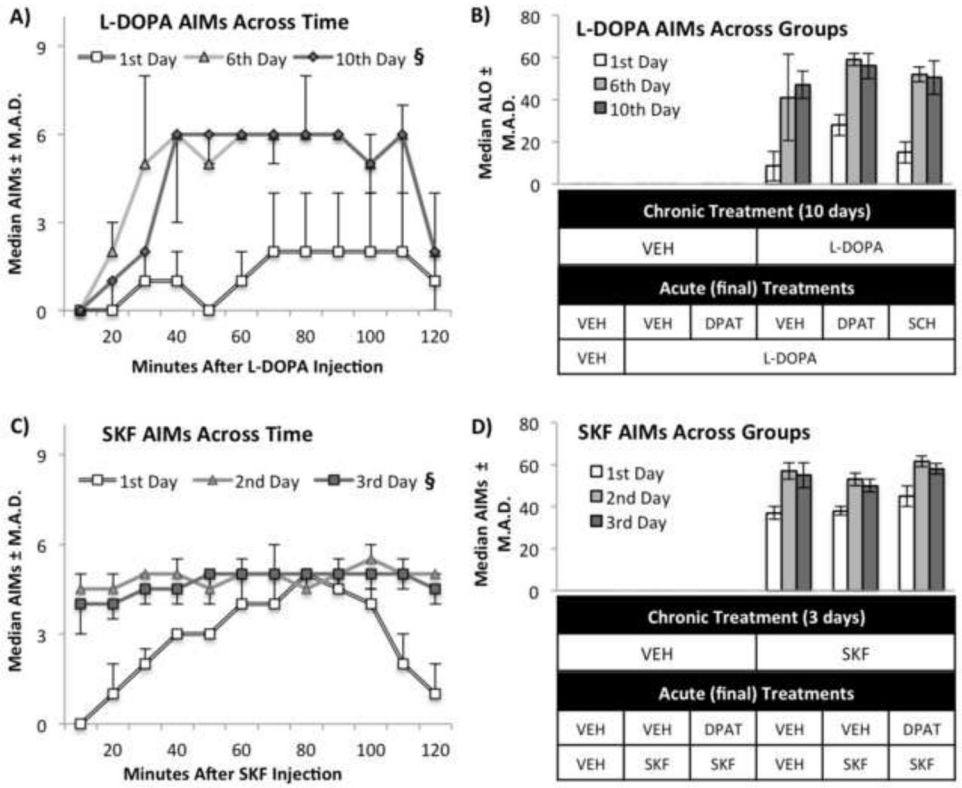

Figure 2.

Results from behavioral testing during the chronic treatment phase for experiments 1 and 2. **A)** Timecourse of dyskinesia development among all rats injected with chronic L-DOPA (6 mg/kg) in experiment 1. **B)** Median AIMs on each test day for each of the six treatment groups in experiment 1. **C)** Dyskinesia development timecourse for all rats that received chronic SKF (0.8 mg/kg) treatment in experiment 2. **B)** Median AIMs on each test day for each of the six treatment groups in experiment 2. **§** $p < .05$ vs. 1st day.

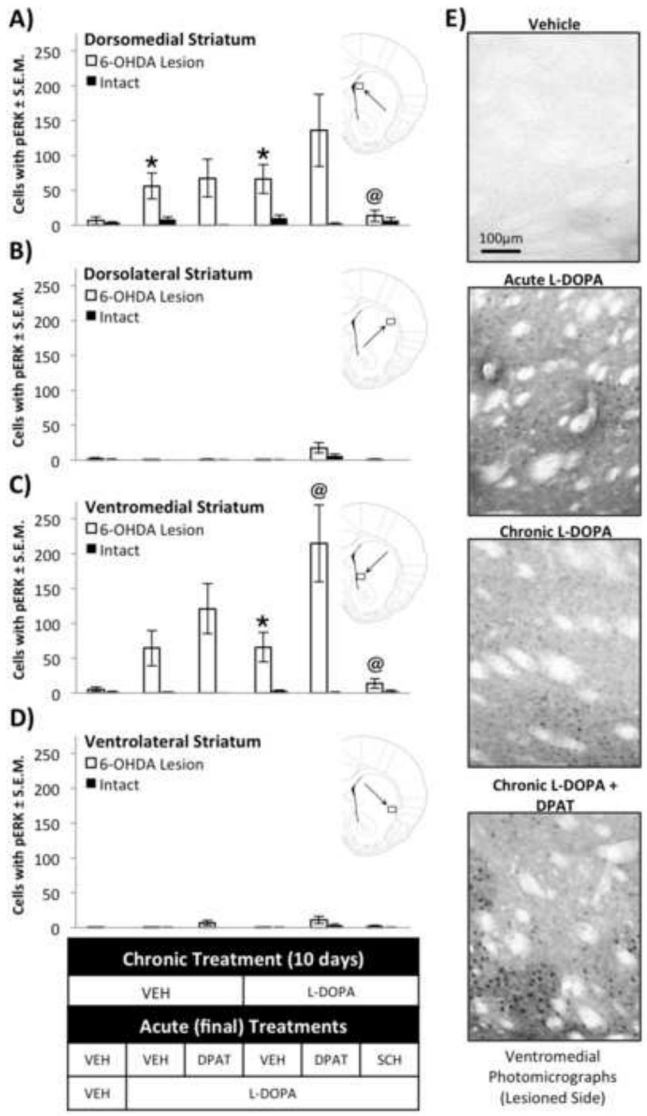

Figure 3.

Immunohistological analysis of the number of striatal cells expressing pERK for rats in experiment 1. Rats were treated daily with L-DOPA (6 mg/kg) or VEH for 10 days. The next day, each rat was given two of the following treatments and sacrificed 20 min later: L-DOPA, the 5-HT_{1A}R agonist DPAT (1 mg/kg), the D_1R antagonist SCH (1 mg/kg) or VEH. Photomicrographs were analyzed from four regions of each striatal hemisphere: **A)** dorsomedial **B)** dorsolateral **C)** ventromedial and **D)** ventrolateral. The location of each photomicrograph is indicated by a black box in the schematic insets to the bar graphs (created using a section from the brain atlas of Paxinos and Watson [1998] at 1 mm anterior to bregma). **E)** Samples of representative histology from the lesioned-side ventromedial striatum. * p<.05 vs. chronic VEH and VEH+VEH; @ p<.05 vs. chronic L-DOPA and VEH +L-DOPA.

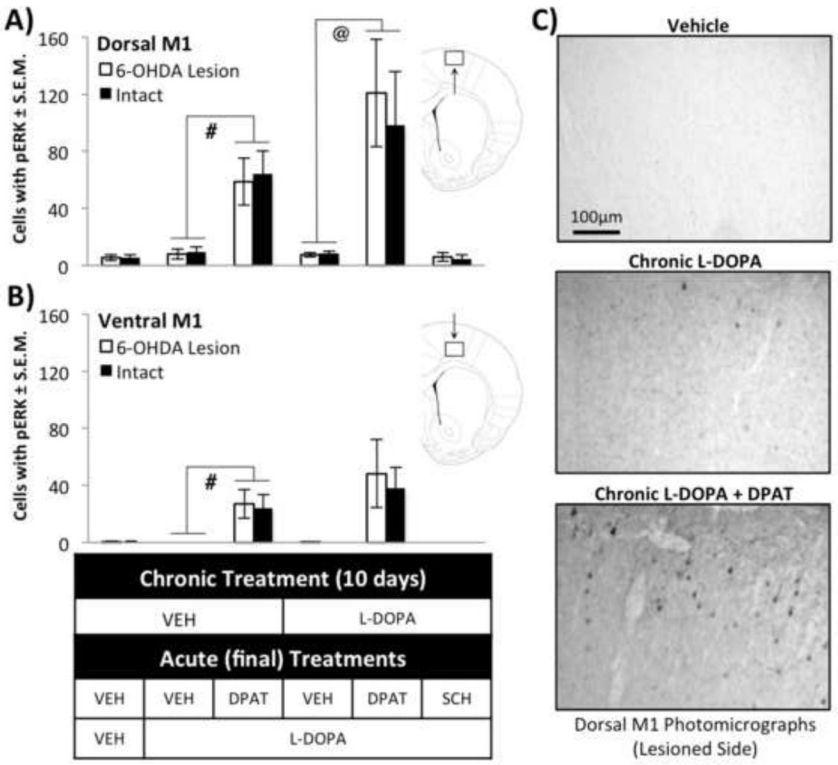

Figure 4.

Immunohistological analysis of the number of M1 cells expressing pERK for rats in experiment 1. Rats were treated daily with L-DOPA (6 mg/kg) or VEH for 10 days. The next day, each rat was given two of the following treatments and sacrificed 20 min later: L-DOPA, the 5-HT_{1A}R agonist DPAT (1 mg/kg) the D_1R antagonist SCH (1 mg/kg) or VEH. Photomicrographs were analyzed from two regions of each M1 hemisphere: **A)** dorsal and **B)** ventral. The location of each photomicrograph is indicated by a black box in the schematic insets to the bar graphs (created using a section from the brain atlas of Paxinos and Watson [1998] at 1 mm anterior to bregma). **C)** Samples of representative histology from the lesioned-side dorsal M1. Only ¼ of each photomicrograph is shown in order to make cells visible. # $p<.05$ vs. chronic VEH and VEH+L-DOPA; @ $p<.05$ vs. chronic L-DOPA and VEH+L-DOPA.

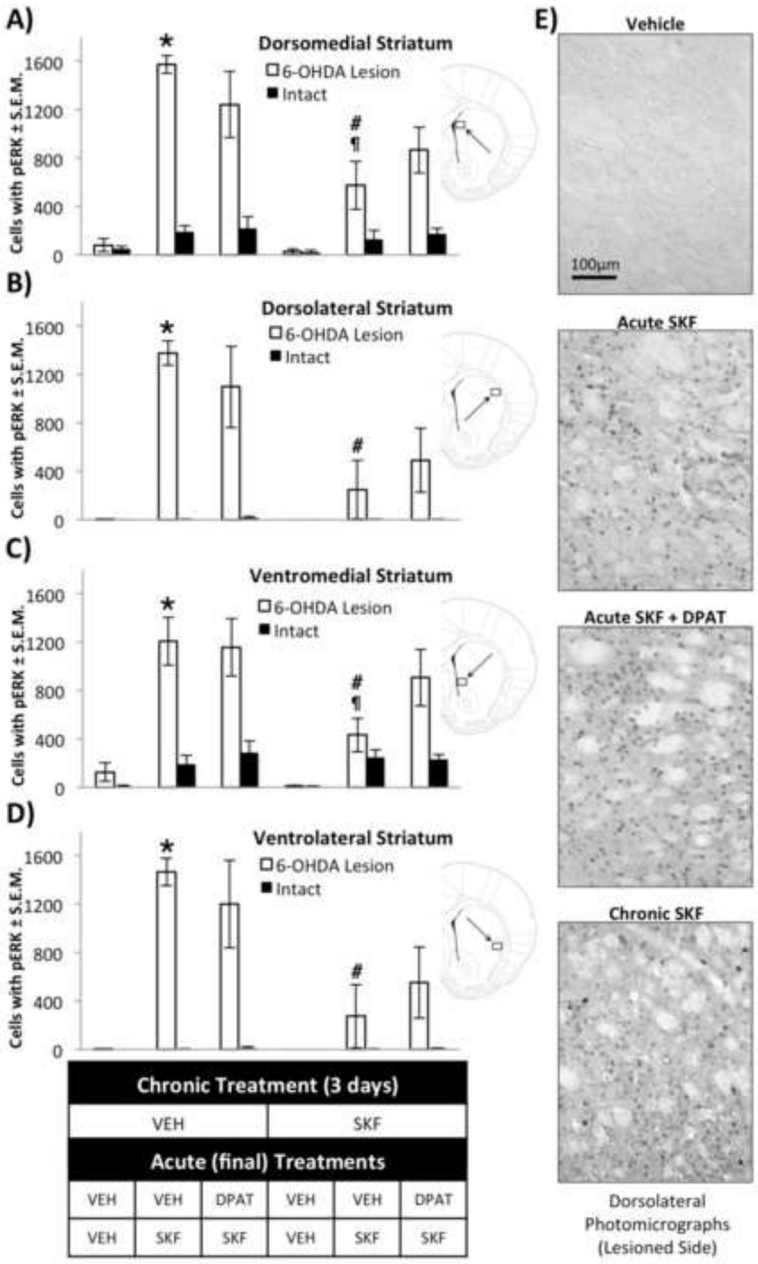

Figure 5.

Number of striatal cells positively-labeled for pERK among rats in experiment 2. Rats were treated with SKF (0.8 mg/kg) or VEH for 3 days. The next day, each rat was given two of the following treatments and sacrificed 20 min later: SKF, the 5-HT_{1A}R agonist DPAT (1 mg/kg) or VEH. Four photomicrographs were quantified from each striatal hemisphere, covering the **A)** dorsomedial **B)** dorsolateral **C)** ventromedial and **D)** ventrolateral regions. The location of each photomicrograph is indicated by a black box in the schematic insets to the bar graphs (created using a section from the brain atlas of Paxinos and Watson [1998] at 1 mm anterior to bregma). **E)** Samples of representative histology from the lesioned-side dorsolateral striatum. * p<.05 vs. chronic VEH and VEH+VEH; # p<.05 vs. chronic VEH and VEH+SKF; ¶ p<.05 vs. chronic SKF and VEH+VEH.

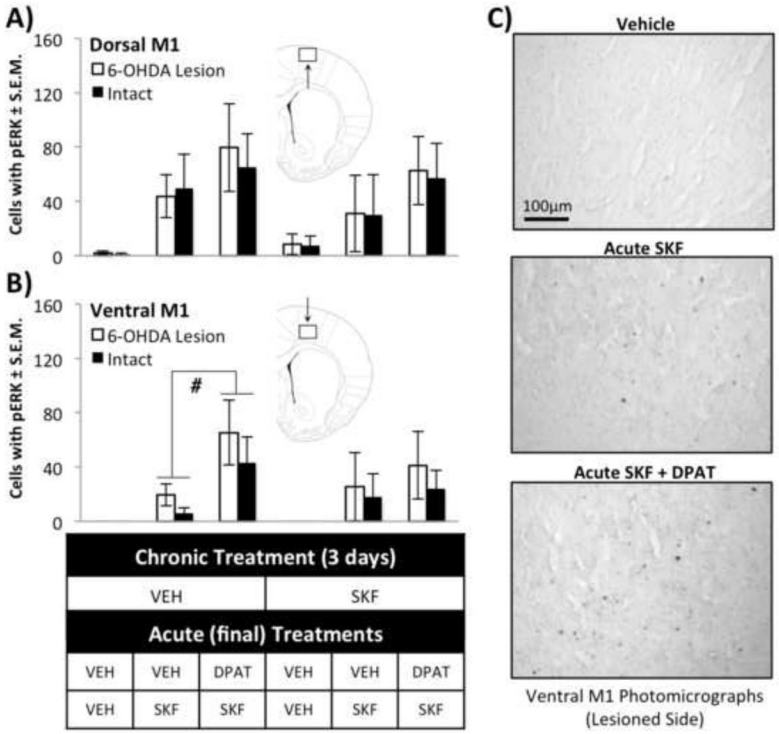

Figure 6.

Number of M1 cells positively-labeled for pERK among rats in experiment 2. Rats were treated with SKF (0.8 mg/kg) or VEH for 3 days. The next day, each rat was given two of the following treatments and sacrificed 20 min later: SKF, the 5-HT_{1A}R agonist DPAT (1 mg/kg) or VEH. Photomicrographs were analyzed from two regions of each M1 hemisphere: **A)** dorsal and **B)** ventral. The location of each photomicrograph is indicated by a black box in the schematic insets to the bar graphs (created using a section from the brain atlas of Paxinos and Watson [1998] at 1 mm anterior to bregma). **C)** Samples of representative histology from the lesioned-side ventral M1. Only ¼ of each photomicrograph is shown in order to make cells visible. # $p<.05$ vs. chronic VEH and VEH+SKF.