A role for mitogen- and stress-activated kinase 1 in L-DOPA-induced dyskinesia and ΔFosB expression

Supplemental Information

Supplemental Methods and Materials

Chromatin preparation and immunoprecipitation

Fixed striatal punches were homogenized in a nuclear extraction buffer (10 mM Tris (pH 8.0), 100 mM NaCl, 2 mM $MgCl_2$, 0.3M sucrose, 0.25% IGEPAL CA-630) containing protease inhibitors (1 mM PMSF, 0.1 mM aprotinin, 0.1 mM leupeptin) and phosphatase inhibitors (okadaic acid and NaF), by douncing 15 times using a 2 ml loose grind pestle, followed by 30 min incubation on ice. The homogenate was further dounced 50 times, followed by 10 min centrifugation at 2400 g to pellet the nuclei. The extracted nuclei were then lysed in a lysis buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1% (wt/vol) SDS and protease/phosphatase inhibitors, diluted in chromatin immunoprecipitation (ChIP) dilution buffer (10 mM Tris-HCl (pH 7.5), 140 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% (vol/vol) Triton-X-100, 0.1% (wt/vol) SDS, 0.1% (wt/vol) Na-deoxycholate) and sonicated to an average size of 300-500 bp (10 cycles 30 sec ON, 30 sec OFF, highest setting) using a Bioruptor standard device (Diagenode; Belgium).

ChIP was performed as described (1), with some modifications. Three µg anti-H3 (“GERA”, antigen sequence: CGIQLARRIRGERA), 10 µg anti-rabbit IgG (DAKO; Denmark) or 2 µg anti-phospho-histone H3 (Ser10) (17-685, Millipore; Billerica, MA) was incubated at 4°C with

Dynabeads protein A (Invitrogen; Waltham, MA) in ChIP dilution buffer in a total volume of 100 µl. The bead-antibody complexes were washed and incubated at 4°C for 2 hrs with 20 µl chromatin in a total volume of 250 µl. The beads were then washed in 3x ChIP dilution buffer and 1x TE (10 mM Tris-HCl, pH 8.0, 10 mM EDTA) After washes, DNA was eluted from beads and de-crosslinked in 20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 50 mM NaCl. 1% (wt/vol) SDS and 50 µg/ml protease K, at 68°C overnight. For input 20 µl chromatin was de-crosslinked in 20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 50 mM NaCl 68°C overnight. ChIP and input DNA was then purified and eluted using Minelute PCR purification kit (Qiagen; Netherlands). Epitope enrichment on chromatin was assessed by qPCR (3 technical replicates; 7500 Fast, Applied Biosystems) using a dilution series of input chromatin as reference. The following primers were used for the *fosB* promoter region: forward 5´-agttccaatcctgtcgaagc-3´, reverse 5´-ctttctgtgggcttctggat-3´.

Western blotting

Aliquots (5 µl) were used for protein quantification with the bicinchoninic acid assay kit (Pierce; Rockford, IL, USA). Western blotting was carried out as previously described (2, 3), using primary antibodies against phospho-Ser10 of histone H3 (1:1000; Millipore, Billerica, MA), phospho-Thr34 of dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) (1:1000, PhosphoSolutions; Aurora, CO), and phospho-Thr202/Tyr204 of ERK (1:1000, Cell Signaling; Danvers, MA). Phosphorylated values were normalized to corresponding values of total protein detected by antibodies against total histone H3 (1:80000, Abcam; Cambridge, England), total DARPP-32 (1:20000, gift from Paul Greengard), and total ERK (1:8000, Cell Signaling; Danvers, MA). Levels of actin and GAPDH, detected using specific antibodies

(1:10000, Abcam; Cambridge, England), were determined as loading controls. An antibody against TH (1:3000, Millipore; Billerica, MA) was used to assess the extent of the lesion. The protein signal was visualized by enhanced chemiluminescence (Pierce; Rockford, IL) and quantified using Quantity One software (Bio-Rad; Hercules, CA). All antibodies produced the expected number of bands at the expected molecular weight. We did not observe any cross-reactivity within a range of at least 10 kDa above or below the expected molecular weight.

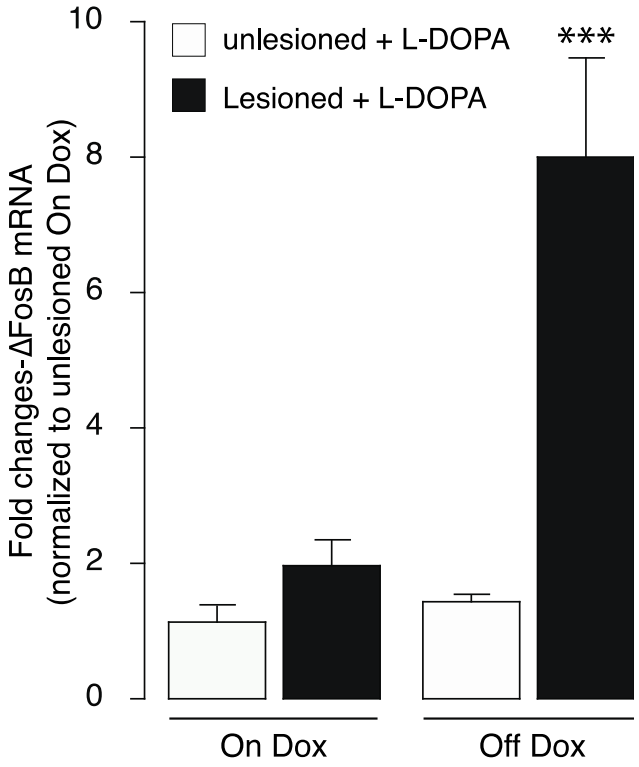
6-OHDA lesion

Mice were anesthetized with a mixture of fentanyl citrate (0.315 mg/ml), fluanisone (10 mg/ml) (VetaPharma, Leeds, UK), midazolam (5 mg/ml) (Hameln Pharmaceuticals, Gloucester, UK), and water (1:1:2 in a volume of 10 ml/kg), and mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) equipped with a mouse adaptor. 6-OHDA was dissolved at a concentration of 3 μg of free base 6-OHDA/μl. Each mouse received two unilateral injections of 6-OHDA (2 μl/injection) into the right striatum as previously described (3), according to the following coordinates (mm) (4): antero-posterior (AP), +1; medio-lateral (ML), -2.1; dorso-ventral (DV), -3.2; and AP +0.3; ML, -2.3; DV, -3.2. Animals were allowed to recover for 3 weeks before behavioral evaluation and drug treatment were carried out. All animals except one, which died immediately post-surgery, survived through the recovery period and the experimental procedures. At the end of the experiments, the extent of the lesion was evaluated by measuring the levels of tyrosine hydroxylase (TH) in the striata ipsilateral and contralateral to the lesion by Western blotting. Only mice with a TH depletion of 80% or more were included in the study. Above this point we have not observed any correlation between the severity of dyskinesia and the extent of the lesion (3). Furthermore, we did not observe any statistically significant

difference in TH depletion between experimental groups (two tailed unpaired t-test: $t_{(15)} = 1.22$, $p > 0.05$ for wild-type vs. MSK1 KO mice; $t_{(15)} = 1.15$, $p > 0.05$ for on- and off-Dox ΔFosB and $t_{(16)} = 0.21$, $p > 0.05$ for on- and off-Dox ΔcJun transgenic mice).

Supplemental References

1. Dahl JA, Collas P (2008): A rapid micro chromatin immunoprecipitation assay (microChIP). *Nat Protoc*. 3:1032-1045.
2. Santini E, Alcacer C, Cacciatore S, Heiman M, Herve D, Greengard P, et al. (2009): L-DOPA activates ERK signaling and phosphorylates histone H3 in the striatonigral medium spiny neurons of hemiparkinsonian mice. *J Neurochem*. 108:621-633.
3. Santini E, Valjent E, Usiello A, Carta M, Borgkvist A, Girault JA, et al. (2007): Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *J Neurosci*. 27:6995-7005.
4. Franklin KBJ, Paxinos G (1997): *The Mouse Brain in Stereotaxic Coordinates*. San Diego: Academic Press.



Suppl. Fig. 1. The effect of L-DOPA on ΔFosB mRNA is potentiated by inducible overexpression of ΔFosB in striatonigral MSNs. Mice that inducibly overexpress ΔFosB (Off Dox) and control littermates (On Dox) were lesioned unilaterally with 6-hydroxydopamine and treated for 14 days with 20 mg/kg of L-DOPA (in combination with 12.5 mg/kg of benserazide). The graphs show the levels of ΔFosB mRNA in the striata ipsilateral (lesioned) and contralateral (unlesioned) of control (On Dox) and ΔFosB overexpressing (Off Dox) mice, determined 24 hr after the last drug administration. Note the increase in ΔFosB mRNA in the lesioned Off Dox mice treated with L-DOPA, in comparison to On Dox mice. Data are shown as means ± SEM. Two-way ANOVA indicated significant interaction between treatment (lesioned+L-DOPA) and genotype ($F_{(1, 33)} = 14.30$, $p < 0.001$); *** $p < 0.001$ vs. all other experimental groups (post-hoc Bonferroni-Dunn test).