J Neural Transm (2009) 116:1571–1578
DOI 10.1007/s00702-009-0304-z

BASIC NEUROSCIENCES, GENETICS AND IMMUNOLOGY - ORIGINAL ARTICLE

Accumulation of phosphorylated tyrosine hydroxylase into insoluble protein aggregates by inhibition of an ubiquitin–proteasome system in PC12D cells

Ichiro Kawahata · Hirofumi Tokuoka · Hasan Parvez · Hiroshi Ichinose

Received: 29 June 2009 / Accepted: 22 August 2009 / Published online: 12 September 2009

Abstract Tyrosine hydroxylase (TH) is a rate-limiting enzyme for the biosynthesis of catecholamines including dopamine. The relationship between proteasomal dysfunction and the etiology of Parkinson's disease has been suggested, but it is unknown if TH protein is affected by proteasomal dysfunctions. Here, we examined the effect of inhibition of ubiquitin–proteasomal pathway on biochemical characteristics of TH protein in the neuronal cells. Inhibition of 20S or 26S proteasome by proteasome inhibitor I, or MG-132 in NGF-differentiated PC12D cells induced dot-like immunoreactivities with the anti-^{40}Ser-phosphorylated TH (p40-TH) antibody. These dots were tightly co-localized with ubiquitin and positive to Thioflavine-S staining. These dot-like immunoreactivities were not obvious when immunostaining was performed against total-TH or choline acetyltransferase. Western blotting analysis showed time-dependent increase of p40-TH in the Triton-insoluble fractions. We also examined the effect of okadaic acid, an inhibitor of protein phosphatase 2A, which is a phosphatase acting on p40-TH. Okadaic acid increased the amount of insoluble p40-TH. These data suggest that p40-TH is prone to be insolubilized and aggregated by dysfunction of an ubiquitin–proteasome system in PC12D cells.

Keywords Tyrosine hydroxylase · Ubiquitin–proteasome system · MG-132 · Okadaic acid · Parkinson's disease

Abbreviations

ChAT	Choline acetyltransferase
DA	Dopamine
NGF	Nerve growth factor
OA	Okadaic acid
PD	Parkinson's disease
PKA	Cyclic AMP-dependent protein kinase (protein kinase A)
PP2A	Protein phosphatase 2A
TH	Tyrosine hydroxylase
p40-TH	Tyrosine hydroxylase phosphorylated at ^{40}Ser

Introduction

Tyrosine hydroxylase (tyrosine 3-monooxygenase, TH; EC 1.14.16.2), is a rate-limiting enzyme for biosynthesis of catecholamines (Nagatsu et al. 1964). The TH activity is strictly regulated by many factors to control the amount of dopamine. Activation by phosphorylation of the TH protein and inactivation by the binding of its end product dopamine to its active site are the two major mechanisms for regulation of the TH activity (Fujisawa and Okuno 2005). On depolarization, cyclic AMP-dependent protein kinase (PKA) and calcium-calmodulin dependent protein kinase II (CaMKII) are activated, then PKA phosphorylates TH at ^{40}Ser and

Electronic supplementary material The online version of this article (doi:10.1007/s00702-009-0304-z) contains supplementary material, which is available to authorized users.

I. Kawahata · H. Tokuoka · H. Ichinose (✉)
Graduate School of Bioscience and Biotechnology,
Tokyo Institute of Technology, 4259-B7, Nagatsuta-cho,
Midori-ku, Yokohama 226-8501, Japan
e-mail: hichinos@bio.titech.ac.jp

H. Parvez
CNRS, Institut Alfred Fessard de Neurosciences,
French National Research Centre, Gif sur Yvette, France

CAMKII phosphorylates TH at ^{19}Ser (Haycock 1990; Haycock and Haycock 1991). Phosphorylation at ^{40}Ser leads TH to liberate dopamine from its active site and change the conformation to the high-specific activity form (Daubner et al. 1992). Cytosolic free dopamine can bind to the active site of TH, and make TH inactive to suppress overproduction of dopamine (Okuno and Fujisawa 1985). It was reported that the phosphorylated form of TH was highly labile, whereas the dopamine-bound form was stable (Royo et al. 2005). Phosphorylated TH at ^{40}Ser (p40-TH) can be dephosphorylated by protein phosphatases such as protein phosphatase 2A (PP2A) (Haavik et al. 1989; Leal et al. 2002). Previously, we demonstrated that the bacterially expressed and purified TH protein, which is free from dopamine and reported to be in a similar conformation with p40-TH (Le Bourdellès et al. 1991), forms insoluble aggregates in the presence of BH4 (Urano et al. 2006).

Recent studies suggest proteasomal dysfunction and accumulation of unnecessary proteins may be one of the causes of neurodegenerative disorders such as Parkinson's disease (PD) (McNaught et al. 2001; McNaught and Olanow 2003). However, it is yet to be known if TH protein, a key enzyme of DA synthesis, is affected by proteasomal dysfunction.

In the present study, we investigated the effect of proteasomal inhibition on the TH protein using PC12D cells. We especially focused on p40-TH, because the phosphorylation on this site is more directly associated with the catalytic activity than other phosphorylation sites, Ser8, Ser19 and Ser31 (Dunkley et al. 2004). We found that proteasomal inhibition leads to accumulation of insoluble p40-TH and formation of p40-TH-containing intracellular aggregates which tightly co-localized with ubiquitin. Furthermore, inhibition of PP2A increased the amount of the p40-TH in insoluble fractions.

Materials and methods

Cell culture

PC12D cells (Katoh-Semba et al. 1987) were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma, MO, USA) containing 5% heat-inactivated fetal bovine serum (Biowest, Nuaillé, France) and 10% horse serum (JRH, KS, USA). Cells were cultured on plastic culture dishes (Falcon, NJ, USA) and incubated at 37°C with 5% CO_2. For the differentiation of PC12D cells, cells were cultured with 50 ng/ml nerve growth factor (NGF; Wako, Osaka, Japan) in the culture medium for 7 days. A 26S proteasome inhibitor, MG-132 (Calbiochem, Darmstadt, Germany), a 20S proteasome inhibitor, PSI (Calbiochem, San Diego, USA) and PP2A inhibitor, Okadaic acid (Wako, Osaka, Japan), were prepared as 250 μM or 10 mM stock solutions in dimethyl sulfoxide (DMSO). As a control, we added the same volume of DMSO.

Immunocytochemistry of cell culture

NGF-differentiated PC12D cells were incubated in culture media containing 250 nM MG-132, 10 μM PSI or DMSO for 12, 24, and 48 h. Cells were cultured on Poly-D-lysine coated culture slides (BD Biosciences, CA, USA). For double-labeled immunofluorescence analysis, PC12D cells were washed with PBS and fixed in 4% paraformaldehyde for 15 min at room temperature (RT). After washing with PBS, cells were permeabilized with 0.1% Triton X-100 in PBS for 25 min and washed with PBS. After pre-blocking in 1% BSA/PBS for 90 min, cells were incubated overnight at 4°C with the following primary antibodies; anti-TH polyclonal antibody (1:500 dilution, Millipore AB152, MA, USA), anti-^{40}Ser phosphorylated TH antibody (1:500 dilution, Cell Signaling Technology #2791S, MA, USA), anti-ubiquitin monoclonal antibody (1:100 dilution, Santa Cruz Biotechnology sc-8017, CA, USA), or anti-choline acetyltransferase (ChAT) polyclonal antibody (1:1,000 dilution, Millipore AB143). After washing with PBS, cells were incubated with Alexa Fluor 488- or 546-conjugated secondary antibodies (1:2,000 dilution, Invitrogen, CA, USA). Images were acquired by fluorescent microscopy or confocal laser microscopy (BioRad, Göttingen, Germany) using a 100× oil-immersion objective. All experiments were repeated at least three times.

Quantitative analysis of fluorescence intensity was performed using NIH ImageJ 1.39 software. First, the background signal intensities were measured from regions without any cells and subtracted from all the images. The remaining signals of cells were used to define total cell areas. We defined the inclusions as dot-like signals 3SD above the background levels and measured the areas.

Thioflavine-S staining

For Thioflavine-S staining, cells were fixed and subjected to indirect immunofluorescence staining as described above. Before mounting, cells were treated with 0.005% Thioflavine-S in 70% ethanol for 5 min. Thereafter, chambers were washed three times in 70% ethanol, once in water, and then mounted.

Western blot analyses

Naïve or NGF-differentiated PC12D cells were exposed to MG-132 (250 nM), OA (concentrations indicated in each experiment), or DMSO (control) for 2–48 h. Cells were collected using a cell scraper, washed with PBS, and

resuspended in lysis buffer consisting of 25 mM HEPES (pH 7.4), 5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, and 0.5% Triton X-100 with 1 μg/ml each of pepstatin and leupeptin, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 25 mM sodium fluoride. After incubation on ice for 20 min, the insoluble material was pelleted by centrifugation at 100,000×g for 20 min at 4°C, resuspended in SDS-sample buffer, and boiled for 10 min. Protein concentration of both detergent-soluble and -insoluble fractions was determined by the Bradford method (BioRad, CA, USA). Twenty micrograms of protein from both fractions was separated by SDS–PAGE (10%) and transferred to a PVDF membrane, blocked in 5% non-fat milk for 1 h, and incubated overnight at 4°C with the following primary antibodies; anti-TH polyclonal antibody (1:1,000 dilution, Millipore AB152), anti-^{40}Ser phosphorylated TH antibody (1:1,000 dilution, Cell Signaling Technology #2791S), anti-ubiquitin monoclonal antibody (1:200 dilution, Santa Cruz Biotechnology sc-8017), or anti-β-actin monoclonal antibody (1:10,000 dilution, Sigma A5441). Immunoreactive bands were visualized with horseradish peroxidase-conjugated secondary antibodies (1:30,000 dilution, GE Healthcare Life Sciences, Uppsala, Sweden) and the chemiluminescent HRP substrate (Millipore, Massachusetts, USA). Visualized bands were detected by LAS-3000 mini (Fujifilm, Tokyo, Japan).

Quantitative analysis by densitometry was performed using Multi Gauge Ver.2.11 software (Fujifilm). Intensity of the bands between MG-132 treated and vehicle treated PC12D cells were compared after subtracting background signals (arbitrary unit per square mm, a.u./mm^2). All experiments were repeated four times. Statistical significance was calculated according to Student's t test.

Results

Proteasomal inhibition leads to formation of p40-TH positive insoluble aggregates

We first investigated the effects of proteasomal inhibition on the TH protein by immunocytochemistry in NGF-differentiated PC12D cells. Cells were cultured for 7 days with NGF, then exposed to a 26S proteasome inhibitor, MG-132, at 250 nM for up to 48 h. Under the condition, cells showed no drastic morphological alterations (data not shown). We stained the cells with an antibody against p40-TH to examine a possible alteration in the cellular localization of p40-TH. We found that the p40-TH antibody showed dot-like immunoreactivities from 12 h after MG-132 treatment, suggesting the generation of protein aggregates containing p40-TH (Fig. 1a, p40-TH). In double-staining with the p40-TH antibody and the antibody against ubiquitin, most of p40-TH positive signals were tightly co-localized with the ubiquitin signals (Fig. 1a, p40-TH/Ubi). The p40-TH immunopositive spots increased their size in a time-dependent manner (Fig. 1b, top). Images with lower magnification were shown in supplementary figure (Suppl Fig. 1). We also examined the effect of proteasomal inhibition with another reagent PSI, an inhibitor of 20S proteasome, and the similar trends were observed under the same condition as MG-132 experiments (Suppl Fig. 2).

When we examined the immunoreactivity with the polyclonal antibody against total-TH protein, the signal accumulated more slowly than that of p40-TH, and finally formed larger signals than the p40-TH immunoreactivities (Fig. 1a, TH/Ubi). Total-TH positive areas were larger than those of ubiquitin, whereas most of ubiquitin positive ones were TH immunoreactive (Fig. 1a, TH/Ubi). Areas of p40-TH immunoreactivities were smaller in size, but their signals were stronger and denser than those of TH by fluorescence quantification (data not shown).

In contrast, immunoreactivities against ChAT showed no dot-like signals as seen in the p40-TH (Fig. 1a, ChAT/Ubi). Furthermore, ChAT did not co-localize with any ubiquitin marker (Fig. 1a, ChAT/Ubi). We also performed the experiments in the NGF-untreated cells and the similar results were obtained (data not shown).

To further characterize the p40-TH immunopositve signals, we counter-stained the MG-132 treated cells with Thioflavine-S, an insoluble aggregate marker. Most p40-TH immunoreactivities were co-localized with Thioflavine-S (Fig. 2), suggesting the p40-TH positive dot-like signals were protein aggregates.

Taken together, these data suggest that p40-TH is more preferentially incorporated to Thioflavine-S- and ubiquitin-positive aggregates by proteasome inhibition, than non-phosphorylated-TH and ChAT.

Proteasomal inhibition leads to time-dependent increase in the amount of insolubilized p40-TH

We then examined if the p40-TH-containing aggregates, which was detected by immunocytochemistry, is detergent-soluble or not by Western blotting. After treatment with MG-132, cells were homogenized in presence of 0.5% Triton X-100, separated to soluble and insoluble fractions by ultra-centrifugation at 100,000 × g, and subjected to Western blot analyses. The intensity of p40-TH immunoreactive band in the Triton-X soluble component was slightly increased in MG-132 treated cells. In contrast, the p40-TH positive signal in the insoluble fraction was detected 12 h after MG-132 treatment, and increased dramatically in a time-dependent manner, though almost no signal was detected in the control insoluble fractions (Fig. 3a, p40-TH). Inhibition of proteasomal activity by MG-132 was confirmed by the accumulation of ubiquitin-positive signals in

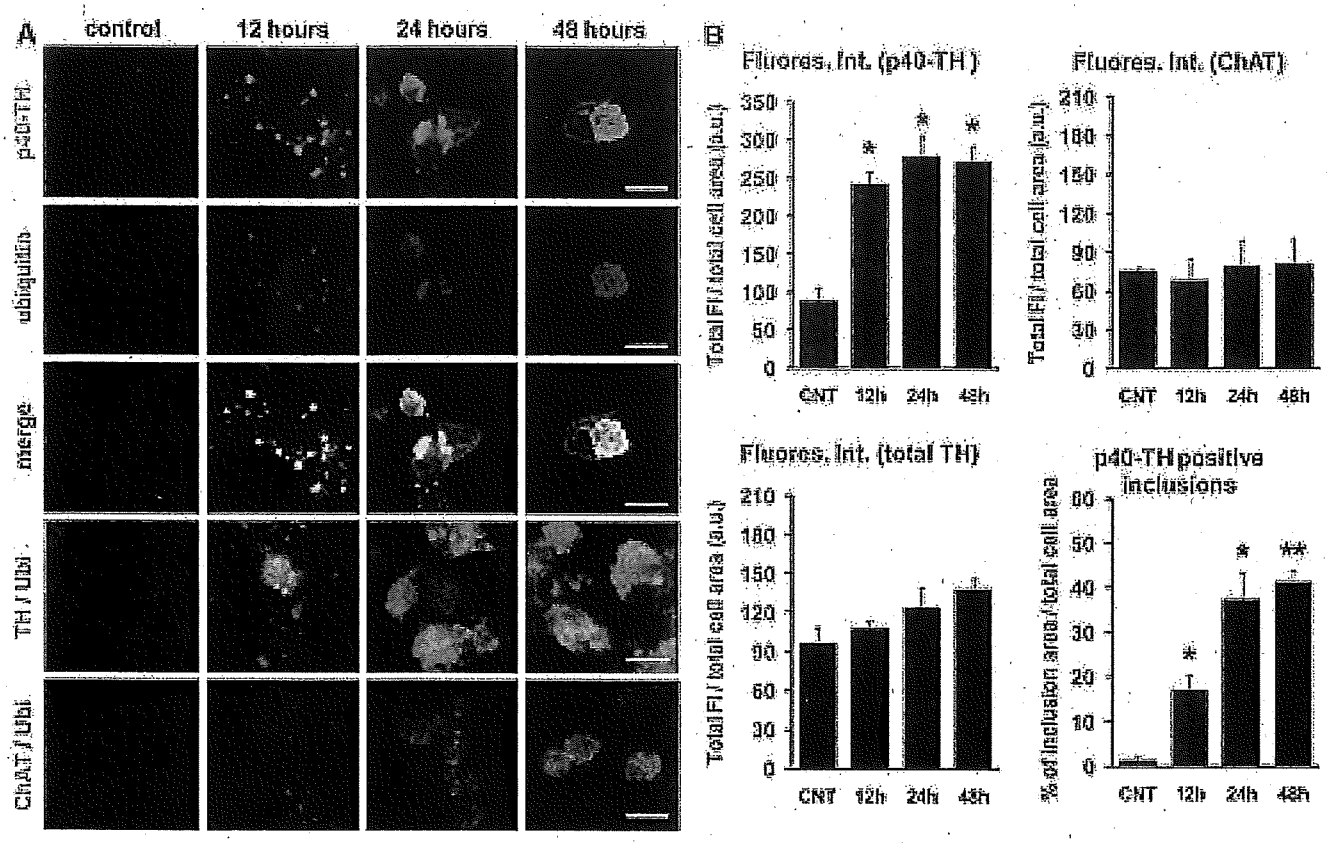

Fig. 1 26S proteasomal inhibition induces formation of p40-TH positive cytoplasmic aggregates. **a** Representative immunofluorescence staining of MG-132-treated PC12D cells. MG-132 treatment induced aggregation of p40-TH and formation of its positive inclusions. Immunoreactive spots of p40-TH tightly co-localized with those of ubiquitin. Ubiquitin immunoreactive signals were smaller than TH positive ones and most of them were included in the TH immunoreactive area. The antibody against ChAT did not show the formation of aggregates and no specific co-localization with any ubiquitin marker. Scale bars, 10 μm. **b** Quantification of fluorescence intensities shown in **a**. Fluorescence intensities per cell area for p40-TH, total-TH, and ChAT, and the ratios of strong p40-TH positive areas (beyond 3 SD of control signal) per total cell area were shown. The areas of p40-TH positive inclusions were increased time-dependently, but ChAT positive ones were not. Measurement for the area was carried out with NIH ImageJ software. *CNT* control, *V* vehicle treatment (DMSO). Values represent mean ± SD; $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to control. All cells were differentiated with 50 ng/mL NGF for 7 days and exposed to 250 nM MG-132 for indicated hours

the insoluble fractions (Fig. 3a, ubiquitin). The alteration in the amount of total-TH protein was not drastic (Fig. 3a, TH). No significant change was observed in the β-actin levels (Fig. 3a, β-actin). Quantitative analysis revealed that insoluble component of p40-TH was increased to about 15-fold of the control 48 h after the addition of MG-132, but total-TH showed no significant difference (Fig. 3b). We also calculated the ratios for the Triton-insoluble to Triton-soluble components of p40-TH and total-TH in Fig. 3c. These data indicate that p40-TH is prone to be insolubilized compared with non-phosphorylated TH under the condition of proteasomal dysfunction.

PP2A inhibition also leads to accumulation of insoluble p40-TH

In order to clarify whether the occurrence of insoluble p40-TH aggregates was caused by the characteristics of p40-TH itself or by the effects of other proteins which were accumulated due to the inhibition of proteasome, we treated PC12D cells with okadaic acid (OA), an inhibitor of PP2A, which reportedly dephosphorylates p40-TH (Haavik et al. 1989; Leal et al. 2002). First, PC12D cells were exposed to different concentrations of OA for 24 h, and then homogenates were separated to Triton-insoluble and -soluble components by ultra-centrifugation and analyzed by Western blotting. By increasing the concentration of OA from 10 to 250 nM, the amount of p40-TH in Triton-insoluble components increased in a dose-dependent manner (Fig. 4a, c, p40-TH). Next, cells were treated with 250 nM OA for various hours. Insoluble p40-TH was detected 6 h after the addition of OA, and the amount of insoluble p40-TH increased time-dependently (Fig. 4b, d, p40-TH). The amount of total-TH protein showed no significant alteration in both the Triton-soluble and -insoluble fractions (Fig. 4a–d, TH). The proportion of insoluble component to soluble component was

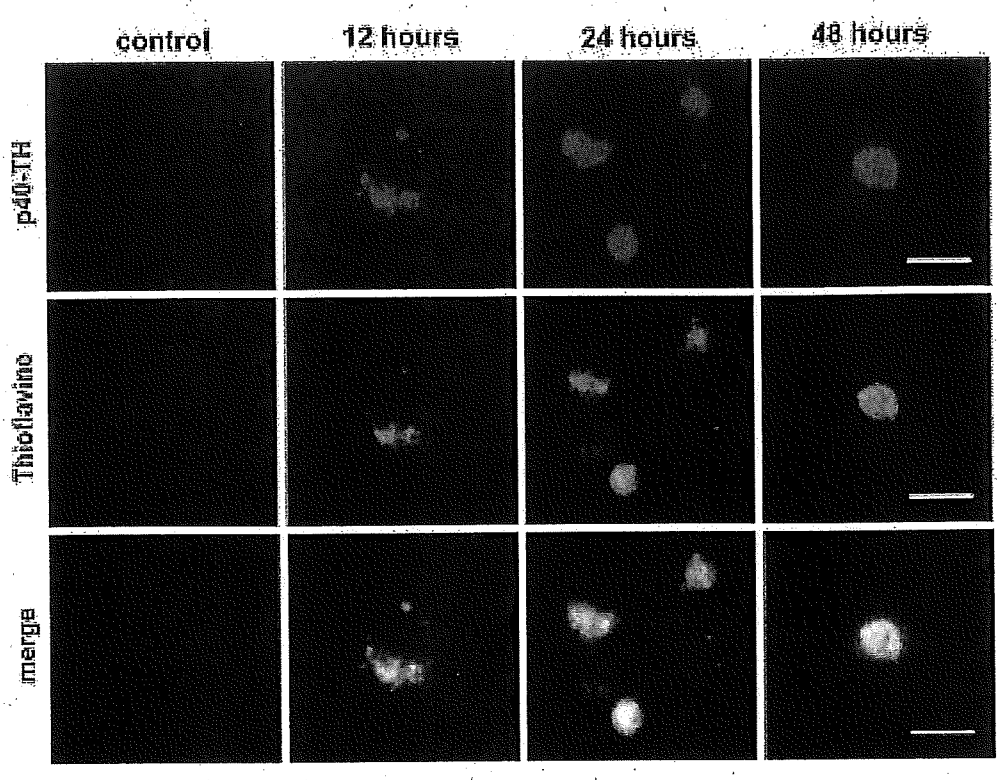

Fig. 2 Localization of p40-TH immunoreactivity and Thioflavine-S positive aggregates in NGF-differentiated PC12D cells. PC12D cells were grown with NGF to differentiate for 1 week then exposed to 250 nM MG-132 for indicated hours. Thioflavine-S positive aggregates appeared in 12 h, and they are smaller than p40-TH immunoreactive spots and partially co-localized. Tight co-localization of p40-TH immunoreactivity and Thioflavine-S positive aggregates was observed from 24 h. *Scale bars* 10 μm

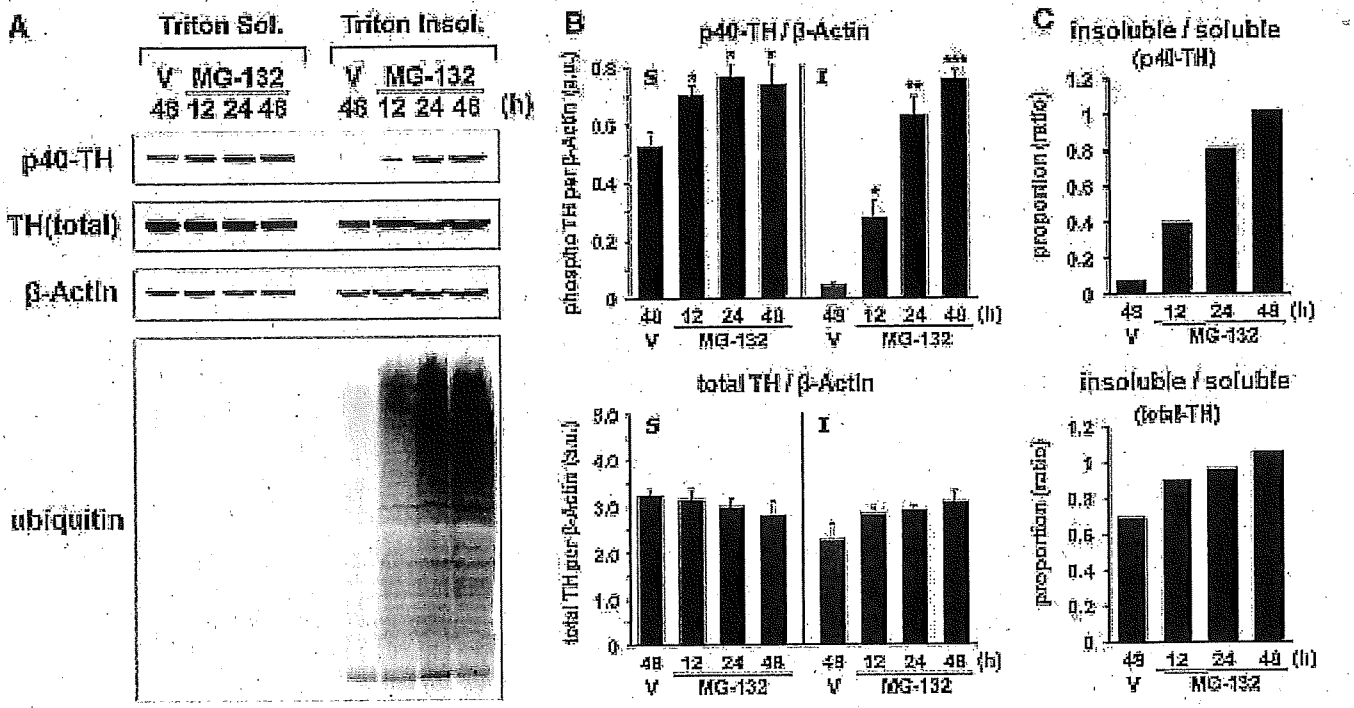

Fig. 3 Western blot analysis of the effect of 26S Proteasomal inhibition on p40-TH solubilization. **a** NGF-differentiated PC12D cells were treated with MG-132 for indicated hours, fractionated to Triton X-100-soluble and -insoluble fractions, and subjected to Western blotting. **b** Summary of quantification for the immunoreactivities on panel **a**. Without MG-132, most p40TH was in soluble fractions, while treatment with MG-132 lead to accumulation of p40-TH in insoluble fractions in a time-dependent manner. No significant alteration was observed with total-TH and β-actin though modest increase of insoluble component was observed in total-TH. Values represent mean ± SD; $n = 3$ in all experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ compared to control "V". *V* vehicle treatment, *S* Triton X-100-soluble fraction, and *I* triton-insoluble fraction, respectively. **c** The proportion for the triton-soluble and -insoluble component in p40-TH and total-TH under the basal conditions and in the presence of the MG-132, a 26S proteasomal inhibitor

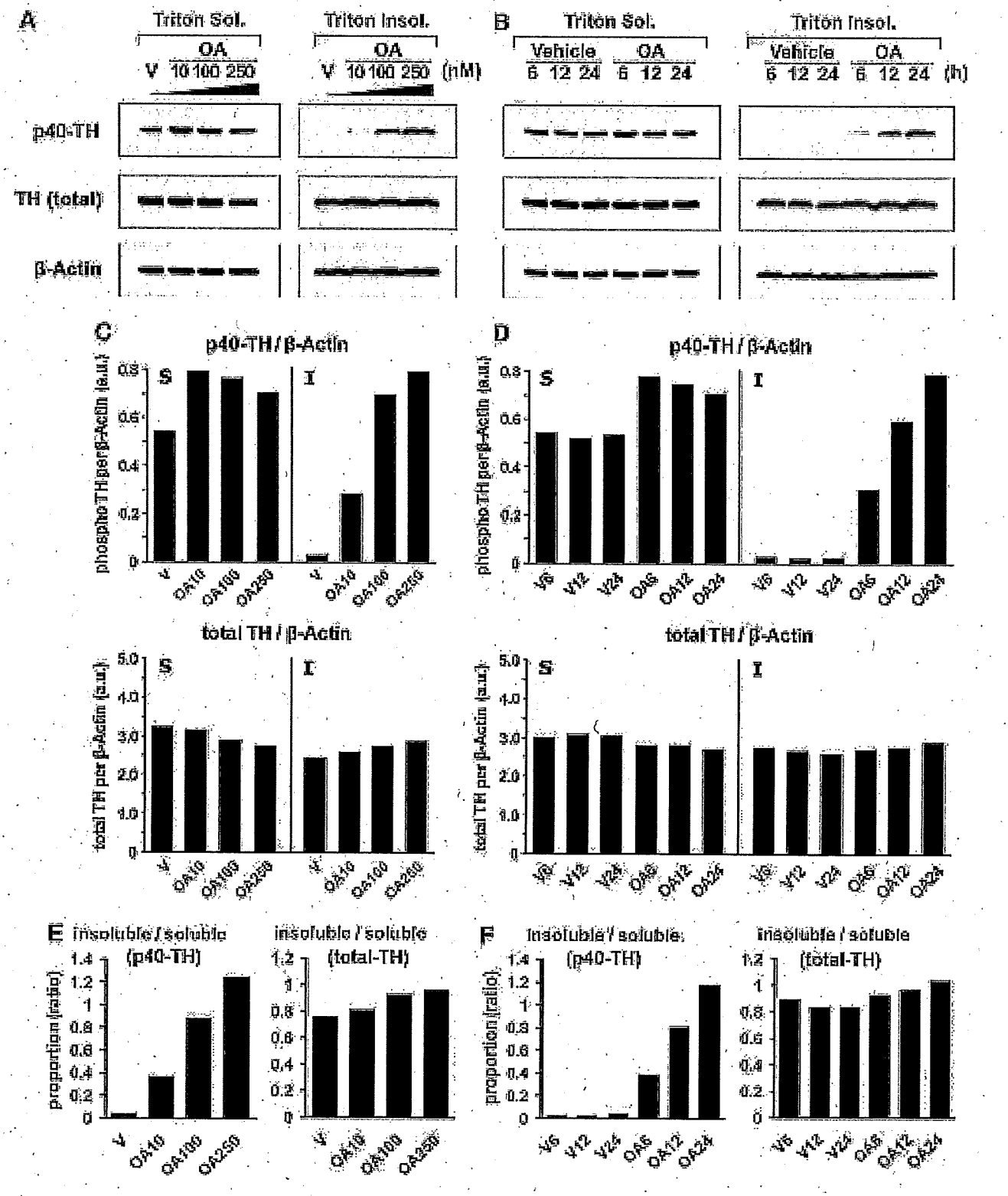

Fig. 4 Increase in insoluble component of p40-TH by PP2A inhibition with OA. **a** PC12D cells were treated with indicated concentrations of OA for 24 h, and subjected to Western blotting analysis. PP2A inhibition induced a dose-dependent accumulation of insoluble p40-TH, as observed in MG-132 treatment. **b** PC12D cells were treated with 250 nM OA for indicated hours, and subjected to Western blotting analysis. Insoluble p40-TH was accumulated time-dependently. No apparent alteration was observed in total-TH level. Vehicle, *OA* okadaic acid. (**c**, **d**) Summary of quantification for the immunoreactivities shown in **a** and **b**. (**e**, **f**) The proportion for the triton-soluble and -insoluble component in p40-TH and total-TH under the basal conditions and in the presence of okadaic acid, an inhibitor of PP2A

compared between p40-TH and total-TH, and it indicates that the increase rate of insolubilization was more drastic in p40-TH (Fig. 4e, f). These results suggest that p40-TH protein tends to be easily insolubilized and accumulate in the cell.

Discussion

In this study, we investigated the effect of proteasomal dysfunction on the TH protein in PC12D cells. We demonstrated for the first time that p40-TH was insolubilized and aggregated in living neuronal cells by proteasomal inhibition and PP2A inhibition. These aggregates were positive for Thioflavine-S and co-localized with ubiquitin. Our present data show that p40-TH is prone to make insoluble aggregates in the cells, although it is not clear whether the aggregates are constituted of p40-TH alone or with many other proteins.

Our group previously showed that recombinant TH protein expressed in *Escherichia coli* had tendency to be easily aggregated in vitro (Urano et al. 2006). It has been reported that the bacterially expressed TH protein showed enzymatic properties that were similar to p40-TH, because both p40-TH and bacterially expressed TH are free from the bound dopamine (Le Bourdellès et al. 1991; Fujisawa and Okuno 2005). Thus, our results on insoluble aggregates of p40-TH in PC12D cells are in good accordance with our previous in vitro results, and suggest that the aggregation of the p40-TH protein may occur in living neuronal cells.

Phosphorylation sites of TH are within the first 40 amino acid residues in the flexible N-terminal domain (Dunkley et al. 2004), and deletion of the N-terminal region was reported to stabilize the TH protein (Nakashima et al. 2005). These findings suggest that a conformational change induced by the phosphorylation at ^{40}Ser may make the TH protein labile and prone to be aggregated.

Increases in the amount of soluble p40-TH were not prominent in the cells treated with okadaic acid (Fig. 4). We treated the cells with okadaic acid for relatively longer period (6–24 h), because we did not detect any insoluble p40TH less than 6 h and we thought that it might take time to form triton-insoluble inclusions in the cells. Thus, it would be possible that a feed-back regulation to reduce the excess amount of p40TH happened in the cells. It would be noteworthy that slight increase in the amount of p40-TH and/or alteration in the metabolism of p40-TH resulted in the increases of insoluble p40-TH.

We showed inhibition of 20S or 26S proteasome induced the accumulation of insoluble component of p40-TH. This result raises possibility that p40-TH may be degraded by ubiquitin–proteasome system. Interestingly, mono-ubiquitination of TH protein was reported using recombinant TH protein, which is presumably DA-free like p40-TH (Døskeland and Flatmark 2002). Thus, p40-TH may be ubiquitinated under normal condition, and inhibition of proteasome may cause accumulation of excess p40-TH which in turn forms insoluble aggregates.

26S proteasome-mediated protein degradation can be impaired by α-Syn protofibrils (Zhang et al. 2008). In addition, the impairment of proteasome function is supposed to be age-dependent (Carrard et al. 2002; Keller et al. 2002). Such failure of the ubiquitin–proteasome system, which would induce an accumulation and aggregation of cytoplasmic proteins, may play a major role in the pathogenesis of both familial and sporadic forms of PD (McNaught et al. 2001; McNaught and Olanow 2003), perhaps mediated by cellular inflammation (Wersinger and Sidhu 2002). Further studies will clarify the possible relevance of the regulation of TH phosphorylation and degradation with pathogenesis of neurodegenerative disorders.

Several reports showed that Lewy bodies were immunopositive to anti-TH antibody in locus ceruleus (Nakashima and Ikuta 1984), cerebral cortex (Kuljis et al. 1989) and substantia nigra (Kawahata et al. 2009). α-Synuclein (α-Syn) is a major component of Lewy bodies, and the deposition of α-Syn is a hallmark of a subset of neurodegenerative disorders including PD (Ueda et al. 1993; Spillantini et al. 1997, 1998). Since α-Syn is expressed not only in the nigrostriatal dopaminergic neurons, but also in the cerebral cortex and other cholinergic neurons (Maroteaux et al. 1988), this alone cannot explain the selective neuronal degeneration in PD. Given that TH is expressed selectively in catecholaminergic neurons, the tendency of p40-TH protein to form insoluble aggregates may be relevant to the selective degeneration of dopaminergic neurons in PD. It would be important to investigate the interaction of α-Syn and phosphorylated TH, although we could not examine it in this study because PC12D cells express little amount of α-Syn. We recently found p40-TH positive immunoreactivities in the substantia nigra from an autopsied brain of a PD patient (Kawahata et al. 2009). In the future studies, we would explore whether presence of the p40-TH protein may make dopaminergic neurons vulnerable to damages caused by proteasomal dysfunction.

Acknowledgments This work was supported by the Research Grant (18A-2) for Nervous and Mental Disorders from the Ministry of Health, Labor and Welfare of Japan; by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; and by JST, CREST.

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Parkinsonism and Related Disorders 16 (2010) 153–162

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Review

Identification and management of deep brain stimulation intra- and postoperative urgencies and emergencies☆

Takashi Morishita [a,c], Kelly D. Foote [b], Adam P. Burdick [b], Yoichi Katayama [c,d], Takamitsu Yamamoto [c,d], Steven J. Frucht [e], Michael S. Okun [a,b,*]

[a] Department of Neurology, University of Florida College of Medicine/Shands Hospital, Movement Disorders Center, McKnight Brain Institute, Gainesville, FL, USA
[b] Department of Neurosurgery, University of Florida College of Medicine/Shands Hospital, Movement Disorders Center, McKnight Brain Institute, Gainesville, FL, USA
[c] Department of Neurological Surgery, Nihon University School of Medicine, Tokyo, Japan
[d] Division of Applied System Neuroscience, Department of Advanced Medical Science, Nihon University School of Medicine, Tokyo, Japan
[e] Department of Neurology, Columbia University College of Physicians and Surgeons/Columbia-Presbyterian Medical Center, New York, NY, USA

ARTICLE INFO

Article history:
Received 30 June 2009
Received in revised form
23 September 2009
Accepted 1 October 2009

Keywords:
Deep brain stimulation
Movement disorders
Emergency
Differential diagnosis
Adverse event

ABSTRACT

Deep brain stimulation (DBS) has been increasingly utilized for the therapeutic treatment of movement disorders, and with the advent of this therapy more postoperative urgencies and emergencies have emerged. In this paper, we will review, identify, and suggest management strategies for both intra- and postoperative urgencies and emergencies. We have separated the scenarios into 1 – surgery/procedure related, 2 – hardware related, 3 – stimulation-induced difficulties, and 4 – others. We have included ten illustrative (and actual) case vignettes to augment the discussion of each issue.

Contents

☆ The review of this paper was entirely handled by the Co-Editor-in-Chief, Zbigniew Wszolek.

* Corresponding author at: Department of Neurology, McKnight Brain Institute, Medical Director National Parkinson Foundation, 100 S Newell Dr, Room L3-101, Gainesville, FL 32610, USA. Tel.: +1 352 273 5550; fax: +1 352 273 5575.
E-mail address: okun@neurology.ufl.edu (M.S. Okun).

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doi:10.1016/j.parkreldis.2009.10.003

1. Introduction

Neurosurgical procedures for basal ganglia disorders may result in urgent or emergent management issues. Postoperative urgencies and/or emergencies should be identified and treated in an expeditious manner. Deep brain stimulation (DBS) has been increasingly utilized for addressing neurologic and neuropsychiatric disorders [1,2], and with the increasing number of DBS cases being performed each year, there has been a commensurate increase in the number of issues relating to the surgical, the procedural, and to the stimulation-related phenomena. Some of these issues have manifested themselves as movement disorders (e.g. dyskinesia, ballism, dystonia), although the majority have presented in other ways [3–5]. In this paper, we have separated the potential scenarios into 1 – surgery/procedure related, 2 – hardware related, and 3 – stimulation-induced phenomena. The discussion has been augmented by the use of clinical vignettes which illustrate the diagnosis and management of both urgent and emergent situations. Complications of DBS have unique manifestations, and diagnostic criteria and management have not been fully established in some cases. Therefore, it is possible that clinicians may overlook DBS-induced complications, and delay the appropriate management. This delay may unnecessarily result in secondary complications. The aim of this paper is to review urgent and emergent DBS-associated situations to provide recommendations for appropriate management.

2. Methods

Complications with DBS-specific manifestations have been specifically selected for this review, and a PubMed based literature search was performed for each issue. We queried the Institutional Review Board (IRB) approved DBS database of University of Florida Movement Disorders Center (UFMDC) for the illustrative cases from the period between July 2002 and June 2009.

2.1. Surgery/procedure related urgencies/emergencies

2.1.1. Intracranial hemorrhage

Case 1. A 73-year-old man with a 16-year-history of Parkinson's disease (PD) underwent unilateral subthalamic nucleus (STN) lead implantation. There was no history of hypertension, diabetes mellitus (DM), or coronary artery disease (CAD). Following the procedure, he became somnolent, and a postoperative computed tomography (CT) scan revealed a hematoma in the left lateral ventricle (Fig. 1B). There was involvement of the third ventricle and the Sylvian aqueduct. The patient developed acute obstructive hydrocephalus that necessitated emergent ventriculostomy. He convalesced for one week postoperatively, and then developed a deep venous thrombosis, an aspiration pneumonia, atrial fibrillation, a urinary tract infection, and sepsis. The total hospitalization was extended to 40 postoperative days. Following eight months of rehabilitation and anticoagurant therapy he has recovered, and implantation of Implantable Pulse Generator (IPG) was scheduled.

Hemorrhage is an emergent adverse event that may be seen following DBS, and may result in significant morbidity or rarely even death. Hemorrhages following DBS may include intracerebral (ICH), intraventricular (IVH), subdural (SDH), subarachnoid (SAH) and epidural (EDH) (Fig. 1). Hemorrhagic complications have been assumed to be due to damage to the blood vessels by the microelectrode recordings (MERs) and/or macrostimulation passes, and it has been discussed that multiple MERs and/or macrostimulation passes may increase the incidence of hemorrhagic complications (debated but generally accepted among the experts) [6–8]. Intracranial hemorrhage can be diagnosed by CT scan which may be sought in the postoperative period and is usually performed as a result of a mental status change and/or a focal neurological deficit. The incidence of hemorrhage varies from 0.6 to 3.3% [7,9–13]. Intracranial hemorrhage can precipitate secondary complications such as pneumonia, pulmonary embolus, and urinary tract infection. A recent German multicenter study revealed an overall 30-day postoperative mortality rate of 0.4% (5 of 1183 patients), and mortality due to hemorrhage in 2 of 5 patients [14]. Delay of identification and management of ICH can result in significant morbidity, therefore emergent care should be employed to prevent both primary and secondary complications. When ICH is encountered it mandates immediate neurosurgical consultation, preferably by the neurosurgeon who implanted the DBS system, although this is not always possible. Most patients can be managed conservatively, however if operative intervention for evacuation is deemed necessary every attempt should be made not to remove the DBS hardware (Table 1). Patients requiring evacuation as well as those not requiring surgery have the potential for good recovery.

2.1.2. Venous infarction

Case 2. A 60-year-old man with PD underwent a staged unilateral globus pallidus interna (GPi) DBS. He was discharged on postoperative day #1 following an uncomplicated hospital course, but later that day he began to develop left-sided weakness, lethargy, and confusion which peaked on postoperative day #2. He presented to the emergency room (ER) on postoperative day #4. A head CT scan revealed hemorrhage spreading from the center of the DBS lead. The region was surrounded by edema (Fig. 1D). The diagnosis of venous infarction was made and he was conservatively managed. Following several months, his neurological status returned to baseline, and his DBS was effectively programmed to address both motor fluctuations and parkinsonism.

Venous infarction has been associated with damage to a cortical vein that may occur during DBS surgery. Cerebral edema and hemorrhage may slowly develop and are usually the result of venous stasis or venous hypertension. These phenomena are thought to occur as a result of venous obstruction which usually traces to the damaged cortical vein. Venous infarction can be characterized by a delayed clinical onset with edema and possibly hemorrhage along the path of the DBS lead [15]. These features may be absent in some cases. Importantly, the head CT may not reveal an obvious lesion in the immediate postoperative period, and a repeat CT may be necessary to confirm diagnosis. The prognosis is usually positive in venous infarction occurring post-DBS [7,16]. Careful preoperative targeting using a high quality contrasted MRI will aid in avoiding cortical veins, and may prevent this complication [7]. If the diagnosis of venous infarction is made postoperatively, the management should include optimizing the venous return (e.g. elevate the head of the bed), managing blood pressure, avoiding dehydration, and initiating early rehabilitation (Table 1).

2.1.3. Dyskinetic storm

Postoperative dyskinesia is a relatively common phenomenon associated with STN DBS, especially in PD patients who preoperatively suffered from severe medication-induced dyskinesia [4]. Microelectrode recording, cannula placement and/or lead placement may all induce dyskinesias especially in patients with PD. Acute and severe exacerbation of dyskinesias (dyskinetic storm) in the operative setting has been previously reported and may be a feature associated with a positive prognosis [3]. In severe cases, dyskinesia may be associated with dyspnea and rhabdomyolysis [17,18], and emergent administration of sedative agents (such as IV propofol) may be required [3] (Table 1). Dyskinesia may also be induced by DBS placement in a delayed fashion [19], and dyskinetic storm may be encountered in the clinical setting following DBS programming. Management of dyskinesia in the clinical settings will also be discussed in the "*Stimulation-related motor symptoms*" section of this review.

2.1.4. Postoperative behavioral and cognitive problems

Postoperative cognitive and behavioral decline is a common DBS-related adverse event. It is usually temporary, but in patients with preoperative cognitive dysfunction it may persist. The incidence of confusion has been recently reported as 5% in a large single center study [20], but rates may vary depending on preoperative comorbidities, target site, and whether staging of operative sides is employed (i.e., as opposed to same-day simultaneous bilateral DBS implantation) [4,21–23]. The incidence of behavioral and cognitive problems may be higher in STN DBS when compared to other targets [4,23–25]. A recent randomized double-blind study revealed a higher incidence of cognitive adverse events in patients with STN DBS when compared to GPi DBS [23]. Also verbal fluency seemed worse in STN and the change was reported as more surgery-related rather than a stimulation-related effect (i.e. it occurred in the off STN condition as well as the on STN DBS condition during blinded testing) [23].

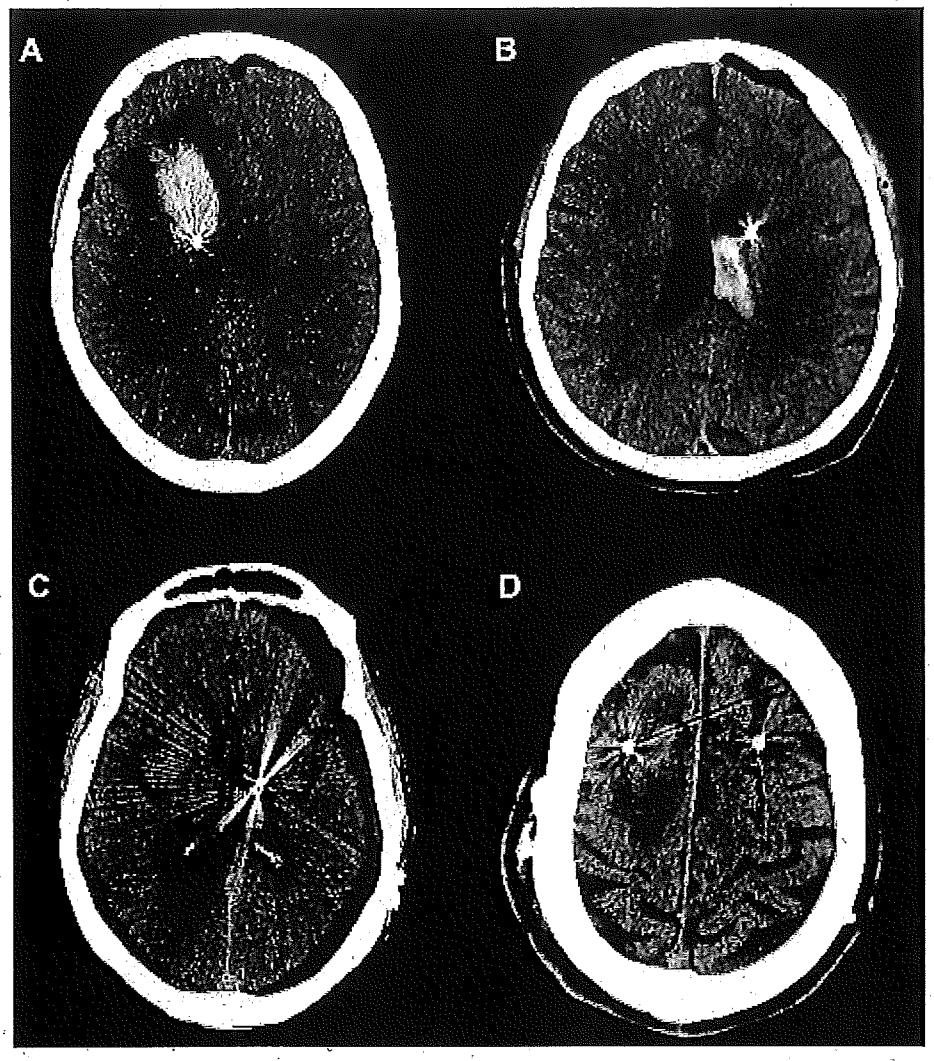

Fig. 1. Computed tomography (CT) scan images of the many types of hemorrhage that may be encountered following DBS lead implantation. This panel of CT images reveals examples of intracerebral hemorrhage (A), intraventricular hemorrhage (B), subdural hemorrhage (C), and venous infarction (D).

Anticholinergics (including not only anti-Parkinsonian medications but also medications for neurogenic bladder) may also increase the risk of postoperative cognitive problems, and may need to be discontinued [26]. It is important for clinicians to keep in mind that advanced age and/or pre-existing neurological compromise may predispose patients to mental status changes following DBS, and this is a compelling reason for centers to perform preoperative neuropsychological screening [21].

The management of postoperative behavioral and cognitive problems should include a diagnostic workup of potential underlying causes that may exacerbate and/or contribute to the clinical condition. These may include urinary tract infections, hemorrhage or medications related phenomena. If no underlying treatable cause can be identified, the clinician should utilize pharmacotherapy and a multi/interdisciplinary approach to manage the behavioral change(s). This approach may be facilitated in select cases by an inpatient admission.

Patients may become restless and violent postoperatively due to hallucinations/delusions, and this situation may require urgent/emergent care. In these cases, conventional neuroleptics are usually contraindicated, however using selective dopaminergic blockers such as quetiapine or clozapine may be useful [27] (Table 1). Non-selective dopaminergic blockers (e.g. olanzapine, risperidone, haloperidol, etc.) that have been commonly employed for the treatment of behavioral emergencies have also been observed to lead to drug-induced parkinsonism as well as other movement disorders [28,29].

2.1.5. Suicide attempt or ideation

Case 3. A 54-year-old woman with PD and depression who had a left DBS implantation two years prior to presentation was brought to the emergency room following a suicide attempt by drug overdose. Passive suicidal ideation was noted on her psychiatric evaluation prior to DBS.

Several reports have revealed cases of attempted and completed suicide occurring following DBS [30–32]. DBS may increase the risk of suicide when compared to the general population, but not necessarily when compared to a PD population (without DBS) [31,33]. A recent multicenter study revealed that preoperative history of impulse control disorders or compulsive medication use, postoperative depression, postoperative apathy, and being single were strongly associated with suicide attempts [33]. Previous suicide attempts, younger age of the patient, and younger onset of PD were also revealed to be associated with suicide attempt within the same study cohort. Therefore, screening for suicidal ideation following DBS should be routine, and if discovered, it should be treated as an emergency. Clinicians should admit patients to the hospital for multi/interdisciplinary care, which may include cognitive behavioral therapy, counseling, and/or medication/stimulation adjustment(s) (Table 1). Whether DBS results in disinhibition or impulsiveness and ultimately contributes to suicidal ideation or suicide remains controversial [34]. Vigilant pre and postoperative screening for depression and suicidal ideation are recommended. Preoperative neuropsychological and psychiatric evaluation is highly recommended as a preventative measure [21,32]. Advanced PD patients are likely to have cognitive and/or mood disturbances, and neuropsychologists can play an important role for screening out these issues. Recently, there have been several reports of suicide in dystonia DBS highlighting that this issue may not be solely related to PD [30,31,35].

2.1.6. Myocardial infarction

Case 4. A 58-year-old male with PD, coronary artery disease (CAD) (previously treated with angioplasty), hypertension, diabetes mellitus (DM), and hyperlipidemia underwent unilateral STN DBS placement. An implantable pulse generator (IPG) was placed four weeks following the DBS lead. Following IPG implantation

Table 1
Postoperative surgery and hardware related urgencies and emergencies.

Issue	Routine/urgent/emergent	Management
Intracerebral hemorrhage	Emergent	If the hemorrhage is very large, an emergent craniotomy may need to be performed.
Intraventricular hemorrhage	Emergent	Ventriculostomy if necessary may be performed for obstructive hydrocephalus.
Subdural hematoma	Emergent	If acute and symptomatic, an emergent craniotomy may be performed in select cases. If chronic, a burr hole irrigation may be performed, or it may be watched conservatively.
Epidural hematoma	Emergent	An emergent craniotomy should be performed immediately in severe cases.
Venous infarction	Urgent/emergent	Conservative therapy. An emergent craniotomy may be performed if hemorrhage is life threatening.
Dyskinetic storm	Urgent	Sedative agents may be administered in select cases. Reducing the dopaminergic medication may help. In some cases ICU care is necessary.
Behavioral/cognitive issues	Urgent/emergent	Identify and treat the underlying issues. Selective dopamine blockers (e.g. quetiapine, clozapine) may be used, but non-selective blockers should be avoided if possible.
Suicide ideation/attempt	Emergent	Evaluation for an underlying issue such as battery life and/or unintended on/off. Admit the patient to the hospital for multidisciplinary care including behavioral therapy, counseling, medication adjustment and/or stimulation adjustment.
Air embolus	Emergent	Wax edges of the burr hole, lower patient's head, jugular venous compression, administer oxygen
Infection-UTI	Routine/urgent	Hydration and appropriate antibiotics, case should be taken to adjust PD medications if necessary as levels may be altered by antibiotics.
Infection-lead	Emergent	The lead should be removed and appropriate antibiotics should be administered.
Infection-IPG	Urgent	The IPG and usually the extension cable should be removed and appropriate antibiotics should be administered.
Lead fracture	Urgent	Lead replacement, if an appropriate candidate.
Lead electrical short	Urgent	Lead replacement, or potentially reprogramming at a different contact.
Lead migration	Urgent	Lead replacement, surgical alteration of lead position, or potentially reprogramming at a different contact.
Lead misplacement	Urgent	Lead replacement.
IPG malfunction	Urgent	IPG replacement, manage potential rebound symptoms.

the patient died in his sleep on postoperative day one from a myocardial infarction.

Assessment of medical comorbidities must be performed on all patients undergoing DBS surgery [21]. Clinicians should be aware that patients taking medications that can affect the cardiovascular system such as bromocriptine, and tricyclic antidepressants (TCAs) may be at increased risk when undergoing general anesthesia [36]. The incidence of angina and arrhythmia following DBS surgery has been reported as 0.3% in a recent large single center study [20]. A history of CAD may increase the perioperative risk of MI and angina. High risk patients should be carefully monitored (pre and postoperatively), and they may even require extra monitoring following general anesthesia. Cardiac or pulmonary symptoms in the postoperative period should be treated as an urgent, and possibly even emergent complications. Although this complication can be encountered following any surgical procedure, chest pain in the region surrounding the IPG DBS skin incision may be erroneously attributed to a DBS-related issue rather than to a cardiac related issue [37]. Clinicians should be aware that the risk of comorbidities and medications, and that postoperative chest pain following DBS/IPG implantation can present or evolve into urgent or emergent issues.

2.1.7. Air embolus

Air embolus a relatively uncommon complication of neurosurgical procedures. However, clinicians should be aware that the incidence of air embolus during DBS surgeries has been reported to be as high as 3% in a recent report, and DBS-related air embolus may manifest differently from other neurosurgical procedures, since during DBS the procedure is performed awake rather than under general anesthesia [38,39]. When the neurosurgeon is preparing the burr hole for microelectrode recording and/or macrostimulation, air embolus may occur with tachycardia, a rise in the end tidal CO_2, and cough. This reaction is typical of entrainment of air into the venous system. It is important to preoperatively position the patient's head as close to supine as possible to minimize this complication. In a recent series, Hooper et al. reported the potential use of an external Doppler device to enhance monitoring for air embolus during DBS [39]. These authors also noted that the cough was the best clinical indicator to pick up an air embolus. When encountered, the head position should be adjusted (lower the patient's head), bone edges of the burr hole should be waxed, the surgical field rigorously irrigated, and the patient vigorously supported from a cardiopulmonary standpoint. Additionally, having the neurologist, nurse or physiologist temporarily compress the neck (to inhibit venous return) may aid the neurosurgeon in identifying the problematic region, and in quickly correcting the situation (Table 1).

2.2. Hardware-related urgencies/emergencies

2.2.1. Hardware infection

Case 5. A 43-year-old man with a nine-year-history of PD underwent unilateral STN DBS. He arrived for a routine clinic appointment and staple removal on postoperative day seventeen. Following the staple removal there was purulent drainage from the cranial incision site, and the pectoral incision revealed tender erythema (Fig. 2). He was admitted to the hospital urgently, and the IPG and the extension wire were both removed. A course of intravenous antibiotics was completed prior to re-implantation.

Case 6. A 71-year-old man with a history of medically refractory essential tremor (ET) underwent a unilateral thalamic DBS implantation. Four weeks following surgery, the patient reported to the clinic for routine follow up care. Headache and progressive dysphagia were the chief complaints, and a head CT scan revealed a brain abscess along the DBS lead tract. The CT scan demonstrated an edematous lesion surrounding the DBS lead which was enhanced with contrast media (Fig. 3). He was admitted for emergent craniotomy, DBS lead removal and abscess drainage.

Hardware related infections are not uncommon in DBS. The incidence of infection and/or erosion following device implantations has been reported to range between 0 and 15.2% [10,40–46]. Even the most vigilant surgical technique cannot guarantee the absence of postoperative infectious complications. The devices in these scenarios may require emergent removal, in contrast to the management of ICH, which may not require lead removal (Table 1). Cultures should be sent anytime hardware is removed or a potentially infectious pocket aspirated. Although several factors may be related to infection rate, pre and postoperative prophylactic antibiotics may prevent hardware infection, however the evidence base for their use is

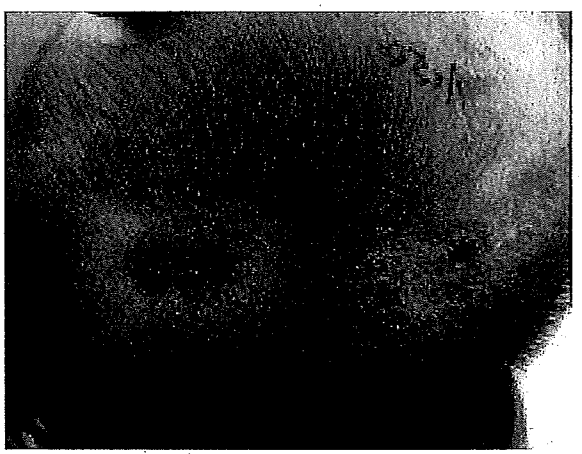

Fig. 2. Infection in the cranial skin incision. The illustration reveals purulent drainage from the cranial incision.

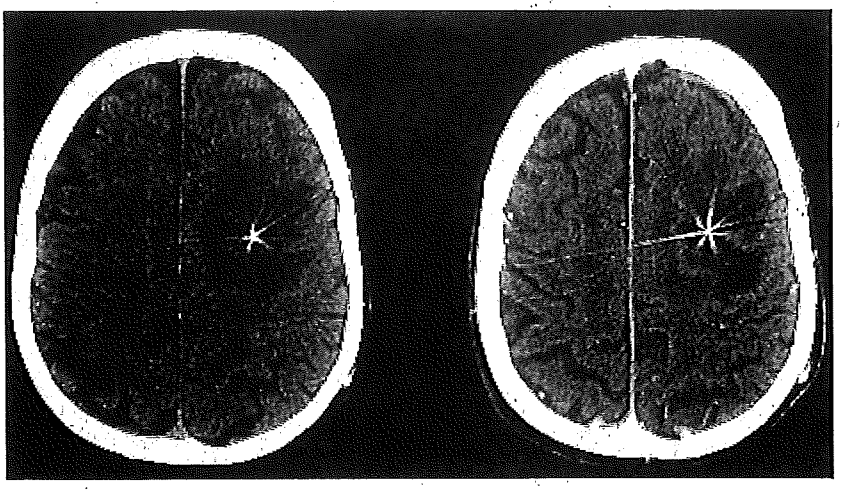

Fig. 3. Computed tomography (CT) scan images of a brain abscess following DBS lead implantation. A CT scan image without contrast (left) revealed a low density area which indicated an edematous lesion surrounding the DBS lead. The lesion was enhanced with contrast media (right).

weak [44,46]. One recent study did however report a reduction in the infection rate by locally injecting anti-staphylococcal antibiotics (e.g. neomycin, polymixin) directly into the operative wound [46].

There are several factors that may impact the management of a DBS infection. These include 1 – whether the infection is deep or superficial, 2 – whether the brain lead is involved, and 3 – whether there are single or multiple sites of involvement [40]. A superficial infection may be managed in select cases in a non-operative and conservative fashion, however a deep infection may require emergent hardware removal. When the brain lead and/or multiple sites are involved, many DBS teams elect to remove all hardware (lead, extension, and IPG). In cases where only the IPG or extension cable appears infected, there is an option to remove only the purported infected hardware and to attempt to preserve the brain lead. Following a course of 6–8 weeks of IV antibiotic therapy, device(s) re-implantation may be possible.

2.2.2. Hardware malfunction

Case 7. A 76-year-old woman with a history of essential tremor (ET) who underwent a left thalamic DBS implantation three years prior presented to clinic with sudden tremor recurrence. A few days before her presentation, following a mammogram she experienced a tingling sensation in the right upper extremity with an abrupt loss of benefit in her right upper extremity tremor. When the device was checked the impedance was discovered to be greater than 2000 Ω, and a chest x-ray revealed a flipped IPG and a twisted extension cable (Fig. 4). A fracture of the extension cable was suspected, and replacement of the cable resolved the issue.

When a DBS patient reports sudden loss of efficacy, the clinician should consider hardware malfunction [4,47]. Mechanical stress to the device may result in lead fracture, a break in the extension cable, or an IPG failure. Blomstedt et al. reported 7 of 8 broken electrodes in their cases were encountered in patients with ET, and they speculated head tremor may have contributed to the adverse event(s) [44]. Compulsive manipulation of the IPG device, referred to as "Twiddler's syndrome", has also been reported to result in extension cable fractures [48].

The DBS programming/interrogation device should be used to measure the impedance and current drain for each of the four lead contacts. This procedure will assist in verifying the physical integrity of the DBS system. A high impedance along with a low current drain may be consistent with a lead fracture or with an extension cable break. Alternatively a low impedance with possible high current drain may be supporting evidence for a short circuit. In short circuits the patient will frequently complain of a shock-like sensation when palpating the IPG or when pressing along the extension cable tract. A plain film x-ray should be obtained to search for a fracture along the course of the lead or extension wire (Fig. 5). When the location of the problem cannot be precisely identified, the next step is to replace the extension wire and to retest impedances in the operating room setting. This procedure may save replacement of the intracranial lead in select cases [47]. Clinicians should always keep in mind that contacts with normal impedances/current

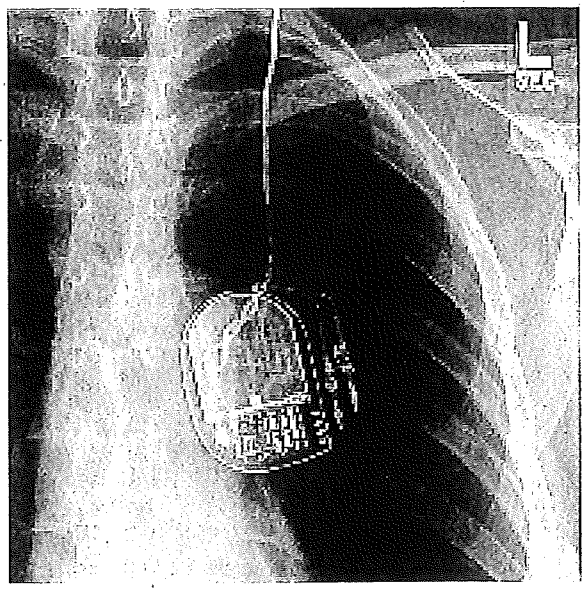

Fig. 4. A chest x-ray revealed a twisted extension cable and flipped IPG following a mammography. These features have been referred to as the twiddler syndrome.

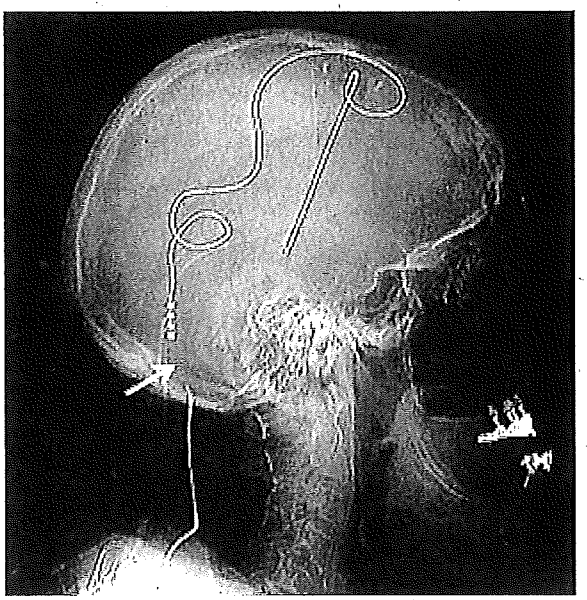

Fig. 5. A skull x-ray revealed an extension cable fracture.

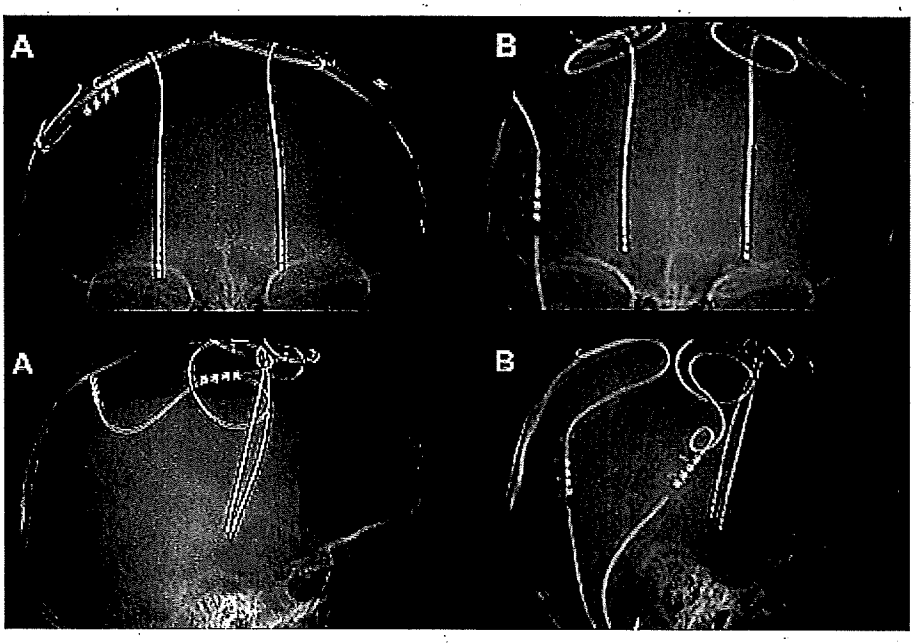

Fig. 6. Dorsal lead migration shown by serial x-rays. A) A skull x-ray at one month post-implantation. B) A skull x-ray at eight months pre-repeat implantation. Left and right leads had deviated from baseline.

drain values, are potentially programmable. An attempt to reprogram these functioning contacts should be sought prior to recommending replacement (Table 1).

2.2.3. Lead migration

Case 8. A 7-year-old boy with DYT-1 positive generalized dystonia underwent bilateral GPi DBS. After an initial dramatic response, his benefit deteriorated over the first year. Measurement and comparison of his DBS leads revealed dorsal lead migration (Fig. 6). His head circumference was measured and found to be 51.5 cm preoperatively and 53 cm 30 months later. Repositioning the DBS leads recaptured benefits.

Case 9. A 26-year-old man developed tardive dystonia following exposure to a neuroleptic drug used to address his severe depression. He subsequently underwent bilateral GPi DBS. Preoperatively he suffered severe and painful retrocollic head jerks. Postoperatively his subjective pain and head jerking clinically improved (pain approximately 50+% and movement disorders approximately 40–50%). Six months following the operation the benefits waned, and a CT scan revealed that the left and the right leads had migrated 15.6 mm and 4.6 mm ventrally from their initial position (Fig. 7). The patient underwent successful lead replacements.

Lead migration, either dorsal or ventral, can result from a malfunction of anchoring devices, skull growth, or vigorous head movements [47,49]. Yianni et al. reported that 3 of 133 patients (2.3%) experienced lead migration [49]. All three patients had dystonia, and the authors hypothesized that axial movements contributed to lead migration. When ventral lead migration is noted in a patient with GPi DBS, clinicians should be cautious as the ventral lead migration may result in severe mood changes due to the spread of the stimulation to other regions such as but not limited to the amygdala [50]. Skull growth in children is another cause of lead migration as illustrated by case 7. When lead migration is noted, changing the active contact (deeper or shallower depending on the direction of migration) should be attempted in most cases prior to surgical revision [51] (Table 1). This adverse event highlights the importance of examining postoperative imaging.

2.2.4. Lead misplacement

Case 10. A 60-year-old man with a history of PD underwent bilateral STN DBS at an outside institution 5 years prior to presentation to our clinic. He reported a lack of benefit from DBS, and repeated programming in the past had not improved his situation. An MRI scan revealed lead misplacement (Fig. 8). The patient was unwilling to undergo a lead replacement because of a combination of claustrophobia and fear of the surgical suite.

Lead misplacement is a not uncommon complication of DBS surgery and has been reported to be associated with technical error, intraoperative brain shift

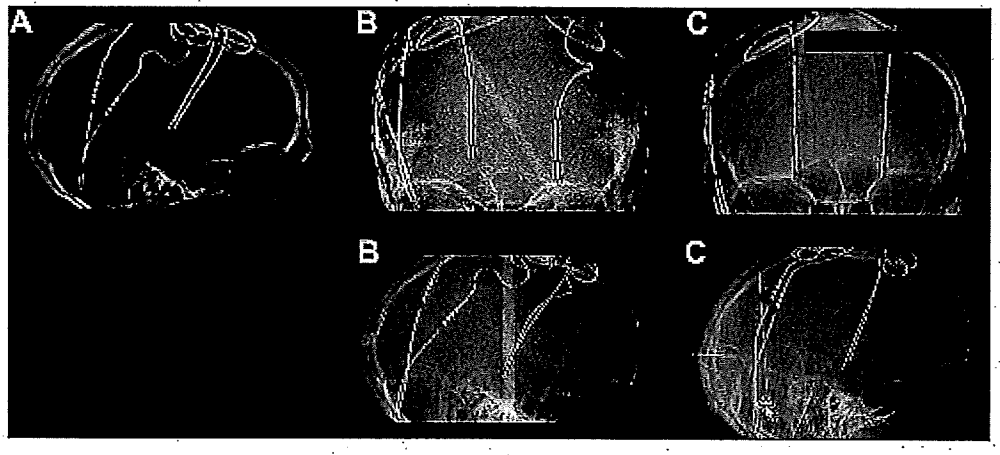

Fig. 7. Ventral lead migration shown by serial x-rays. A) A skull x-ray at one month post-implantation. B) A skull x-ray at fourteen months following first operation. The left and right leads had moved approximately 16 mm and 5 mm downwards from the initial position, respectively. C) A skull x-ray at one month following lead replacement.

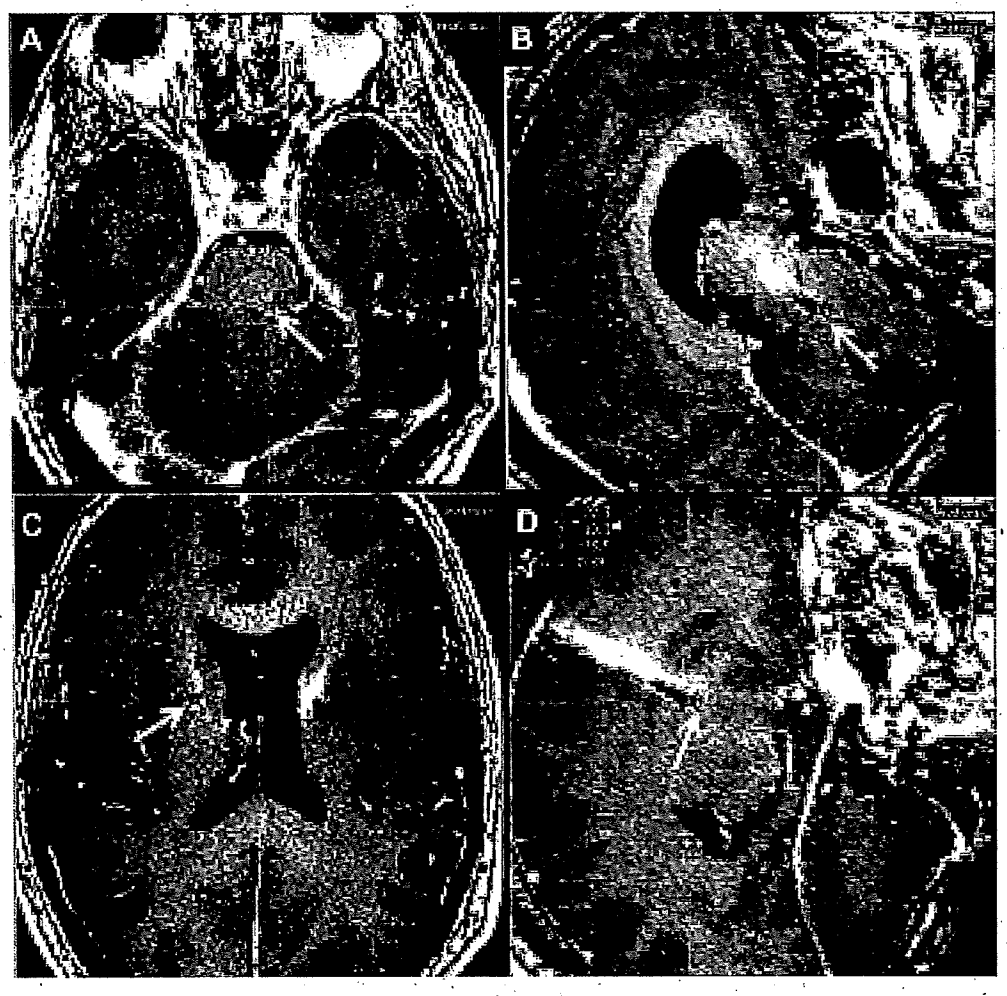

Fig. 8. Lead misplacement. Arrows indicate the tip of DBS leads. An axial slice (A) and a sagittal slice (B) of a T1 weighted magnetic resonance imaging (MRI) scan revealed the tip of the left DBS lead located too far posterior and deep for the subthalamic nucleus (STN). An axial slice (C) and a sagittal slice (D) of the same MRI scan revealed too shallow location of the left DBS lead.

(usually the result of a cerebrospinal fluid (CSF) leak [5]), and/or a failure in devices designed to secure the lead [49]. A suboptimal outcome from DBS surgery and/or low thresholds for stimulation-induced side effects (when the device is interrogated) may suggest lead misplacement. DBS leads placed in suboptimal locations may not be able to be corrected by programming [47]. Stimulation-induced side effects can lead to an urgent or an emergent situation, and in extreme cases can result in severe and sometimes unexpected symptoms. Even slight variations (sometimes only millimeters) of the location of a DBS lead may alleviate negative symptoms and lead to a more optimal therapeutic benefit [51] (Table 1).

2.3. *Stimulation-related urgencies/emergencies*

2.3.1. *Stimulation-related motor symptoms*

Stimulation-related motor symptoms include dyskinesia, chorea/ballism, gait disturbances, motor pulling, verbal fluency problems (verbal fluency has motor and non-motor/cognitive components), dysarthria, and hypophonia (Table 2). Many stimulation-induced motor symptoms resolve following reprogramming of the voltage, pulse width, and/or frequency.

If dyskinesia or chorea/ballism was induced by stimulation, reducing the amplitude/voltage of stimulation, or reducing the levodopa equivalent dose may alleviate the issue. Severe stimulation-induced dyskinesia/ballism in the clinic setting should alert the DBS programmer that voltage adjustment should be performed very slowly (sometimes in 0.1–0.2 V increment increases over many weeks). When stimulation-induced hyperkinesia is encountered, the ultimate outcome for patients is usually excellent. One important exception is underlying infection (e.g. pneumonia, UTI, etc.) which may exacerbate dyskinesia. We suggest an evaluation for an infectious process should be sought in cases where medical management proves difficult [18].

Although pre-existing gait and/or speech problems (e.g. on medication freezing, dysarthria, and hypophonia) do not typically respond to stimulation [23,52], stimulation-induced gait and speech issues may be improved in select cases with reprogramming, sometimes into a bipolar configuration. Patients themselves may discover relief by switching one or both devices (utilizing a remote control) to an off position when speaking. Additionally recent reports have revealed changing high frequency (>100 Hz) to lower frequency (<100 Hz) programming settings may improve gait, voice and other clinical features [53,54]. More research into DBS settings that may have the potential to improve or enhance clinical symptoms will be required, as some of the current low frequency settings seem to provide only temporary relief [53,54].

2.3.2. *Stimulation-related non-motor symptoms*

When stimulation spreads to surrounding neuronal regions, and to limbic and associative regions within grey matter structures, symptoms such as unpleasant feelings, paresthesias, behavioral complaints, and cognitive issues may emerge (Table 2). Pseudobulbar laughter and crying (mood incongruent) have been reported with stimulation, and both have been reportedly addressed by the use of antidepressant medications and by DBS reprogramming [55–57]. Two of the most worrisome stimulation-induced issues are depression and mania [32,58–62]. Both may require medication changes, reprogramming, verification of lead locations and potential hospitalization [47]. Depression should be carefully followed as it can result in suicidal ideation or suicidal attempt [32]. Several reports have linked stimulation of the substantia nigra region to acute depression in patients with STN DBS [58,59]. Abrupt cessation or reduction of dopaminergic medications can also result in apathy or depression [52,63]. If depression follows induction of stimulation, reprogramming to a more dorsal contact may be one solution. The lead location should be checked as misplacement into non-motor regions is one explanation for stimulation-induced non-motor features. Useful strategies include administration of antidepressants/antipsychotics, titrating neuropsychiatric medications to optimal doses, and completely optimizing dopaminergic medications [52]. Inpatient care including multi/interdisciplinary approaches (including psychiatrists, psychologists, neurologists, neurosurgeons and other health professionals) and behavioral therapies may also prove useful.

Table 2
Postoperative stimulation-induced urgencies and emergencies.

Issue	Routine/urgent/emergent	Management
Chorea/ballism	Routine/urgent	Try to program slowly (e.g. slow increase of voltage over many weeks/months). May try dorsal contact. May also reduce dopaminergic medications.
Dyskinesia	Routine/urgent	Reducing the dopaminergic medication may help. Try to program slowly (e.g. slow increase of voltage over many weeks/months). May try dorsal contact.
Motor pulling	Urgent	Try to reduce voltage or pulse width. Try bipolar stimulation, or possibly another lead contact. Some situations may require lead replacement. Check lead location.
Gait disturbance	Routine/urgent	Try another setting (e.g. another contact, reducing pulse width, voltage or frequency). Low frequency (60 Hz) with higher voltage or pulse width may help.
Verbal fluency problem	Routine	Try another contact, perhaps a more dorsal contact on the DBS lead. Try changing stimulation to bipolar or decrease pulse width, voltage or frequency.
Dysarthria/dysphagia	Routine	Try another contact. Try changing stimulation to bipolar or decrease pulse width, voltage or frequency. Check lead location. Prescribe speech therapy.
Hypophonia	Routine	Try another contact. Try changing stimulation to bipolar or decrease pulse width, voltage or frequency. Prescribe speech therapy.
Cognitive decline	Routine	This problem may be disease progression, surgery-related, or stimulation-related. Seek neuropsychological testing, consider reprogramming to a dorsal contact. Check lead location.
Mania/hypomania	Routine/urgent	Adjust medications. Consider discontinuation of dopamine agonist and use of quetiapine or clozapine. Consider moving to a dorsal contact and/or decreasing the pulse width, voltage or frequency. Check lead location. Consider admission for multi/interdisciplinary management.
Impulse control	Urgent	Adjust medications. Consider discontinuation of dopamine agonist and addition of clozaril or seroquel. Consider moving to a dorsal contact and/or decreasing the pulse width, voltage or frequency. Check lead location. Consider admission for multi/interdisciplinary management.
Suicide ideation/attempt	Emergent	Admit the patient to the hospital for multi/interdisciplinary care, and treat underlying cause. May need both medication adjustment and programming. Check lead location.
Anxiety/fear	Urgent	Consider more frequent and higher doses of dopaminergics, and altering DBS contacts, perhaps moving more dorsal. Check lead location. Consider admission for multi/interdisciplinary management.
Severe depression	Emergent	Behavioral therapy, counseling, medication adjustment and/or stimulation adjustment. Check lead location. Consider admission for multi/interdisciplinary management.
Postoperative mania	Urgent	Behavioral therapy, counseling, medication adjustment and/or stimulation adjustment. Check lead location. Consider admission for multi/interdisciplinary management.
Pseudobulbar cry/laughter	Urgent	SSRI, TCA or dextromethorphan.
Autonomic features	Urgent	May habituate on own, try stimulation parameter adjustments, or change contact if continues to be troublesome.
Sensory phenomena	Urgent	Try reduce voltage or pulse width. Try bipolar, or possibly other contact.
Accidental on/off	Urgent/emergent	Turn on the IPG, keep a diary to identify the problem.
Symptom rebound (motor and/or non-motor)	Emergent	DBS hardware workup including impedance check, battery check, x-ray study, and assess for tolerance.

2.3.3. Accidental on/off

When the DBS device unpredictably turns off, the clinician must investigate potential environmental triggers (the device has a duty log to assist in documenting these occurrences). Exposure to magnetic forces (e.g., a magnetized ice freezer or store security devices) is the most commonly reported etiology [47]. Prescribing "rechecking" of the DBS device on a regular or semi-regular schedule may prove useful (utilizing the patient issued remote control). Additionally, having the patient document and describe activities and relevant environments may yield the source of the problem. The patient should be educated to avoid strong magnetic fields, and to have their remote device with them at all times in order to recheck on/off status, and to learn prevention strategies for accidental on/off's. Most patients who undergo DBS surgery do not have any issues with accidental on/off's during the lifetime of their devices.

If more than one IPG is utilized to power multiple DBS leads, the chest pacemakers must be placed a minimum of six inches apart. Failure to separate the IPG's in space may result in cross-communication of the devices, and a result in an automatic and unintended reset to the factory default stimulation settings. We have observed this phenomenon in a single case, a boy with generalized dystonia and two chest IPGs (author observations). Interestingly when lying supine this boy's devices were six inches apart, but when leaning forward in a chair for programming sessions the distance was cut to only four inches. It is therefore important when programming to make sure (especially in children) that patients sit back in the chair, or alternatively lie in a supine position.

2.3.4. Symptom rebound

Several cases of severe symptom rebound following battery failure have been reported following DBS [64,65]. The more beneficial DBS is for clinical symptoms, the more dramatic the rebound symptoms may be. Symptom rebound may include both motor and non-motor manifestations such as tremor, gait problems, stiff legs and suicidal ideation (author observations), as well as severe depression. We have observed rebound of motor symptoms with battery failures in cases of dystonia and Parkinson's disease, but also rebound of non-motor symptoms including depression and suicidal ideation with battery failure (author observations). Sudden worsening of symptoms should always prompt a battery status check by an experienced DBS programmer. If the device is off, resuming stimulation may be all that is necessary (see Section 2.3.3). If the device is on, checking impedances and current drain at each of the four DBS contacts may provide useful information for evaluation of lead integrity (hardware malfunction) as discussed in the "hardware malfunction" section of this paper (Table 2).

3. Others

3.1. Dystonic storm

Dystonic storm or status dystonicus is a rare but possibly life-threatening condition which presents with severe generalized and possibly painful hyperkinetic dystonic spasms. Patients with an underlying history of primary or secondary dystonia are prone to this condition, and stressors such as trauma, infection or surgical intervention can trigger the dystonic spasms. The optimum treatment for this condition is not established, but a reasonable strategy is to use an aggressively increasing approach beginning with oral medications, then graduating to intravenous and intrathecal medications, then switching to deep sedation or anesthesia, and finally culminating with surgery [66]. Dopamine blockade with non-selective agents such as pimozide, risperidone, olanzapine or haloperidol and sedation with propofol and midazolam, have all been reported successful for the short term control of symptoms, and for improving quality of life. Manji et al. has reported that triple therapy with oral tetrabenazine, high-dose benzhexol, and pimozide is effective especially in children [67,68]. However, dystonic

storms may be unusually refractory to some oral medications. Patients with dystonic storms should be admitted to the intensive care unit (ICU) due to the possibility of accompanying respiratory compromise, hyperthermia, dehydration, and rhabdomyolysis resulting in potential renal failure. Deep sedation or anesthesia with endotracheal intubation may be required for refractory cases. Additionally, there has been at least one case of a patient with a dystonic storm successfully treated with intrathecal baclofen [69]. DBS or pallidotomy may be an option of last resort in cases where symptoms continue for many weeks/months [70,71]. Clinicians should be aware that postoperative infections and certain medications (dopamine blockers, antiemetics, etc.) can postoperatively induce movement disorders especially in patients with pre-existing basal ganglia damage.

4. Conclusion

Knowledge of potential DBS urgencies and emergencies can in many cases enhance outcomes. More intra- and postoperative urgencies and emergencies continue to be identified as the DBS field expands. Clinician's handling DBS in their practices should be versed in the identification and management of surgery/procedure related, hardware related, and stimulation-induced issues.

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Development/Plasticity/Repair

Nurr1 Is Required for Maintenance of Maturing and Adult Midbrain Dopamine Neurons

Banafsheh Kadkhodaei,[1] Takehito Ito,[2] Eliza Joodmardi,[1] Bengt Mattsson,[3] Claude Rouillard,[1] Manolo Carta,[3] Shin-Ichi Muramatsu,[4] Chiho Sumi-Ichinose,[5] Takahide Nomura,[5] Daniel Metzger,[6] Pierre Chambon,[6] Eva Lindqvist,[7] Nils-Göran Larsson,[8,10] Lars Olson,[7] Anders Björklund,[3] Hiroshi Ichinose,[2] and Thomas Perlmann[1,9]

[1]Ludwig Institute for Cancer Research, Stockholm Branch, SE-171 77 Stockholm, Sweden, [2]Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 226-8501, Japan, [3]Wallenberg Neuroscience Center, Lund University, SE-221 84 Lund, Sweden, [4]Department of Neurology, Jichi Medical University, Tochigi 329-0498, Japan, [5]Department of Pharmacology, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan, [6]Department of Functional Genomics Institut de Génétique et Biologie Moléculaire et Cellulaire, 67404 Illkirch, France, Departments of [7]Neuroscience, [8]Laboratory Medicine, and [9]Cell and Molecular Biology, Karolinska Institutet, SE-171 77 Stockholm, Sweden, and [10]Max Planck Institute for Biology of Ageing, D-50931 Cologne, Germany

Transcription factors involved in the specification and differentiation of neurons often continue to be expressed in the adult brain, but remarkably little is known about their late functions. Nurr1, one such transcription factor, is essential for early differentiation of midbrain dopamine (mDA) neurons but continues to be expressed into adulthood. In Parkinson's disease, Nurr1 expression is diminished and mutations in the *Nurr1* gene have been identified in rare cases of disease; however, the significance of these observations remains unclear. Here, a mouse strain for conditional targeting of the *Nurr1* gene was generated, and *Nurr1* was ablated either at late stages of mDA neuron development by crossing with mice carrying Cre under control of the dopamine transporter locus or in the adult brain by transduction of adeno-associated virus Cre-encoding vectors. *Nurr1* deficiency in maturing mDA neurons resulted in rapid loss of striatal DA, loss of mDA neuron markers, and neuron degeneration. In contrast, a more slowly progressing loss of striatal DA and mDA neuron markers was observed after ablation in the adult brain. As in Parkinson's disease, neurons of the substantia nigra compacta were more vulnerable than cells in the ventral tegmental area when *Nurr1* was ablated at late embryogenesis. The results show that developmental pathways play key roles for the maintenance of terminally differentiated neurons and suggest that disrupted function of Nurr1 and other developmental transcription factors may contribute to neurodegenerative disease.

Introduction

Adaptation to a changing environment requires plasticity in the adult CNS. However, to ensure that neurons are properly maintained, such plasticity must be balanced against mechanisms that counteract phenotypic instability. Studies of how neurons develop may help to unravel functions important for the stability of nerve cells as factors promoting their differentiation may also contribute to their maintenance. Indeed, many transcription factors identified for their critical roles during neuronal development continue to be expressed in the postnatal nervous system, raising the possibility that they contribute to the integrity of already differentiated neurons (Hendricks et al., 1999; Vult von Steyern et al., 1999; Kang et al., 2007; Alavian et al., 2008). However, the consequences of adult gene ablation of any of these factors have not yet been reported, and very little is known of their functions in differentiated neurons.

From a clinical perspective, it is of particular interest to identify factors that maintain stability of neurons that are affected in neurodegenerative disorders as loss of phenotype would likely cause or contribute to disease. Parkinson's disease (PD) is characterized by progressive pathology of midbrain dopamine (mDA) neurons of substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), typically involving deposition of α-synuclein-rich cytoplasmic protein aggregates termed Lewy bodies. During development, early signaling events induce transcription factors that control the specification and differentiation of mDA neurons (Smidt and Burbach, 2007). Several of these factors, including Nurr1, Lmx1a, Lmx1b, Pitx3, FoxA2, and En1/2, continue to be expressed in the postnatal and adult brain (Zetterström et al., 1996; Smidt et al., 1997, 2000; Alberi et al., 2004; Simon et al., 2004; Kittappa et al., 2007). Nurr1, belonging to a family of ligand-independent nuclear receptors (Wang et al.,

Received Aug. 11, 2009; revised Oct. 17, 2009; accepted Oct. 28, 2009.

This work was supported by grants from the Michael J. Fox Foundation (T.P., A.B., L.O.), Vetenskapsrådet via Linné Center DBRM (T.P.), Grants-in-Aid for Human Frontier Science Program (P.C., D.M., H.I.), Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, the Ministry of Health, Labor, and Welfare of Japan, and the Japan Science and Technology Agency, Core Research for Evolutional Science and Technology (S.-I.M., H.I.), Vetenskapsrådet (L.O.), Swedish Brain Power (L.O.), Swedish Brain Foundation (L.O.), and the Swedish Parkinson Foundatin (L.O.). We are grateful to Johan Ericson and members of the Perlmann and Ericson laboratories for valuable discussions. We thank Andrée Dierich and Jean-Marc Bornert for their help in mouse mutagenesis, Noriko Ihira for help in construction of the targeting vector and screening of ES clones, Björn Anzelius and Naomi Takino for their help with AAV vectors, Ulla Jarl for help with histology, and Marie-Louise Alun for advice on mice handling.

Correspondence should be addressed to either of the following: Thomas Perlmann, Department of Cell and Molecular Biology, Karolinska Institutet, SE-171 77 Stockholm, Sweden, E-mail: thomas.perlmann@licr.ki.se; or Hiroshi Ichinose, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 226-8501, Japan, E-mail: hichinos@bio.titech.ac.jp.

DOI:10.1523/JNEUROSCI.3910-09.2009

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2003; Perlmann and Wallén-Mackenzie, 2004), becomes expressed in developing mDA neurons that have just exited the cell cycle and is essential for mDA neuron development because mDA neurons of both the SNc and VTA fail to express dopaminergic markers and newborn *Nurr1*-null mice lack mDA neuron cell bodies and their striatal projections (Zetterström et al., 1997; Castillo et al., 1998; Saucedo-Cardenas et al., 1998). How Nurr1 regulates target genes in mDA neuron development remains essentially unknown but may involve a functional interaction with the homeobox transcription factor Pitx3 (Jacobs et al., 2009).

Determining the role of Nurr1 also in the adult brain is of particular importance because previous studies suggested an association of this protein with PD pathology. Nurr1 expression is diminished in neurons with α-synuclein inclusions in postmortem PD brain tissue, and *Nurr1* mutations and polymorphisms have been identified in rare cases of PD (Xu et al., 2002; Le et al., 2003; Zheng et al., 2003; Grimes et al., 2006). However, the significance of genetic lesions remain unclear (Wellenbrock et al., 2003; Hering et al., 2004; Tan et al., 2004). These observations emphasize the importance of elucidating the role of Nurr1 in more mature mDA neurons by analyzing the consequences of conditional *Nurr1* gene ablation in mice.

Materials and Methods

Conditional Nurr1 gene-targeted mice. Mouse 129SV genomic library constructed in bacterial artificial chromosome (BAC) was screened by PCR. A BAC clone containing the entire Nurr1 gene was selected, and a BamHI–MunI fragment containing exon 1 to exon 5 was recloned into a pBluescript II vector. A floxed neomycin cassette was inserted into an internal EcoRI site located in intron 3, and a synthetic loxP sequence was inserted at SalI site located in intron 2. Mouse embryonic stem (ES) cells were electroporated with the targeting vector, and the homologously recombinated clones were screened by PCR and Southern blot analysis. ES clones with three loxP sites were selected, and a plasmid expressing Cre DNA recombinase was transiently transfected into the cells. ES cells with two loxP sites without a neomycin cassette were selected by PCR and used for production of chimeric mice.

Animals. Mice were kept in rooms with controlled 12 h light/dark cycles, temperature, and humidity, with food and water provided *ad libitum*. All animal experiments were performed with permission from the local animal ethics committee. The generation of dopamine transporter (*DAT*)–*Cre* mutant mice has been described previously (Ekstrand et al., 2007). Mice were mated during the night, and the females were checked for vaginal plugs in the morning [day of vaginal plug considered as embryonic day 0.5 (E0.5)].

L-3,4-Dihydroxyphenylalanine treatment. Methyl L-3,4-dihydroxyphenylalanine (L-DOPA) hydrochloride and the peripheral DOPA decarboxylase inhibitor benserazide-HCl (Sigma-Aldrich) were dissolved in Ringer's solution immediately before use. L-DOPA was intraperitoneally given every second day at the dose of 2.5 mg/kg combined with 0.625 mg/kg benserazide. Chronic treatment with L-DOPA/benserazide was administered for 50 d, starting at postnatal day 15 (P15). During this period, the mice were carefully observed and weight was measured regularly. Reported hyperactivity was observed in $cNurr1^{DATCre}$ mice when given a single higher dose of L-DOPA (25 mg/kg L-DOPA, 6.25 mg/kg benserazide).

Histological analyses. At embryo stages, embryos were fixed for 2–24 h in 4% phosphate-buffered paraformaldehyde (PFA), cryopreserved in 30% sucrose before being embedded in OCI (Sakura Finetek), and cryosectioned at a thickness of 10–20 μm onto slides (SuperFrostPlus; Menzel Gläser). For the isolation of brains for immunolabeling at P15 and onward, animals were anesthetized with Avertin (tribromoethanol; 0.5 mg/g) and perfused through the left ventricle with body-temperature PBS, followed by ice-cold 4% PFA. The brains were dissected and post-fixed overnight in 4% paraformaldehyde and subsequently cryoprotected for 24–48 h in 30% sucrose at 4°C. The brains were serially sectioned on a cryostat or sliding microtome at 10–30 μm. Littermates were used in an all comparative experiments.

For immunohistochemistry, sections were preincubated for 1 h in blocking solution containing either 10% normal goat sera or 5–10% bovine serum albumin, 0.25% Triton X-100, and 0.01% Na-azide in PBS. Primary antibodies diluted in blocking solution were applied overnight at 4°C. After rinses with PBS, biotinylated- or fluorophore-conjugated secondary antibodies diluted in PBS were applied for 1 h at room temperature. Biotinylated secondary antibodies were followed by incubation with streptavidin–horseradish peroxidase complex (ABC elite kit, Vectastain) for 1 h and subsequent exposure to diaminobenzidine (DAB kit; Vector Laboratories). Primary antibodies and dilution factors were as follows: rabbit anti-Nurr1 (1:100; M196; Santa Cruz Biotechnology), anti-Nurr1 (1:250; E20; Santa Cruz Biotechnology), rabbit anti-tyrosine hydroxylase (TH) (1:500; Pel-Freeze), rat anti-DAT (1:2000; Millipore Bioscience Research Reagents), mouse anti-TH (1:200; Millipore Bioscience Research Reagents), rabbit anti-vesicular monoamine transporter (VMAT) (1:500; Millipore Bioscience Research Reagents), rabbit anti-L-DOPA decarboxylase (AADC) (1:500; Millipore Bioscience Research Reagents), rabbit anti-Cre (1:10,000; Covance Research Products), guinea pig anti-Lmx1b (1:1000) (Andersson et al., 2006), and rabbit anti-Pitx3 (Smidt et al., 2004). In some cases (anti-AADC, anti-VMAT, and anti-Nurr1), the blocking steps were performed after antigen retrieval (Dako). Finally, expression was detected by secondary antibodies from Jackson ImmunoResearch. Section images were collected by confocal microscopy (Leica DMIRE2) and bright-field microscopy (Eclipse E1000K; Nikon). Cell counting was performed by counting all SNc DA neurons detected by immunohistochemistry (DAB) in a total of three sections per animal (every 12th tissue section) within the ventral midbrain of animals taken at 4 months after vector injection in both wild-type wt^{AAVCre} and $cNurr1^{AAVCre}$ animals. The mean of counted cells per animal was established from both the injected and non-injected sides in each animal, and the relative decrease was calculated as a percentage as described in Results.

AAV–Cre injections. Two and a half- to 5-month-old animals received one unilateral stereotaxic injection in the right striatum using a 10 μl Hamilton microsyringe fitted with a glass pipette tip. The animals were anesthetized with isoflurane, 1 μl was injected during 5 min, and the cannula was left in place for an additional 2 min before being slowly retracted. The anteroposterior and mediolateral coordinates from bregma were −2.8 and −1.1 mm, respectively, and the dorsoventral coordinates from the dura were −4.3 mm. Animals were killed 0.5, 1.5, and 4 months after injection, and the brains were isolated.

Measurement of tissue content for dopamine, serotonin, and their metabolites. In supplemental Tables 1–3 (available at www.jneurosci.org as supplemental material), tissues were collected from P1, P7, P14, and adult (P48) wt^{DATCre} and $cNurr1^{DATCre}$ mice. One- to 14-d-old mice were killed by decapitation, and P48 mice were killed by cervical dislocation. Brains were rapidly removed, chilled in saline (4°C), dissected, frozen on dry ice, and stored at −80°C until use. To process tissues for HPLC and electrochemical detection of monoamines and metabolites, samples were homogenized by sonication in 5 vol or in 30 μl of 0.1 M perchloric acid, followed by centrifugation. Endogenous levels of noradrenaline, DA, 3,4-dihydroxyphenylacetic acid, homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid were determined in the supernatants. A reverse column (BAS, C-18, 100.0 × 3.2 mm, 3 μm particle diameter) was used for separation. The mobile phase consisted of 0.05 M sodium phosphate/0.03 M citric acid buffer containing 0.1 mM EDTA and was adjusted with various amounts of methanol and sodium-L-octane sulfonic acid. The flow rate was 0.3 ml/min. Monoamines and metabolites were detected using a glassy-carbon electrode detector, which as set at +0.7 V versus an Ag/AgCl reference electrode. Resultant peaks were measured and compared with repeated control samples containing fixed mixed amounts of compounds of interest.

In supplemental Table 4 (available at www.jneurosci.org as supplemental material), all animals were killed, and striata and cortex were rapidly dissected out, frozen on dry ice, and stored at −80°C. To determine monoamines, tissue was homogenized in 0.1 M perchloric acid and centrifuged at 10,000 rpm for 10 min before filtering though minispin filters for an additional 3 min at 10,000 rpm. The tissue extract were then analyzed by HPLC as described previously (Carta et al., 2007) with minor