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REVIEW

Transcription factor Pitx3 mutant mice as a model for Parkinson's disease

Fu-Ming Zhou (✉)[1], Li Li[1], Juming Yue[2], John A. Dani[3]

[1] *Department of Pharmacology, University of Tennessee College of Medicine, Memphis, TN 38103, USA*
[2] *Department of Pathology, University of Tennessee College of Medicine, Memphis, TN 38103, USA*
[3] *Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA*

BACKGROUND: Parkinson's disease (PD) is a common, age-dependent degenerative neurological disorder impairing motor control function and cognition. A key pathology of PD is a degeneration of the nigrostriatal dopamine system, leading to a severe dopamine denervation in the striatum and dysfunction of the striatal neural circuits.
OBJECTIVE: To better understand the pathophysiology of the nigrostriatal dopamine denervation and to discover better treatments, animal PD models are needed.
METHODS: The authors' original research on the transcription factor Pitx3 null mutant mice and the relevant literature were reviewed.
RESULTS: An important feature of an animal PD model is the severe, PD-like nigrostriatal dopamine denervation. This feature is provided in the transcription factor Pitx3 null mutant mice. These mice have a severe and bilateral nigral dopamine neuron loss and dopamine denervation in the dorsal striatum, while the dopamine neuron loss in the ventral tegmental area and dopamine denervation in the ventral striatum are moderate, creating a dorsal-ventral dopamine loss gradient and mimicking the dopamine denervation pattern in PD. Pitx3 null mice show motor function deficits in the balance beam and pole tests and these deficits are reversed by L-3,4-dihydroxyphenylalanine (L-dopa). These mice also show impaired cognitive functions as indicated by reduced motor learning and avoidance memory. L-dopa, D1 agonists and, to a lesser extent, D2 agonists, induce normal horizontal movements (walking) and also dyskinesia-like movements consisting of vertical body trunk movements and waving paw movements.
CONCLUSIONS: The easy-to-maintain Pitx3 null mice with an autogenic, consistent and gradient dopamine denervation are a convenient and suitable mouse model to study the consequences of dopamine loss in PD and to test dopaminergic replacement therapies for PD.

Keywords Animal model, basal ganglia, L-3, 4-dihydroxyphenylalanine (L-dopa), dopamine receptor supersensitivity, 6-hydroxydopamine (6-OHDA), Parkinson's disease, Pitx3, striatum, substantia nigra

Introduction

Parkinson's disease (PD) is a common degenerative neurological disorder caused by a severe loss of the nigral dopamine neurons and the nigrostriatal dopaminergic projection, leading to the cardinal motor deficits and symptoms including resting tremor, a slowness and paucity of movements, muscle rigidity and postual imbalance (Parkinson, 1817; Kish et al., 1988; Hornykiewicz, 2001; Braak et al., 2004; Kordower et al., 2013; Del Tredici and Braak, 2016). Epidemiological studies indicate that the PD prevalence is similar in the same age groups in developed and developing countries; as the world's population grows in size and age due to increased births and life expectancy, the PD population is also increasing (Samii et al., 2004; de Lau and Breteler, 2006). Current pharmacological therapies for the motor symptoms are not ideal and can induce significant side effects (Bastide et al., 2015; Beaulieu-Boire and Lang, 2015). There is no effective treatment for cognitive deficits. Thus, better treatments are needed to better control the motor and nonmotor (cognitive) symptoms in PD and also to slow the disease process. This requires the determination of pathophysiological consequences of the nigrostriatal DA

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Correspondence: Fu-Ming Zhou
E-mail: fzhou3@uthsc.edu

denervation and understanding how the PD symptoms are produced at molecular, cellular and circuit levels. It also requires testing new compounds/potential anti-PD drugs. All these lines of research and developlemt require animal models. Thus, suitable animal models are important. Currently, there is no perfect animal model that can mimic all aspects of PD and has both face, construct, and predictive validity (Chesselet and Richter, 2011). Hence, selection of animal models depends on the experimental questions, for example, to study the consequences of the severe DA denervation in PD, the animal model must have consistent, severe DA loss.

The striatum and its dopamine system

The striatum is a large subcortical structure. While a countinous nucleus in rodents, the striatum in primates including humans comprises the putamen and the caudate nucleus, but the striatal neuron types and the neuronal properties are similar or identical in rodents and primates (Gerfen and Bolam, 2010; Oorschot, 2010). The striatum receives excitatory glutamatergic inputs from motor and somatosensory cortices and other cortical areas (Doig et al., 2010; Gerfen and Bolam, 2010; Huerta-Ocampo et al., 2014; Deng et al., 2015) and also from the thalamus (Gerfen and Bolam, 2010; Huerta-Ocampo et al., 2014; Smith et al., 2014). 90%-95% of the neurons in the striatum are the GABAergic medium spiny neurons (MSNs). MSNs are the projection neurons of the striatum. One group of MSNs heavily express dopamine D1 receptors (D1Rs) and project to and inhibit the high-frequency firing GABAergic neurons in the globus pallidus internal segment (GPi) and the substantia nigra pars reticulata (SNr)-the 2 output nuclei of the basal ganglia, forming the direct pathway (Gerfen and Bolam, 2010; Zhou, 2016). The other group of MSNs heavily express D2Rs and project to and inhibit the high frequency firing GABAergic neurons in the globus pallidus external segment (GPe), forming the indirect pathway. Evidence indicates that D1-MSN activity and the consequent striatonigral output facilitate movement, while D2-MSN activity and the striatopallidal output inhibit movement (Kravitz et al., 2010; Redgrave et al., 2010; Sano et al., 2013; Friend and Kravitz, 2014; Chiken et al., 2015). Evidence also indicates that MSN activity is critical to cognition (Simpson et al., 2010; Rothwell et al., 2014), habit learning and formation (Graybiel and Grafton, 2015), and emotional and motivational regulation (Haber and Knutson, 2010; Sesack and Grace, 2010; Lobo et al., 2013, Révy et al., 2014; Francis et al., 2015; Ikemoto et al., 2015).

A distinct feature of the striatum is the dense axon terminals arising from the substantia nigra DA neurons projecting to the dorsal striatum and the ventral tegmental area DA neurons projecting to the ventral striatum (Fig. 1). Though the number of cell somata is quite small (German and Manaye, 1993, Nelson et al., 1996; Oorschot 1996, 2010; Hardman et al., 2002), the projection axons of these DA neurons bifurcate repeatedly in the striatum, eventually forming an extremely dense DA axon network in both rodents and primates including humans (Levey et al., 1993; Ciliax et al., 1999; Lewis et al., 2001; Matsuda et al., 2009; Ding et al., 2015); matching the dense DA innervation, the expression levels of D1Rs and D2Rs in the striatum are also extremely high, the highest in the brain (Levey et al., 1993; Yung et al., 1995; Hurd et al., 2001), providing the anatomical substrate for intense DA signaling in the striatum and for DAs profound behavioral effects.

Motor and behavioral functions of the striatum and the nigrostriatal DA system

The motor function of the striatum is most vividly demonstrated when the striatum becomes dysfunctional and loses its normal function, e.g., a key symptom of Huntington's disease is the increased abnormal choreic movements (Walker, 2007), likely due to the loss of striatal medium spiny neurons, those in the indirect pathway in particular (Mitchell et al., 1999; Glass et al., 2000; Walker, 2007; Obeso et al., 2014). Consistent with these clinicopathological data, experimental ablation or inactivation of indirect pathway medium spiny neurons increases motor activity (Sano et al., 2003; Durieux et al., 2009, 2012; Bateup et al., 2010; Sano et al., 20013; Chiken et al., 2015). HD patients also have behavioral and congitive deficits, indicating behavioral and cognitive functions of MSNs, although the severe cognitive deficits in late stage HD likely involve cortical abnormalities (Walker, 2007).

DA activity in the striatum is required for normal motor function in both rodents and primates. This is demonstrated by the fact that local drug infusion into the striatum to block striatal DA receptors is sufficient to induce PD-like akinesia (Franco and Turner, 2012). Local toxin infusion into the striatum to lesion the striatal DA innervation also leads to motor deficits in animals (Lee et al., 1996; Kirik et al., 1998; Bagga et al., 2015). These studies confirm the classic view that the striatal DA denervation is the main cause of PD motor symptoms, consistent with the fact that DA innervation and DA receptor expression are highly concentrated in the striatum (Fig. 2). Thus, the dopamine system's main motor-promoting function is mediated by D1-MSNs and D2-MSNs including their projections, although dopaminergic activity in neurons in other brain areas may contribute additional complexity to motor control and parkinsonism. These conclusions are further supported by the facts that a total inhibition of L-dopa synthesis in brain DA neurons leads to akinesia and being to unable to feed and drink, and thus is leathal when exogenous L-dopa is not provided (Zhou and Palmiter, 1995). Inhibition of DA release by blocking action potential propagation of the nogrostriatal DA axons in the

medial frorebrain bundle also induces akinesia (Galati et al., 2009). Ablation of D1-MSNs reduces motor activity, whereas ablation of D2-MSNs increases motor activity (Sano et al., 2003; Durieux et al., 2009, 2012; Sano et al., 2013; Révy et al., 2014; Chiken et al., 2015).

PD animal models

Animal models are needed to understand the etiology, pathogenesis and pathophysiology of human diseases and also to test therapeutic drugs, as amply demonstrated by the discovery of L-dopa treatment for PD (Carlsson, 2001; Fahn, 2015; Lees et al., 2015). Ideally, an animal PD model needs to have face validity to mimic PD's clinical symptoms and DA denervation and tau pathology, construct validity to mimic the etiology of DA denervation and tau pathology, and predictive validity to predict the therapeutic effects of the drug based on its effect in the animal model. However, due to the unknown etiology and the complexity of PD, no animal PD model meets all these requirements. For example, in the classic 6-OHDA unilateral nigrostriatal DA lesion model, L-dopa induces a striking, contraversive or asymmetric rotation toward the DA intact side (Ungerstedt, 1971;Schwarting and Huston, 1996), providing a motor phenotype or face validity for studying PD pathophysiology and testing new therapeutic motor-stimulating dopaminergic drugs (Lane et al., 2006; Marin et al., 2006; Thiele et al., 2011). However, toxin models do not mimic the disease mechanism of sporadic PD and hence lack construct validity. Furthermore, 6-OHDA lesion requires manual stereotaxic intracranial injection that is not only time-consuming but also has unavoidable variations in the surgery among individual animals such that post-mortem neurochemical and immunohistochemical examinations are needed to verify the extent and severity of the DA lesion (Schwarting and Huston, 1996; Thiele et al., 2011), another time-consuming procedure. Thus a simpler animal model with a predefined, autogenic and consistent DA denervation can complement the 6-OHDA model, would make more effective use of research resources, and render the testing of motor-stimulating compounds for DA replacement therapy more efficient and reliable.

Genetic models such as mutant alpha synuclein over-expression partially mimic the etiology of sporadic PD (Chen et al., 2015) but do not mimic the severe parkinsonian DA denervation such that dopaminergic drugs produce little motor stimulation (Chen et al., 2015). Thus, the consensus is that there is currently no perfect animal PD model, but insights, knowledge, therapeutic testing can still be obtained in imperfect animal models when the chosen model matches the experimental question (Chesselet and Richter, 2011), for example, the 6-OHDA lesion model is suitable for testing a dopaminergic drug's motor stimulating effect but useless for studying the etiology of DA neuron degeneration. Conversely, alpha synuclein overexpression model may be useful for studying the etiology of PD but not useful for testing a dopaminergic drug's motor stimulating effect because the residual striatal DA in the striatum is $\geqslant 50\%$ of the normal level, and dopaminergic drug does not produce any major motor stimulation (Chen et al., 2015) due to the compensation of the residual DA innervation (Schwarting and Huston, 1996).

The discovery of the Pitx3 null mutant mouse

Working at The Jackson Laboratory in Bar Habor, Maine, Varnum and Stevens (1968) reported the spontaneously occurred mutant aphakic or eyeless mice, first discovered by their laboratory assistant Hannah Cunningham, in a colony of 129/Sv-SlJ mice. Because the offspring of the original 129/Sv-SlJ aphakic mice were weak and had reduced fertility, Varnum and Stevens (1968) outcrossed the original aphakic male mouse to a C57BL/6 female mouse, eventually leading to the current C57BL/6 mouse-based aphakic mice maintained at The Jackson Laboratory (Jackson stock number 000942) (Fig. 1). The discovery of aphakic mice is another example that careful and reliable observations are of the paramount importance and where these observations are published really does not matter.

Thirty years after the initial discovery of Varnum and Stevens (1968), the molecular genetic basis for the spontaneous eyeless mutation was established as a null mutation of the homeobox transcription factor Pitx3 gene in these eyeless, i.e, $Pitx3^{-/-}$, mice (Semina et al., 1997, 1998, 2000). Pitx3 has a high degree of homolog to the pituitary transcription factors Pitx1 and Pitx2, but unlike Pitx1 and Pitx2, the pituitary is not the main expression site for Pitx3. Soon, it was found that in the mouse brain, the Pitx3 gene is selectively expressed in midbrain DA neurons in the brain (Smidt et al., 1997).

Since they are aphakic, $Pitx3^{-/-}$ (Pitx3 null) mice are easily and reliably identified (Fig. 1). The genotypes can also be determined by PCR-based genotyping to identify WT, homozygotes, and heterozygotes, using the published PCR primer sequences (Li et al., 2013). Since Pitx3 gene is recessive, heterozygous $Pitx3^{+/-}$ mice and WT $Pitx3^{+/+}$ mice are identical in appearance and cannot be separated visually; the nigrostriatal dopamine system is normal in $Pitx3^{+/-}$ mice (Hwang et al., 2003; Nunes et al., 2003; van den Munckhof et al., 2003; unpublished data of FM Zhou); the motor response to dopaminergic stimulation (systemic injection of L-dopa) in heterozygous $Pitx3^{+/-}$ mice is minimal, if any, like the L-dopa response in in WT mice, due to the fact that there is large amounts of endogenous L-dopa in the striatum in $Pitx3^{+/-}$ mice such that exogenous L-dopa does not increase DA release.

Based on our experience, C57BL/6-based Pitx3 null mice are robust and fertile without the need of any special care. $Pitx3^{-/-}$ parents produce about 8 $Pitx3^{-/-}$ male and female

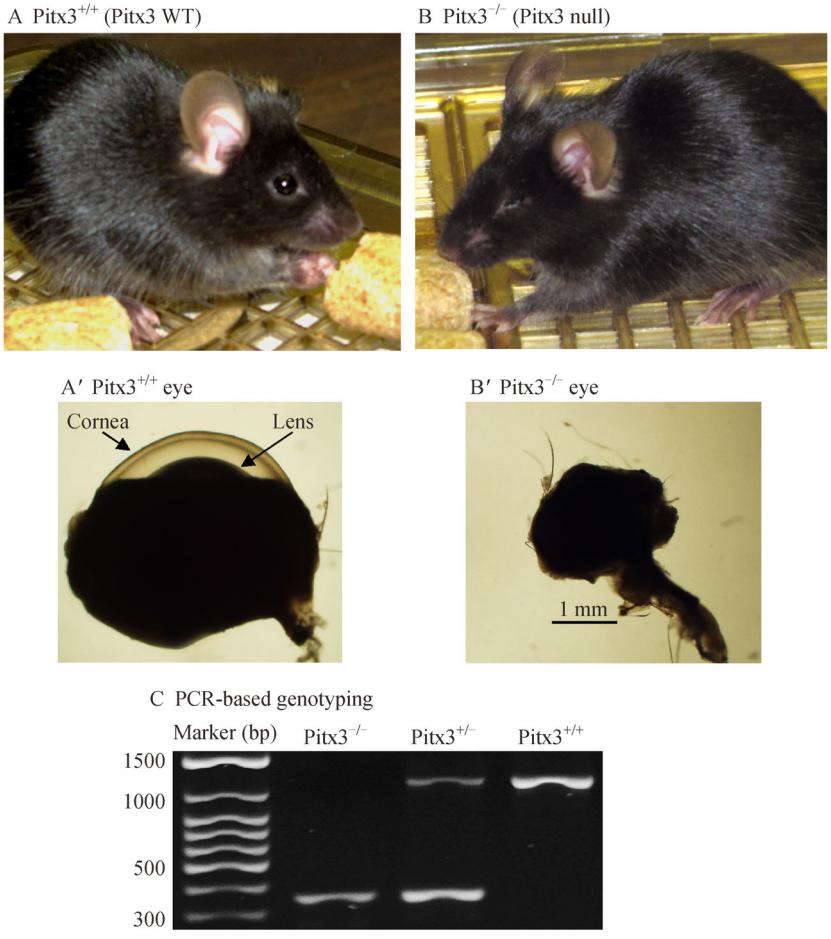

Figure 1 Visual identification of Pitx3$^{+/+}$ (Pitx3 WT) mice and Pitx3$^{-/-}$ mutant (Pitx3 null) mice. (A) Pitx3 WT mouse. (B) A Pitx3 null mouse. These 2 example mice were of 35 days old. Pitx3 WT and Pitx3 null mice look identical except that Pitx3 null mice have malformed eyes that clearly identified Pitx3 null mice on or after postnatal day 14 when Pitx3 WT and Pitx3 null mice open their eyes. A' and B' show that, compared with the Pitx3 WT mouse, the eye of the Pitx3 null mouse is much smaller. The scale in B' applies to A'. However, Pitx3Null mice eat, drink, are fertile, and do not need any special care. (C) PCR-based genotyping identifies Pitx3 WT, Pitx3$^{-/-}$ homozygotes, and Pitx3$^{+/-}$ heterozygotes using the following primers: TTCTACCGAGGAAAGCTGGA and TGCTTTGCTGGA-CATGGTAG. A-B from Wei et al., 2013, C from Li et al., 2013.

pups per birth, all pups survive and grow into adults, although Pitx3$^{-/-}$ pups, juveniles, young adult and mature adults are about 20% smaller than age-matched Pitx3$^{+/-}$ and Pitx3$^{+/+}$. Before 18 months of age, the mortality of Pitx3 null mice is similar to WT mice, but after 20 months of age, Pitx3$^{-/-}$ mice have a higher mortality rate; about 50% of Pitx3$^{-/-}$ mice can live to 30 months of age.

In humans, homozygous Pitx3 null mutation is apparently rare such that so far there is only one report of Pitx3 null mutation in humans (Bidinost et al., 2006). This report documented 2 young adult Pitx3 null Lebanese brothers of consanguineous parents: besides eye malformation, these 2 brothers were intellectually impaired and wheelchair bound; they showed postural imbalance when forced to stand upright; they also showed choreic movements and other abnormalities in motor control. These clinical observations in humans indicate that Pitx3 may have more functions and effects in the human brain than in the mouse brain, although it is also possible that adverse effects from Pitx3 null mutation are more difficult to detect in mice, for example, impaired intellect is more difficult to detect in mice than in humans.

The nigral DA neuron loss in Pitx3 null mice

Six years after the discovery of the selective Pitx3 expression in midbrain DA neurons in the mouse brain (Smidt et al., 1997), 4 laboratories published almost simultaneously that midbrain DA neurons are selectively lost in Pitx3$^{-/-}$ mice (Hwang et al., 2003; Nunes et al., 2003; van den Munckhof et al., 2003; Smidt et al., 2004). Specifically, these studies indicated that when the Pitx3 null mouse reaches a juvenile age, most nigral DA neurons are lost, while about 50% of DA neurons in the VTA survive (Hwang et al., 2003; Nunes et al., 2003; van den Munckhof et al., 2003; Luk et al., 2013),

producing a DA loss pattern that resembles that in PD (McRitchie et al., 1997; Damier et al., 1999; Hornykiewicz 1998, 2001; Kordower et al., 2013). Further, van den Munckhof et al. (2003) reported that only Pitx3-expressing DA neurons (most ventral nigral DA neurons and about 50% VTA DA neurons) are lost in Pitx3 null mice, while VTA DA neurons not expressing Pitx3 survive in Pitx3 null mice, indicating a differential Pitx3 expression in mouse midbrain DA neurons with some DA neurons in the dorsal substanita nigral and 50% VTA DA neurons being Pitx3-independent. It has also been reported that Pitx3 is expressed in human midbrain DA neurons (Reyes et al., 2013) and Pitx3-expressing DA neurons were lost in human PD brains (Smidt et al., 1997), though quantitative data were not provided and a differential loss of nigral vs. VTA DA neurons was not examined in that study (Smidt et al., 1997).

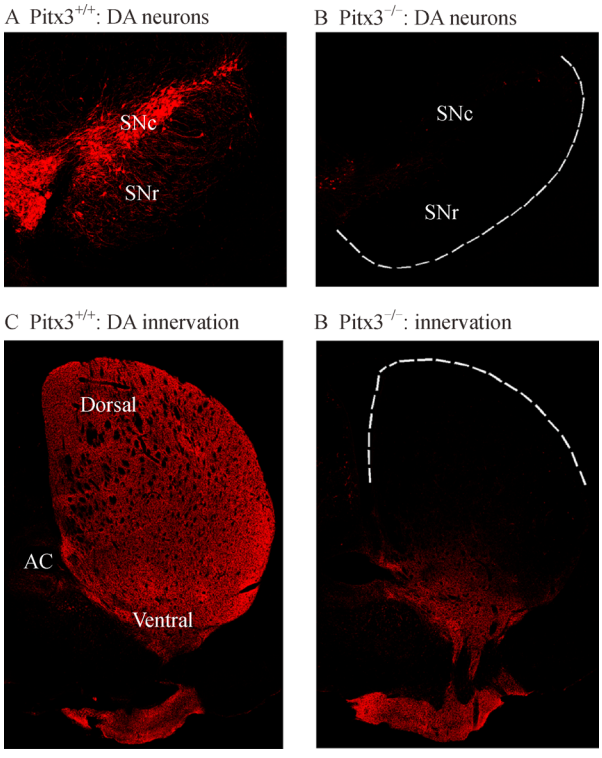

Figure 2 The PD-like DA neuron loss pattern in Pitx3 null mice. (A) Tyrosine hydroxylase (TH)-immunostained midbrain DA neurons in a Pitx3 WT mouse. Note the dense DA dendritic network in the substantia nigra pars reticulate (SNr) in Pitx3 WT (wild-type) mice. (B) TH-immunostained mibrain DA neurons in a Pitx3 null mouse. (C) Dense DA innervation in the entire striatum in a Pitx3 WT mouse. AC, anterior commissure. (D) Severe (95-99%) DA axon loss in the dorsal striatum in a Pitx3 null mouse. The ventral striatum has about 50% residual DA axons. Modified from Ding et al., 2015.

Some issues and inconsistency remain about the expression of Pitx3 in DA neurons. Reyes et al. (2013) reported that in the mouse brain, 95% of the DA neurons in the substantia nigra and 80% of the DA neurons in the VTA express Pitx3 (Reyes et al., 2013), and there was no Pitx3 differential expression in among nigral DA neurons and between nigral DA neurons and VTA DA neurons. Korotkova et al. (2005) reported similar results in mice. Further, it was reported that 95% of DA neurons in the dorsal and ventral substantia nigra and in the VTA express Pitx3 in postmortem human brains of ~ 80 years old indivuduals without PD or other neurodegenerative or neuropsychiatric disorders, and there was no Pitx3 differential expression in nigral and VTA DA neurons (Reyes et al., 2013), partially contradicting the previously reported differential Pitx3 expression (van den Munckhof et al., 2003). Thus, further studies are needed to determine the potential differential expression of Pitx3 in DA neurons in the dorsal and ventral substantia nigra and in the VTA DA neurons in animals, and more importantly in PD brains in humans; and it is also needed to determine if Pitx3-expressing DA neurons are selectively lost in PD and the underlying cellular and molecular mechanisms. Furthermore, genetic epidemiological studies suggest genetic vairants of Pitx3 may confer a risk factor to PD (Guo et al., 2011), while other studies reported negative results (Jiménez-Jiménez et al., 2014). One clinical report on 2 brothers with homozygous Pitx3 mutation clearly indicates that the human brain does not function properly when Pitx3 function is lost, leading to severe motor and cognitive deficits (Bidinost et al., 2006). In the absence of detailed brain pathological examination, the potential Pitx3 loss-induced structural abnormalities in the brains of these 2 patients and hence the normal Pitx3 function in the normal human brain are unknown.

Regardless, the selective loss of the nigrostriatal DA neurons in Pitx3 null mice still has a face validity for the nigrostriatal DA denervation in PD, i.e., these mice can be used to study the consequences of DA loss and screen for dopaminergic motor-stimulating drugs.

The parkinsonian-like, gradient striatal DA denervation in Pitx3 null mice

Postmortem studies in PD patients have established that when akinesia first appears in early stage PD patients, 80% of the tissue DA content in the striatum (the putamen) is already lost; smaller DA losses do not lead to akinesia; the DA loss in the putamen is at 88% level for mild akinesia and at a 99% level for marked akinesia (Hornykiewicz, 2001). In late stage PD, the DA loss in the dorsal putamen is a staggering 99%, leading to severe akinesia (Hornykiewicz, 2001; Kordower et al., 2013), whereas the nucleus accumbens retains up to 50% of the normal DA level (Kish et al., 1988; Hornykiewicz, 1998, 2001). These clinicopathological data not only indicate a strong compensatory mechanism in the striatal DA system, but also suggest that an animal PD model with severe DA loss offers a good face validity. This is consistent with experimental animal data that show that 90% DA loss in the striatum is required to produce motor deficits and DA

supersensitivity (Shwarting and Huston, 1996); recent studies indicate that only 5% normal DA level in the striatum can maintain motor function (Darvas and Palmiter, 2009; Golden et al., 2013). Postmortem studies in PD patients have also established that the DA denervation is more severe in the dorsal putamen and less severe in the ventral striatum (Kish et al., 1988; Hornykiewicz, 2001; Kordower et al., 2013). Thus, a severe DA denervation in the dorsal striatum needs to be a key component of the face validity of PD animal models.

Using tyrosine hydroxylase (TH) immunoreactivity as a marker for DA axons, studies have detected a gradient loss of DA innervation with a virtually complete DA fiber loss in the dorsal striatum, a substantial DA fiber loss in the middle striatum, and only a moderate DA fiber loss in the ventral striatum (Fig. 2) (van den Munckhof et al., 2003, 2006; Ding et al., 2007, 2011; Beeler et al., 2009; Li et al., 2013; Li and Zhou, 2013;Wei et al., 2013). Compared with PitxWT mice, quantitative anatomical studies have estimated that the DA fiber loss is 99%, 90% and 59% in the dorsal, middle and ventral striatal subregions. Quantitive neurochemical analysis with HPLC has indicated a similar gradient DA loss pattern in the striatum in Pitx3 null mice: tissue DA content in the dorsal (1.01 ± 0.08 μmole/g wet tissue in Pitx3 WT vs. 0.02 ± 0.00 μmole/g in Pitx3 null), middle (0.93 ± 0.10 μmole/g in Pitx3 WT vs. 0.09 ± 0.02 μmole/g in Pitx3 null) and ventral striatum (0.84 ± 0.11 μmole/g in Pitx3 WT vs. 0.34 ± 0.07 μmole/g in Pitx3 null) (Wei et al., 2013). These results, together with other studies in Pitx3 null mice (van den Munckhof et al., 2003, 2006; Ding et al., 2007, 2011; Beeler et al., 2009), establishing that Pitx3 null mice have a PD-like, gradient striatal DA denervation, a unique feature among existing animal PD models.

Motor deficits in Pitx3 null mice

In experimental animals and accidentally poisoned humans, inhibition of DA release or synthesis, or toxin destruction of the nigrostriatal DA projection quickly leads to loss of motor function or akinesia, a key symptom of PD (Ungerstedt, 1971; Ballard et al., 1985; Zhou and Palmiter, 1995; Galati et al., 2009; Willard et al., 2015). In monkeys, local infusion of DA receptor antagonists into the striatum also induces PD-like motor deficits (Franco and Turner, 2012). These data confirm the classic view that loss of striatal DA function (DA denervation or DA receptor blockade) is the main cause for PD motor deficits, although extrastriatal DA denervation may add additional complexity to the symptoms in PD patients. In PD patients and MPTP poisoned patients, the precursor to DA, L-dopa, strongly stimulates motor activity (Ballard et al., 1985; Olanow et al., 2009; Nutt et al., 2010). Thus, motor deficits are an important component of the face validity for a animal PD model.

Probably due to the residual DA innervation in the middle and ventral striatum (Fig. 2), Pitx3$^{-/-}$ mice do not show overt motor deficit in their home cages and open field test boxes (Hwang et al., 2005; Beeler et al., 2009). This is demonstrated by a small dose (40 mg/kg) of alpha methyl-p-tyrosine (AMPT, an inhibitor of tyrosine hydroxylase) that produces a clear reduction in motor activity or akinesia in Pitx3$^{-/-}$ mice but no clear effect in WT mice; 200 mg/kg AMPT is required to produce a similar akinesia in WT mice (Li and Zhou, 2013). This result indicates that the residual DA maintains the basic open field motor function but is close to the threshold of being insufficient such that a small dose of a DA synthesis inhibitor brings the DA level below that threshold.

Studies indicate that when Pitx3 null mice are placed under a demanding condition, their motor deficits are revealed (Hwang et al., 2005). For example, in the challenging beam test, Pitx3 null mice take a longer time to traverse the increasingly narrow beam (Hwang et al., 2005; Li and Zhou, 2013); in the pole test test, Pitx3 null mice take a longer time to orient themselves (Hwang et al., 2005). L-dopa reversed these deficits (Hwang et al., 2005; Li and Zhou, 2013). Pitx3 null mice also show deficits in the rotarod test (Le et al., 2015). These results indicate that dorsal striatum-DA denervated Pitx3$^{-/-}$ mice have motor deficits, consistent with the classic view that the dorsal striatum and its DA innervation are critical to motor function (Alexander et al., 1986; Kish et al., 1988; Franco and Turner, 2012; Tremblay et al., 2015).

To further characterize the motor deficits, future studies will need to use additional tests such as the adhesive removal test and the adjusting step tests (Glajch et al., 2012) to further determine the motor function impairments due to the DA denervation in the dorsal striatum in Pitx3 null mice. The results will not only further establish Pitx3 null mice as a useful PD model but also provide additional information on the motor and sensory deficits caused by a severe DA denervation in the dorsal striatum.

The dopaminergic drug-induced movements in Pitx3 null mice are potentially highly useful for testing drugs' anti-PD effects. These tests commonly use the classic unilateral 6-OHDA model and the dopaminergic drug-induced asymmetric rotation is used as a measured for the drug's anti-PD power (Lane et al., 2006; Marin et al., 2006). Since the rotational movement in the unilateral 6-OHDA DA lesion model is equivalent to the horizontal movement in the bilateral DA lesion model (genetic or toxin) (Li et al., 2013), Pitx3 null mice can be used for these tests and are more convenient, time-saving and consistent than the unilateral 6-OHDA DA lesion model.

Cognitive deficits in Pitx3 null mice

Cognition generally refers to the brain's ability of memory, thinking, reasoning, computation, understanding, attention, decision making, planning, language, visual recognition, and learning new knowledge and skills including motor and

procedural skills. The huaman brain clearly has the most highly developed cognitive functions. Impairements of cognitive functions in humans are relatively easy to detect and recognize via human-to-human communication, whereas in non-human animals, cognitive functions and their impairments are more difficult to monitor because human-to-animal communication is obviously difficult and even impossible. Further, in many ways, cognitive and motor functions are intricately intertwined because cognition functions are often required to learn motor skill and guide movements. Contributions to cognition from the striatal subregions and the intense striatal DA system are likely mediated by the extensive cortical input to the striatum and the cortico-basal ganglia-thalamo-cortical loop (Haber, 2016).

The cognitive functions of the striatum were not initially recognized. For example, James Parkinson stated that the cognitive functions of his PD patients were normal (Parkinson, 1817). Two factors contribute to Parkinson's not seeing cognitive deficits. First, he examined only 5 relatively young patients whose age ranged 55-72 years and who might indeed have no detectable cognitive deficits. Second, Parkinson might have missed cognitive decifits in some of his PD patients because he examined 2 of these 5 patients causually in the street and he just watched his 6th patient from a distance in the street. Today, clincial evidence indicates that 10%-25% of early stage PD patients have mild cognitive impairments (MCI), for example, verbal fluency/languge ability and task-set switch/cognitive flexibility are impaired in PD patients (Gotham et al., 1988; Cools et al., 2001). With increased life expectancy for PD patients, most PD patients will eventually develop cognitive impairements and even dementia (Katzenschlager et al., 2008; Aarsland and Kurz, 2010; Aarsland et al., 2010; Svenningsson et al., 2012; Robbins and Cools, 2014; Yarnall et al., 2014; Weintraub et al., 2015). The mechanism for MCI is not clear but may involve DA denervation in the striatum, at least partially, that may lead to basal ganglia-cortical circuit dysfunction (Siepel et al., 2014). This possiblity is further supported by the finding that the general intellectual and cognitive functions were impaired in DA toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-poisoned, young humans without Lewy pathology in the cerebral cortex (Stern and Langston, 1985, 1990). Dementia in PD is probably due to widespread Lewy pathology in cerebral cortical areas (Goedert et al., 2013; Del Tredici and Braak, 2016; McCann et al., 2016). Although MCI does not substantially degrade the patient's quality of life, dementia is disabling and causes immense suffering to the patient, suffering and burden to the family and society. Currently, there is no pharmacotherapy for cognitive deficits in PD. Thus, delineation of potential contribution to cognitive deficits from dorsal and ventral striatal DA denervation can help the development of treatments for MCI. Hence, animal models with selective dorsal and/or ventral striatal DA denervation are useful in this regard.

Published data indicate that Pitx3 null mutant mice with dorsal striatal DA denervation have deficits in the rotarod motor-sensory learning test and T-maze visuospatial learning and memory test (Ardayfio et al., 2008). These mice also displayed deficits in the passive inhibitory avoidance test to assess long-term memory for the footshock training (Ardayfio et al., 2008). These results indicate that the dorsal striatum and its regulation by the DA system contribute critically to cognitive functions, and these dorsal striatum-DA-dependent cognitive functions may be impaired in PD; consequently, adequate dopaminergic treatment may improve these striatum-originated cognitive deficits. The substantial intellectual impairment in Pitx3 null humans (Bidinost et al., 2006) is also consistent with the idea that the dorsal striatum and its DA innervation are important to cognitive function. Thus, because of its predominantly dorsal striatal DA denervation, Pitx3 null mice will be useful in studying the mechanisms of striatum-originated cognitive deficits and its treatments, e.g., for screening for compounds before clinical trials. An advantage of using Pitx3 null mice to study cognitive dysfunction is that the potential dysfunctions in Pitx3 null mice are due to DA loss in the striatum, not due to DA loss in cortical areas because the VTA-riginated cortical DA innervation is relatively intact (50% intact), not due to cortical neurodegeneration.

Dopaminergic stimulation of motor activities in Pitx3 null mice

Small and clinical relevant doses (0.5-6 mg/kg, IP injection, together with the dopa decarboxylase inhibitor benserazide at 5 mg/kg) of L-dopa consistently and roburstly stimulate the normal horizontal movements (walking) in Pitx3 null mice, starting in the very first injection (Li et al., 2013; Li and Zhou, 2013). This stimulation starts about 5-10 min after injection and lasts for 1-2 h, the effect of a high dose starts faster and lasts longer; the stimulation intensity is also dose dependent. No rotational movements or any asymmetric movements are induced, probably due to the bilaterally symmetric DA denervation. This motor stimulation occurs on the first injection of L-dopa. In contrast, these doses of L-dopa and benserazide have no overt effect on the motor activity in WT mice. These results of L-dopa stimulation of horizontal movements in Pitx3 null mice indicate that DA denervation in the dorsal striatum produces the necessary conditions (molecular changes) for L-dopa to stimulate motor activity. Certainly, the use of the open field test in rodent PD models, mouse models in particular, faces the problem that the baseline open field movements are often normal even with extensive and severe striatal DA denervation, as in Pitx3 null mice, whereas similar DA denervation would cause overt parkinsonism in humans. This species difference is at least partially due to the fact that mice are light and small such that motor deficiency does not show well and is hard to detect compared with big, heavy animals such humans. Thus,

despite these imperfections of the baseline motor parameter in rodent PD models, dopaminergic stimulation of open field motor activity is a valid and important measure of a drug's anti-PD potency.

In addition to the open field test, challenging beam and pole tests have also been used to determine the dopaminergic motor stimulation in Pitx3 null mice (Hwang et al., 2005; Li and Zhou, 2013). These mice show baseline deficiencies in these tests, i.e., these mice need a longer period of time to traverse the challenging beam and to orient themselves downward than WT mice. L-dopa injection reverses these dificiencies. These results not only demonstrate that the severe DA denervation in the dorsal striatum causes motor deficits in Pitx3 null mice, but show that L-dopa can reverse these deficits, further establishing Pitx3 mice as a model for studying the consequences of DA denervation and for testing anti-PD drugs.

In addition to the normal movements, the very first IP injection of L-dopa (1-20 mg/kg with 5 mg/kg benserazide, IP) also dose-dependently induces dyskinesia-like trunk, limb and paw movements in Pitx3 null mice (Ding et al., 2007; Li et al., 2013; Li and Zhou, 2013). In PitxWT mice, L-dopa injection, even at high doses, does not induce any dyskinesia-like movements. Thus, Pitx3 null mice are useful for studying L-dopa-induced dyskinesias.

The perinatal timing of DA loss in Pitx3Null mice is not a determining factor for the motor behavioral consequence

Since most nigral DA neurons are lost during the perinatal period in Pitx3Null mice (van den Munckhof et al., 2003), there is a concern that a developmental abnormality may be responsible for the baseline motor deficits, cognitive deficits and the L-dopa-induced normal and abnormal movements, potentially confounding data interpretation and limiting the utility of this mouse model. To resolve this concern, in a recent study, 6-OHDA symmetric DA denervation was induced in the dorsal striatum in both hemispheres in adult Pitx3WT mice (Li et al., 2013). This study found that normal Pitx3WT mice with adult-onset 6-OHDA-induced symmetric, bilateral DA denervation in the dorsal striatum produced normal and abnormal movements, upon L-dopa stimulation, that are similar to the L-dopa-induced normal and abnormal movements in Pitx3Null mice that have a perinatal-onset symmetric bilateral DA denervation in the dorsal striatum. These data clearly establish that the L-dopa-induced normal and abnormal movements in Pitx3 null mice are due to DA denervation, not due to the perinatal timing of DA loss, and do not require additional developmental abnormalities, thus eliminating this concern about the validity of Pitx3 null mice as a model animal to study the consequences of DA loss (Li et al., 2013). The L-dopa-induced motor activities in Pitx3 null mice are also similar also to those in Mitopark mice with adult onset, progressive DA loss when the DA loss levels are similar (Ekstrand et al., 2007; Gellhaar et al., 2015), indicating that DA loss severity, not progressivity, determines the dopaminergic response, and further validating Pitx3 null mice as a useful animal model for studying PD.

Conclusions

The studies discussed in this review establish that Pitx3 null mutant mice have a severe nigral DA neuron loss and a severe DA denervation in the dorsal striatum, providing a robust face validity mimicking the severe nigral DA neuron degeneration and dorsal putaminal DA denervation in PD. The motor and cognitive deficits provide additional face validity for Pitx3 null mice to serve as a PD model. The robust dopaminergic motor stimulation in Pitx3 null mice indicate that these mice can predict the motor stimulating effect of dopaminergic drugs for PD. Pitx3 null mice offer several key advantages: besides being the maintenance-free, the DA denervation in these mice is autogenic without the need of surgery and consistent among individual Pitx3 null mice.

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Compliance with ethics guidelines

Fu-Ming Zhou, Li Li, Juming Yue, John A. Dani declare that they have no conflict of interest. This article is a literature review.

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