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Research Report

Motor deficits and altered striatal gene expression in *aphakia* (*ak*) mice

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ABSTRACT

Like humans with Parkinson's disease (PD), the *ak* mouse lacks the majority of the substantia nigra pars compacta (SNc) and experiences striatal denervation. The purpose of this study was to test whether motor abnormalities in the *ak* mouse progress over time, and whether motor function could be associated with temporal alterations in the striatal transcriptome. *Ak* and *wt* mice (28 to 180 days old) were tested using paradigms sensitive to nigrostriatal dysfunction. Results were analyzed using a linear mixed model. *Ak* mice significantly underperformed *wt* controls in rotarod, balance beam, string test, pole test and cotton shred tests at all ages examined. Motor performance in *ak* mice remained constant over the first 6 months of life, with the exception of the cotton shred test, in which *ak* mice exhibited marginal decline in performance. Dorsal striatal semi-quantitative RT-PCR for 19 dopaminergic, cholinergic, glutaminergic and catabolic genes was performed in 1- and 6-month-old groups of *ak* and *wt* mice. Preproenkephalin levels in *ak* mice were elevated in both age groups. Drd1, 3 and 4 levels declined over time, in contrast to increasing Drd2 expression. Additional findings included decreased Chrnα6 expression and elevated VGluT1 expression at both time points in *ak* mice and elevated AchE expression in young *ak* mice only. Results confirm that motor ability does not decline significantly for the first 6 months of life in *ak* mice. Their striatal gene expression patterns are consistent with dopaminergic denervation, and change over time, despite relatively unaltered motor performance.

1. Introduction

Chronic levodopa therapy in patients with Parkinson's disease (PD) frequently results in motor complications that limit the utility of the drug (Olanow et al., 2006; Linazasoro, 2007). These arise from the combined effects of progressive dopaminergic denervation and chronic levodopa therapy (Olanow et al., 2006). Both result in adaptive changes in striatal neurochemistry that, together, are thought to result in motor fluctuations and dyskinesias seen in treated patients (Jenner, 2000; Calon

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Abbreviations: Ache, acetylcholinesterase; *ak*, aphakia; DA, dopamine; Drd, dopamine receptor; Chrm, cholinergic muscarinic; Chrn, cholinergic nicotinic; Comt, catechol-o-methyl transferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Maoa, monoamine oxidase A; MPTP, 1-methyl 4-phenyl 1,2,3,6 tetrahydropyridine; PD, Parkinson's disease; Penk1, preproenkephalin 1; SNc, substantia nigra compacta; Tac1, tachykinin 1; VGluT, vesicular glutamate; VTA, ventral tegmental area

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Table 1 – Descriptive statistics in *ak* and *wt* mice

Test	*ak*			*wt*		
	n	Mean	SE	*n*	Mean	SE
Rotarod	60	31.5	0.99	54	43.7	1.39
Balance beam	60	3.4	0.16	54	2.8	.011
Pole test	59	16.5	1.21	54	12.1	0.61
String test	60	26.0	1.23	54	12.7	0.59
Cotton shred	57	0.36	0.05	43	0.59	0.06
Body weight	60	18.92	0.97	54	24.23	0.99

Means of balance beam, pole test and string test are given as time (seconds) taken to complete the task. Rotarod results are reported as the mean time (seconds) on the rod before falling. Cotton shred results describe the mean percentage of cotton shredded after 6 h. Body weight means are given in grams. Data were collected from *ak* mice (mean age: 66 days) and *wt* mice (mean age: 64 days).

and Di Paolo, 2002; Guigoni et al., 2005; Hurley and Jenner, 2006; Linazasoro, 2007).

Striatal denervation in PD is accompanied by a host of secondary neurochemical alterations in dopaminergic receptors (Hurley and Jenner, 2006) as well as in striatal glutaminergic (Kashani et al., 2007), serotonergic (Di Giovanni et al., 2006), cholinergic (Zhou et al., 2003) systems and neuropeptides (Backman et al., 2007). The Pitx3 –/– or aphakia (*ak*) mouse is a promising model of striatal denervation in PD as it exhibits the cell specificity of neurodegeneration observed in humans (Hwang et al., 2003; Nunes et al., 2003; Smidt et al., 2004), similar neuroadaptive phenomena at the level of the striatum (Smits et al., 2005; van den Munckhof et al., 2006) and locomotor deficits that are rescued by levodopa (Hwang et al., 2005; van den Munckhof et al., 2006). Pitx3 is a member of the pituitary family of bicoid type homeobox transcription factors (Semina et al., 1997). It is expressed in midbrain dopaminergic neurons by E11.5 (Smidt et al., 1997), where it facilitates differentiation of the A9 cell group constituting the substantia nigra pars compacta (SNc; Chung et al., 2005). In homozygous *ak* mice, genomic deletions within the promoter and exon1 of the Pitx3 gene (Rieger et al., 2001; Semina et al., 2000) result in incomplete lens development and aphakia. Undetectable expression of Pitx3 protein in the midbrain is accompanied by failed development of approximately 90% of SNc neurons and severe reduction of dorsal striatal dopaminergic innervation (Hwang et al., 2003; Nunes et al., 2003; Smidt et al., 2004; van den Munckhof et al., 2003). The *ak* mouse retains expression of Pitx3 in skeletal muscle (L'Honore et al., 2007). In addition, low levels of Pitx3 expression in the eye can be detected by PCR (Rieger et al., 2001), indicating that the *ak* mouse represents a cell-specific hypomorph of Pitx3. Deletion of Pitx3 using recombinant technology (L'Honore et al., 2007) results in a similar ocular and midbrain phenotype.

Heterozygous *ak* mice are normal (Nunes et al., 2003). In homozygote *ak* mice, SNc cell loss (particularly in the ventral nigra) is almost complete by birth, whereas the ventral tegmental area (VTA) undergoes progressive postnatal cell loss (Nunes et al., 2003; van den Munckhof et al., 2003) so that approximately 50% of this region is lost by 100 days (van den Munckhof et al., 2003). *Ak* mice exhibit relatively subtle behavioral and motor deficits (Hwang et al., 2003, 2005; van den Munckhof et al., 2003, 2006), and some conflicting data exist in this regard (Smidt et al., 2004; Hwang et al., 2005). It is not known whether motor signs progress with age in *ak* mice. Additionally, it is unclear how the striatal transcriptome responds to persistent dopaminergic denervation over time. Studies have reported increased, decreased or unchanged mRNA expression levels of dopamine receptors and neuropeptides in *ak* mice (Smidt et al., 2004; Smits et al., 2005; van den Munckhof et al., 2006). These inconsistencies may lie in differing methodologies used to assess striatal expression of these proteins or their transcripts.

The *ak* mouse appears to be a good model for exploring the effects of long-term levodopa or other treatments on motor function and adaptive mechanisms within the striatum. However, the effect of the primary gene defect on these phenomena in the *ak* mouse is incompletely described. The objective of this study was to perform a comprehensive study of motor function in *ak* mice using tests sensitive to nigrostriatal dysfunction and to determine whether their motor impairments progress with age. In addition, we assessed whether striatal gene expression for markers of dopaminergic, cholinergic and glutaminergic systems was altered by dopaminergic denervation and whether this pattern changed over time.

2. Results

2.1. *Body weight*

ak mice were significantly ($p<0.0001$) lighter than *wt* mice (Table 1). This difference was significant in both genders, with female and male *ak* mice being 3.72 g and 6.51 g lighter on average respectively than their *wt* counterparts. In both genotypes, males were significantly heavier than females ($p<0.0001$). Therefore, to control for the effects of body weight and sex, these factors were included in all multivariable models.

2.2. *Overall, ak mice underperform wt mice in all tests of motor function*

As only one previous report described the performance of *ak* mice in motor tests typically used to assess fine motor function

Table 2 – Effect of genotype on motor performance comparing *ak* to *wt* (reference) mice

Test	Coefficient	SE	*p* value
Rotarod	−18.76	1.62	<0.0001
Balance beam	0.96	0.21	<0.0001
Pole test	16.76	1.5	<0.0001
String test	9.45	1.3	<0.0001
Cotton shred	−0.81	0.15	<0.0001

Data were analyzed using a repeated measures linear regression model, adjusted for age, sex and body weight. The coefficients reflect the performance of *ak* mice compared to *wt* mice. The negative values of coefficients reflect shorter time (seconds) on the rotarod or fewer (grams) cotton shredded by *ak* mice, and positive coefficients reflect longer times (s) taken by *ak* mice to traverse the balance beam or complete the pole and string tests. The unit of measurement in all the tests is seconds (except grams for cotton shred).