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Motor and cognitive deficits in the heterozygous *leaner* mouse, a $Ca_v2.1$ voltage-gated Ca^{2+} channel mutant

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Abstract

The *leaner* mutation in mice affects the $Ca_v2.1$ voltage-gated calcium channel α_{1A}-subunit gene (*Cacna1a*), causing a reduction in calcium currents predominantly in Purkinje cells. This reduction in calcium currents causes severe progressive cerebellar ataxia, beginning around postnatal day 10, in homozygous *leaner* mice (tg^{la}/tg^{la}), while their heterozygous littermates (tg^{la}/+) present no obvious behavioral deficits. In humans, heterozygous mutations in the *Cacna1a* orthologous gene produce a broad range of neurological manifestations. To evaluate the phenotypic status of the tg^{la}/+ animals, we assessed motor performance and cognition, at different ages, in these mutant mice. We were able to observe age-dependent impairment in motor and cognitive tasks; balance and motor learning deficits were found in demanding tasks on the rotarod and on the hanging wire test, while spatial learning and memory impairment was observed in the Morris water maze. Progressive dysfunction in escape reflexes, indicative of neurological impairment, was also present in tg^{la}/+ animals. Although not presenting major motor alterations, tg^{la}/+ mice show age-dependent motor and cognitive deficits.

Keywords: Rotarod test; Water maze test; Natural mutant; Calcium currents; Memory; Learning

1. Introduction

The *leaner* mutant (tg^{la}) mouse was first described in the 60s, as a cerebellar mutant (Sidman et al., 1965). Homozygous animals (tg^{la}/tg^{la}) are severely ataxic due to cerebellar atrophy, resulting from gradual degeneration of granule, Purkinje, and Golgi cells (Frank et al., 2003; Herrup and Wilczynski, 1982; Lau et al., 2004). Molecular analysis has shown a mutation in the splice donor consensus site of the *Cacna1a* gene, encoding the highly conserved brain specific $Ca_v2.1$ voltage-gated calcium channel (VGCC) α_{1A}-subunit, which is highly expressed in the cerebellum and hippocampus (Day et al., 1996; Volsen et al., 1995). This mutation results in the truncation of the normal open reading frame beyond repeat IV and the expression of two novel α_1 subunits with truncated carboxyl tails (Doyle et al., 1997; Fletcher et al., 1996). Although the homozygous mutants are extremely affected, their heterozygous littermates (tg^{la}/+) present no evident phenotypic abnormalities. Thus, the neurologically abnormal phenotype has been considered by several authors as inherited in an autosomal recessive manner (Fletcher et al., 1996; Meier and MacPike, 1971; Tsuji and Meier, 1971). Functional studies showed a reduction of approximately 30% in Ca^{2+} conductance in tg^{la}/+ Purkinje cells. Channels harboring the *leaner* mutation in homozygosity showed a greater reduction in the calcium currents (Lorenzon et al., 1998).

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Reports on the expression of α_{1A}-subunit levels are conflicting, with some studies reporting a reduction on *Cacna1a* transcript levels (Doyle et al., 1997), while others failed to observe differences in mRNA or protein expression amounts (Lau et al., 1998). This mutant has a diminished Ca^{2+} buffering ability, attributed to reduced uptake by the endoplasmic reticulum and decreased expression of Ca^{2+}-binding proteins (Dove et al., 2000).

In humans, mutations in the *Cacna1a* orthologous gene (*CACNA1A*) have been implicated in three dominantly inherited disorders, with overlapping clinical features: the progressive spinocerebellar ataxia type 6 (SCA6), familial hemiplegic migraine type 1 (FHM1) and episodic ataxia type 2 (EA2) (Ophoff et al., 1996; Zhuchenko et al., 1997). At the mutational level, missense, nonsense and splice-site mutations, as well as a small $(CAG)_n$ expansion, are involved in these disorders, and the same molecular alteration may be associated with more than one phenotype in a single family (Alonso et al., 2003).

Calcium is a highly versatile and ubiquitous intracellular messenger, responsible for controlling, directly or indirectly, diverse cellular processes, including cell differentiation and proliferation, neurotransmitter release, transcription factor activation, apoptosis and synaptic plasticity (Berridge et al., 2000). Calcium plays a key role in the induction of activity-dependent synaptic plasticity in various central synapses, as a result of incoming information (Fitzjohn and Collingridge, 2002).

Modeling neurodegenerative disorders has provided important insights into protein function and disease pathogenic mechanisms. Animal model studies have also shown that usually mutation overexpression is required to produce detectable phenotypic alterations during the mouse lifespan (Watase and Zoghbi, 2003). Furthermore, the high diversity of clinical presentations shown in humans carrying the same *CACNA1A* mutation (Alonso et al., 2003), and the role of calcium homeostasis in normal brain function, have raised the hypothesis of subtle phenotypic alterations in tg^{la}/+ mice. To address this, we applied a battery of behavioral tests to assess motor and cognitive functions of tg^{la}/+ mice, throughout aging.

2. Methods

2.1. Animal husbandry

All the procedures described were approved by the local Ethics Committee and the Portuguese Chief Veterinary Office (Direcção Geral de Veterinária).

Breeder pairs of heterozygous tg^{la}/+ mice were acquired from The Jackson Laboratory (Bar Harbor, Maine, USA) and

Table 1
Number of animals used in each protocol

Behavioral test	Age (months)	wt		tg^{la}/+	
		Male	Female	Male	Female
Rotarod first protocol	6	5	5	5	5
	12	6	5	4	4
	22	5	5	5	5
Rotarod second protocol	6	10	10	10	10
	12	10	10	9	10
	22	9	7	8	6
Water maze—acquisition	6	10	10	10	10
	12	10	10	9	10
	22	9	7	8	6
Water maze—distractor cue	6	10	10	10	10
	12	10	10	9	10
Water maze—cued	6	10	10	10	10
	12	10	10	9	10
Hanging wire	6	5	5	5	5
	12	5	5	7	5
	22	4	2	4	2
Clasping behavior	6	5	5	5	5
	12	5	7	8	8
	22	4	2	4	2