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Research Report

Increased excitability and altered action potential waveform in cerebellar granule neurons of the Ts65Dn mouse model of Down syndrome

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ABSTRACT

Down syndrome (DS) is characterized by intellectual disability and impaired motor control. Lack of coordinated movement, poor balance, and unclear speech imply dysfunction of the cerebellum, which is known to be reduced in volume in DS. The principal cause of the smaller cerebellum is a diminished number of granule cells (GCs). These neurons form the 'input layer' of the cerebellar cortex, where sensorimotor information carried by incoming mossy fibers is transformed before it is conveyed to Purkinje cells and inhibitory interneurons. However, it is not known how processing of this information is affected in the hypogranular cerebellum that characterizes DS. Here we explore the possibility that the electrical properties of the surviving GCs are changed. We find that in the Ts65Dn mouse model of DS, GCs have a higher input resistance at voltages approaching the threshold for firing, which causes them to be more excitable. In addition, they fire narrower and larger amplitude action potentials. These subtly modified electrical properties may result in atypical transfer of information at the input layer of the cerebellum.

1. Introduction

Down syndrome (DS) describes a collection of disabilities that include mental retardation and motor incoordination. It is due to the inheritance of an additional copy of all or part of chromosome 21 (trisomy 21; OMIM ID: 190685) and occurs in different populations in 1 per 370 to 1700 live births (Cocchi et al., 2010; O'Nuallain et al., 2007; Parker et al., 2010). Impaired motor coordination in DS is evident as limited fine motor control, delays in the acquisition of gross and fine motor skills, dysarthria (the unclear articulation of words), strabismus (squint), nystagmus (oscillating eye movements), and altered balance and gait (Frith and Frith, 1974; Henderson et al., 1981; Spano et al., 1999; references in Galante et al., 2009).

The lack of coordination and poor balance implicate dysfunction of the cerebellum, a key brain structure involved in the control of movement. This inference is supported by the finding that in individuals with DS, the volume of the cerebellum and the density of GCs therein are reduced by one third and one quarter respectively (Aylward et al., 1997; Baxter et al., 2000; Jernigan and Bellugi, 1990; Pinter et al., 2001; Raz et al., 1995). Moreover, modeling of the triplication of genes on

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Abbreviations: GC, granule cell; DS, Down syndrome; PC, Purkinje cell; AP, action potential; EPSC, excitatory postsynaptic current; P, postnatal day; R_{in}, input resistance; C_{in}, input capacitance
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human chromosome 21 in DS, by triplication of differing numbers of orthologous genes in mice, generates different mouse models (for example, Ts65Dn, Ts1Cje, Ts1Rhr, Tc1) with varying degrees of decreased cerebellar volume, lower GC density and altered behavior (Dierssen et al., 2009; Galante et al., 2009; Haydar and Reeves, 2011; Lana-Elola et al., 2011; Moldrich et al., 2007). These changes may be accompanied by changes in cerebellar gene expression (Laffaire et al., 2009; Moldrich et al., 2007) and in the number and morphology of Purkinje cells (PCs), the class of cerebellar neuron that integrates input from GCs, as well as other cells, and produces the sole output from the cerebellar cortex (Baxter et al., 2000; Necchi et al., 2008). It is not known if these anatomical and transcriptional modifications are accompanied by alterations in the function of the surviving GCs, which constitute the first stage at which sensorimotor signals transmitted to the cerebellum by mossy fibers (MFs) are processed (Arenz et al., 2009).

We investigated the input–output characteristics of GCs in the young adult Ts65Dn mouse, a model which replicates the deficit of GCs observed in DS and is the most widely studied model of DS (Baxter et al., 2000; Dierssen et al., 2009; Haydar and Reeves, 2011). We find that these cells fire action potentials (APs) in response to smaller current input and that the APs are narrower and have a higher overshoot. These differences may alter GC processing of signals conveyed to the cerebellum by MFs.

2. Results

2.1. *Cerebellar GCs are larger in Ts65Dn mice*

Whole-cell patch-clamp recording was used to determine if the electrical properties of mature cerebellar GCs (P40–60) are altered in the hypogranular cerebellum that characterizes DS. The data presented were obtained from slices derived from 10 Ts65Dn mice and 15 wild-type mice, which were littermates of the Ts65Dn mice. Measurements of input capacitance (C_{in}) indicated that the surface area of the GCs recorded in this study was ~25% greater for cells from Ts65Dn DS mice than wild-type mice (median and inter-quartile values calculated from voltage deflections evoked by negative current jumps in current-clamp, wild-type, 3.0 (2.4, 4.0) pF, $n=48$; Ts65Dn, 3.8 (3.1, 4.4) pF, $n=40$, $p=0.008$, Mann–Whitney U test; median and inter-quartile values of amplifier-readout after cancelation of current transients in voltage-clamp, wild-type, 2.1 (1.7, 3) pF, $n=48$; Ts65Dn, 2.9 (2.5, 3.3) pF, $n=40$, $p=0.033$, Mann–Whitney U test). The increase in size of Ts65Dn GCs suggested by the difference in C_{in} is consistent with reports of a lower packing density of GCs in the Ts65Dn cerebellum (Baxter et al., 2000; Roper et al., 2006). As we did not anticipate a difference in C_{in}, we did not examine cell morphology by filling cells with a dye during recording in order to determine if the increased C_{in} was due to enlargement of the soma or dendrites.

2.2. *Increased excitability of Ts65Dn GCs*

As described previously for wild-type cerebellar GCs (Brickley et al., 2001; Cathala et al., 2003; D'Angelo et al., 1995, 1998), current-clamp recording revealed a non-linear dependence of subthreshold membrane voltage on injected current in wild-type GCs (Figs. 1A and B). The relationship was also non-linear in Ts65Dn cells, but it was not identical to that in wild-type cells (Figs. 1A and B). While there was no difference in resting membrane potential (Fig. 1B, wild-type, -80.0 ± 0.3 mV, $n=38$; Ts65Dn, -79.7 ± 0.5 mV, $n=21$; $p=0.607$, Student's t-test) or in voltage changes caused by hyperpolarizing currents, depolarizing currents caused greater voltage changes in Ts65Dn than in wild-type GCs (Fig. 1B). Hence, input resistance (R_{in}) varied with membrane potential in both types of cells but R_{in} at depolarized membrane potentials was higher in Ts65Dn than in wild-type GCs. This divergence is more apparent when the mean R_{in}, derived from the mean voltage–current relationship (Fig. 1B), is plotted against membrane potential (Fig. 1C).

If the higher C_{in} was the only difference between Ts65Dn and wild-type GCs, the R_{in} of Ts65Dn cells would be lower than that of wild-type cells at all membrane potentials. That this was not the case (Fig. 1C) indicates that the resistance of a unit area of membrane is higher in Ts65Dn GCs, and hence the density of open ion channels is lower. In order to compare membrane resistance, injected currents were normalized by C_{in}, a measure of surface area, and expressed as current-density (pA/pF). Plots of subthreshold voltage against current-density were constructed (Fig. 1D), and the first derivative of the curve fitted to each of the mean voltage–current density relationships was plotted against membrane potential (Fig. 1E). These revealed the higher specific resistance in Ts65Dn GCs at voltages approaching the threshold for firing of APs (Fig. 1E), which resulted in a lower rheobase (size of the sustained current required to initiate AP firing, Fig. 1F). This was not accompanied by a difference in the voltage at which APs were triggered (Fig. 1G). These findings show that, once normalized for size, GCs fire more readily in Ts65Dn than in wild-type mice.

2.3. *AP accommodation is unaltered in Ts65Dn GCs*

Once depolarization exceeded AP threshold, increasing depolarizing current pulses increased the frequency of APs in both wild-type and Ts65Dn GCs (Fig. 2A). Equal increments in current-density caused a similar rise in firing frequency (Fig. 2B), indicating that a change in the steepness of the input/output relationship does not accompany the lower rheobase of Ts65Dn GCs outlined above. There was also no difference in AP accommodation, as deduced from comparisons of the attenuation of AP amplitude and instantaneous frequency during maintained depolarization. Fig. 2C shows heights of APs expressed as a fraction of the first AP for current injections that evoked a minimum of 4, 22 and 46 events. In both cell types, there was little change in the size of the 4 APs evoked near rheobase, but during suprathreshold depolarizations there was a marked decrease in amplitude between the first and second APs, which was followed by a gradual decline of subsequent APs, as observed previously in wild-type GCs (Brickley et al., 2001, 2007; D'Angelo et al., 1998; Hamann et al., 2002). Close superposition of the plots (Fig. 2C) demonstrates that attenuation of AP height during prolonged stimulation is not different in wild-type and Ts65Dn GCs. There was also no difference in firing pattern, as illustrated by close superposition of plots of instantaneous frequency