Passive Models of Neurons in the Deep Cerebellar Nuclei: the Effect of Reconstruction Errors

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Abstract

The goal of this study was to determine how the fit of passive parameters in a compartmental model varies depending on the precise morphological reconstruction of the neuron. We performed whole cell recordings of deep cerebellar nucleus neurons in brain slices, reconstructed the neuronal morphologies and converted them into detailed compartmental models. A genetic algorithm was used to find the best fit of specific capacitance C_M, membrane resistance R_M and axial resistivity R_A of the model with recordings from the same cell. We then introduced morphological alterations that represented the likely consequence of shrinkage artefacts and reconstruction errors. We found that the optimal fits of passive parameters change as much as 173% with such morphological alterations. In addition, dendrites cut during slicing could affect the value of R_M, but not C_M or R_A.

Key words: Deep Cerebellar Nuclei, Passive Neuron Model, Morphology, Reconstruction, Genetic Algorithm.

Introduction

The deep cerebellar nuclei (DCN) provide the main output from the cerebellum. Three different types of DCN neurons have been described: large glutamatergic neurons which project to the thalamus, red nucleus and other brain stem nuclei, smaller GABAergic neurons which carry feedback signals to the inferior olive, and even smaller interneurons which colocalise both GABA and

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glycine [3,1,10]. Here, we describe the construction of passive models of large DCN neurons. The effect of reconstruction errors and dendrites which have been cut during slicing are studied in detail.

Construction of the Passive Model

Whole cell patch-clamp recordings were made from somata of large DCN neurons in slices from 14-17 day old rats, using an Axoclamp IIB amplifier (Axon Instruments, Inc.). To construct a passive model, voltage-gated ion channels and synaptic inputs were blocked with (in mM) TTX (0.001), TEA (10), 4-AP (2), Cd^{2+} (0.2), Ni^{2+} (2), Cs^{+} (5), amiloride (0.5), CNQX (0.01) and picrotoxin (0.02). The voltage responses to long (1s) current pulses were checked for linear scaling [7]. Neurons that behaved passively had an average steady state input resistance of $R_N = 452 \pm 165 M\Omega$ (n = 15).

In these neurons, voltage responses to short current pulses were recorded (+0.5nA and -1nA $\times$ 0.5ms). The prepulse voltage baseline was subtracted, the traces were scaled by 1/current and filtered with a time dependent Gaussian filter ($\sigma = 0.05t$) [6]. Very noise traces were rejected, the grand average of all voltage traces was calculated, and the time constant $\tau_0 = R_M C_M$ was used to obtain an initial estimate of R_M for compartmentalisation (assuming a standard value of $C_M = 1\mu F/cm^2$).

During the recordings, the neurons were filled with biocytin. After fixation, staining and mounting, two cells with a soma diameter $\geq$ 20 μm were recon-

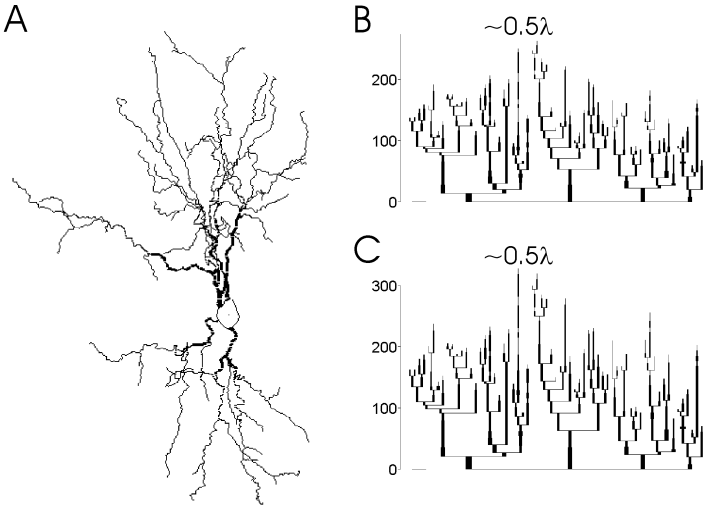

Fig. 1. Morphology (A) and dendrograms (B,C) of the large DCN cell cn0106c. B shows the original dendrogram, in C a shrinkage correction factor of 2 has been applied along the z-axis. The horizontal bars in B and C indicate $10\mu m$.

structed using Neurolucida (MicroBrightField, Inc.); the morphology of one of the cells is shown in Fig. 1A. Neither of the cells had any spines, but a few filiform appendages were found that were included in the reconstruction [10]. One of these cells, *cn0106c*, was reconstructed independently by two persons. The CVAPP software (Robert Cannon, see *www.compneuro.org*) was used to convert the Neurolucida files into cell parameter files for the neural simulator GENESIS [2]. The voltage responses of the reconstructed cells to short current pulses were simulated in GENESIS.

Using a genetic algorithm (GA) with uniform crossover, fitness ranking and a constant mutation probability of 0.1, the values of C_M, R_M and R_A were optimised for each reconstructed cell by matching the simulated response with electrophysiological data from the same cell. The fitness criterion for the GA was the negative root mean square error (RMSE) of the model response compared with the experimental response, and a model was considered fit enough if the RMSE was $\leq 1\%$ of the average voltage response. The GA constrained the parameters to the following ranges: $0.5\mu F/cm^2 \leq C_M \leq 2.5\mu F/cm^2$, $5k\Omega cm^2 \leq R_M \leq 200k\Omega cm^2$ and $20\Omega cm \leq R_A \leq 300\Omega cm$. Separate GAs were run assuming non-uniform specific membrane resistances, but in no case did this result in an improvement of the final fit.

The final values for the first reconstruction of *cn0106c* were $C_M = 1.70\mu F/cm^2$, $R_M = 32.7k\Omega cm^2$ and $R_A = 262\Omega cm$. As it is very easy to introduce errors in dendritic diameters [5], the cell was reconstructed by somebody else and the fitting repeated, resulting in $C_M = 1.43\mu F/cm^2$, $R_M = 38.8k\Omega cm^2$ and $R_A = 300\Omega cm$. A second cell that was reconstructed had passive parameters in a similar range ($C_M = 1.63\mu F/cm^2$, $R_M = 34.4k\Omega cm^2$ and $R_A = 299\Omega cm$).

Effect of Reconstruction Errors and Cut Dendrites

The relative differences of C_M, R_M and R_A between the two reconstructions of *cn0106c* were 19%, 19% and 14.5%, respectively. Because of these large differences, we decided to study the effect of diameter errors on passive cable parameters more systematically. The largest and smallest dendritic diameters in the first reconstruction were 3.84 μm and 0.35 μm, compared to 4.47 μm and 0.23 μm in the second reconstruction. This corresponds to a relative difference of 16% and 52% for the largest and smallest dendrite, respectively. To reproduce the larger relative error for smaller dendrites, we ran GA based fits for a range of cell parameter files that had been generated by changing all dendritic diameters to:

$$d_{new} = d_{old} + f_d \frac{d_{old}}{d_{old} + k} \quad (1)$$

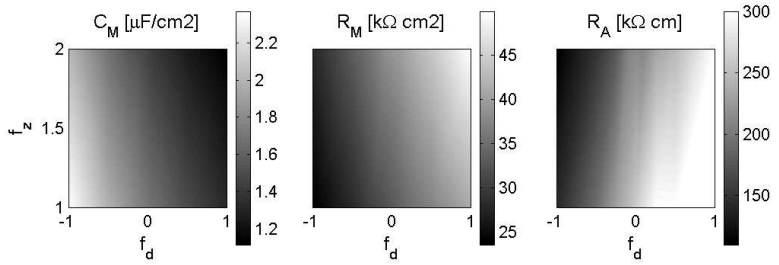

Fig. 2. Range of values of C_M, R_M and R_A for different diameter change factors f_d and z-axis correction factors f_z that have been applied to the reconstruction of *cn0106c*.

where $k = 2\mu m$, $-1 \leq f_d \leq 1$ is the diameter correction factor and d_{old} are the dendritic diameters in the first reconstruction of *cn0106c*. For $f_d = 1$, this introduced a maximum diameter error of $0.67\mu m$ for $d = 4\mu m$ dendrites and $0.11\mu m$ for $d = 0.25\mu m$ dendrites, both of which are in a realistic range. Varying dendritic diameters in this way resulted in passive parameter values in the range between $C_M = 1.32\mu F/cm^2$, $R_M = 41.2k\Omega cm^2$, $R_A = 300\Omega cm$ (for $f_d = 1$, i.e. thick dendrites) and $C_M = 2.37\mu F/cm^2$, $R_M = 23.6k\Omega cm^2$, $R_A = 142\Omega cm$ (for $f_d = -1$, i.e. thin dendrites). Thus, realistic errors in dendritic diameters can introduce errors of up to 111% in passive cable parameters. The same results were obtained in a set of simulations where the dendritic diameter change factor f_d was made an additional parameter optimised by the GA.

Another factor that can have a significant impact on passive parameters is tissue shrinkage during fixation and mounting of slices. Although the shrinkage in x and y direction is usually negligible [4,9,10], shrinkage by factors of 3.7 and 2.8 in z direction has recently been reported in morphological studies of DCN neurons [10] and cortical pyramidal cells [4]. In our preparation, the thickness of slices after coverslipping was found to be reduced by factors of up to 2. We studied the effect of shrinkage by applying correction factors f_z between 1 and 2 along the z-axis of our first reconstruction of *cn0106c*. A z-axis correction of $f_z = 2$ resulted in values of $C_M = 1.44\mu F/cm^2$, $R_M = 39.1k\Omega cm^2$ and $R_A = 213\Omega cm$. Dendrograms of the original reconstruction of *cn0106c* and the shrinkage corrected version with $f_z = 2$ are shown in Fig. 1 B and C. In both cases, the electrotonic length of the longest dendrite is approximately 0.5λ, indicating an electrically compact cell.

Although a large range of passive cable parameter values can be found in the literature [8,6], values of the z-axis corrected reconstruction were closer to more recently published numbers [9]. The combination of shrinkage and diameter errors could lead to values for C_M between 1.11 and $2.37\mu F/cm^2$, $R_M = 23.6 - 49.9k\Omega cm^2$ and $R_A = 110 - 300\Omega cm$ (Fig. 2). Thus, faulty shrinkage correction and diameter measurement errors can introduce errors of up to 173% in the passive cable parameters.

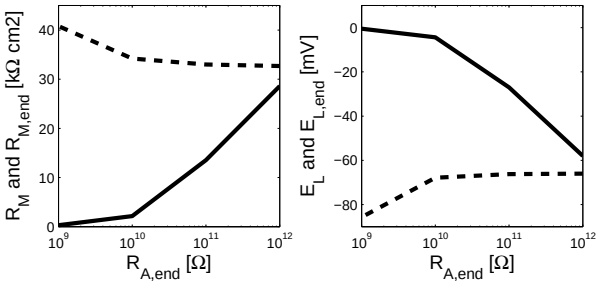

Fig. 3. Effect of $R_{A,end}$ in a partially resealed cut dendritic end on membrane resitivity and total leakage reversal potential in the dendritic compartment (solid) and the whole cell (dashed).

Finally, it can not be excluded that dendrites are cut during slicing which is expected to affect the passive cable parameters. As it is not known whether a cut dendrite fully reseals, we studied the effect of a cut dendritic end by adding an additional leakage current with a reversal potential of $0mV$ and a variable resistance $R_{A,end}$ to a dendritic compartment $70\mu m$ ($\approx 0.1\lambda$) from the soma. The total leakage current in the affected compartment (with length l and diameter d) then becomes:

$$I_{L,end} = \frac{\pi l d}{R_M}(V - E_L) + \frac{V}{R_{A,end}} = \frac{\pi l d}{R_{M,end}}(V - E_{L,end}) \tag{2}$$

with an effective leakage reversal potential and membrane resistance in the cut compartment which are given by:

$$E_{L,end} = E_L\,(1 + \frac{R_M}{\pi l d R_{A,end}})^{-1} \text{ and } R_{M,end} = (\frac{1}{R_M} + \frac{1}{\pi l d R_{A,end}})^{-1} \tag{3}$$

In order to get a good fit, E_L had to be made an additional parameter in the GA. The effect of $R_{A,end}$ on the passive parameters E_L, $E_{L,end}$, R_M and $R_{M,end}$ in the cut compartment and the whole cell is shown in Fig. 3. No fits could be obtained for $R_{A,end} \leq 1G\Omega$, indicating that cut dendrites reseal to a large extent. The values of C_M and R_A were not affected.

Conclusions

The construction of a passive model is an important first step in building a realistic computational model of a neuron with active conductances. We have developed passive models of DCN neurons and studied the effect of reconstruction errors and dendrites which have been cut during slicing. We found that realistic diameter measurement errors and shrinkage in z-direction can lead to errors of almost 200% in all passive cable parameters. In contrast, cut dendritic ends seem to reseal to a large extent and only affect the value of

the specific membrane resistance, but not the specific capacitance or the axial resistivity. The consequence of the uncertainty in estimating passive cable parameters on the predictions made by active neuronal models should be an interesting subject for future research.

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