Morphological Correlates of Intrinsic Electrical Excitability in Neurons of the Deep Cerebellar Nuclei

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Aizenman, Carlos D., Eric J. Huang, and David J. Linden. Morphological correlates of intrinsic electrical excitability in neurons of the deep cerebellar nuclei. J Neurophysiol 89: 1738–1747, 2003; 10.1152/jn.01043.2002. To what degree does neuronal morphology determine or correlate with intrinsic electrical properties within a particular class of neuron? This question has been examined using microelectrode recordings and subsequent neurobiotin filling and reconstruction of neurons in the deep cerebellar nuclei (DCN) of brain slices from young rats (P13–16). The neurons reconstructed from these recordings were mostly large and multipolar (17/21 cells) and were likely to represent glutamatergic projection neurons. Within this class, there was considerable variation in intrinsic electrical properties and cellular morphology. Remarkably, in a correlation matrix of 18 electrophysiological and 6 morphological measures, only one morphological characteristic was predictive of intrinsic excitability: neurons with more spines had a significantly slower basal firing rate. To address the possibility that neurons with fewer spines represented a slowly maturing subgroup, recordings and reconstructions were also made from neurons at a younger age (P6–9). While P6–9 neurons were morphologically indistinguishable from P13 to 16 neurons, they were considerably less excitable: P6–9 neurons had a lower spontaneous spiking rate, larger fast AHPs, higher resting membrane potentials, and smaller rebound depolarizations. Thus while the large projection neurons of the DCN are morphologically mature by P6–9, they continue to mature electrophysiologically through P13–16 in a way that renders them more responsive to the burst-and-pause pattern that characterizes Purkinje cell inhibitory synaptic drive.

INTRODUCTION

The deep cerebellar nuclei (DCN) are at the center of the cerebellar circuitry and form its primary output structure. In the DCN, a variety of excitatory and inhibitory projections, representing several streams of sensory-motor information, converge. Purkinje cells, which constitute the sole output of the cerebellar cortex, send GABAergic inhibitory projections to the DCN. Additionally, the DCN receive glutamatergic excitatory inputs from various precerebellar nuclei and the inferior olive via mossy fibers and climbing fibers, respectively. The firing patterns of the DCN neurons will therefore reflect the sum of all the neural computations that are performed in the cerebellar system that are then translated into a variety of motor outputs. There is increasing evidence that the DCN and an analogous structure, the vestibular nuclei, play an important role in several types of motor learning, including associative eyelid conditioning and adaptation of the vestibulo-ocular reflex (see Bear and Linden 2000; Hansel et al. 2001; Mauk 1997; Raymond et al. 1996).

The cells of the DCN are heterogeneous, consisting of both large and small projection neurons and local circuit neurons. Their anatomical properties have been well described. The large projection neurons have fusiform or multipolar shaped somata, with diameters ranging from 15 to 35 μm, and typically 2–5, thick primary dendrites. They may either be spiny or smooth (see Beitz and Chan-Palay 1979a,b; Chan-Palay 1977; Voogd et al. 1996), are immunoreactive for glutamate (Batini et al. 1992), and project to a variety of premotor centers. In addition, there are smaller projection neurons in the DCN that are GABAergic (Batini et al. 1992; Kumoi et al. 1988) and send axons to the inferior olive (Fredette and Mugnaini 1991). The small projection neurons are also fusiform or multipolar, but their somata range from 5 to 20 μm in diameter. There are also a small number of GABAergic local circuit neurons that project within the DCN and also have small somata (5–15 μm). These different cell types are heterogeneously distributed throughout the DCN (Batini et al. 1992; Beitz and Chan-Palay 1979a,b; Chan-Palay 1977; Kumoi et al. 1988). It is not clear whether these morphologically defined cell types have different electrophysiological characteristics, and few attempts have been made to correlate the two measures (Czubayko et al. 2001).

The electrophysiological properties of DCN neurons have been studied in a variety of preparations using intracellular recordings (see Sastry et al. 1997 for review). DCN neurons are very active at rest and either spike regularly or burst spontaneously (Aizenman and Linden 1999; Czubayko et al. 2001; Gardette et al. 1985b; Jahnson 1986a,b; Llinas and Muhlethaler 1988; Raman et al. 2000). One important characteristic is that DCN neurons will exhibit a pronounced rebound depolarization (RD) that typically triggers a series of action potentials immediately after the offset of a hyperpolarizing pulse or a train of inhibitory postsynaptic potentials (IPSPs). Underlying this RD are several conductances including low-threshold, voltage-gated Ca2+ channels, Ih and a persistent Na+ conductance (Aizenman and Linden 1999; Jahnson 1986a,b; Llinas and Muhlethaler 1988; Raman et al. 2000). This depolarizing envelope drives Na+ spiking, which in turn recruits high-threshold Ca2+ channels and results in a large Ca2+ transient (Aizenman et al. 1998; Gauck et al. 2001; Muri and Knopfel 1994). The RD is terminated in part by the activation of
Ca\(^{2+}\)-sensitive K\(^+\) channels of the SK type (Aizenman and Linden 1999).

The Ca\(^{2+}\) transient associated with the RD can drive several forms of use-dependent plasticity including long-term potentiation and depression (LTP and LTD) of the Purkinje cell-DCN inhibitory synapse (Aizenman et al. 1998, 2000; Morishita and Sastry 1993, 1996; Ouardouz and Sastry 2000) as well as a novel form of nonsynaptic plasticity in which the intrinsic excitability of the DCN neuron is persistently increased (Aizenman and Linden 2000; W. Zhang, J. H. Shin, and D. Linden, unpublished observations).

One important limitation to these plasticity studies has been that the identity of the DCN neurons involved has not been determined. Because these studies have used a “blind” recording technique and because there is no apparent ordering to the different anatomical subclasses of DCN neurons, it has been impossible to select particular cell types for recording. Thus our initial motivation for the present study was simply to determine the degree of morphological variability represented in our microelectrode recordings using conditions that matched our previous studies of use-dependent plasticity. However, by performing current-clamp recordings from these same neurons, it has also been possible to measure the degree to which DCN neuronal morphology is correlated with intrinsic electrical properties.

METHODS

Slice preparation

Coronal slices from 6- to 16-day-old Sprague-Dawley rat cerebellum were cut at a thickness of 400 \(\mu\)m for microelectrode recordings as previously described (Aizenman et al. 1998). Briefly, rats were decapitated, and their brains promptly were removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF, see following text). The cerebellum was then blocked and prepared for slicing. Slices were cut using a Vibratome 100. The slicing stage was filled with ice-cold standard ACSF containing (in mM) 126 NaCl, 5 KCl, 2 CaCl\(_2\), 2 MgSO\(_4\), 26 NaHCO\(_3\), 1.25 NaH\(_2\)PO\(_4\), and 20 d-glucose, pH 7.4, 321 mosM, and continuously bubbled with 95\% O\(_2\)-5\% CO\(_2\). After the slices were cut, they were mounted onto small nylon mesh squares (60 \(\mu\)m grid size, Small Parts) and incubated for 30 min at room temperature in a custom-made interface chamber.

Microelectrode recordings

Slices were transferred to a Haas style interface chamber maintained at 33\(^\circ\)C, perfused with standard ACSF at a rate of 2 ml/min. Borosilicate glass microelectrodes (80–150 M\(\Omega\)) were filled with 3 M KAc and were advanced into the DCN using a Leitz mechanical micromanipulator. Recordings were made either from the medially or laterally located DCN as no differences have been observed between the two nuclei. Membrane voltage was recorded using an Axoclamp 1D amplifier (Axon Instruments) in bridge mode, and digitized at 5 kHz using an ITC-16 interface (Instrutech) controlled with Axodat 1.2.2 software. Data were analyzed off-line using Axograph 4.5 and Igor Pro.

Neurobiotin fills, histology, cell reconstruction

Recordings made for subsequent morphological analysis included 8\% neurobiotin (Vector Labs) in the microelectrode. After recording, DCN neurons were filled with tracer for 15–25 min, using repetitive pulses of positive current (approximately 1 nA, 200-ms duration, applied at 3 Hz). After a recovery period of \(\approx\)15 min, the slices were then fixed for \(>1.5\) h in 4\% paraformaldehyde in 1X PBS (pH 7.6). Because the filled cells were often buried within the 400 \(\mu\)m thick slices, resectioning was necessary for their proper staining and visualization. The fixed slices were placed in 30\% sucrose in 1X PBS overnight at 4\(^\circ\)C for cryoprotection. A sliding microtome was then used to resection frozen slices at 60 \(\mu\)m in the coronal plane. Resected slices were washed in 0.5 M Tris, 0.1\% Triton-X100 (4 \(\times\) 15 min each at room temperature on a rotating shaker), incubated in Vectastain Elite ABC solution (Vector Labs) for 1 h and washed in 0.5 M Tris (4 \(\times\) 15 min). To react the peroxidase and DAB, sections were preincubated for 20 min in DAB solution without H\(_2\)O\(_2\), and then incubated for 9 min in the dark in DAB solution with H\(_2\)O\(_2\). Nickel was added to enhance the color of the precipitate. The sections were then washed in the dark three times with 0.5 M Tris and then sequentially mounted, dehydrated, and coverslipped. In Fig. 3R, neurons were filled with Lucifer yellow delivered by alternating pulses of positive and negative current and subsequently imaged using a Noran OZ laser-scanning confocal microscope.

Neurobiotin-filled neurons were drawn and analyzed with the aid of a light microscope and a drawing tube under \(\times400\) magnification. Neurons in which the soma, dendrites, and axon were found in more than one section were reconstructed according to the section’s orientation on the slide. Drawings represent a two-dimensional projection of the neuronal structure. A variety of morphological parameters were calculated for each cell. These included: soma size (long and short axis); number of primary dendrites, defined as those emerging directly from the soma; branch index, which is the number of branches intersecting a circle with a radius of 30 \(\mu\)m centered on the cell soma divided by the number of primary dendrites (this measure provides a normalized index of how many times the dendrites have branched within this distance); average distance from the soma to the first branch point; and spine index, a qualitative scale that indicated how spiny and tortuous the dendrites are. This index utilized integers from 1 to 4 to score the spinniness and tortuosity of the dendrites. A neuron with an index of 1 had very straight and smooth dendrites and almost no spines; 2: slightly bending dendrites and few spines; 3: somewhat tortuous dendrites but relatively few spines; 4: dendrites that were extremely gnarled and tortuous and an abundance of spines. To ensure consistency in determining the spine index, three observers independently rated each cell under \(\times1,000\) magnification, and their scores were averaged. Observers were blind to the electrophysiological properties of the cells.

All reagents for preparing ACSF and for electrophysiology were from Sigma. Reagents for neurobiotin labeling were purchased from Vector Labs.

RESULTS

Recordings were made from the deep cerebellar nuclei in coronal slices from young rats in two age ranges: P6–9 and P13–16. We recorded from a total of 32 neurons using sharp microelectrodes (11 at P6–9 and 21 at P13–16) and subsequently filled them with neurobiotin before fixing the tissue. Twenty-eight neurons were successfully recovered (7 at P6–9 and 21 at P13–16), and their morphology was reconstructed by camera lucida drawing to allow for comparison with measures of intrinsic electrical excitability.

DCN neurons from P13–16 rats

The intrinsic electrophysiological characteristics of neurons recorded from cerebellar slices of P13–16 rats are summarized in Table 1. These include the basal firing rate, the width and amplitudes of spontaneous single action potentials, and their associated fast AHP. Several responses evoked by hyperpolar-
TABLE 1. Electrophysiological properties of large DCN neurons

<table>
<thead>
<tr>
<th></th>
<th>P13–16</th>
<th>Range</th>
<th>P6–9</th>
<th>Range</th>
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<tbody>
<tr>
<td>Spontaneous</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>“Resting” membrane potential, mV</td>
<td>−58.84 ± 1.71</td>
<td>−71.59 ± 47</td>
<td>−51.90 ± 1.86</td>
<td>−62.32 ± 43</td>
</tr>
<tr>
<td>Spike threshold, mV</td>
<td>−68.58 ± 2.41</td>
<td>−95.55 ± 85</td>
<td>−58.00 ± 2.24</td>
<td>−70.50 ± 85</td>
</tr>
<tr>
<td>Spontaneous spike frequency, Hz</td>
<td>37.26 ± 4.85</td>
<td>11–83</td>
<td>12.28 ± 4.46</td>
<td>0–25</td>
</tr>
<tr>
<td>Spike width, ms</td>
<td>2.13 ± 0.33</td>
<td>1.1–2.6</td>
<td>6.76 ± 3.03</td>
<td>2.6–27.5</td>
</tr>
<tr>
<td>Fast AHP amplitude, mV</td>
<td>10.42 ± 0.66</td>
<td>6.9–17.1</td>
<td>14.56 ± 1.72</td>
<td>7.1–22.9</td>
</tr>
<tr>
<td>Hyperpolarizing pulse responses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( R_{\text{input}} ), MΩ</td>
<td>59.26 ± 2.97</td>
<td>39–80</td>
<td>52.80 ± 3.73</td>
<td>45–78</td>
</tr>
<tr>
<td>Sag, %</td>
<td>87.21 ± 2.15</td>
<td>71–100</td>
<td>94.20 ± 1.74</td>
<td>86–100</td>
</tr>
<tr>
<td>RD duration, ms</td>
<td>473.16 ± 58.45</td>
<td>114–855</td>
<td>126.40 ± 35.61</td>
<td>18–372</td>
</tr>
<tr>
<td>RD number of spikes</td>
<td>4.94 ± 0.93</td>
<td>2–12</td>
<td>1.34 ± 0.15</td>
<td>1–2</td>
</tr>
<tr>
<td>RD peak frequency, Hz</td>
<td>30.68 ± 9.60</td>
<td>4–156</td>
<td>35.50 ± 28.17</td>
<td>7–120</td>
</tr>
<tr>
<td>Time to first RD spike, ms</td>
<td>58.13 ± 7.53</td>
<td>23–128</td>
<td>50.20 ± 4.91</td>
<td>26–81</td>
</tr>
<tr>
<td>RD first interspike interval, ms</td>
<td>95.51 ± 18.67</td>
<td>6.1–280</td>
<td>106.33 ± 33.18</td>
<td>8.3–144</td>
</tr>
<tr>
<td>Depolarizing pulse responses</td>
<td></td>
<td></td>
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<tr>
<td>Slow AHP peak amplitude, mV</td>
<td>3.14 ± 0.39</td>
<td>0.5–6.7</td>
<td>2.30 ± 0.55</td>
<td>1–6.35</td>
</tr>
<tr>
<td>First interspike interval, ms</td>
<td>18.87 ± 2.43</td>
<td>2.6–39.2</td>
<td>19.70 ± 7.36</td>
<td>5.3–26.6</td>
</tr>
<tr>
<td>Peak spike frequency, Hz</td>
<td>106.39 ± 21.77</td>
<td>7.5–384</td>
<td>90.25 ± 35.51</td>
<td>39–189</td>
</tr>
</tbody>
</table>

Spontaneous measures refer to the spontaneous spiking properties of deep cerebellar nuclei (DCN) neurons. “Resting” membrane potential (RMP): average interspike membrane potential measured without injection of bias current. Spike threshold: membrane potential at which the neurons began to spontaneously fire spikes. Spontaneous spike frequency: frequency of spiking at RMP. Spike width: width of spike measured at half-maximal spike amplitude. Fast AHP amplitude: peak amplitude of afterhyperpolarization (AHP) immediately after a single spontaneous spike, measured relative to \( V_m \) immediately prior to spike onset. Hyperpolarizing pulse responses measures were made during and following a \( −0.5 \) nA current pulse given while the cell was held at a \( V_m \) just below spike threshold with a continuous bias current. \( R_{\text{input}} \): input resistance. Sag: the amplitude of the \( \Delta V_m \) evoked by the hyperpolarizing pulse expressed as a percent ratio of the timepoint immediately prior to repolarization relative to the peak. Rebound depolarization (RD) duration: duration of the total depolarizing envelope immediately following the offset of the test pulse. RD number of spikes: average number of spikes elicited during an RD. RD peak frequency: derived from the shortest inter-spike interval during an RD. Time to first RD spike: delay between the offset of the hyperpolarizing pulse and the first spike of the RD. RD first interspike interval: delay between the first 2 spikes during an RD. Depolarizing pulse measures were made during and following a \( +0.5 \) nA current pulse given while the cell was held at a \( V_m \) just below spike threshold. Slow AHP peak amplitude: amplitude of the large AHP after the offset of the depolarizing pulse measured relative to \( V_m \) immediately prior to depolarizing current injection. First inter-spike interval: interval between 2 spikes during the depolarizing test pulse. Peak spike frequency: derived from the shortest inter-spike interval during the depolarizing test pulse. \( n = 17 \) and 9 for P13–16 and P6–9, respectively.

DCN neuron physiology falls along a continuum of spiking behaviors, and it has been shown that their place in the continuum can be pharmacologically modified (Aizenman and Linden 1999; McCormick and Pape 1990), and aspects of the RD including width, number of spikes, and peak and average firing rate. Spiking evoked by depolarizing current injection was also quantified resulting in measures including latency, peak and average spike frequency, accommodation index as well as the amplitude of the subsequent slow AHP measured after the termination of the depolarizing pulse. Excluded from this table are four neurons that had unusual electrophysiological and morphological properties and are discussed in the next section.

DCN neuron physiology falls along a continuum of spiking behaviors, and it has been shown that their place in the continuum can be pharmacologically modified (Aizenman and Linden 1999). In general, DCN neurons had a large amount of spontaneous activity, spiking regularly at 11–83 spikes/s. Overall we observed a continuum between less active, regularly spiking cells and very active bursting cells (compare Figs. 1, A and B, which illustrate the range of activity). Cells responded to depolarizing pulses by increasing their firing frequency, often firing an initial burst of action potentials. Cells with higher spontaneous firing rates tended to produce bigger and longer bursts.

A hyperpolarizing current pulse was often followed by a rebound depolarization (RD) which evoked a number of action potentials. This RD has been shown to be mediated, at least in part, by activation of low-threshold Ca\(^{2+}\) channels (Aizenman and Linden 1999; Gauck et al. 2001; Llinás and Mühlethaler 1988; Muri and Knopfel 1994). Regularly spiking cells had RDs with few spikes (see Fig. 1A, bottom) at relatively low frequencies, whereas bursting cells had large RDs with several high-frequency spikes (Fig. 1B, bottom). It remains unclear whether the cells in these populations belong to a single or to several morphologically distinct subclasses or whether there is any correlation between their electrophysiological and morphological characteristics.

Morphological data for these cells are summarized in Table 2. The soma diameter of the filled cells ranged from 15 to 34 \( \mu \)m, with a mean of 23.6 ± 1.3 \( \mu \)m (SE) (age = P13–16). By comparison with the work of other labs that have identified cells with antibodies to glutamate and GABA (Batini et al. 1992; Kumoi et al. 1988), ~85% of the cells in with these soma sizes have been shown to be glutamatergic and hence project to premotor centers. In agreement with studies of glutamatergic projection neurons (Batini et al. 1992; Chandar et al. 1977; Kumoi et al. 1988), the filled DCN cells range from very spiny to completely aspiny and have two to six primary dendrites (4.0 ± 1.1) with multipolar somata. A sample of these is shown in Fig. 2, A–D. The extent of the dendritic tree as well as the thickness and spine index of the primary dendrites varied greatly but was not necessarily correlated. For example, compare the cells in Fig. 2, A and B or D and C. The spine index of the reconstructed cells was scored using a scale ranging from 1 to 4 (see METHODS). Cells with a spine index of 1 were aspiny, with smooth dendrites (Fig. 2A), while a spine index of 4 was assigned to cells that had very spiny and tortuous dendrites (Fig. 2D). This rating was performed independently by three observers and then averaged (see Table 2).

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In some cells, an axon was seen originating from the soma, and this usually could be followed to the edge of the cerebellar slice, suggesting that these are projection neurons (Fig. 2, B and D). Shown in Fig. 3 are micrographs of dendritic segments of smooth and spiny cells similar to those illustrated by drawings in Fig. 2. Figure 3A has examples of neurobiotin filled neurons and Fig. 3B shows confocal images of cells filled with Lucifer yellow.

**DCN neuronal types rarely encountered with microelectrodes**

While most DCN neurons encountered with a microelectrode seem to fit within a particular range of morphologies, there were a few examples of cells that did not fit either the electrophysiological (Table 1) or morphological (Table 2) profiles described in the preceding text. Of 21 neurons we reconstructed in P13–16 rats, 4 neurons had atypical morphologies. Two neurons had an axon that branched extensively near the cell body, with small varicosities along it, forming a cloud-like structure that enveloped the soma and dendrites (Fig. 4A). The soma diameters of these cells were 12 and 24 \( \mu m \) (short axis), and both had thick, spiny dendrites. These are likely to be local circuit neurons. They had relatively large but fast AHPs after each action potential and a larger than normal sag was present during a hyperpolarizing pulse (Fig. 4A). In addition, the RD consisted of a few spikes but no obvious underlying depolarizing envelope. Although they could spike at high frequencies, these cells could not be made to burst with injection of depolarizing current. Unfortunately, quantitative comparisons with larger projection neurons were not possible due to the limited sample size. Another two neurons exhibited a bipolar morphology (Fig. 4B), with small elongated cell bodies and primary dendrites emerging from both ends. Electrophysiological properties which fell between these 2 extremes.

### Table 2. Morphological properties of large DCN neurons

<table>
<thead>
<tr>
<th></th>
<th>P13–16</th>
<th>Range</th>
<th>P6–9</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma, long axis, ( \mu m )</td>
<td>23.57 ± 1.27</td>
<td>15–34</td>
<td>21.86 ± 0.96</td>
<td>18–25</td>
</tr>
<tr>
<td>Soma, short axis, ( \mu m )</td>
<td>16.86 ± 1.02</td>
<td>12–26</td>
<td>16.57 ± 0.95</td>
<td>13–21</td>
</tr>
<tr>
<td>Number of primary dendrites</td>
<td>4.21 ± 0.22</td>
<td>3–6</td>
<td>4 ± 0.31</td>
<td>3–5</td>
</tr>
<tr>
<td>Branch index</td>
<td>1.89 ± 0.17</td>
<td>1–3.25</td>
<td>1.96 ± 0.17</td>
<td>1.3–2.75</td>
</tr>
<tr>
<td>Distance to first branch, ( \mu m )</td>
<td>24.24 ± 3.51</td>
<td>9.8–57.3</td>
<td>22.77 ± 6.86</td>
<td>9–60</td>
</tr>
<tr>
<td>Spine index</td>
<td>2.45 ± 0.25</td>
<td>1–4</td>
<td>2.72 ± 0.41</td>
<td>1.3–4</td>
</tr>
</tbody>
</table>

The morphological properties of large DCN neurons from 2 different age ranges were derived from neurobiotin fills and subsequent fixation, resectioning and reconstruction as described in methods. \( n = 17 \) and 7 for P13–16 and P6–9, respectively.
ologically, these cells were indistinguishable from the large projection neurons (Fig. 2), making it unclear whether they truly constitute a separate cell type. We do not believe that these bipolar cells were simply regular large cells that were missing some of their dendrites because the somata of these cells were totally contained within the thick slice. Because of the small number of these cells in the sample, the rest of our analysis has been focused on the large, presumably glutamatergic, projection neurons.

**Correlations between morphology and electrophysiology**

We calculated correlations using a Spearman rank order correlation statistic for all 18 measures of intrinsic electrical excitability listed in Table 1, and 5 of the 6 morphological measures listed in Table 2 (the measure “number of primary dendrites” was excluded over concern that some primary dendrites might have been severed and unrecovered for neurons at the cut edge of the brain slice). Interestingly, of these 108 correlations, only one reached statistical significance at the level of $P < 0.03$. In general, these data indicate that while a variety of morphologically distinct neurons may be encountered using a microelectrode, they are mostly similar in terms of their intrinsic electrical excitability. In addition, there is little systematic relationship between the structure of the DCN neuron as revealed by light microscopy and its intrinsic electrical excitability. The one morphological characteristic that was predictive of certain aspects of intrinsic excitability was spine index. Cells with a higher spine index (more spines) had a significantly slower basal firing rate (Fig. 5A; $r_s = -0.63, P < 0.03$). Spinnier cells also tended to have larger fast AHPs (Fig. 5B; $r_s = 0.46, P > 0.05$) as well as more negative resting membrane potentials, although neither of these two measures were statistically significant in our sample (Fig. 5C; $r_s = -0.49, P > 0.05$). It is possible that all three of these correlations result from a single underlying alteration such as an alteration in the conductance or voltage dependence of voltage-gated $K^+$ channels in neurons with more spines.

**DCN neurons from P6–9 rats**

One possibility is that neurons with a lower spine index recorded in slices from P13–16 rats represent a population that is maturing slowly. To address this possibility, recordings were made from DCN cells of P6–9 rats. Recordings were obtained from 11 neurons, 7 of which were successfully recovered for reconstruction. Morphologically, these cells were indistin-

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**FIG. 2.** Camera lucida drawings of P13–16 DCN neurons. Examples of camera lucida drawings of DCN neurons of various sizes with dendrites ranging from smooth and aspiny (A) through intermediate morphologies (B and C) to tortuous and very spiny (D). Note variations in shape of the soma, branching patterns of the dendrites and thickness of the primary processes. Cells of various morphologies were found throughout the DCN with no apparent layering or spatial organization.
guishable from those of P13–16 animals (Fig. 6A, Table 2). For example, soma sizes ranged from 13 to 25 μm, the spine index was 2.7 ± 0.4, and number of primary dendrites was 4 ± 0.3 with a branching index of 2 ± 0.5.

By contrast, the intrinsic electrophysiological properties of DCN cells changed considerably between P6–9 and P13–16 (summarized in Table 1). A typical example of electrophysiological traces from a P7 cell is shown in Fig. 6B. Note how a depolarizing pulse generated only one spike that was riding on a small transient depolarization. A hyperpolarizing pulse generated a brief RD with only one spike. This was unlikely to reflect a difference in the recording quality between older and younger cells because \( R_{\text{input}} \) remained comparable (\( P = 0.28 \) Kolmogorov-Smirnov test). The resting membrane potential tended to become more negative in older animals but was not significantly different (\( P = 0.09 \) K-S test) and younger cells had slower spontaneous spiking frequencies (\( P = 0.01 \) K-S test) as well as short RDs that evoked relatively few spikes (\( P < 0.001 \) K-S test). Taken together, these measures indicate that the intrinsic excitability of DCN neurons increased substantially between P6–9 and P13–16.

**FIG. 3.** Smooth and spiny dendrites in P13–16 DCN neurons. Examples of dendritic arbor types in neurobiotin-filled neurons (A) and confocal images of neurons filled with Lucifer yellow (B). Arrowheads indicate some locations where spines can be seen.
DISCUSSION

There are three main findings that have emerged from this work. First, in slices from P13–16 rats, most of the neurons reconstructed (17 of 21 cells) had large cell bodies (Fig. 2; Table 2), suggesting by comparison to previous experiments that have used glutamate and GAD immunohistochemistry (Kumoi et al. 1988; Batini et al. 1992) that these are mostly glutamatergic neurons that project to premotor centers. This finding is useful because it allows for the cellular attribution of three different forms of use-dependent plasticity recorded in the DCN under identical conditions [LTP and LTD of inhibitory Purkinje neuron-DCN synapses (Aizenman et al. 1998) and persistent use-dependent increases in intrinsic excitability (Aizenman and Linden 2000)].

Second, while these large projection neurons had diverse morphology (characterized by 6 measures; see Table 2) and different degrees of intrinsic excitability (characterized by 18 measures; see Table 1), only one morphological characteristic was predictive of intrinsic excitability: neurons with more spines had a significantly slower basal firing rate (see Fig. 5A). Perhaps, this represents a homeostatic mechanism by which neurons with more spines reduce their excitability to normalize the effects of greater synaptic drive and thereby maintain a constant functional output (Turrigiano and Nelson 2000; Hansel et al. 2001). It will be interesting to determine whether neurons in the DCN with a higher spine index really do receive more synaptic drive and, if so, whether it is glutamatergic or GABAergic. Alternatively it is possible that cells with a higher spine index receive different types of synaptic inputs than those with smooth dendrites, and thus the changes in spontaneous activity reflect alterations in background synaptic activity and not in the intrinsic properties of the cells. However, dendritic spines are traditionally associated with excitatory synapses, and it would be expected that having more spines would lead to increased spiking due to increased background excitation rather than the decreased firing rate we observed. Another possibility is that having more synaptic inputs would cause a decrease in the overall input resistance of the cell, thus causing
an apparent change in intrinsic properties. This is also not likely the case because input resistance measurements do not correlate with neither spine index nor spontaneous firing rate.

In only 2 of 21 neurons reconstructed from P13–16 rats was a substantially different overall electrophysiological phenotype observed. Both of these neurons had moderate to large soma sizes (12- and 24-μm short axis) but had a locally projecting axon, indicating that they are interneurons (Fig. 4A). It is likely that blind microelectrode recording from brain slices introduces a sampling bias in favor of larger cells that are presumably easier to impale. One other study has attempted to correlate morphology and electrophysiology in the DCN of slices from P12–21 rats by specifically targeting cell somata of different sizes using a visualized whole cell patch technique (Czubayko et al. 2001). This study also found that the majority of the neurons recorded in this manner had electrophysiological profiles similar to that described herein for P13–16 neuron (they call these type I cells). Additionally, they also found that ~10% of the neurons had a different electrophysiological profile that closely matched that of the presumed local interneurons recorded herein (which they call Type II cells). The two type II cells reconstructed by Czubayko et al. (2001) had small somata and small dendritic arbors, leading these authors to suggest that they were interneurons (they were unable to reconstruct the axon). It is interesting to note that both the present study, using blind microelectrode impalement, and that of Czubayko et al. (2001), using a visualized approach with infrared DIC optics, yielded about the same fraction of presumed interneurons (~10%). This value is likely to be an underestimation of the total number of interneurons in the DCN (Batini et al. 1992; Beitz and Chon-Palay, 1979b).

Third, neurons recorded in brain slices of still younger rats (P6–9), while morphologically indistinguishable from those in P13–16 slices (Fig. 6A; Table 2), were much less excitable (Fig. 6B; Table 1). Younger cells had lower spontaneous spiking frequencies, wider action potentials, larger AHPs, and a smaller hyperpolarizing sag (indicating smaller $I_h$). Furthermore, the RD evoked by a hyperpolarizing pulse was of shorter duration and smaller amplitude and hence was associated with the generation of far fewer spikes. These findings confirm and extend previous observations by Gardette et al. (1985b). Using horseradish peroxidase labeling, Bourrat and Sotelo (1986) determined that the main stages of dendritic differentiation in the rat DCN occur between E16 and E19. At birth, the dendritic morphology of these neurons is almost fully developed. In late prenatal life, Purkinje cell inputs begin to innervate the DCN (Wassef and Sotelo 1984; Wassef et al. 1985), although synaptic maturation does not occur until early in the first postnatal week (Gardette et al. 1985a; Garin and Escher 2001). Thus in large projection neurons of the DCN, dendritic morphology and (at least some aspects of) synaptic function appear to mature before intrinsic electrophysiological properties.

Perhaps the most striking aspect of electrophysiological maturation that was observed over the period P6–9 to P13–16 was the development of the RD response to hyperpolarizing pulses. In the intact cerebellum, this response is typically triggered when a DCN neuron receives a burst of IPSPs followed by a pause. This burst and pause is the pattern of activation that is evoked in Purkinje neurons by climbing fiber activation and that is then conveyed to DCN neurons by GABAergic synapses, where it is a particularly effective trigger for RDs (Aizenman et al. 1999). Climbing fibers are known to fire during motor errors and their signals have been implicated in several forms of cerebellar motor learning including adaptation of the vestibulo-ocular reflex and associative eyelid conditioning (Raymond et al. 1996). Analysis of associative eyelid conditioning in young rats has shown that the ability to acquire conditioned responses emerges in the third postnatal week and is associated with an increased firing rate evoked by the periorbital shock unconditioned stimulus (Freeman and
Nicholson 2000, 2001; Stanton et al. 1992). In associative eyelid conditioning, the unconditioned stimulus is conveyed to the cerebellum by climbing fiber activation. Therefore it is possible that the developmental maturation of the RD observed herein may underlie the ability of DCN neurons to detect the sequelae of the unconditioned stimulus and respond with a postsynaptic Ca$^{2+}$ transient that can trigger persistent changes in synaptic and nonsynaptic efficacy (Aizenman and Linden 2000; Aizenman et al. 1998; see Hansel et al. 2001 for review).

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