“Hairy Baskets” Associated With Degenerative Purkinje Cell Changes in Essential Tremor

Cordelia R. Erickson-Davis, BA, Phyllis L. Faust, MD, PhD, Jean-Paul G. Vonsattel, MD, Sachin Gupta, MD, Lawrence S. Honig, MD, PhD, and Elan D. Louis, MD, MSc

Abstract

Essential tremor (ET) is one of the most common neurologic diseases. Increased numbers of torpedoes and Purkinje cell (PC) loss have been documented in the brains of patients with ET. We recently observed a dense and tangled appearance (“hairiness”) of the basket cell axonal plexuses that surround PC soma in Bielschowsky preparations of cerebellar cortex in ET brains. Here, we assessed basket cell “hairiness” in 37 ET (32 cerebellar ET; 5 Lewy body variant ET), 21 nondisease control, and 48 disease control brains using a semiquantitative scale. In 8 cerebellar ET cases (25%), there were high basket scores (rating = 3), whereas no Lewy body variant ET, 1 nondisease control (4.8%), and 2 diseased controls (4.2%) had high basket scores (p = 0.001). The hairy basket scores correlated with numbers of torpedoes (p < 0.001) and inversely with numbers of PCs (p = 0.06). Axonal plexus density obtained by image analysis of basket cell processes traced from digitized images was higher in ET than in nondisease control brains (p = 0.016). Closely spaced sites of synaptic contact between basket cell processes and PCs were identified by electron microscopy in ET cases. These data indicate that structural changes are not restricted to PCs in ET, and that other neurons within their functional network may be involved in its pathogenesis.

Key Words: Basket cells, Cerebellum, Essential tremor, Neurodegenerative, Pathology, Pathophysiology, Purkinje cells.

INTRODUCTION

Essential tremor (ET) is a chronic brain disease, the most recognizable feature of which is a 4- to 12-Hz action tremor (1, 2). It is one of the most common neurologic disorders present in 4.0% of individuals 40 years or older (3), and in as many as 21.7% of individuals aged 95 years or older (4). The tremor is usually progressive (5) and produces functional disabilities (6). Recent evidence also suggests an associated increased risk of mortality (7).

A wealth of clinical data suggests that ET is a disorder of cerebellar dysfunction. Intention (i.e. “cerebellar”) tremor of the hands occurs in 44% of ET cases (8). Abnormalities in tandem gait and balance have been repeatedly described in ET patients (9–11), and ET patients with intention tremor may also have other cerebellar signs such as dysdiadochokinesia (12). Eye movement abnormalities that indicate cerebellar dysfunction have also been described in ET (13). Unilateral cerebellar stroke has been reported to abolish ipsilateral arm tremor in ET (14), and cerebellar outflow pathways are the targets of deep brain stimulation surgery, which is effective in treating ET (15, 16). Numerous neuroimaging studies using functional magnetic resonance imaging (17), positron emission tomography (18, 19), [¹H] magnetic resonance spectroscopic imaging (20, 21), diffusion-tensor imaging (22), and voxel-based morphometry (23) have provided evidence that cerebellar structure and function are abnormal in ET.

Until recently, there had been few postmortem studies of ET (24). Studies that explore the pathological anatomy and improve our understanding of the pathophysiology of ET are critically important as there is no cure for ET and first-line medications, of which there are only two, are estimated to be ineffective in as many as 50% of patients (25). In each of 2 recent large postmortem series, the presence of degenerative changes in the cerebellum has become evident (24, 26–28). To date, these changes consist of an increased number of torpedoes (5- to 6-fold greater than seen in age-matched control brains) and a mild, approximately 40%, loss of Purkinje cells (PCs) (27, 29). Whether the structural changes in ET are restricted to PCs or whether they involve other neurons in their functional network has not been investigated.

Recently, we observed an unusual dense and tangled (we now term “hairy”) appearance of the basket cell axonal plexus surrounding PC soma in Bielschowsky preparations of cerebellar cortical sections in ET cases. Basket cells are γ-aminobutyric acid–ergic inhibitory interneurons found in the molecular layer; they send out axonal collaterals to form a pericellular basket around PC perikarya. To our knowledge, there are only 2 previous reports describing a dense and tangled appearance of basket cell axonal plexus profiles occurring with cerebellar degeneration: a single case of Creutzfeldt-Jakob disease (30) and an early study examining...
brains of individuals diagnosed with “senile dementia” (31). Because this pathologic finding may provide important mechanistic insights into the pathogenesis of ET, we assessed basket cell changes in ET, other neurodegenerative diseases, and nondiseased control cases.

**MATERIALS AND METHODS**

**Brain Repository**

This study was conducted at the Essential Tremor Centralized Brain Repository at the New York Brain Bank, Columbia University (32). The 106 brains included 37 from ET cases; 31 (83.8%) of these were collected prospectively beginning in 2003, and 6 were archival. All 37 had been diagnosed by their treating neurologist, and all diagnoses were confirmed using Essential Tremor Centralized Brain Repository criteria (27). Essential tremor cases were divided into 2 groups based on the presence or absence of brainstem Lewy bodies on postmortem examination (27, 29, 33, 34). Essential tremor brains without Lewy bodies have an array of cerebellar abnormalities that include increased numbers of torpedoes and PC loss (27, 29). The Lewy body–free brains have been referred to as cerebellar ET and brains with Lewy bodies as Lewy body variant of ET in previous publications (24, 32). Demographic and clinical information were collected on each brain; the severity of tremor on Archimedes (24, 32). Demographic and clinical information were collected on each brain; the severity of tremor on Archimedes

**Tissue Processing**

All brains underwent complete neuropathologic assessment at the New York Brain Bank (26, 27, 32, 33, 36). Brains received ratings of neurofibrillary tangles using Braak and Braak staging (37, 38) and Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) ratings (39) for neuritic plaques. National Institute on Aging–Reagan Institute criteria for AD (40) were also assigned. Postmortem interval (PMI) was the number of hours between death and placement of the brain in a cold room or on ice.

A standard 3 × 20 × 25-mm parasagittal tissue block from the neocerebellum was harvested from each brain as described (26, 27, 29, 33). Seven-micrometer-thick paraffin sections, impregnated using a modified Bielschowsky silver technique, were used for semiquantitative and quantitative analyses of basket cell processes and quantification of torpedoes. Sections were also stained with Luxol fast blue-hematoxylin and eosin (LH&E) for quantification of torpedoes and PCs (26, 27, 33). For immunohistochemical analyses, sections were incubated with mouse monoclonal SMI-31 (Covance, Princeton, NJ), as previously described (41). In each brain, torpedoes in an entire LH&E section and an entire Bielschowsky preparation were counted, and PCs in five 100 × LH&E fields were counted and averaged, as previously described (27).

**Semiquantitative Rating of Hairy Baskets**

A semiquantitative rating of the appearance of the basket cell plexus surrounding PC bodies throughout Bielschowsky

**TABLE 1.** Demographic and Clinical Characteristics of Cases and Controls

<table>
<thead>
<tr>
<th></th>
<th>Cerebellar ET</th>
<th>LBVET</th>
<th>AD</th>
<th>PD/DLBD</th>
<th>PSP</th>
<th>Dystonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>32</td>
<td>5</td>
<td>20</td>
<td>15</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>83.6 ± 7.6</td>
<td>86.8 ± 4.7</td>
<td>82.9 ± 5.2</td>
<td>78.1 ± 6.7</td>
<td>73.4 ± 7.2*</td>
<td>68</td>
</tr>
<tr>
<td>Female sex</td>
<td>(26.2%)</td>
<td>(20.0%)</td>
<td>(60.0%)</td>
<td>(60.0%)</td>
<td>(40.0%)</td>
<td>(41.7%)</td>
</tr>
<tr>
<td>(n = 20)</td>
<td>20</td>
<td>12</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PMI (hours)</td>
<td>4.7 ± 4.4</td>
<td>7.7 ± 7.3</td>
<td>7.7 ± 4.8</td>
<td>8.0 ± 7.8</td>
<td>7.1</td>
<td>7.8 ± 8.4</td>
</tr>
<tr>
<td>Brain weight (g)</td>
<td>1,211 ± 138</td>
<td>1,184 ± 131</td>
<td>1,097 ± 119</td>
<td>1,128 ± 272</td>
<td>1,164 ± 110</td>
<td>1,632</td>
</tr>
<tr>
<td>CERAD score</td>
<td>0.9 ± 1.1</td>
<td>1.2 ± 1.3</td>
<td>3.0 ± 0.0*</td>
<td>1.5 ± 1.1</td>
<td>0.4 ± 0.7</td>
<td>0</td>
</tr>
<tr>
<td>Braak AD score</td>
<td>2.0 ± 1.2</td>
<td>2.2 ± 0.8</td>
<td>5.8 ± 0.4*</td>
<td>1.3 ± 1.0</td>
<td>1.2 ± 0.9</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are either mean ± SD or number (percentage).

*AD, Alzheimer disease; DLBD, diffuse Lewy body disease; ET, essential tremor; LBVET, Lewy body variant of ET; PD, Parkinson disease; PMI, postmortem interval; PSP, progressive supranuclear palsy.

© 2010 American Association of Neuropathologists, Inc.

263
preparations was performed by a senior neuropathologist (P.L.F.) who was blinded to all clinical information. The following scale was used: 0 (few or no discernible processes); 1 (sparse number of processes); 2 (moderate number of processes); and 3 (dense tangle of processes). In some instances, the rater used intermediate values (0.5, 1.5, and 2.5). Hence, the hairy basket rating ranged included the values 0, 0.5, 1, 1.5, 2, 2.5, and 3.

Quantitative Analysis of Basket Cell Plexus

Quantification of the density of the basket cell axonal plexus formations was performed on a subsample of 10 brains (including 5 randomly selected cerebellar ET brains and 5 randomly selected nondiseased control brains) by a single trained physician who was blinded to clinical information. Digital images of the Bielschowsky neocerebellum preparations were obtained using a Zeiss Axioplan 2 microscope fit with an Axiocam HR digital camera (20× objective lens). For image acquisition, slides were oriented with the dentate nucleus situated on the right and the folia branches extending leftward. Folia were labeled numerically, and the 2 folia located most centrally in the slide were selected. Within each of these folia, 3 smaller subregions consisting of 1 to 2 distal peaks (gyri) and 1 to 2 distal troughs (sulci) were chosen such that there were 6 subregions photographed per brain. In each photograph, the basket cell processes surrounding each visible PC perikaryon were traced using Adobe Photoshop 5.0 (approximately 40 PCs/brain). Images of the basket cell tracings were then inverted and imported into Image J (NIH), where the integrated density was assessed (i.e. a function of the program whereby each pixel of the basket tracing was assigned a gray value using a variable gray scale and then summed).

Ultrastructural Analysis

Flat Eponate 12 (Ted Pella, Redding, CA) embedded vibratome sections of cerebellar cortical tissue were dissected into 3 × 4–mm sections and re-embedded in the same resin. Semithin sections were then stained with toluidine blue and examined by light microscopy for PCs. Once PCs were identified on semithin section, the block was further trimmed around the PCs to the size required for ultrathin sectioning. Ultrathin sections were stained with uranyl acetate and lead citrate and then examined by transmission electron microscope (JEOL 1200EXII, Peabody, MA), as described (41).

Statistical Analyses

All analyses were performed in SPSS (version 16.0). As previously noted, a priori, the focus of our interest was cerebellar ET cases rather than Lewy body variant of ET cases. Demographic and clinical features of diagnostic groups (e.g. cerebellar ET vs controls) were compared using N2 tests and analysis of variance (ANOVA) with Tukey post hoc comparisons (Table 1).

The hairy basket rating included the values 0, 0.5, 1, 1.5, 2, 2.5, and 3. In nondiseased controls, the correlates of the hairy basket rating were assessed using Pearson correlation coefficients (e.g. hairy basket rating by age). To facilitate further analyses, hairy basket ratings were then collapsed into a smaller number of rating categories as follows: low (0 or 1,
and also including any ratings of 0.5 and 1.5); intermediate (2 and also including any ratings of 2.5); and high (3). Diagnostic groups were compared with regard to hairy basket rating category (Mantel-Haenszel $\chi^2$ test; Table 2). For several analyses, diagnostic groups were combined (AD + PD/DLBD + PSP + dystonia = diseased controls; diseased

**FIGURE 2.** Immunohistochemical analysis of basket cell processes. (A, B) Vibratome sections (100-µm-thick) immunostained with monoclonal antibody SMI-31 demonstrate prominence of phosphorylated neurofilaments in basket cell processes (long arrows). (A) A dense and tangled, or “hairy,” appearance of basket cell axonal plexus formation in a section from a cerebellar essential tremor case with a hairy basket rating of 3. (B) A control case with a rating of 0. Torpedoes (short arrows) are also seen.

**FIGURE 3.** Quantification of basket cell axonal plexus density. (A–D) Examples of tracings scored according to a semiquantitative scale are shown: (A) 0 (few, or no discernible processes); (B) 1 (sparse processes); (C) 2 (moderate numbers of processes); and (D) 3 (dense tangle of processes). (E) Basket cell plexus densities were higher in cerebellar essential tremor (ET) cases (closed circles; median value = 15.10) than in controls (open squares; median value = 6.98; $p = 0.016$).
controls + nondiseased controls = all controls; Table 2). Because of the modest sample size, basket cell plexus density was compared across diagnostic groups using the Mann-Whitney test.

In an unadjusted logistic regression model, diagnosis (cerebellar ET vs all controls) was the outcome variable. In this model, the hairy basket rating category was the independent categorical variable (high, intermediate, and low, as previously described). In adjusted regression models, covariates that were associated with either the outcome or independent variable or for which a priori evidence was considerable that the variable may be a confounder were included.

The correlates of the hairy basket rating in cerebellar ET cases and nondiseased controls and in cerebellar ET cases alone were determined using Pearson correlation coefficient when both variables were normally distributed; otherwise, Spearman correlation coefficient was used. The degeneration index was defined as the number of torpedoes on Bielschowsky section divided by the mean number of PCs per 100× LH&E-stained high-power field. High values indicated large numbers of torpedoes and/or low PC counts (i.e. PC loss) (42).

RESULTS

ET, Nondiseased Controls, and Diseased Controls

Cerebellar ET cases were similar to Lewy body variant of ET, AD, and PD/DLBD cases and nondiseased controls with respect to age and PMI. However, when diseased controls (AD + PD/DLBD + PSP + dystonia) were combined and all controls were combined (diseased controls + non-diseased controls), cerebellar ET cases were older by an average approximately 5 years, and mean PMI was shorter (Table 1). All groups were similar with respect to gender and brain weight (Table 1). Consortium to Establish a Registry for Alzheimer’s Disease scores and Braak AD scores were higher in AD cases than cerebellar ET cases (Table 1). Braak AD scores were higher in cerebellar ET cases than nondiseased controls (Table 1).

Basket Cell Plexus Alterations in Cerebellar ET

Bielschowsky sections of cerebellar neocortex revealed variable appearances of basket cell axon collaterals that surround the PC perikarya. Figure 1 illustrates examples of the semiquantitative scores of the basket cell plexus morphologies. “Hairy baskets” (rating = 3, the severe end of the spectrum) were defined as a dense and tangled axonal plexus (Fig. 1D). These hairy baskets generally surrounded viable-appearing PCs, rather than PCs that had previously died (i.e. “empty baskets”). In each of the 8 ET cases with hairy basket scores of 3, 20 hairy baskets were assessed (160 hairy baskets). Purkinje cell somata were visible in 141 of these (88.1%). Immunohistochemistry highlighted the neurofilament-rich basket cell axonal processes that comprise the hairy baskets and PC axonal torpedoes (Fig. 2).

We also assessed whether the hairy basket rating was similar in different neocerebellar regions within the same brain. For this analysis, 1 Bielschowsky section was examined from 3 separate neocerebellar blocks in 7 brains with hairy basket ratings ranging from 0 to 3. The agreement between ratings was high (Block 1 vs Block 2, Pearson r = 0.91, p = 0.005; Block 1 vs Block 3, Pearson r = 0.95, p = 0.001; and Block 2 vs Block 3, Pearson r = 0.96, p < 0.001). This indicated that the data...
from a single block were likely to be broadly representative of the entire neocerebellum.

The hairy basket rating was not correlated with age (Pearson $r = 0.24$, $p = 0.31$), PMI (Pearson $r = -0.04$, $p = 0.87$), brain weight (Pearson $r = -0.20$, $p = 0.41$), CERAD score (Pearson $r = -0.09$, $p = 0.71$), or Braak AD score (Pearson $r = 0.05$, $p = 0.83$) in nondiseased controls. It also did not differ by gender in nondiseased controls ($1.5 \pm 0.9$ in males vs $1.1 \pm 0.8$ in females, $p = 0.34$).

Hairy basket rating category was higher in cerebellar ET cases than in AD, PSP, dystonia, PD/DLBD (marginal difference), and nondiseased controls (Table 2). When control groups were combined, the differences with cerebellar ET were more marked. In 8 of 32 ET cases (25.0%), there was a hairy basket rating of 3, whereas only 4.2% of diseased controls and 4.8% of nondiseased controls had a score of 3 (Table 2). In an unadjusted logistic regression model in which diagnosis (cerebellar ET vs all controls) was the outcome variable, brains in the high (rating = 3) hairy basket rating category were 10 times more likely to be cerebellar ET than controls (OR = 2.44; 95% CI = 0.92–6.46; $p = 0.07$). In a logistic regression model that adjusted for age, gender, PMI, brain weight, CERAD score, and Braak AD score, these values were as follows: OR = 18.95 (95% CI = 1.88–191.56; $p = 0.01$) and OR = 3.52 (95% CI = 0.82–15.02; $p = 0.089$).

To provide a quantitative measure of these basket cell plexus alterations, the axonal plexuses surrounding extant PCs in 5 cerebellar ET cases and 5 nondiseased controls were traced from Bielschowsky preparation images (Fig. 3A–D). When the densities of the tracings were determined, the average basket cell plexus in cerebellar ET cases was approximately twice the density of the average plexus in nondiseased controls (mean = 13.97 ± 3.56 [median = 15.10] vs 6.98 ± 2.20 [median = 6.68]; Mann-Whitney $z = 2.40$; $p = 0.016$; Fig. 3E). Basket cell plexus density was highly correlated with the hairy basket rating (Pearson $r = 0.73$; $p = 0.018$). Basket cell plexus density was also strongly associated with the number of torpedoes (Spearman $r = 0.79$; $p = 0.007$), marginally inversely with the number of PCs (Spearman $r = -0.25$; $p = 0.49$), and with the degeneration index (Spearman $r = 0.76$; $p = 0.01$).

**Ultrastructural Analysis of the Basket Cell Plexus**

We performed ultrastructural studies to compare basket cell processes in ET and control cases. In an ET case with a hairy basket rating of 3, there were several layers of neurofilament-rich basket cell processes adjacent to the PC soma that intertwined at various angles (Fig. 4A, C–E). In contrast, in a nondiseased control with a hairy basket rating of 0, only 1 or 2 basket cell processes having a somewhat thinner diameter were found adjacent to the PC soma (Fig. 4B).

The identity of these neurofilament-rich processes as basket cell fibers was further confirmed in the ET case by the identification of synapses with the PC soma and its initial axon segment. In several instances, contacts with the PC somata were marked by multiple, closely spaced synaptic sites within 1 basket cell process (Fig. 5A–C; arrows). A basket fiber axon was also identified making contact with the axon hillock of the PC. In the basket axon, there were multiple synaptic sites marked by vesicle accumulations beneath the axonal membrane (Fig. 5D, d′). These findings further corroborate and extend our light microscopic findings of basket cell plexus morphology.

**Correlates of the Hairy Basket Rating**

In cerebellar ET cases and nondiseased controls, the hairy basket rating was robustly correlated with number of torpedoes (Spearman $r = 0.49$, $p < 0.001$; Fig. 6A), inversely with the number of PCs (Spearman $r = -0.27$; $p = 0.06$; Fig. 6B), and inversely with the degeneration index (Spearman $r = 0.48$; $p = 0.001$; Fig. 6C), indicating that these basket cell changes were associated with torpedo formation and PC loss. Within cerebellar ET cases, the hairy basket rating was associated with age (Pearson $r = 0.35$; $p = 0.048$) and marginally with disease duration (disease duration was known in 19 cases and Pearson $r = 0.35$; $p = 0.19$). Hairy basket rating was associated with number of torpedoes (Spearman $r = 0.49$; $p = 0.005$) but not with number of PCs (Pearson $r = 0.01$; $p = 0.98$); however, it was associated with the degeneration index (Spearman $r = 0.45$; $p = 0.01$). Hairy

---

**FIGURE 6.** Correlations of hairy basket scores in cerebellar essential tremor (ET) and nondiseased control cases. (A–C) Hairy basket score correlated with number of torpedoes (A) (Spearman $r = 0.49$; $p < 0.001$), inversely with the number of Purkinje cells (B) (Spearman $r = -0.27$; $p = 0.06$), and degeneration index (C) (Spearman $r = 0.48$; $p = 0.001$).
Hairy Baskets Associated With Essential Tremor

Recent postmortem studies have documented degenerative changes in the cerebellum of ET cases, as manifested by increased numbers of torpedoes and loss of PCs (24, 27–29, 42). These postmortem data complement a rich history of clinical, physiologic, and imaging studies that have long implicated cerebellar and cerebellotegmental circuitry abnormalities in ET (8–15, 19–21, 43). Although previous pathologic studies focused on degenerative changes in PCs, the relationship between ET and cerebellar interneurons has remained unexplored. We examined archival and prospectively acquired cerebellar tissue of ET cases and both diseased and nondiseased controls and found that the axonal plexuses of basket cells surrounding PCs in cerebellar ET brains are significantly “hairier” as compared with controls.

Basket cells are γ-aminobutyric acid–ergic inhibitory interneurons that receive input from parallel fibers and, to a limited extent, from climbing and mossy fibers. In humans, up to 50 axon collaterals from neighboring basket cells descend from the molecular layer and combine to form a complex basket structure around the PC soma to which the basket cells’ entire axonal output is devoted (44). Reductions in cerebellar basket cell axonal plexuses have been reported in several disease states, including hypothyroidism (44), ataxia telangiectasia (45), and Menkes kinky hair disease (46). Preservation of basket cell plexuses in the setting of PC death (i.e. “empty baskets”) has been reported in Creutzfeld-Jakob disease (47) and spinocerebellar ataxia Type 1 (48), but a dense and tangled appearance of the axonal plexus profile has not, to our knowledge, been documented in the ataxia literature. Curiously, an early 20th century study describing PC abnormalities in 16 brains of individuals diagnosed with “senile dementia” noted increased basket cell fiber “tangles and masses” alongside PC soma showing signs of degeneration in 3 cases (31). Another more recent study qualitatively examined basket cells in Creutzfeld-Jakob disease and noted empty baskets with abnormally dense phosphorylated neurofilament positivity in a single case (31). Here, we documented that individuals with a diagnosis of cerebellar ET were more than 10 times as likely as controls to have a hairy basket rating of 3 (the marked or severe end of the spectrum). In addition, the median axonal plexus density in cerebellar ET was approximately 2 to 3 times higher than in controls. “Hairiness” of basket cell axonal plexuses in these independent semiquantitative and quantitative measures was positively associated with number of torpedoes and the degeneration index, and inversely with number of PCs, suggesting that hairy baskets and torpedoes may be concomitant features of cerebellar degeneration in ET.

The mechanism by which this increased “hairiness” occurs is unknown. One possible explanation is that the increased profiles observed in our study represent an accumulation of converging basket cell processes recruited from neighboring PCs that have been damaged or died. Although there has been little investigation of such a phenomenon in the human cerebellum, selective preservation and reorganization of basket cell axonal processes has been demonstrated in basket cells in the CA1 and CA3 region of the hippocampus (49, 50). These cells form baskets around hipocampal pyramidal cells and function as local circuit inhibitory γ-aminobutyric acid–ergic interneurons, analogous to the relationship between cerebellar basket cells and PCs. These disease-resistant hippocampal basket cells undergo extensive reorganization in the setting of pyramidal cell death (51). In a few cases, basket cell plexuses displayed increased density adjacent to areas with severe pyramidal cell loss and no basket formations, suggesting that these basket cell processes might be converging on and reorganizing around remaining pyramidal cells (51).

The molecular mechanisms that underlie the degenerative changes observed in the ET cerebellum are not clear. The array of cellular changes within the complex neuronal network in cerebellar cortex have yet to be fully catalogued in ET; thus, further work with human postmortem tissue is needed. In addition, genes have not been identified for ET, and no transgenic mice currently exist that provide an animal model for ET. The most widely used animal model for ET is based on the administration of neurotoxins (e.g. harmaline and ibogaine) to rodents and other mammals. These toxins induce synchronous firing of inferior olivary neurons at an 8- to 12-Hz frequency and sustained glutamatergic stimulation of PCs via their climbing fiber afferents (52–54), triggering a massive increase in intracellular calcium and an excitotoxic-mediated PC degeneration and cell death (55–57). However, this toxin-based animal model produces an acute, reversible action tremor. Most animals shake for a matter of hours, and there is more severe destruction of cerebellar cortex in readily identifiable linear bands. Although these latter clinical and pathologic features clearly differ from those in ET, this model demonstrates the functional significance of the olivocerebellar system and a potential role for excitotoxic-mediated PC death in the generation of this type of tremor. It is intriguing that harmane (a tremor-producing, indole-alkaloid structurally similar to harmaline) has been found in the blood of some ET patients at levels up to 50% higher than age-matched controls (58). Although additional studies are needed to establish whether this exposure is of etiologic importance in ET, we postulate that the PC loss documented in ET (whether excitotoxic or not) leads to a reorganization of the cerebellar interneuron network, with sprouting and/or accumulation of neighboring basket cell processes on surviving PC bodies.

Ultrastructural analysis revealed the presence of multiple, closely spaced sites of synaptic contact between the dense, multilayered basket cell processes and PC soma in ET. The neurophysiologic abnormality in ET is not clear, but is thought to be the result of decreased cerebellar PC inhibitory output. Our observations raise the possibility that the increased density of basket cell processes identified in a significant number of ET cases contribute to this inhibitory effect on PC output. Recent studies have demonstrated that climbing fiber responses in
PCs are modulated through cerebellar interneuron networks that include the basket cell (59). Basket cell output is also inhibited by the axon collaterals of PCs and excited by both parallel fibers and collaterals of climbing fibers (60). Cerebellar interneurons are also coupled by dendro-somatic, dendro-dendritic, and somato-somatic gap junctions, which may lead to synchronization of firing in groups of organized neural networks (61). Specialized axo-axonic septate junctions electrically couple basket fiber processes in the axon hillock region (62). Thus, the disease process in ET may cause an imbalance in the interactions between cerebellar afferents, PCs and their axon collaterals, and the cerebellar interneuron network that includes basket cells. Additional studies on other cerebellar neurons and their interactions in ET are clearly needed to understand the basis for tremor generation in this disorder.

Despite being the largest series of ET brains reported to date, the sample size was modest. Despite this limitation, we were able to detect significant case-control differences, indicating that the sample was sufficient. This study had several strengths. This was the first investigation of a relatively novel pathologic finding in a large series of ET brains. We examined basket cell profiles both semiquantitatively and quantitatively and, furthermore, compared ET brains against both nondiseased control brains and a group of diseased controls with a variety of neurodegenerative pathologies.

In summary, the degenerative changes in PCs that characterize cerebellar ET are accompanied, in some cases, by a dense and tangled, or “hairy,” appearance of basket cell axonal plexus formations. This relatively novel finding was not observed to any degree in either diseased controls or normal control brains. This finding provides initial evidence that structural changes in ET are not restricted to the PC but also involve their functional network. The presence of hairy baskets is a heretofore uninvestigated phenomenon, and further exploration is warranted to understand its relationship to PC degeneration and its role in the pathogenesis of ET.

ACKNOWLEDGMENT

The authors thank Hong Yi (Emory University) for assistance with preparation of tissue for ultrastructural studies.

REFERENCES

31. Uyematsu S. A study of some peculiar changes found in the oxons and dendrites of the Purkinje cells. Arch Neurol Psych 1921;5:231–69.