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Purkinje Cell Loss is a Characteristic of Essential Tremor

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Abstract

The study of the neuropathological underpinnings of essential tremor (ET) is a relatively new undertaking. The purpose of this paper is three-fold. The first is to comment on methodological problems in a recently-published paper by Rajput et al, the major one being the small sample size of that study, which resulted in a Type II statistical error. Hence, one cannot conclude based on their data that there is no Purkinje cell (PC) loss in ET. Secondly, we comment on conceptual problems with that study, which suggested that PC loss might not be a featured characteristic of ET because it is also found in other disease states. We discuss why this is an erroneous conclusion. Our third purpose is to more broadly discuss the role of the cerebellum in ET, giving consideration to the wealth of clinical and postmortem data that have accumulated over recent years. In this discussion, we make the following points: (1) it is now generally recognized that ET is a disease of cerebellar systems dysfunction, (2) given the nature of the postmortem work, revealing the presence of several types of structural-anatomical changes within the cerebellum and absence of detectable changes in other brain regions, the most empirically-based explanation is that the primary problem in ET is in the cerebellum itself, (3) that the collection of cellular changes in the cerebellum in ET are also present in other cerebellar degenerations should add to rather than detract from the notion that ET is a disease of cerebellar degeneration.

Keywords

Essential tremor; cerebellum; Purkinje cells; neuropathology; neurodegeneration

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This paper began as a letter to the editor, commenting on several methodological and conceptual problems with the paper by Rajput et al. [1]. We were asked by the editors to expand the paper to include a more general discussion of the role of the cerebellum in essential tremor (ET). The paper is thus compartmentalized into these two main sections.

1. Comments on Rajput et al. [1]

We read with interest the paper by Rajput et al.[1] regarding Purkinje cell (PC) loss and ET and would like to comment on several issues.

First, they write: “The hypothesis that PC loss is pathognomonic of ET [here they cite papers by our group] is not supported by our data”. “Pathognomonic” implies that a particular feature is so specific to or distinctly characteristic of a disease that it makes the diagnosis. We have not put forth the hypothesis that PC loss is only found in the ET brain. Rather, we found that PC loss is characteristic of the ET brain in comparison with normal aging, and that it is one of a constellation of cerebellar changes that distinguish the ET brain from normal aging [2,3].

Second, PC loss may also be seen in other diseases, and they write that this is “inconsistent with the hypothesis that, [sic] progressive PC loss is characteristic of ET”. To the contrary, a pathological finding may be characteristic of more than one disease. Thus, that neuronal loss in the cerebellar dentate nucleus is characteristic of progressive supranuclear palsy makes it no less a characteristic feature of some forms of spinocerebellar ataxia and, in both situations, distinguishes them from healthy aged brains. Suggesting that PC loss is not a featured characteristic of ET because it is also found in other disease states is an erroneous conclusion.

Third, there is the major issue of study power. In statistical hypothesis testing, there are two types of incorrect conclusions or “errors” that may be drawn, namely Type I and Type II errors. A Type II error (a false negative) occurs if the null hypothesis is incorrectly accepted when it should in fact be rejected (i.e., when an investigator concludes that there is no difference between groups when one truly exists). Studies with small sample sizes (i.e., under-powered studies) are particularly prone to Type II errors. Their conclusion that there is no difference in PC number between ET cases and normal controls, or between ET cases and diseased controls (PD) reflects a Type II error. The study enrolled only 7 ET cases, 6 Parkinson’s disease (PD) cases, and 2 controls. Hence, their statistical hypothesis testing involved comparisons of 7 vs. 6 (ET vs. PD) and 7 vs. 2 (ET vs. controls). As shown below (Table), PC counts were lower in ET cases than controls using each of their three counting methods, with the reduction ranging from 5.8% to 23.7%. While a 5.8% reduction is marginal, a 23.7% reduction represents a drop by nearly one-quarter, and is likely to have clinical consequences. The study lacked the power to determine whether any of these percentage differences were statistically significant. Power is ideally $\geq 80\%$ in any study, but in their study ranged from only 13.7%–17.6% (i.e., the probability of rejecting the null hypothesis in the setting of a true case-control difference was only 13.7% – 17.6%). For the ET vs. PD comparison, PC counts were lower in ET than PD cases using two of their three methods (Table), with the reduction ranging from 9.3% to as high as 31.4%. Here again, a 31.4% difference is clinically significant but the study lacked the statistical power (31.2% – 41.2%) to determine whether differences were statistically significant. In summary, with differences across the board as well as differences of 23.7% that were not found to be significant, the small sample size of their study resulted in a Type II error, and hence, one cannot conclude based on their data that there is no case control difference. They write that their study “militates against” there being a difference despite the fact that the study does not have the statistical power to assess case-control differences.

Their argument that the sample size was adequate because Axelrad et al. [3] enrolled a similar number of ET cases is not valid for two reasons. First, it is the number of cases and controls (i.e., the total sample size) that determines the study power. Axelrad et al. [3] enrolled 14 ET cases and 11 controls; thus, the total sample size was nearly double that of the study by Rajput et al. [1]. Second, sample size is a moot point in a study that rejects the null hypothesis (e.g. Axelrad et al. [3]), and is only an issue in studies that fail to reject the null hypothesis (e.g., Rajput et al. [1]), as the issue is whether the latter represent true null studies (i.e, proof of absence) or rather, merely inconclusive, underpowered studies (i.e., absence of proof) that make Type II errors.

Finally, they noted that there was no correlation between PC counts and tremor severity/duration, arguing that this provides evidence that PC depletion is of dubious significance in ET. There are several problems with this conclusion. With their small sample size, the study was unable to detect a statistically significant age-associated reduction in PCs (only an insignificant trend was present), showing that even well-established biological associations went statistically undetected in their study. More importantly, the study argues that clinical features and pathological changes must by necessity correlate in neurodegenerative diseases, but this is not the absolute rule. In PD, for example, it has been shown that Braak Lewy body stage does not correlate with clinical (Hoehn and Yahr) stage [4], and in Alzheimer's disease (AD), plaque counts have been shown not to correlate with mini mental status test scores [5]. In brain bank samples, one is often dealing with end-stage disease, making such clinical-pathological correlations challenging. Furthermore, plaques, Lewy bodies, or PC loss may be mere markers of subtler and/or more central pathophysiological changes that are likely to better correlate with clinical scores. While cerebellar PC loss is certainly not pathognomonic of ET, its presence may well be a remnant of a slowly progressive PC dysfunction, which certainly warrants further investigation. We now turn to a more general discussion of the role of the cerebellum in ET, in light of work conducted over recent years.

2. General Discussion of the Role of the Cerebellum in ET

A. It is Generally Recognized that ET is a Disease of Cerebellar Systems Dysfunction

Given the sheer wealth of accumulating clinical, physiological, neuroimaging, surgical-therapeutic, and postmortem data (far too numerous to cite here)[6,7], it would be difficult to argue that ET is anything other than a disease (or a set of related diseases) primarily of cerebellar system dysfunction. This is important to recognize as it provides both a physiological and a loose anatomical framework with which to begin to conceptualize ET as well as a focus through which to advance our studies of this disease/disease complex.

B. Where in the “Cerebellar System” is the Primary Problem in ET?

Whether the principal problem in ET is in the cerebellum itself or in cerebellar relay systems (i.e., input and/or output pathways) has been a subject of debate [8]. Given the nature of the postmortem work, revealing the presence of several types of structural-anatomical changes within the cerebellum and absence of detectable changes in other brain regions (see section C below), the most empirically-based explanation is that the primary problem in ET is in the cerebellum itself. Beginning with the pioneering work of Morgagni in the mid-1700s and advancing through to studies of PD in the last century, a basic tenet in modern medicine is that diseases arise from pathological changes in tissue, and the detection of these changes is an important step for identifying the locus of disease. Acceptance of such tissue-based evidence can be slow, even in situations where the evidence now seems unquestionable; thus, early deniers of the role of the substantia nigra in PD claimed, despite the presence of clear pathological change, that it was too small to be of any physiological significance [9]. In terms of ET, if the identified structural-anatomical changes in the cerebellum are not

primary, the alternative premise is that they are merely secondary to some other primary process. The problem with this premise is the near absence of empiric evidence to support it. A postulate that has been perpetuated in the tremor literature for decades is that the tremor of ET *could be* the result of abnormal intrinsic oscillations originating in the inferior olivary nuclei and spreading throughout the olivocerebellar network [10]. Somehow, over time, “could be” has evolved into “is”, and this notion is often uncritically accepted. Yet there is no direct evidence to substantiate this model in any human studies. Indeed, the sole support seems to be derived from the harmaline animal model of tremor. Harmaline, a neurotoxin, induces synchronized rhythmic activity of inferior olive neurons by increasing their electrical coupling. When administered to laboratory animals under experimental conditions it produces an artificial, acute, reversible, total body tremor. While this animal-toxin-induced tremor shares several features with the tremor seen in the human disease ET (e.g., tremor frequency), it also shares clinical features with the tremor of hyperthyroidism, lithium, and a variety of other tremor types. The tremor in the laboratory animals also differs in several fundamental respects from that seen in humans with ET (e.g., its very short duration and wide-spread bodily distribution). We must be careful not to draw uncritical parallels between this experimental model of tremor and the specific human disease ET. While it is possible that the structural-anatomical changes in the ET cerebellum are merely secondary, one must then contend with the absence of an empirically-based alternative that is confirmed in human studies.

C. There are Structural-Anatomical Changes in the ET brain

As late as 2004, large uncontrolled (i.e., no age-matched comparison brains) studies were published without a quantitative approach to cerebellar microscopic pathology [11]. The need for controlled, quantitative studies of ET was apparent. With such studies, we have learned that there is an approximate 7-fold increase in number of damaged PC axons (i.e., PC axonal swellings or “torpedoes”), an approximate 30–40% reduction in the number of PCs, an increase in the number of heterotopic (displaced) PCs, structural-anatomical changes in surrounding neurons (i.e., hypertrophy of basket cell processes) and glial cells (Bergmann gliosis in some studies) and, in some ET cases, extension of these cortically-based changes to the underlying cerebellar dentate nucleus [2,6]. The latter as well as the presence of basket cell changes indicates that ET is not merely a disease of PCs. It is equally important to emphasize that these studies utilized age-matched control tissue, thereby indicating that the changes in ET are disease-associated and not merely age-associated. The studies also adjusted for other potential confounders (e.g., ethanol intake). Studies have demonstrated that ET brains with more damaged PC axons (i.e., more torpedoes) also have fewer PCs, which makes mechanistic sense, as do the findings that brains with more displaced (heterotopic) PCs have fewer surviving PCs and that hypertrophic changes in basket cell processes are correlated with numbers of damaged PCs (i.e., torpedoes) [2,6,12]. In other words, the microscopic changes are not occurring in random, senseless isolation but seem to be part of a cascade of mechanistically inter-related primary and secondary cellular changes. These studies included tissue collected prospectively as well as tissue from several brain banks, including tissue kindly provided by Dr. Rajput’s group, the latter of which revealed PC loss and excessive torpedoes, as discussed elsewhere [6]. Our published studies represent only a start, as current investigations begin to explore alterations in PC dendritic morphology, extent of abnormal PC recurrent collateral formation and other structural disarrangements within the complex, cerebellar cortical microenvironment in ET.

D. ET is not the only Disease with Underlying Degenerative Changes in the Cerebellum

Cerebellar degenerations share many of the same set of microscopic pathological features (torpedoes, PC loss), indicating that there is a certain bland commonality to the manner in which the cerebellum degenerates [6]. Yet none would argue that SCA-2 is not a cerebellar

degeneration because the collection of cellular changes is rather typical of other cerebellar degenerations, sharing many of the same features. One must apply the same logic to ET as well.

Nonetheless, the cerebellum does not always degenerate in exactly the same manner. As reviewed previously [6], SCA-2 differs from SCA-3 in that SCA-2 is characterized by severe atrophy of PCs, while in SCA-3, the PCs are intact. Similarly a rapidly progressive and subtotal/total destructive cerebellar degenerative process (e.g., several of the SCAs) would not be expected to exhibit the same exact changes as a slowly progressive subtotal process, as occurs in ET. The important challenge is to try to understand the nature of the underlying molecular disarrangement that sets these cellular cerebellar changes in motion.

E. Summary

As we move forward with our understanding of the biology of ET, it will be important to question and debunk old myths, carry out careful, quantitative, adequately-powered, controlled studies to collect a set of empiric data, and to build a logical conceptual framework around these data.

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Table

Power of study by Rajput et al.[1]

	Reduction (%) in number of Purkinje cells in ET cases vs. controls (Rajput et al. [1])	Sample size used by Rajput et al.[1]	Power to detect a 20% reduction in number of Purkinje cells in ET cases vs. controls	Sample size needed for adequate power to detect a 20% reduction in Purkinje cells in ET
Purkinje cell count 1 (sectioned Through any part of nucleolus)	23.7%	7 (ET) vs. 2 (controls)	15.2%	7 (ET) vs. 17 (controls)
Purkinje cell count 2 (sectioned Through any part of nucleus)	11.3%	7 vs. 2	17.6%	7 vs. 15
Purkinje cell count 3 (sectioned Through any part of cell body)	5.8%	7 vs. 2	13.7%	7 vs. 19
	Reduction (%) in number of Purkinje cells in ET cases vs. PD cases (Rajput et al.[1])	Sample size used by Rajput et al.[1]	Power to detect a 20% reduction in number of Purkinje cells in ET cases vs. PD cases	Sample size needed for adequate power to detect a 20% reduction in Purkinje cells in ET
Purkinje cell count 1 (sectioned Through any part of nucleolus)	0.0%	7 (ET) vs. 6 (PD)	35.1%	7 (ET) vs. 17(PD)
Purkinje cell count 2 (sectioned Through any part of nucleus)	31.4%	7 vs. 6	41.2%	7 vs. 15
Purkinje cell count 3 (sectioned Through any part of cell body)	9.3%	7 vs. 6	31.2%	7 vs. 19