CEREBELLAR PURKINJE NEURONS WITH ALTERED TERMINAL DENDRITIC SEGMENTS ARE PRESENT IN ALL LOBULES OF THE CEREBELLAR VERMIS OF AGEING, ETHANOL-TREATED F344 RATS

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Abstract — Previous studies from this Laboratory have shown that cerebellar Purkinje neurons (PN) in ageing, ethanol-fed Fischer 344 rats may have terminal dendritic segments that are longer than in control rats. They also showed that the longer terminal segments represented a toxic effect of ethanol on PN, because their increase in length resulted from an ethanol-induced deletion of other terminal dendritic segments and not from dendritic growth. The purpose of the present study was to determine whether this effect of ethanol was localized to specific lobules or was widely distributed within all lobules of the cerebellar vermis. Twelve-month-old male Fischer 344 rats were treated with a liquid ethanol diet for 48 weeks. Age- and weight-matched controls were pair-fed with an isocaloric control diet. Terminal dendritic segments in Golgi–Cox-stained PN in four groups of lobules in the cerebellar vermis of control and ethanol-fed rats were measured for treatment-related changes in length. Results from these measurements showed that ethanol-exposed PN with significantly longer terminal segments were present in all groups of lobules, i.e. they were widely distributed and not confined to specific lobules. Results from these measurements also confirmed in a large sample of neurons (40 neurons per rat) that the topologically distinct unpaired terminal segments were characteristically longer than the paired terminal segments in PN of control and ethanol-fed rats and that both types of terminal segments were longer in the ethanol-fed rats than in the controls.

INTRODUCTION

Cerebellar Purkinje neurons (PN) are known to be sensitive to acute and chronic elevations of ethanol in circulating blood (Victor et al., 1959; Walker et al., 1981; Pentney, 1982, 1995; Torvik et al., 1982; Tavares et al., 1983a,b, 1987a,b; Phillips, 1985; Torvik and Torp, 1986; Pentney et al., 1989; Pentney and Quackenbush, 1990, 1991; Lin et al., 1992; Karhunen et al., 1994; Wu et al., 1995; Dlugos and Pentney, 1997). Previous studies from this Laboratory have shown several important effects of long-term intake of intoxicating amounts of ethanol by ageing Fischer (F)344 rats on PN. These effects included an increase in the individual lengths of terminal segments (Pentney et al., 1989; Pentney and Quackenbush, 1990, 1991), a decrease in the median path length of dendritic arbors (Pentney, 1995) and a decrease in the total number of synapses on PN dendritic arbors (Dlugos and Pentney, 1997). These results were consistent with a model of dendritic degeneration in which ethanol induced deletions of terminal dendritic segments at their junctions with their parent branches (Pentney, 1995). The deletion of terminal segments was also shown recently to be the most probable cause of the decrease in median path lengths of the dendritic arbors and of the loss of synapses on the dendritic arbors (Tabbaa et al., 1999).

Several of the above studies utilized procedures based on quantitative data from entire dendritic arbors. Two major drawbacks of that type of analysis result from the stringent criteria that must be used for selection of entire dendritic arbors. Those criteria yield only a limited number of PN for study in each subject, and the lobular subdivisions of the cerebellar vermis are not represented equally or extensively. An important conclusion, that all of the ethanol-induced modifications in PN of ageing F344 rats involved terminal segments of the PN arbors, did emerge from those early studies, however. Furthermore, even in the restricted cell samples in those studies, only 30% of the PN had ethanol-modified dendritic arbors (Pentney, 1995), suggesting that the effects of ethanol might be localized, rather than widespread throughout the cerebellar vermis. It has been known for some time that, during perinatal development, rodent PN demonstrate a regional sensitivity to ethanol’s effects related to their maturational status (Ward and West, 1992). The question of regional differences in sensitivity of PN to ethanol within the cerebellar vermis has not been addressed previously in the ageing cerebellum.

The study presented here used only terminal segments of a large number of PN dendritic arbors in each subject for quantitative analysis. The exclusion of all dendritic segments, except terminal segments, offered the advantage of increasing the number of cells that could be sampled in each subject. The sampling procedure was also designed to obtain equal samples of PN from all subdivisions of the cerebellar vermis in order to address the question of PN regional sensitivity to ethanol. The main purpose of this analysis was to determine whether the effect of ethanol on PN terminal dendritic segment lengths was expressed broadly throughout the vermis or was localized to specific lobules of the vermis. A secondary outcome of this study was that two key results from previous studies, i.e. a consistent significant difference between the mean lengths of paired and unpaired terminal dendritic segments in the arbors and consistent ethanol-related increases in terminal segment lengths (Pentney et al., 1989), were also corroborated.

METHODS

Animal model

Sixteen 12-month-old, male F344 rats (NIA colony, Harlan Sprague Dawley, Inc.) were used in this study. The rats were assigned randomly to ethanol and control treatment groups.

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Rats in the ethanol-fed group received a vanilla-flavoured Sustacal-based liquid diet (Mead-Johnson Nutritional Division, Evansville, IN, USA). Each 100 ml of the diet also contained 0.3 g of vitamins, 0.7 g of salts (Vitamin Diet Fortification Mixture and U.S.P. Salt Mix 14, ICN Nutritional Biochemicals), and 6.25 g of ethanol (diet formulated by Freund, 1980). Rats in the control group received an isocaloric diet lacking ethanol. Rats in the control group were matched by weight with rats in the ethanol group for the pair-feeding protocol, i.e. ethanol-fed rats had daily ad libitum access to the ethanol diet and the pair-fed rats received the volume of diet consumed by their ethanol-fed partners on the previous day. In our previous studies, an additional chow control group was used. Results from those studies showed that the terminal dendritic segments in ethanol-fed rats were significantly longer than corresponding terminal segments in chow-fed and pair-fed control rats, whereas there were no significant differences between the lengths of terminal segments in the two types of controls (Pentney and Quackenbush, 1990, 1991). For this reason, only one control group, the liquid diet control group, was used for measurements of dendritic length in this study.

Treatment conditions were identical for the control and ethanol-treated rats. They were housed in a room dedicated to this study in the Laboratory Animal Facilities of the School of Medicine and Biomedical Sciences at the University of Buffalo. Lighting was on a 12 h light/12 h dark cycle (lights on at 06:00), and temperature was maintained at 22 ± 2°C. The rats were housed individually in wire cages suspended from racks. They were weighed weekly to monitor their health during treatment with the liquid diets. The Institutional Animal Care and Use Committee (IACUC) of the School of Medicine and Biomedical Sciences approved all procedures used for treatment and care of the animals.

**Blood alcohol concentrations**

After 6 weeks of ethanol treatment, blood samples for measurements of circulating blood alcohol levels (BAL) were collected from tail veins at 23:00, 13 h after fresh diet was placed on the cages and 5 h after the start of the dark period. Blood samples were also collected from three control rats. The samples were analysed by gas chromatography with n-propanol as an internal standard.

**Tissue preparations**

Following 48 weeks of dietary treatment, each rat was given a lethal dose of chloral hydrate (400 mg/kg, intraperitoneal). Once the rat was deeply anaesthetized, the cervical spinal cord was severed, and the brain was freed gently from the skull. The cerebellum was then isolated from the brainstem, and the vermis was separated from the cerebellar hemispheres and placed immediately in the Golgi–Cox fixative. Details of the fixation and block-staining procedures have been described in detail previously (Pentney, 1986). The blocks of tissue were subsequently dehydrated, embedded in 10% celloidin, and placed immediately in the Golgi–Cox fixative. Details of the fixation and block-staining procedures have been described in detail previously (Pentney, 1986). The blocks of tissue were subsequently dehydrated, embedded in 10% celloidin, and sectioned parasagittally at 120 μm. All tissue blocks were coded prior to the embedding step to avoid investigator bias during selection of cells for analysis.

**Quantitative procedures**

PN selected for measurements of dendritic segment lengths were completely filled with the Golgi–Cox precipitate, had unbroken terminal dendritic segments (Figs 1 and 2A), and could be brought into sharp focus with a ×100 oil immersion objective lens. In a bifurcating arbor, such as that of PN, two-thirds of the spiny terminal segments will be at the peripheral tips of the dendritic branches. These segments were arranged in pairs, or rarely as triplets, at the ends of the bifurcating branches (Fig. 2A, arrowheads). The remaining third of the spiny terminal segments, the unpaired terminal segments, branched singly from second- or higher-order segments within the arbors (Fig. 2A, arrow). It was shown previously that the mean lengths of paired and unpaired terminal dendritic segments were significantly different (Pentney et al., 1989). For that reason, the paired and unpaired terminal segments were measured and analysed separately.

As the purpose of this study was to determine whether the effect of ethanol on the lengths of terminal segments occurred in PN in all lobules or only in specific lobules of the vermis, it was necessary to select neurons from all lobules of the vermis and to ensure that all regions of the vermis were represented equally in the cell sample. To accomplish these objectives in an efficient manner, we categorized the lobules of the vermis into four groups: lobules I–II, III–V, VI–VIII and IX–X (Fig. 3). In each rat, measurements of terminal dendritic segment lengths were made in 10 neurons in each of the four groups for a total of 40 neurons per rat. Cell selection was begun at the midline, with adjacent sections to the right and left of the midline being used alternately until 40 neurons had been selected. Only one of the 10 neurons in each lobular group was selected from a single section, ensuring also that a minimum of 1200 μm, 600 μm on each side of the midline, was sampled along the longitudinal axis of the lobules. In some rats, it was necessary to sample neurons over greater distances (up to 3600 μm, 1800 μm on each side of the midline) in order to obtain measurements from 40 neurons.

Random samples of 15 pairs of terminal dendritic segments and 15 unpaired terminal dendritic segments were measured in each neuron. The procedures for selection of these segments were as follows. The pairs of terminal segments were numbered in an exact clockwise sequence from the origin of the primary dendrite. A random numbers table was used to determine which of the pairs of terminal segments would be the first pair to be measured. The image of each terminal dendritic segment, viewed with a ×100 oil immersion objective lens, was projected through a Zeiss optical microscope drawing tube (final magnification was ×1760) onto a flat drawing surface. A line passing from the junction of the terminal segment with the parent segment through the centre of the dendrite to its free tip was then made and used for measurement of the segment length. Additional pairs of terminal dendritic segments were drawn in sequence until drawings of a complete sample of 15 pairs of terminal segments had been made. The above procedure was then repeated for an independent selection of 15 unpaired terminal segments. In this way, 1200 paired terminal segments and 600 unpaired terminal segments were selected for measurement in each rat. Digitized measurements of the linear tracings were then made on a Numonics digitizing tablet with AutoCAD, version 12.0.

**Statistical analysis**

Mean values for the lengths of paired and unpaired terminal dendritic segments were initially determined for each PN.
Subsequently the data were grouped in two different ways. First, the mean lengths of all paired terminal segments and of all unpaired terminal segments in each PN arbor were determined. From the mean values in each cell, means for the group of 40 PN arbors in each rat were then determined, yielding a single mean value for each type of segment in each rat. Mean values for each treatment group of eight rats were then determined. A split-plot analysis of variance (ANOVA) was used to test for significant differences between the mean values for the lengths of the two types of terminal dendritic segments (paired and unpaired) in the control and ethanol-fed rats. This modified ANOVA adjusted for the fact that measurements of

Fig. 1. A representative Golgi–Cox-stained Purkinje neuron from a 24-month-old F344 rat, treated chronically with ethanol for 48 weeks. The extensive dendritic arbor is composed of internal and external segments. All external segments are terminal segments, i.e. they have a free tip. Paired terminal segments are attached to and extend from the terminal bifurcation point (junction) of a dendritic branch. Unpaired terminal segments are attached to non-terminal bifurcation points within the dendritic arbor. Arrowheads point to the pial surface of the cerebellar lobule. Paired and unpaired terminal segments enclosed by the frame on the left edge of the dendritic field are shown in greater detail in Fig. 2. Calibration bar = 20 μm.
pair ed and unpaired terminal segment lengths were from the same rats.

Second, the data from the 40 PN in each rat were separated according to the lobular location of the cells to determine mean values for the ten cells in each of the four groups of lobules. From the values determined for each lobular group in each rat, mean values were subsequently determined for each treatment group. Two-way ANOVA was used to determine whether there were significant differences in the lengths of terminal segments in the four groups of lobules in the vermis of the control and ethanol-fed rats. Separate tests were performed for the paired and unpaired terminal segment values. Main effects identified in the two-way ANOVA were analysed further by a simple ANOVA and localized with a post hoc Duncan multiple range test, as necessary. In all tests an alpha level less that 0.05 was accepted as significant.

RESULTS

Measurements of body weights

The initial body weights of rats in the ethanol and control groups (means ± SEM) were 367.9 ± 21.3 g and 411.1 ± 23.4 g, respectively. The terminal weights of rats in the ethanol and control groups were 454.2 ± 27.4 g and 508.0 ± 25.3 g, respectively. All ethanol and control rats gained weight during the 48 weeks of dietary treatment. In spite of the fact that equal calories were consumed over the course of treatment by all ethanol and pair-fed partners, however, the average
increase in body weight per control rat was 10.4 g more than that of its ethanol-fed partner.

**Measurements of BAL**

The mean BAL of the ethanol-treated rats was 90.3 ± 34.3 mg/dl, confirming that intoxicating levels of circulating ethanol were present in many of the rats when the blood samples were collected. The volume of the ethanol diet consumed by each rat prior to blood collection was recorded, but the time between blood collection and the last feeding by each rat was not known. It could not be determined, therefore, whether the BAL measured at 23:00 h in individual rats actually corresponded to peak BAL in these rats. Alcohol levels from metabolic sources in blood samples from the control rats ranged from 0 to 3 mg/dl.

**Lengths of terminal segments in control and ethanol-fed rats**

The mean length of the unpaired terminal segments in all rats was significantly longer than the corresponding mean for the paired terminal segments ($F_{1,28} = 98.765, P < 0.001$, Fig. 4). There was also a main effect of chronically consumed ethanol on the mean lengths of all terminal dendritic segments in PN, regardless of terminal type. The mean length of all terminal segments in PN of the ethanol-treated rats was significantly longer than the corresponding mean in the controls ($F_{1,28} = 17.274, P < 0.005$, Fig. 5).

**Effect of ethanol versus lobular location**

The values in Table 1 are group means derived from the animal means in each treatment group. There was a significant effect of ethanol on terminal dendritic segment length with the paired terminal segments in the ethanol-treated rats being consistently longer than those in the control rats ($F_{1,14} = 6.65, P < 0.025$). There was no significant difference in the mean lengths of paired terminal segments relative to their location within specific groups of lobules ($F_{3,42} = 2.31, P > 0.05$, Fig. 6).

A similar analysis of the lengths of unpaired terminal segments also confirmed that there was a significant effect of
ethanol on the mean lengths of the unpaired terminal segments. Those in the ethanol-treated rats were consistently longer than those in the controls ($F_{1,14} = 10.64$, $P < 0.01$). This test also indicated that there was a significant effect of PN location within specific lobules ($F_{3,42} = 5.75$, $P < 0.005$), but the validity of this effect was questionable for the following reasons. Localization of the effect was not achieved in subsequent tests. A simple ANOVA of the unpaired terminal segment lengths in ethanol-fed rats showed that there were definitely no significant differences relative to the lobular location of the unpaired terminal segments in that treatment group ($F_{3,28} = 0.79$, $P = 0.51$). A simple ANOVA of the measurements of unpaired terminal segment lengths in control rats did yield an $F$-value that approached significance ($F_{3,28} = 2.50$, $P = 0.08$) but did not reach the acceptable alpha level of $<0.05$. Further tests were not carried out.

DISCUSSION

Results presented here corroborated and extended earlier reports from this laboratory relative to the effects of ethanol on cerebellar PN. Those that corroborated earlier reports confirmed that: (1) unpaired terminal dendritic segments were significantly longer than paired terminal dendritic segments (Pentney et al., 1989; Woldenberg et al., 1993), a topological characteristic that was not disrupted by ethanol treatment; (2) paired and unpaired terminal dendritic segments were longer in ethanol-fed, than in control, rats (Pentney et al., 1989; Pentney and Quackenbush, 1990, 1991). When initially reported, the ethanol-induced lengthening of terminal dendritic segments was believed to represent a type of compensatory dendritic growth (Pentney, 1991) but is now understood to result from deletion of other closely associated terminal segments (Pentney, 1995; see also in the present work, Fig. 2). Most importantly, results from this study provided new information showing that PN that are sensitive to the effects of ethanol are present in all lobules of the cerebellar vermis. The use of randomly selected dendritic terminal segments for measurements of dendritic lengths was unquestionably a more efficient approach to data collection than was the use of entire dendritic arbors for measurements in our earlier studies (Pentney et al., 1989; Pentney and Quackenbush, 1990, 1991; Pentney, 1995). It must be acknowledged, however, that justification for the use of a random selection of only terminal dendritic segments for measurements was tied directly to results from measurements of entire dendritic arbors. The latter measurements indicated strongly that terminal dendritic segments were the primary targets of the actions of ethanol.

Whereas PN that are sensitive to ethanol were shown here to be distributed widely within all lobules of the cerebellar vermis, it would not be correct to deduce that terminal segments in all PN were equally sensitive to ethanol. In fact, this appears not to be true from the previous study in which 30% of randomly sampled PN in the ethanol-fed rats were markedly sensitive to the actions of ethanol, with the remaining 70% appearing to be insensitive (Pentney, 1995). Those results had suggested that PN sensitive to ethanol might be distributed within the vermis in a localized, rather than a

Fig. 6. A histogram showing that the mean lengths of the paired terminal segments in Purkinje dendritic arbors were longer in the ethanol-fed rats than in the controls in all lobular groups.

There were no significant differences between lobular groups ($P > 0.05$).

Fig. 7. A histogram showing that the mean lengths of the unpaired terminal dendritic segments were significantly longer in the ethanol-fed rats than in the controls.

A significant effect of lobular location was also indicated ($P < 0.005$). With further testing, this effect was shown not to be attributable to the ethanol. The effect appeared to be within the control group but it could not be localized. An attempt to do so produced an $F$-value that exceeded the acceptable alpha level ($P = 0.08$).
widespread, pattern but results presented above refute this suggestion. The full extent of dendritic damage that can be induced by ethanol in affected PN before that damage becomes lethal for the cell is not known. In our studies, the dendritic damage could be completely reversed by a sufficiently lengthy withdrawal period (Pentney and Quackenbush, 1991; Pentney, 1995; Dlugos and Pentney, 1997), suggesting that the ethanol-induced dendritic damage detected in our model remained sublethal for lengthy periods of time. Other investigators have reported that, in Sprague Dawley rats, chronic ethanol intake during 80% of their life span was required to produce a significant loss of PN (Tavares et al., 1987a). It may be that PN have such expansive dendritic arbors that they may sustain large amounts of dendritic damage before that damage proves to be lethal.

As shown above, PN with longer terminal dendritic segments were present in all lobules of the vermis in ethanol-fed rats. Given that ~90% of the vermis was sampled in the present study, we conclude that PN sensitive to ethanol were distributed widely throughout the vermis. It appears likely, therefore, that the sensitivity of mature PN to ethanol may be independent of particular sources of afferent input to PN or particular efferent targets of PN, since it is independent of particular lobular locations. PN sensitive to ethanol may also be widely distributed within the cerebellar hemispheres, but the hemispheres were not included in the present study.

It is important to note that there is a potential caveat to the above conclusion that could not be addressed in this study. It is possible that PN sensitive to ethanol might be restricted to specific cerebellar microzones, functionally organized units of the cerebellum (Oscarsson, 1979). PN within specific sagittally oriented microzones might share a sensitivity to ethanol, whereas PN in adjacent microzones might be insensitive. It appears unlikely that sensitivity to ethanol itself would be a characteristic feature of microzonal organization, but the possibility that certain biochemical characteristics of PN that are features of microzonal organization may predispose PN to be sensitive to elevated levels of ethanol cannot be ruled out at present. To our knowledge, the sensitivity of PN to ethanol has not been studied in relation to microzonal organization, and microzones of the vermis cannot be identified in Golgi–Cox preparations.

Some regional differences have been shown, e.g. PN in the posterior lobe of the cerebellum were shown to have significantly reduced synaptic input in rats that consumed an ethanol diet for long periods of time (Dlugos and Pentney, 1997). Earlier studies of synaptic organization in the molecular layer of ethanol-treated rats had not examined differences in the numbers of synapses in different lobes (Phillips, 1985; Tavares et al., 1987b). The only studies in which PN that were sensitive to ethanol were found to be limited to specific lobules of the cerebellum vermis were studies of the effects of ethanol on the developing cerebellum during the immediate postnatal period (Bauer-Moffett and Altman, 1975, 1977; Pierce et al., 1989; Bonthius and West, 1990). In these studies, a lobule-related sensitivity was shown to stem from differences in PN maturation in different lobules at the time of exposure to ethanol.

Maturational differences between PN are not likely to be an important cause of the sensitivity of PN to ethanol in adult subjects. It could be expected, however, that age-related neuronal degeneration might be important. The normal life span of F344 rats is ~25 months (data supplied in Harlan Product Guide, effective 1 July 1999), and the rats used in the study reported here were 24–25 months of age. Currently available data show that the numbers of PN in rodent cerebella are stable between 3 and 27 months of age (Drüge et al., 1986; Bakalian et al., 1991; Dlugos and Pentney, 1994). Several other morphometric parameters of the cerebellum are altered by ageing, however. The volume of the cerebellar molecular layer (Dlugos and Pentney, 1994), the numbers of PN terminal dendritic segments (Woldenberg et al., 1993), and the total volume of anterior lobe PN arbors (Dlugos and Pentney, 1997) all decline with normal ageing. It was also shown some time ago that PN dendritic terminal segments shortened significantly by 18 months of age in F344 chow control rats (Pentney, 1986). Nonetheless, PN dendritic terminal segments of 18-month-old F344 rats treated with ethanol between 12 and 18 months of age were unchanged in length (Pentney and Quackenbush, 1991). We cannot dismiss the possibility that an age-related shortening of terminal dendritic segments and an ethanol-related lengthening of terminal dendritic segments (through deletion events) may fortuitously balance out to produce normal mean values for terminal segment lengths in 18-month-old ethanol-fed rats. Only after an additional lengthy period of alcohol consumption, however, was there significant evidence of an effect of the ethanol on dendritic terminal segment lengths. These results suggest that the ageing process per se did not sensitize PN dendritic arbors to the effects of ethanol. Previous studies have shown that there are interactions between dietary treatment and treatment duration and between dietary treatment and recovery (Pentney and Quackenbush, 1991), but not between dietary treatment and ageing. It can be presumed that rats that survive to 24 months of age represent naturally healthier subjects in our treatment groups, yet PN dendritic terminals in 24-month-old rats were not immune to the effects of a sufficiently lengthy ethanol treatment (Pentney and Quackenbush, 1991).

There was a difference between weights of ageing, control and ethanol-treated F344 rats after extended treatment with liquid diets in this study, as was also noted previously (Pentney and Quigley, 1987; Pentney and Quackenbush, 1991; Dlugos and Pentney, 1997). A possible influence of body weight on terminal dendritic segment lengths was considered in several earlier studies, but none was detected. It was also shown previously that there were no direct relationships between body weights and brain weights (Dlugos and Pentney, 1997) or between diet-induced changes in body weights and ethanol-induced changes in dendritic length (Pentney and Quigley, 1987; Pentney et al., 1989; Pentney and Quackenbush, 1991).

Considering that during ageing PN dendritic terminal segments shortened by dying back from their free tips and that following ethanol intake some terminal dendritic segments lengthened as others lost contact with their junctions, we conclude that different mechanisms were probably responsible for the age-related and the ethanol-related changes in terminal segment length. Predictably, each age-related or ethanol-induced event must result eventually in the loss of entire terminal segments, but they do so within different time frames, as noted above.

There is abundant evidence that ethanol alters membrane protein functions, and that it does so at blood concentrations
measured in this study (Fadda and Rosetti, 1998). The actions of ethanol on membrane proteins commonly disrupt membrane regulation of calcium. A major and extensive organelle involved in calcium regulation within PN dendrites is the smooth endoplasmic reticulum (SER), and there are important indications that structural and functional features of the SER may be altered by ethanol. It is known, for example, that the average relative volume of the SER in dendrites of PN is greatly increased in young Wistar rats after only 3 months of ethanol treatment (Lewandowska et al., 1994). It has been reported further that mRNA levels of inositol 1,4,5-triphosphate receptor 1, a major component of SER membranes, were reduced in C57BL/6J mice following ethanol treatment for 4 weeks (Simonyi et al., 1996). More recently, preliminary data from this laboratory showed that many SER profiles in PN dendritic shafts and spines of ageing, ethanol-treated F344 rats were significantly enlarged after 40 weeks of ethanol treatment (Dlugos and Pentney, 1998). A frequent association between excessive calcium influx into neurons and unusual expansion of the endoplasmic lumen (Garthwaite et al., 1992) suggested that dilatation of SER profiles results from imbalances in intracellular calcium that are produced by the effects of ethanol on membrane proteins and receptors. As indicated above, the ethanol-induced dilatation of SER profiles was present in dendritic shafts and spines, but the effect was more robust in dendritic shafts than in dendritic spines. The link between SER dilatation in dendritic shafts and selective vulnerability of dendritic branch points involving terminal segments has yet to be delineated, however. A number of vital pieces of information relative to effects of ethanol on specific membrane proteins, receptors, signal-transducing proteins, and calcium-binding proteins of the SER may all be needed to complete such delineation.

There is no reason to suppose that age-related and ethanol-related dendritic degeneration cannot progress simultaneously in the same PN, but, as alluded to above, we have not obtained evidence of interactions between age-related and ethanol-related dendritic degeneration in our model. It appears from our studies that some PN are more sensitive to ethanol, and therefore sustain more dendritic damage, than others (Pentney, 1995; Dlugos and Pentney, 1997). Nonetheless, the distribution of PN that have been altered morphometrically by ethanol-induced mechanisms does not appear to be determined by their level of sensitivity to ethanol.

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