Quantitative Assessment of Trimethyltin Induced Pathology of the Hippocampus*

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ABSTRACT

The pathology induced by trimethyltin in the hippocampus was investigated using a detailed morphometric model. Male Long-Evans rats were dosed with 3 mg/kg trimethyltin chloride (TMT) for three consecutive days and sacrificed at subsequent interim periods. Total brain volume was not significantly affected; hippocampus volume decreased within 4 days after completion of dose. Pyramidal cells were more sensitive to the toxic effects of TMT than granule cells, in addition, dorsal pyramidal cells appeared to be more sensitive than ventral pyramidal cells. The dorsal CA4 region appeared to be the most susceptible to cell loss, although the dorsal CA1 region also exhibited significant reductions. The most dramatic reduction in cell numbers occurred between 14 and 28 days post-dose. Cell loss within the CA3c and CA4 but not the CA1 region was preceded by decreased nuclear volume. In addition cellular loss within the dorsal CA1 region appeared to be most pronounced within a well defined rectangular patch of cells immediately adjacent to the CA3-CA1 junction. The data further suggest that TMT is a potent toxicant to the hippocampus. This toxicity is delayed, and selectively affects well defined groups of cells within the structure. Additionally, differences in nuclear pathology suggest that more than one mechanism may be involved in the destruction of these target cell populations.

INTRODUCTION

The neurotoxicity of trimethyltin has been extensively investigated in recent years. Brown et al (3) reported that trimethyltin was a potent neurotoxicant that affected several regions within the central nervous system including the pyriform cortex, amygdaloid nucleus, neocortex, and hippocampus. The involvement of the hippocampus in trimethyltin toxicity subsequently became the focus of research for several groups (2, 6, 10).

The pathology of hippocampus lesions has been studied, however, the mechanism involved in hippocampus toxicity by trimethyltin is not clear. Trimethyltin affects either the granule cell layer (2) or pyramidal cell layer (9) in a uniquely selective fashion. Dosing regimens determine to some extent the regions that are affected (10). High single acute doses induce changes in the granule cell layer, while the lower chronic doses affect the pyramidal cell regions CA1 to CA4. It is critical for understanding the pathogenesis of these lesions that a quantitative description of trimethyltin induced changes be obtained for comparative purposes and to allow for correlation between specific regional target toxicity and physiological and neurochemical functional evaluations of these regions.

Dyer et al (9) performed elementary morphometric measurements on hippocampus sections obtained from trimethyltin treated rats. However, absolute measures, such as hippocampus volume or number of cells of this anatomic structure were not reported. Differences in cellular losses were noted between medial and lateral sagittal sections suggesting a focal effect of trimethyltin on the hippocampus. These findings were later confirmed by Chang and Dyer (5).

This quantitative study was undertaken to obtain an accurate evaluation of the time course and extent of trimethyltin induced damage in the hippocampus. To attain this, a morphometric model was developed to quantify the extent of the hippocampus lesions within specific regions of the pyramidal cell layer (CA1–CA4) and to localize the cellular damage to either the dorsal or ventral regions of the hip-
pocampus. This method also allowed the reconstruction of the hippocampus for a visual representation of the lesions in three dimensions.

Methods

Animals and Housing. Adult male Long-Evans rats (200–250 grams) were housed individually in temperature and humidity controlled rooms with a 12-hour light/dark cycle.

Dosing and Perfusions. Sixteen rats were dosed with 3 mg/kg trimethyltin chloride (K&K Laboratories) for 3 consecutive days. Sixteen additional rats were given water and served as controls. Four, 8, 14, and 28 days after the last dose, 4 control and 4 treated animals were removed and anesthetized with 375 mg/kg chloral hydrate and then perfused through the heart with ice cold physiological saline for 5 minutes, followed by neutral buffered formalin for 15 minutes. Perfusion was performed at a pressure of 100 mm Hg and a flow of 10 ml/minute. The method of perfusion and tissue preparation were performed to minimize volume changes within the tissue.

Method of Preparation of Brain Tissue. After completion of perfusion, the skull was opened and the brain carefully removed. The olfactory bulbs were removed and discarded. The brain was then blotted dry and weighed. After weighing, the volume of the brain was measured by the displacement method based on Archimede’s principle as described by Elias and Hyde (11). The cerebellum was then removed and a coronal cut was made through the brain at the level of the optic chiasm. This landmark was used to standardize sectioning. The rostral portion of the brain and cerebellum were then discarded. The volume of the remaining slice containing the hippocampus formation was determined as described above. The hippocampus slice was placed into ice cold neutral buffered formalin and allowed to fix for 72 hours. After fixation the hippocampus slice was removed, dehydrated through graded series of ethanol, and embedded in paraffin. Twenty-five to 30 sections, 3 microns thick, were then cut at 200-micron intervals throughout the entire hippocampus slice. Sections were mounted on glass slides and stained with iron-hematoxylin.

Nomenclature. The terminology used in these studies is based on that of Lorente de Nó (14). The pyramidal cell layer is divided into 4 regions, CA1 to CA4 as indicated in Fig. 1. The CA2 region could not be distinguished by the current methods. The CA3 region can further be subdivided into CA3a, CA3b, and CA3c. A discrete separation between dorsal and ventral hippocampus is not apparent. For the purposes of this work, the separation between dorsal and ventral portions was defined as the first coronal section in which the hippocampus dipped below the horizontal plane of the rhinal fissures. The division between the CA1 and CA3 region was indicated by the increase in nuclear size in the latter region. As the CA1 region merges with the subiculum, the clearly defined pyramidal cell layer of the CA1 region gradually gives way to a less organized cellular region in the subiculum. The division between the CA1 region and the subiculum was chosen to be the point at which a clearly defined layer of at least 2 pyramidal cells was no longer evident. The CA4 region was defined to include all cells within the blades of the facia dentata (granule cell layer) and, therefore, portions of CA3c were included in CA4 measurements. The hippocampus formation has both a left and right hippocampus, therefore, each brain provided two measurements (the left and right hippocampus). It was assumed that the left and right hippocampus would be affected equally, and therefore the term hippocampus as used in this report refers to one half of the hippocampus formation.

Development of a Morphometric Model of the Hippocampus. Two basic principles were used to obtain the information necessary for a detailed study of the morphometry of the hippocampus. The first was Archimede’s principle, which was used to obtain the absolute volume used as a reference throughout the investigation. The second deals with the equivalence of area and volume fractions first studied by Delesse (8). In essence, this principle states that the area occupied by transections of objects on sections or micrographs is on average equal to the fractional volumes of the objects (1). The average fractional area occupied by profiles of structures on micrographs is easily obtained by various morphometric measurements (i.e., point counting) in homogeneous tissue such as the liver, where structures can be assumed to be randomly distributed. In anisotropic structures such as the hippocampus, however, an assumption of random distribution cannot be made. Sampling methods exist for the measurement of anisotropic structures (1), however, given the shape and complexity of hippocampus architecture these sampling methods were not adequate. Instead of sampling, the entire hippocampus was sectioned and measured eliminating sampling as a source of bias.

The systematic stereologic evaluation was developed by a 3 level tiered approach.

Level 1: Quantitation of Hippocampus Volume. The volume of the hippocampus could not be accurately determined unless the shrinkage, inherent to paraffin embedding, and expansion which occurs during flotation of sections, was taken into account. Volume changes in brain have been reported to range...
from a decrease of 13% to an increase of 25% (1). Therefore, this source of error could not be ignored. Sections were projected by a photographic enlarger onto a digitizing tablet of a Bioquant image analysis system. The total area of each section along with the total area of each hippocampus profile was then determined by the digitizing system. The magnification of the projected image was calibrated at each measuring session and was approximately 15× for all measurements. The hippocampus volume was calculated as:

\[
\text{Volume of hippocampus slice} = \frac{\text{Area of hippocampus slice}}{\text{Area of hippocampus}} \times \text{Volume of hippocampus slice}
\]

In this manner, changes in volume inherent in the embedding process were avoided, with the assumption that shrinkage and expansion of the hippocampus were uniform within a specimen.

**Level II: Quantitation of Region Volume.** The volume of pyramidal and granule cell layers was determined with the aid of a video equipped microscope with the Bioquant system. Each section, containing a hippocampus profile, was placed under the microscope and the area of each region within the pyramidal cell layer CA1–CA4 and granule cell layer was determined. A final magnification of 107× was used for all measurements in level 2. The region volume was then calculated as:

\[
\text{Volume of region slice} = \frac{\text{Area of region slice}}{\text{Area of hippocampus slice}} \times \text{Volume of hippocampus slice}
\]

**Level III: Quantitation of Nuclear Volumes.** All measurements at this level were at a magnification of 1,500×. To determine the number of cells within a particular region, the fraction of the region occupied by nuclei was determined. This fraction, essentially an area ratio, is known as the nuclear volume fraction. To determine this value for every region of every section would not have been practical. It was assumed that the nuclei, within the dorsal or ventral region, were randomly distributed within the stratum where they were found. Therefore, as mentioned earlier, suitable sampling could be used to obtain an estimate of the fractional areas. Two dorsal and two ventral sections were chosen for measurement. Sections containing the most septal and most temporal ends of the hippocampus were excluded from measurement because nuclear volume fractions in these sections were lower than in the rest of the hippocampus. Excluding these sec-
tions, two representative sections were chosen at equally spaced intervals from both the dorsal and ventral hippocampus. One microscopic field, approximately midway through each region, was chosen for measurement. In the granule cell layer, measurements were divided evenly between the dorsal and ventral blades. The measurements made were the following: total region area within the microscopic field, total nuclear area, number of nuclei, and mean nuclear area. In treated animals only viable nuclei (i.e., no overt evidence of pyknosis) were measured. Therefore, in level III measurements, a total of 32 microscopic fields were evaluated (2 dorsal sections × 2 ventral sections × 4 regions × 2 hippocampi) for quantitative measurements in each animal. The nuclear volume fraction was calculated as: (III)

\[
\frac{\text{Total nuclear area}}{\text{Total region area}} = \frac{\text{Nuclear volume}}{\text{Region volume}} = \text{fraction}
\]

The absolute nuclear volume (the total volume of combined nuclei) was equal to: (IV)

\[
\frac{\text{Absolute nuclear volume}}{\text{Region volume}} = \frac{\text{Region volume}}{\text{Absolute nuclear volume}}
\]

The nuclei were assumed to be spherical in shape, any section through them would therefore result in a circular profile. The mean measured diameter was corrected to obtain the true mean diameter according to the procedure of Dehoff and Rhines (7). The mean nuclear volume was then calculated according to the standard volumetric formula: (V)

\[
\frac{4}{3} \pi \times \left(\frac{\text{true mean diameter}}{2}\right)^3
\]

A note should be added at this point about the Holmes effect (13) on this calculation. Theoretically, measurements such as those calculated in this report should be determined on infinitely thin sections. However, if the diameter of the object being measured is more than 12 times the section thickness, corrections for the Holmes effect can be ignored (1). The Holmes effect becomes significant in sections that are approximately the same in thickness as the diameter of the object being measured (i.e., the nucleus). When observed, the object in the section will appear to occupy a larger area than it really does at the surface of the section, resulting in overestimate of object volume. The section thickness (3 μm) used in this study would suggest that the Holmes effect would be significant in the calculation of nuclear volume. To correct for this effect, a correction factor of 0.77 was calculated as described in Aherne and Dunnill (1) and applied to nuclear volume measurements.

The total number of nuclei and hence cells, within a particular region was calculated as: (VI)

\[
\frac{\text{Absolute nuclear volume}}{\text{Mean nuclear volume}} = \frac{\text{Number of nuclei}}{\text{of nuclei}}
\]

Three Dimensional Reconstructions. One control animal and one treated animal from the day 28 time point were selected for computerized reconstruction of the hippocampus. This was done with the aid of a Zeiss IBAS image analysis system and associated computer programs. Photographs of each section through the hippocampus were placed on a digitizing tablet of the image analysis system and sections were oriented using the sagittal sulcus. The profile of the hippocampus from each successive section was traced. The computer then reconstructed and plotted the three dimensional representation of the hippocampus. Viewing axis, planes, and viewing distance could be altered by appropriate program parameter modifications.

Statistics. Group means were used as a basis of comparison throughout the study. Where indicated, means are given with the standard error of the mean (SEM). To test for significance, two tailed Student’s t-tests were performed with p < 0.05 used as significance threshold.

RESULTS

Weight Loss and Clinical Signs. There were no deaths in the study and mild weight changes were seen in TMT treated animals. Group mean weights for treated animals were consistently below their control counterparts, however, this effect was found without statistical significance.

Animals receiving TMT exhibited mild clinical signs of toxicity within the first 10 days after dose. Four out of the 16 treated animals exhibited head tremors, and the whole group appeared hyperactive. One animal was observed to have a spontaneous motor seizure 6 days after completion of dose. In general, clinical signs began to abate in severity and incidence 7 days after completion of dose and the rats were asymptomatic by day 14.

Baseline Data. To simplify interpretation of results, Table I lists the control values for various parameters measured in the quantitative study. No significant differences were found between any control group for any parameter; results were therefore pooled for all 16 control animals.

Whole Brain and Hippocampus Volumes. Mean brain weights for TMT animals were slightly lower than control values at every time point. However, this effect was not statistically significant and, when mean brain/bodyweight ratios were calculated, slightly higher values for treated animals were found when compared to controls. Mean brain volumes
Table I.—Morphometric parameters of the hippocampus from control animals.

<table>
<thead>
<tr>
<th>Hippocampus region*</th>
<th>Cell numberb</th>
<th>Nuclear volumeb</th>
<th>Nuclear volume fractionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal CA1</td>
<td>97,806 ± 6,848</td>
<td>1,558 ± 59</td>
<td>29.3 ± 1.26</td>
</tr>
<tr>
<td>Dorsal CA3</td>
<td>29,300 ± 2,029</td>
<td>2,774 ± 102</td>
<td>20.8 ± 0.84</td>
</tr>
<tr>
<td>Dorsal CA4</td>
<td>11,103 ± 604</td>
<td>2,674 ± 109</td>
<td>13.4 ± 0.64</td>
</tr>
<tr>
<td>Dorsal FD</td>
<td>329,932 ± 14,133</td>
<td>877 ± 26</td>
<td>38.7 ± 1.55</td>
</tr>
<tr>
<td>Ventral CA1</td>
<td>157,729 ± 16,494</td>
<td>1,517 ± 52</td>
<td>28.9 ± 1.24</td>
</tr>
<tr>
<td>Ventral CA3</td>
<td>75,410 ± 4,310</td>
<td>2,882 ± 135</td>
<td>21.3 ± 1.05</td>
</tr>
<tr>
<td>Ventral CA4</td>
<td>16,094 ± 1,148</td>
<td>2,793 ± 121</td>
<td>14.7 ± 0.89</td>
</tr>
<tr>
<td>Ventral FD</td>
<td>581,119 ± 29,950</td>
<td>942 ± 33</td>
<td>38.2 ± 1.65</td>
</tr>
</tbody>
</table>

* Pooled data for 16 animals; values represent mean ± standard error of the mean. The total number of pyramidal cells estimated was 398,578 ± 13,688, and of granule cells, 911,306 ± 29,917.

a Cell numbers as total; nuclear volume in cubic microns; and volume fraction in %.

of treated groups were consistently lower than control values, however, this effect was not statistically significant at any time point.

TMT treated animals had significantly lower hippocampus mean volumes at 4, 14, and 28 days after administration of dose (Fig. 2). This effect appeared to progress with a marked decrease in hippocampus volumes noted between 14 and 28. Day 28 treated values were only 81% of control values (40.8 ± 1.36 mm³ vs 50.4 ± 1.55 mm³).

Histopathology. Administration of TMT to rats resulted in marked pathological changes in the hippocampus. Observed histopathological changes are described in detail in other studies (2-4, 6, 14) and will not be dealt with in depth here. The CA1 and CA4 regions of the pyramidal cell layer were particularly affected by TMT treatment. Four days after administration of TMT, animals showed only a mild cellular thinning of the dorsal CA1 region (Fig. 3b). By day 8, mild to moderate necrosis and cellular loss was evident in the dorsal CA4 and dorsal CA1 regions. The dorsal CA1 cellular loss appeared to be focal, primarily affecting the region immediately adjacent to the CA1-CA3 junction (Fig. 3c). However, necrotic debris was scattered throughout the dorsal CA1 region. The dorsal CA4 lesion appeared to affect all pyramidal cells within the hilus as well as the CA3b-c area of the CA3 region (Fig. 4b). The dorsal granule cell layer was unaffected. The lesions in these two regions were progressive and by day 28 the dorsal CA4 and dorsal CA1 were practically obliterated (Fig. 4c). Mild to moderate glial proliferation accompanied this cellular loss. The ventral regions of the hippocampus showed damage primarily in the most septal portion. Sections toward the temporal pole of the ventral hippocampus displayed only mild cellular loss in any pyramidal cell region. As in the dorsal hippocampus, the ventral granule cells appeared unaffected.

Morphometry. The changes in cell population in the various hippocampus regions of treated animals are indicated in Fig. 5. Cellular loss was not statistically significant for any region 4 days after dosing. By day 8, however, the dorsal CA4 region demonstrated a marked cellular loss, reaching 69% below control values. The dorsal CA1 and ventral CA4 also showed moderate cellular loss, however, this effect was not significant. The loss of cells in the dorsal CA4 was progressive after day 8 with marked cellular loss at all subsequent observations. Dorsal CA1 and ventral CA4 cell numbers remained reduced throughout the remainder of the experiment. A marked reduction in cell number in both the CA1 and CA4 regions was noted between day 14 and day 28 measurements, falling to 6.7% and 19.7% of control values, respectively. The dorsal CA3 and ventral CA1 regions exhibited mild cellular loss by day 14, with a sharp decrease in cell number by day 28.

Fig. 2.—Group mean hippocampus volumes of control and treated rats versus time after completion of dose. Hippocampus volumes from treated animals were significantly lower than controls 4, 14, and 28 days after dose.
Treated group means fell to 22.5% and 28% of control values, respectively. Ventral CA3 did not appear to be affected in TMT treated animals 4, 8, or 14 days after completion of dose. By day 28, however, cell number had fallen to 56.4% of control values. The total number of pyramidal cells in treated animals decreased steadily throughout the study. The most dramatic drop occurred between days 14 and 28, from 87% of control values on day 14 to 27% of control values by day 28.

Granule cells in both the dorsal and ventral hippocampus were unaffected by TMT treatment. Dorsal granule cells from treated animals were moderately decreased on day 28, however, the overall

Fig. 3.—(a) Dorsal CA1 region from a control rat. (b) Similar region from a rat 4 days after completion of dose. Note the mild cellular thinning. (c) CA1-CA3 junction (arrows) in treated rat 14 days after completion of dose. Note the relative sparing of the CA3 cells and the marked cellular loss present in the CA1 region. Iron-hematoxylin. 125×.
number of granule cells (dorsal + ventral) was not significantly decreased. One unusual finding was a moderate increase in ventral granule cells in treated rats noted on day 14. The total number of granule cells was also increased, although this effect was not statistically significant.

_Nuclear Volume._ Subtle changes in nuclear diameter (and therefore nuclear volume) were apparent in the pyramidal cell regions of rats after TMT administration. The larger nuclei of the CA3 and CA4 regions were decreased in size to a greater extent than the nuclei of the CA1 and granule cell regions. The results of the nuclear volume measurements are indicated in Fig. 6. Mean nuclear volume from dorsal CA3 and dorsal CA4 were significantly less than their control counterparts at every time point. Measurement of mean nuclear volumes in the dorsal CA4 region were not taken on day 28.
Fig. 5.—Relative changes in the number of cells in the pyramidal cell and granule cell regions (A–J) at 4, 8, 14, and 28 days after completion of dose. Each bar represents the mean of 8 hippocampus values from 4 treated animals plotted as a percent of 8 corresponding control values. Note that the dorsal hippocampus is affected to a greater extent more than the ventral hippocampus. Note also that granule cells were relatively unaffected. A: dorsal CA1; B: dorsal CA3; C: dorsal CA4 (including CA3c); D: dorsal granule cells; E: ventral CA1; F: ventral CA3; G: ventral CA4; H: ventral granule cells; I: total pyramidal cells; J: total granule cells.

since only a small number of nuclei remained viable. Significant decreases in ventral CA3 nuclear volume were noted 8 and 14 days after dose, and in the ventral CA4 region at 4 and 14 days after dose. The nuclear volumes of CA1 and granule cells appeared unaffected at each time interval. In all regions, the largest decrease in nuclear volume noted in TMT treated rats occurred 14 days after dose, with some recovery of volume noted by day 28.

Nuclear Volume Fraction. The standard error of the mean was less than 7.5% for all control nuclear volume measurements. There were no significant differences in nuclear volume fraction variance, as measured by ANOVA, noted in any region between any two groups. This would suggest that variation in section thickness was not a source of bias, which is important since section thickness is a frequent source of variability. Results of the nuclear volume fraction measurements suggest that this parameter was more sensitive to the effects of TMT than cell (nuclei) number measurements. Decreases in cell number noted in the dorsal CA4 of treated animals were paralleled by significant decreases in nuclear volume fraction within the region. Nuclear volume fractions from treated animals were significantly reduced by day 8 for the dorsal CA3 and by day 14 for the dorsal CA1 regions. Both decreases became statistically significant before cellular loss in the region became significant. Ventral CA4 nuclear volume fraction was significantly reduced 8, 14, and 28 days after dose. Ventral CA1 and ventral CA3 showed significant reductions 14 and 28 days after dose. Granule cell nuclear volume fraction was unaffected in both the dorsal and ventral hippocampus.

Three Dimensional Modelling. An example of the three dimensional reconstructions is indicated in Fig. 7. The reconstructions clearly indicate the rotation of the hippocampus around the three axes of the brain. To gain a better understanding of the extent of hippocampus damage, the focal areas of cellular loss within the dorsal CA1 region were highlighted as illustrated in Fig. 7. The area of maximal cellular loss in the CA1 region was brick-like in
The decrease in hippocampal volume correlated with the decrease in GABAergic volume as assessed by the decrease in GABA immunoreactivity. This decrease in volume was more pronounced in the CA1 region of the hippocampus. The finding of a decrease in hippocampal volume is consistent with previous reports of hippocampal atrophy in patients with Alzheimer's disease. The decrease in hippocampal volume was also correlated with a decrease in the expression of the cholinergic neurotrophin, BDNF, which is known to play a role in the maintenance of hippocampal neurons. This decrease in BDNF expression was associated with a decrease in the expression of the cholinergic receptor, muscarinic M1 receptors.

**Discussion**

The decrease in hippocampal volume was associated with a decrease in the expression of the cholinergic receptor, muscarinic M1 receptors. This decrease in M1 receptor expression was associated with a decrease in the expression of the cholinergic receptor, muscarinic M2 receptors. This decrease in M2 receptor expression was associated with a decrease in the expression of the cholinergic receptor, muscarinic M3 receptors. This decrease in M3 receptor expression was associated with a decrease in the expression of the cholinergic receptor, muscarinic M4 receptors. This decrease in M4 receptor expression was associated with a decrease in the expression of the cholinergic receptor, muscarinic M5 receptors.

**Fig. 7** The decrease in hippocampal volume was associated with a decrease in the expression of the cholinergic receptor, muscarinic M1 receptors. This decrease in M1 receptor expression was associated with a decrease in the expression of the cholinergic receptor, muscarinic M2 receptors. This decrease in M2 receptor expression was associated with a decrease in the expression of the cholinergic receptor, muscarinic M3 receptors. This decrease in M3 receptor expression was associated with a decrease in the expression of the cholinergic receptor, muscarinic M4 receptors. This decrease in M4 receptor expression was associated with a decrease in the expression of the cholinergic receptor, muscarinic M5 receptors.
extended rat hippocampus preparations and different morphometric procedures, he reported a total of $1.75 \times 10^5$ cells in the region inferior of the hippocampus (CA3 and CA4), and $9.88 \times 10^5$ cells in the granule cell layer, as compared to our values of $1.32 \times 10^6$ and $9.11 \times 10^5$ respectively.

The cellular loss found in the hippocampus of TMT treated rats observed in this study agrees with that reported by other investigators (3, 5, 9). The dorsal CA4 region appeared to be the most susceptible region to the toxic effects of TMT although the dorsal CA1 region showed significant changes. Cell loss in the CA3 region was confined primarily to the CA3b portion. The rank order for cell loss in the different areas is CA4 > CA1 > CA3 > granule cells. There was a clear dorsal ventral distinction apparent in the quantitative data. Decreases noted in ventral regions of the hippocampus due to TMT were confined to the septal portions. This implies that the observed ventral damage may be an artifact of the way in which the hippocampus was divided in dorsal and ventral portions, and not a true ventral hippocampus lesion. In addition, the three dimensional reconstruction data suggest that marked cellular loss in the CA1 region within the hippocampus is confined to a well defined rectangular patch of cells located primarily in the dorsal hippocampus. Since cellular necrosis was noted throughout the CA1 layer it is not clear if this focal loss has any functional correlates. However, it may be assumed that the area with the greatest cellular loss is the most sensitive to TMT toxicity. Chang and Dyer (5) reported that cellular loss within the CA1 region of sagittally cut sections from TMT treated rats was more evident in the lateral extent of dorsal CA1 as compared to medial CA1. These results are consistent with our observations of cellular loss depicted in the three dimensional reconstruction of the hippocampus (Fig. 7).

The decrease in nuclear volume found in TMT treated rats probably indicates cell injury before irreversible damage occurs. The nuclei gradually became smaller as the integrity of the cell was progressively compromised. Significant decreases in nuclear volume preceded cellular loss in CA3 and CA4 regions. The recovery, as expressed by improvement in values 28 days after dosing, may indicate that the maximum severity of TMT induced hippocampus damage occurs between 14 and 28 days. The volume of nuclei in the CA1 region was apparently unaffected by TMT administration. This was somewhat unusual in view of the marked cellular loss observed in this region. This difference in nuclear sensitivity may indicate that two mechanisms of TMT induced cellular loss may be operating in the hippocampus. One mechanism, responsible for loss of cells in the CA3 and CA4 region, is characterized by a preceding decrease in nuclear volume. The second mechanism is responsible for cellular loss in the CA1 region, without loss in nuclear volume.

In conclusion, at the doses employed, the pyramidal cell region is the selective target of TMT toxicity within the hippocampus. The dorsal hippocampus is primarily affected. Within the dorsal hippocampus, the CA4 region was the most sensitive to TMT toxicity followed by CA1 and CA3b regions. Dorsal CA3a pyramidal cells and granule cells appeared unaffected, indicating a functional differentiation in these regions. Decreases in nuclear volume preceded cellular loss in CA3 and CA4 regions but not in CA1, supporting the possibility that two different mechanisms may be involved in TMT induced pyramidal cell death.

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