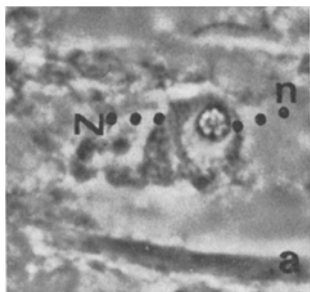


cytological controls. All observations were made with positive phase-contrast microscopy.

**Results and discussion.** The control tissue showed the typical spindle-shaped nuclei with the spherical-shaped homogeneous-appearing nucleoli as observed previously<sup>4,5</sup>. Marked nucleolar enlargement as well as the formation of highly refractile nucleolar inclusions observed in previous investigations<sup>3,4</sup> were also evident here in the explants incubated in the stabilized and unstabilized liquid paraffin (Figure). There was no evidence of microbial contamination in any of the explants incubated in the unsterilized stabilized and unstabilized liquid paraffin (see also reference No. 6).

The levels of BHT employed as an antioxidant in foodstuffs (ca. 0.01%) show no detectable toxic effects in animals<sup>7-9</sup>. Impairment of growth and of phospholipid synthesis and liver damage have been found to occur in rats at higher levels of BHT (ca. 0.2%)<sup>8,9</sup>. The 10 ppm of BHT employed in the liquid paraffin used in this investigation is the maximum allowable concentration listed in the British Pharmacopoeia<sup>1</sup> for medicinal grade liquid paraffin and is well below the concentrations of BHT considered to be non-toxic and toxic in animal feeding experiments<sup>7-9</sup>. The results of this investigation show that nucleolar enlargement and the formation of refractile nucleolar inclusions were not inhibited by the 10 ppm of BHT in the liquid paraffin used here. In addition, endogenous starch formation was also evident in the sac cells of juice vesicles incubated in the stabilized and unstabilized liquid paraffin (unpublished observation; see also reference No. 6).



48-h-old explant incubated in stabilized light grade liquid paraffin showing an enlarged nucleolus with prominent refractile inclusions.  $\times 1600$ . N, nucleus; n, nucleolus.

It is not known whether the lemon fruit explants would have responded differently to higher concentrations of the antioxidant BHT. However, the ability of non-growing lemon fruit explants to manifest certain endogenous cytological and physiological phenomena which are also found in growing explants by incubating them in non-aqueous oily media<sup>4-6</sup> may provide a tissue system for examining the effects of fat-soluble antioxidants and their oxidation products on these endogenous cytological and physiological phenomena.

**Sumario.** Explantas de fruta de limón manifiestan semejante nucleolar comportamiento, cuando están incubadas en parafina líquida con, y sin auto-oxidantes.

H. A. KORDAN

Department of Botany, University of Birmingham, Birmingham B15 2TT (England), 23 November 1972.

<sup>1</sup> *British Pharmacopoeia* (The Pharmaceutical Press, London 1968), p. 709.

<sup>2</sup> *United States Pharmacopoeia XVI* (Mack Publishers Co., Easton, Pennsylvania 1960), p. 517.

<sup>3</sup> *The Merk Index*, 7th edn. (Merck and Co., Inc., Rahway, New Jersey 1960) p. 788.

<sup>4</sup> H. A. KORDAN, *Experientia* 28, 107 (1972).

<sup>5</sup> H. A. KORDAN, *Z. Pflphysiol.* 76, 311 (1972).

<sup>6</sup> H. A. KORDAN, *Phytochemistry* 11, 2743 (1972).

<sup>7</sup> R. J. WARD, *Chemy. Ind., Lond.* 1st edn. 1959, 498°.

<sup>8</sup> D. V. PARKE, *The Biochemistry of Foreign Compounds*, 1st edn. (Pergamon Press, Oxford 1968), p. 166.

<sup>9</sup> L. FRIEDMAN and S. I. SHIBKO, in *Toxic Constituents of Plant Foodstuffs* (Ed. E. LIENER; Academic Press, New York and London 1969), p. 349.

<sup>10</sup> R. F. CRAMPTON, in *Metabolic Aspects of Food Safety* (Ed. F. J. C. ROE; Blackwell Scientific Publications, Oxford and Edinburgh 1970), p. 59.

<sup>11</sup> Stabilized and unstabilized light, medium, and heavy grades of liquid paraffin were generously furnished by Mr. R. K. BYFIELD and Mr. T. WILSON, Dalton and Co. Ltd., Derby, England. Stabilized and unstabilized liquid paraffin in any of these 3 grades can be obtained from Dalton and Co. Ltd. Stabilized and unstabilized medicinal grade liquid paraffin were generously furnished by Mr. N. NIX, Boots The Chemists, Nottingham, England.

<sup>12</sup> H. A. KORDAN, *Science* 149, 1382 (1965).

<sup>13</sup> A. G. E. PEARSE, *Histochemistry, Theoretical and Applied*, 2nd edn. (Little, Brown and Co., Boston 1960), p. 788.

## Synaptic Boutons in the Hippocampus: Changes are Produced by Age and Experience

For many years researchers investigating the fine structure of the central nervous system have been concerned with the mechanisms by which postnatal structural changes in neurons occur morphologically. It has been suggested that morphological changes in the cortex of the rat after day 20 can be accounted for by means of increased dendritic branching<sup>1</sup>. Furthermore<sup>2,3</sup>, researchers have reported that environmental manipulations alter the fine structure of the dendritic plexus. Based upon these data it seems reasonable to conclude that a possible morphologic site of change exists in the dendritic arborizations of neurons in the central nervous system. Such changes would be expressed by arborization changes within not only the dendritic plexuses but also the axonal endings. Specifically these would include increased dendritic spines, increased axonal arborizations,

greater numbers of dendrites, etc. Suggestions such as these have been offered by several investigators<sup>4-6</sup>. Taken together, these morphological alterations suggest that the most sensitive measure of change in dendro-axonal relationships is the number of synaptic boutons

<sup>1</sup> J. T. EAYRS and B. GOODHEAD, *J. anat.* 93, 385 (1959).

<sup>2</sup> A. GLOBUS and A. B. SCHEIBEL, *Expl Neurol.* 19, 331 (1967).

<sup>3</sup> F. VALVERDE, *Expl Brain Res.* 3, 337 (1967).

<sup>4</sup> M. C. DIAMOND, D. KRECH and M. R. ROSENZWEIG, *J. comp. Neurol.* 123, 111 (1964).

<sup>5</sup> M. C. DIAMOND, F. LAW, H. RHODES, B. LINDNER, M. R. ROSENZWEIG, D. KRECH and E. L. BENNETT, *J. comp. Neurol.* 128, 117 (1966).

<sup>6</sup> R. N. WALSH, O. E. BUDTZ-OLSEN, J. E. PENNY and R. A. CUMMINS, *J. comp. Neurol.* 137, 361 (1969).

present. The present experiment reports alterations found in the number of synaptic boutons of control animals used in an experiment concerned with possible relationships between synaptic distributions and hippocampal theta activity.

Six 150 g male Long-Evans rats were divided into 2 groups of 3 each. Group 1 was sacrificed prior to the experiment. Group 2 animals were treated as controls for the duration of the main experiment. Daily handling included being placed in a small circular (1 foot diameter) open field for 15 min, during which time the animals were not handled. Upon completion of the experiment (approximately 60 days later), group 2 animals were sacrificed. All animals were anesthetized with chloral hydrate and under deep anesthesia, perfused transcardially with buffered 10% formalin preceded by a saline exsanguination. The brains were removed and fixed for an additional 2 weeks in formalin. Following this the brains were blocked and separated into left and right sides. The tissue was treated with the RASMUSSEN technique<sup>7</sup> for demonstration of synaptic boutons. The tissue was embedded in paraplast and sectioned in the horizontal plane at 10  $\mu$ m. All tissue was coded and subsequent data analysis was carried out without knowledge of group designation. The molecular layer of the dentate gyrus of the hippocampal formation was divided into a superficial outer zone and a deeper inner zone. Synaptic boutons were counted on 5 dendrites in each zone. Counting was done under oil immersion ( $\times 900$ ) and the number of boutons/10  $\mu$ m length of dendrite calculated for all 6 brains (12 hippocampi). The mean number of boutons is shown in the Table.

A three-factor analysis of variance<sup>8</sup> with repeated measures revealed that the number of boutons for the 2 zones was not different within each group ( $F=1.931$ ,

with 1 and 4 degrees of freedom,  $p > 0.10$ ). However, between group comparisons demonstrated that the number of boutons was greater in group 2 animals (late sacrifice) than in group 1 animals ( $F = 21.09$ , with 1 and 4 degrees of freedom,  $P < 0.02$ ). None of the interaction terms proved to be significant.

Within the context of the present findings, it is difficult to dissociate between age changes and environmental influences, such as handling, as the factors responsible for the bouton differences. It seems reasonable to conclude that both factors contributed to the change in synaptic bouton density within the dentate gyrus.

The distribution of bouton changes is interesting. Although there was not a difference between the inner and outer zones of the gyrus, the two zones are morphologically distinct. The outer layer receives ipsilateral fibres while the inner one receives commissural fibres<sup>9,10</sup>. In addition, the inferior aspect of the deep or inner zone has a horizontal axonal plexus (supragranular axonal plexus of Cajal) consisting of primary axon terminals of the polymorphic cells of the hilus of the dentate gyrus. The present data suggest that a non-specific (i.e., independent of input-output systems) increase occurred in the synaptic bouton density of the dentate gyrus.

The findings described here support the view that the hippocampus is a plastic system that is adaptable to change whether the change be of ontogenetic, environmental, or combined origin. These data are particularly interesting since, to our knowledge, they represent the first demonstration of increases in synaptic boutons in mature animals as a function of age and/or experience. These observations support the hypothesis that morphological changes occur at the level of the synapse complex.

*Zusammenfassung.* Erstmaliger morphometrischer Nachweis bei 2 Gruppen reifer, altersunterschiedlicher Ratten, dass die Zahl der Synapsen im Hippocampus mit dem Alter zunimmt, wobei die effektive Steigerungsrate auch mit Umwelteinflüssen zusammenhängen könnte.

R. B. CHRONISTER, J. J. BERNSTEIN,  
S. F. ZORNETZER and L. E. WHITE JR.<sup>11</sup>

*Center for Neurobiological Sciences and  
Department of Neuroscience, University of Florida,  
Gainesville (Florida 32601, USA); and  
Division of Neuroscience, University of South Alabama,  
Mobile (Alabama 36600, USA), 12 October 1972.*

Mean number of boutons/10  $\mu$ m length of dendrite of molecular layer of dentate gyrus

	Superficial zone (mean $\pm$ standard error of the mean)	Deep Zone (mean $\pm$ standard error of the mean)
Group 1 (early)		
Animal No. 31 right	20.76 $\pm$ 0.83	26.90 $\pm$ 1.92
left	24.28 $\pm$ 2.31	19.62 $\pm$ 2.44
Animal No. 32 right	28.20 $\pm$ 1.49	23.66 $\pm$ 3.18
left	35.96 $\pm$ 5.35	25.12 $\pm$ 2.96
Animal No. 33 right	20.50 $\pm$ 2.91	19.12 $\pm$ 1.45
left	21.68 $\pm$ 1.30	24.26 $\pm$ 3.29
Group 2 (late)		
Animal No. 28 right	27.14 $\pm$ 1.67	29.50 $\pm$ 3.02
left	34.44 $\pm$ 2.74	42.86 $\pm$ 5.17
Animal No. 29 right	31.76 $\pm$ 1.00	34.70 $\pm$ 1.93
left	33.70 $\pm$ 0.73	37.56 $\pm$ 4.38
Animal No. 30 right	43.70 $\pm$ 2.81	36.44 $\pm$ 2.07
left	36.12 $\pm$ 5.68	33.22 $\pm$ 2.31

<sup>7</sup> G. L. RASMUSSEN, in *New Research Techniques of Neuroanatomy* (Thomas, Springfield 1957), p. 27.

<sup>8</sup> B. J. WINER, *Statistical Principles in Experimental Design* (McGraw-Hill, New York 1962).

<sup>9</sup> T. W. BLACKSTAD, *J. comp. Neurol.* 105, 417 (1956).

<sup>10</sup> T. W. BLACKSTAD, *Acta anat.* 35, 202 (1958).

<sup>11</sup> This work was supported by grants from the U.S. National Institutes of Health to JJB (No. NS-06164) and to LEW, JR., (No. NS-09358) and funds from the Center for Neurobiological Sciences of the University of Florida (No. NIH-MH-10320).

## Lamellar Bodies in Oocytes of *Xenopus laevis* and their Relation to the Mode of Fixation

Lamellar bodies, which are frequently called 'myelin-like figures', have been described as being present in a great number of different cell types, such as hepatocytes<sup>1,2</sup> myoblasts<sup>3</sup>, neuroblasts<sup>4,5</sup>, immature ovarian cells<sup>6</sup>, etc. (see DIDIO<sup>7</sup> for a more complete reference list on the

subject). Various morphogenetic or physiological features have been attributed to these structures, e.g. the formation of mitochondria<sup>4</sup>, or the Golgi apparatus<sup>8</sup>, or participation in the transformation of glycogen into lipid<sup>2</sup>. By some authors the lamellar bodies have been