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[7] for demonstration of synaptic boutons. The tissue was enbedded in paraplast and sectioned in the horizontal plane at 10 μm. 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 (× 900) and the number of boutons/10 μm length of dendrite calcuted for all 6 brains (12 hippocampi). The mean number of boutons is shown in the Table.

A three-factor analysis of variance[8] 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.

Mean number of boutons/10 μm length of dendrite of molecular layer of dentate gyrus

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

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[9,10]. 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 gryrus.

The findings described here support the view that the hippoccampus 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.

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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[1,2] myoblasts[3], neuroblasts[4,5], immature ovarian cells[6], etc. (see DIDIO[7] 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[4], or the Golgi apparatus[6], or participation in the transformation of glycogen into lipid[2]. By some authors the lamellar bodies have been

reported to be merely artefacts; others showed them on their micrographs without commenting on them at all.

During studies on amphibian oogenesis, lamellar bodies were found to be present in oocytes of *Xenopus laevis* under certain circumstances. Therefore the present study was undertaken to elucidate their origin and if possible their nature.

After regular fixation in glutaraldehyde buffered with Na-cacodylate followed by Osmium-tetroxide fixation, myelin-like whorls (lamellar bodies LB) were found in oocytes of *Xenopus laevis* (Figure 1). These LBs were preferentially located in the cortical region of the oocyte, or they were in direct contact with lipid droplets. In some cases they could be found in the zona radiata or even in direct contact with the follicular epithelial cells. This, however, was more the case in previtellogenic than in vitellogenic oocytes. Astonishingly enough, the LBs could only be detected in oocytes which had either just entered the vitellogenic phase or were still in the previtellogenic stage of development. In oocytes larger than approximately 650 µm, i.e. oocytes which possess already well developed yolk platelets, the lamellar bodies had totally disappeared again. The size of the LBs ranged widely between 0.1 and 3.0 µm. The thickness of the individual lamellae ranged between 68 and 700 Å. The smallest lamellar bodies consisted of one lamella only, which was spirally arranged. Sometimes the lamellar bodies consisted of numerous vesicles which were encircled by a larger vesicle (Figure 2). As reported in a previous paper[8], oocytes in the previtellogenic or early vitellogenic stage of development are most actively engaged in the synthesis or accumulation of lipid. Thus the presence of probably unstable lipid coincides with the presence of myelin-like figures, i.e. lamellar bodies.

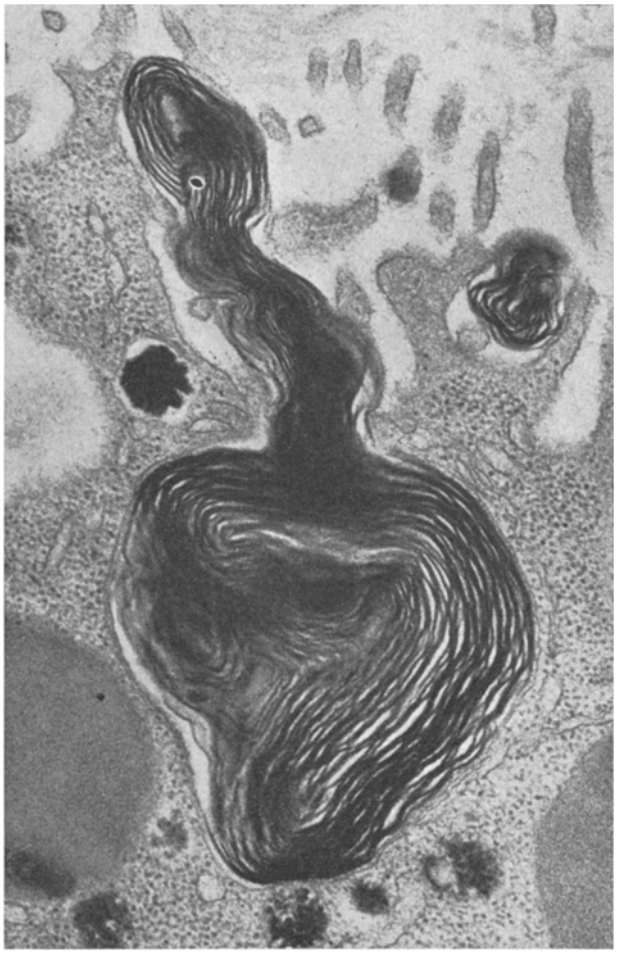

Fig. 1. Lamellar body in the cortex of an early vitellogenic oocyte extending partly into the zona radiata. ×31,000.

With a different mode of fixation, e.g. using either glutaraldehyde, osmium tetroxide or potassium permanganate as the sole fixative, the number of lamellar bodies found was drastically diminished. The ones that were still present after the employment of one of the three above-mentioned fixatives were much smaller than after combined fixation with glutaraldehyde and osmium-tetroxide.

As expected, oxidation of ultrathin sections with H_5JO_6 or H_2O_2 not only resulted in a pronounced decrease of electron density (Figure 3) but also in reduction in width of the individual lamellae. This, and the fact that remaining lamellae closely resembled the very few lamellae which could be produced with glutaraldehyde as a single fixative, indicated that the LBs were to a great extent made up of lipid. In order to test the lamellar structure which remained after oxidation with H_5JO_6 or H_2O_2, the sections were floated on 0.5% pronase for approximately 2 h. As a result of this treatment, the lamellar bodies disappeared altogether, leaving free spots of embedding medium behind (Figure 4).

To a great extent the formation of lamellar bodies can certainly be attributed to impurities of the commercially available glutaraldehyde. According to ANDERSON[9], these impurities, which in UV-spectrophotometry result in a second absorption maximum at 235 nm, either consist of acrolein, glutaric acid or a polymer of glutaraldehyde. With the use of highly purified glutaraldehyde, distilled according to the procedure proposed by ANDERSON[9] (supplied by Th. Schuchardt Ltd. Munich) which has been employed for over a year in this laboratory the formation of LBs could no longer be observed. Similar results were obtained with the use of 'regular' glutaraldehyde which had been washed with activated charcoal at least 4 times.

The formation of tubular endoplasmic reticulum after prefixation with glutaraldehyde, as has been described for oocytes of *Rana temporaria* and *Rana esculenta*[10], was not found to be dependent upon the purity of the employed glutaraldehyde.

From the above the following can be concluded: 1. The formation of whorls is initiated through the action of impurities of the commercially available regular glutaraldehyde. 2. The lamellar bodies are formed through the action of osmium tetroxide. 3. The lamellar bodies consist mainly of lipid and to a certain extent of protein.

As a consequence from 1 and 2 it would appear very probable that the observed structures are merely an artefact caused through improper fixation. However, the fact remains that the lamellar bodies have mainly been found in younger oocytes, i.e. those which are most actively engaged in the synthesis of lipid. It therefore

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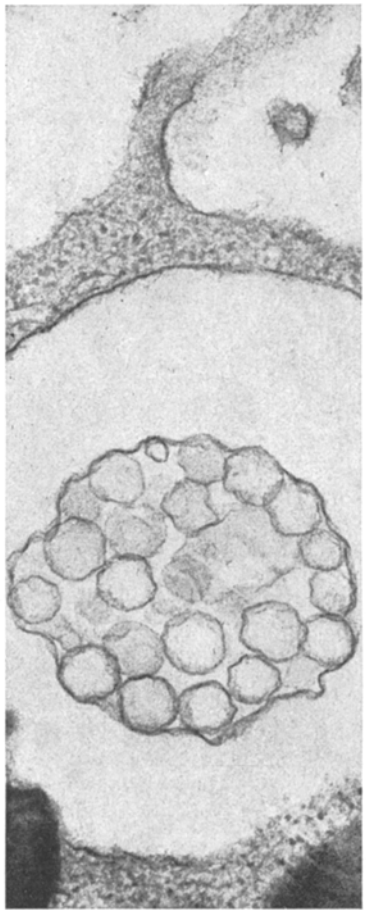

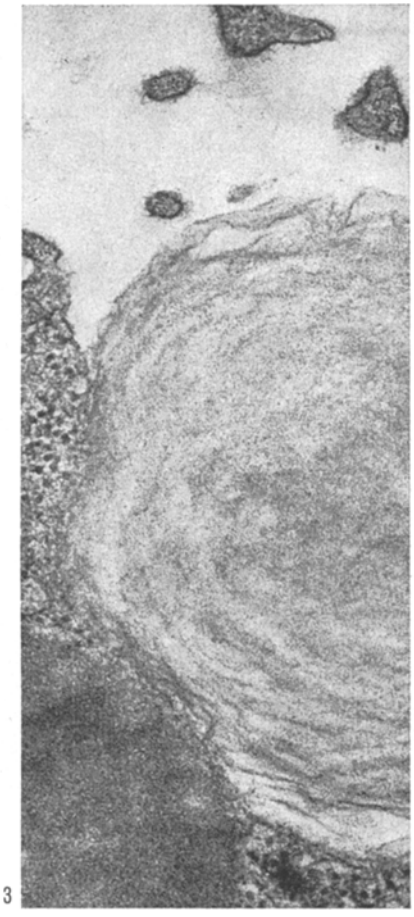

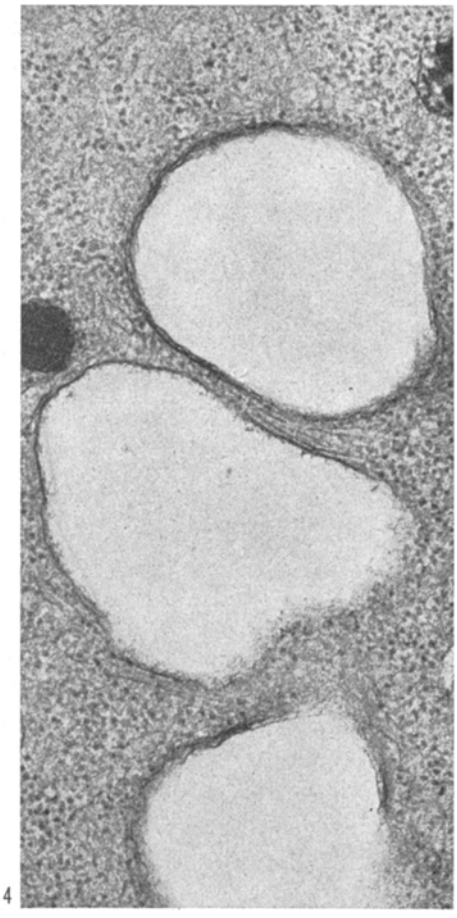

Fig. 2. Lamellar body of the vesicular type after oxidation on H_2O_2. ×39,000.
Fig. 3. Lamellar body after oxidation on H_5JO_6. ×39,000.
Fig. 4. Vacuoles in the cortex of the oocyte produced by oxidation of lamellar bodies followed by enzymatic digestion with pronase. ×31,000.

appears more likely that the lamellar bodies, though morphologically an artefact, biochemically represent the presence of a certain lipoprotein complex, the composition of which, rather than its solubility, is altered through the application of commercially available regular glutaraldehyde. This is consistent with results reported by SHARMA et al.[11], who found that after stimulation of isolated adrenal cortex cells of the rat with ACTH the number of lamellar bodies was significantly increased, which, however, was not associated with an increase in solubility of the lipid.

The fact that lamellar bodies occur after application of osmium tetroxide as the sole fixative, although not very many, indicates that the same type of lipid as is formed through the action of 'regular' glutaraldehyde is present to a certain extent in cells under normal conditions. The term 'normal conditions' should, however, in this case be used with care, since most of the lamellar bodies or myelin-like figures that have been found so far were present in embryonic or regenerating cells, that is synthetically highly active cells.

Considerations as put forward by RUBY and WEBSTER[6], such as the fact that lamellar bodies were only found in immature cells of the bat ovary, are therefore not convincingly against an artificial nature of the LBs in terms of morphology. Even though it has become evident during the course of this study that the lamellar bodies can only be regarded as an artefact from a morphological point of view, the results reported in this paper emphasize again the necessity of employing several different fixatives in ultrastructural studies, since only a differential analysis of several ultrastructural fixation patterns can ensure unequivocal results.

Zusammenfassung. Nach Doppel-Fixierung der Ovarien von *Xenopus laevis* sind in Oocyten kurz vor und in der frühen Dotterbildungsphase lamelläre Einschlusskörper («myelin-like figures») zu finden. Es wird gezeigt, dass Verunreinigungen des Glutaraldehyds für das Zustandekommen der «myelin-like figures» verantwortlich sind, diese selbst jedoch erst durch die nachfolgende Einwirkung von OsO_4 in Erscheinung treten. Bei Verwendung von gereinigtem Glutaraldehyd sind die lamellären Einschlusskörper nicht nachzuweisen. Sie bestehen wahrscheinlich aus einem Lipoprotein-Komplex und finden sich nur in lipidsynthetisch stark aktiven Oocyten. Daraus wird geschlossen, dass diese Körper morphologische Artefakte sind, die auf Grund einer normalen physiologischen Aktivität entstehen.

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