

# “Epileptic” Brain Damage in Rats Induced by Sustained Electrical Stimulation of the Perforant Path. II. Ultrastructural Analysis of Acute Hippocampal Pathology

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OLNEY, J. W., T. DEGUBAREFF AND R. S. SLOVITER. “Epileptic” brain damage in rats induced by sustained electrical stimulation of the perforant path. II. Ultrastructural analysis of acute hippocampal pathology. *BRAIN RES BULL* 10(5) 699-712, 1983.—Sustained electrical stimulation of the perforant path evokes granule cell population spikes and epileptiform discharges, abolishes recurrent inhibition in the granule cell layer and induces a reproducible pattern of hippocampal damage (see preceding paper, this volume, for electrophysiological and light microscopic findings). Electron microscopic findings described here reveal that the hippocampal damage is identical in pattern and cytopathological detail to that associated with sustained limbic seizures induced by chemical convulsants such as kainic acid, folic acid and dipiperidinoethane. Acutely swollen dendritic segments distributed in a laminar pattern corresponding closely with the termination of putative glutamate or aspartate-containing fibers, including those of the perforant path, were a conspicuous finding. Cell bodies of CA1 and CA3 pyramidal neurons and various interneurons in the hilus and elsewhere displayed degenerative changes ranging from mild to severe. Both the dendritic and somal degenerative changes closely resemble the “excitotoxic” type of damage that the putative transmitters glutamate and aspartate are known to cause. It is proposed, therefore, that sustained electrical stimulation of the perforant path results in excessive synaptic release and accumulation of glutamate (or aspartate) at numerous dendrosomal receptors in the hippocampus with consequent degeneration of the dendrosomal structures housing these receptors. Early excitotoxic effects on interneurons that mediate recurrent inhibition may play an important role in the observed loss of recurrent inhibition and in the evolution of subsequent excitotoxic degeneration in the hippocampus.

Epilepsy      Hippocampus      Epileptic brain damage      Perforant path stimulation  
Glutamate/aspartate excitotoxicity      Kainate/folate-like toxicity  
Hippocampal ultrastructure

THE preceding paper presented a light microscopic analysis of hippocampal structural changes caused by sustained electrical stimulation of the perforant path [28]. The highly selective pattern of damage involved cells in the dentate

hilus, at the granule cell hilar border and in the CA3 and CA1 pyramidal cell layers. CA2 pyramidal cells and dentate granule cells were relatively unaffected. Also noted were irregular clear spaces surrounding somata which have been

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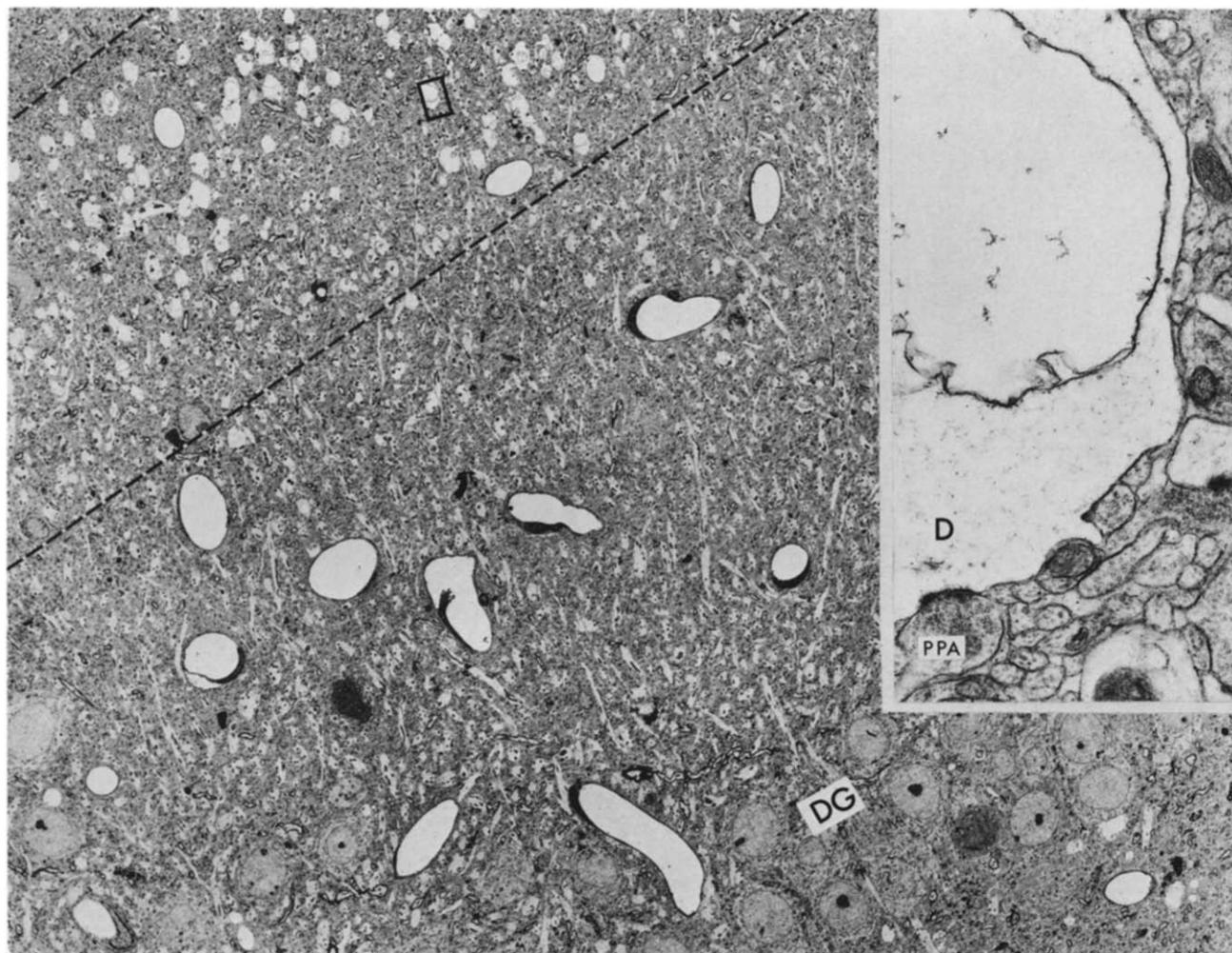


FIG. 1. A scene from the rostral dentate gyrus after 24 hr intermittent perforant path stimulation showing massively dilated segments of granule cell dendrites (between dashed lines) confined to the distal portion of the molecular layer where perforant path axons terminate. Dentate granule neurons (DG) are present at lower right. The inset (from boxed region) presents a magnified view of a swollen dendrite (D) which contains a massively dilated profile probably of mitochondrial origin. It is contacted by a normal-appearing axon (PPA) of presumptive perforant path origin (overview  $\times 375$ ; inset  $\times 11250$ ).

identified as acute glial swellings in rats given chemical convulsants [7, 18-20, 27]. This pattern of damage caused by electrical stimulation is, by light microscopy, virtually identical to the pattern of damage caused by the chemical convulsants kainic acid [20,30], dipiperidinoethane [19] and folic acid [21]. The present electron microscopic analysis of the acute damage was undertaken to: (1) compare ultrastructurally the hippocampal damage caused by sustained electrical stimulation of the perforant path with the similar damage caused by chemical convulsants; (2) discriminate neuronal and glial changes in morphology; and (3) evaluate the effects of electrical stimulation on intracellular organelles and synaptic structure not visible at the light microscopic level.

#### METHOD

The electrophysiological experiments and perfusion fixations were performed by one of us (RSS) and the brains from

these experiments were then sent to JWO and TdeG for post-fixation and subsequent analysis by electron microscopy.

A total of 14 male Sprague-Dawley descendant rats (Charles River Labs; 250-400 g) were used in this study. Rats were housed on a 12 hr light/dark cycle and given free access to food and water. Rats were anesthetized with urethane (1.25 g/kg IP) and placed in a stereotaxic apparatus. Rectal temperature was monitored continuously and maintained at  $37 \pm 1^\circ\text{C}$  with a warm water coil placed under the animal.

Control and experimental rats used in this study were treated the same as the rats in the preceding paper [28]. Briefly, two perforant path stimulation paradigms were used. One was the 24 hr intermittent paradigm consisting of continuous stimulation with twin pulses (40 msec apart) at 2 Hz for 24 hr. In addition, a 10 sec train of single stimuli at 20 Hz was delivered through the same electrode every minute throughout the 24 hr period (1,440 trains). The second

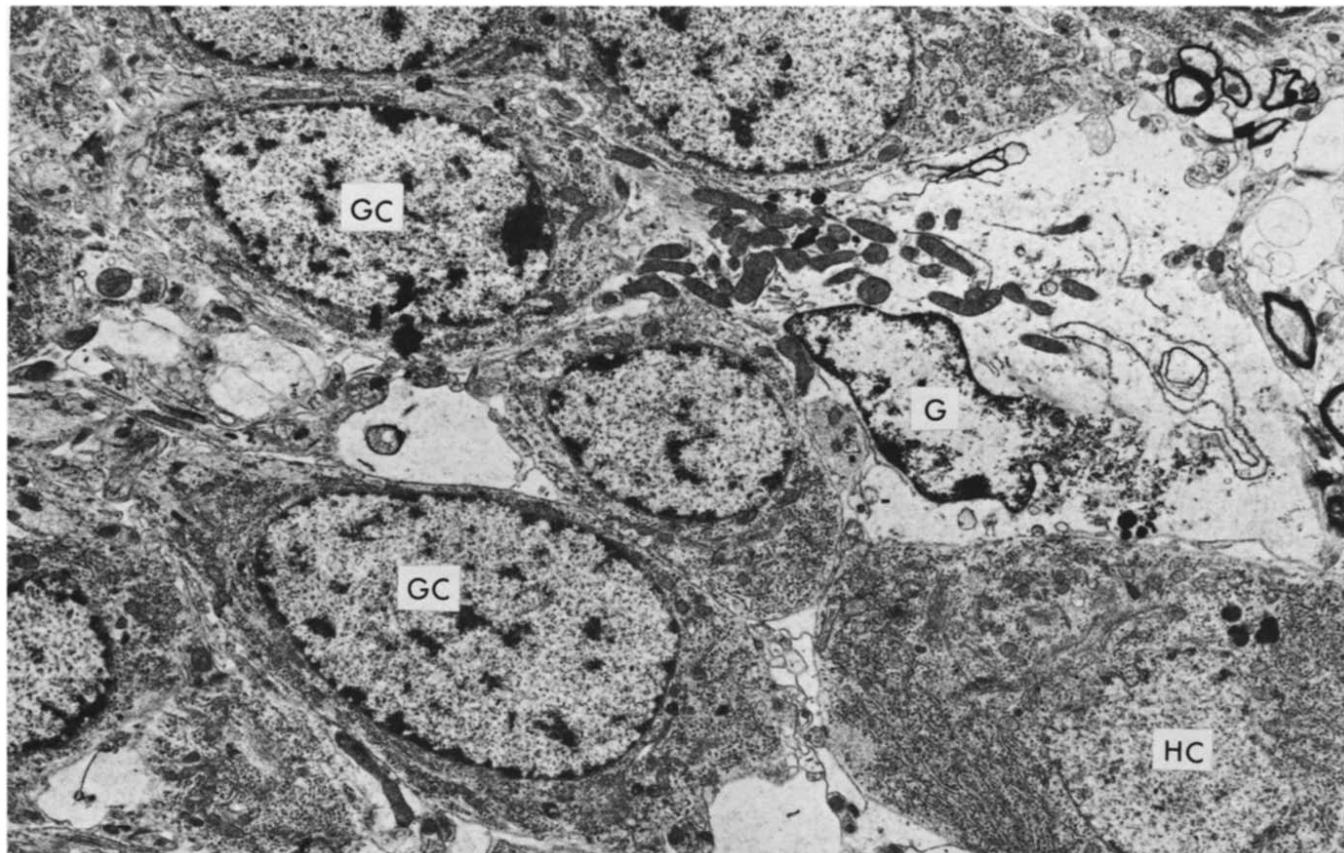


FIG. 2. A scene of the dentate granule cell layer following 2 hr of continuous perforant path stimulation. Granule cells (GC) appear normal except for a possible increase in the coarseness of nuclear chromatin. The pattern of nuclear chromatin in granule cells of control rats is relatively coarse but not as coarse as is shown here after 2 hr of perforant path stimulation. A glial cell (G) and a hilar cell (HC) are shown in the infragranular zone at right. Note the edematous swelling of the glial cell, the disarray of its endoplasmic reticular membranes and the apparent rupture of its nuclear envelope with dispersal of nuclear contents into the cytoplasm. Edematous processes scattered about the scene are from glial cells. It is noteworthy that the hilar cell, which appears entirely normal, has no synaptic contacts on its somal surface. This is in contrast to the majority of hilar cells which receive dense axosomatic innervation and undergo acute degeneration following either 2 hr or 24 hr perforant path stimulation (see Figs. 9b and 10) ( $\times 6000$ ).

paradigm used was a shorter one consisting of 2 hr of continuous 20 Hz stimuli to the perforant path. All continuously stimulated rats responded to all 20 Hz perforant path stimuli with large amplitude (20–40 mV) granule cell population spikes throughout the 2 hr stimulation period (approx. 144,000 spikes). Control groups for each paradigm consisted of rats treated identically and stimulated identically except that the tips of the same stimulating electrode were placed in the cortex at the minimum distance above the perforant path which did not evoke a granule cell potential.

Hippocampal granule cell activity was evoked and recorded and recurrent inhibition was evaluated as described in the preceding paper [28]. At the end of the stimulation period, rats were removed from the stereotaxic apparatus and perfused through the heart with saline and then fixative (1.5% glutaraldehyde/1% paraformaldehyde in phosphate buffer). In all cases, the motor manifestation of aldehyde fixation began 75–90 sec after the last perforant path stimulus. Brains were removed from the skull and placed in perfusate. Post-fixation began approximately 20 hr after perfusion. Brains were processed for histopathological evalua-

tion by combined light and electron microscopy as described previously [15,20].

## RESULTS

Rats from both experimental groups, i.e., 24 hr/intermittent and 2 hr/continuous, exhibited a loss of recurrent inhibition after stimulation as described and illustrated in Fig. 3 of the preceding paper [28]. Conversely, control rats did not exhibit loss of recurrent inhibition, also as described previously [28].

Semi-thin ( $1 \mu$ ) araldite sections for light microscopy corroborated that a pattern of hippocampal pathology similar to that observed in rats treated with chemical convulsants [19–21] was reproduced by either 24 hr intermittent or 2 hr continuous electrical stimulation of the perforant path. The pattern consisted of relatively conspicuous changes concentrated in the infragranular hilar zone and extending through the hilus to include CA3 and 1 (but not CA2) pyramidal regions in a reaction characterized by acute intracellular edema affecting certain cellular elements and dark cell de-

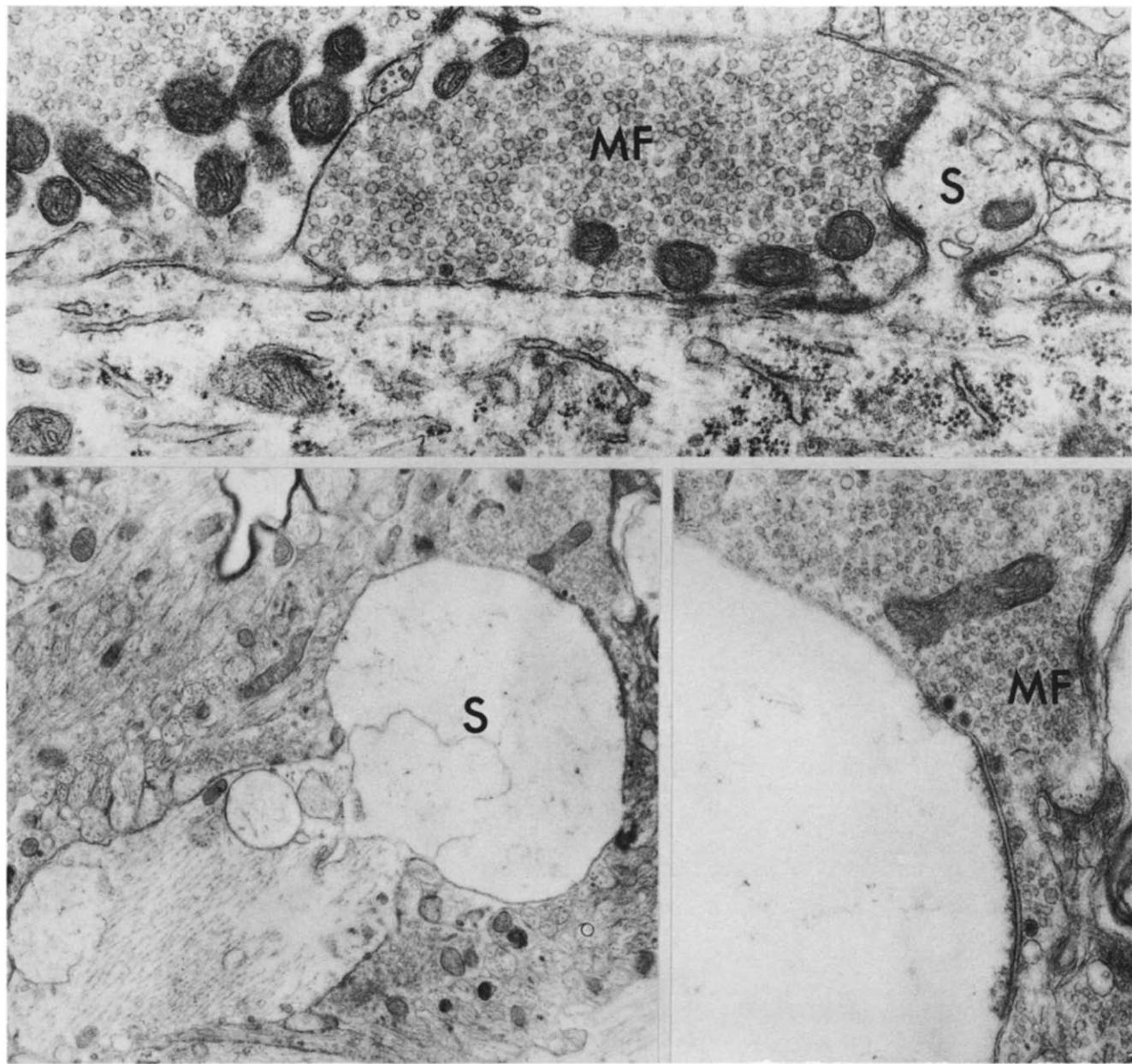


FIG. 3. At top is a mossy fiber (MF) terminal making synaptic contact with a spine (S) given off by the mainstem dendrite of a CA3 pyramidal neuron from control brain. Below at left is a mossy fiber terminal synapsing with a CA3 dendritic spine which has undergone massive dilatation after 24 hr of intermittent perforant path stimulation. At lower right a magnified view of the synaptic contact region reveals that the presynaptic mossy fiber terminal (MF) displays no pathological changes while the postsynaptic CA3 dendritic spine (S) is acutely degenerating (top  $\times 32000$ ; bottom left  $\times 16000$ ; bottom right  $\times 30000$ ).

generation affecting others. The acute edematous type of reaction was relatively more prominent in the 2 hr continuously stimulated brains but all components of the pattern of damage previously reported in kainate [20,27] or d-piperidinoethane [19] treated rats were evident following either mode of stimulation. In addition to the more conspicuous features of this reaction, there were relatively subtle changes in specific sublaminae of certain dendritic fields

including distal dentate molecular, distal apical CA3 and distal basilar CA1 zones.

By electron microscopy, a conspicuous component of the acute edematous reaction was attributable to massively swollen processes and cell bodies of specific astroglia that co-mingle intimately with the cell bodies of dentate granule and CA3 and 1 pyramidal neurons (Figs. 2 and 6). This response of glia has been described previously [7, 18, 27] as a

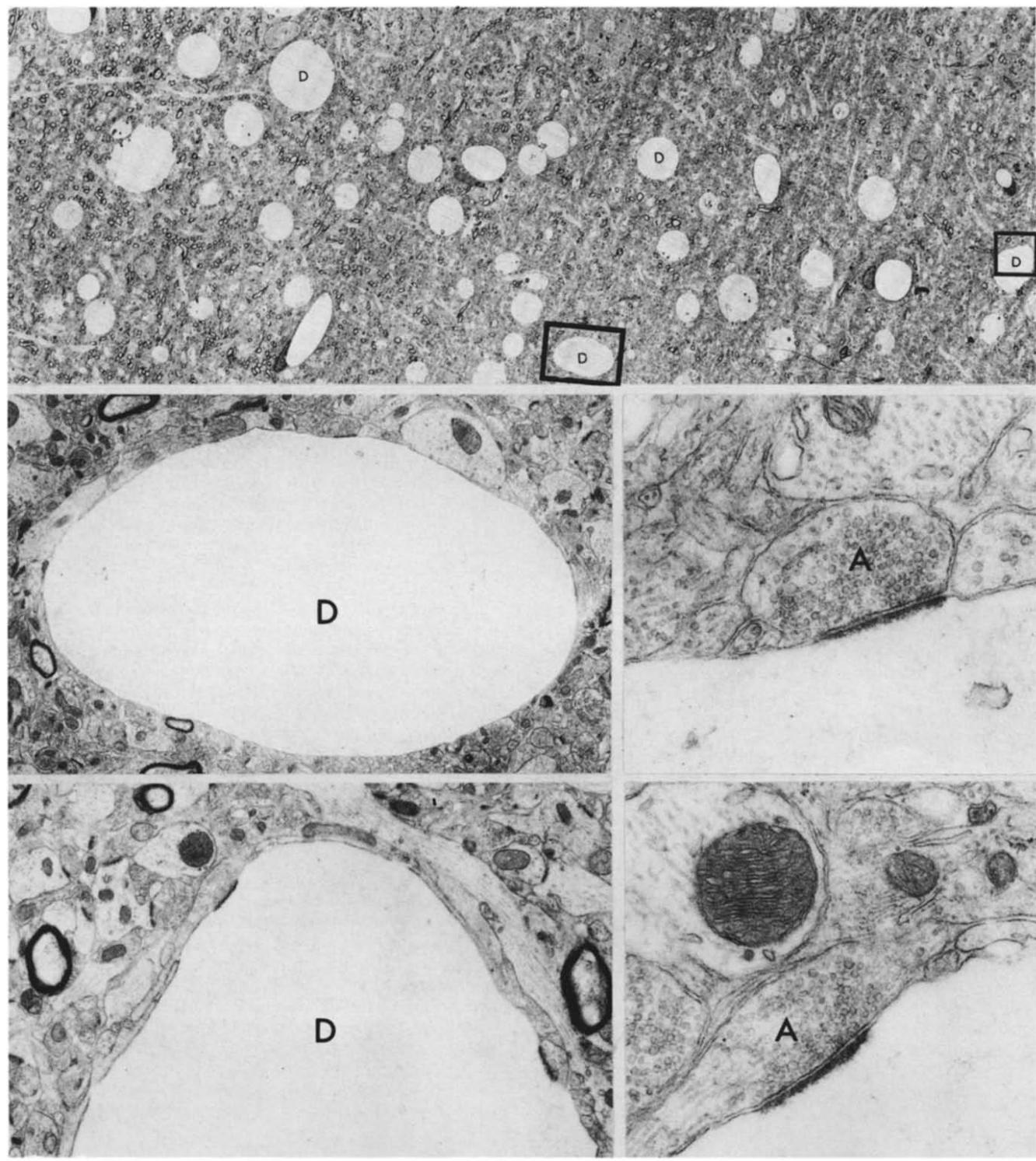


FIG. 4. At top is a survey view of the distal apical CA3 dendritic field showing numerous massively dilated dendritic segments after 24 hr intermittent stimulation. The swollen dendrites (D) in the boxed regions are progressively magnified in the middle and lower panels to show the normal appearance of axon (A) terminals that make asymmetric synaptic contact (arrowheads) with the abnormal dendritic processes. Note that the pathological reaction in this region is entirely confined to specific dendritic structures that receive these synaptic contacts which are presumably excitatory and of perforant path origin. The surrounding neuropil is well preserved and shows no degenerative changes (top  $\times 425$ ; left, both panels  $\times 9000$ ; right, both panels  $\times 36000$ ).

prominent feature of the acute hippocampal response to kainate treatment.

The second major component of the acute edematous response was largely confined to specific dendritic segments. Typically, this included scattered dendritic processes in the dentate hilar region and a more laminar pattern of dilatations affecting (a) distal dentate granule cell dendrites (Fig. 1), (b) the mossy fiber-invaginated spines given off by the proximal dendritic shafts of CA3 pyramids (Fig. 3), (c) distal apical branchlets of CA3 dendrites (Fig. 4), (d) distal basilar branchlets of CA1 dendrites (Fig. 5) and (e) dendritic branchlets in the CA1 apical field. Of the above changes, (b), (c) and (d) were most consistently observed. This pattern and relative consistency of involvement is the same as has been observed in kainate or dipiperidinoethane-treated rats [19,20]. Axon terminals making presynaptic contact with swollen dendrites characteristically appear normal in kainate or dipiperidinoethane-treated rats [19,20]. This was also the case following perforant path stimulation (Figs. 1 and 3-5) with the possible exception that some terminals, primarily in 2 hr continuously stimulated rats, appeared at least partially depleted of vesicle content. Confirmation of this impression awaits evaluation by a more quantitative approach.

The additional major feature of the hippocampal response to perforant path stimulation was seen in the cell bodies and mainstem components of several neuronal cell types. For descriptive convenience, these changes will be discussed as two separate processes (dark cell degeneration and non-dark cell degeneration) although both types of change sometimes occurred simultaneously in the same degenerating cell. In neurons which degenerated without assuming a dark cell appearance, cytoplasmic organelles displayed distinctive pathological changes ranging from mild to extreme. Conspicuous swelling was exhibited frequently by mitochondria in dendritic processes (Fig. 1) but infrequently by those in the somal cytoplasm. The latter tended to undergo a condensation and rounding-up process in which septal membranes became thickened and the matrix more dense as the organelle, without swelling, was transformed from its naturally oblong shape to a sphere (Figs. 7 and 8). The rough endoplasmic reticulum underwent a series of degenerative changes including the transformation of saccular reticular membranes into membrane-bound vacuoles and the dispersal of ribosomes diffusely throughout the cytoplasm (Figs. 7 and 8). Nuclear changes consisted of clumping and coalescing of nuclear chromatin, first into small aggregates then into progressively larger dense masses causing the nucleus eventually to have the condensed dark appearance referred to in the light microscopic literature as nuclear pyknosis (Figs. 6, 8, 9).

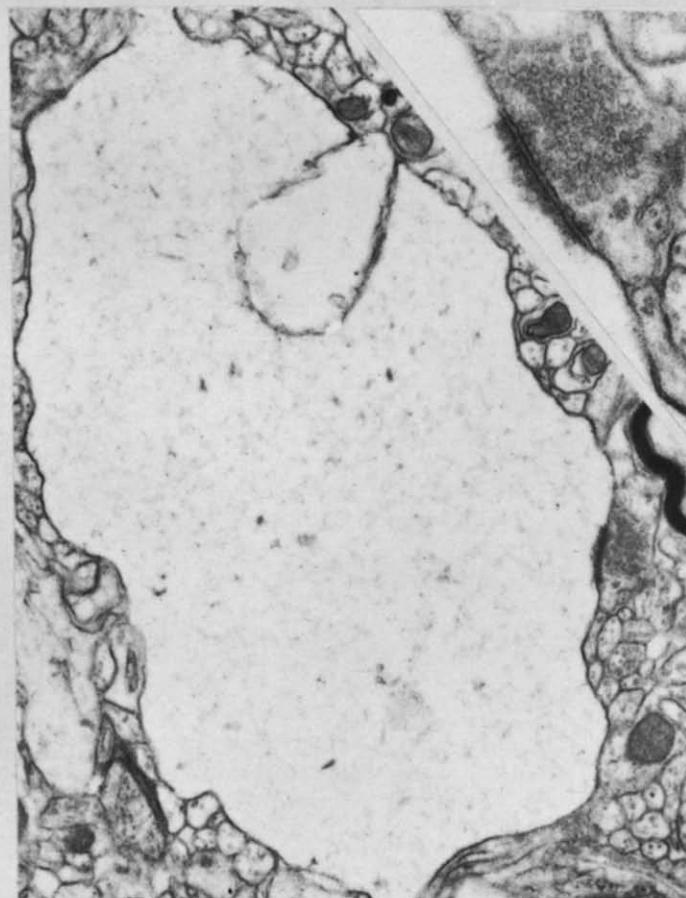
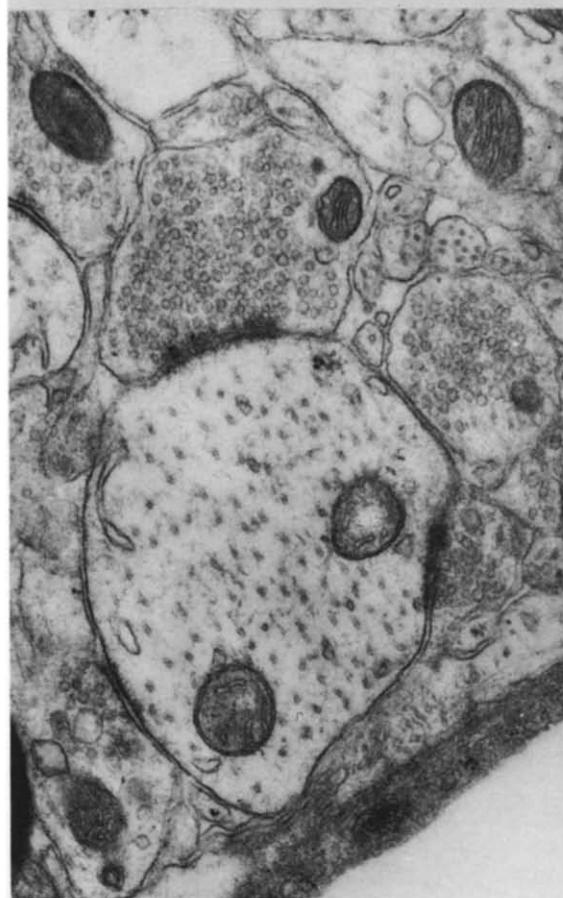
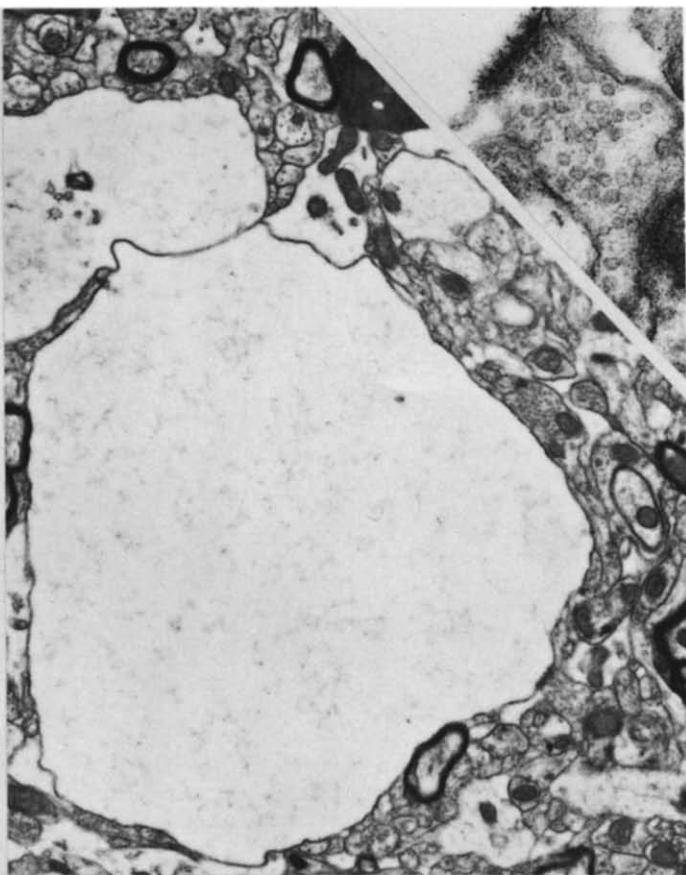
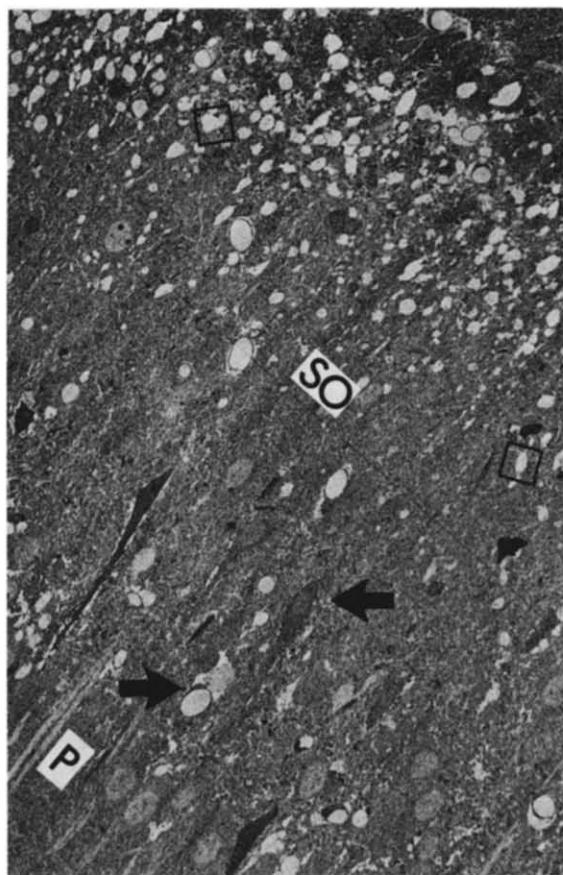
Dark cell transformation, a frequently encountered and most conspicuous consequence of perforant path stimulation, involves shrinkage of the cell body, apparently due to

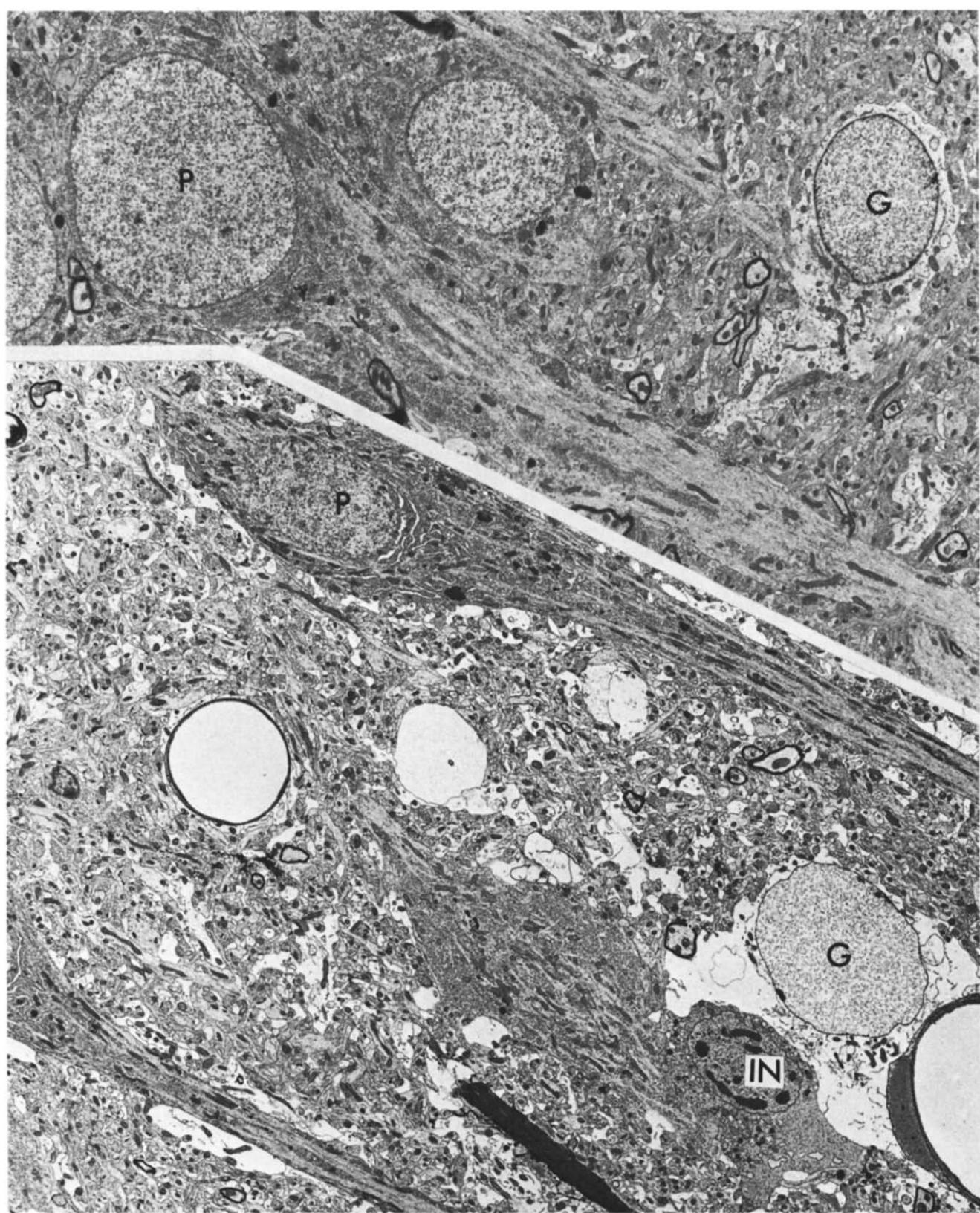
loss of intracellular fluid, with both the cytoplasmic and nucleoplasmic compartments assuming a dense compacted appearance. Vacuolar spaces bounded by endoplasmic reticular membranes develop in the cytoplasm as an early concomitant of dark cell transformation. A satisfactory description of pathological changes in other intracellular structures is precluded by the dark cell transformation process itself which compresses the internal contents of the cell into a dense homogeneous mass thereby rendering fine distinctions in structural detail impossible (Fig. 9). Although dark cell changes have been described as a manifestation of preparation artifact in poorly processed brain tissue, artifact is an unlikely explanation for our findings since our control and experimental brains were perfused and processed in an identical manner and hippocampal dark cell changes occurred only in experimental (perforant path stimulated) rats. Also arguing against poor perfusion fixation as an explanation for dark cell changes is the fact that tissue components displaying excellent preservation were typically observed in the immediate vicinity of neurons exhibiting extreme dark cell degeneration (e.g., see Fig. 9). Both dark cell degeneration and the non-dark cell degenerative changes described here have been observed consistently as a feature of the seizure-related neurodegenerative reaction in rats treated with kainate or dipiperidinoethane [8, 16, 19] and have also been described as a feature of the acute cytopathology induced in retina or the hypothalamus by systemic administration of glutamate [14,15].

The type of neuron undergoing degeneration could not be ascertained in all cases, especially in the hilar zone where cells of polymorphous description are distributed in a scattered pattern. However, cells fitting the description [24] of the pyramidal basket cell, a GABA-containing interneuron [25] which usually lies in the hilus immediately subjacent to the granule cell layer, were easily identified in control hippocampi (Fig. 9a) and cells of this type displaying pathological changes were found in perforant path stimulated brains (Figs. 9b and 10). In either control or experimental brains, the dendrosomal surfaces of these interneurons usually were densely covered with synaptic contacts having the morphological appearance of excitatory synapses (Figs. 9 and 10). This is consistent with the description of this cell type by Ribak and Anderson [24] who, in fact, did not describe any sub-population of basket cells lacking such dense innervation. It is noteworthy, therefore, that while we found numerous examples in our experimental material of degenerating basket cells with abundant axosomatic synapses (Figs. 9a and 10), we also found in experimental brains an occasional basket cell that both lacked such innervation and lacked any signs of degeneration (Fig. 2). In addition to degenerating pyramidal basket cells, we observed either dark cell or non-dark cell degeneration of pyramidal CA3 (Fig. 8) or CA1 (Figs. 6 and 7) neurons or of interneurons in CA3 and CA1

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FIG. 5. At upper left is a survey electron micrograph of the CA1 pyramidal cell layer (P) and stratum oriens dendritic field (SO) after 2 hr continuous perforant path stimulation. Massive dilatation of dendritic segments in the distal portion of the stratum oriens is evident. The arrows point to a scene which is shown at higher magnification in Fig. 6. The boxed regions at top are shown at higher magnification in the panels at right (top and bottom) with insets to illustrate that the presynaptic axon terminal forming a presumptive excitatory synapse on the surface of the degenerating dendrite appears normal. At lower left is a scene from the CA1 stratum oriens region of control brain showing the same kind of synaptic contacts in which both pre- and post-synaptic elements appear normal (top left  $\times 375$ ; top and bottom right  $\times 12000$ ; insets  $\times 50000$ ; bottom left  $\times 36000$ ).





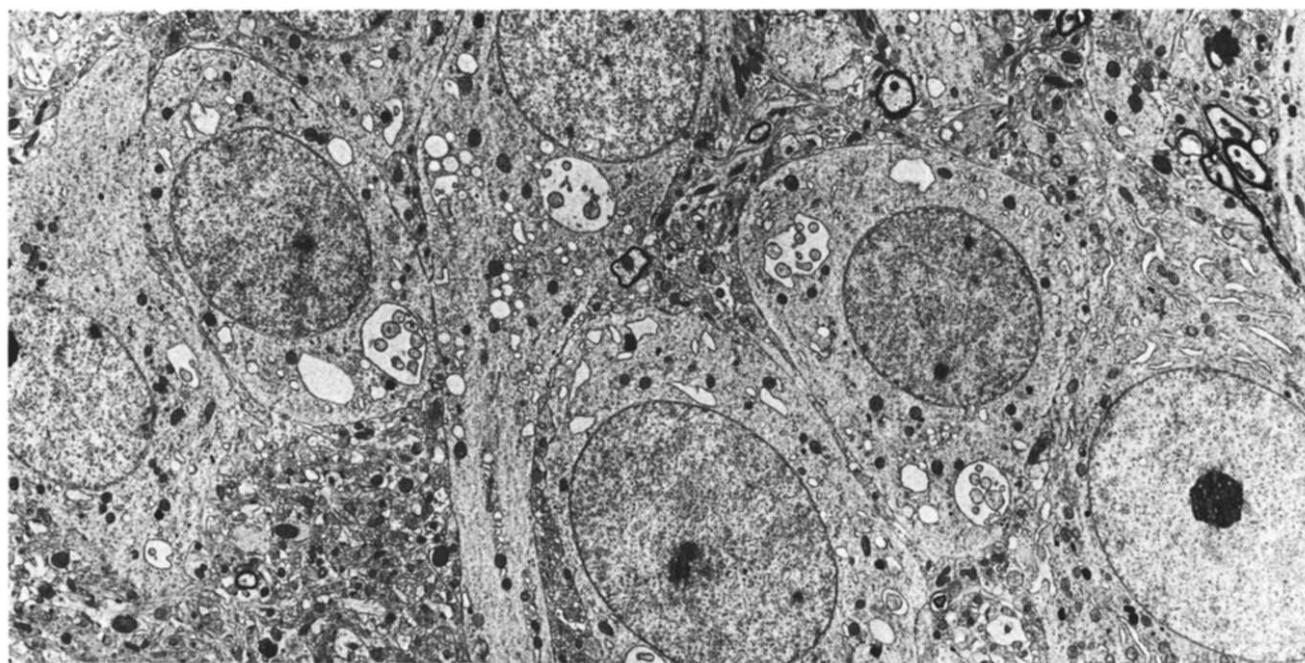


FIG. 7. A group of CA1 pyramidal neurons following 24 hr intermittent perforant path stimulation. By light microscopy these neurons appear normal except for the vacuoles which can be detected in the cytoplasm. By electron microscopy several pathological changes of a moderately severe degree can be identified. Saccules of endoplasmic reticulum have formed membrane-bound vacuolar compartments within which islands of cytoplasmic matrix are undergoing dissolution, an apparent auto-digestive process. The endoplasmic reticular system throughout the cytoplasm has lost its organizational pattern and is largely represented by dispersed ribosomal particles. All mitochondria have become small, dense spherical structures. Crenulation of the nuclear envelope, a pathological change that usually precedes clumping of nuclear chromatin and more advanced stages of nuclear pyknosis, is evident. It is not clear whether the changes in these CA1 pyramidal neurons following 24 hr intermittent stimulation differ in kind or only degree from those in CA1 neurons (Fig. 6) following 2 hr continuous stimulation ( $\times 3528$ ).

hippocampal regions (Fig. 6) and occasional dark cell transformation of dentate granule cells (not shown). It should also be noted that dentate granule neurons after 2 hr continuous stimulation but not 24 hr intermittent stimulation displayed an unusually coarse pattern of nuclear chromatin; these cells otherwise appeared normal (Fig. 2).

#### DISCUSSION

The gliotoxic changes observed here after sustained electrical stimulation of the perforant path are indistinguishable from those associated with sustained limbic seizure activity induced by any of several chemical convulsants [17-22].

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FIG. 6. At top is a normal appearing CA1 pyramidal neuron (P) from control brain and an adjacent glial cell (G) which also appears normal. Below is a scene from the CA1 hippocampal region following 2 hr perforant path stimulation showing a pyramidal neuron (P), glial cell (G) and presumptive interneuron (IN). This is a magnified view of a scene (see arrows) from Fig. 5. The pyramidal neuron is showing early signs of the dark cell transformation process in which portions of the cytoplasm become increasingly more dense except for endoplasmic reticular saccules which continue to outline clear spaces that eventually develop into vacuoles. The glial cell (G) which, in this scene borders on a blood vessel, is markedly edematous and most of the additional edematous structures distributed about this scene are swollen glial processes. Immediately beside the glial cell is a neuron which, after only 2 hr of stimulation, is in such an advanced stage of necrosis that most of its structural components are barely recognizable. At higher magnification (not shown) all mitochondria are small, dense and spherical, the endoplasmic reticulum is reduced to fragmented strands and a few bloated vacuoles, ribosomal material is dispersed diffusely throughout the cytoplasm and clumping of nuclear chromatin is so pronounced that it can readily be identified as a nuclear pyknosis process. We tentatively identify this cell as an interneuron (IN) because of its location in the middle of the stratum oriens dendritic field (both top and bottom  $\times 3920$ ).

Thus, the ability of chemical convulsants to induce such changes does not imply a chemo-specific toxic action of these agents upon glia but rather suggests that some feature of sustained hippocampal discharge activity—whether induced electrically or by chemical agents—affects glia detrimentally. Moreover, since in the present instance an electrical stimulus was applied to neural elements outside the hippocampus (perforant path axons) and these elements are connected synaptically with neurons (but not glia) within the hippocampus, it is reasonable to assume that this type of toxic action upon glia is neuronally mediated. High extracellular  $K^+$  concentrations generated by repetitive neuronal discharge activity provide a possible explanation for the

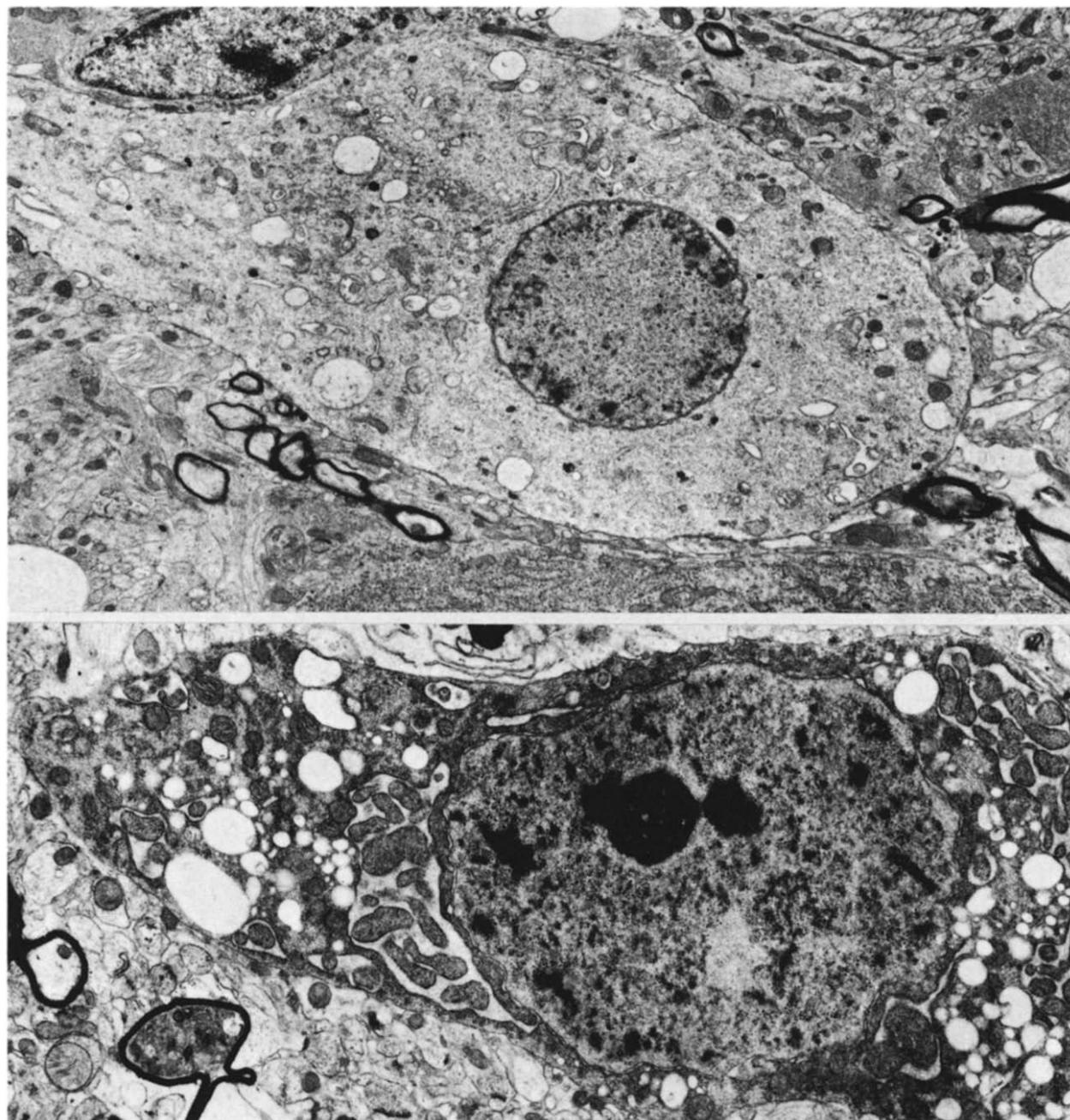


FIG. 8. The degenerating neurons in both the top and bottom views are CA3 pyramidal neurons following 24 hr intermittent perforant path stimulation. The type of degenerative process manifested by the cell at top is identical to that shown by CA1 neurons in Fig. 7 but is representative of a moderately more advanced stage of degeneration. The neuron in the lower panel displays a combination of non-dark cell changes like those in the cell above and dark cell changes like those in the cell shown in Fig. 10 (both figures  $\times 6000$ ).

gliotoxic changes observed since increased extracellular  $K^+$  reportedly has a membrane depolarizing action on cultured glia [11] and induces glial swelling in primate brain *in vivo* [3].

In a previous description of hippocampal changes associated with subcutaneous kainate treatment [20], we noted that many of the degenerating elements receive innervation from putatively glutameric fibers and suggested, since

kainate is a structural analog of glutamate, that the pathological reaction may reflect a direct toxic interaction between kainate and glutamate synaptic receptors. The following more recent observations, however, suggest an alternate interpretation: (1) dipiperidinoethane, which is not structurally related to either kainate or glutamate, reproduces kainate-like changes in hippocampus following subcutaneous administration; (2) intra-amygdaloid injection of folic acid re-

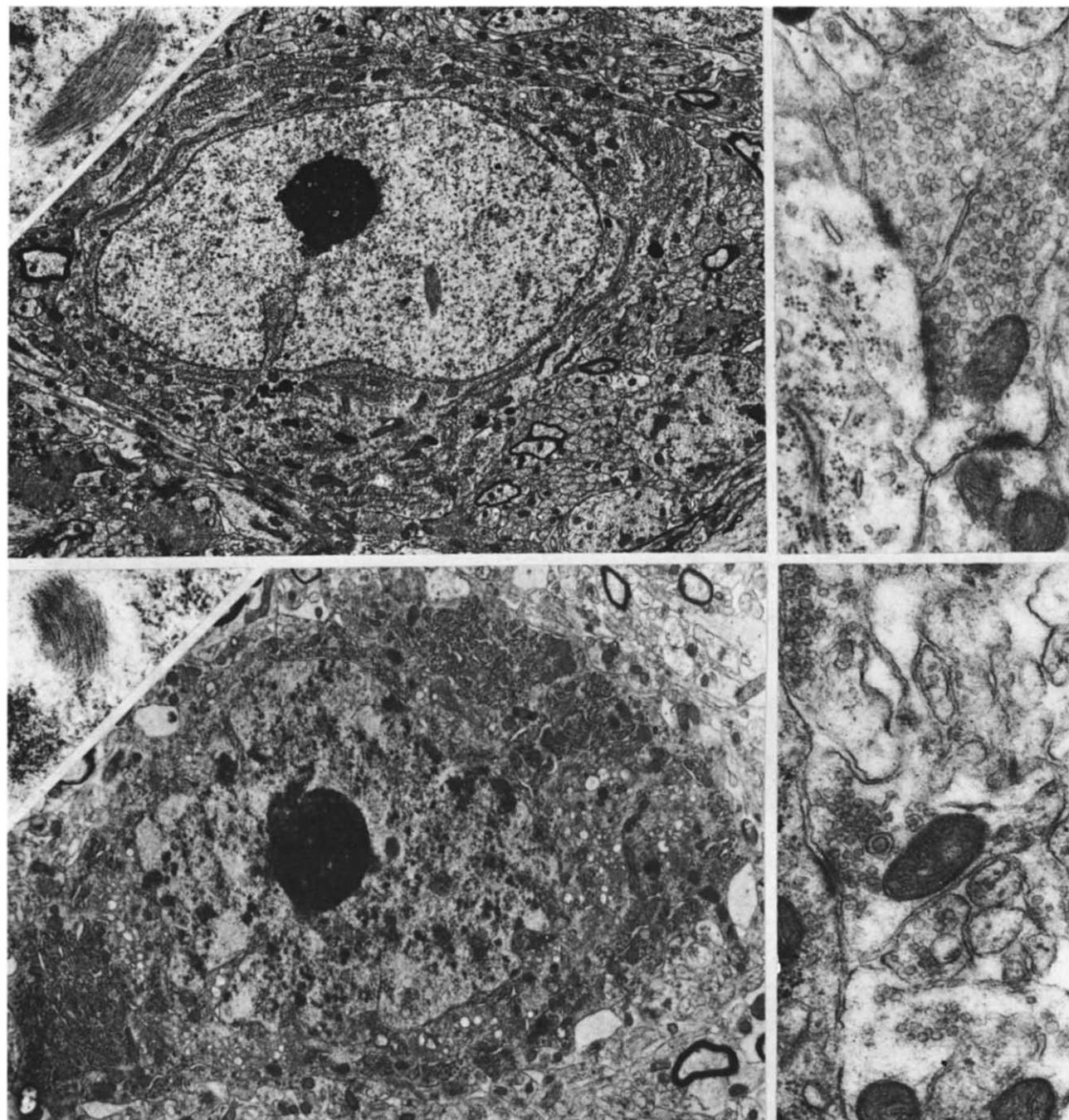
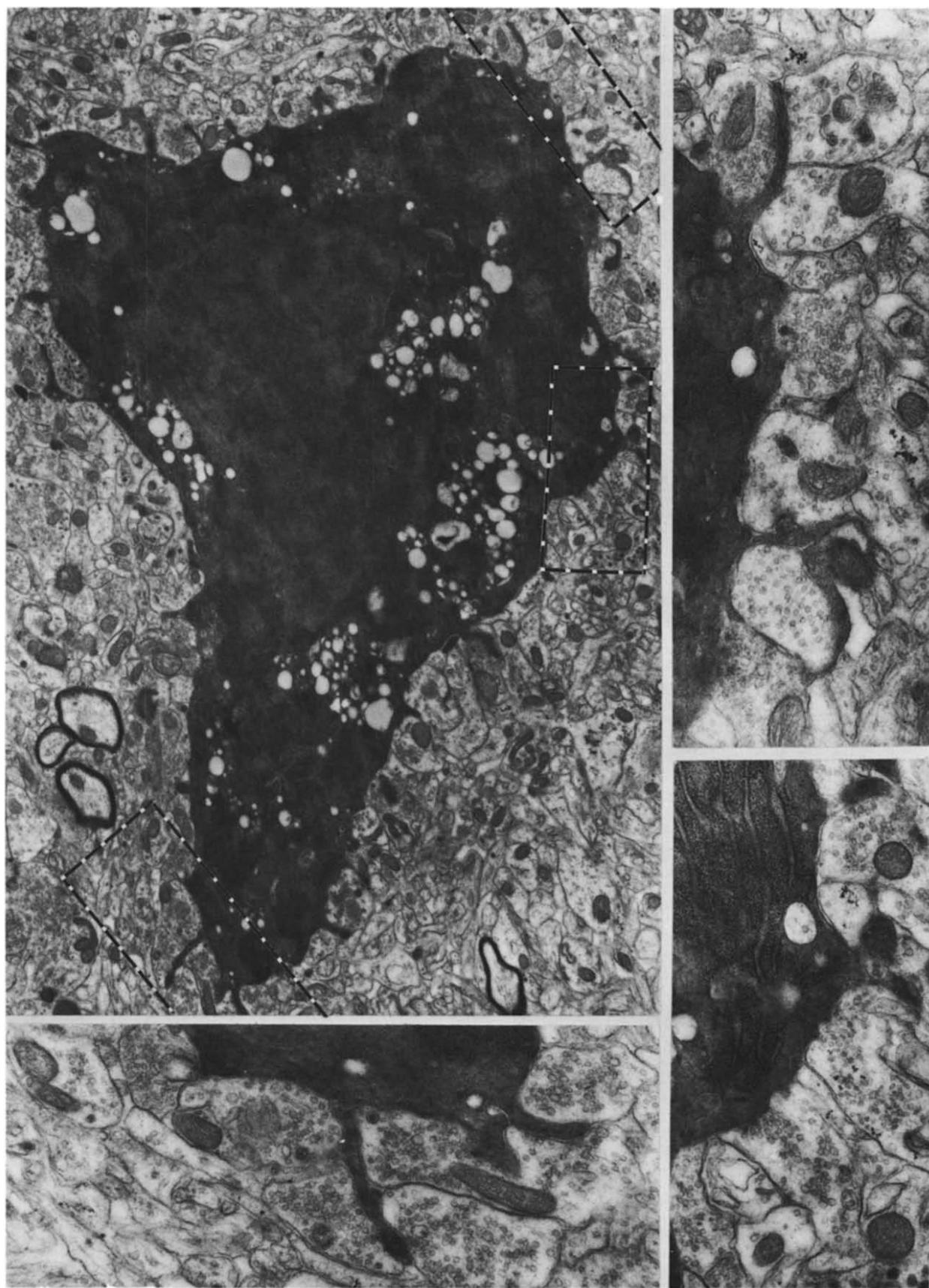


FIG. 9. At top is a control neuron located immediately subjacent to the granule cell layer which receives numerous axosomatic synaptic contacts of the type abstracted at right, has abundant rough endoplasmic reticulum, an infolded nucleus and a nuclear rod (inset), characteristics of presumed pyramidal basket cells [24]. Below is a presumed pyramidal basket cell following 24 hr intermittent stimulation displaying relatively mild signs of degeneration. Amidst abnormal clumps of chromatin, a nuclear rod (inset) is distinguishable in the nucleoplasm. Numerous small vacuoles have formed apparently from Golgi membranes, although the rough endoplasmic reticulum also contributes to the vacuolization process. Abstracted at right is one of the several axon terminals in synaptic contact with the somal surface of this cell. Both the cytoplasm and nucleoplasm are dense and the cell appears slightly shrunken which suggests it may be in an early stage of dark cell degeneration (overviews  $\times 5000$ , magnified views  $\times 32000$ ).



produces the kainate type of seizure-related hippocampal damage [21] even though folic acid lacks the direct neuron-necrotizing action [22] which glutamate-type excitotoxins mediate through glutamate receptors; (3) kainate, dipiperidinoethane and folic acid all produce the same kind of seizure activity as well as the same pattern of seizure-associated brain damage and the brain damage in each case is prevented by pretreatment with the anticonvulsant diazepam [2, 7, 8]. (4) Electrical perforant path stimulation causes not only kainate-like electrophysiological changes in the hippocampus—decreased recurrent inhibition and granule cell seizure activity—but kainate-like histopathological changes [28-30] (confirmed herein by electron microscopy). Items 1-3 above suggest that the major common denominator among kainate, folic acid and dipiperidinoethane is the ability of these agents to induce sustained limbic seizures and that the hippocampal damage ensues as a consequence of the seizure activity. Item 4 strongly reinforces this interpretation by showing that the type of hippocampal damage induced by these chemical convulsants can be reproduced, in the absence of such convulsants, by electrically induced (and maintained) discharge activity in hippocampal circuits. Thus, our findings support the hypothesis [2, 13, 16, 17, 28-30] that some factor(s) intrinsic to discharge activity in specific hippocampal pathways may be responsible for the neurodegenerative changes induced by any of these experimental approaches.

Human epileptics are often found at autopsy to have neuronal cell loss in several brain regions, including the hippocampus [6, 12]. Because a similar pattern of brain damage occurs secondary to anoxia, and cerebral oxygenation is sometimes compromised during seizure episodes, this type of brain damage has been traditionally classified as ischemic damage [12]. It seems unlikely that oxygen deficiency plays a significant role in the type of seizure-linked brain damage reported here, however, since perforant path stimulation does not interrupt respiration and it has been demonstrated that both the arterial oxygen concentration and cerebral blood flow are increased in KA or DPE treated rats during the time interval while they are sustaining seizure-related brain damage [4]. Moreover, even if a relative oxygen deficiency could be documented in local brain regions undergoing damage [1], this would not explain why presynaptic axon terminals, which must have a high oxygen requirement due to repetitive firing, are spared while postsynaptic dendrites and neuronal cell bodies are damaged. Indeed, Garcia *et al.* [9] showed recently that ischemia caused axonal swelling but did not report dendritic pathology. Since glutamate or aspartate may be the excitatory transmitters released at the synaptic sites where postsynaptic degeneration is occurring and these agents are known [15-17, 23] to cause this type of cytopathology (excitotoxic degeneration of dendrites and cell bodies with axonal sparing), excessive release of endogenous glutamate or aspartate at hippocampal synapses may be the mechanism leading to postsynaptic degeneration. Moreover, since the dramatic glutamate-like dendritic swell-

ings we observe in the hippocampus following perforant path stimulation closely resemble the dendritic pathology demonstrated by Scheibel *et al.* [26] in surgically excised tissue from the hippocampi of human epileptics, it is reasonable to postulate a role for glutamate or aspartate in the hippocampal damage associated with human epilepsy.

Because of the highly efficient re-uptake mechanisms in brain for terminating the excitatory activity of glutamate or aspartate, it may be questioned whether increased release of these agents by itself would have neurotoxic consequences. However, repetitive firing of glutamate-containing axon terminals which house the glutamate/aspartate re-uptake transport system may entail expenditure of so much energy for membrane repolarization that the energy needs of the re-uptake system are compromised. Glia are also thought to possess a glutamate/aspartate uptake system which presumably would prevent an accumulation of glutamate or aspartate in the synaptic cleft even if the intra-axonal system were to fail. Glial uptake of glutamate or aspartate from the synaptic cleft, however, may be less effective than axonal re-uptake because the glial system is not in such intimate contact with the cleft. Moreover, in the course of either sustained limbic seizures or sustained perforant path stimulation, glial cells manifest signs of toxicity which may impair either their uptake capacity or their intracellular ability to metabolize and inactivate glutamate or aspartate. Thus, in addition to the excessive release of glutamate or aspartate into the synaptic cleft, mechanisms for removal of the excitotoxin from the cleft may be impaired by the seizure process (if sufficiently sustained) and this combination of circumstances may lead to excitotoxic consequences for dendritic or somal elements that are postsynaptic to glutamate/aspartate terminals. The recent demonstration by Griffiths *et al.* [10] that calcium is accumulated intracellularly in hippocampal neurons undergoing degeneration as a consequence of allylglycine-induced status seizures is consistent with this interpretation, i.e., sustained seizure activity in glutamate or aspartate pathways results in excessive depolarization and pathological permeability changes in postsynaptic dendrosomal membranes which, in turn, lead to influx and toxic intracellular accumulation of calcium.

Basket cells or other interneurons which perform an inhibitory function in the hippocampus are relatively sparse compared to the granule and pyramidal cell populations which they inhibit. Thus, at least some individual interneurons presumably receive recurrent collaterals from numerous granule or pyramidal cells and this may explain why we found the dendrosomal surfaces of degenerating interneurons densely covered with synaptic terminals. If these terminals use glutamate or aspartate as transmitter and large numbers of them fire simultaneously and repetitively upon a small number of interneurons, as would occur during sustained limbic seizures or electrical perforant path stimulation, such interneurons might succumb early to the excitotoxic effects of these transmitters. When glutamate, aspartate or kainate are iontophoresed upon central neurons, it

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FIG. 10. A hilar cell showing advanced dark cell degeneration following 24 hr intermittent stimulation. This cell was located immediately below the granule cell layer at the apex of the hilus. The uniformly dark color and crowding together of structures within the cell make it difficult to assess the condition of individual cellular components. Numerous vacuoles are present in the cytoplasm. Cytoplasmic extensions into the neuropil assume the appearance of pseudopodia. The surfaces of the cell and of these extensions are covered with synapses (see magnified view of boxed regions) (overview  $\times 9000$ , magnified view  $\times 28000$ ).

results, depending on dose, in either reversible depolarization or irreversible depolarization block and electrical silence [5]. If inhibitory interneurons in the dentate hilus region, by virtue of receiving dense glutamate/aspartate innervation, are silenced early, this would explain the early loss of recurrent inhibition which in the previous study [28] was found to give rise to disinhibited firing of dentate granule neurons. If interneurons that inhibit the firing of CA1 or CA3 pyramidal neurons also receive dense glutamate/aspartate collateral innervation, these interneurons may also be silenced relatively early by an excitotoxic mechanism, the result being generalized disinhibited discharge activity in limbic circuits and, quite likely, a more extensive pattern of cytopathological damage than would occur under conditions of unimpaired recurrent inhibition. We propose, therefore,

that much of the cytopathological damage induced in the hippocampus by sustained seizures or perforant path stimulation can be understood in terms of the abnormally sustained nature of the stimulatory events, the use of excitotoxic transmitters by both the perforant path and intrahippocampal circuits, the potential of these transmitters, when excessively released, to abolish recurrent inhibition and the augmented release of excitotoxins at many hippocampal synapses that occurs when recurrent inhibition has been abolished.

#### ACKNOWLEDGMENTS

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