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Focal Cortical Seizures Cause Distant Thalamic Lesions

Abstract. *Topical application of convulsants to the rat sensorimotor cortex in concentrations sufficient to cause repetitive focal motor seizures resulted in acute neuropathology (dark cell neuronal degeneration and spongiform neuropil changes) involving both the cortical seizure focus and certain thalamic nuclei within seizure pathways. Changes in the cortex were localized primarily in layer IV and those in the thalamus in nuclei having reciprocal connections with the cortical focus. The spongiform neuropil changes consisted of massively dilated presynaptic axon terminals in the cortex and postsynaptic dendrites in the thalamus. The dendritic and dark cell changes resemble the excitotoxic damage caused by glutamate and aspartate. Since these putative transmitters may be released locally from recurrent collaterals and remotely from corticothalamic axons, excessive release of glutamate or aspartate may account for the changes in both sites. The abnormal axons in sensory cortex appear to be terminals of thalamocortical neurons. Swelling of these axons may be caused by excessive anti- and orthodromic firing in the course of focal motor seizures.*

Focal epilepsy in man is the clinical expression of a pathological lesion of neocortex causing excessive neuronal firing through local and long circuits. Early studies in which depth electrodes were used to trace seizure spread have recently been extended with [^{14}C]deoxyglucose autoradiography (*1*). During convulsions, there is a marked increase in metabolism in seizure pathways in subcortical sites. In thalamus, in particular, a neocortical focus can cause both orthodromic and antidromic activation of thalamocortical relay (TCR) cells (*2*). Changes in neuronal firing rate, increases in extracellular potassium, and changes in glucose and amino acid metabolism can be as intense in the thalamus as in the neocortical focus (*1*, *3*).

Pathological studies of experimental focal epilepsy have emphasized changes in cortex, particularly the swelling of processes identified as dendrites (*4*). Experimental seizures induced in the limbic system by convulsants or repetitive electrical stimulation are associated with acute lesions distant from the focus (*5*). Distant lesions, or "double pathology" occur in human temporal lobe epilepsy and probably play a role in the expression of clinical convulsions (*6*). We undertook to assess neuropathological changes associated with focal sensorimotor seizures in local and distant seizure pathways.

Sprague-Dawley rats were anesthetized with halothane, and an epidural well was prepared over the forelimb sensorimotor cortex (*7*). After the animal recovered from anesthesia, one of the following convulsants was infused into the well: 18 m*M* folic acid, 100 m*M* sodium penicillin, 2.5 m*M* bicuculline methiodide, or 0.3 m*M* picrotoxin. The concentrations were chosen to cause an equivalent state of repetitive focal seizures. In control rats, the well was infused with vehicle. At the end of a seizure period (2 hours or more), animals were killed with barbiturate, and brains were fixed in situ by perfusion with 1.5 percent glutaraldehyde and 1 percent paraformaldehyde buffered with phosphate (*p*H 7.3 to 7.4) for light and electron microscopy (*8*).

Animals were alert and freely mobile during focal cortical discharges (Fig. 1a). Concomitant jerking of the contralateral forelimb did not interrupt ventilation. Animals displaying brief recurrent seizures for longer than 2 hours had characteristic lesions in the cortex and thalamus (Figs. 1b and 2).

Histological examination of the cortex revealed two patterns of damage. Superficial laminae (layers I to III) of the motor cortex and the sensory cortex beneath the well exhibited signs of injury, including swelling of cell bodies and processes of both glia and neurons, scattered dark neurons, and edema of layer I. Similar, but milder, changes were observed in controls, suggesting an artifact resulting from surgical trauma. The other pattern occurred only in animals with recurrent seizures and consisted of conspicuous dark cell and spongiform changes mainly in layer IV of the adjacent forelimb sensory cortex (Fig. 2a),

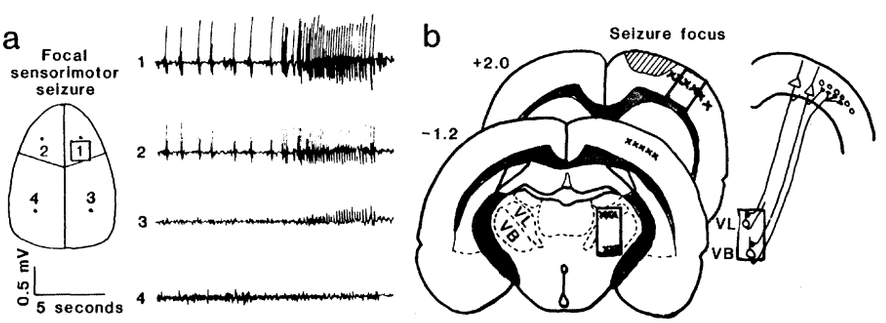

Fig. 1. (a) The site and relative size of the epidural well (1) over the forelimb sensorimotor cortex is indicated on a dorsal view of the rat skull. Wire electrodes were inserted into the epidural space at the focus, contralateral homotopic cortex (2), and bilateral occipital cortices (3 and 4). A characteristic electroencephalogram shows spikes and short seizures (one every 1 to 5 minutes) with 2.5 m*M* bicuculline methiodide in the epidural well. This general type of pattern occurred with each convulsant. (b) Neuropathological changes (xxx) occurred principally in middle cortical layers within the posterolateral extension of the focus and in subcortical terminal fields having a reciprocal relation with the focus. Numbers indicate approximate anteroposterior coordinates relative to bregma. Abbreviations: *VB*, ventral-basal complex; *VL*, ventralis lateralis.

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with minor spread into deeper layers of the sensory and motor cortex. Dark cell degeneration seemed to be occurring primarily in nonpyramidal neurons, but pathological changes precluded more definitive cytological classification in this material. With electron microscopy, we found that the spongiform neuropil changes consisted of massively swollen axon terminals (Fig. 2b). Occasionally, a swollen preterminal axon emerging from a myelin sheath was also seen. The dilated terminals typically contained round, clear synaptic vesicles and made synaptic contact with small dendritic spines, but did not receive presynaptic contacts from other processes. Since the axons of TCR neurons from the thalamic ventrobasal complex form axospinous synapses confined largely to cortical layers IIIb and IV (*9*), and since these thalamic neurons also showed changes (see below), we suspect that the swollen axon terminals belong to these thalamic cells. Definitive identification awaits further experimentation with radioactive tracers.

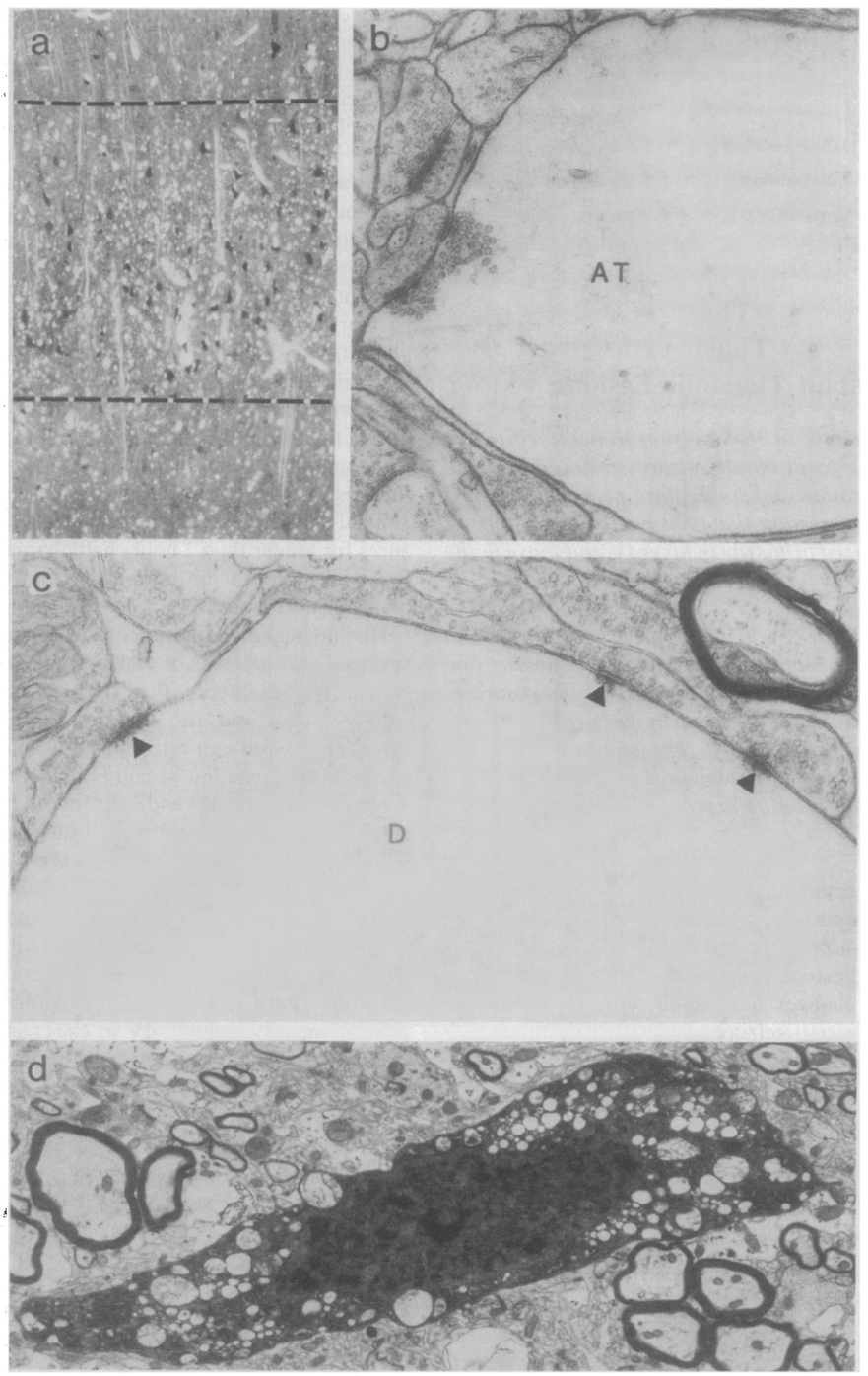

Fig. 2. (a) Light micrograph showing the sensory cortex of a rat after 2 hours of seizure activity induced by topical application of picrotoxin (0.3 m*M*). A laminar band of pathological changes (dilated processes and dark cell degeneration) primarily restricted to layer IV (between dashed lines) is evident (×50). (b) A dilated process from the midlaminar field in (a) magnified by electron microscopy to reveal that it is a presynaptic axon terminal (*AT*). A collection of synaptic vesicles can be seen in the dilated terminal near the synaptic complex (×20,000). (c) A similarly dilated process, but this is a massively edematous dendrite (*D*) from the VL thalamus after 2 hours of seizures induced by topical application of folic acid (18 m*M*) to the cortex. Arrowheads indicate the synaptic complexes where normal appearing axon terminals make presynaptic contact with the swollen dendrite (×22,000). (d) A thalamic VL neuron displaying an advanced stage of dark cell degeneration after focal motor seizures (topical bicuculline methiodide, 2.0 m*M*) for 6 hours (×4000).

Spongiform changes were also seen ipsilaterally in two thalamic nuclei—ventralis lateralis (VL) and the forelimb sector of the ventrobasal complex (VB) (Fig. 1b). Previous autoradiographic studies in this model have shown an intense increase in metabolism in these two sites (*1, 2*). With electron microscopy, we found that the spongiform changes were due to massive swelling of small dendrites (Fig. 2c). These profiles were identified as dendritic, since they were in asymmetric postsynaptic contact with normal-appearing axonal boutons containing clear spherical vesicles (presumptive excitatory synapses). Dilatation of axon terminals, the characteristic acute change noted in cortical layer IV, was not a feature of the thalamic lesion. Large thalamic neurons—presumed to be TCR cells—exhibited cytoplasmic vacuolization after 2 hours of seizure activity and dark cell degeneration after 4 to 6 hours (Fig. 2d). These dark cell changes were indistinguishable from those in the cortex, which developed within 2 hours of seizure activity. Other brain regions, including the contralateral thalamus, did not display either dark cell changes or swollen processes.

Since all convulsants used in the study caused the same pathological changes, we conclude that these are a reflection of the epileptic process and are independent of triggering mechanisms. Strong seizures in experimental primates and in man interrupt ventilation, and hypoxemia appears to play a role in neurodegenerative changes. However, hypoxemia is not a likely explanation for the cytopathology we observed, since our animals remained alert and breathed normally. Moreover, local consumptive hypoxia would not explain selective axonal changes in one locus and dendritic changes in another.

Excessive burst firing of pyramidal neurons is a major electrophysiological event underlying focal sensorimotor sei-

zures. Deep cortical pyramids send short axon collaterals in part into terminal fields in cortical layer IV (*10*) and long corticofugal fibers to VL or VB thalamic neurons. The transmitter released by pyramidal cells is probably glutamate (*11*), which in high concentration causes swelling of dendrites and swelling or dark cell degeneration of neuronal cell bodies (*8, 12*). We suggest that swelling of dendrites in the thalamus and dark cell degeneration in both the cortex and thalamus reflect a seizure-induced, excitotoxic process mediated by excessive release of glutamate at synaptic receptors. Such damage to local inhibitory interneurons could release control over neighboring cortical pyramids (*13*) and disrupt the temporal and spatial containment of the focus. Permanent excitotoxic damage to cortical interneurons could underlie the loss of γ-aminobutyric acid–dependent inhibition found in other models of focal epilepsy (*14*). Excessive release of glutamate at receptor sites in the thalamus (motor cortex → VL; sensory cortex → VB) would have excitotoxic consequences for the dendritic structures containing these receptors as well.

Swelling of axon terminals in the cortex cannot be explained by a glutamate-mediated mechanism. A clue to the cause of this morphological change may lie in evidence that thalamocortical axons that project to layer IV are driven both ortho- and antidromically in the course of focal sensorimotor seizures (*2*). Excessive epileptic firing in these terminals might cause a focal derangement in ionic or metabolic membrane homeostasis that would be manifested as acute swelling.

Our findings support the thesis that sustained seizures can result in neuronal degeneration in the seizure focus and elsewhere within seizure pathways by mechanisms other than anoxia. If an excitotoxic mechanism underlies important components of the neuropathology of experimental seizures, it is possible that brain damage in human epilepsy may in part have a similar basis. If so, the recent discovery of agents that powerfully block both the excitatory and neurotoxic actions of glutamate excitotoxins provides hope for the chemoprophylactic approach to the management of epilepsy-related brain damage (*15*).

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Discrimination and Imitation of Facial Expressions by Neonates

Abstract. *Human neonates (average age, 36 hours) discriminated three facial expressions (happy, sad, and surprised) posed by a live model as evidenced by diminished visual fixation on each face over trials and renewed fixations to the presentation of a different face. The expressions posed by the model, unseen by the observer, were guessed at greater than chance accuracy simply by observing the face of the neonate, whose facial movements in the brow, eyes, and mouth regions provided evidence for imitation of the facial expressions.*

Facial expressions of emotions such as happiness, sadness, and surprise have been observed in the very young infant (*1*) and in several cultures (*2*). Because of their early appearance and their apparent universality, these basic facial expressions may reflect innate processes (*3*). We have investigated whether neonates can discriminate and imitate these facial expressions. Projected photographs of facial expressions are discriminated as early as 3 months of age in a visual habituation paradigm (*4*). The young infant is also physically capable of reproducing these expressions; all but one of the discrete facial muscle actions of adults have been identified in the neonate (*5*). Although a debate continues on what processes may be involved (*6*), imitations of facial movements such as lip protrusion, mouth widening, and tongue thrusting have been reported for 12- to 21-day-old babies (*7*). We now have evidence for both the discrimination and imitation of facial expressions at an even younger age, shortly after birth.

In this study, a series of three facial expressions (happy, sad, and surprised) were modeled by an adult for 74 neonates (mean age, 36 hours) (*8*). The model held the neonate upright with the newborn's head supported in one hand and torso in the other hand. The two faces were separated by approximately 10 inches. The neonate's visual fixations on the adult's face and the neonate's facial movement patterns were recorded by an observer who stood behind the model in order to see the infant's face but remained unaware of the expression being modeled. Split-screen videotaping of the neonates' and model's faces provided checks on the reliability of coding by observer and face presentation by model (*9*).

To sustain alertness and to elicit the

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