

## DENERVATION AND REINNERVATION OF AMYGDALOID NEURONS IN DRUG-REFRACTORY TEMPORAL LOBE EPILEPSY

### Cellular Pathology of Amygdala Neurons in Human Temporal Lobe Epilepsy

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The amygdala complex substantially contributes to the generation and propagation of focal seizures in patients with temporal lobe epilepsy (TLE). A cellular substrate for increased excitability in the human amygdala, however, remains to be identified. We analyzed the three-dimensional morphology of 264 neurons from different subregions of the amygdaloid complex obtained from 17 en bloc-resected surgical specimens by using intracellular Lucifer Yellow (LY) injection and confocal laser scanning microscopy. Autopsy samples from unaffected individuals ( $n = 3$ ; 20 neurons) served as controls. We have identified spine-laden, spine-sparse, and aspiny cells in the lateral, basal, accessory basal, and granular nuclei. Semiquantitative analysis points to significant changes in neuronal soma size, number of dendrites, and spine densities in specimens from epilepsy patients compared with controls. Neuronal somata in the epilepsy group were smaller compared with those of controls ( $P < 0.01$ ); neurons had fewer first-order dendrites ( $P < 0.01$ ), whereas the maximal density of spines per dendritic segment in these cells was increased in TLE patients ( $P < 0.01$ ). Dendritic alterations such as focal constrictions or spine bifurcations also occurred. These changes were consistent between amygdaloid subregions. The dendritic morphology of amygdaloid neurons in TLE patients points to substantial changes in synaptic connectivity and would be compatible with altered neuronal circuitries operating in the epileptic human amygdala. Although the morphologic alterations differ from those described in hippocampal subregions of a similar cohort of TLE patients, they appear to reflect a characteristic pathological substrate associated with seizure activity/propagation within the amygdaloid complex.

### COMMENTARY

The emotional center of the brain, the amygdala, is composed of 13 different nuclei and cortical regions. As previous studies show, amygdaloid damage is not uncommon in patients with temporal lobe epilepsy (TLE). According to magnetic resonance imaging (MRI) studies, it is present in ~20% to 30% of patients with drug-refractory TLE and, based on autopsy studies, in 27% to 76% of TLE patients with hippocampal damage. The little that is known about the distribution of neuronal loss and gliosis in epileptic amygdala is based largely on Nissl and glial staining. These studies have consistently revealed that one of the most vulnerable regions of the amygdala is the lateral nucleus. Via the lateral nucleus, most of the sensory information from various cortical regions enters the amygdala and becomes further distributed to other amygdaloid regions via extensive intraamygdaloid pathways. The other damaged region is the parvocellular division of the basal nucleus, which is the amygdaloid gateway to the hippocampus. Unlike in the epileptic hippocampus, no data are available about the axonal or dendritic alterations in the epileptic amygdala in experimental or human epilepsy.

Aliashkevich and colleagues filled 264 amygdaloid neurons with a fluorescent dye, Lucifer Yellow, in 250- $\mu$ m-thick slices obtained from 17 patients who had drug-refractory TLE. The majority of the neurons were in the lateral nucleus ( $n = 169$ ), but the authors also successfully filled neurons in the basal ( $n = 81$ ), accessory basal ( $n = 7$ ), and granular nucleus ( $n = 7$ , corresponding to the medial aspect of the paralaminar nucleus in monkey and human amygdala). Thus amygdaloid nuclei, which are known to be damaged in epileptic brain, were all included in the present analysis. Data from epileptic brain were compared with three autopsy controls. Patients had an average of 5.3 seizures/month, mean age at onset of epilepsy was 13.8 years, and the mean duration of the disease was 19.8 years. All patients had complex partial seizures. Unfortunately, no details about the clinical symptoms of seizures or depth electrode recordings were provided, and therefore putative amygdaloid involvement in seizure onset is unknown. No description was given of MRI findings of the amygdala or data about the appearance of amygdaloid damage in routine pathology. Instead, the authors mention that 15 of 17 patients had Ammon's horn

sclerosis, one had dual pathology consisting of dysembryoplastic neuroepithelial tumor in the cortex of the superior temporal gyrus and hippocampal sclerosis, and one had diffuse gliosis in cortical and subcortical regions.

Analysis of 3D-reconstructed neurons revealed that neurons in the epileptic amygdala were small and had fewer dendrites. The remaining dendrites appeared thicker and carried several irregularities, including constrictions, swelling of dendritic shafts, and bifurcation of spines. As the authors speculate, lack of dendritic branching could relate to the lack of afferent inputs. No data exist about the deafferentation of epileptic amygdala occurring as a result of atrophy of brain areas that provide inputs to the amygdala. One may only speculate whether, for example, damage to the perirhinal and entorhinal cortex, or to the hippocampus proper, may have reduced the number of inputs to the spiny neurons in the lateral and basal nuclei, which were included in the analysis. The histopathologic analysis revealed the occurrence of hippocampal damage in most of the patients. In a chronic patient group such as this, it is likely that a substantial percentage of patients also would have damage in the parahippocampal cortical areas, as the MRI studies from recent literature suggest, and therefore a reduced number of projections targeted to the amygdala.

Studies on mossy fiber sprouting in the dentate gyrus have revealed that axonal sprouting is associated with increased density of granule cell spines. Currently, no single study has demonstrated axonal sprouting in the epileptic amygdala. Unlike in the hippocampus, where mossy fiber sprouting readily can be demonstrated with Timm staining or dynorphin immunohistochemistry, no reliable marker can demonstrate axonal sprouting in the amygdaloid pathways. The increase of spine density from 1.8 to 2.9 spines/10  $\mu\text{m}$  in the epileptic amygdala could relate to

increased axonal sprouting associated with activity-dependent plasticity in the epileptic amygdala. Keeping in mind that a large majority of patients had hippocampal pathology that was likely associated with mossy fiber sprouting, the present data support the idea that reorganization of neuronal circuits is not limited to the hippocampus in drug-refractory patients with TLE.

Perhaps one of the most dramatic findings of the study was the variability of abnormalities in dendritic shafts, including nodular varicosities, focal dendritic shaft swellings or constrictions, and spine bifurcations. As the authors suggest, these abnormalities could relate to alterations in microtubular arrangements, accumulation of cytoskeletal filaments, or increased number of mitochondria. These data are of interest as they relate to recent microarray data from animals that have experienced prolonged seizures induced chemically or electrically. Array data show that expression of many of the genes that mediate structural alterations or reorganization of cell cytoskeleton is altered. Whether these alterations persist in the chronic epileptic state associated with frequent, spontaneous seizures is a new and interesting field of research.

The study by Aliashkevich and co-workers provides the first, and we hope not the last, analysis of cellular morphology in epileptic human amygdala. As the data show, many similarities are found to hippocampal pathology. This work is an important addition to the constantly expanding list of studies showing that neuronal pathology extends beyond the hippocampus in drug-refractory TLE. It is time to focus our microscope on regions located outside the hippocampus in the search for neuronal substrates of epileptogenesis and ictogenesis in human TLE. The amygdala is an excellent candidate.

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