Epileptic baboons have lower numbers of neurons in specific areas of cortex

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Epilepsy is characterized by recurrent seizure activity that can induce pathological reorganization and alter normal function in neocortical networks. In the present study, we determined the numbers of cells and neurons across the complete extent of the cortex for two epileptic baboons with naturally occurring seizures and two baboons without epilepsy. Overall, the two epileptic baboons had a 37% average reduction in the number of cortical neurons compared with the two nonepileptic baboons. The loss of neurons was variable across cortical areas, with the most pronounced loss in the primary motor cortex, especially in lateral primary motor cortex, representing the hand and face. Less-pronounced reductions of neurons were found in other parts of the frontal cortex and in somatosensory cortex, but no reduction was apparent in the primary visual cortex and little in other visual areas. The results provide clear evidence that epilepsy in the baboon is associated with considerable reduction in the numbers of cortical neurons, especially in frontal areas of the cortex related to motor functions. Whether or not the reduction of neurons is a cause or an effect of seizures needs further investigation.

primates | plasticity | neuronal density

Epilepsy is associated with structural changes in the cerebral cortex (e.g., refs. 1–6), and partial epilepsies (i.e., seizures originating from a brain region) may lead to loss of neurons (7) and altered connectivity (8). The cerebral cortex is a heterogeneous structure comprised of multiple sensory and motor information-processing systems (e.g., refs. 9 and 10) that vary according to their processing demands, connectivity (e.g., refs. 11 and 12), and intrinsic numbers of cells and neurons (13–16). Chronic seizures have been associated with progressive changes in the region of the epileptic focus and in remote but functionally connected cortical or subcortical structures (3, 17). Because areas of the cortex are functionally and structurally different, they may also differ in susceptibility to pathological changes resulting from epilepsy.

The relationship between seizure activity and neuron damage can be difficult to study in humans. Seizure-induced neuronal damage can be convincingly demonstrated in animals using electrically or chemically induced status epilepticus (one continuous seizure episode longer than 5 min) to reveal morphometric (e.g., refs. 18 and 19) or histological changes (e.g., refs. 20 and 21). Subcortical brain regions are often studied for vulnerability to seizure-induced injury (21–27); however, a recent study by Karbowski et al. (28) observed reduction of neurons in cortical layers 5 and 6 in the frontal lobes of rats with seizures. Seizure-induced neuronal damage in the cortex has also been previously demonstrated in baboons with convulsive status epilepticus (29).

The goal of the present study was to determine if there is a specific pattern of cell or neuron reduction across the functionally divided areas of the neocortex in baboons with epilepsy. Selected strains of baboons have been studied as a natural primate model of generalized epilepsy (30–36) that is analogous to juvenile myoclonic epilepsy in humans. The baboons demonstrate generalized myoclonic and tonic-clonic seizures, and they have generalized interictal and ictal epileptic discharges on scalp EEG. Because of their phylogenetic proximity to humans, baboons and other Old World monkeys share many cortical areas and other features of cortical organization with humans (e.g., refs. 9 and 10). Cortical cell and neuron numbers were determined using the flow fractionator method (37, 38) in epileptic baboon tissue obtained from the Texas Biomedical Research Institute, where a number of individuals develop generalized epilepsy within a pedigreed baboon colony (31–36). Our results reveal a regionally specific neuron reduction in the cortex of baboons with naturally occurring, generalized seizures.

Results

We present cell and neuron numbers for small blocks of tissue across all cortical areas and regions in four baboons: two epileptic baboons and two neurologically normal controls. We focused only on neocortex cell and neuron numbers in the present report. One hemisphere from each case was used to determine cell and neuron numbers. The brain of case 09-27 was purchased from the University of Washington National Primate Research Center. Cases 11-31, 10-04, and 11-45 were provided by the Texas Biomedical Research Institute. All baboons were female, between 12 and 17 y of age, and with body weights between 17.8 and 23.4 kg. Table S1 summarizes specific details on the age, sex, size, and perfusion method for each case. Cases 09-27 and 11-31 were neurologically normal, and cases 10-04 and 11-45 were epileptic. Case 10-04 experienced multiple seizures at age 13 y, based upon witnessed seizure activity and craniofacial injury typically related to seizure activity. The first reported craniofacial injury occurred at age 10 y. Two witnessed seizures were reported

Significance

We examined the variability of neuron packing densities across cortical regions and areas in two baboons with spontaneous, untreated epilepsy and two baboons without epilepsy. The two baboons without epilepsy had the distribution of neocortical neurons expected for Old World monkeys and baboons, whereas the baboons with untreated epilepsy had reduced numbers of cortical neurons overall, with the greatest reductions in motor and frontal areas of the cortex, and with little or no reduction in the primary visual cortex. The results suggest that neuron loss may follow untreated seizure activity, and this loss is greatest in areas of the cortex related to motor functions.

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in case 11-45, the first occurring at age 7 y after the delivery of an infant, and at age 16 y. Case 11-45 also underwent a scalp electroencephalogram at age 12 y, indicating generalized interictal epileptic discharges, but no evidence of photosensitivity. Both epileptic baboons were clinically otherwise unremarkable, and neither had experienced an episode of status epilepticus. There were no reports of motor impairment or any other type of behavioral impairment. Neither epileptic baboon was treated with antiepileptic medications.

Overall Reduction of Cell and Neuron Number in the Cortex with Epilepsy. One cortical hemisphere from each of two normal baboons and two epileptic baboons was manually flattened. Flattened cortex varied in surface area between 186 and 242 cm^2. Each flat cortex was viewed on a light box to identify borders of myelin-dense primary sensory areas [primary visual cortex (V1), primary sensory cortex (S1), and primary motor cortex (M1)] (15, 16, 39). Cortex was dissected into tissue pieces that were processed as 142 (09-27; average surface area = 1.307 cm^2), 194 (11-31; average surface area = 1.201 cm^2), 177 (10-04; average surface area = 1.389 cm^2), and 428 (11-45; average surface area = 0.4478 cm^2) samples. Tissue pieces for case 11-45 were dissected with a smaller area in an effort to further localize specific regions or areas of cell or neuron loss. For each dissected tissue sample, we determined the total number of cells and percentage of neurons per piece. Cell and neuron densities for each tissue piece were calculated by dividing the numbers of cells and neurons by square centimeter of cortical surface area. Table S2 summarizes the data for each case.

The numbers of cells and neurons across the entire cortical sheet were remarkably consistent between the two neurologically normal baboon cases. For case 09-27, there were 4.67 billion cells in the cortex, of which 2.36 billion (51%) were neurons. Similarly, for case 11-31, there were 4.29 billion cells in the cortex, of which 2.27 billion (52%) were neurons.

The overall numbers of neurons across the entire cortical sheet were markedly lower in epileptic baboons (Fig. 1*B′*) relative to normal baboons (Fig. 1*A′*) (U = 1826.0; $P = 0.0001$), and the overall number of cells per cortical hemisphere was also lower (Fig. 1 *A* and *B*) (U = 1436.0; $P = 0.0001$). For epileptic baboon 10-04, there were 4.00 billion cells in the cortex, of which 1.61 billion (39%) were neurons; and for epileptic baboon 11-45, there were 4.24 billion cells across the cortex, of which 1.79 billion (41%) were neurons. Epileptic baboons had a lower range of cells overall (4.00–4.24 billion cells) relative to control baboons (4.29–4.67 billion cells). In contrast, the reduced neuron number was evident, with a range of 2.26–2.39 billion cortical neurons in neurologically normal baboons, and 1.61–1.79 billion cortical neurons in epileptic baboons. Thus, epileptic baboon 10-04 had ∼48% fewer neurons across the extent of the cortex, and epileptic baboon 11-45 has ∼26% fewer cortical neurons (mean reduction of ∼37%).

Cell and Neuron Reduction Associated with Epilepsy Is Specific to Cortical Area. We previously showed that the neuron density and the ratio of neurons to nonneuronal cells (mainly glia cells) vary to a great extent across cortical areas in a nonuniform pattern (15) (Fig. 2). In all cases, there was a caudal-to-rostral decrease in the numbers of cells and neurons across the cortical sheet, with primary sensory areas having the highest neuron densities, which is consistent with previous reports of normal primates (Figs. 1 *A* and *A′*, and 2 *A* and *B*) (15, 16). In normal baboons, V1 was ∼25% more cell dense than S1 (U = 114.0, $P = 0.006$) and M1 (U = 262.0, $P = 0.108$), and almost twice as neuron dense as S1 (U = 68.0, $P = 0.0001$) and M1 (U = 56.0, $P = 0.0001$), which is evident in Fig. 2. M1 and S1 were similarly cell dense (U = 59.0, $P = 0.152$), whereas M1 was 19% less neuron dense than S1 (U = 54.0, $P = 0.093$). Epileptic baboons also demonstrated a caudal-to-rostral decrease in cell and neuron densities across the cortical sheet (Figs. 1 *B* and *B′*, and 2 *C* and *D*) despite the overall reduction in the number of neurons. In epileptic baboons, V1 was on average, ∼43% more cell dense than both S1 (U = 115.0, $P = 0.0001$) and M1 (U = 207.0, $P = 0.0001$). V1 was on average ∼3.75-times more neuron dense than S1 (U = 14.0, $P = 0.0001$), and ∼5.63-times more neuron dense than M1 (U = 0.0, $P = 0.0001$). Cell densities in S1 and M1 were similar (U = 160.0, $P = 0.813$). However, M1 was roughly half as neuron dense as S1 (U = 99.0, $P = 0.042$).

We found that cell (U = 1457.0, $P = 0.304$) and neuron (U = 1483.0, $P = 0.377$) densities in V1 were similar in normal and epileptic baboons. The average cell density in V1 in normal baboons was 27.2 million cells/cm^2 vs. 26.9 million cells/cm^2 in epileptic baboons; the average neuron densities were 19.9 million neurons/cm^2 and 18.5 million neurons/cm^2, respectively (Fig. 2). Reductions in cells and neurons were evident outside of V1, particularly in the frontal lobe. In the primary somatosensory area S1, the average cell density was 23% lower in epileptic baboons relative to normal baboons but was not statistically significant (U = 38.0, $P = 0.091$). There was a 51% drop in S1 neuron density in epileptic baboons (5.32 million neurons/cm^2 vs. 11.0 million neurons/cm^2) that was statistically significant (U = 13.0, $P = 0.001$). The primary area most affected by cell and neuron reduction was M1. There was a 35% loss in cell density in M1 of epileptic baboons (15.0 million cells/cm^2) relative to normal baboons (23.2 million cells/cm^2) (U = 38.0, $P = 0.0001$). There was a drastic reduction in neuron density within M1, with a 65% reduction in neuron density in epileptic baboons (3.21 million neurons/cm^2) compared with normal baboons (8.91 million neurons/cm^2) (U = 0.0, $P = 0.0001$).

Neuron Reductions Within M1 Are Not Uniform. We found that the most substantial neuron reduction within M1 in epileptic baboons was localized to the most lateral aspect of the primary motor cortex, which generally corresponds to the locations of hand- and face-movement representations. In each baboon case, we estimated the boundaries of M1 and the subregions of M1 for movement representations of body parts based on visible M1 boundaries, sulcal landmarks, and reference to previously published depictions of M1 subdivisions (16), and dissected these regions accordingly. Cell densities across movement representations within M1 were found to be consistent within individuals. The data in Fig. 3 shows the distribution of neuron densities within the estimated representations. In normal baboons, the hand representation was found to be more neuron dense than the upper limb (U = 0.000, $P = 0.050$) and trunk (U = 0.000, $P = 0.050$) movement representations located in the more medial aspect of M1. Neuron density of the hand was not found to be different from the lower limb (U = 1.000, $P = 0.077$) or face representations. Conversely, in epileptic baboons, the hand representation was less neuron dense than the trunk representation (U = 2.000, $P = 0.012$). Averaged for both normal baboons, the face representation was ∼35% higher in neuron density relative to the upper limb, trunk, and lower limb representations. The hand representation of normal baboons was 24% more neuron dense than the upper limb, trunk, and lower limb regions. Conversely, averaged neuron densities for both epileptic baboons showed that the face representation was 13% lower than neuron densities of the upper limb, trunk, and lower limb regions. The hand representation of epileptic baboons was 36% lower compared with the upper limb, trunk, and lower limb regions. These data suggest that lateral M1, which is typically the most neuron-dense region of M1 (16), is the region that is most affected by neuron loss in epileptic baboons.

Cell and Neuron Densities Within Representations of V1 and S1. We found that foveal representation areas were consistently higher in cell and neuron densities than peripheral representation areas

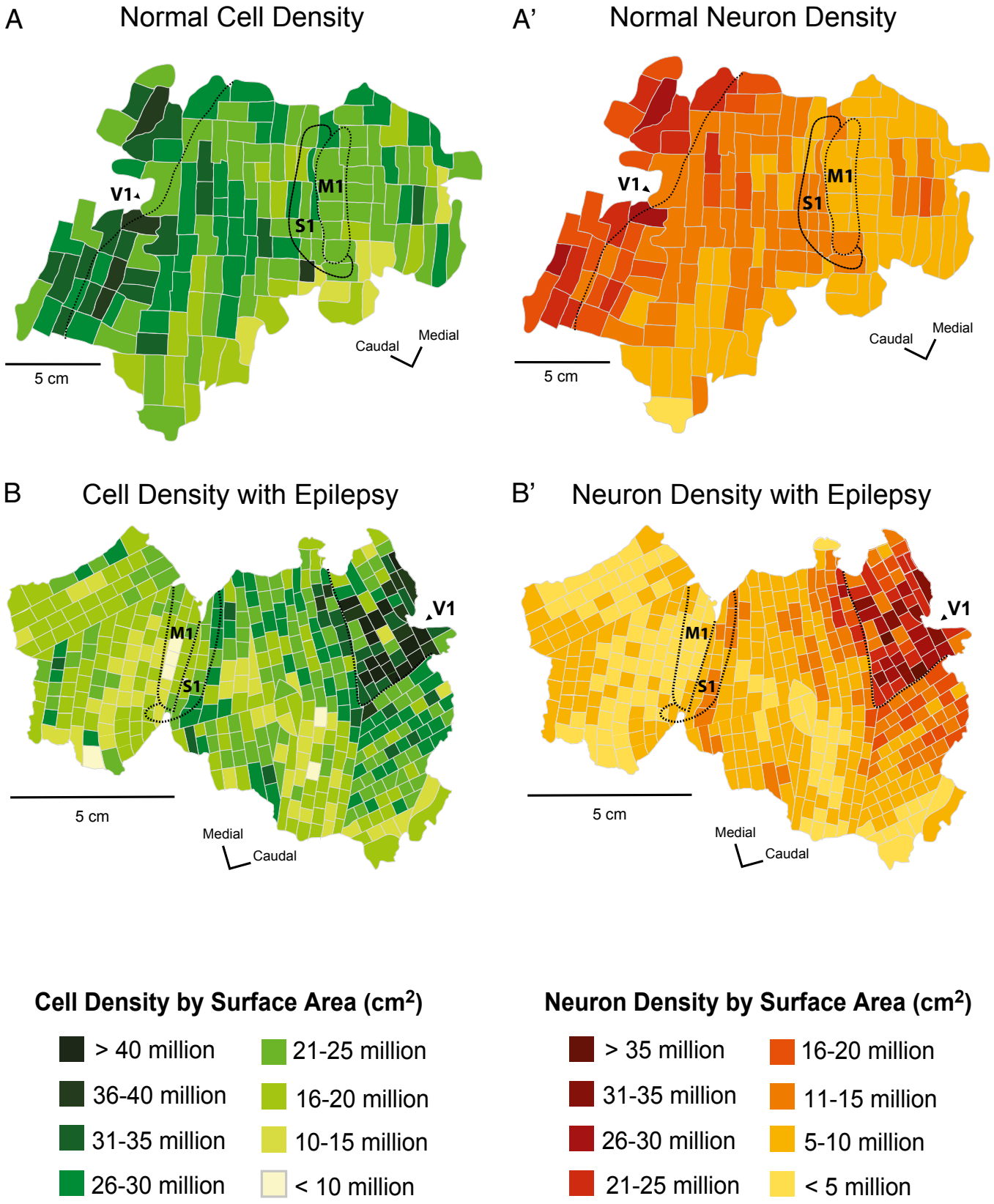

Fig. 1. Cell and neuron density maps from a normal baboon (case 09-27) and an epileptic baboon (case 11-45). The normal cell (*A*) and neuron (*A'*) distribution in baboons shows a general caudal-to-rostral decrease in cells and neurons across the cortical sheet, with the highest cell and neuron densities located within primary sensory areas, which is consistent with findings from other primates. Epileptic baboons have consistently lower cell (*B*) and neuron (*B'*) densities relative to control. Neuron reduction appears to be regional specific with the most neuron loss observed in cortex rostral to the central sulcus, including M1.

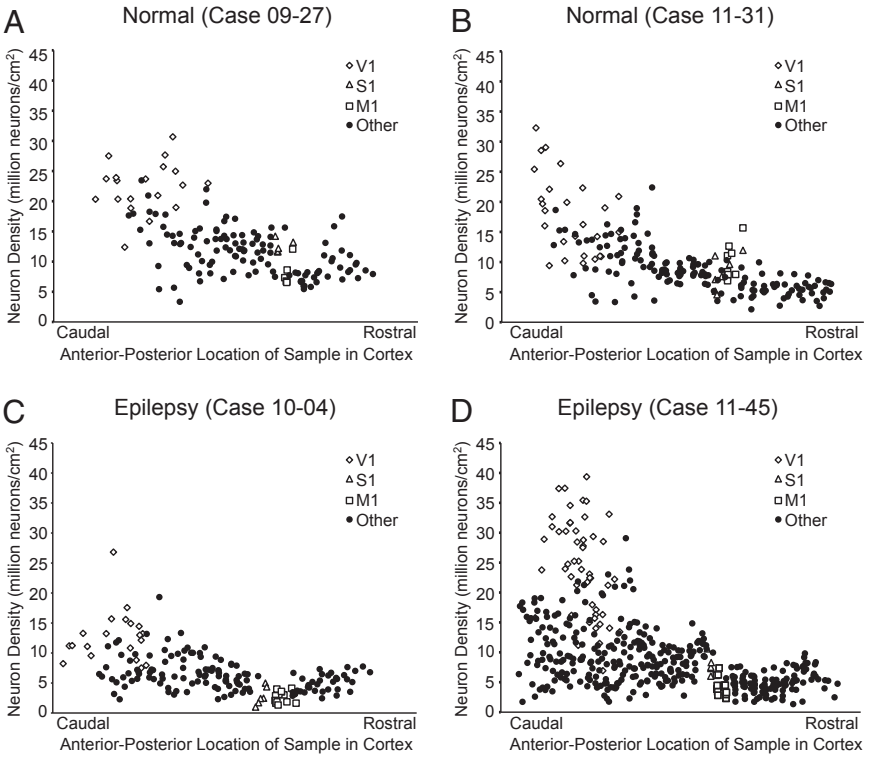

Fig. 2. Neuron density versus the anterior-posterior dimension was plotted for each case. All flattened hemispheres were dissected into tissue pieces, and each piece was assigned an anterior-posterior coordinate by generating centroid measures. Normal neuron distribution (*A* and *B*) is shown to follow the caudal-to-rostral decrease in cortical neuron density that is typical of primates. Epileptic baboons show a reduction of neurons within this distribution, particularly in cortex rostral to the central sulcus (*C* and *D*).

within V1, but that there was no overall difference related to epileptic or nonepileptic conditions. The average cell density in central vision areas in normal and epileptic baboons was ~32 million cells/cm^2. The average cell density for peripheral regions was 26.0 million cells/cm^2 in epileptic baboons, and 22.2 million cells/cm^2 in neurologically normal baboons. Foveal regions contained 22.9 million neurons/cm^2 (normal) and 21.8 million neurons/cm^2 (epileptic), and peripheral regions contained 17.9 million neurons/cm^2 (normal) and 15.5 million neurons/cm^2 (epileptic). No consistent internal variation according to somatotopic location was found within S1 in any case examined in this study.

Discussion

Using the flow fractionator method of counting cells and neurons (37, 38), we were able to demonstrate general and area-selective reductions in cortical neuron numbers and packing densities in the brains of epileptic compared with normal baboons. Although the numbers of cells and neurons were relatively equivalent in the primary visual cortex, they were dramatically reduced in the primary somatosensory and primary motor cortices. Differences in neuronal reduction were noted between functional regions of the motor cortex, with lateral M1 (subsuming face and hand functions) were more affected than medial M1 (proximal upper extremity, trunk, and lower extremities). This pattern of neuron reduction may have important implications in the etiology and pathophysiology of this natural model of genetic epilepsy.

Although epilepsy is associated with neuron loss and associated atrophy of cortical and subcortical structures, these effects have been described only following the prolonged and continuous seizure activity that characterizes status epilepticus (29). Neither of the two epileptic baboons in this study suffered from status epilepticus, and both appeared to have sporadic seizures. The baboon with the greatest neuronal reduction did experience more frequent seizures before euthanasia, but had no evidence of acute brain injury or infection to account for the seizure increase. Clinical reports (e.g., refs. 40 and 41) and behavioral studies (e.g., refs. 2 and 42) have reported impaired movement dexterity with epilepsy. There were no reports of motor impairment in either epileptic baboon, but it must be noted that motor dexterity was not explicitly studied. Behavioral studies are needed to carefully evaluate motor performance.

The reduction in neurons may account for the decrease in sulcal areas, particularly of the depths of cortical sulci in the epileptic baboons, most prominently revealed for the central, intraparietal, and cingulate sulci in MRI images (33). Although there was no decrease in cortical thickness, the reduction of cortex in cortical fissures suggests an overall reduction in cortical volume, consistent with the present evidence of neuron reduction in epileptic baboons. Decreased neuronal counts could be associated with decreased axonal and dendritic connections, particularly in U-fiber pathways that span the sulci. It is possible that neuron hypertrophy contributes to retention of some cortical thickness as microscopic examination of neocortex of temporal lobe epilepsy patients reported a 28% increase in neuron size with 3D unbiased stereology (43). Dendritic hypertrophy may also contribute to the retention of cortical thickness; however, it is dependent on cortical region and layer examined and the severity and the number of seizures (1, 43). These potential mechanisms can be further evaluated histologically and by diffusion weighted imaging.

Localized reduction in neuron numbers likely reflects regional differences in cortical microcircuitry. Although reduced numbers of neurons were found in M1 and S1, the number of neurons in V1 remained relatively constant. Laminar differences between V1 and M1 are readily apparent. In V1, a thick layer 4 contains densely packed, small neurons, and fewer pyramidal neurons. In

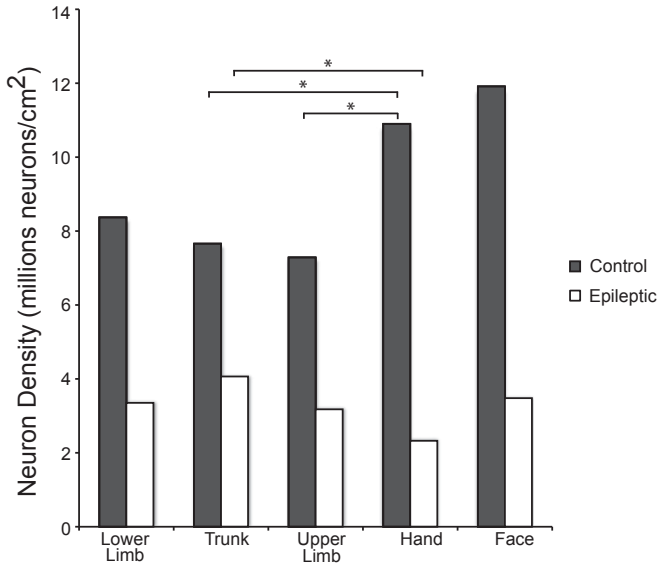

Fig. 3. Histograms of neuron density in M1. Movement representation boundaries within M1 were estimated and dissected as described by Young et al. (16), and neuron densities by surface area were plotted for each case. There is neuron reduction within M1 of epileptic baboons relative to normal baboons. Lateral M1, which contains the face and hand representations, was the most neuron dense M1 region in normal baboons. In epileptic baboons, there is a substantial reduction of neurons in M1 in both cases, with the hand movement representation being the least neuron dense region of motor cortex.

contrast, M1 is defined by large pyramidal neurons in layers 2/3 and layer 5 (e.g., refs. 44–47), and a nearly absent layer 4. There is regional variation in the structural complexity of cortical pyramidal neurons that have implications for plasticity. V1 pyramidal neurons have the simplest dendritic arbors and fewest synaptic spines (48), whereas those in the frontal cortex have the most complex dendritic arbors (49), in support of more complex patterns of connectivity necessary for plasticity (50). With repeated seizures, there are decreased hyperpolarization-activated currents of layer 5 pyramidal neurons (51), a persistent expansion of movement representations areas (52), a decrease in movement threshold (53) in layer 5 of the motor cortex, and an increase in the polysynaptic component of the callosal-neocortical evoked potential (52), reflecting a source in superficial layer 5 and a sink in deep layer 5 (54). There are also a greater number of efficacious excitatory synapses (2) and alterations in hypertrophy of basilar dendrites (1), both in layer 5, which probably support the generation and maintenance of epileptic networks. The basal dendritic territories of pyramidal neurons of layer 5 in the somatosensory cortex also contain the largest number of parvalbumin-immunoreactive cell bodies (55), an inhibitory population of interneurons important for seizure propagation control (56), which is reduced in the cortex of epileptic patients (e.g., ref. 57) and the motor cortex of rats with seizures (58). In epileptic baboons, M1 and S1 maximally express interictal epileptic discharges, both spontaneous and elicited by intermittent light stimulation (e.g., ref. 36). Myoclonic seizures, which are the most common seizure types in the baboon, mainly affect the face and upper extremities (59), the somatopic regions most affected by neuron reduction. Motor cortex hyperexcitability in juvenile myoclonic epilepsy has been linked to impaired functioning of intracortical inhibition in transcranial magnetic stimulation studies (60). Further study of the intact cortical architecture of M1 is needed to determine what specific cell types are affected and their laminar distribution.

Materials and Methods

All brains were flushed with 0.9% phosphate buffer saline (PBS) and shipped overnight in the same solution. Upon arrival, one hemisphere from each was manually flattened. Readily identifiable cortical areas were dissected from the cortical sheet with a scalpel, including the primary visual cortex (V1), primary sensory cortex (S1), and primary motor cortex (M1). The locations of dissection cuts were drawn onto a high-resolution photograph of the cortex. The surface area was measured for each piece using Image J software (National Institutes of Health). This software was also used to assign an anterior-posterior coordinate by generating a centroid measure for the location of each tissue piece. Each piece was processed for cell and neuron density using the flow fractionator method for cell and neuron counting (37, 38). The overall number of cells and neurons were compared for epileptic and neurologically normal conditions using a Mann–Whitney test. Comparisons were also made between V1, S1, and M1 between the two conditions using the Mann–Whitney test. Statistical significance was set at $P < 0.05$, and was analyzed using SPSS.

Additional methodological details are given in *SI Materials and Methods* (including Figs. S1 and S2).

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