chondria reverts to a more elementary state with hypoxic exposure. Examples of the former process have been described in fetal or postnatal mitochondria. For example, there are numerous reports of a perinatal increase in the specific activities of many Krebs cycle and electron chain components (*12*). These changes are usually attributed to mitochondrial proliferation, with increases in inner membrane and enzyme synthesis (*13*).

The possibility of promitochondria is an old suggestion that arose from studies on yeast, which reversibly lose well-developed mitochondria and cytochromes aa_3, b, c, and c_1 (*14*) during anaerobic cultivation. Heyman-Blanchet *et al.* (*15*) reported the isolation of mitochondria-like particles from anaerobic yeast. Wallace and Linnane (*16*) reported that reexposure to O_2 in yeast was associated with numerous electron-transparent vesicles that finally evolved into mitochondria. This finding was supported by some studies and refuted by others.

Even in yeast the possibility of a promitochondrion is not in the main stream of mitochondrial studies. It has not to our knowledge been at all considered in mammalian systems.

Our data do not permit the resolution of the mechanisms of the changes that have been observed, but it is clear that the present findings dictate some modification of current views concerning the basic regulation of mitochondrial composition. The results are inconsistent with individual regulation of mitochondrial proteins and are inconsistent with regulation of mitochondrial number by a simple balance between rates of mitochondrial replication and degradation.

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Intracellular Study of Human Epileptic Cortex: In vitro Maintenance of Epileptiform Activity?

Abstract. *Intracellular recordings were obtained in the in vitro slice preparation from neurons of lateral and mesial temporal cortex removed from human epileptics suffering from intractable temporal lobe seizures. Spontaneous rhythmic synaptic events, which were capable of triggering action potential discharge, were observed in many neurons, particularly in mesial tissue slices. Such activity may reflect the epileptogenic capacity of this human cortex.*

The difficulties involved in investigating basic mechanisms underlying epileptic activity include the ethical and technical problems of obtaining intracellular electrophysiological data from human epileptic brain. Although intracellular data can be collected from animal models (*1*), the questionable relevance of any one model to human epilepsy makes animal results difficult to interpret. Extracellular recordings have been obtained from the human epileptic brain (*2*), but these data cannot be used to reveal underlying mechanisms of abnormal discharge. Development of the in vitro slice preparation (*3*) has made it possible to use intracellular techniques to study human cortical tissue excised

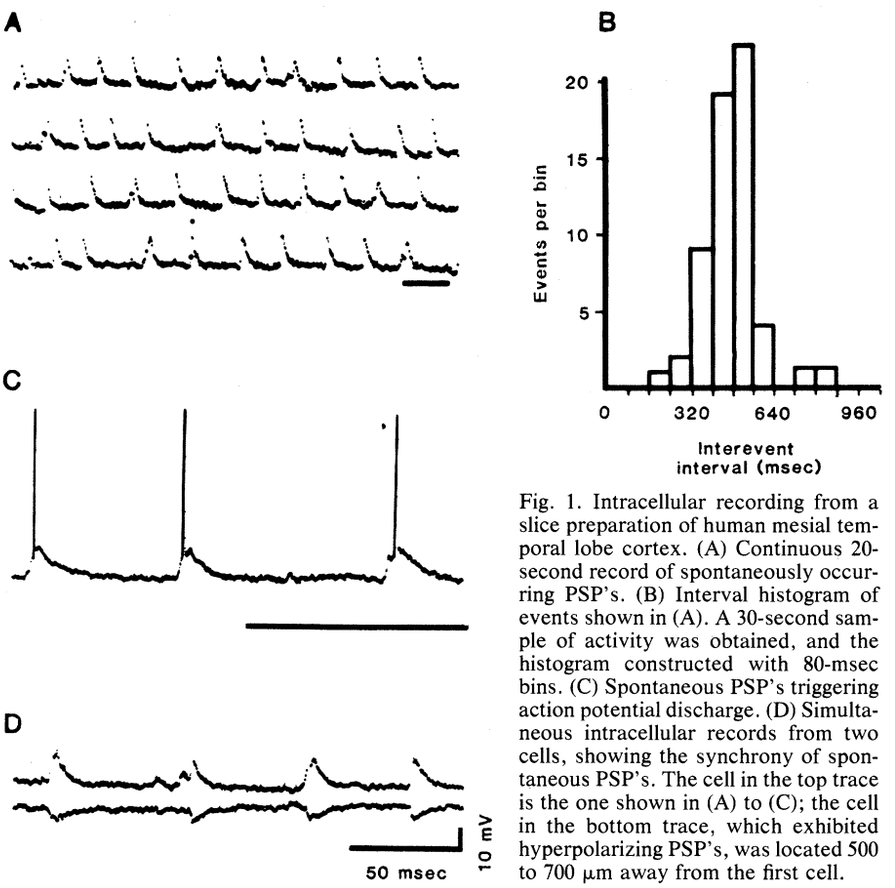

Fig. 1. Intracellular recording from a slice preparation of human mesial temporal lobe cortex. (A) Continuous 20-second record of spontaneously occurring PSP's. (B) Interval histogram of events shown in (A). A 30-second sample of activity was obtained, and the histogram constructed with 80-msec bins. (C) Spontaneous PSP's triggering action potential discharge. (D) Simultaneous intracellular records from two cells, showing the synchrony of spontaneous PSP's. The cell in the top trace is the one shown in (A) to (C); the cell in the bottom trace, which exhibited hyperpolarizing PSP's, was located 500 to 700 μm away from the first cell.

Table 1. Clinical and experimental findings.

Patient	Electroencephalogram and electrocorticogram	Computed tomogram	Pathology
1	Basal and anterolateral temporal sharp waves (suspect mesial focus)	Mesial temporal atrophy	Lateral: mild diffuse gliosis of white matter Mesial: focal gliosis of cerebral white matter and hippocampus
2	Diffuse lateral and mesial spiking (suspect mesial focus)	Mesial temporal herniation and atrophy	Lateral: normal Mesial: dense gliosis of hippocampus
3	Anterolateral temporal and mesial temporal spiking (suspect mesial focus)	Mesial herniation	Lateral: diffuse gliosis Mesial: moderate to focally dense and marked gliosis of the hippocampus
4	Inferior temporal gyrus spiking		Deep inferior temporal neuroglial hamartoma
5	Diffuse lateral and mesial spiking (greater mesial)		Diffuse white matter gliosis
6	Predominant mesial temporal spiking	Mesial temporal atrophy	Lateral: normal Mesial: hippocampal sclerosis

during surgery for intractable seizures (*4, 5*). These in vitro studies have been discouraging, however, inasmuch as the excised tissue did not seem to retain appreciable epileptiform characteristics (*6*). We now report findings obtained in in vitro studies of human epileptic cortex that suggest that human cortical epileptogenesis, as reflected by synchronous cellular events, can be studied in vitro. These results also implicate mesial temporal structures, rather than lateral temporal cortex, as the prevalent sites of epileptogenesis.

Human cortical tissue was studied from six patients in which intractable temporal lobe epilepsy required resection of the anterior temporal lobe (*7*). Tissue samples (ranging in size from about 0.5 to 3 cm^3) from both lateral temporal cortex and mesial temporal structures (which included the hippocampus in three patients) were removed from regions that showed active electroencephalographic spiking during corticography (Table 1). The tissue was put immediately into cold bathing medium and was trimmed and sliced (750 μm thick) within 2 minutes of removal. These slices were transferred to slice incubation chambers and maintained and studied as previously described for both human and animal slices (*6, 8*). Slices from lateral and mesial samples were studied for five of six patients; slices from each location were studied independently in two electrophysiology laboratories.

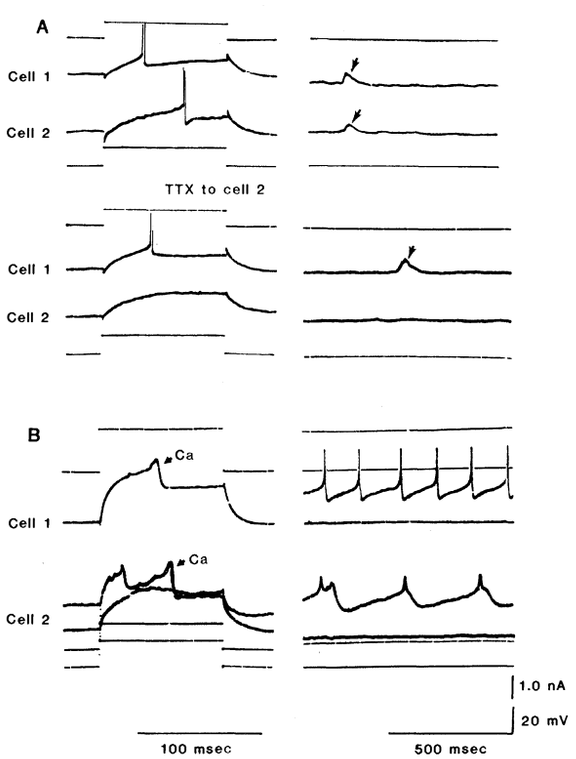

Fig. 2. Effects of TTX on spontaneous PSP's and action potentials in two hippocampal neurons recorded simultaneously. (A) Under normal conditions, depolarizing current pulses elicited narrow sodium spikes in these cells; spontaneous synchronously occurring PSP's were also observed (unlabeled arrows). A droplet containing $10^{-4}M$ TTX was applied near cell 2; spiking and spontaneous PSP's were blocked in cell 2 but not initially in cell 1. (B) After TTX had spread to cell 1, normal sodium spikes were blocked in cell 1 as well as in cell 2. Higher intensity depolarizing current pulses evoked long-lasting all-or-none spikes in both neurons. Tonic membrane depolarization also produced trains of these presumed calcium spikes.

Cellular activity in both lateral and mesial tissue samples was apparent almost immediately in most slices. No spontaneous field potential events were observed, but cells exhibited resting action potential discharge. Intracellular penetrations, obtained in all tissue samples, showed a background of spontaneous synaptic input (unitary synaptic events) in many neurons. Stimulation in white matter underlying the site of cell penetration resulted in evoked synaptic drive which consisted of both excitatory and inhibitory postsynaptic potentials (PSP's). In lateral cortical neurons, average resting potential was -58.6 ± 4.9 mV ($N = 26$), input resistance was 34.6 ± 13.4 megohms ($N = 22$), and action potential amplitude was 68.7 ± 11.0 mV ($N = 27$). In mesial tissue slices, comparable values were -55.2 ± 12.3 mV ($N = 62$), 38.2 ± 13.5 megohms ($N = 46$), and 67.4 ± 10.3 mV ($N = 66$). Manipulation of cell resting potential by current injection resulted in a determination of inhibitory PSP reversal potential averaging about -59 mV.

Although these baseline measures did not differ significantly in lateral and mesial cortical neurons, one outstanding feature did appear differentially. Spontaneous events occurred in a large proportion of mesial neurons (64 of 83 neurons as opposed to 8 of 32 neurons in lateral tissue). These events occurred rhythmically (Fig. 1A), with an average interevent interval of about 500 msec (Fig. 1B). They were considered to be PSP's because: (i) Current injections to manipulate membrane potential led to appropriate changes of event amplitude, rever-

Table 1 (continued).

Slice sample location	Occurrence of spontaneous events	Surgical result
Mesial: temporal cortex (exact site unknown)	Mesial: most cells	Significant reduction in seizure frequency
Lateral: temporal neocortex Mesial: hippocampus and adjacent cortex	Lateral: none Mesial: most cells	Eight months seizure-free, then seizures return; seizure-free after subsequent callosal section
Lateral: anterior temporal neocortex Mesial: temporal cortex (exact site unknown)	Lateral: about half the cells Mesial: about half the cells	Significant reduction in seizure frequency
Lateral: inferior temporal gyrus Mesial: hippocampus and adjacent cortex	Lateral: none Mesial: none	Seizure-free
Lateral: anterolateral temporal neocortex Mesial: anterior cortex (exact site unknown)	Lateral: none Mesial: about half the cells	Significant reduction in seizure frequency
Lateral: anterior temporal neocortex Mesial: hippocampus and adjacent cortex	Lateral: none Mesial: about one-quarter of cells	Seizure-free

sal of event polarity, or both. These changes were the same as seen during current manipulations of evoked activity resulting from stimulation of orthdromic input; (ii) current manipulation of potential waveform did not affect the rate of occurrence of these events, indicating that these events could not be due to voltage-dependent, intrinsic mechanisms; (iii) these events were blocked by local tetrodotoxin (TTX) applications to cell dendrites (microdrop, $10^{-4}M$) (Fig. 2A). The PSP's were extremely complicated, often with both depolarizing and hyperpolarizing components. Similar "average" reversal potentials were found for spontaneous and evoked PSP's, but no single reversal potential could be determined for spontaneous events, since different components of the PSP complex reversed at different membrane potential levels (Fig. 3). When cell resting potential was near threshold, these spontaneous events could trigger action potential discharge in some neurons (Fig. 1C); the event was primarily hyperpolarizing in other neurons (lower trace in Fig. 1D). Repetitive stimulation at rates of 0.2 to 10 Hz "recruited" the occurrence of spontaneous events; the spontaneous rhythm resumed, however, promptly after the stimulus train.

In the tissue from mesial temporal lobe in which these spontaneous rhythmic PSP's were recorded, simultaneous intracellular recordings from two independent electrodes showed that the spontaneous rhythmic PSP's were occurring approximately synchronously over a wide area (millimeters) of a given slice (Figs. 1D and 3). The waveform of the event in the two recordings could differ appreciably; when electrodes were widespread (> 200 μm apart), the synchrony typical of closely recorded neurons was lost, but a consistent phase relationship was maintained. There was no field potential reflection of these cellular events, perhaps because of the inexact synchrony and variable waveforms among neurons. In cell pairs showing approximately synchronous activity, tests for direct synaptic connections (chemical or electrotonic) failed to reveal coupling. This result suggested that each cell of the pair was receiving synaptic input from another, common source.

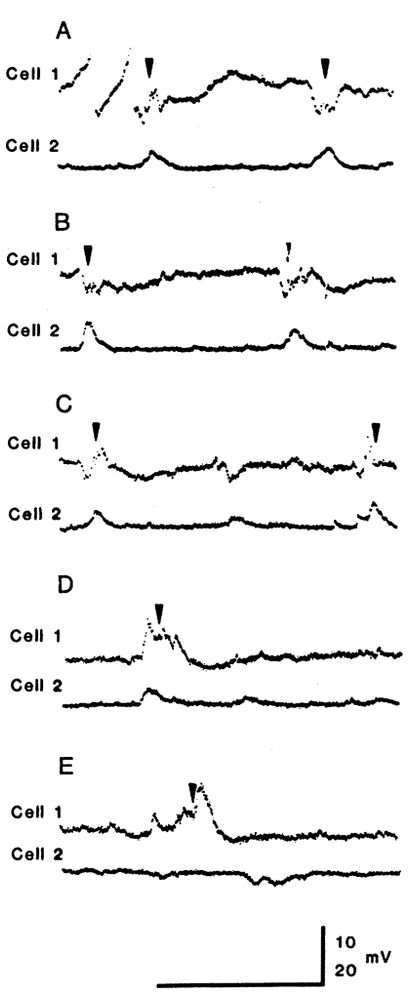

Fig. 3. Simultaneous recording from two neurons while manipulating membrane potential of one of them (cell 1 of each pair) with intracellularly injected current. (A) to (C) Depolarization of cell 1. (D) Resting potential (−51 mV for cell 1). (E) Hyperpolarization of cell 1. Voltage gain for cell 2 is half that for cell 1; cell 2 membrane potential was not manipulated, but marked the occurrence of spontaneous PSP's (arrowheads). Hyperpolarization of cell 1 (to −57 mV) increased PSP amplitude (E). Depolarization (to −48 mV) first inverted only one component of the PSP (C); further depolarization [to −39 mV (B) and to −36 mV (A)] turned over the entire PSP, although multiple components were still obvious.

Intracellular recordings from lateral temporal cortex also occasionally showed evidence of similar spontaneous rhythmic postsynaptic activity occurring synchronously through the slice. However, the proportion of lateral slices exhibiting this activity (25 percent) was much lower than that of mesial slices (77 percent), and the number of involved cells within each slice was smaller. Such rhythmic spontaneous activity had not been seen in a previous study of lateral cortex (*6*) when slices were cut only 500 μm thick. These results indicate that, contrary to our conclusions based on the study of 500-μm thick lateral cortex slices (*6*), epileptiform activity may be maintained in excised slices of cortical tissue. This activity takes the form of spontaneous, rhythmic, synchronized postsynaptic activity, and it is most often seen in mesial temporal lobe tissues. The mesial locus of this activity is consistent with recent clinical findings regarding the most likely "site" of temporal lobe

foci (9). If most temporal lobe foci can be localized to mesial structures, the interpretation of electrocorticographic results must be carefully evaluated; laterally placed electrodes may show vigorous epileptic spiking that is primarily a result of projected activity.

The actual mechanism underlying the spontaneous events—how this rhythmic activity is initiated and why it is maintained—remains to be elucidated. The cells display a high threshold calcium conductance and may even generate calcium spikes (Fig. 2B); some cells may be capable of generating intrinsic paroxysmal depolarizing shift discharge such as described by Prince and Wong (5). However, most cells display only synaptic events which result from a mixture of excitatory and inhibitory input. The importance of maintaining an intact minimal circuitry for generating these rhythmic events is underscored by the absence of such activity in thin (500-μm) slices. Thicker slices preserve more circuitry, which consequently may give rise to spontaneous rhythmic postsynaptic potentials. The pacemaking mechanism for these events is unclear, but the synaptic drive certainly seems capable of initiating the cell burst firing patterns previously found in in vivo extracellular recordings from human epileptic foci (2).

Interpretation of the activity seen in our slice studies as "epileptic" must, of course, be conditional, since normal tissue from mesial temporal cortex was not available for study as a control. It is possible that cells in normal mesial tissue produce spontaneous rhythmic activity. Nevertheless, the dissociation of mesial and lateral temporal regions with respect to this activity suggests that the spontaneous activity is not simply a normal discharge pattern. Further, the likelihood of observing these potentials seems loosely correlated with the abnormality of the tissue (Table 1). Thus, these spontaneous, rhythmic PSP's may reflect an underlying pathology of epileptic tissue.

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Ribose Intervention in the Cardiac Pentose Phosphate Pathway Is Not Species-Specific

Abstract. *Ribose is cardioprotective in the rat in a variety of pathophysiological conditions. The metabolic basis for this effect is the low capacity of the oxidative pentose phosphate pathway in the myocardium. Ribose bypasses this pathway, elevates the available pool of 5-phosphoribosyl-1-pyrophosphate, and thus stimulates the biosynthesis of adenine nucleotides. In the study reported here the activity of glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the oxidative pentose phosphate shunt, was very low in the human heart and was of the same order of magnitude in the myocardium of various animal species. Furthermore, ribose had a similar stimulating effect on myocardial adenine nucleotide biosynthesis in the guinea pig, in which hemodynamic parameters are different from those in the rat. It is concluded that the metabolic basis for the effectiveness of ribose is similar in all species investigated.*

A special metabolic feature of the myocardium is the low capacity of the pentose phosphate pathway (1). In the oxidative branch of this shunt ribose-5-phosphate is produced that is converted into 5-phosphoribosyl-1-pyrophosphate, an essential substrate for the synthesis of purine and pyrimidine nucleotides. As a consequence, the rates of these synthetic processes are very low in the myocardium (2). In searching for the rate-limiting step, Eggleston and Krebs (3) found that glucose-6-phosphate dehydrogenase (G6PDH), the first enzyme of the oxidative pentose phosphate pathway, exerts a tight control in the liver. In the myocardium the activity of this enzyme is lower than in the liver and in most other organs (4), and the available pool of 5-phosphoribosyl-1-pyrophosphate is smaller than in the liver and kidney (5). Hence, the rate of adenine nucleotide biosynthesis is minute in the heart compared with other organs (2).

The limited pool of 5-phosphoribosyl-1-pyrophosphate and the low rate of adenine nucleotide biosynthesis can be overcome by ribose in the heart, both in the control state (5) and in various pathophysiological conditions. Ribose enhances cardiac adenine nucleotide biosynthesis in catecholamine-treated rats (6) and in animals recovering from oxygen deficiency (7) and experimental

Table 1. Activities of the first two enzymes of the oxidative pentose phosphate pathway in the myocardium of different species. Heart tissue was homogenized in ice-cold 0.15*M* KCl containing 8 ml of 0.02*M* $KHCO_3$ per liter. Centrifugation, dialysis of the supernatant, and measurements of enzyme activities at 25°C were done in accordance with the methods of Glock and McLean (4), and protein concentration in the dialyzate was determined with the biuret reaction. The human papillary muscles, which were kept in the ice-cold solution, were homogenized within 2 hours after the samples were obtained during cardiac surgery. When rat hearts were maintained in the same solution for this period of time after excision, enzyme activities were not altered. Values are means ± standard errors.

Species	*N**	Enzyme (units per gram of protein)		*P*†
		G6PDH	6-Phosphogluconate dehydrogenase	
Guinea pig	8	6.0 ± 0.46	11.6 ± 0.36	< 0.0005
Rat	28	4.3 ± 0.15	11.2 ± 0.25	< 0.0005
Rabbit	4	3.4 ± 0.77	12.2 ± 1.21	< 0.0005
Dog	6	1.5 ± 0.19	8.8 ± 0.43	< 0.0005
Calf	4	1.6 ± 0.35	9.3 ± 0.50	< 0.0005
Monkey	5	2.4 ± 0.59	5.0 ± 0.56	< 0.0250
Human	16	3.4 ± 0.15	5.9 ± 0.27	< 0.0005

*Number of hearts or heart samples. †Determined with Student's *t*-test for unpaired data.