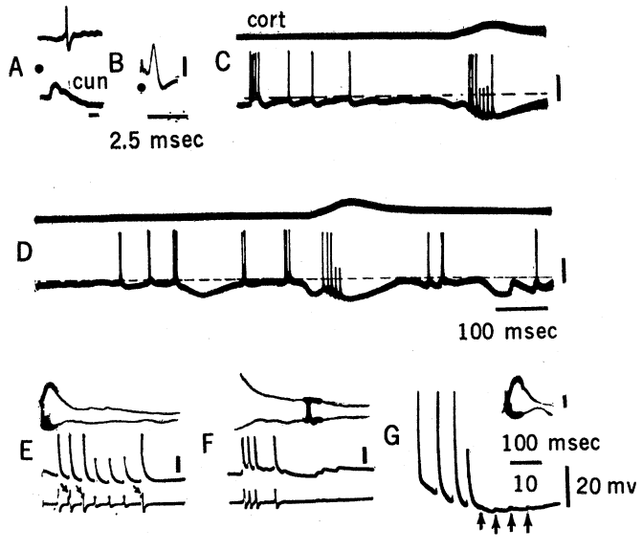
Thalamocortical Relay Neurons: Antidromic Invasion of Spikes from a Cortical Epileptogenic Focus

Abstract. *Thalamocortical relay neurons whose axons project into a penicillin-induced cortical epileptogenic focus generate bursts of action potentials during spontaneous interictal epileptiform discharges. These bursts originate in intracortical axons and propagate antidromically into thalamic neurons. Repetitive spike generation in cortical axons and presynaptic terminals could produce a potent excitatory drive and contribute to the generation of the large depolarization shifts which are seen in cortical elements during focal epileptogenesis.*

Penicillin is a widely studied epileptogenic agent; however, the mechanisms which underlie its convulsant action remain obscure. Cortical intracellular recordings have revealed only one consistent abnormality in neurons within the epileptogenic focus. This is a depolarization shift, best described as a "giant" excitatory postsynaptic potential (EPSP) (*1*), which occurs during the electrocorticographically recorded surface interictal discharge. One possible mechanism for generation of excessive postsynaptic depolarizations is an abnormality in intracortical axons or presynaptic terminals which would cause them to fire repetitively during penicillin epileptogenesis (*2*). Such effects have been demonstrated with a variety of other drugs in different invertebrate and vertebrate preparations (*3*). We now report that penicillin produces similar presynaptic effects when applied to cat cortex.

Reasoning that action potentials spontaneously generated in an axon or terminal exposed to penicillin would be detectable as antidromic spikes in the parent cell body, we recorded intracellularly in the ventrobasal thalamic nuclei of cats, from neurons whose axons project into a penicillin-induced cortical epileptogenic focus. Animals were lightly anesthetized with pentobarbital. Standard techniques were used for stimulation and recording and for the creation of an epileptogenic focus in the forepaw area of the somatosensory cortex (*4*). The procedures employed for thalamic intracellular recording have been described by others (*5*).

Cells selected for study were identified as thalamocortical relay (TCR) neurons on the basis of short, fixed latency synaptic activation following contralateral peripheral (forepaw) stimulation (Figs. 1A and 2A) and antidromic invasion following ipsilateral cortical stimulation (Figs. 1B and 2B). Bursts of spikes were recorded extracellularly in 26 TCR cells coincident with the spontaneous surface epileptiform discharge. Stable intracellular recordings during these bursts were obtained from five neurons in four preparations. Figure 1, C and D, and Fig. 2C show typical intracellular recordings from two such identified TCR neurons during focal epileptogenesis. In these cells spontaneous action potentials arose from depolarizing prepotentials at a constant firing level, as indicated by the dotted lines. With each cortical epileptiform transient, an inhibitory postsynaptic potential (IPSP) lasting



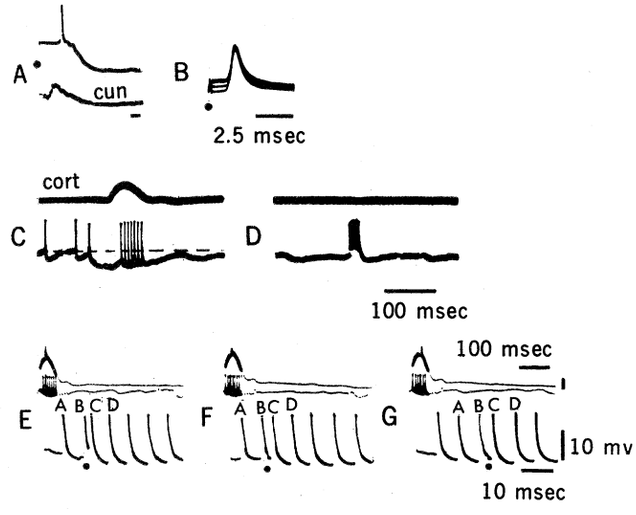


Fig. 1 (left). Recordings from a TCR cell. (A) Extracellular recording of response to electrical stimulation of contralateral forepaw (at dot). Lower trace: monitor of the afferent volley in cuneate nucleus. (B) Response to direct stimulation of forepaw area of sensorimotor cortex (at dot). (C, D) Spontaneous activity in same cell after impalement by microelectrode. Dotted line indicates spontaneous firing level. Upper trace from ipsilateral cortex. Note burst of spikes, independent of firing level and without prepotentials, which is generated during IPSP associated with cortical interictal discharge. (E, F) Upper two traces as in C and D at slow sweep speed. Third trace is expanded sweep of cellular activity during intensified portion of upper traces. Bottom trace is electrically differentiated to show fractionation of spikes into IS and SD components (arrows). (G) Upper two traces as in C and D at slow sweep speed. Bottom trace: expanded sweep of cellular activity during intensified portion of upper traces. Microelectrode gain increased to show small potentials (arrows) which are unmasked when SD and IS spikes are blocked at the peak of IPSP negativity. Polarity: cortical and cuneate traces, positivity down; microelectrode traces, positivity up. Time calibration in G is for E, F, and G (100 msec for upper traces, 10 msec for others); in D, for C and D. Amplitude calibration in B is for A and B, 5 mv. All other amplitude calibrations are 20 mv. Fig. 2 (right). Recordings from TCR cell. Parts A, B, C, and D are as in Fig. 1, A, B, C, and D, respectively. Note in D that injury-related high-frequency discharge is associated with a depolarizing slow potential and is not similar to epilepsy-related burst in C. Parts E, F, and G are as in Fig. 1G. Dots indicate stimulation of ipsilateral cortex evoking antidromic spike *C* during course of epilepsy-related burst. Polarities and time calibrations are as in Fig. 1. All amplitude calibrations, 10 mv.

100 to 200 msec was generated which usually had a brief EPSP superimposed early in its course (Fig. 1, C and D, and Fig. 2C). During these IPSP's, bursts of spikes arose without prepotentials from a baseline of membrane hyperpolarization which was up to 15 mv negative to the usual firing level, and they reached levels of depolarization close to those of spontaneous spikes (Fig. 1, C and D, and Fig. 2C).

We considered the possibility that these potentials were associated with injury to the neuronal membrane. However, this seems unlikely since they only occurred during surface epileptiform events and in neurons that had stable resting potentials. Even in neurons that showed signs of depolarization, spikes associated with cortical epileptogenesis (Fig. 2C) did not resemble high-frequency bursts of injury discharges (Fig. 2D).

It is therefore evident that these impulses actively invaded the soma from a distant zone of spike initiation. Three sites remote from the initial segment trigger area might be considered: the dendrites of the TCR cell, the thalamic terminals of TCR cell axon collaterals, and the cortical endings of TCR cell axons.

Maekawa and Purpura (*6*) have reported that spikes may be generated in the dendrites of ventrobasal thalamic neurons. However, these are revealed in intrasomatic recordings as fast prepotentials which may be seen in isolation or may trigger full-sized spikes, indicating that they are electrotonically propagated to the cell body. The spikes that we observed were not associated with such rapid depolarizing prepotentials. Moreover, rhythmic bursts of action potentials invading the soma from a dendritic spike generator have not been reported. It is therefore unlikely that these bursts are of dendritic origin. On the other hand, several lines of evidence indicate that they invade the soma antidromically from the intracortical endings of thalamic fibers.

Spikes during the bursts were identical in amplitude and duration to cortically evoked antidromic potentials in the same cell (for example, Fig. 2, E–G). Figure 1, C and D, shows that during the IPSP negativity, these spikes were often fractionated into components in a manner similar to the IS-SD spikes of motoneurons (*7*). Electrically differentiated records in Fig. 1E show that a progressive delay in the generation of the large (SD) spike component developed as membrane polarization increased until the SD spike failed and the isolated IS spikes remained. Such fractionation of spikes, dependent upon the level of soma polarization, has been described by Andersen *et al.* (*8*) for cortically evoked antidromic spikes in TCR neurons. Figure 1F shows that spontaneous spikes in the same cell did not show this double configuration even though they fired at high frequency.

In the cell illustrated in Fig. 1, both IS and SD components were often blocked at the peak of the IPSP. In these instances, low-amplitude potentials occurred at the same frequency as the preceding spikes in the burst (Fig. 1G, arrows). These potentials resembled the M spikes of motoneurons, which represent the antidromic invasion of the medullated axon (*9*).

By cortically eliciting antidromic spikes during the course of spontaneous bursts, we obtained data which indicate that the spike bursts originated in the intracortical axons of TCR cells. Interspike intervals during spontaneous bursts were quite regular (for example, Figs. 1E and 2C). In the cell illustrated in Fig. 2, they measure from 6.5 to 8 msec in ten bursts. Figure 2, E–G, shows that by evoking an antidromic action potential during the course of a burst, we were able to reset the rhythm of spike firing. Impulses that were produced by direct stimulation of the cortex during interictal discharge are labeled *C*. Interposition of this spike led to a delay in the generation of the next impulse, such that the interval between spike *A* and spike *B* was approximately equivalent to the interval between spike *C* and spike *D*. This is best seen in Fig. 2G, where interval *B-D* is lengthened to 12.5 msec and intervals *A-B* and *C-D* are approximately 7.2 msec. In order to thus alter the interspike intervals, the cortically evoked antidromic potential (which was identical to the other burst spikes) must have invaded to the site of burst initiation.

In Fig. 2E, the interval between the spontaneous burst spike *B* and the evoked antidromic spike *C* was 2 msec. Since the antidromic conduction time for this neuron was 1.3 msec (Fig. 2B), spike *C* was initiated 0.7 msec after the initiation of spike *B*. Thus, it would not have been possible for spike *B* to originate in the thalamic cell body or its dendrites and spike *C* in the cortex without occlusion occurring in the axon, in which case only spike *B* would have been recorded by the intrasomatic electrode (*10*). The same argument would rule out the thalamic terminals of TCR cell axon collaterals as sites of burst initiation. Golgi studies have shown that these collaterals branch from the main axon at a point close to the TCR cell body (*11*), and it is unlikely that the conduction time from their terminals to the cortex is shorter than the antidromic latency to the soma.

These data lead us to conclude that thalamic fibers or terminals in the penicillin-induced cortical epileptogenic focus are altered in some way so that during surface epileptiform activity they generate bursts of action potentials which propagate antidromically into cell bodies in the ventrobasal nuclei. We can only speculate regarding the mechanisms by which the burst discharges are initiated. Since axon-axonal synapses have not been described in sensorimotor cortex (*12*), it is unlikely that these spikes are generated by synaptic depolarization of presynaptic terminals. Several nonsynaptic mechanisms could account for this phenomenon, such as "ephaptic" depolarization produced by large field potentials (*13*) or the effects of the large increase in extracellular potassium concentration which probably occurs within the focus during interictal discharge (*14*).

Whatever the mechanism of their generation, these impulses could also propagate orthodromically to invade presynaptic terminals. This would produce a prolonged and potent release of transmitter onto the cells of the focus, contributing to the generation of depolarization shifts in cortical neurons. Antidromic invasion into afferent pathways and their collaterals might produce widespread disturbances of cortical and subcortical function as a result of focal interictal discharge (*15*).

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15. Since preparation of this manuscript an abstract has appeared which reports the occurrence of extracellularly recorded bursts of action potentials in lateral geniculate neurons during interictal activity in a penicillin-induced epileptogenic focus in visual cortex. The authors of the abstract suggest that these spikes are antidromic [A. D. Rosen and E. F. Vastola, *Trans. Amer. Neurol. Ass.* **96**, 297 (1971)].
16. This investigation was supported by PHS grant NS 06477 to D.A.P. and PHS postdoctoral fellowship No. 42766 to M.J.G. We thank D. Kennedy and P. Schwartzkroin for helpful suggestions and comments on the manuscript and Pamela Vario for secretarial assistance.

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Deficiency of α-L-Fucosidase

Abstract. *A new form of α-L-fucosidase deficiency has been found in a 20-year-old severely retarded male. Additional signs include angiokeratoma corporis diffusum and anhydrosis. The skin lesion is due to an accumulation of residual bodies, presumably containing oligosaccharides and glycoproteins, in endothelial cells and fibrocytes. The enzyme activity in blood relatives indicates that the disease is inherited as a simple autosomal recessive trait that segregates according to Mendelian principles. Because the enzyme activity in the heterozygotes was consistently below that of normal controls, the carriers of the trait in this family could be ascertained.*

A significant decrease in, or absence of, activity of a lysosomal hydrolase in all tissues of an individual is the biochemical hallmark of lysosomal diseases. In some of these diseases, the predominant or exclusive substrate for the defective enzyme accumulates selectively, such as in acid maltase deficiency (Pompe's disease) (*1*) and in many of the sphingolipidoses (*2*). In other lysosomal diseases, for example the mucopolysaccharidoses, the defective enzyme normally hydrolyzes several biological substrates, and therefore different nondegradable biochemicals accumulate in residual bodies. The α-L-fucosidase deficiency presumably belongs in the latter category. Four children with this biochemical defect have been described: two with the clinical picture of Hurler's syndrome, and two with symptoms that were less distinct (*3*, *4*). All four suffered from severe mental and physical retardation, and a hyperhidrosis.

We now report on a patient with a deficiency of α-L-fucosidase, who also suffers from severe mental and physical retardation. However, although our patient is afflicted with angiokeratoma corporis diffusum, a skin lesion characteristic of Fabry's disease, the α-galactosidase activity in our patient is normal whereas it is below normal in Fabry's disease (*5*).

The patient, a 20-year-old white male, was the product of an uneventful pregnancy and delivery. His parents are not known to be related to each other by blood, nor are his grandparents. His early development was unremarkable, but at the age of 14 months mental and motor retardation became obvious with his failure to walk and talk; he did not learn to understand the spoken word. At the age of 24 months, muscular weakness and hypotonia were noted, and his physical growth, being within normal limits up to this time, began to slow down. At age 16, he weighed only 75 pounds, was 4 feet tall, and showed severe kyphoscoliosis and a pigeon chest. He never displayed organomegaly nor did he present radiographic evidence of skeletal abnormalities compatible with mucopolysaccharidoses. Since the age of 2 years the patient has been bedridden, and has experienced frequent episodes of respiratory tract infections.

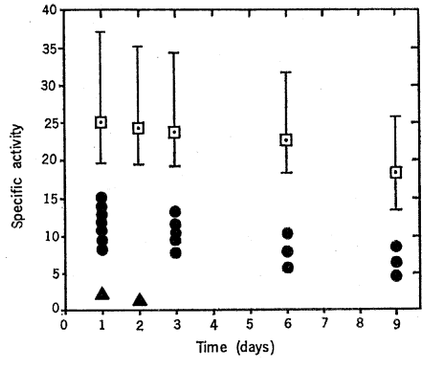

Fig. 1. Effect of storage time at 4° to 5°C on leukocytic α-fucosidase activity (nanomoles of *p*-nitrophenol liberated per milligram of protein per hour). ⊡, mean of ten controls with total range indicated by bar; ●, carriers; ▲, patient. No significant age-dependent differences (from 2 to 60 years) for leukocytic α-fucosidase activity were observed.

At age 4, he developed blue-brown, pinhead-sized, raised skin lesions, first over the abdomen and back, and then involving the lower, and finally the upper extremities, producing the characteristic picture of angiokeratoma corporis diffusum. Histological examinations revealed multiple teleangiectasias. Anhidrosis and inability to control body temperature developed synchronously with the skin lesions and necessitated confinement of the patient to an air-conditioned room for the last 10 years. For the past 2 years, the patient has suffered from convulsions at the rate of approximately one per month. The electroencephalographic tracings showed low voltage activity throughout with occasional paroxysms of 3- to 4-hz rhythmic waves, but no spike-wave discharges.

Because of the characteristic clinical and histological aspects of the skin lesion, a tentative diagnosis of Fabry's disease was made. However, we recognized that the severe mental and physical retardation did not fit this diagnosis nor did the normal renal functioning.

We measured (*6*) the activity of various lysosomal hydrolases in leukocytes, prepared according to a minor modification (*7*) of the procedure of Snyder and Brady (*8*). Urinary hydrolases were assayed as follows. Urine was collected over a period from 12 to 24 hours and was filtered; the proteins were precipitated with ammonium sulfate in a molal concentration between 1.5 and 4.5, were dissolved in 5 m*M*