Chronic Focal Epileptiform Discharges Induced by Injection of Iron into Rat and Cat Cortex

Abstract. *A single injection of 5 or 10 microliters of ferrous or ferric chloride into rat or cat sensorimotor cortex resulted in chronic recurrent focal paroxysmal electroencephalographic discharges as well as behavioral convulsions and electrical seizures. Recurrent focal epileptiform discharge caused by cortical injection of iron salts suggests that the development of human posttraumatic epilepsy may depend, in part, on the neurochemical alterations induced by the principal metallic ions found in whole blood.*

The development of a truly representative model of the human epileptogenic focus is essential for the better understanding and to more rational prophylaxis and therapy of human posttraumatic epilepsy. Among experimental methods used to induce epileptiform seizures of focal origin, topical or intracerebral application of alumina gel to primate cerebral cortex has been the one which best fulfills the crucial criterion of prolonged "spontaneous" recurrence resembling that seen in man (*1*). The alumina gel model has the disadvantage of being unpredictable or ineffective in species other than subhuman primates. In addition, alumina gel application induces the formation of an alumina granuloma which has no histopathological analogy in human epilepsy (*2*). Other metals applied topically, such as cobalt, are effective in inducing active focal epileptogenic lesions for periods up to several days in a variety of species, but they fail to produce a long-lasting chronic epileptogenic lesion (*3*). We have found a simple, reproducible experimental model of human focal epilepsy that appears to overcome many of the disadvantages of previous models and at the same time has the important potential of reflecting the pathogenetic mechanisms responsible for the posttraumatic epilepsies in man.

Metallic ions, such as cobalt, applied to the brain by pial iontophoresis cause acute epileptiform activity (*4*). In the course of screening metallic salts from the transition series for convulsant effects, we observed that abrupt and predictable focal spike discharges were induced by the pial application of the salts of iron (*5*). Since hemosiderin deposition is a prominent histopathological feature of the human posttraumatic epileptic focus, we asked whether recurrent seizure discharge would develop after subpial injection of aqueous solutions containing the principal metallic compound found in whole blood.

Five stainless steel extradural screw electrodes were implanted in the calvarium of each Sprague-Dawley rat (200 to 300 g) and domestic cat used in this experiment. While the rats were under pentobarbital anesthesia, we injected them subpially with 5 μl of aqueous solutions containing 100 mmole of $FeCl_2$ or $FeCl_3$, or 0.9*N* NaCl (*6*). The cats were injected with 10 μl of aqueous iron only. After recovery from anesthesia, cortical electroencephalographic recordings were taken daily for 1 week and weekly thereafter. Fifteen rats were tested with ferrous chloride, 20 with ferric chloride, and 14 with saline. Two cats were injected with ferrous chloride and two with ferric chloride.

Both ionic salts of iron caused focal

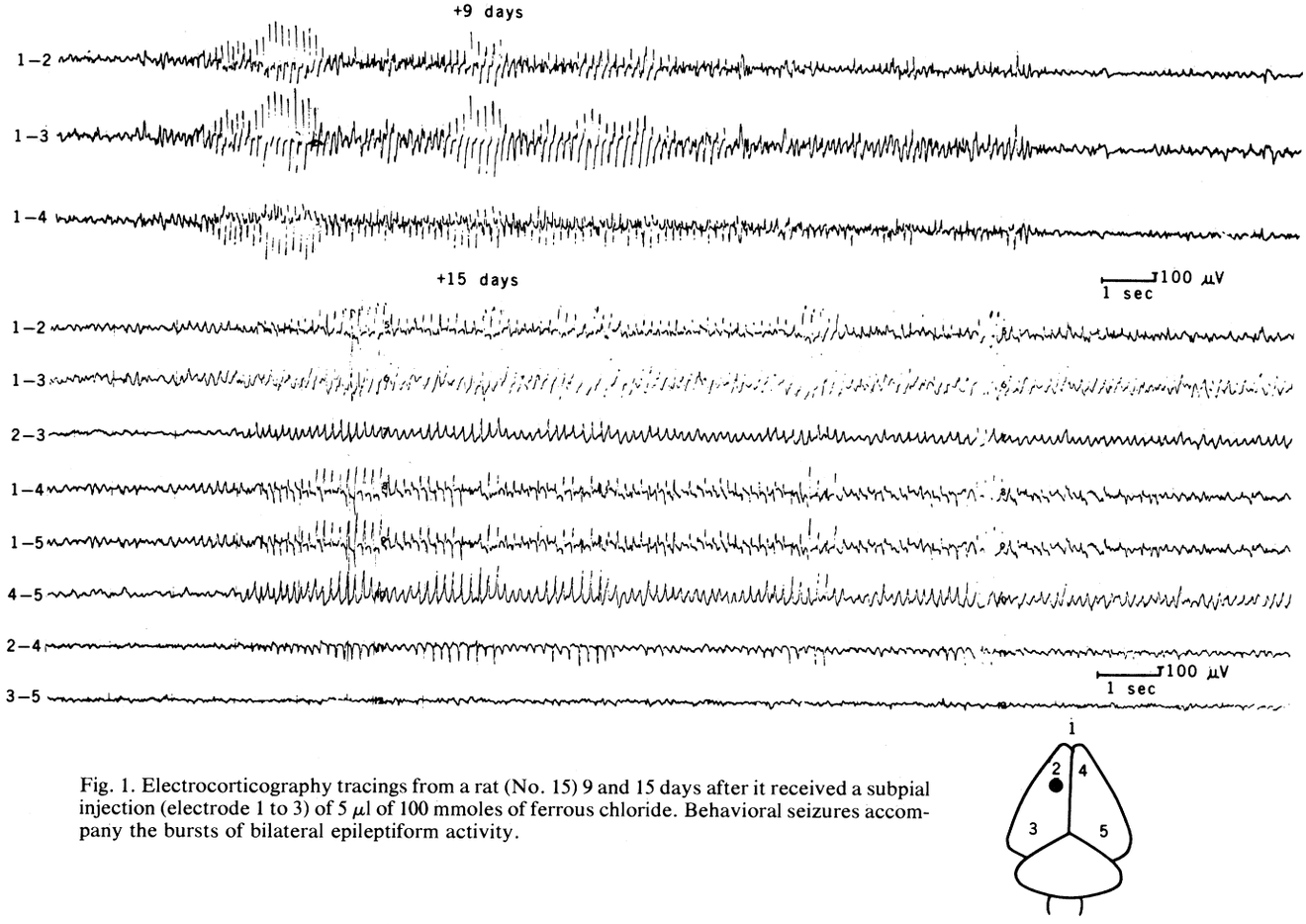

Fig. 1. Electrocorticography tracings from a rat (No. 15) 9 and 15 days after it received a subpial injection (electrode 1 to 3) of 5 μl of 100 mmoles of ferrous chloride. Behavioral seizures accompany the bursts of bilateral epileptiform activity.

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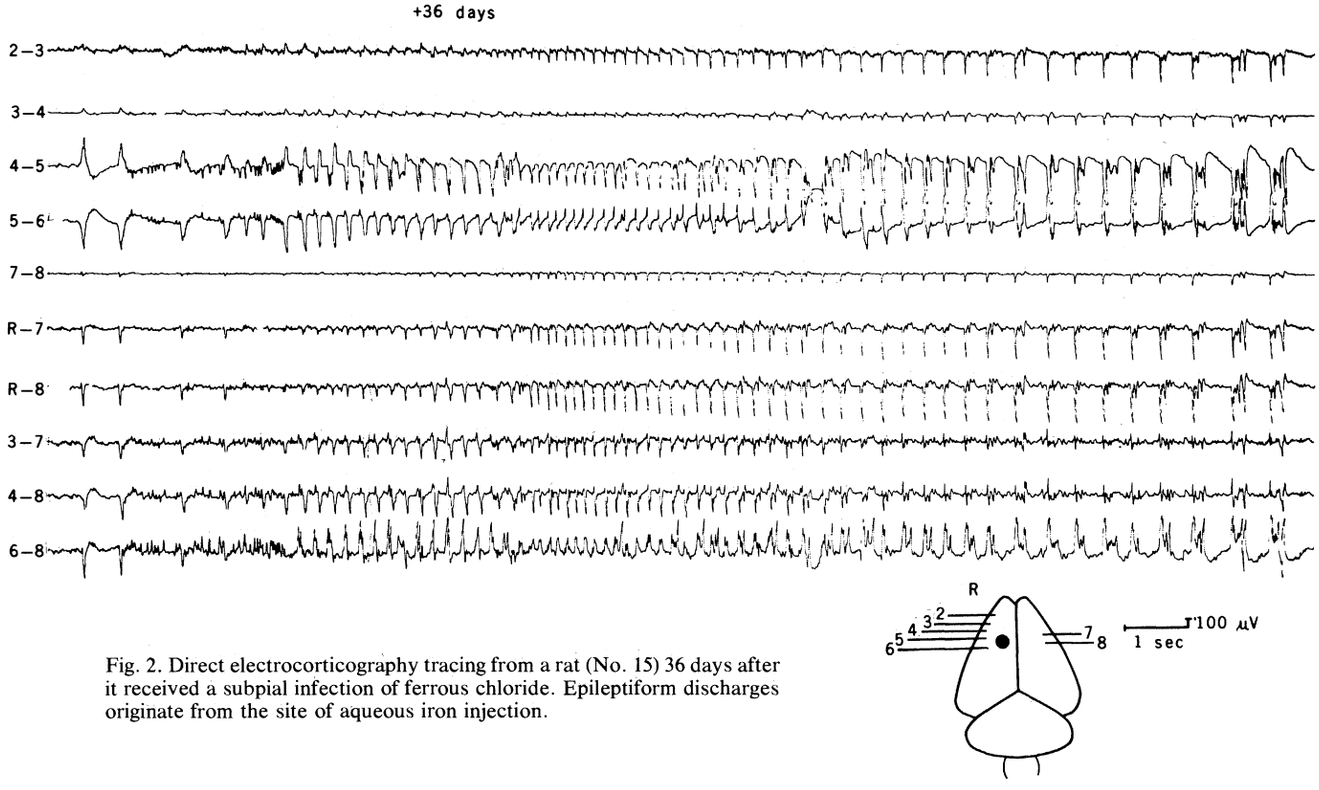

Fig. 2. Direct electrocorticography tracing from a rat (No. 15) 36 days after it received a subpial infection of ferrous chloride. Epileptiform discharges originate from the site of aqueous iron injection.

spiking activity within 48 hours, with spread of this epileptiform activity contralaterally, in both animal species. Frequent and sustained bursts of epileptiform activity developed within 10 days (Fig. 1). Behavioral convulsions (*7*) and electrocorticographic discharges (Fig. 2) continued to persist beyond 12 weeks in 94 percent of the iron-injected rats, and in all of the iron-injected cats (*8*). Four of 14 animals injected with NaCl exhibited transient focal spike activity lasting less than 14 days. Selected rat and cat brains were examined histologically 6 weeks after iron injection (*9*). A minute indentation was observed at the site of iron injection. Sections through the focus showed a narrow cavity within the cortical mantle, contiguous with the needle tract, lined with numerous iron-filled macrophages extending into the neuropil. Differential histochemical staining identified ferric ions within the injection site regardless of the original salt of iron injected. Proliferated glial fibers surrounded the iron focus.

Iron is a transition metal (*10*) occurring in valence states of 2+ and 3+. The ions of iron will form numerous coordination complexes with nitrogen, oxygen, and sulfhydryl ligands; ferric ions also hydrolyze in aqueous solution, liberating protons and forming microcrystalline polynuclear species (*11*). Both ferric and ferrous ions stimulate respiration of tissue suspensions of brain (*12*), inhibit brain Na^+- and K^+-dependent adenosinetriphosphatase, bind strongly with adenosine triphosphates, and have an affinity for catecholamines (*13*). Although biologically active, the mechanism by which iron salts induce epilepsy remains unknown.

Biological iron is normally protein-bound in transferrin and hemoglobin and isolated from the brain by vascular endothelium. Head injury or hemorrhagic cortical infarction will result in extravasation of blood and deposition of iron within the neuropil. Accompanying hemosiderin deposition is a high percentage of development of late epilepsy in patients with intracerebral hematoma, cortical laceration, or subarachnoid hemorrhage (*14*).

The creation of a chronic epileptic focus in the rat and cat by subpial injection of metallic ions found in whole blood rather than foreign metals or chemicals is one major advantage of the ferric-ferrous technique. A second advantage is that the creation of a truly chronic epileptic focus in the ubiquitous and genetically homogeneous albino rat and the domestic cat, provides a means for the ready assessment of pharmacologic methods for the prophylaxis of focal seizures and drug screening. A third advantage of the technique is that it will permit the study of the evolution of the chronic epileptic focus in a variety of animal species by morphological, biochemical, and physiological techniques. However, the most important advantage of this method is, in our opinion, that a chronic epileptic focus can now be induced by quantitative chemical methods that reflect the events considered possible after head injury and cerebral infarct in humans.

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References and Notes

1. A. A. Ward, Jr., in *Experimental Models of Epilepsy*, D. D. Purpura, J. K. Penry, D. Tower, D. M. Woodbury, R. Walter, Eds. (Raven, New York, 1972), pp. 1-35.
2. A. B. Harris, *Exp. Neurol.* **49**, 691 (1975); H. Payan, M. Toga, M. Berard-Badier, *Epilepsia* **11**, 81 (1970).
3. R. S. Dow, A. Fernandez-Guardiola, E. Manni, *Electroencephalogr. Clin. Neurophysiol.* **14**, 399 (1962); E. Y. Henjyoji and R. S. Dow, *ibid.* **19**, 152 (1965); G. D. Dawson and O. Holmes, *J. Physiol. (London)* **185**, 455 (1966); H. M. Payan, *J. Neurosurg.* **28**, 146 (1967).
4. L. J. Willmore, P. M. Fuller, A. B. Butler, N. H. Bass, *Exp. Neurol.* **47**, 280 (1975); P. A. Schwartzkroin, Y. Shimada, Y. B. Bromley, *ibid.* **55**, 353 (1977).
5. L. J. Willmore, G. W. Sypert, J. B. Munson, *Neurosci. Abstr.* **3**, 148 (1977).
6. Animals were anesthetized with pentobarbital and placed in a David Kopf small-animal stereotaxic apparatus. A burr hole 1 mm in diameter

was made in the calvarium over the left sensorimotor cortex. A 30-gauge needle attached to a microinjection syringe held rigidly in a stereotaxic micromanipulator was inserted into the cortex 1.2 mm below the exposed dura. Aqueous solutions were injected in 5 μl volumes over 5 minutes. Cats were restrained and anesthetized in a similar fashion and injected with 10 μl of aqueous iron solution; two animals were injected in the postcruciate gyrus and two in the sigmoid gyrus.

7. During generalized electrographic seizures, rats interrupted cage exploration. Piloerection and rhythmic twitching of the vibrissae and neck musculature accompanied each spike discharge. Cats with left postcruciate lesions displayed intermittent focal motor seizures originating in the limb musculature with occasional secondary major motor generalization.
8. Animals were lightly anesthetized with ether, paralyzed with *d*-tubocurarine (3.0 mg/kg, subcutaneously), and artificially ventilated by way of a tracheostomy. All wound margins were infiltrated with 2 percent lidocaine. Bilateral craniectomies were performed and a recording array of six Nichrome wires was placed on the isocortex along the site of iron injection parallel to the sagittal suture. Two electrodes were placed on the contralateral homotopic cortex. The electrocorticogram was recorded with bipolar and referential montages with a Grass model 6 electroencephalograph.
9. Animals were anesthetized with pentobarbital and killed by transcardiac perfusion with neutral buffered formalin. Selected brains were embedded in paraffin, and coronal sections (10 μm) were taken from an area extending 2 mm anterior and 2 mm posterior to the site of injection. Alternate sections were stained with phosphotungstic acid–hematoxylin, Masson's trichrome, hermatoxylin-eosin, and cresyl violet. Other brains were mounted in a freezing microtome and sectioned at 25 μm. Alternate sections were stained with cresyl violet, hematoxylin-eosin, Perl's Prussian blue stain, and Turnbull's reaction.
10. Iron occurs in group VIII of the periodic table of the elements.
11. P. M. Harrison, *Clin. Toxicol.* **4**, 529 (1971).
12. F. Panimon, M. K. Horwitt, R. W. Gerard, *J. Cell. Comp. Physiol.* **17**, 1 (1941).
13. A. Schaefer, A. Seregi, M. Komlos, *Biochem. Pharmacol.* **23**, 2257 (1974).
14. E. P. Richardson, Jr., and P. R. Dodge, *Epilepsia* (third series) **3**, 49 (1954); B. Jennett, *J. Neurol. Neurosurg. Psychiatry* **38**, 378 (1975); W. F. Caveness, *J. Neurosurg.* **20**, 570 (1963); H. A. Kaplan, *Epilepsia* **2**, 111 (1961); W. R. Russell and C. W. M. Whitty, *J. Neurol. Neurosurg. Psychiatry* **15**, 93 (1952).
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Intellectual Status of Working-Class Children Adopted Early into Upper-Middle-Class Families

Abstract. *Failure rates observed (13 ± 6 percent for school failures, 17 ± 5 percent for scores below 95 on a collective IQ test) were far below those expected from the social class of birth (55 percent, 51 percent) or observed in a control group (56 ± 8 percent, 49 ± 9 percent) but close to those expected from the social class of adoption (15 percent, 15 percent).*

There have been a certain number of speculations recently that there may be a genetic origin for the "educational lag of disadvantaged children" (*1*), that possibly "the class structure of modern society is essentially a function of the innately differing intellectual and other qualities of the people making up these classes" (*2*), or that we may be faced with "a future in which social classes not only continue but become ever more solidly built on inborn differences" (*3*). Part of the confusion surrounding these speculations stems from a failure to distinguish clearly between questions about individual variations and questions about group differences and also from the rarity of directly relevant observations (*4*). Our study contributes in a direct manner to the question of group differences in educational failures within primary school.

For questions concerning group differences, empirical answers can only come from the study of subjects who have been reared since birth by adoptive parents belonging to a different group from that of the biological parents (*5*). We present here the principal results of a study of this type, in which we have examined the intellectual failures of working-class children adopted early into upper-middle-class families. School curricula and IQ scores were obtained for an unbiased sample of 32 such subjects, all of them Caucasians. Comparisons were made with a control group of children of the same biological mothers, as well as with groups of children of the general population studied by others (*6, 7*).

The 32 adopted (A) subjects were obtained from the files of six public agencies from various parts of France. We examined the files of all the children who had been abandoned at birth between 1962 and 1969. We focused our attention on cases where the absence of professional qualification was known for both biological parents. For one-fifth of these cases, the children turned out to have been placed before the age of 6 months into a family of high socio-professional status (*8*). We succeeded in locating the children and in obtaining school curricula and IQ scores for all 32 subjects.

For 28 of these 32 A subjects, the biological mother could eventually be traced. Among these 28 mothers, 20 turned out to have children of school age who had not been abandoned (*9*). The 20 sibships contained 39 biological (B) subjects.

For each of the 20 sibships, one B subject to be tested was defined before the school curricula were known as the closest one in age to the corresponding A subject. Extensive search and the subsequent examination of school records made it unlikely that any B subject of school age had been missed and we were eventually able to obtain the school curriculum of every single B subject. This permitted both an unbiased estimate of the rate of school failures among B subjects and an a posteriori check on the absence of bias in the B subjects tested for IQ.

For the 20 B subjects tested and for 95 percent of the A subjects (*10*), we obtained two independent IQ scores, usually by administering a French collective test ECNI (*6*) as well as the full WISC (*11*). For school curricula (*12, 13*), a distinction was made between relatively mild failures (the repetition of grades within primary school) and serious failures (placement in a class with a simplified curriculum). For IQ tests, a reasonably comparable metric was obtained by defining mild failure as scoring below 95 and serious failure as scoring below 85. The comparison between A and B groups is presented in Table 1.

The contrast in intellectual status between the two groups is considerable, especially for serious failure—that is, for those failures that are likely to have the greatest impact on the future social and professional life of the subjects.

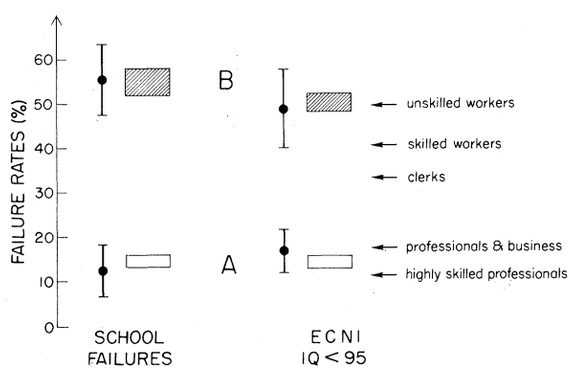

Fig. 1. Comparison of failure rates of A and B groups (circles with standard error bars) with rates expected for A children on the basis of adoptive parents (open rectangles) or biological parents (shaded rectangles) and with rates observed in the general population for five groups of schoolchildren (arrows).

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