Spontaneous seizures generated in rats by kindling: A preliminary report

JOHN P. J. PINEL, R. F. MUCHA, and A. G. PHILLIPS
University of British Columbia, Vancouver, British Columbia, Canada

Daily amygdaloid stimulation administered to rats at current levels initially too low to produce a motor response but high enough to produce an electrographic afterdischarge (AD) resulted in the progressive development and intensification of stimulus-induced epileptic activity (kindling). In contrast to previous studies, however, stimulation was continued long after the point where the exacerbation of motor seizures (MSs) seemed complete. Several animals were stimulated for up to 7 months and, with continued stimulation, an epileptic syndrome characterized by spontaneous motor seizures was observed to develop.

There is a gradual development of epileptogenicity when daily bipolar electrical stimulation is applied to the brain of rabbits (Tanaka, 1972), mice (Leech, 1972), rats (Goddard, 1967), cats (Goddard & Morrell, 1971), or monkeys (Goddard, McIntyre, & Leech, 1969) at current levels which are initially too low to evoke any behavioral response. With each successive stimulation, there is a progressive development and subsequent intensification of stimulus-induced epileptic activity until eventually each stimulation reliably produces a bilateral clonic motor seizure (MS). This "permanent" increase in the effectiveness with which local electrical stimulation can induce MSs has been termed "the kindling effect."

There are striking parallels between this kindling phenomenon and the progressive development of epileptic symptoms in human patients. In untreated clinical cases, as in kindled animals, there is a progressive development of symptoms within a particular attack and from one fit to the next (Hughlings Jackson, 1870). The potential of the kindling paradigm as a model of this clinical epileptogenesis has been emphasized by others (cf. Goddard et al., 1969; Morrell, 1973) but there is still a major question concerning the usefulness of such an approach. By definition, human epilepsy is a spontaneously recurring self-sustained paroxysmal dysfunction of the brain; however, as Goddard et al. (1969) have pointed out, although intermittent focal stimulation reliably produces a gradual exacerbation of stimulus-induced seizures in rats, no spontaneous seizures have been observed to develop. Thus, the major shortcoming of kindled seizures in rats as a model of epileptogenesis is that kindling does not seem to induce a truly epileptic condition. Accordingly, the purpose of the present study was to determine whether kindling

would eventually lead to the development of spontaneous seizures in rats if the regimen of stimulation were sufficiently long.

METHODS

Twenty-one days following implantation in the right medial amygdala of a bipolar electrode (.01-insulated nichrome wire), each of 18 male 400-450 g hooded rats (Canadian Breeding Laboratories, La Prairie, Quebec) were subjected to repeated brain stimulation. Electrographic activity was monitored for 60 sec prior to each stimulation through low noise, shielded Microdot cables which conducted the signal to a Grass Model 78B polygraph. Then a 1-sec, 60-Hz sine-wave current was applied through the recording electrode and electrographic and motor activity were monitored for at least 60 sec following the termination of any afterdischarge (AD) or MS activity. Switching from the recording to the stimulation mode and back again was done automatically by a specially constructed device which also isolated the polygraph amplifiers during current delivery to reduce poststimulation interference in the recording channel. Motor activity was recorded from a single insulated wire that was fastened to the recording lead and fed into a channel of the polygraph. Movement of the wire by the animal produced changes in potential proportional to the magnitude of the motor activity. The rats were initially stimulated three times per day at 400 microA with at least a 2.5-h interstimulation interval. Following 15 days under this regimen, stimulations were administered only once a day for 22 days. At the end of this 22-day period, all animals were manifesting bilateral clonic motor seizures but only those animals which had no more than a 20% difference on any 2 consecutive days in either MSs or ADs on the last 10 days were kept in the experiment. Additional periodic stimulation was administered to these 14 stable animals for a period of about 6 months in an attempt to develop spontaneous seizures. Since, at the time, it was not known whether long-term stimulation would produce epileptic animals, various stimulation parameters were used. Interstimulation intervals varied from 2.5 to 72 h and current intensities ranged from 1 microA to 400 microA, but intensities other than 400 microA were used only infrequently. Stimulation was permanently curtailed in a subject when spontaneous seizures were first observed.

At the termination of the experiment, the animals were asphyxiated and their brains were removed for storage in a buffered formalin solution. The brains were subsequently embedded in paraffin wax, sectioned at 20 microns, and stained according to a modification of the Kluver-Barerra technique.

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For reprints, write to John Pinel, Department of Psychology, University of British Columbia, Vancouver, B. C. V6S 1W5.

RESULTS

Stimulation at the various electrode sites in the amygdala produced the same progressive development of seizures that had previously been reported (cf. Goddard et al., 1969). At first, each stimulation produced no behavioral response and either no or a very brief AD, but on ensuing trials MSs and ADs developed and increased in duration with each successive stimulation. Accompanying the increased duration of the MSs and ADs was an intensification of the MS pattern through the five stages previously described by Racine (1972): (1) facial movements only, (2) facial movements and head nodding, (3) facial movements, head nodding, forelimb clonus, and rearing, (5) facial movements, head nodding, forelimb clonus, rearing, and falling.

Of the 14 stable animals which began the 6-month period of stimulation, only four completed this procedure. The other 10 animals dislodged their electrodes. This high attrition rate was a direct result of our attempts to produce spontaneous seizures. As stimulations progressed, the stimulation-induced epileptic activity became more violent and interictal spiking punctuated the electrographic records of all animals. At this stage, the general health of the animals declined, infections often developed, and the electrodes were lost, usually during particularly violent seizures. We did not employ antibiotics because of the possibility that they might interact with the convulsive effects of the brain stimulation.

Nevertheless, four animals did complete the additional 6 months of stimulation and two of these did develop spontaneous motor seizures. The first animal to develop spontaneous seizures lived for over 2 months before status epilepticus. During this period, it displayed a broad range of epileptic activity ranging from mild running fits and myoclonic jerking to Class 5 seizures similar to those previously produced only by brain stimulation in kindled animals. During some periods,

seizures would occur every few minutes for several hours; at other times, the animals remained seizure free for considerable periods of time. Figure 1 is comprised of the electrographic record of this subject during a spontaneous Class 5 seizure. The second rat to develop spontaneous epileptic activity displayed Classes 3 and 4 seizure activity for about a week after stimulation was curtailed. It eventually recovered.

The antecedents of spontaneous convulsive activity was the same for both animals. First there was the development and proliferation of "interictal" spiking observed in the prestimulation electrographic records. Second, the seizures elicited in these animals grew very unstable. Although both animals had suffered over 100 Class 5 seizures, they began to occasionally demonstrate a seizure pattern of a lower class with a much-abbreviated duration, and in some cases they failed to show any response to the stimulation at all. Thirdly, shortly before the first observation of spontaneous seizures, both animals began to display myoclonic jerking in conjunction with interictal spikes of particularly high amplitude.

Histological examination revealed that all of the electrodes did in fact terminate in the amygdaloid nuclei, and, like previous investigators, we were unable to correlate our results with the placement within this area. On the basis of histology, we discounted gross cellular damage, infection, and edema as factors in the development of spontaneous seizures since there was no gross damage in the epileptic animals and the two animals which completed the experiment without becoming epileptic did have extensive damage along the electrode track. However, there was a marked reduction in the number of normal cell bodies at the stimulation site of the epileptic animals in comparison to the number in adjacent tissue, at the homotopic sites in the contralateral hemisphere, or in other noninfected, nonepileptic subjects.

DISCUSSION

The purpose of this preliminary investigation was to show that rats could be kindled to the point where they would display a recurring self-sustained paroxysmal dysfunction (epilepsy), and, since two subjects developed such an epileptic syndrome after several months of repeated amygdaloid stimulation, our goal was clearly accomplished. Why have previous investigators studying the kindling phenomenon failed to observe the development of spontaneous seizures? Although, on the basis of the present investigation, we are in no position to identify the factors which will facilitate or retard the development of spontaneous seizures, we suspect that the production of spontaneous seizures in rats by periodic brain stimulation may simply be a matter of continuing the regimen of stimulations

Figure 1. Electrographic and motor activity of a subject during a spontaneous Class 5 motor seizure.

for a sufficiently long time. Most previous studies of the kindling phenomenon have been curtailed at the point where full (Class 5) motor seizures have developed, or shortly thereafter.

The present experiment also has important implications for the use of local stimulation of the brain for therapeutic purposes (cf. Heath, 1970). Goddard (1971) has previously pointed out the relevance of the kindling effect to this procedure. He pointed out that repeated electrical stimulation of a particular site will produce an irrevocable increase in the susceptibility of that structure to seizures produced by subsequent stimulation even when the electrical stimulation produces no evidence of seizure activity during initial applications. The present findings and those of Wada and Sato (1973) illustrate that the risks involved are greater than first expected. Repeated stimulation to various sites in the brain cannot only lead to a condition where further stimulation can induce seizures, but to a condition where paroxysms may occur spontaneously. Thus, the possible dangerous consequences of therapeutic brain stimulation deserves even greater emphasis.

Although the present results certainly raise more questions than they answer, they do comprise the first demonstration that an epileptic syndrome can be systematically induced in rats with repeated brain stimulation. A similar observation has recently been reported in cats by Wada and Sato (1973) and, together, these results emphasize the potential of the kindling paradigm as a valuable model for the systematic study of epileptogenesis.

REFERENCES

- Goddard, G. V. Development of epileptic seizures through brain stimulation at low intensity. *Nature*, 1967, 214, 1020-1021.
- Goddard, G. V. The kindling effect: A precautionary note on therapeutic applications of localized brain stimulation. In D. V. Reynolds and A. E. Sjöberg (Eds.), *Neuroelectric research: Electroneuroprosthesis, electroanesthesia and non-convulsive electrotherapy*. Springfield: Thomas, 1971.
- Goddard, G. V., & Morrell, F. Chronic progressive epileptogenesis induced by focal stimulation on the brain. *Neurology*, 1971, 21, 393.
- Goddard, G., McIntyre, D., & Leech, C. A permanent change in brain function resulting from daily electrical stimulation. *Experimental Neurology*, 1969, 25, 295-330.
- Heath, R. G. Perspectives for biological psychiatry: Presidential address. *Biological Psychiatry*, 1970, 2, 81-88.
- Jackson, J. H. A study of convulsions. In: J. Taylor (Ed.), *Selected readings of John Hughlings Jackson* Vol. 1. 1870. Reprinted New York: Basic Books, 1958.
- Leech, C. K. Comparison of audiogenic seizure development and kindled convulsions in seven strains of mouse. Doctoral Dissertation, Dalhousie University, 1972.
- Morrell, F. Goddard's kindling phenomenon: A new model of the "mirror focus." In: H. C. Sabelli (Ed.), *Chemical modulation of brain function*. New York: Raven, 1973.
- Racine, R. J. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalography and Clinical Neurophysiology*, 1972, 32, 281-294.
- Tanaka, A. Progressive changes of behavioral and electroencephalographic responses to daily amygdaloid stimulation in rabbits. *Fukuoka Acts Medica*, 1972, 63, 152-164.
- Wada, J. A., & Sato, M. Generalized convulsive seizure state induced by daily stimulation of the amygdala in split-brain cats. Paper presented at the 27th annual meeting of the American Electroencephalographic Society, Boston, 1973.

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