Kindling Rates in Sprague Dawley Rats as a Function of Copper and Nichrome Electrodes

Martin B. Wolske, Eliza Hui Chien Lian and Curtis K. Leech
Department of Psychology
Anderson College
Anderson, Indiana 46012

Introduction

Kindling is the process of developing experimental epilepsy through repeated electrical brain stimulation over a period of time (4). It has been found to be a generalized phenomenon, occurring in a variety of animals (e.g. frogs, mice, rabbits, rats, cats, monkeys, and for therapeutic reasons, humans) (11). Because of its reliability, kindling has been widely used as a dependent variable in studies that have ranged from the effects of epileptic medications (11) to the testing of kindling as a neurological model of normal brain function (4, 6). It appears that many changes that occur in neural activity are reliably reflected in kindling rates (11).

The Wistar, Royal Victoria and Long Evans strains of rat have been the primary targets of these investigations. However, the Sprague Dawley rat strain, which has not been used very often, has great appeal because of its gentle disposition (appreciated by students not used to handling rats), and its wide use in behavioral studies. Preliminary research in our laboratory has found that the Sprague Dawley strain has been somewhat more refractory to kindling procedures than the other strains. However, Leech (5) reported pilot data that found the Sprague Dawley strain kindled faster than the other strains. One primary difference between the two studies was that Leech (5) had used nichrome wire electrodes, whereas copper wire had been used in the second study because of its availability and economy. The present study was undertaken as a first step in establishing normative data on kindling rates in Sprague Dawley rats, and determining if copper wire can be used effectively in brain stimulation studies.

Method

Twenty-six male Sprague Dawley rats were obtained from Holtzman Co. (Madison, Wisconsin). They weighed approximately 300 grams at the time of surgery. They were individually housed in a room that was kept at approximately 24 degrees Celsius and had lights that turned on at 6:00 AM and turned off at 6:00 PM. Purina Lab Chow and water were available at all times, except 12 hours prior to surgery when food was removed. Tetracycline was added to their water prior to and following surgery to avoid respiratory infection.

After one week of regular handling, the rats were anesthetized with Nembuta (50mg/kg, i.p.). Bipolar electrodes, composed of either enamel coated copper wire (.0213 cm in diameter) or diamel coated nichrome wire (.0217 cm in diameter) were fabricated by soldering the wire to Amphenol pins (220-PO2). These were then twisted, dipped into epoxylite for additional insulation, and baked for 6 hours at 120 degrees Celsius. The wires were then tested for continuity, cut to length, and were stereotaxically implanted in the amygdala-piriform region of either hemisphere.

One week following surgery, both the nichrome and copper groups were subdivided into "threshold" and "control" groups, each of which received a different treatment on the first day. The threshold group was tested for after discharge (AD) threshold utilizing a procedure similar to that outlined by McIntyre et al. (7). Each rat was stimulated at increasing intensities, with an intertrial rest period of one minute between stimulation onsets. The procedure started with a two second train of 60-Hz, monophasic square wave (1 msec pulse duration) at 35 uA, delivered from a Grass SD9 stimulator fed thorugh

a Grass constant current unit, where the polarity was reversed daily. The entire sequence was: 35, 50, 75, 100, 150, 200, 300 uA. If AD was observed on a trial, the animal was not stimulated again that day. To reduce the risk of brain damage, 300 uA was not exceeded. The control group, by contrast, received the same stimulation parameters as the threshold group, but only at an intensity of 50 uA. Twenty-four hours later, and every twenty-four hours thereafter, both groups were stimulated at 50 uA. This was continued for 30 days or until 6, stage 5 convulsions (rearing and falling with clonus) were exhibited (11).

Following the 30 day stimulation period, AD thresholds were reassessed on the 8 rats in the threshold group which did not achieve 6, stage 5 convulsions.

Animals that had not kindled after the 30 day period were randomly divided into two groups. One group was given 200 uA (other stimulation parameters were the same as previously described) for 10 days to determine if their prior level of current was insufficient to provoke kindling. The other group was held as a control group for possible brain damage differences due to the stimulation at 200 uA for 10 days.

Following completion of testing, the rats were perfused with 10% formalin. The brains were then frozen and sectioned through the areas of the electrode tract. Selected sections were mounted on slides, stained with thionin, and examined microscopically.

Results

Histological analysis indicated that three rats had electrodes outside of the amygdaloid-piriform cortex area, and were eliminated. Three additional rats were eliminated due to a short circuit in the electrode, death following surgery, or the electrode becoming detached from the head.

The median number of stimulations (days) needed to produce AD, stage 1 convulsions (e.g. eye blinking and repetitive mouth movements), and stage 5 convulsions, for copper and nichrome electrode groups, appear in Table 1. Medians were used because not all subjects developed convulsions. As can be seen, the nichrome electrode group had a lower median number of daily stimulations than the copper electrode group for each effect listed in Table 1. However, only the fourth comparison of the number of days of AD needed to produce the first stage 5 convulsion, using the Mann-Whitney U test, was statistically significant ($z = 2.485$, $p < .05$). The second comparison of daily stimulations needed to produce a second AD had borderline statistical significance ($z = 1.774$, $p < .1$). The first and third comparisons were not statistically significant.

TABLE 1. Medians and ranges for afterdischarge (AD) and convulsion measures for copper and nichrome groups. Please note that "30+" should be read as exceeding the 30 day limit when testing was terminated.

	Copper		Nichrome	
Measurement	Median	Range	Median	Range
Days to first AD	25	3-30+	6	1-30+
Days to second AD	+	20-30+	7	2-30+
Days of AD to first stage 1	+	1-30+	5	2-30+
Days of AD to first stage 5	+	+-30+	8	7-30+

Table 2 shows the same measures as Table 1 for the animals that were later run at 200 uA. None of the comparisons between copper and nichrome groups were statistically significant at this current level.

Comparisons of AD threshold measurements between copper and nichrome groups showed borderline significance for the first threshold assessment while the second threshold

assessment that followed the 30 day stimulation period was not significant ($z = 1.8473$, $p < .1$; $z = 1.51807$, $p \geq .129$, respectively).

TABLE 2. Medians and ranges for afterdischarge (AD) and convulsion measures for copper and nichrome groups that received 200 uA after 30 days at 50 uA. Please note that "40+" should be read as exceeding the 40 day limit when testing was terminated.

Measurement	Copper		Nichrome	
	Median	Range	Median	Range
Days to first AD	25	3-40+	32	6-34
Days to second AD	33	20-40+	33	7-35
Days of AD to first stage 1	2	1-40+	2	1-5
Days of AD to first stage 5	11	6-40+	7	5-40+

Where possible, kindling rates for the Sprague Dawley strain were then compared with data on Wistar rats, obtained from a study by McIntyre and Roberts (8). These data are listed in Table 3. Comparisons using t-tests found that the Sprague Dawley rats had significantly higher initial AD thresholds ($t(20) = -6.507$, $P < .001$) than the Wistar rats. Further, the Sprague Dawley rats showed a significantly shorter AD duration ($t(20) = 2.832$, $p < .05$) and duration of clonus ($t(20) = 3.704$, $p < .001$) than the Wistar rats. The kindling rate in days could not be statistically compared because McIntyre & Roberts used means, whereas the present study used medians.

TABLE 3. Afterdischarge (AD) threshold and kindling rate (number of days of AD to first stage 5 convulsion) for Wistar and Sprague Dawley strains.
Note: Only the median was available for the kindling rate in Sprague Dawley.

Group	N	AD threshold (uA)		Kindling rate (No. of stim)	
		X	SD	X	SD
Wistar	18	43.9	4.8	8.7	1.9
S.D.	4	112.5	47.9	5	

TABLE 4. Mean time, in seconds, for after discharge (AD) duration, latency to clonus, and duration of clonus for Wistar and Sprague Dawley Strains. Data from the last five convulsions were used.

Group	N	AD duration		Latency to clonus		Duration of clonus	
		X	SD	X	SD	X	SD
Wistar	18	78.1	15.2	7.5	2.1	45.1	4.2
S.D.	4	56.3	21.0	4.9	1.0	35.6	6.6

Discussion

The results clearly show that copper electrodes are less effective than nichrome for kindling convulsions. This was indicated by the significantly fewer number of stimulations required to produce stage 5 convulsions in rats with nichrome wire, and the lower AD thresholds found in the nichrome group. The most plausible reason for this appears

to be that copper wire causes neural damage that retards the kindling process. Babb and Kupfer (1), found that copper deposits resulted in necrosis and phagocytosis in the neural tissue of rats. Although the present study found no apparent gross tissue damage in the histologies of the rats, the possibility of necrotic and phagocytic damage could not be ruled out.

Further, several studies have suggested that kindling is the result of tissue damage caused by the kindling procedures (11). The present study suggests that this damage could not be due to the necrotic and phagocytic damage found by Babb and Kupfer from copper electrodes, since these electrodes were the more refractory to the kindling procedures. Indeed, damage produced by necrosis and phagocytosis seems to retard the kindling process, as evidenced by the increased number of stimulations required to produce convulsions in the copper group as compared to the nichrome group.

The present study also suggests that the Sprague Dawley rats are less consistent than the Wistar rats in their manifestations of kindling, although as Leech (5) reported, they kindle more rapidly at 50 uA. However, the difference in the kindling rates suggested by the means, includes the differences in the initial AD threshold between these two strains. Measures from the first AD to the first stage 5 convulsion are needed for the Wistars to make this a more meaningful comparison. A further difference between strains is seen in the shorter latency to clonus and shorter durations of AD and clonus for the Sprague Dawley rats. This would seem to suggest faster breakdown of inhibitory circuits, but also faster recovery after that breakdown. However, some Sprague Dawley rats occasionally have "bimodal" or even "trimodal" distributions for both AD and convulsive activity. In other words, there is occasionally a break in AD and/or convulsion activity (ranging from 2 to over 75 seconds) before a recurrence of the AD or convulsion activity. This may be again followed by a break and a third recurrence of AD or convulsive activity. This variability occurs often enough in Sprague Dawley rats to consider it typical of the strain, and appears different from the occasional post convulsive episodic bursts of spiking activity described by Goddard et al. (4). The neurological differences suggested by these different convulsive patterns is intriguing and warrants further study. Also worth mentioning was the occasional spiking seen in 5 of the rats after the start of the experiment, but before stimulation on any given day. Racine (10) noted a similar occurrence in 20% of his rats. It is interesting that this was found only in the copper group (50% of the copper group had this occur at least once). Several times when this occurred the rats were left on the EEG machine for up to 20 minutes prior to stimulation. During that time, there was no evidence of the spiking activity abating.

In conclusion, the present study found that copper wire was less efficacious than the nichrome wire for producing kindling, and should, therefore, be avoided. Further, The Sprague Dawley strain was more inconsistent in its manifestations of kindling than the Wistar strain. It is also suggested that these strain differences reported here may serve as a useful independent variable in studies exploring inhibitory processes, but may not be preferable to the inbred strains of mice used by Leech (5) and Leech and McIntyre (6).

Acknowledgment

Special thanks is directed to Drs. William Farmen and G. Lee Griffith for their valuable assistance in data analysis. Both also provided statistical help, encouragement, advice and constructive criticism. This research was partially supported by the Center for Public Service, The Psychology Department and a Faculty Development Grant to C.K. Leech, all at Anderson College.

Literature Cited

1. Babb, T.L. and Kupfer, W. 1984. Phagocytic and metabolic reactions to chronologically implanted metal brain electrodes. *Experimental Neurology* 86:171-182.
2. Carrington, C.D., Ellinwood Jr., E.H. and Krishnan, R.R. 1984. Effects of single and repeated alcohol withdrawal on kindling. *Biological Psychiatry* 19(4):525-537.
3. Frank, R.A. and Stutz, R.M. 1982. Behavioral changes induced by basolateral amygdala self-stimulation. *Physiology and Behavior* 28:661-665.
4. Goddard, G.V., McIntyre, D.C. and Leech, C.K. 1969. A permanent change in brain function resulting from daily electrical stimulation. *Experimental Neurology* 25(3):295-330.
5. Leech, C.K. 1972. Rate of development of electrically kindled convulsions compared to audiogenic seizures and learning ability in six inbred mouse strains. Ph.D. Dissertation, University of Waterloo.
6. Leech, C.K. and McIntyre, D.C. 1976. Kindling rates in inbred mice: An analog to learning? *Behavioral Biology* 16:439-452.
7. McIntyre, D.C., Nathanson, D. and Edson, N. 1982. A new model of partial status epilepticus based on kindling. *Brain Research* 250:53-63.
8. McIntyre, D.C. and Roberts, D.C.S. 1983. Long-term reduction in beta-adrenergic receptor binding after amygdala kindling in rats. *Experimental Neurology* 82:17-24.
9. McNamara, J.O., Peper, A.M. and Patrone, V. 1980. Repeated seizures induce long-term increase in hippocampal benzodiazepine receptors. *Proc. Natl. Acad. Sci.* 77(5):3029-3032.
10. Racine, R. 1972. Modification of seizure activity by electrical stimulation: II. motor seizure. *Electroencephalography and Clinical Neurophysiology* 32:281-294.
11. Racine, R. 1978. Kindling: The first decade. *Neurosurgery* 3(2):234-252.
12. Shavit, Y., Caldecott-Hazard, S. and Liebeskind, J.C. 1984. Activating endogenous opioid systems by electroconvulsive shock or footshock stress inhibits recurrent kindled seizures in rats. *Brain Research* 305:203-207.
13. Welsh, K.A. and Gold, P.E. 1984. Attenuation of epileptogenesis: Proactive effect of a single ipinephrine injection on amygdaloid kindling. *Behavioral and Neural Biology* 40:179-185.