Journal of Neuroscience Methods, 27 (1989) 109–113
Elsevier

NSM 00905

A non-stereotaxic method for olfactory bulb kindling reveals distinct kindling rates among inbred mouse strains

Robert C. Green and Benjamin J. Elizondo

Departments of Neurology and Neuropathology, The Children's Hospital and Harvard Medical School, Boston, MA 02115 (U.S.A.)

(Received 2 June 1988)
(Revised 23 September 1988)
(Accepted 26 September 1988)

Key words: Epilepsy; Seizure; EEG; Limbic system

A simple method for olfactory bulb kindling in the unrestrained mouse is described. Twisted wire electrodes attached to a dip-socket head assembly were implanted in the olfactory bulb. A ground lead was secured to a single skull screw and implantation was visually guided into the olfactory bulb. In all properly implanted preparations, stimulation produced an afterdischarge with behavioral responses which progressed to fully kindled convulsions. Location within the olfactory bulb did not affect the kindling rate.

With this technique, mice from the C57BL/6J, C3H/He and DBA/2 inbred strains were kindled. Within each strain, kindling parameters were closely distributed about the group mean. Kindling rates in C3H and DBA inbred strains were significantly different from each other, but not significantly different from those reported in amygdala kindling.

Introduction

Kindling is a powerful animal model of epilepsy in which the repeated administration of subconvulsive electrical stimulation produces a progressive and predictable series of stimulus-induced motor seizure responses, culminating in a generalized seizure (Goddard et al., 1969). The kindling phenomenon has been demonstrated in many species and with stimulation in a number of neuroanatomic areas, but the most popular technique is stereotaxic implantation of electrodes into the amygdala or entorhinal cortex of the rat (McNamara et al., 1983). However, kindling parameters may differ widely depending upon which specific anatomic structure is implanted (Goddard et al., 1969), and histological verification is required to confirm the location of the implanted electrodes in most studies.

Olfactory bulb kindling has been elicited in the rat (Goddard et al., 1969; Cain, 1977; Attia et al., 1984; Lupica and Berman, 1988) and in the rabbit (Deluca, 1975). In these studies, stereotaxic implantation was used. But in smaller mammals, such as mice, stereotaxic surgery may be less accurate, particularly when different murine strains have slightly different stereotaxic coordinates (Slotnick and Leonard, 1975). In the course of kindling experiments in mutant mice, we have developed a simple and rapid technique for electrode implantation in the olfactory bulb which permits the acquisition of highly reproducible kindling data. In this report, we describe this method and demonstrate the consistency of kindling rates within each of several inbred strains of mouse.

Correspondence: R.C. Green, Emory Neurobehavioral Unit, Wesley Woods Hospital, 1821 Clifton Road, NE, Atlanta, GA 30029, U.S.A.

Methods

Bipolar electrodes were constructed from two 0.008 inch diameter twisted stainless steel wires, Teflon-insulated except at the cut tips (Plastic Products, Norfolk, VA) which were soldered onto the female ends of a screw mount dip socket IC series (Samtec, New Albany, IN). The wires were bent as shown in Fig. 1 and the ends were cut at a slight angle and separated by 0.5 mm. Mice were anesthetized with 0.02 ml/g intraperitoneal Avertin (tribromoethanol). Skull holes were drilled without injury to the dura and the electrode assembly was lowered into place under visual guidance. The implant was glued to the skull with dental acrylic. Attachment was facilitated by interposing a nylon mesh square (1000 μm, Small Parts, Miami, FL) between the skull surface and the electrode assembly (Fig. 1). A single 0–80 $\times$ 1/16 round head screw (Plastic Products, Norfolk, VA) provided additional stabilization for the implant as well as a grounding site for the electrode assembly (Fig. 1).

Stimulations began 4–7 days after implantation. The stimulus consisted of a 1 s train of 1 ms biphasic square-wave pulses at a frequency of 60 Hz (Grass Model S8 Stimulator). Stimulating electrodes were also used to record electrical activity from the olfactory bulb on a Grass Model 6 polygraph. Afterdischarge (AD) threshold (the lowest current required to elicit an AD) was determined by beginning with an initial stimulation of 100 μA and increasing in 50 μA increments. Each subsequent stimulation was delivered at 50 μA over the AD threshold. Experimental preparations which did not produce an AD at stimulation current levels of less that 800 μA were excluded from the study. Staging of the behavioral responses followed a modification of those established by Racine (1972): Stage 1, mouth and facial movements; Stage 2, repetitive vertical head and neck movements; Stage 3, forelimb clonus; Stage

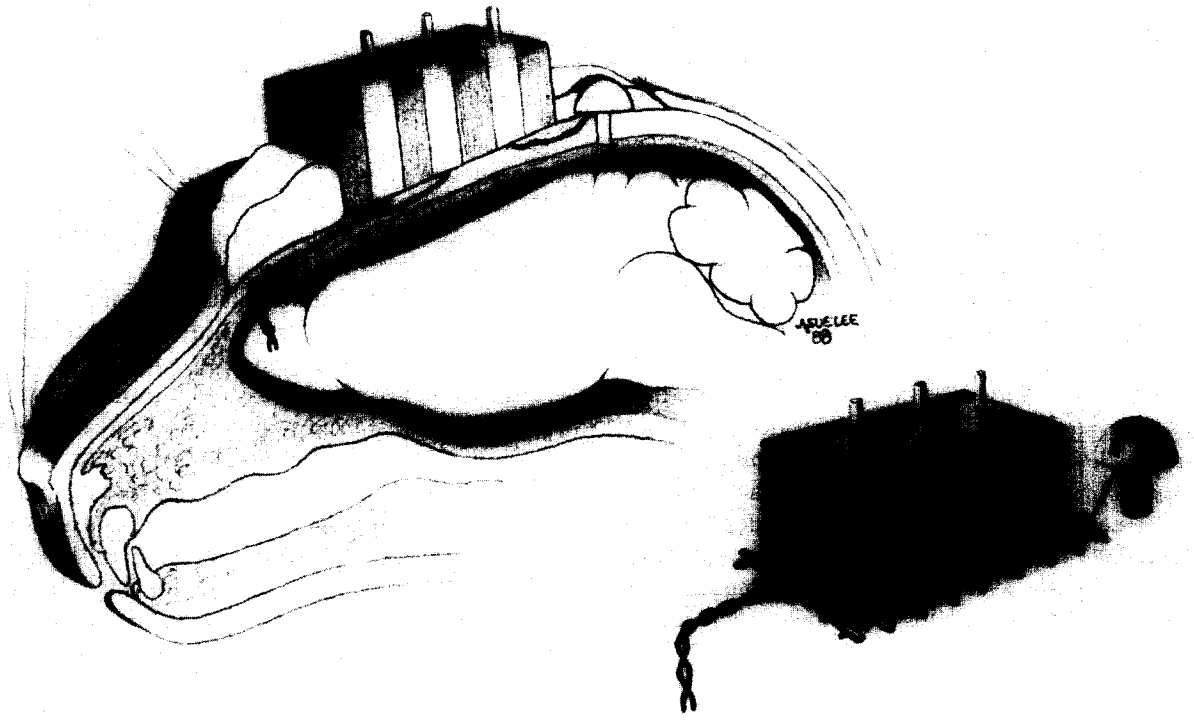

Fig. 1. Cutaway drawing on the left illustrates the placement of head assembly and implanted wires within the olfactory bulb. The intact assembly, including nylon mesh square, is shown on the right.

4, bilateral forelimb clonus and rearing; Stage 5, rearing, falling (loss of postural control) and/or hindlimb clonus.

Recorded measures included the AD threshold and duration, and the kindling rates. AD duration described the time from stimulus onset to the end of the electrographic AD. Kindling rates reflected the number of stimulations required to produce the first Stage 5 convulsion and the number of stimulations required to produce 6 consecutive Stage 5 convulsions. A description of the behavioral response was noted for each stimulation.

Nine animals were lesioned with 40 μA direct current for 60 s (Grass Model LM5) and sacrificed 3 days later for histology. The placement of electrodes in these animals was verified by histological examination of the olfactory bulb.

All statistical comparisons were made using Student's two-tailed, unpaired *t*-test.

Results and Discussion

Histological characteristics of effective implantation

After a brief experimenter training period, an afterdischarge potential was obtained in approximately 80% of implantations and all preparations which produced an AD were fully kindled. The head assembly was lost in less than 5% of the preparations during the kindling period. Examination of animals in whom ADs were successfully produced revealed lesions indicating electrode placement within the olfactory bulb in the internal granular layer (as in Fig. 2), with occasional overlap into the glomerular and external plexiform layers. In all cases where kindling was unsuccessful, the electrode tips were not located within the olfactory bulbs, but had penetrated too deeply or at an angle, so that they had passed through the parenchyma of the bulbs altogether. These observations suggest that placement anywhere within the parenchyma of the mouse olfactory bulb is sufficient for successful kindling.

Consistency of kindling parameters in a population of inbred mice

Olfactory bulb implantation was used to kindle 13 inbred mice of the C57BL/6J strain of both sexes and ranging from 2–9 months of age. Table I describes the mean AD threshold, AD duration and number of stimulations required to produce the first and sixth consecutive stage 5 seizure. The results reveal standard error variations of 12.8% about the group mean for the AD threshold, and variations of 5% or less for all other measures. Thus, olfactory bulb kindling within an inbred strain of mice can produce consistent results.

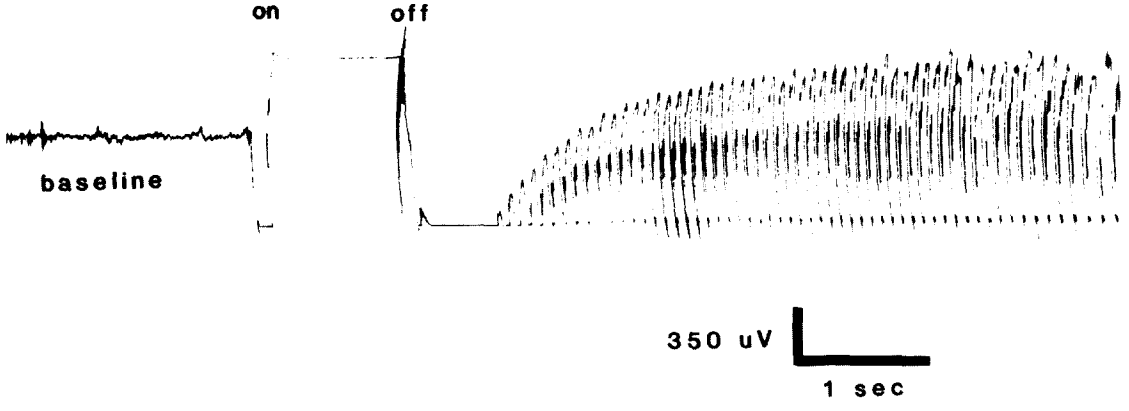

Fig. 2. This electrographic recording reveals the low-voltage baseline prior to stimulation and the start of a typical afterdischarge after stimulation. The first second stimulation is designated by the on/off labels. The flattened portion of the tracing at the start of the afterdischarge represents a brief saturation of the amplifiers.

TABLE I

KINDLING PARAMETERS OF THE C57BL/6J INBRED STRAIN

Values are the means ± the standard error ($n = 13$). Values in parentheses are the percentage deviation of the standard errors from the means.

AD threshold (μA)	AD duration at first Stage 5 (s)	Stimulations to first Stage 5	Stimulations to sixth consecutive Stage 5
475.0 ± 60.8 (12.8%)	20.8 ± 1.038 (5%)	8.7 ± 0.308 (3.5%)	13.8 ± 0.207 (1.5%)

Comparison of olfactory bulb kindling rates to amygdala kindling rates

The only previously reported study of kindling rates in mouse strains revealed significant differences in amygdala kindling rates between C3H/He and DBA/2 strains (Leech and McIntyre, 1976). We compared their results with olfactory bulb kindling in the same strains (Table II). As with amygdala kindling, olfactory bulb kindling to the first stage 5 seizure required significantly fewer trials in DBA/2 mice than in C3H/He mice ($P < 0.001$). Comparisons with the Leech and McIntyre data revealed no significant difference between the rates of amygdala kindling and olfactory bulb kindling within each inbred strain.

In the rat, both Goddard et al. (1969) and Cain (1977) described olfactory bulb kindling rates that were different from those in the amygdala. However, Goddard et al. (1969) counted non-AD producing stimulations in very few animals as the basis for their comparison, while Cain (1977) re-

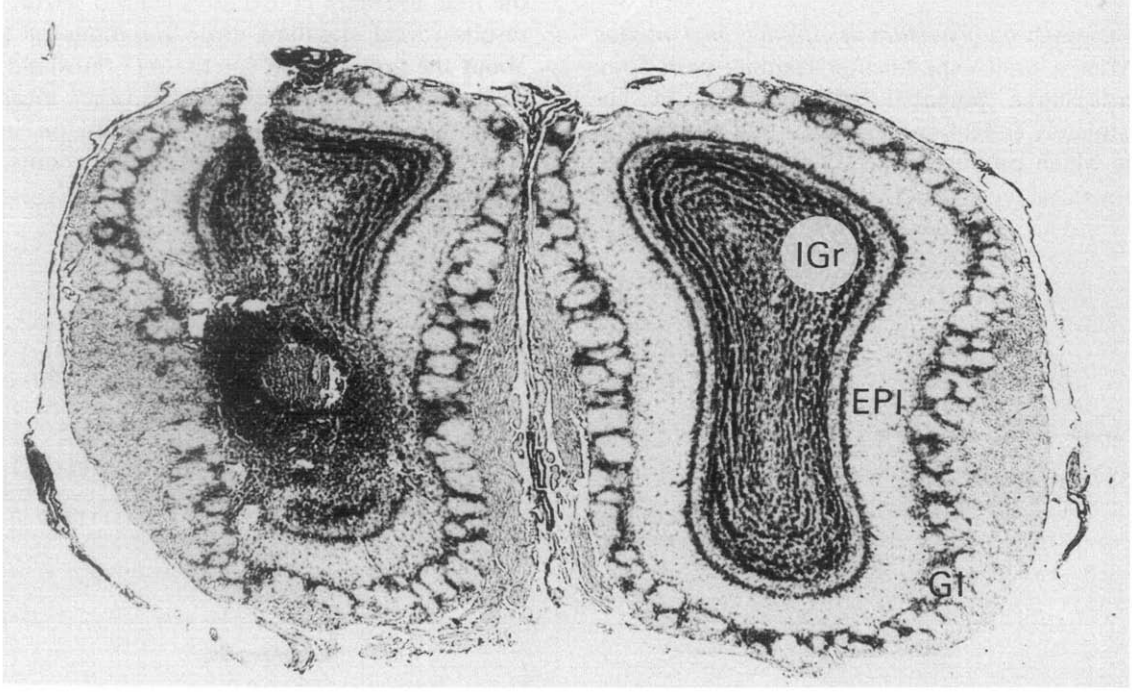

Fig. 3. Electrode placement was histologically verified by serial coronal section and Nissl staining of the olfactory bulbs in selected animals. In this example, a circle of reactive cells marks the site of the electrode tips within the left internal granule cell layer. Olfactory bulb kindling was successfully obtained with implantations in the different portions of the internal granule cell layer (IGr) with lesions extending into the external plexiform layer (EPl) and glomerular layer (Gl).

TABLE II

COMPARISON OF OLFACTORY BULB TO AMYGDALA KINDLING RATES IN C3H/He AND DBA/2 INBRED STRAINS

Strain	Amygdala stimulations to first stage 5 (Leech and McIntyre, 1976)	OB Stimulations to first Stage 5
C3H/He	17.1 ± 1.637 ($n = 26$)	21.8 ± 3.543 ($n = 6$)
DBA/2	5.0 ± 1.52 ($n = 22$)	4.0 ± 0.577 ($n = 6$)

Values are the means $\pm$ the standard error. Amygdala vs OB kindling rate in C3H/He, $p = 0.19$ Amygdala vs OB kindling rate in DBA/2, $p = 0.74$.

ported a smaller difference (in the opposite direction); and neither offered statistical comparisons. Equivalent kindling rates from olfactory bulb and amygdala in the mouse might be expected, in light of the direct and reciprocal connections between these two structures which have been demonstrated in a number of rodent species (Powell et al., 1965). We conclude that olfactory bulb kindling provides a simple, rapid and highly reproducible alternative to amygdala kindling in the mouse.

Acknowledgements

This work was supported by the Penfield Fellowship of the Epilepsy Foundation of America (RCG) and NIH grant NS 20820 (Dr. Sidman). We appreciate the generous loan of equipment from Mrs. Ellen Grass and the Grass Corporation. We thank Jim Cote and Clair Kerr for technical assistance and Craig Applegate for helpful suggestions.

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