KINDLING IN GENETICALLY ALTERED MICE

Implications for the Role of LTP in Kindling

Donald P. Cain

Department of Psychology and
Graduate Program in Neuroscience
University of Western Ontario
London, Ontario N6A 5C2, Canada

1. INTRODUCTION

Kindling is a neuroplastic process that appears to involve the lasting alteration of synaptic function. This implies that kindling may require a neural growth process for some aspect of its establishment. The fact that inhibition of protein synthesis blocks kindling without affecting the epileptiform afterdischarge (AD) that normally leads to its development [8] is consistent with that suggestion, as are reports of kindling-induced neural alterations and sprouting at the ultrastructural level [17,18,51].

If a neural growth process is involved in kindling, it seems likely that this would require the transcription of information from genes for the subsequent synthesis of protein. Activity-induced gene expression has been proposed as a potential mechanism for the conversion of short term neural activity, such as epileptiform AD, into long term changes in the nervous system, such as kindled seizure susceptibility [15,43]. One method of evaluating this possibility is by measuring gene expression during and after kindling. This approach has shown that kindling is associated with the expression of immediate-early genes [11,15,52]. Although this method is useful, it relies fundamentally on correlative data, and it is not clear whether any of the genes studied so far are crucial for kindling, and if they are crucial, what their role is in the mechanism of kindling.

In addition to measuring gene expression, it is now possible to engineer a defined mutation into a mouse germ line by gene-targeting, thereby creating mutant mice that can be neurologically evaluated [22]. An advantage of gene-targeting is that the interpretation of the seizure phenotype can be simplified by the prior knowledge of the neural expression pattern and biochemical function of the targeted gene, which may provide insights into the molecular mechanisms of epilepsy.

One form of gene-targeting involves a gene knockout technique that creates a mutated version of a targeted gene, rendering it inactive. The result is a mouse that fails to
express the product of the inactivated gene. A second form of gene-targeting involves modification of a gene in order to increase or decrease its activity. Such transgenic mouse strains express the gene product, but at levels higher or lower than normal. Knockout and transgenic strains have the potential to allow a functional analysis of a gene's role in biological processes.

Among the knockout and transgenic mouse strains developed to date, those developed for the study of long term potentiation (LTP) and learning are of particular interest. LTP is a lasting increase in neural transmission at specific synapses that results from the application of brief high-frequency trains of electrical pulses through electrodes. NMDA-mediated LTP has been studied as a laboratory model of the kind of plastic change that could underlie learning and memory [3], and NMDA/LTP mechanisms have been hypothesized to underlie kindling [2,12,14,30,31,33,34,48,53]. Therefore, it would be of interest to examine kindling in the same strains of mouse that harbor genetic alterations known to affect the induction of LTP. The strains examined in the experiments discussed here harbor genetic alterations in the Fyn tyrosine kinase gene [21], the CaMKII serine-threonine protein kinase gene [32], and the GluR2 subunit of the AMPA receptor gene [27]. Each of these strains has been shown to exhibit both altered LTP and altered behavior. We chose to study electrical kindling in the amygdala, a structure that readily kindles in the mouse [8].

2. GENERAL METHODS

Surgery for the bilateral implantation of indwelling amygdaloid electrodes was accomplished under pentobarbital anesthesia (60–80 mg/kg) after preadministration of 10 mg xylazine. Electrodes were constructed of twisted teflon-insulated Nichrome wire 127 μm in diameter soldered to gold-plated connector pins. Implantation was into the basolateral amygdala at the following coordinates, using standard stereotaxic techniques determined using pilot animals: Anterior-posterior + 2.5 mm relative to interaural zero; medial-lateral 3.3 mm; 5.3 mm ventral to skull surface. A miniature connector and leads attached the pins to brain stimulation and recording equipment. Histological examination confirmed the accuracy of the electrode placements.

After 10 days recovery, trains of biphasic square wave pulses (1.0 msec each, 60 pulse-pairs per sec, for 1.0 sec) were applied through the electrode at increasing intensity to determine AD threshold. Once-daily stimulation at 110% AD threshold was applied beginning 24 hr later until 3 generalized convulsions occurred. Stimulation was applied between 10:00 am and 2:00 pm at the same time for all groups. Electrographic records were obtained from the electrodes before and after each stimulation using a Grass polygraph. AD occurred in response to each suprathreshold stimulation. Kindling sessions of some mice were videotaped, and the tapes were replayed for scoring of the convulsions using Racine's categories [37], with stage 1 indicating brief behavioral immobility with ear flattening or twitching of the facial musculature and stage 5 indicating generalized convulsions. In cases in which videotapes were not made, convulsive behavior was scored by observation of the mice during kindling. After expression of 3 stage 5 convulsions or 30 kindling sessions with AD, mice were allowed 3 to 4 weeks without stimulation and then rekindled with the same electrical stimulation as a measure of retention of kindling.

Spontaneous behavioral activity was measured in some mice using an automated behavior monitoring device (Digiscan, Omnitech) that consisted of 6 monitors (40 × 40 × 30 cm), each of which contained 2 tiers of infrared sensors to measure horizontal (locomotion) and vertical (rearing) movements. A total of 12 behavioral activity variables was