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Genetic resistance to kindling associated with alterations in circuit function

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ABSTRACT

How a seizure spreads from a focal onset zone to other regions of the brain is not well understood, and animal studies suggest that there is a genetic influence. To understand how genetic factors may influence seizure spread, we examined whether the kindling resistance of WAG/Rij rats, which are slow to develop kindled motor seizures, is independent of the site of seizure induction and thus a global phenomenon, or whether it is circuit specific.
We compared the kindling rates (number of stimulations to induce kindled motor seizures) of WAG/Rij rats to the rates of kindling in Sprague Dawley rats. Both groups underwent a standard hippocampal kindling protocol and a separate group was kindled from the medial dorsal nucleus of the thalamus, a site that has been previously demonstrated to result in the very rapid development of motor seizures. To examine whether there were differences in the interaction in a circuit involved with the motor seizures, evoked responses were obtained from the prefrontal cortex following stimulation of the subiculum or medial dorsal thalamic nucleus.
The WAG/Rij rats once again demonstrated resistance to kindling in the hippocampus, but both strains kindled rapidly from the medial dorsal nucleus. In the WAG/Rij rats there was also a reduction in the duration of the afterdischarge in the frontal cortex during hippocampal stimulation, but there was no reduction during thalamic kindling. The prefrontal cortex evoked responses were reduced following stimulation of the subiculum in the WAG/Rij rats, but the evoked responses to thalamic stimulation were the same in both strains.
These findings suggest that there are genetic influences in the strength of the input from the subiculum to the prefrontal cortex in WAG/Rij rats that could explain the resistance to limbic kindling because of reduced excitatory drive onto a key target region.

1. Introduction

The process by which seizure activity spreads from one part of the brain to another is not well understood. A common mechanism hypothesized for seizure spread is the progressive recruitment of adjacent regions from a single seizure onset zone, the so called Jacksonian march. There are times, however, when seizure activity appears simultaneously in areas that are not adjacent, an observation that is not consistent with Jacksonian march (Rektor et al., 2009). A significant literature reports that subcortical regions can influence the spread of seizure activity (Gale, 1992; Garant et al., 1993; Moshe et al., 1995). These observations suggest a more complex network interaction. Clinically it has been noted that some individuals with the same seizure syndrome (i.e. limbic epilepsy) differ in their propensity to have secondarily generalized convulsions (Thom et al., 2010). This observation raises the possibility that there are differences in the underlying circuits supporting seizure spread.

The kindling model of focal seizures (Goddard et al., 1969) in which seizures evolve from non-convulsive to convulsive, can help elucidate the mechanisms of seizure spread. In previous work we and others have shown that the midline thalamic nuclei are a key circuit component of limbic seizures and that modulating these nuclei can influence seizure spread (Cassidy and Gale, 1998; Miller and Ferrendelli, 1990; Bertram et al., 2001, 2008). These thalamic nuclei are closely connected to the hippocampus, entorhinal cortex and amygdala as well as the prefrontal cortex (Herkenham, 1978; Yanagihara et al., 1987; Su and Bentivoglio, 1990; Bertram and Zhang, 1999; Zhang and Bertram, 2002; Sloan and Bertram, 2009: Wouterlood et al., 1990; Turner and Herkenham, 1991; Van Groen and Wyss, 1990; Berendse and Groenewegen, 1991). Kindling stimulation of this midline thalamic region has been the fastest route to generalization of limbic seizures (Bertram and Williamson, 2005). Subsequent studies demonstrated that the medial dorsal thalamic nucleus (MD) plays a key role in seizure initiation and spread as part of a divergent convergent excitation

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amplification circuit (Sloan et al., 2011a, 2011b). Others have shown that the responses in the frontal cortex from hippocampal stimulation can be gated through the MD (Floresco and Grace, 2003).

Rats with genetic absence seizures are resistant to developing convulsions during limbic kindling (Eskazan et al., 2002; Aker et al., 2006; Onat et al., 2007; Akman et al., 2008; Akman et al., 2010) as well as audiogenic kindling (Vinogradova, 2008). This resistance makes them a potential tool for elucidating the neurobiology of seizure spread. To explore the question of whether resistance to seizure generalization was global or circuit specific, we used our previous results showing very rapid kindling from the midline region of the thalamus to determine whether WAG/Rij rats were kindling resistant to thalamic stimulation. Continued resistance would suggest a more global circuitry issue. Our finding in this study that they kindle as rapidly from the midline nuclei as Sprague-Dawley rats suggest that there are differences between the strains in connectivity of the limbic system to other regions. To begin that evaluation we examined the interaction of key circuit components in seizure spread to determine whether there were alterations in the connections among potential targets of seizure spread. In this study we demonstrate for the first time that there are genetically related differences in the strength of neuronal circuits that influence the process of seizure spread.

2. Materials and methods

All animals were used under protocols approved by the Animal Care and Use Committee (ACUC) of the University of Virginia. Animals were maintained under standard laboratory conditions on a 12/12 hour light/dark cycle and were allowed free access to food and water.

2.1. Groups for study

2.1.1. Sprague-Dawley rats

Adult rats (male and female) were obtained from Harlan (Indianapolis, Indiana, USA). The animals were age matched to the WAG/Rij rats.

2.1.2. WAG/Rij

Adult male and female rats were obtained from a breeding colony at the University of Virginia that descended from two breeding pairs that came from the Netherlands. All animals were between 4 and 6 months of age, at which point they had developed spike wave discharges. The presence of the spike wave discharges was confirmed prior to kindling to assure that each rat had the EEG phenotype.

2.2. Kindling studies

This set of experiments was performed with two goals: 1) to confirm that WAG/Rij rats were resistant to standard limbic kindling and 2) to evaluate whether kindling resistance was also found when kindling in the MD.

Kindling resistance in this study is defined as the inability to achieve a fully kindled state (three consecutive Racine stage 4 or 5 seizures) after 50 induced afterdischarges. In Sprague-Dawley rats it typically requires 20 to30 induced afterdischarges to achieve full kindling with our protocol. The MD was chosen as the site of comparison as previous studies have shown that Sprague-Dawley rats typically have convulsions very rapidly (5 or fewer stimulations) when stimulated in this nucleus. We had also shown that the MD was consistently involved in the initial stages of seizures arising in the limbic system (Bertram et al., 2001).

2.3. Surgery

Under 2% isoflurane anesthesia rats were placed in a multi-arm stereotaxic frame (Kopf Instruments). Body temperature was maintained by a heated water blanket. Teflon-coated, twisted-pair, stainless steel, bipolar electrodes were implanted either in the MD, [from bregma, −2.3 mm anteroposterior (AP), +1.0 mm mediolateral (ML), −5.8 mm dorsoventral (DV), 10° angle], or in the CA1 of the mid-ventral hippocampus (from bregma, −5.1 mm AP, +4.9 mm ML, −5.0 mm DV). The thalamic electrodes were placed so that the two tips were in an anterior-posterior relationship. There were also two monopolar cortical electrodes placed over the frontal cortex (from bregma, +3.0 mm AP, ±2.0 mm ML) or over the frontal and parietal cortices (parietal electrode position −1.5 mm AP, +2.0 ML). All electrodes were secured with dental acrylic and skull screws. Coordinates were obtained from a standard stereotaxic atlas (Paxinos and Watson, 1998).

2.4. EEG recording

After 7 days of recovery animals were placed in our rodent epilepsy monitoring unit that has been standard at our institution for over 20 years (Bertram et al., 1997; Bertram, 2006). Baseline EEG was recorded for up to 24 h from WAG/Rij rats to confirm the presence of spike wave activity. Each rat was placed in custom plexiglass containers and connected to the stimulator and EEG through a cable and electrical swivel. The electrodes for stimulation were connected to the EEG through a manual switch which was activated to alternate between the EEG machine and stimulator (A-M Systems Model 1260) without disturbing the rats or moving the wires. Signals were amplified through a Grass Model 12 analog amplifier and recorded with Harmonie digital EEG software (Stellate Systems, Montreal). Behavioral data were video recorded, digitized and synchronized with the EEG through the software.

2.5. Hippocampal kindling

In this set of experiments we wished to confirm the previously reported kindling resistance of WAG/Rij rats and to determine if there were differences in the recruitment of the neocortex into seizure activity.

WAG/Rij (n = 9) and SD (n = 9) rats were kindled with 6 stimuli (50 Hz, 200 μA peak to peak biphasic square wave in 2 second stimulation trains) at one hour intervals on Mondays, Wednesdays and Fridays (6 stimuli per day), until they experienced three consecutive stage 4 or 5 seizures, or they completed 50 stimulations. This protocol is a modification of the Lothman rapid kindling protocol that has been standard in the laboratory (Lothman and Williamson, 1993). Afterdischarge duration for each of the recording sites was measured (in seconds) and the behavioral seizure score (Racine 5 point scale) was determined (Racine, 1972).

2.6. Thalamic (MD nucleus) kindling

MD kindling was performed on WAG/Rij (n = 7) and SD (n = 5) rats. The kindling protocol was the same as for hippocampal kindling except that the stimulation intensity was increased to 400 μA peak to peak and the stimulation duration increased to 10 s because the afterdischarge threshold in the MD is significantly greater than in the hippocampus. A separate set of rats (SD = 5 and WAG/Rij = 4) were evaluated for frontal cortex involvement during MD kindling. This step was taken because of the differences found in cortical involvement following hippocampal kindling. An MD stimulating electrode was placed as above and left and right recording electrodes were placed over the prefrontal cortex (Bertram and Williamson, 2005).

2.7. In vivo physiology

Because the results from the kindling experiments indicated that there was a difference between the two strains with regard to the recruitment of the prefrontal cortex during hippocampal kindling we wished to determine whether there were differences in limbic-prefrontal and thalamic-prefrontal circuits between WAG/Rij and SD rats.