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Research Report

Pontine reticular formation neurons are implicated in the neuronal network for generalized clonic seizures which is intensified by audiogenic kindling

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Abstract

The caudal pontine reticular formation nucleus (cPRF) is implicated in seizure propagation to the spinal cord in several forms of generalized convulsive seizures, including audiogenic seizures (AGS). Focal microinjection studies implicate cPRF as a requisite neuronal network site subserving generalized AGS in the moderate severity substrain of genetically epilepsy-prone rats (GEPR-3s). AGS in GEPR-3s culminate in generalized clonus, but daily repetition of AGS (AGS kindling) results in an additional seizure behavior, facial and forelimb (F and F) clonus, not seen prior to kindling. This study examined cPRF neuronal firing changes and seizure behaviors during AGS in GEPR-3s. We examined extracellular cPRF neuronal responses to acoustic stimuli (12 kHz) and observed neuronal firing during AGS. cPRF neurons exhibited onset responses to acoustic stimuli before and after AGS kindling. After AGS kindling, increased neuronal firing occurred, and response latencies were prolonged. Tonic neuronal firing occurred during generalized clonus, which changed to burst firing after AGS kindling. Burst firing also occurred during F and F clonus. Increased neuronal firing and the change from tonic to burst firing suggest that AGS kindling involves increased cPRF excitability. These data support an important role for cPRF neurons in generation of generalized clonus in unkindled GEPR-3s, which is increased by AGS kindling. The increased cPRF response latency might reflect a greater role of rostral components of the AGS neuronal network in transmission of acoustic responses to cPRF. This study also suggests that cPRF neurons may be involved in F and F clonus, which was unexpected since F and F clonus is thought to originate primarily in forebrain structures.

Theme: Disorders of the nervous system
Topic: Epilepsy: human studies and animal models

Keywords: Audiogenic seizure; Kindling; NMDA receptor; Convulsion; Pontine reticular formation; Genetically epilepsy-prone rat

1. Introduction

Genetically epilepsy-prone rats (GEPRs) exhibit abnormal sensitivity to many seizure-inducing treatments and display convulsive audiogenic seizures (AGS) in response to acoustic stimuli [8,37]. Two substrains of GEPRs exist, GEPR-9s that exhibit tonic hind limb extension and GEPR-3s that exhibit generalized clonic seizures. The numerical value in each substrain designation refers to an AGS score based on the scale of Jobe et al. [22]. The neuronal networks subserving AGS in both substrains have been examined, and major similarities and certain differences in the participation of certain brain sites have been observed [10,34–36].

The caudal pontine reticular formation nucleus (cPRF) is implicated in seizure propagation to the spinal cord in several forms of generalized convulsive seizures, including AGS in both GEPR-3s and GEPR-9s [12,13,31,34]. An important role for the cPRF was also shown in GEPR-3s, since microinjection of NMDA receptor antagonists in this site completely blocks AGS [34]. The cPRF is implicated in the AGS network in GEPR-9s, and neurons in the cPRF of GEPR-9s exhibit a precipitous onset of neuronal firing at lower acoustic intensities as compared to normal rats. GEPR-9s display a rapid tonic neuronal firing pattern

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immediately prior to the tonic hind limb extension phase of AGS [12]. A significantly lower intensity electrical stimulus in the cPRF of GEPR-9s is required to produce seizures similar to AGS as compared to normal rats [3]. After continued and repeated ethanol administration, ethanol withdrawn (ETX) rats often exhibit generalized clonic seizures behaviorally similar to those in GEPR-3s, and these AGS are also completely blocked by focal microinjection of a NMDA receptor antagonist into cPRF. In ETX rats, cPRF neurons exhibit elevated neuronal responsiveness to acoustic stimuli. The role of cPRF in generalized clonic seizure behavior observed in GEPR-3s is unknown, and examination of neuronal firing changes in GEPR-3s was a major goal of the current study.

Repeated induction of AGS (AGS kindling) in GEPR-3s results in prolongation of seizures and the appearance of facial and forelimb (F and F) clonic seizure behavior, which is not seen in GEPR-3s prior to AGS kindling [29,35,36]. F and F clonus is also induced in Wistar AGS-susceptible rats by AGS kindling [25,27]. F and F clonus is a common feature of several types of forebrain seizures [25,33,39]. However, the neuronal mechanisms that mediate F and F clonic behavior and whether brainstem pathways play a role in the seizure network for these convulsions are unclear. Recent studies in GEPR-3s indicated that AGS kindling induced major changes in the neuronal firing exhibited by neurons in the amygdala, and perirhinal cortex [35,36]. However, whether changes in neuronal firing occur in the cPRF as a result of AGS kindling is unknown. Therefore, the present study examined cPRF neuronal firing in GEPR-3s before and after AGS kindling. Extracellular action potentials in the cPRF of behaving GEPR-3s were recorded in response to acoustic stimuli and during AGS to examine the relationship between neuronal firing pattern changes and seizure behaviors.

2. Materials and methods

2.1. Animals

GEPR-3s (250–400 g) of either sex were used in this study. All animals were screened for AGS susceptibility at weekly intervals for 3 weeks according to the established screening procedures for these animals [37]. Data from 9 non-kindled and 8 kindled GEPR-3s were involved in the study. The experimental protocols used in this study were approved by the Laboratory Animal Care and Use Committee of Southern Illinois University School of Medicine.

2.2. AGS kindling

GEPR-3s were kindled by inducing an audiogenic seizure (AGS) (described later) once daily between 8:00 AM and 10:00 AM for 21 days [29].

2.3. Microwire electrode implantation

Microwire electrodes consisted of an amphenol connector (Dale Electronics, Yankton, SD, USA) connected to a bundle of six 13 μm Teflon-coated tungsten wires (Resistance of ~1 MΩ, California Fine Wire Company, Grover City, CA, USA), used previously to record extracellular neuronal activity in response to acoustic stimuli and during seizure activity [1,17,35,36]. The microwires were adhered together before implantation by coating them with polyethylene glycol, which melted after implantation as it reached brain temperature, allowing a small amount of movement to the microwire tips at the implantation site. The head was placed in the flat skull orientation, based on setting the incisor bar at −3.3 mm. The electrodes were stereotaxically implanted using the following coordinates: (Posterior: 10.8 ± 0.5 from bregma; Lateral to the midline: ±0.8; Ventral from brain surface: 8.6) according to the atlas of Paxinos and Watson [32]. Animals were given tetracycline (1 g/l) in drinking water for 1 week during the recovery period. At least 1 week of recovery time between surgery and recording of neuronal activity was given for non-kindled animals and 10 days (based on recovery time for AGS susceptibility) for AGS-kindled animals.

2.4. Acoustic stimulation

Acoustic stimuli were generated by Grason Stadler logic modules and delivered through a speaker placed 40 cm from the center of a transparent plastic chamber and calibrated to dB SPL (re:0.0002 dyn/cm^2). cPRF neuronal activity was recorded in response to 12 kHz pure tone bursts, which have been shown to be most effective for induction of AGS [14]. Pure tone burst stimuli (12 kHz frequency, 100 ms duration, 5 ms rise and fall) were presented at acoustic intensities ranging from 70 to 110 dB SPL in 5 dB SPL increments.

2.5. Neuronal data acquisition and analysis

The animals were placed in a transparent cylinder (40 cm diameter) within a sound-attenuating chamber (Industrial Acoustics, Bronx, NY, USA), and a cable attached to a differential OP-AMP was connected to the microwire assembly implanted on the animal. To record neuronal activity in response to acoustic stimuli, 12 kHz tone bursts were given at a rate of 0.5 Hz. To induce seizures, a stimulation rate of 4.0 Hz was needed. The neuronal firing and the seizure behavior of each animal were recorded simultaneously on a split-screen video for correlation of the seizure behavior with the pattern of neuronal firing. Neuronal firing was recorded on a magnetic tape, and single unit responses were analyzed off-line by generating poststimulus-time histograms (PSTH) (1 ms bin width, 200 ms scan length), using a Macintosh Quadra 800 and MacLab 4 channel recorder. Single unit activity was displayed using a Macintosh-based digital oscilloscope