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LATERAL ASYMMETRY OF EARLY SEIZURE MANIFESTATIONS IN EXPERIMENTAL GENERALIZED EPILEPSY

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Abstract—Reorganization of seizure networks during epileptogenesis involves cortico-subcortical and interhemispheric interactions. In the audiogenic kindling (AK) model of generalized tonic–clonic seizures, upstream seizure propagation along ascending brainstem-to-forebrain pathways determines progressive intensification of repeated sound-induced convulsions. Full-blown audiogenic seizures are bilaterally symmetric and their repetition results in bisynchronous recruiting the cortex in secondary epileptogenesis. The present study describes lateral asymmetry of initial behavioral and EEG manifestations of audiogenic seizures and AK in Wistar and WAG/Rij rats with acoustic hypersensitivity. These rats exhibit consistent individual lateralization of running seizures (run directionality) induced by repeated binaural stimulation. Since this initial preconvulsive running reflects seizure onset in the auditory brainstem, the running asymmetry suggests non-symmetric early epileptic activation of brainstem substrates by sound in these rats. Repetition of the asymmetric brainstem seizures led to asynchronous recruiting the cortex into seizure network and lateralization of running seizures was predictive for asymmetry of early cortical seizure manifestations in Wistar and WAG/Rij rats. Both electrographic markers of AK, spreading depression (SD) and post-running afterdischarge, first appeared in the cortex ipsilateral to run direction, suggesting lateralized brainstem-to-forebrain seizure generalization during AK. At the population level, no bias in lateralization of running and SD was found in Wistar and WAG/Rij rats but incidence of secondary cortical seizures varied, depending on strain and run laterality. Among Wistar rats, cortical seizures developed more rarely in right-runners than in left-runners, suggesting enhanced resistance of the right hemisphere to epileptogenesis in rats of this strain. WAG/Rij rats with mixed (absence and audiogenic) epilepsy showed weak lateralization of early cortical seizures and no left–right difference in their incidence during AK. Present findings suggest (1) lateralized brainstem-to-forebrain seizure propagation and hemispheric difference in its facility in Wistar rats, (2) alterations of intra- and interhemispheric seizure propagation in WAG/Rij rats with genetic absence epilepsy.

Key words: lateralization, audiogenic seizure, kindling, spreading depression, absence seizures, epileptogenesis.

INTRODUCTION

Epileptic seizures are network phenomena reflecting abnormal activation of spatially restricted (focal seizures) or widely distributed (generalized seizures) neuronal networks (Engel, 2006). Lateral asymmetry of seizure manifestations is an important clinical sign in epilepsy; seizures limited to one hemisphere are referred to as focal ones while bilateral epileptogenic abnormalities are markers of generalized epilepsy. But growing evidence points to a lack of clear dichotomy between focal and generalized epilepsies and indicates that focal activation of bilateral networks may underlie generalized seizures (Meeren et al., 2002; McNally and Blumenfeld, 2004; Engel, 2006). Furthermore, lateral asymmetry of cortical hyperexcitability, lateralized EEG and motor ictal manifestations have been reported in human and experimental generalized epilepsy (Myslobodsky and Rosen, 1979; Blume, 1998).

Cortico-subcortical interactions have been shown to play an important role in expression of generalized seizures: cortico-thalamic oscillations underlie generalized absence seizures (Meeren et al., 2002; Coenen and van Luijtelaar, 2003), brainstem regions are involved in generalized tonic–clonic seizure network (Browning et al., 1985; Faingold, 2004; Blumenfeld et al., 2009). A valuable experimental approach to investigate brainstem–forebrain network interactions underlying generalized tonic–clonic seizures is the audiogenic kindling (AK) model in which prolongation of repeated audiogenic seizures depends on propagation of epileptic activity along ascending pathways from the brainstem to forebrain (Marescaux et al., 1987; Faingold, 2004; Jobe and Browning, 2006). Primary focus of these seizures, triggered as a reflex response to acoustic stimulation of susceptible rodents, localizes in the auditory brainstem, specifically in the inferior colliculus (IC) (Krushinsky et al., 1970; Browning et al., 1985; Garcia-Cairasco et al., 1993; Faingold, 2004). The forebrain is not involved in expression of acutely induced audiogenic seizures but becomes recruited secondarily after repeated seizures. This secondary epileptogenesis associated with development of additional clonic seizures and epileptiform discharges in the cortex depends on upstream seizure propagation along brainstem-to-forebrain projections.

Although a full-blown audiogenic convulsion represents non-lateralized tonic/clonic seizure reflecting bilateral epileptic activation of brainstem seizure

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Abbreviations: AD, afterdischarge; AK, audiogenic kindling; IC, inferior colliculus; SWDs, spike-wave discharges; SD, spreading depression.

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substrates, the seizure appears not to be generalized from the start. Audiogenic convulsions always begin with a brief period of running which reflects the earliest seizure activation in the IC before widespread excitation of brainstem seizure networks responsible for development of generalized convulsions (Jobe and Browning, 2006). A motor asymmetry of the initial running behavior, suggesting lateralized seizure onset at the brainstem level, has been reported in mice (Ward and Collins, 1971) and audiogenic Wistar rats (Pierson, 1992; Garcia-Cairasco et al., 1993; Vinogradova, 2010).

Repetition of full-blown audiogenic seizures with bilaterally symmetric pattern evokes bisynchronous secondary recruitment of the cortex into epileptic network (Simler et al., 1999) and studies on AK produced by full-blown audiogenic seizures do not usually distinguish seizure responses of the two hemispheres. However, in the mild AK paradigm produced by repeated running seizures, non-symmetric epileptic activation of the cortex has been recently described (Vinogradova, 2010). Unidirectional running and unilateral cortical spreading depression (SD) are highly reproducible lateralized phenomena observed upon repeated binaural sound stimulation of audiogenic Wistar rats.

SD, a wave of transient cellular depolarization, is known as a hallmark of neuronal hyperexcitation and a frequent consequence of seizures (Leao, 1944; Bures et al., 1974; Somjen, 2001). Being triggered in one hemisphere, cortical SD non-synaptically propagates over the cortex of the hemisphere at a speed of about 3 mm/min but never invades the contralateral cortex. During AK in Wistar rats cortical SD appears as a unilateral event indicating non-synchronous recruitment of the cortex of the two hemispheres into audiogenic seizure network (Vinogradova et al., 2009). Similar unilateral pattern of cortical SD, implying asymmetric secondary activation of the cortex, has been previously recorded during AK in WAG/Rij rats with mixed (absence and audiogenic) generalized epilepsy (Vinogradova et al., 2006).

WAG/Rij rats derived from Wistar strain as a genetic model of absence epilepsy exhibit spontaneous bisymmetrical non-convulsive spike-wave discharges (SWDs) in the EEG (Coenen and van Luijtelaar, 2003). A population of these rats has a mixed form of generalized epilepsy, displaying both spontaneously occurring SWDs and acoustically induced convulsive seizures. Therefore, these rats represent a useful model for investigation of mechanisms underlying coexistence of different seizure types and overlapping seizure networks. Our previous studies have shown identical patterns of audiogenic seizures in Wistar and WAG/Rij rats (Vinogradova, 2008) that suggest independent function of thalamo-cortical networks responsible for SWD generation and brainstem networks driving audiogenic seizures. On the other hand, rats with genetic absence epilepsy have been shown to exhibit a resistance to secondary seizure generalization during electrical kindling of the amygdala (Eskazan et al., 2002) that indicates a negative interaction between thalamo-cortical and limbic circuits generating absence and kindled seizures, respectively. However, rats with absence epilepsy show more widespread bilateral activation of the forebrain at the early stages of amygdala kindling than non-epileptic rats (Carcak et al., 2008). To address the question of whether susceptibility to primarily generalized absence seizures influences the lateralized features of AK epileptogenesis, the present study compared individual and population lateral asymmetry of AK manifestations in Wistar rats susceptible to audiogenic seizures and WAG/Rij rats susceptible to both absence and audiogenic seizures. We suggest that separate analysis of seizure responses of the two hemispheres in the course of the upstream seizure generalization may shed light on network interactions during epileptogenesis.

EXPERIMENTAL PROCEDURES

Animals

Adult male Wistar and WAG/Rij rats (2–3 months, 250–350 g) were used. After preliminary screening for audiogenic susceptibility rats predisposed to sound-induced seizures were selected from general laboratory populations of Wistar and WAG/Rij rats. Intact rats (without implanted electrodes) were housed in groups of five per cage under controlled environmental conditions (a 12-h light–dark cycle, lights on at 7:00 A.M., 20–23 °C) with free access to food and water. Rats with implanted electrodes were housed in individual cages under the same conditions. The experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and our protocol was approved by the Institutional Animal Care Committee. All efforts were made to minimize animal suffering.

Audiogenic kindling procedure

Audiogenic seizures were induced by standard broad-band sound stimulation (13–85 kHz, 50–60 dB) produced by a purpose-made mechanical vibro-device. The sound lasted until the onset of running. If no convulsions occurred, the sound was applied for 60 s. Each rat was exposed to the sound stimulation once a day at 3–4-day intervals between repetitions. Twenty-five running episodes were induced in each rat with a reproducible seizure response. Rats with unstable seizures were subjected to 25 sound stimulations.

In 25 Wistar and 15 WAG/Rij rats implanted with cortical electrodes, both behavioral and electrographic manifestations of repeated audiogenic seizures were recorded. In 100 Wistar and 50 WAG/Rij intact rats, only behavioral profile of AK was studied.

The kindling procedures were identical for implanted and intact rats. Each animal was individually placed in a wooden square experimental chamber with a transparent wall ($60 \times 60 \times 60$ cm^3 for intact rats, $60 \times 50 \times 40$ cm^3 for implanted rats) and exposed to the acoustic stimulus. Sound-induced behavior was videotaped for off-line analysis. Latency to seizure onset and duration of each seizure phase were recorded. Appearance of post-running clonus and increase in its severity were used as behavioral markers of AK progression. The clonus severity was scored according to the scale of Racine (1972): 1 – facial/ear clonus; 2 – head nodding; 3 – forelimb clonus; 4 – forelimb clonus with rearing; 5 – forelimb clonus with rearing and falling.

Surgery

Rats were anesthetized with chloral hydrate (360 mg/kg, i.p.) and treated locally with 2% novocaine. Stainless steel screws positioned over the left and right parietal cortices were used for