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NEOCORTICAL SLICES FROM ADULT CHRONIC EPILEPTIC RATS EXHIBIT DISCHARGES OF HIGHER VOLTAGES AND BROADER SPREAD

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Abstract—Much of the current understanding of epilepsy mechanisms has been built on data recorded with one or a few electrodes from temporal lobe slices of normal young animals stimulated with convulsants. Mechanisms of *adult*, *extratemporal*, *neocortical chronic* epilepsy have not been characterized as much. A more advanced understanding of epilepsy mechanisms can be obtained by recording epileptiform discharges simultaneously from multiple points of an epileptic focus so as to define their sites of initiation and pathways of spreading. Brain slice recordings can characterize epileptic mechanisms in a simpler, more controlled preparation than *in vivo*. Yet, the intrinsic hyper-excitability of a chronic epileptic focus may not be *entirely* preserved in slices following the severing of connections in slice preparation. This study utilizes recordings of multiple electrode arrays to characterize which features of epileptic hyper-excitability present in *in vivo* chronic adult neocortical epileptic foci are preserved in brain slices. After tetanus toxin somatosensory cortex injections, adult rats manifest chronic spontaneous epileptic discharges both in the injection site (primary focus) and in the contralateral side (secondary focus). We prepared neocortical slices from these epileptic animals. When perfused with 4-Aminopyridine in a magnesium free medium, epileptic rat slices exhibit higher voltage discharges and broader spreading than control rat slices. Rates of discharges are similar in slices of epileptic and normal rats, however. Ictal and interictal discharges are distributed over most cortical layers, though with significant differences between primary and secondary foci. A chronic neocortical epileptic focus in slices does not show increased spontaneous pacemakers initiating epileptic discharges but shows discharges with higher voltages and broader spread, consistent with an enhanced synchrony of cellular and synaptic generators over wider surfaces.

Key words: chronic epilepsy, neocortical epilepsy, brain slices.

INTRODUCTION

Epilepsy is a disease involving the electrical activity of the brain and it manifests with waves of neuronal excess synchrony that propagate over the cortex and disrupt the normal function of the brain. A normal brain can generate epileptic discharges after exposure to pro-convulsant stimuli but in an epileptic brain, epileptic discharges can occur spontaneously without apparent stimulation. The current understanding of basic epilepsy mechanisms has been built mostly through studies on temporal lobe slices of normal young animals stimulated with convulsants and with recording performed through one or a few electrodes.

Recordings from *ex vivo* brain slices offer the opportunity of characterizing epileptic mechanisms in a simpler, and more controlled preparation than *in vivo*. Several studies indicate that features of the intrinsic hyper-excitability of an epileptic brain still persist in brain slices despite the severing of connections of slice cutting both in the temporal lobe (Mody et al., 1988; Empson and Jefferys, 1993; Smith et al., 1998; Gabriel et al., 2004; Carter et al., 2011) and in the neocortex (Prince and Tseng, 1993).

Mechanisms of *adult*, *extratemporal*, *neocortical chronic* epilepsy have not been studied as much as those of mesial temporal lobe acute epilepsy of immature brains. In addition, a more detailed understanding of epilepsy mechanisms can be gained by recording epileptiform discharges simultaneously from multiple points of an epileptic focus, rather than with a single electrode (see Kohling et al., 1999; Chang et al., 2011; Gonzalez-Sulser et al., 2011, 2012). Recordings simultaneously from multiple points can help to define sites of initiation and pathways of spreading.

In the present paper we are using Multiple Electrode Arrays to characterize the features of epileptic discharges evoked in the neocortical slices of chronic epileptic adult rats.

The mechanisms generating epileptic discharges can be classified into two subsequent steps. *First*, epileptic discharges may be initiated by neurons with a spontaneous bursting pacemaker activity, and *second* the initial burst recruits a large number of adjacent neurons firing together into a large paroxysmal electrical transient signal (Chagnac-Amitai and Connors, 1989; Richardson et al., 2007). In principle, intrinsic hyper-excitability of chronic epilepsy may reflect one or both of these mechanisms, with an enhancement of spontaneous

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burst firing and/or enhancement of neuronal connectivity leading to broader recruitments of synapses.

The data presented in this paper show that in brain slices from chronic epileptic animals mechanisms of intrinsic epileptic excitability are spared. Slices from epileptic animals do not exhibit higher rates of burst discharges, but rather they show higher voltage discharges and broader spreading. In comparison with normal tissue, epileptic cortex pacemaker bursting recruits a much higher number of excitatory synapses into firing together summing up into an epileptic discharge of higher voltages and also spread through broader surfaces. The neurophysiological findings presented here corroborate the hypothesis that molecular mechanisms underlying epilepsy may facilitate neuronal synaptic inter-connections, too (Barkmeier et al., 2012; Beaumont et al., 2012).

EXPERIMENTAL PROCEDURES

Intra-cortical injection of tetanus toxin and EEG recordings

All studies were carried out with institutional approval (AIC protocol A01-09-06) on 4-month-old male Sprague–Dawley rats kept on a 12-h light/dark cycle and implanted with six skull based recording screws (Small Parts, Inc., part#TX00-2-C) after tetanus toxin was stereotactically injected into the somatosensory cortex as previously described (Brener et al., 1991; Nilsen et al., 2005); (AP −1 mm, L 3.5 mm, as measured from bregma, depth 1.5 mm). Three screws were placed over each hemisphere at AP +4 mm, −1 mm and −6 mm, L 3.5 mm relative to the bregma. A reference screw was also placed over the nasal sinus. Tetanus toxin (Sigma, catalog# T3194; 1 L at 100 ng/μL in 0.01 M sodium phosphate) was injected in the left somatosensory cortex. The dose varied from 65 to 100 ng for each batch of toxin based on a dose response study to produce the same level of spiking. Recordings were made using a Stellate-Harmonie recording system at 200 Hz either for one-hour periods at the same time of day or every other day for 24-h periods using video EEG monitoring.

Slice preparation

After intracortical injection of Tetanus Toxin *in vivo*, EEG epileptiform activity is evident 1–2 days after the injection. It reaches its highest expression at 2–3 weeks and typically persists for several months (Brener et al., 1991; Barkmeier et al., 2012). Animals were euthanized 14–20 days after the intracortical tetanus toxin injection and after *in vivo* EEG recordings in the preceding days had confirmed the presence of robust spontaneous epileptiform discharges.

At the time chosen to prepare slices the animal is anesthetized with isoflurane and euthanized by decapitation. The head is immediately placed in a low-calcium high-magnesium saline (NaCl 126 mM; KCl 3.5 mM; $CaCl_2$ 0.1 mM; $MgSO_4$ 10.0 mM; $NaHCO_3$, 26 mM; NaH_2PO_4 1.25 mM; Glucose 10 mM) at 2–3 °C, bubbled with 95% O_2/5% CO_2. The skull is opened and the brain is removed and incubated in a recovery chamber at 2–3 °C for 4–5 min. The parietal lobe is affixed to a stage with cyanoacrylate glue. The chamber is filled with ice cold saline at 2–5 °C.

Coronal slices (370 μm) of hemispheres were cut between sections corresponding to plate 33 (Bregma 0.00) to plate 47 (Bregma −1.72) of Paxinos and Watson atlas (2007). The lateral sectum protrusion into the ventricles was a prominent structural landmark easily visible to identify the section at which coronal slices were to be cut. Starting from the posterior part of the sectum, per each animal we cut only three to four coronal slices, each 370-μm thin to facilitate slice oxygenation (Alger et al., 1985; Jiang et al., 1991). The distance between the most anterior and the most posterior slice section was about 1.5 mm along the anterior-to-posterior axis or about 6% of the anterior-to-posterior length of the hemisphere. In our preliminary data we could not identify any obvious differences in patterns of epileptic activity between slices of more anterior level versus those corresponding to a more posterior level. Slices were transferred on a holding chamber at room temperature with physiological saline bubbled with 95% O_2/5% CO_2.

Extracellular field recordings in adult brain neocortical slices by electrode arrays

Recordings are performed between 2 and 12 h after the dissection. Recording solution was: NaCl 126 mM, KCl 3.5 mM, $CaCl_2$ 1.1 mM, $MgSO_4$ 1.0 mM, $NaHCO_3$ 26 mM, NaH_2PO_4 1.25 mM, glucose 10 mM.

The array is at the bottom of the chamber (like in Steidl et al., 2006) and is a 10 × 6 matrix (10 columns, six rows) of planar titanium electrodes, of 30 μm diameter, 500 μm inter-electrode distance and 30–50 k Ohm impedance (Multichannel Systems, 60MEA500/30iR-Ti, Reutlingen, Germany). The outlet cable was interfaced into a Stellate Harmonie system (64 channels E2 amplifier).

Slices were positioned in the chamber. Excess fluid was removed and slice position was adjusted with a needle. A platinum wire anchor with a mesh kept the slice in position. The solution inflow was 2 ml/min. A digital camera picture of the slice position was taken at the end of the recording.

Adult brain is more resistant to epileptiform activity, and the somatosensory cortex has a lower susceptibility to epileptiform activity (Salanova, 2012). Differences between adult somatosensory epileptic cortex, likely resistant to epileptiform activity, and corresponding normal tissue may be resolved if slices are exposed to pro-convulsant stimulation of adequate intensity such as with *two* pro-convulsant stimuli rather than one stimulus. Accordingly, slices were perfused with a solution (i) without magnesium and (ii) containing 100 μM 4-AP.

Experimental design: rationale for the choice of control group

Our goal *in this study* is assessing whether slices from chronic epileptic rats do maintain features of epileptic hyper-excitability despite the loss of connections due

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