Neuroscience 284 (2015) 153–164

CHANGES IN LONG-RANGE CONNECTIVITY AND NEURONAL REORGANIZATION IN PARTIAL CORTICAL DEAFFERENTATION MODEL OF EPILEPTOGENESIS

M. KUŚMIERCZAK,[a] F. LAJEUNESSE,[a] L. GRAND[a†] AND I. TIMOFEEV[a,b*]

[a] *Centre de recherche de l'Institut universitaire en santé mentale de Québec (CRIUSMQ), Université Laval, Québec G1J 2G3, Canada*

[b] *Department of Psychiatry and Neuroscience, Université Laval, Québec G1V 0A6, Canada*

Abstract—Severe brain injuries can trigger epileptogenesis, a latent period that eventually leads to epilepsy. Previous studies have demonstrated that changes in local connectivity between cortical neurons are a part of the epileptogenic processes. In the present study we aimed to investigate whether changes in long-range connectivity are also involved in epileptogenesis. We performed a large unilateral transection (undercut) of the white matter below the suprasylvian gyrus in cats. After about 2 months, we either injected retrograde tracer (cholera toxin, sub-unit B, CTB) or performed Golgi staining. We analyzed distribution of retrogradely labeled neurons, counted dendritic spines in the neocortex (Golgi staining), and analyzed dendritic orientation in control conditions and after the injury. We found a significant increase in the number of detected cells at the frontal parts of the injured hemisphere, which suggests that the process of axonal sprouting occurs in the deafferented area. The increase in the number of retrogradely stained neurons was accompanied with a significant decrease in neocortical spine density in the undercut area, a reduction in vertical and an increase in horizontal orientation of neuronal processes. The present study shows global morphological changes underlying epileptogenesis. An increased connectivity in the injured cortical regions accompanied with a decrease in spine density suggests that excitatory synapses might be formed on dendritic shafts, which probably contributes to the altered neuronal excitability that was described in previous studies on epileptogenesis.

Key words: epileptogenesis, cholera toxin, retrograde staining, sprouting.

INTRODUCTION

Penetrating cortical wounds induce acute seizures that last for hours or days (Dinner, 1993). Then seizures terminate. Vietnam and Croatia postwar epidemiological studies report that 10–15 years after the trauma about 50% of patients with penetrating cranial wounds develop epilepsy (Salazar et al., 1985; Marcikic et al., 1998). The set of latent processes that are triggered by cortical insult and that can lead to the development of epilepsy is called epileptogenesis. The epilepsy itself, on the other hand, is characterized by unprovoked seizures (Rakhade and Jensen, 2009; Timofeev, 2011). The acute phase of epileptogenesis usually occupies the first 7 days after the injury, and this early phase can be characterized with acute seizures (Beghi et al., 2010), immediate early genes response or changes in ion concentration (Rakhade and Jensen, 2009).

The cortical undercut is a well-established model of post-traumatic epileptogenesis; it consists in the partial deafferentation of the neocortex and hence imitates a penetrating brain injury. This model has been used in humans, monkeys, cats, and also in rodents, both *in vivo* and *in vitro* (Echlin et al., 1959; Echlin and Battista, 1963; Hoffman et al., 1994; Prince and Tseng, 1994; Nita et al., 2006, 2007; Xiong et al., 2011). In chronic conditions, the anatomical changes in the undercut cortex involve cortical flattening, reduction in cortical depth and delamination (Avramescu et al., 2009). In our previous experiments with the use of undercut in the suprasylvian gyrus of cats, paroxysmal activity could be observed within hours after the injury (Topolnik et al., 2003a,b) and then acute seizures stopped. Electrographic activities starting around the undercut cortex were recorded within tens of hours in cortical isolation experiments (Nita et al., 2007; Timofeev et al., 2013). Within the first 2 months there was a progressive increase in the cortical territories involved in the generation of electrographic paroxysmal activities, which eventually led to a development of full-blown electrographic and behavioral seizures (Nita et al., 2007; Timofeev et al., 2013). Because of the progressive increase in the cortical tissue involved in the paroxysmal activities, we hypothesized

*Correspondence to: I. Timofeev, Centre de recherche de l'Institut universitaire en santé mentale de Québec (CRIUSMQ), Université Laval, Québec G1J 2G3, Canada. Tel: +1-418-663-5747; fax: +1-418-663-8756.

E-mail addresses: magda.kusmierczak.1@ulaval.ca (M. Kuśmierczak), francis.lajeunesse.1@ulaval.ca (F. Lajeunesse), Laszlo.Grand@libd.org (L. Grand), Igor.Timofeev@phs.ulaval.ca (I. Timofeev).

† Present address: The Lieber Institute for Brain Development, 855 North Wolfe Street, Suite 300, Baltimore, MD 21205, USA. Tel: +1-240-423-5103; fax: +1-410-955-1000.

Abbreviations: ANOVA, analysis of variance; CTB, cholera toxin subunit B; EEG, electroencephalogram; EPSPs, excitatory postsynaptic potentials; PBS, phosphate-buffered saline.

http://dx.doi.org/10.1016/j.neuroscience.2014.09.079

that the traumatized area might create conditions leading to an increase of connectivity with the rest of the brain.

So far, reports pertaining to morphological changes in the cortical undercut model of epileptogenesis mainly showed a local (hundreds of microns) increase in connectivity (Salin et al., 1995; Jin et al., 2006, 2011; Avramescu and Timofeev, 2008), while it remains unknown whether there are any longer range changes in neocortical connectivity. Because in the undercut model of trauma the seizures were not initially generated in the area lying directly above the transection, but in the surrounding areas, and then, with a delay of only a few milliseconds, they invaded also the injured region, (Nita et al., 2007), we hypothesized that excitatory cells in the surrounding areas would form new direct connections with the undercut. Using retrograde tracer cholera toxin (sub-unit B, CTB), we found a significant increase in connectivity of the undercut cortex with more anterior cortical regions. However, within the undercut region, neurons showed a reduced spine density on secondary and higher order dendritic branches, which suggests that new synapses are not formed on dendritic spines, but on dendritic shafts. We also observed a shift in cortical organization from a mainly vertical orientation of neuronal processes in control conditions to a mainly horizontal in the undercut cortex. Together with an overall reduction of neurons, in particular inhibitory interneurons (Avramescu et al., 2009), our results point to major morphological changes of the undercut cortex that contribute to the altered excitability of the epileptogenic tissue.

EXPERIMENTAL PROCEDURES

Undercut surgery

Experiments were performed in accordance with the guideline of the Canadian Council on Animal Care and approved by the Université Laval Committee on Ethics and Animal Research. All efforts were made to minimise the number of animals used and their suffering. The surgery was performed under sterile conditions. Six male cats (8–12 months old) were initially anesthetized with IM administration of a mixture of ketamine (15 mg/kg), buprenorphine (0.01 mg/kg), acepromazine (0.3 mg/kg) and glycopyrrolate (0.011 mg/kg). The anesthesia was maintained with isoflurane inhalation (3–4% for induction and 0.7–2% throughout the surgery), and lactated Ringer's solution was continuously delivered IV at a rate of 5–10 ml/kg/h. Lidocaine/marcaine (0.50/0.25%) was used for additional local analgesia at all pressure points and incision lines. The level of anesthesia was monitored on the basis of heart rate (aiming 90–110 beats/min). Oxygen saturation of the arterial blood (aiming over 90%) and end-tidal CO_2 (~3.5%) were also monitored. The body temperature was maintained between 37 and 39 °C with a heating pad. In order to match our morphological data to the available physiological description of the epileptogenesis leading to spontaneous seizure activities (Nita et al., 2006, 2007; Avramescu and Timofeev, 2008), the experiments were performed on the suprasylvian gyrus. A craniotomy was performed in the left hemisphere and a large undercut of the white matter below the suprasylvian gyrus (13–15 mm postero-anteriorly and 3–4 mm mediolaterally) was used to produce partial cortical deafferentation as previously described (Topolnik et al., 2003b; Nita et al., 2007). A custom-designed knife was inserted at the back of the suprasylvian gyrus, perpendicularly to its surface and at a depth of 3–4 mm, then rotated 90° and advanced rostrally along the gyrus parallel to its surface for a total distance of 13–15 mm, then moved back, rotated 90° and removed from the same place where it entered the cortex. Thus, the cut provided conditions of partial cortical deafferentation, with the anterior part of the neocortex in the suprasylvian gyrus being relatively intact, while in the posterior part of the gyrus the white matter was transected. The surface of the brain was then covered with a quick drying silicone elastomer (Kwik-Sil, World Precision Instruments, Sarasota, FL, USA) that prevents the leakage of cerebrospinal fluid and tissue growth on the dura. Dental acrylic cement was then placed over the opening and the neighboring bones and the scalp was sutured. To prevent infection, an antibiotic, cefazolin (50 mg/kg), was injected and for post-operational recovery anafen (2 mg/kg, 2–3 times for 3 days), baytril (5 mg/kg, 1 time for 7 days), metoclopramide (0.4 mg/kg) and cyproheptadine were given to the animals.

Retrograde labeling

Tracer injection. The injections of CTB (low salt, List Biological Laboratories, Campbell, CA, USA) were performed 50–69 days after the undercut surgery. The procedures of injection and immunohistochemical reactions were similar to those recently described (Ahmed et al., 2012). Briefly, the animals were anesthetized with an IM injection of ketamine–xylazine (15 mg/2 mg respectively, 0.22 ml/kg) and then were continuously infiltrated IV with ketamine in lactated Ringer's solution (0.45% NaCl and 2.5% dextrose at an approximate rate of 10–15 ml/kg/h) throughout the whole experiment. Frequency of ketamine–xylazine booster IV injections (1/3 of the initial dose) was estimated for each animal separately on the basis of electroencephalogram (EEG) recording and heart rate. An anesthesia booster was injected whenever there was a slight tendency of the EEG pattern to become activated or when the heartbeat increased. The range of time intervals between boosters was approx. 0.5–2 h. Animals were artificially ventilated and paralyzed with 2% gallamine triethiodide; glycopyrrolate (0.011 mg/kg) was administered IV every 6 h.

After opening the scalp and removing the dental acrylic, a craniotomy was performed over the right intact hemisphere to match the size of the opening over the left undercut cortex. The dura matter was removed from both left and right gyri. Cholera toxin in a concentration of 1% (water solution) was injected once in the left and right suprasylvian gyri, approximately 1 cm to the front from the point of knife insertion for the undercut. The tracer was injected with a microinjector (Nanoliter 2000, World Precision Instruments, FL, USA), with a glass micropipette with a diameter of around 40–50 μm