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Dentate gyrus progenitor cell proliferation after the onset of spontaneous seizures in the tetanus toxin model of temporal lobe epilepsy

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ABSTRACT

Temporal lobe epilepsy alters adult neurogenesis. Existing experimental evidence is mainly from chronic models induced by an initial prolonged status epilepticus associated with substantial cell death. In these models, neurogenesis increases after status epilepticus. To test whether status epilepticus is necessary for this increase, we examined precursor cell proliferation and neurogenesis after the onset of spontaneous seizures in a model of temporal lobe epilepsy induced by unilateral intrahippocampal injection of tetanus toxin, which does not cause status or, in most cases, detectable neuronal loss. We found a 4.5 times increase in BrdU labeling (estimating precursor cells proliferating during the 2nd week after injection of toxin and surviving at least up to 7 days) in dentate gyri of both injected and contralateral hippocampi of epileptic rats. Radiotelemetry revealed that the rats experienced 112±24 seizures, lasting 88±11 s each, over a period of 8.6±1.3 days from the first electrographic seizure. On the first day of seizures, their duration was a median of 103 s, and the median interictal period was 23 min, confirming the absence of experimentally defined status epilepticus. The total increase in cell proliferation/survival was due to significant population expansions of: radial glial-like precursor cells (type I; 7.2×), non-radial type II/III neural precursors in the dentate gyrus stem cell niche (5.6×), and doublecortin-expressing neuroblasts (5.1×). We conclude that repeated spontaneous brief temporal lobe seizures are sufficient to promote increased hippocampal neurogenesis in the absence of status epilepticus.

Introduction

The generation of new neurons, neurogenesis, in the adult brain continues in mammals, including humans, throughout life (Eriksson et al., 1998; Gage et al., 1998). Neurogenesis is regulated by a variety of physiological stimuli and newly born dentate gyrus neurons integrate into the circuitry of the adult hippocampus, leading to theories for roles in learning and memory (Deng et al., 2010).

In animal models, hippocampal neurogenesis is affected by a wide range of pathological conditions including of focal epilepsies, in particular those induced by initial status epilepticus (Bender et al., 2003; Bengzon et al., 1997; Gray and Sundstrom, 1998; Jessberger et al., 2005, 2007; Nakagawa et al., 2000; Parent et al., 1997). Despite the difficulties in assessing neurogenesis in adult humans, where robust controlled methods for detection cannot be implemented ethically, evidence from surgically excised human tissue supports the presence of altered neurogenesis in clinical temporal lobe epilepsy (TLE) (Blumcke et al., 2001; Crespel et al., 2005; Fahrner et al., 2007; Parent et al., 2006) especially in patients with poor memory performance preoperatively (Coras et al., 2010). In animal models, which allow the detection of changes with a greater temporal fidelity, hippocampal neurogenesis is initially enhanced following the induction of status epilepticus (Bengzon et al., 1997; Gray and Sundstrom, 1998; Jessberger et al., 2005, 2007; Parent et al., 1997) and later declines in some (Hattiangady and Shetty, 2008) but not all models (Bonde et al., 2006). However, while previous studies have examined the differential effects of severe convulsive versus less severe status epilepticus on hippocampal neurogenesis (Bouilleret et al., 1999;

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Hung et al., 2012; Mohapel et al., 2004; Yang et al., 2008), there have been no studies to our knowledge examining the effects of repeated spontaneous seizures without a prior status. The fully convulsive status epilepticus based models of TLE used previously (induced by kainate, pilocarpine or electrical stimulation) are typically associated with substantial morphological changes, in the form of neuronal loss and sprouting of axonal collaterals. Few studies have demonstrated seizure-induced cell proliferation and/or neurogenesis in the absence of status epilepticus. Bengzon et al. (1997) found single evoked afterdischarges could increase neurogenesis 3-fold and a rapid kindling protocol (40 stimuli at 5 min) increased it 6-fold, in both cases with a concomitant increases in apoptosis (TUNEL staining) of newly generated neurons/precursor cells. Smith et al. (2005) found increased neurogenesis for at least 21 days (respectively) following rapid amygdala kindling, without neuronal loss, in contrast with Bengzon et al. (1997). Conventional kindling, with daily or twice-daily stimulation, increased neurogenesis for several days following stage 5 seizures, but not in the earlier stages of kindling or after single afterdischarges (Nakagawa et al., 2000; Scott et al., 1998, 2010). Ferland et al. (2002) observed a transient increase in BrdU labeled cells 3 days after a single flurothyl seizure or up to 7 days after 8 daily seizures, in this case without evidence of neuronal death. Single febrile seizures at P10 did not induce increased neurogenesis, although more prolonged seizures induced by kainic acid did (Bender et al., 2003). However, none of these paradigms can model the effect of spontaneous onset seizures on neurogenesis of potential relevance to the 60% of patients with TLE in whom there is no antecedent history of prolonged febrile seizures (Waruiru and Appleton, 2004).

Intrahippocampal tetanus toxin induces epilepsy with spontaneous and recurrent seizures but without major morphological changes (Jefferys et al., 1992; Jiruska et al., 2010; Mellanby et al., 1977), and notably without status epilepticus at any stage (Finnerty et al., 2000; Hawkins and Mellanby, 1987; Jiruska et al., 2010). While the majority of rats gain seizure remission in this model after 6–8 weeks, they retain abnormal cellular pathophysiology (Vreugdenhil et al., 2002), permanent cognitive and other behavioral impairments (Brace et al., 1985; Mellanby, 1982), and a minority continues to seize (Mellanby, 1993). This model provides a means of testing the effect of early repeated spontaneous seizures on cell proliferation and neurogenesis independently of prolonged status epilepticus.

Materials and methods

Animals

Sixteen adult male Sprague-Dawley rats weighing approximately 250 g were housed under standard conditions in a room with controlled temperature (22 ± 1 °C) and 12/12 h light/dark cycle. The animals had ad libitum access to food and water. All animal procedures were licensed and performed in strict accordance with the Animal Scientific Procedures Act (1986) of the United Kingdom and with Institutional Ethical Review.

Surgery, recording and BrdU injections

Surgical preparation was performed under ketamine/methibromide or isoflurane anesthesia. Small trephine openings were drilled symmetrically over both hippocampi at coordinates 4.1 mm caudal to bregma and 3.9 mm either side of the midline using the atlas of Paxinos and Watson (1998). Using a Hamilton microsyringe and infusion pump (KD Scientific Inc., USA) 1 μl of tetanus toxin (Sigma-Aldrich, UK) solution was injected into the stratum radiatum of the right hippocampal CA3 area. Tetanus toxin solution contained 25 ng of tetanus toxin in 1 μl of 0.05 M phosphate buffered saline (PBS; Sigma-Aldrich, UK) and 2% bovine serum albumin (Sigma-Aldrich, UK). Tetanus neurotoxin solution was injected at 200 nl/min. The microsyringe was left in hippocampus for 5 min after the injection ended to avoid the solution leaking back through the injection track. Control animals were injected with 1 μl of 0.05 M PBS with 2% bovine serum albumin. Following the injections, silver ball electrodes were inserted into both openings epidurally over both cortices and fixed to the skull using dental acrylic. Electrodes were connected to single channel bipolar telemetric transmitters (Data Sciences International, s'Hertogenbosch, Netherlands) which were implanted subcutaneously over the dorsal aspect of the thorax and secured with sutures. Following surgery, animals were housed in single cages and allowed to recover for two days. Continuous synchronized video-electrocorticography monitoring started on the 4th day, which precedes the onset of spontaneous seizures (Jefferys and Walker, 2006) and continued until the end of the experiments. Electrocotricography was recorded using Dataquest A.R.T. 4.3 aquisition system (Data Sciences International, s'Hertogenbosch, Netherlands) and sampled at 100 Hz. Video was recorded synchronously using digital infra-red cameras (Y-cam Solutions Ltd, Richmond, UK) and Spike2 software (Cambridge Electronic Design, Cambridge, UK). Recorded signals were exported, reviewed and analyzed using Spike2, to verify the development of spontaneous seizures and to determine seizure frequency and duration. Seizures were classified as secondary generalized when they progressed into falls followed by generalized convulsions.

On day 10 (while still being continuously monitored), rats received the first of seven daily intraperitoneal injections of bromodeoxyuridine solution (BrdU, 50 mg/kg, 10 mg/ml in 0.007 M NaOH/0.9% sterile saline; Sigma-Aldrich, UK). On day 17, 24 h after the last BrdU injection, animals were humanely overdosed with ketamine and then perfused using 0.9% saline followed by 4% paraformaldehyde. Brains were extracted and postfixed in 4% paraformaldehyde.

Immunohistochemistry

The brains were sectioned along the coronal plane at 40 μm intervals through the entire hippocampus on a vibratome (Leica VT1000M, Leica Microsystems Ltd, Milton Keynes, UK). Each slice was transferred to an individual well (of a 24 well plate) containing PBS for storage. All immunohistochemistry was performed on systematically sampled tissue, with the initial section selected randomly and subsequent sections being taken at constant intervals thereafter, ensuring the entire dentate gyrus was sampled. BrdU immunostaining was performed on 12 sections per animal. Caspase-3 labeling and doublecortin (Dcx) labeling were performed on 6 systematically sampled sections from each animal. For double stain immunohistochemistry of BrdU and Dcx, sections were incubated in 2 M HCl at 37 °C for 30 min, followed by washing and incubation with 3% H_2O_2–10% methanol for 30 min. Saturation of non-specific binding sites was achieved in 5% donkey serum in 0.25% Triton X-100 (1 h). This was followed by overnight incubation of primary antibodies to BrdU (rat monoclonal 1:1000; Oxford Biotech, UK) and Dcx (goat polyclonal 1:200; Santa Cruz, CA, USA). The final step was incubation with secondary antibodies Alexa 448/594 raised in donkey (1/500, Invitrogen).

Rabbit polyclonal cleaved caspase-3 primary antibody was used at 1:200 (New England Biolabs, Hitchen, UK). Triple immunohistochemistry studies for BrdU, Sox2 (1:500 Goat polyclonal Santa Cruz Biotechnology, CA, USA) and anti-rabbit GFAP (1/500, DAKO) were also conducted to look at the proliferation of both type I and type II/III precursor cells in subgranular zone (Encinas et al., 2006). Secondary antibodies were Alexa 488, Alexa 594, and Alexa 647 (1:500, Invitrogen, Life Technologies Ltd, Paisley, UK) all were raised in donkey and matched the primary combinations. Finally the slices were mounted on glass microscope slides with Mowiol mounting medium (Harlow Chemicals, Harlow, Essex, UK) and stored in the dark at 4 °C to delay damage from exposure to ultraviolet light. Non-specific secondary antibody binding was excluded by the lack of immunostaining in control experiments omitting the primary antibody.