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EXPERIMENTAL FEBRILE SEIZURES INDUCE AGE-DEPENDENT STRUCTURAL PLASTICITY AND IMPROVE MEMORY IN MICE

K. TAO, J. ICHIKAWA, N. MATSUKI, Y. IKEGAYA AND R. KOYAMA *

Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan

Abstract—Population-based studies have demonstrated that children with a history of febrile seizure (FS) perform better than age-matched controls at hippocampus-dependent memory tasks. Here, we report that FSs induce two distinct structural reorganizations in the hippocampus and bidirectionally modify future learning abilities in an age-dependent manner. Compared with age-matched controls, adult mice that had experienced experimental FSs induced by hyperthermia (HT) on postnatal day 14 (P14-HT) performed better in a cognitive task that requires dentate granule cells (DGCs). The enhanced memory performance correlated with an FS-induced persistent increase in the density of large mossy fiber terminals (LMTs) of the DGCs. The memory enhancement was not observed in mice that had experienced HT-induced seizures at P11 which exhibited abnormally located DGCs in addition to the increased LMT density. The ectopic DGCs of the P11-HT mice were abolished by the diuretic bumetanide, and this pharmacological treatment unveiled the masked memory enhancement. Thus, this work provides a novel basis for age-dependent structural plasticity in which FSs influence future brain function.

Key words: febrile seizure, hippocampus, dentate gyrus, memory, structural plasticity.

INTRODUCTION

Follow-up surveys of children who experienced febrile seizures (FSs) have revealed no association between early-life FSs and global cognitive dysfunctions such as academic progress, intellect, or behaviors compared to children without FS at school (Ellenberg and Nelson, 1978; Verity et al., 1998); however, these studies did not specifically investigate whether FSs induce memory dysfunction. In another population-based study that examined the effects of FSs on working memory, it was shown that school-aged children with FS experiences performed significantly better than age-matched control children in learning, consolidation, memory retrieval, and delayed recognition (Chang et al., 2000, 2001). Importantly, however, those children with an onset of FSs before 1 year-of-age had deficits in these properties, which suggests an age-dependent modulation of memory performances. To date, the mechanisms whether and how age-dependent experiences of FSs affect differently on later memory function remains unclear.

The dentate gyrus (DG) is one of the most susceptible brain regions against seizures and epilepsy (Sloviter, 1994). Both in temporal lobe epilepsy (TLE) individuals and their animal models, the dentate granule cells (DGCs) exhibit several distinct structural abnormalities such as aberrant axonal sprouting (Sutula et al., 1988) and ectopic somal positioning (Scharfman et al., 2007). Importantly, it has been reported that the FS-induced emergence of ectopic DGCs during postnatal days contributes to the future development of epilepsy in a rat model of severe FSs (Koyama et al., 2012).

DG filters and outputs the neural activity from the entorhinal cortex to the hippocampus as the first layer of the hippocampal trisynaptic circuit, play an important role in the hippocampus-dependent learning and memory (Treves and Rolls, 1994). Especially, DGCs are involved in the hippocampus-dependent episodic memory via mediating pattern separation and pattern completion (Nakashiba et al., 2012). Further, structural plasticity of the large mossy fiber terminals (LMTs), which are the primary synaptic connections from the DGCs to the dendrites of CA3 pyramidal cells (Claiborne et al., 1986), has a critical role for the formation and maintenance of context-dependent spatial memory (Ruediger et al., 2011). Therefore, we hypothesized that early-life FSs would induce structural reorganization of DGCs and exert subsequent effects on the DG-dependent learning and memory performances in adulthood.

EXPERIMENTAL PROCEDURES

Animals

Mice from the reporter line Thy1-mGFP (Lsi1; a generous gift from Dr. Pico Caroni) were maintained under

*Corresponding author. Address: Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel: +81-3-5841-4782; fax: +81-3-5841-4786.
E-mail address: rkoyama@mol.f.u-tokyo.ac.jp (R. Koyama).

Abbreviations: ANOVA, analyses of variance; BDNF, brain-derived neurotrophic factor; DG, dentate gyrus; DGCs, dentate granule cells; FS, febrile seizure; HBSS, Hanks' balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HT, hyperthermia; LMTs, large mossy fiber terminals; MEM, minimal essential medium; PB, phosphate buffer; PKA, protein kinase A; Trk, tropomyosin receptor kinase; ZnT3, zinc transporter 3.

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controlled temperature and light schedule conditions and provided with unlimited food and water. All the experimental procedures conformed to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and to the guidelines issued by the University of Tokyo.

Pharmacological agents

The pharmacological agents were applied at the following concentrations *in vitro*: Rp-adenosine-3′,5′-cyclic monoph osphorothioate (Rp-cAMPS: competitive antagonist of the cyclic nucleotide-binding domains of PKA, Sigma, St Louis, MO, USA), 100 μM; KT-5720 (PKA antagonist, Sigma), 10 μM; K252a (Trk receptor antagonist, Wako), 300 nM; and an anti-BDNF antibody Calbiochem), 10 μg/ml.

The pharmacological agents were administered intraperitoneally (i.p.) at the following concentrations *in vivo*: bumetanide at 0.1 mg/kg (Sigma) and pentobarbital at 37 mg/kg (Sigma).

Prolonged experimental FSs

Prolonged experimental FSs were induced as previously described (Koyama et al., 2012) with some modifications. Briefly, male mouse pups at P11 (n = 60) and P14 (n = 54) were placed in a glass jar, and their core temperature was raised using a regulated stream of moderately heated air. Their rectal temperatures were measured at baseline, seizure onset and every 2 min during the seizures. Hyperthermic temperatures (40.0–42.0 °C) were maintained for 30 min, and the occurrence and duration of seizures, which consisted of an acute sudden arrest of hyperthermia (HT)-induced hyperactivity, such as running, followed by oral automatism (biting and chewing) and often by body flexion. After the periods of HT, the mice were placed on a cool surface, monitored for 15 min and then returned to their home cages. The mice in the control (normothermic) groups were treated identically to those in the hyperthermic groups with the exception that they were not exposed to HT; the control mice were placed in a glass jar, and their rectal temperatures were measured every 2 min for 40–50 min.

Early environmental enrichment

The enriched environment designed to induce complex sensory-motor stimulation in neonatal mice was configured based on previous protocols (He et al., 2010; Kondo et al., 2012; Liu et al., 2012) with some modifications and consisted of a large cage (28 × 45 × 20 cm) containing a running wheel, tunnels and wooden blocks. The objects were repositioned once per day. Two pregnant mice (embryonic days 16–20) were housed with two additional filler females. The litters were housed with their mother until P21 and then transferred to a standard cage.

Behavioral experiments

The open-field test was conducted using a square, white, expanded polystyrene box (47 × 47 × 19 cm) with an open top and a floor that was covered with clear acrylic sheeting. The arena of the open field included a center zone (26 × 26 cm). The test was performed at day 0 for 10 min.

Preexposure-dependent contextual fear conditioning was performed over three consecutive days. On the preexposure day (day 1), the mice were placed in a rectangular chamber (18 × 15 × 15 cm) that contained a metal grid floor connected to a shock scrambler (SGS-003DX; Muromachi Kikai, Tokyo, Japan) for 3 min. On the conditioning day (day 2), the mice were placed in the chamber and then subjected to a single foot shock (2 s, 1 mA) 10 s or 180 s after placement. After the shock, the mice remained in the chamber for 30 s before being returned to their home cage. On the test day (day 3), the mice were placed in the chamber and kept there for 300 s. Fear memory was assessed as the percentage of time spent freezing (defined as a complete lack of movement with the exception of respiration). All the sessions were video recorded for automated scoring of the freezing behavior. For the anatomical analysis, the mice were perfused with 4% paraformaldehyde (PFA) dissolved in 0.1 M PB 90 min after the test.

Organotypic slice culture

Hippocampal slice cultures were prepared from P8 or P12 mice based on a previous protocol (Gogolla et al., 2006) with some modifications. Briefly, dissected hippocampi placed on an agarose block were sliced into 400-μm-thick transverse sections using a DTK-1500 vibratome (Dosaka) in aerated, ice-cold Gey's balanced salt solution (GBSS) containing 25 mM glucose. The slices were incubated for 60 min at 4 °C in cold incubation medium containing minimal essential medium (MEM) and Hanks' balanced salt solution (HBSS) at a ratio of 2:1, 10 mM Tris, 25 mM HEPES and 5 mM $NaHCO_3$. For the P12 mice, 1 mM kynurenic acid was added to the incubation medium. The slices were placed on Omnipore membrane filters (JHWP02500; Millipore, Bedford, MA, USA) (Koyama et al., 2007) in a solution containing 50% MEM, 25% horse serum (HS), 25 mM HBSS, 10 mM Tris, 25 mM HEPES and 5 mM $NaHCO_3$ supplemented with 33 mM glucose. Finally, the slices were cultured at 37 °C in a humidified incubator with 5% CO_2 and 95% air.

Sample preparation and immunohistochemistry

The experimental animals were deeply anesthetized and transcardially perfused with cold PBS followed by 4% PFA. The brain samples were post-fixed using 4% PFA at 4 °C for 24 h. Horizontal sections thicknesses of 200 μm were generated using a Zero-1 vibratome (Dosaka). For slice cultures, the samples were fixed using 4% PFA at 4 °C for 24 h under agitation. The fixed slices were rinsed 3 times with 0.1 M phosphate buffer (PB). Next, the slices were permeabilized overnight at 4 °C in 0.1 M PB with 0.1% Triton X-100 and 5% goat serum. After extensive washing with 0.1 M PB, the slices were incubated in 5% goat serum in 0.1 M PB at room temperature for 60 min under agitation.