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Long-term modifications of epileptogenesis and hippocampal rhythms after prolonged hyperthermic seizures in the mouse

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ABSTRACT

Complex febrile seizures are often reported in the history of patients with mesio-temporal lobe epilepsy (MTLE) but their role in its physiopathology remains controversial. We postulated that prolonged hyperthermic seizures might, as a "single-hit", modify the hippocampal rhythms, facilitate epileptogenesis and influence subsequent epilepsy when a second-hit already exists or subsequently occurs. To test this hypothesis, we examined the effects of hyperthermic seizures (30 min at 40–41 °C) at postnatal day 10 on hippocampal activity in C57BL/6J mice in comparison to their littermates in sham conditions (22 °C), with or without another insult. Using local field potential, we observed an asymmetry in the hippocampal susceptibility to seize in hyperthermic conditions. When these mice were adult, an asymmetrical increase of low frequency power was also recorded in the hippocampus when compared to sham animals. Using two different "two-hit" protocols, no increase in seizures or hippocampal discharge frequency or duration was observed, either in mice with a genetic CA3 dysplasia (*Dcx* knockout), or in mice injected with kainate into the dorsal hippocampus at P60. However, in the latter condition, which is reminiscent of MTLE, the hyperthermic seizures accelerated epileptogenesis and decreased the power in the high frequency gamma band, as well as decreasing the coherence between hippocampi and the involvement of the contralateral hippocampus during hippocampal paroxysmal discharges. Our data suggest that a single episode of prolonged hyperthermic seizures does not induce per se, but accelerates epileptogenesis and could lead to an asymmetrical dysfunction in the hippocampal rhythmicity in both physiological and pathological conditions.

Introduction

Febrile seizures (FS) are the most common childhood seizures, occurring in 2–5% of children between 6 months and 6 years of age (Berg et al., 1992; Verity et al., 1985). In most instances, FS are without any consequences in terms of brain damage or subsequent epilepsy (Hesdorffer et al., 2011). However, the observation that many individuals (up to 60–80%) with mesio-temporal lobe epilepsy (MTLE) had a history of complex febrile seizures has raised the concern that it might adversely and permanently alter the developing brain (French et al., 1993; Hamati-Haddad and Abou-Khalil, 1998). Yet, the mechanisms by which FS lead to, or facilitate, mesio-temporal lobe epileptogenesis remain poorly understood. However, the existence of an additional acquired or developmental anomaly could play a critical role in the development of MTLE (Cendes et al., 1995). Indeed, a prior history of FS was identified in patients with a dual pathology (92%) more often than in those with isolated hippocampal sclerosis (17%) (Fauser et al., 2006). The resulting "two-hit theory" suggests that a brain insult might influence FS themselves or their consequences on the ipsilateral hippocampal formation. However, the order of occurrence of a brain

Abbreviations: EEG, electroencephalography; EcoG, electrocorticography; FS, febrile seizure; GCD, granule cell dispersion; HCN, hyperpolarization-activated cylic-nucleotid modulated cation non-selective channel; HPD, hippocampal paroxysmal discharge; KA, kainate; KA-MTLE model, mice model of MTLE syndrome induced by intra-hippocampal KA injection; LFP, local field potential; MTLE, mesio-temporal lobe epilepsy; NMDA, N-méthyl-D-aspartate.

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insult and FS, and how they influence epileptogenesis and hippocampal sclerosis remains unknown.

Experimental episodes of prolonged hyperthermic seizures can be induced in rat or mouse pups at the age of 10–11 days (P10–11), when hippocampal development corresponds to early childhood in human infants and has been used to model FS (Avishai-Eliner et al., 2002; Baram et al., 1997; Holtzman et al., 1981). Prolonged hyperthermic seizures were shown (i) to involve the hippocampal formation and limbic structures (Dube et al., 2000); (ii) to enhance hippocampal long-term excitability (Dube et al., 2000; Koyama et al., 2012); (iii) to induce transient neuronal injury but not cell death (Bender et al., 2003; Toth et al., 1998); (iv) to lead to altered signal on magnetic resonance imaging T2-weighted sequences in several limbic areas (Dubé et al., 2004; Gibbs et al., 2011) and (v) to alter the expression of several ionotropic receptors or channel subunits (NMDA, GABA, AMPA, Ih or Chloride) (Brewster et al., 2002, 2005; Reid et al., 2012). Hyperthermic seizures were shown to induce the aberrant migration of neonatal-generated granule cells resulting in granule cell ectopia, via the induction of an up-regulation of $GABA_A$ receptors (Koyama et al., 2012). The duration of the hyperthermic seizures appears to be a key factor in generating hippocampal atrophy, but this paradigm did not induce typical hippocampal sclerosis (Gibbs et al., 2008, 2011). In order to model "dual pathology," a focal cortical lesion induced in the neonatal rat was shown to predispose to the development of atypical hyperthermic seizures and subsequent spontaneous recurrent seizures in adult (Scantlebury et al., 2004, 2005). Multiple cortical dysplasia induced by in utero exposure of methylazoxymethanol was also proposed as a model of "dual pathology", in association with hyperthermic seizures, leading also to spontaneous recurrent seizures (Park et al., 2010). Both models suggested that hyperthermic seizures might leave their imprint on the developing brain by altering the way neurons differentiate, connect and communicate with each other, even if such changes may be ultimately compensated for in the absence of a second event.

Here, we explored the consequences of a single episode of prolonged hyperthermic seizures in P10 mice pups as a "single-hit", and secondarily investigated whether such a single episode could aggravate a preceding or subsequent epileptogenic insult, using two models of hippocampal epilepsy: (i) the *Dcx* knockout C57BL/6J mouse that displays a hippocampal dysplasia in the CA3 leading to spontaneous seizures in 30% of the cases (Nosten-Bertrand et al., 2008) and (ii) the MTLE mouse model obtained by intra-hippocampal injection of kainate in C57BL/6J adult mice (Heinrich et al., 2006; Maroso et al., 2010; Riban et al., 2002). In human MTLE, secondary generalized tonic–clonic seizures are rare (Maillard et al., 2004), in contrast, focal mesio-temporal lobe (e.g., hippocampal) seizures remain quite frequent, generally resistant to most anti-epileptic drugs and invalidating for the patients (French et al., 1993; Williamson et al., 1993). Therefore, in the present study, we aimed to reproduce in animal models these focal hippocampal discharges that are not necessarily associated with obvious behavioral changes. This required bilateral bipolar hippocampal local field potential (LFP) recordings of sufficient quality to detect and quantify focal discharges.

Materials and methods

Animals and hyperthermic seizures

All protocols involving animals were accepted by our local ethical committee, in agreement with EU directive 2010/63/EU. C57BL/6J mouse pups (males or females) were bred at the Grenoble Institute of Neuroscience, F-38000 Grenoble, France. *Dcx* knockout ($Dcx^{-/Y}$) (deleted for Dcx exon 3 on the X chromosome) male and heterozygote female ($Dcx^{+/-}$) mouse pups were locally obtained by breeding. This mouse line was generated by using the Cre-loxP site-specific recombination system, as described elsewhere (Kappeler et al., 2006). Mouse pups were produced with wild-type littermate controls by crossing heterozygote *Dcx* females ($Dcx^{-/+}$) with C57BL/6J males (Charles-River, France). Mice were genotyped by PCR, as described previously (Kappeler et al., 2006).

We induced hyperthermic seizures in P10–11 pups using a modified paradigm developed in rats (Baram et al., 1997) and mice (Dubé et al., 2005). Briefly, each half litter (wild-type C57BL/6J and *Dcx* litter ($Dcx^{-/Y}$, $Dcx^{+/-}$, $Dcx^{+/+}$)) was placed for 30 min in a Plexiglas cage and heated at 39.5–41.5 °C while the other half was maintained at 22–24 °C (sham) in the same room. Pups body temperature and behavior were noted every 2 min.

Twelve P10–11 pups (wild-type C57BL/6J) were implanted with electrodes under cryo-anesthesia and finally retained for analyze after histological control (5 were excluded). Bilateral bipolar hippocampal and cortical electrodes were previously positioned using a phantom skull, then embedded in dental acrylic cement, using the lambda as reference. This pedestal was maintained on the pups' skull with cyanoacrylate. During the next 2 h, they were video-EEG recorded before, during and 1 h after the hyperthermic seizure session and then sacrificed to verify the location of the electrodes.

Surgery (see Fig. 1)

Protocol A–B

Once hyperthermic seizures and sham exposed animals (WT and *Dcx* KO) were adult (P60), they were implanted under general anesthesia (4% chloral hydrate; 10 ml/kg, i.p.), with two monopolar electrodes positioned over the left and right anterior cortex; a monopolar electrode over the cerebellum (reference); and bipolar electrodes into both hippocampi [anteroposterior: −1.9; mediolateral: ±1.5; dorsoventral: −1.9 mm] with bregma as the reference (Paxinos and Franklin, 2004). We only used bipolar derivation in the hippocampi in order to ensure precise localization of the signal. All electrodes were made of polyester-insulated stainless steel wires (diameter, 0.125 mm; FE245840, Goodfellow, Lille, France), were soldered to a female connector (BLR150Z; Fischer Elektronik) and were fixed on the skull with cyanoacrylate and dental acrylic cement.

Protocol C

In addition, to compare the consequences of hyperthermic seizures on KA-MTLE model, we injected a separate group of C57BL/6J hyperthermic seizures and sham exposed wild-type adult mice with 50 nl of a 20 mM solution of kainate (KA) (i.e., 1 nmol; Sigma, Lyon, France) into the right dorsal hippocampus [anteroposterior: −1.9; mediolateral: −1.5; dorsoventral: −1.9 mm] as described before (Bouilleret et al., 1999; Heinrich et al., 2006, 2011; Langlois et al., 2010; Pallud et al., 2011; Riban et al., 2002; Suzuki et al., 2005) and positioned the electrodes as described in protocols A and B. Intra-hippocampal injection of kainate induces a focal status epilepticus which spontaneously ends between 15 and 18 h, as described previously (Pernot et al., 2011).

EEG recordings

(For a more detailed justification of each EEG protocol, please report to the Suppl material and methods 1)

Protocols A–B

One week after surgery, we video-EEG recorded the adult wild-type C57BL/6J while freely-moving with a digital video-EEG recording device (Micromed, Treviso, Italy; sampling rate = 1024 Hz) for 4 to 5 sessions of 4–5 h each equally distributed over a one-month period (mice age: P70–P100, randomly repartition of session time: 4 to 5 h am. or pm. for each mouse). We looked for 2 different types of epileptic manifestations: (i) The first type was defined as *focal* hippocampal discharges with hippocampal rhythmicity recruiting poly-spikes of high-amplitude (more than 2 times the background activity) lasting

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