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Hyper-excitability and epilepsy generated by chronic early-life stress

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ABSTRACT

Epilepsy is more prevalent in populations with high measures of stress, but the neurobiological mechanisms are unclear. Stress is a common precipitant of seizures in individuals with epilepsy, and may provoke seizures by several mechanisms including changes in neurotransmitter and hormone levels within the brain. Importantly, stress during sensitive periods early in life contributes to 'brain programming', influencing neuronal function and brain networks. However, it is unclear if early-life stress influences limbic excitability and promotes epilepsy. Here we used an established, naturalistic model of chronic early-life stress (CES), and employed chronic cortical and limbic video-EEGs combined with molecular and cellular techniques to probe the contributions of stress to age-specific epilepsies and network hyperexcitability and identify the underlying mechanisms.

In control male rats, EEGs obtained throughout development were normal and no seizures were observed. EEGs demonstrated epileptic spikes and spike series in the majority of rats experiencing CES, and 57% of CES rats developed seizures: Behavioral events resembling the human age-specific epilepsy infantile spasms occurred in 11/23 (48%), accompanied by EEG spikes and/or electrodecrements, and two additional rats (9%) developed limbic seizures that involved the amygdala. Probing for stress-dependent, endogenous convulsant molecules within amygdala, we examined the expression of the pro-convulsant neuropeptide corticotropin-releasing hormone (CRH), and found a significant increase of amygdalar–but not cortical–CRH expression in adolescent CES rats.

In conclusion, CES of limited duration has long-lasting effects on brain excitability and may promote age-specific seizures and epilepsy. Whereas the mechanisms involved require further study, these findings provide important insights into environmental contributions to early-life seizures.

1. Introduction

Stress is a common precipitant of seizures in individuals with epilepsy (Temkin and Davis, 1984; Neugebauer et al., 1994; Frucht et al., 2000; Spector et al., 2000; Haut et al., 2007; Nakken et al., 2005), and may provoke seizures by several mechanisms including changes in neurotransmitter and hormone levels within the brain (Nakken et al., 2005; Baram, 1993; Joëls, 2009; Danzer, 2012). In addition, an age-dependent epilepsy of infants called infantile spasms (IS), responds to the stress hormones ACTH and prednisone/prednisolone (Hrachovy et al., 1983; Snead et al., 1989; Baram et al., 1996; Mackay et al., 2004). The mechanisms of the anticonvulsant effects of these hormones might involve a suppression of the endogenous proconvulsant stress peptide corticotropin-releasing hormone (CRH) (Baram, 1993; Huang, 2014; Brunson et al., 2001a; Stafstrom et al., 2011). These facts illustrate that there is a complex relationship between stress and seizures, with important clinical implications.

Whereas much remains to be learned about stress, seizures and epilepsy in the mature brain (Joëls, 2009; Danzer, 2012), even less is known about pro-convulsant and pro-epileptic effects of stress early in life (Baram, 1993; Huang, 2014; Hatalski et al., 1998; Velísek et al., 2007). There is epidemiological evidence for increased incidence of epilepsy in children growing up in presumably stressful, resource-poor environments (Shamansky and Glaser, 1979), yet the effects of early-life stress on the risk of developing epilepsy have been little studied in humans (Li et al., 2008; van Campen et al., 2012). In rodent models, stress during development is pro-

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convulsant in several models of seizures and epilepsy: Prenatal maternal restraint increases the severity of status epilepticus (Sadaghiani and Saboory, 2010), enhances the effects of postnatal N-methyl-D-aspartate (NMDA) receptor agonists (Velísek et al., 2007; Chachua et al., 2011), decreases afterdischarge threshold and enhances kindling rates (Edwards et al., 2002). Early postnatal stress increases seizure susceptibility in several models of seizures including amygdala kindling, freeze lesion followed by hyperthermic seizures and chemo-convulsant-induced seizures (Salzberg et al., 2007; Desgent et al., 2012; Schridde et al., 2006).

These data suggest that both prenatal and early postnatal stresses enhance vulnerability to seizures. Perinatal and early-life stresses take place during critical periods of brain development when synapses form, networks get established and environmental signals may have long-lasting effects (Bale et al., 2010). Stress may influence all of these processes and may alter brain circuits, cellular properties, and synaptic connections (Huang, 2014; Brunson et al., 2001b). These changes, in turn, may render the individual more vulnerable to seizures and epilepsy via poorly understood interactions with a number of stress mediators (Joëls, 2009; McEwen, 2007; Baram and Hatalski, 1998; Wang et al., 2001).

In addition to the effects of stress on seizure susceptibility, early-life stress might provoke the emergence of spontaneous seizures (epileptogenesis). This is an important question, because epilepsy, especially childhood-onset epilepsy, is associated with adverse consequences: children with epilepsy perform worse than expected in school, employment, marriage, and parenthood (Camfield et al., 1993; Gaitatzis et al., 2004). They have increase prevalence of depression and intellectual dysfunction (Berg et al., 2008; Cormack et al., 2007). Findings in animal models of seizures support detrimental effects of seizures on cognition and emotion (Holmes et al., 2002; Lynch et al., 2000; Dubé et al., 2009).

To examine directly whether sustained early-life stress promotes hyperexcitability and epilepsy, we used a well characterized model of chronic early-life stress (CES) (Gilles et al., 1996; Avishai-Eliner et al., 2001; Ivy et al., 2008). We then probed the mechanisms underlying these stress-provoked changes to brain-network excitability.

2. Material and methods

2.1. Animals

Subjects were progeny of timed-pregnancy Sprague–Dawley rats. Rats were maintained in quiet facilities under controlled temperatures and light–dark cycle. Cages were monitored every 12 h for the presence of pups and the date of birth was considered postnatal day (P) 0. Pups were mixed among litters, and litter size was adjusted to 12 per dam if necessary, to obviate the potential confounding effects of genetic variables and of litter size. Litters of all experimental groups contained equal numbers of males. When weaned (on P21), male rats were housed 2–3 per cage. All experiments were performed in accordance with the National Institutes of Health (NIH) guidelines on laboratory animal welfare and approved by the University of California–Irvine Institutional Animal Care and Use Committee.

2.2. The chronic early-life stress paradigm

CES was induced in rat pups from P2 to P9 as described previously (Gilles et al., 1996; Ivy et al., 2008; Molet et al., 2014). Stress in pups was provoked by unpredictable and fragmented nurturing behaviors of rat dams (Baram et al., 2012). These behaviors, in turn were induced by equipping the cages with limited nesting material that prevented the dam from constructing a satisfactory nest and altered her behavior (Ivy et al., 2008; Molet et al., 2014). Briefly, on P2, pups (male and female) from several litters were mixed among dams and those assigned to the CES groups were transferred to cages with limited bedding and nesting material. Specifically, cages were fitted with a plastic coated aluminum mesh platform to sit approximately 2.5 cm above the cage floor. Bedding was reduced to only cover the cage floor sparsely, and one-half of a paper towel was provided for nesting maternal. Control dams and pups resided in bedded cages, containing ~0.33 cubic feet of sanitary chips. Control and experimental cages were undisturbed during P2–P9. Maternal nursing behaviors were monitored during the week of CES as described (Molet et al., 2014). At P21, pups were weaned, and male pups were used for the experiments.

2.3. Electrode implantation

Animals ($n = 36$, 23 CES and 13 controls) underwent surgery at P11 – P15. Two EEG systems were used: a tethered and a telemetric. For the tethered setup, a first rat cohort (6 CES and 4 control) was implanted with bipolar stainless steel electrodes (Plastics One, Roanoke, VA) in both hippocampi (coordinates: AP −2.0, L 1.8, V −2.6 mm with reference to Bregma) (Paxinos and Watson, 1998), and with 7 dural screw-electrodes over the parietal frontal and temporal cortices. A second cohort of rats (11 CES and 5 control) was implanted bilaterally with bipolar electrodes in amygdala (coordinates: AP -1.6, L 3.6, V −8.4 mm with reference to Bregma) as well as with one electrode over the right fronto-parietal cortex. The amygdala electrodes (twisted wires) were connected in a sequential manner in a bipolar montage. The electrode going to grid 2 of the first derivation was also connected to grid 1 of the next derivation.

To record from the surface of the cortex, stainless steel insulated electrodes (E363) were used and consisted of electrodes with mounting screw and socket contact. A surface electrode positioned over the cerebellum was used as a ground electrode for all electrode assemblies used here. The intracranial electrodes used in this study (Plastic One; E363/2-2 TW) were made of a stainless steel wire (bare diameter 200 μm) insulated with polyimide (electrode diameter insulated 230 μm) and ending with a female socket contact. The electrodes were supplied twisted together, i.e., bipolar electrodes. The insulation at the tip level was removed for the terminal 0.1 mm, and tips separated by 1 mm. Screws were placed over the cortex and dental acrylic was used to anchor the electrodes to the pedestal. We recorded potentials either between two cortical electrodes, one in the left and one in the right fronto-parietal cortices; or between one of the twisted electrodes in the right amygdala and the right cortical electrode. The electrodes were connected through a pedestal to EEG leads (6 channel electrode cable with 363 plug; Plastics One Roanoke, VA).

For the telemetry system, a third rat cohort (6 CES and 4 controls) was implanted with bipolar electrodes in the right amygdala and with two dural electrodes over the right and left fronto-parietal cortices. The telemetry transmitter allows simultaneous recordings of two channels. Each channel received input from the 2 wires of one intra-amygdala bipolar electrode. The radiotelemetry unit (the two-channel PhysioTel Implantable Transmitter F20-EET; Data Sciences International [DSI], St Paul, MN, USA) was positioned in a pocket created subcutaneously in the rat flank through a scalp incision. All electrodes were fixed in place with dental acrylic and connected to the leads of the radiotelemetry unit, and then covered and fixed to the skull with dental acrylic cement.

For all rats, electrode placement was verified post hoc (Choy et al., 2014), and they were all located in amygdala or hippocampus as intended. Because of the size of the amygdaloid complex in neonatal rats, we can comfortably state only that all electrode tips