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Seizure susceptibility and locus ceruleus activation are reduced following environmental enrichment in an animal model of epilepsy

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Abstract

Alterations in the complexity of social and physical housing environments modulate seizure susceptibility in animal models of epilepsy. The studies described here tested the hypothesis that environmental enrichment would delay seizure onset in the epileptic (El) mouse. Neural activation measured via cFos expression, accumulation of the stress neuropeptide corticotropin-releasing factor (CRF), and behavioral seizure susceptibility were quantified in El mice to better understand the mechanisms of ictogenesis. Enrichment housing of El mice from Postnatal Days 21 to 49 produced a 100% decrease in seizure susceptibility relative to El controls. cFos expression increased in the primary motor cortex, locus ceruleus, and hippocampus of El mice relative to ddY controls, an effect attenuated by enrichment housing. CRF levels were elevated by enrichment in the hippocampus of ddY mice only. This study provides evidence that enrichment housing delays the onset of seizure susceptibility in El mice while altering the neuronal and stress-related responses in seizure-associated regions of the El brain.

Keywords: cFos; El mouse; Environment; Immediate-early gene; Locus ceruleus; Stress

1. Introduction

Rodents exposed to an enriched environment exhibit increased brain weight, cortical thickness, and complexity of dendritic branching, and demonstrate improved performance in learning/memory tasks [1]. Enrichment enhances exploration of new objects, social interactions in a group housing context, exercise using a running wheel, and foraging for food [2]. Much of this morphological and functional plasticity can be demonstrated even when enrichment is instituted in adulthood [3]. Based on these findings, some investigators have proposed that early and substantial environmental enrichment could provide a treatment option for neurological defects that arise in the epileptic brain [4].

Several studies have shown that environmental enrichment protects against seizures. Kainic acid-induced seizures and excitotoxic injury are attenuated in Wistar rats housed from 21 to 42 days of age in enriched cages that provided access to running wheels, tunnels, rubber balls, a maze, a food administration station, and nesting material [5]. Similarly, adult rats housed in an enriched environment consisting of a larger housing area with tubes, tunnels, bridges, running wheels, food treats, and a larger social group, for 28 days prior to and during kindling, required significantly more sessions to achieve a fully kindled state [6]. Thus, environmental enrichment is protective in both chemically and electrically induced seizure models. In contrast to enrichment, social isolation housing consists of housing rodents individually. Isolation housing produces activating behavioral and endocrine consequences in adult rats, increasing reactivity to human handling, nervousness, aggression, and locomotor activity in response to novel situations [7]. The present studies are the first to assess the impact of environmental enrichment or social isolation

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on seizure susceptibility in a genetically predisposed animal model of epilepsy.

In the El mouse, a model of multifactorial temporal lobe epilepsy, both genetic and environmental factors contribute to the onset of the seizure susceptibility [8,9]. Seizure onset in the El mouse can be affected by variables such as age, handling history, gender, maternal/paternal effects, and prior seizures, but in routinely handled, group-housed mice, onset of seizure susceptibility typically occurs around 80–90 days of age [10]. Tail suspension handling at 30 days of age can hasten the onset of seizure susceptibility [10], while decreasing the amount of routine human handling El mice experience can delay the onset of seizure susceptibility [11]. Enriching the early postnatal environment by rearing El pups with non-El dams that provide increased levels of maternal care delays the expression of seizures [12]. Finally, group versus isolation housing over the first 40 days of life alters the adult locomotor phenotype of El mice [13]. Thus, the El mouse represents a noninduced model of epilepsy that exhibits a prolonged time-to-first-seizure interval that is sensitive to multiple external factors including changes in early life environment [14].

As the period leading up to the first epileptic seizure in a vulnerable organism likely involves profound neuroadaptations in the brain, it may be valuable to study modifications in neural connectivity exerted by seizure-modulatory treatments [15]. Expression of the immediate early gene product, cFos, is used as a reliable indicator of seizure-related neural activity [16]. El mice exhibit increased immediate-early gene expression in a variety of brain regions following a seizure [17], or following exposure to tossing up or tail suspension handling, even in mice that have not yet exhibited any seizures [18]. In particular, tail suspension at 40 days of age in El mice upregulates cFos production in the locus ceruleus and primary motor cortex as compared with either El mice that did not experience tail suspension or non-seizure-susceptible ddY mice [19]. This suggests that El mice are hypersensitive to vestibular or stressful stimulus exposure, even prior to the occurrence of seizures. In the present studies, cFos expression was quantified in seizure-prone El mice and non-seizure-susceptible ddY control mice to assess neuroadaptations arising from altered housing conditions, prior to the onset of seizure susceptibility.

In organisms with a predisposition for epilepsy, early-life stress and traumatic events are associated with an increase in the occurrence of seizures [20]. Both the development of the disorder and the repeated occurrence of seizures are facilitated by environmental stressors [21]. Corticotropin-releasing factor (CRF) modulates endocrine, physiological, and behavioral responses to stress, and several studies have shown that central injection of a CRF receptor agonist promotes seizures [22]. In El mice, CRF system impairment delays seizure onset, providing a causal link between the levels of this stress peptide in the brain and seizure susceptibility [23,24]. Thus, one final hypothesis under test in the present studies was that altered reactivity to tail suspension handling resulting from changes in housing conditions in El mice would be reflected by CRF levels in brain.

2. Methods

2.1. Animals and housing conditions

The present study was conducted using El ($n = 66$) and ddY ($n = 66$) mice maintained in a dedicated breeding colony generated from stock donated generously by Dr. Thomas Seyfried (Boston College). Animals were kept under standard conditions in a temperature (22–24 °C)- and humidity (30–31%)-controlled room under a 12-hour reversed light/dark cycle (lights on at 2200). Mice had access to food and water ad libitum, except during seizure susceptibility testing. All animals were housed in specially designed connector cages, which minimized the need for human handling [14]. Briefly, standard polypropylene cages were customized by cutting a circular hole about halfway along one of the long sides of the cage. A threaded insert was inserted into the cage through the hole and covered with a cap. When it was necessary to transfer a mouse to a new clean cage for husbandry, performed by the investigators rather than the vivarium staff, caps were removed and the insert of the home cage was placed next to the insert of a second modified cage. This apparatus allowed mice to move from one cage to another without the need for human handling that is known to influence El seizure susceptibility [11].

Mice were weaned on Postnatal Day 21 (PND 21), ear-punched for identification, weighed, and then allocated to one of three experimental conditions: control (3 or 4 mice per cage, $n = 22$ per strain), enrichment (6 mice per cage, $n = 22$ per strain), or isolation (1 mouse per cage, $n = 22$ per strain). Control and isolation group mice were housed in standard shoebox transfer cages, modified as described above, with Sanichip bedding that was changed once weekly. The enrichment condition consisted of a standard rat cage ($45 \times 24 \times 21$ cm) modified to use the cage connector system, with a wire lid that dipped low enough for the mice to be able to eat from it, a water bottle with an extended nozzle, one running wheel (Comfort Wheel, Super Pet), one polyvinylcarbonate tube (approximately 7 in. long and 2 in. in diameter), two mouse nesting spheres, one cotton nestlet, and food provided on the floor of the cage to allow for foraging. Enrichment cages also contained Sanichip bedding that was changed once weekly. Each enrichment cohort consisted of two groups of three mice drawn from two separate litters born within 1–2 days of each other. Equal numbers of male and female mice were included in each condition, and all group-housed mice were of the same gender. The majority of mice remained in their respective housing conditions until PND 49, except for a subset of mice removed on PND 42 for immunohistochemical analysis. PND 42 was selected from a prior study demonstrating specific changes in neural activation following tail suspension in El mice [19]. Further, environmental manipulations applied up to PND 42 are sufficient to alter adult phenotype in El mice [13]. On PND 49, all remaining animals were returned to control housing conditions. In particular, the control housing condition was unchanged ($n = 3$ or 4/cage), the four remaining mice in the enrichment condition were housed together in one shoebox-sized cage, and mice in the isolation condition were housed together with same-sex littermates ($n = 2$–4/cage).

2.2. Handling-induced seizure susceptibility (HISS) testing

On PND 80, eight mice from each condition were tested using a HISS protocol that involves repetitive tail suspension to simulate exposure to stimuli normally associated with routine cage changing [10]. Each mouse was picked up by the tail, suspended approximately 10–15 cm above the floor of the cage for 30 seconds, and then placed in a clean cage with fresh Sanichip bedding. Two minutes later, the mouse was again picked up by the tail, suspended for 15 seconds, and then returned to the home cage. This same procedure was repeated 30 minutes later. Following the first phase of testing completed on PND 80 (HISS-1), the entire handling procedure was repeated 10 days later on PND 90 (HISS-2); mice were also weighed at the conclusion of HISS-2. Treatment-blind observers detected