Epilepsy & Behavior 44 (2015) 78–85

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Behavioral changes following a single episode of early-life seizures support the latent development of an autistic phenotype

Paul B. Bernard [a], Anna M. Castano [a], Christy S. Beitzel [b], Vivian B. Carlson [a], Tim A. Benke [a,b,c,d,e,*]

[a] Department of Pediatrics, University of Colorado, School of Medicine, USA
[b] Department of Neuroscience Graduate Program, University of Colorado, School of Medicine, USA
[c] Department of Neurology, University of Colorado, School of Medicine, USA
[d] Department of Pharmacology, University of Colorado, School of Medicine, USA
[e] Department of Otolaryngology, University of Colorado, School of Medicine, USA

ARTICLE INFO

Article history:
Received 10 November 2014
Revised 6 January 2015
Accepted 7 January 2015
Available online 4 February 2015

Keywords:
Early-life seizure
Autism
Restricted interest
Repetitive behaviors
Grooming
Social approach
Development
Marble burying

ABSTRACT

We probed the developmental and behavioral consequences of a single episode of kainic acid-induced early-life seizures (KA-ELS) in the rat on postnatal day 7. Correlates of developmental trajectory were not altered, demonstrating that long-term consequences following KA-ELS are not initiated by secondary causes, such as malnourishment or alterations in maternal care. We report reduced marble burying in adult rats, suggestive of restricted interests, a trait common to experimental and clinical autism. We did not detect increased repetitive grooming during habituated cage behavior. However, we did detect reduced grooming in adult KA-ELS rats in the presence of an unfamiliar rat, supporting altered social anxiety following KA-ELS. Reanalysis of a social approach task further indicated abnormal social interactions. Taken together with previous physiological and behavioral data, these data support the hypothesis that KA-ELS lead to a latent autistic phenotype in adult rats not attributable to other early alterations in development.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder affecting approximately 1 in 68 children [1]. Autism spectrum disorder with comorbid intellectual disability (ID) is more often associated with epilepsy [2,3]. The association of epilepsy with ASD (30% of children with autism have epilepsy [3]) has suggested common pathophysiology, though it remains unclear whether epilepsy or the high (>80%) incidence of epileptiform abnormalities [4] is causative or just comorbid [5]. The diagnosis of ASD is behaviorally defined by two core symptom domains: impaired social communication and stereotyped, repetitive behaviors with restricted interests [6]. The clinical (and experimental) severity of these behaviors varies.

While initial studies in immature rats reported minimal long-term consequences (such as morphological damage) following acute seizures, later studies using multiple or more severe seizures demonstrated anatomical changes [7] often correlating with abnormal behavior [8]. Studies using single, mild seizures demonstrate long-term behavioral and physiological changes without anatomical abnormalities which can include features of ASD and ID [9–13] (reviewed in [14]).

We have characterized permanent physiological and behavioral changes in a rat model of KA-ELS [9–12]. These changes are reminiscent of changes observed in clinical and experimental ASD [15], specifically the fragile X mental retardation protein (FMRP) knockout (KO) mouse. These changes include learning deficits, altered socialization, increased mGluR-mediated long-term depression (mLTD), and changes in signaling pathways affected in some types of ASD [9–12]. In order to insure that these long-term effects are a result of KA-ELS rather than a secondary cause, such as malnutrition or altered maternal care, we used a developmental battery to assess growth and developmental trajectory. The necessity to investigate alterations in developmental trajectory following ELS is highlighted as pups display reduced weight gain following ELS in other models [16]. Thus, deficits in weight gain and development could have contributed to the long-term behavioral and physiological deficits reported. Therefore, it was necessary to determine if these long-term deficits are due to ELS itself or if they are a secondary effect of an altered developmental trajectory.

Abbreviations: ASD, autism spectrum disorder; ELS, early-life seizure; FMRP, fragile X mental retardation protein; ID, intellectual disability; KA, kainic acid; KO, knockout; mGluRs, metabotropic glutamate receptors; mLTD, mGluR-mediated long-term depression; P, postnatal day; SA, social approach.

* Corresponding author at: University of Colorado Denver, School of Medicine, 12800 E 19th, MS8102, Denver, CO 80045, USA. Tel.: +1 303 724 3568; fax: +1 303 724 2439.
E-mail address: tim.benke@ucdenver.edu (T.A. Benke).

http://dx.doi.org/10.1016/j.yebeh.2015.01.006
1525-5050/

A core feature of ASD is restricted or repetitive behaviors (DSM-5) [6]. Repetitive behaviors in clinical and experimental ASD encompass a variable range of motor stereotypies, self-injurious behavior, compulsions, persistent occupation with parts of objects, preoccupations or restricted patterns of interest, and inflexible adherence to nonfunctional routines and rituals [17,18]. Burying behavior in rodents refers to the displacement of bedding material using the snout and forepaws in an effort to cover an object [19]. Increased marble burying is generally attributed to increased anxiety [20]. However, more recent evidence suggests that it reflects repetitive, perseverative, obsessive, or compulsive-like behaviors [21]. Excessive grooming, depending on the social situation, can also be a repetitive behavior. We examined this along with stereotypical head-weaving behavior following ELS.

Deficits in social communication are another core feature of ASD [6]. The social approach (SA) task is commonly used to assess social behavior in rodents [22]. Interpretation and analysis of this task can vary [14]. In our previous work, ELS rats spent significantly less total time in the novel rat chamber compared to controls [9], consistent with a social communication deficit. Reanalysis was used to investigate the nature of interactions. Taken together, these results are consistent with the latent development of an autistic phenotype following ELS that cannot be directly attributed to other physical, developmental, nutritional, or maternal factors.

2. Materials and methods

2.1. Animals

All studies conformed to the requirements of the National Institutes of Health *Guide for the Care and Use of Laboratory Rats* and were approved by the Institutional Animal Care and Use subcommittee of the University of Colorado Anschutz Medical Campus. Timed-pregnant Sprague–Dawley rats (Charles Rivers Labs, Wilmington, MA) gave birth in-house. All rodents were housed in microisolator cages with water and chow available ad libitum. Separate cohorts of animals were used for the developmental battery (15 rats), social approach (21 rats), home cage grooming (20 rats), and marble burying (30 rats).

2.2. Seizure induction

Kainic acid (KA) was used to induce limbic-like seizures as done in previous studies [9–12]. Kainic acid administration simulates clinical conditions resulting in glutamatergic overexcitation as may occur in hypoxia–ischemia or other metabolic or genetic derangements [23]. Kainic acid given at P7 (P0 defined as the date of birth) resulted in discontinuous behavioral and electrical seizure activity lasting up to 3 h [24]. Postnatal days 7–10 in the rat are roughly equivalent to the neonatal period in humans [25]; therefore, most models of ELS have seizures on or around this developmental time point. Male rat pups were subcutaneously injected with KA (2 mg/kg; Tocris, Ellisville, MO) on postnatal day (P) 7. Onset of seizure activity occurred within 30 min of injection and was characterized by intermittent myoclonic jerks, generalized tonic–clonic jerks, scratching, "swimming," and "wet-dog shakes." Mortality was less than 3%. Morphological changes (cell loss, axonal sprouting) are not detected in this model, and spontaneous recurrent seizures (SRS) do not occur [9,11,26]. Control male rat pups were injected with an equivalent volume of 0.9% saline. Male pups were chosen in order to eliminate the effects of hormonal cycles on behavior. Rats were then tagged with a microchip (Avid Identification Systems, Norco, CA) so that experimenters remained blind to the treatment. Offspring were returned to their dam after observable seizure activity ceased (approximately 3 h), and dam–pup interactions were periodically observed. The developmental battery was conducted from P5 to P18. Rats were weaned and separated at P20–22. At P60–90, behavioral analyses were undertaken.

2.3. Physical and neurobehavioral developmental battery

Pups were weighed daily from P5 to P18. Incisor eruption was assessed from P5 to criterion (emergence of both lower incisors). Eye opening was assessed from P5 to criterion (a break in the sutures of both eyes). Pinna detachment was assessed from P5 to criterion. Auditory startle response was assessed from P5 to criterion (appearance of startle response). Surface righting ability was measured from P5 to P10: pups were placed in a supine position and positive response was obtained when the animal returned to prone position, with all paws on the ground. Physical, developmental, and neurobehavioral assessments were adapted from the Cincinnati Test Battery and the Barlow and Sullivan Screening Battery [27]. The developmental milestones were evaluated at the same time daily by an observer blinded to treatment groups. A handheld "clicker" (typically used in canine training) was used to produce the sound–startle stimulus. The pup was placed prone on the tabletop, the handheld clicker was positioned 5 cm above the pup, and the "click" was produced. This produced a sudden, loud sound that consistently induced an acoustic startle reflex in adult and adolescent control rats. A sudden retraction of the pup's head and limbs in response to the sound was taken as a positive startle reflex [28].

2.4. Behavioral testing

Prior to testing in the SA (P60) or the marble-burying (P 90) task, all rats were habituated to the holding room (next to testing room) and testing room as well as to the process of transporting the rats from the housing room to the testing room. All rats were also habituated to being handled by the experimenters implementing the testing. Except where noted, a naïve cohort of rats was used for each experiment.

2.4.1. Three-chambered social approach task

The three-chambered SA task, employed as a standard test for assaying sociability in mice [22,29], was adapted for rats by scaling the size of the apparatus [9]. In a three-chambered testing apparatus, preference for a novel rodent versus a novel object is measured. While adapting for size was relatively straightforward, subtleties in performance in the social approach task (and the relationship to social deficits) may vary between species due to more complex social interactions in rats [30]. We reanalyzed previously published data [9]. A subject rat (control or ELS) was placed into the middle chamber of the 3-chambered (99 cm (W) × 162 cm (L) × 41 cm (H)) apparatus. Initially, the subject rat was placed in the center chamber for a ten-minute habituation; for this initial habituation phase, the doors to the other compartments were closed. For the second habituation phase, the sliding doors (10 × 10 cm) were elevated and the subject rat was given free access to the three chambers during a 10-min session. Following the habituation phases, the subject rat was placed back into the center, the sliding doors were closed, and an unfamiliar male target rat (trained and habituated) was placed under one of the two buckets (inverted, wire, medium-sized, golf-range bucket (Western Golf, Thousand Palms, CA)) while a novel object (a rubber cube) was placed under the other bucket; the doors were opened, and the subject rat was permitted to explore the entire apparatus for 10 min for the sociability phase (phase 3). Behavior in each compartment during both sessions was recorded via video (JVC Everio, Bedford, TX) and scored off-line using TopScan (CleverSys, Reston, VA). The apparatus was cleaned with ethanol (70%) between each trial. Total time spent in each chamber as well as time spent exploring (sniffing) the novel rat or object were recorded. A "sniff" was defined as close (3 cm) orientation of the subject's nose toward the novel rat or object. A "sniff" terminated when the subject turned its head (and "attention") away from the rat or object. Low-level white noise with dimmed lighting was present during testing.