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

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Abstract

We probed the developmental and behavioral consequences of a single early life seizure induced by kainic acid (KA-ELS) in the rat on post natal 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.

Keywords

Early Life Seizure; Autism; Restricted Interest; Repetitive Behaviors; Grooming; Social Approach; Development; Marble Burying

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Author responsibilities

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1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder affecting approximately 1 in 68 children [1]. ASD 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 epileptiform phenomenon [4] are causative or just comorbid [5]. Diagnosis of ASD is behaviorally defined by two core symptom domains; impaired social communication and stereotyped, repetitive behaviors with restricted interests [6]. 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) knock out (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 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.

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 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. Re-analysis 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 micro-isolator 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]. KA administration simulates clinical conditions resulting in glutamatergic over-excitation, as may occur in hypoxia-ischemia or other metabolic or genetic derangements [23]. KA given at P7 (P0 defined as the date of birth) resulted in discontinuous behavioral and electrical seizure activity lasting up to three hours [24]. P 7–10 in the rat is roughly equivalent to the neonatal period in humans [25], therefore most models of ELS induce seizures on or around this developmental time point. Male rat pups were subcutaneously injected with KA (2 mg/kg; Tocris, Ellisville, MO) on post natal day (P) 7. Onset of seizure activity occurred within 30 minutes of injection and was characterized by intermittent myoclonic jerks, generalized tonic-clonic jerks, scratching, “swimming,” and “wet-dog shakes.” Mortality was less than 3%. KA given at P7 results in discontinuous behavioral and electrical seizure activity lasting up to three hours [24]. 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 hours) and dam-pups 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 until 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. Pinnae 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, development 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 hand-held “clicker” (typically used in canine training) was used to produce the sound-startle stimulus. The pup was placed prone on the table-top, the hand held 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 (P 60) or the marble burying task (P 90), all rats were habituated to the holding room (next to testing room), 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-Chamber 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 straight forward, 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 re-analyzed previously published data [9]. A subject rat (control or ELS) was placed into the middle chamber of the 3-chamber (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 x 10 cm) were elevated and the subject rat 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 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.

2.4.2. Marble Burying Task, Stereotypical Behavior and Grooming—The marble burying task was used to evaluate repetitive digging behavior [21]. Clean cages (30.5 cm x 45.7 cm x 21.6 cm) were filled with wood chip bedding to a depth of 5.5 cm. Each rat was habituated to this environment for 15 minutes, following which the rat was briefly removed and 16 black marbles (2.5 cm diameter) were placed equidistant in a 4 x 4 arrangement. The number of marbles buried (> 50% covered by bedding material) in 15 minutes was recorded via video and scored off-line. Number of marbles moved, as well as time spent attending to marble was also recorded. White noise (70 dB) with dimmed lighting was present during testing.

While scoring behavioral performance in the marble burying task, it was observed that several rats displayed multiple bouts of repetitive head movements. Baseline behavior was interrupted by several seconds of head swaying from side to side. This pattern of behavior was thought to be consistent and was evaluated with experimenter blind to treatment. Criteria for a positive bout of abnormal head movement was defined as an interruption of normal exploratory behavior for at least 5 seconds, in combination with continual head movement from left to right. The number of episodes meeting criterion during the 15 minute burying phase were recorded.

ASD mouse models are reported to groom more, thought to be indicative of increased anxiety or increased tendency towards repetitive behaviors [31]. Each rat was videotaped (in conjunction with EEG recordings reported previously [9]) while housed individually and fully habituated in a standard rat cage, (46 cm long, 23.5 cm wide, 20 cm high) in dim lighting at the same time each day. Each rat was scored for spontaneous grooming of all body regions for 10 mins. Grooming was also assessed during phase 3 of the SA task (described above).

2.5. Statistics

Data are expressed as mean $\pm$ SEM with n = number of rats for a given treatment. Data are plotted using Sigmaplot 12.0 (Systat, Chicago, IL). Mann-Whitney Rank Sum, ANOVA (Holm-Sidak post-hoc testing), repeated measures ANOVA (Holm-Sidak post-hoc testing) or Student's t-tests were used, where indicated and appropriate using SigmaPlot 12.0 (Systat, Chicago, IL). Significance is reported at $P < 0.05$ unless otherwise stated. All experiments were conducted and scored by experimenters blind to treatment.

3. Results

3.1. Developmental Battery

We did not detect a significant effect of KA-ELS on developmental weight gain ($F(1,13) = 2.30$, $P = \text{NS}$, Repeated Measures ANOVA, Figure 1). Even the day following ELS, weight gain was not altered (control: $22.375 \text{ g} \pm 0.450$, $n = 8$, ELS; $22.875 \text{ g} \pm 0.481$, $n = 7$, $p = \text{NS}$, Student's t-test). Rats were assessed visually up to 4 hours following termination of behavioral ELS. At this time all rats appeared to have normal dam-pup interactions, were feeding normally and had milk in their stomachs. While conducting the pre-described developmental battery, dam-pup interactions were periodically observed in the days and

weeks following ELS. During these observations, dam maternal care was consistent between treatment groups.

Measures of developmental milestones and reflexes were unaffected by KA-ELS (Figure 2): day of first eye opening (control: 13.00 ± 0.378 , $n = 8$, ELS: 13.571 ± 0.202 , $n = 7$, $P = \text{NS}$, Student's t-test), onset of acoustic startle response (control: 12.375 ± 0.183 , $n = 8$, ELS: 12.429 ± 0.202 , $n = 7$, $P = \text{NS}$, Student's t-test), pinnae detachment (control: 14.00 , $n = 8$, ELS: 14.00 , $n = 7$, $P = \text{NS}$, Student's t-test) and incisor protrusion (control: 8.625 ± 0.183 , $n = 8$, ELS: 8.143 ± 0.143 , $n = 7$, $P = \text{NS}$, Student's t-test). Righting ability was not affected by KA-ELS. Prior to KA-ELS on P7, all rats were capable of righting themselves in 2 seconds or less. Following KA-ELS (P8 and beyond) all rats were able to right themselves in approximately 1 second or less. These results are consistent with no interaction of KA-ELS on dam-pup interactions.

3.2. Marble Burying

We investigated marble burying [21] as an assay of repetitive behaviors [31] in adult rats. Clinical ASD is associated with an increase in repetitive behavior, therefore we expected an increase in marble burying as seen in some mouse models of ASD [32] (though not others [33–35]). Following KA-ELS, rats buried fewer marbles (control: 4.24 ± 0.726 , $n = 17$, ELS: 2.29 ± 0.7010 , $n = 13$, $P < 0.05$, Student's t-test). We hypothesized that KA-ELS rats were attending to marbles more, but not successfully burying them. This was not the case as KA-ELS rats moved fewer marbles than their control littermates (control: 8.29 ± 0.882 , $n = 17$, ELS: 4.54 ± 0.94 , $n = 13$, $P = 0.007$, Student's t-test). Further, KA-ELS rats attended (defined as time with marble) to marbles similar to controls (control: $144.21 \pm 18.31\text{s}$, $n = 17$, ELS: $175.08 \pm 33.41\text{s}$, $n = 13$, $P = \text{NS}$, Student's t-test). This supports our hypothesis that KA-ELS rats moved and buried fewer marbles due to focused attention towards specific marbles, while control rats divided their attention more equally over all marbles present.

3.3. Head Movements

While scoring behavioral performance in the marble burying task, it was observed that several rats displayed multiple bouts of repetitive head movements. Baseline behavior was interrupted by several seconds of head swaying from side to side. This pattern of behavior was evaluated with experimenter blind to treatment (see Methods). This behavior was predominantly expressed by KA-ELS rats (episodes per 15 minutes; control: 1.06 ± 0.53 , $n=17$, ELS: 5.39 ± 1.50 , $n=13$, $P<0.006$, Mann-Whitney Rank Sum) (Figure 3d). Some head movements in control rats did also meet our scoring criteria, though infrequent. We speculate head stereotypies in KA-ELS rats may be equivalent to stereotypies, often triggered by stimulation or new situations (such as marble burying) that are associated with clinical ASD. We doubt that these movements are epileptic as we did not detect with prolonged video EEG seizures or epileptiform abnormalities at this age following KA-ELS [9].

3.4. Grooming in habituated cage

Repetitive or excessive grooming is reported to occur in mouse models of ASD (reviewed in [36]) and following ELS (reviewed in [14]). Increased grooming is thought to mimic

repetitive behaviors observed in clinical ASD [36]. Total grooming in habituated cage did not differ between treatment groups (control: 187.40 ± 39.43 , $n = 10$, ELS: 173.80 ± 41.239 , $n = 10$, $P = \text{NS}$, Student's t-test). Number of grooming bouts initiated did not differ (control: 20.40 ± 3.30 , $n = 10$, ELS: 17.70 ± 4.17 , $n = 10$, $P = \text{NS}$, Student's t-test), nor did average length of grooming bouts (control: 9.59 ± 1.38 , $n = 10$, ELS: 10.29 ± 2.24 , $n = 10$, $P = \text{NS}$, Student's t-test)(Figure 4). Establishing equivalent habituated cage grooming was necessary to interpret any differences in grooming that might appear in socialization tasks.

3.5. Altered sociability following KA-ELS

We expanded our previous analysis of the three-chambered SA task in adult rats [9]. We previously found that during the habituation phase (trial #2, Methods), neither control nor KA-ELS rats demonstrated a preference for any of the three chambers and confirmed no side preferences [9]. During trial #3, the sociability phase, KA-ELS rats displayed a reduced preference for spending time in the “novel rat” chamber [9]. Further analysis demonstrated that while KA-ELS rats spent significantly less time in the novel rat chamber, when in the novel rat chamber they spent significantly more time (percentage of time in novel rat chamber) sniffing the target rat compared to controls (control: $26.7 \pm 2.41\%$, $n=9$, ELS: $42.80 \pm 2.79\%$, $n=11$, $P < 0.001$, Student's t-test) (Figure 5). This is consistent with altered socialization following KA-ELS. Time spent sniffing the novel object was also altered (control: $4.10 \pm 1.96\%$, $n=7$, ELS: $12.7 \pm 2.62\%$, $n=10$, $P < 0.03$, Student's t-test). These abnormalities in the three chamber SA task are consistent with ASD features after KA-ELS and other rodent models of ASD [31, 36]. Further, ELS rats spent significantly less time grooming while in the novel rat chamber (control: $12.70 \pm 3.33\%$, $n=11$, ELS: $5.18 \pm 0.805\%$, $n=10$, $P < 0.03$, Student's t-test), suggesting altered social anxiety (Figure 5) [37, 38] given normal habituated cage grooming (Figure 4).

4. Discussion

The KA-ELS model here is distinct from other ELS models since it does not result in SRS [9]. Further, no detectable anatomical changes in hippocampus (cell loss, synaptic reorganization or spine density) have been reported [11, 26], yet KA-ELS rats still have behavioral deficits in learning and memory and social tasks as adults [9, 11, 12, 39]. These behavioral abnormalities correlate with altered mLTD [9]. Many of these findings mimic the behavioral and mLTD changes in the FMRP KO mouse, a prominent model of ASD [40].

The lack of weight gain difference between KA-ELS rats and controls is a very important finding (Figure 1). We have reported many persistent changes following KA-ELS, which are similar to those observed in clinical and experimental ASD [9–11]. We hypothesize that these changes are a direct result of KA-ELS. However an alternative possibility is that these changes occur as a secondary effect. KA-ELS could result in reduced feeding or changes in dam-pup interactions during development that could contribute to the formation of the ASD phenotype. Altered developmental trajectory has been reported in a different ELS model [16] but has not been assessed in the majority of ELS models (reviewed in [14]).

The appearance of developmental milestones (such as acoustic startle, eye opening, and righting ability) is known to be strongly influenced by maternal inputs. For instance, food

deprivation of the nursing mother delays the appearance of developmental milestones in her pups [28]. Results from our developmental battery indicate that developmental weight gain and dam-pup interactions are occurring normally and further supported by other measures of developmental trajectory (Figure 2). Thus our findings highlight the long term effects of KA-ELS in the absence of confounding variables that would be indicative of malnutrition and altered maternal care.

Several behavioral abnormalities have been identified in mouse models of ASD and to a lesser extent in the limited number of rat models of ASD. Often these behavioral abnormalities mimic those observed in clinical ASD. However in some cases, opposite results are observed. Some ASD KO mouse models display increased sociability [41, 42] while others express decreased sociability [29, 43]. Previously, we determined that while KA-ELS rats are still social (they spend more time in the novel rat chamber than the novel object chamber), they are less social than controls [9]. This finding is very similar to what is reported in the FMR1 KO [43]. Other ELS models also report variations in SA performance (reviewed in [14]). We re-assessed rat-rat interactions in SA to provide further evidence of altered social behavior after KA-ELS. While KA-ELS rats spend less time in the novel rat chamber, they spend more time (percent of time in chamber) sniffing the novel rat compared to controls (Figure 5). This may be consistent with reports of abnormally increased sociability in mouse KO models of ASD [41, 42]. KA-ELS rats may have abnormally reduced social anxiety, as KA-ELS rats sniffed the target rat more and groomed less when in the target rat chamber (Figure 5); it is thought that increased grooming, in this context, is indicative of enhanced social anxiety [44]. Elevated anxiety is commonly associated with clinical ASD [45, 46]. Many mouse models of ASD display increased anxiety [47, 48]. However we did not detect changes in anxiety in KA-ELS rats as measured using the open field and elevated plus maze [12]. Results in the ELS model demonstrate the complexity of social behavior in the rodent, which is even more complex in the rat as opposed to the mouse [30]. Socialization deficits are quite heterogeneous in the human population [49], therefore variability in results among various ASD models may reflect a healthy diversity among animal models (mimicking the diversity present in the clinical population) [14].

Alterations in repetitive behaviors vary following ELS from no change in either the marble burying or hole poke tasks in mice [13] to increased grooming [50]. Uncertainty exists with respect to what underlies these changes in grooming: anxiety [51] or compulsive, repetitive behaviors [21]. Our data is consistent with the later, as we found reduced marble burying, yet we have previously determined that anxiety levels were not altered by KA-ELS [12]. We speculate that reduced marble burying is indicative of restricted interest. Restricted interest and cognitive rigidity are a common feature of clinical [52] and experimental ASD [35]. Reduced behavioral flexibility is reported in the fluorothyl ELS model [53]. In the marble burying task KA-ELS rats focus solely on a limited number of marbles and do not shift attention to other marbles compared to control rats. This observation is supported by the fact that while KA-ELS rats buried and moved fewer marbles, they attended to marbles just as long as controls (Figure 3). To further support an ASD phenotype, we found increased stereotypical movements following KA-ELS.

5. Conclusions

Our findings of altered socialization and restricted behavior further link KA-ELS with an ASD and ID neurocognitive phenotype [9, 11, 12]. Severe neonatal seizures, or status epilepticus, both correlate[54] and have been independently associated with an adverse neurocognitive outcome[55, 56]. The odds-ratio of ASD is 3-fold higher in pre-term infants with seizures[57]. In contrast, seizures may simply be a marker of the severity of brain injury [58–60]. Rodent work suggests seizures do not add further neurocognitive deficits to an anatomical insult [61]. Clinical work [62], has suggested that seizures themselves do not necessarily increase the risk of neurocognitive sequelae following hypoxia. While this study found a nearly 2-fold relative risk of neurocognitive sequelae isolated to seizures, it was not statistically significant. Noting this sizable risk, critiques of this study have pointed out that this lack of significance may be due to a lack of statistical power [63, 64]. Clinically, this will be difficult to fully address until there are proven, safe and effective treatments for neonatal seizures. In this vein, seizures have again been implicated as contributing to neurocognitive sequelae in a study following neonatal stroke treated with cooling [65]. Our findings from a rodent model indeed suggest a causal relationship between ELS and ID with ASD. This may have important consequences for clinical treatments for disorders with ID and ASD, particularly those associated with ELS or similar early life insults, where abnormal mLTD and FMRP-mediated signaling may be subsequently present as we have shown [9]. We hypothesize that alterations in mGluR signaling may underlie the long term behavioral alterations in our ELS model; the initial changes after KA-ELS are distinct from the chronic changes but largely unknown [9]. Targeting mGluR-mediated signaling chronically may be one avenue to ameliorate behavioral abnormalities. Our findings and those noted above argue for including in the discussion with families that seizures can independently impact neurodevelopmental outcomes but that there are limitations with acute clinical therapies. These discussions are important to counsel families that given the lack of effective therapies acutely, early educational interventions long-term are crucial.

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Abbreviations

ASD	autism spectrum disorder
ELS	early life seizure
FMRP	Fragile X Mental Retardation Protein
ID	intellectual disability
KA	kainic acid
KO	knock out

mGluR	metabotropic glutamate receptors
mLTD	mGluR mediated long term depression
P	post natal day
SA	social approach

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Highlights

- Early and late effects of kainate-induced early life seizures (KA-ELS) studied in rats.
- KA-ELS do not disrupt dam-pup interactions or early development.
- KA-ELS disrupt social interactions in adult rats.
- KA-ELS cause abnormal repetitive behaviors in adult rats.

Figure 1. Impact of ELS on developmental weight gain

ELS on P7 caused no changes in weight gain, assessed from P5 to P18 ($F(1,13) = 2.30$, $P = \text{NS}$, Repeated Measures ANOVA; control $n = 8$; ELS $n = 7$). Rats were weighed at the same time daily.

Figure 2. Results of developmental battery

A, Onset of acoustic startle occurred on approximately the same day for both ELS rats and controls (control: 12.375 ± 0.183 , $n = 8$, ELS: 12.429 ± 0.202 , $n = 7$, $P = \text{NS}$, Student's t-test). B, Pinna detachment occurred on the same day for both ELS and control rats (control: 14.00, $n = 8$, ELS: 14.00, $n = 7$, $P = \text{NS}$, Student's t-test). C, ELS had no impact on incisor protrusion (control: 8.625 ± 0.183 , $n = 8$, ELS: 8.143 ± 0.143 , $n = 7$, $P = \text{NS}$, Student's t-test). D, ELS did not affect day of first eye opening (control: 13.00 ± 0.378 , $n = 8$, ELS: 13.571 ± 0.202 , $n = 7$, $P = \text{NS}$, Student's t-test).

Figure 3. Marble burying

A, ELS rats buried fewer marbles than age matched controls (control: 4.24 ± 0.726 , $n = 17$, ELS: 2.29 ± 0.7010 , $n = 13$, $P < 0.05$, Student's t-test). B, ELS rats moved fewer marbles than age matched controls (control: 8.29 ± 0.882 , $n = 17$, ELS: 4.54 ± 0.94 , $n = 13$, $P = 0.007$, Student's t-test). C, ELS and control rats spent similar time exploring marbles (control: 144.21 ± 18.31 , $n = 17$, ELS: 175.08 ± 33.41 , $n = 13$, $P = \text{NS}$, Student's t-test). D, Stereotypical head movements were predominantly detected in ELS rats (control: 1.06 ± 0.53 , $n = 17$, ELS: 5.39 ± 1.50 , $n = 13$, $P < 0.006$, Mann-Whitney Rank Sum).

Figure 4. Grooming in habituated cage

A, Total grooming time did not differ between treatment groups (control: 187.40 ± 39.43 , $n = 10$, ELS: 173.80 ± 41.239 , $n = 10$, $P = \text{NS}$, Student's t -test). B, Number of grooming bouts initiated did not differ (control: 20.40 ± 3.30 , $n = 10$, ELS: 17.70 ± 4.17 , $n = 10$, $P = \text{NS}$, Student's t -test). C, ELS did not affect the average length of grooming bouts (control: 9.59 ± 1.38 , $n = 10$, ELS: 10.29 ± 2.24 , $n = 10$, $P = \text{NS}$, Student's t -test).

Figure 5. Social approach task

A, ELS rats spent significantly more time sniffing the novel rat (control: $26.7 \pm 2.41\%$, $n=9$, ELS: $42.80 \pm 2.79\%$, $n=11$, $P<0.001$, Student's t-test). B, ELS rats spent significantly more time sniffing the novel object (control: $4.10 \pm 1.96\%$, $n=7$, ELS: $12.7 \pm 2.62\%$, $n=10$, $P<0.03$, Student's t-test). C, ELS rats spent significantly less time grooming while in the novel rat chamber (control: $12.70 \pm 3.33\%$, $n=11$, ELS: $5.18 \pm 0.805\%$, $n=10$, $P<0.03$, Student's t-test).