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Early-life status epilepticus acutely impacts select quantitative and qualitative features of neonatal vocalization behavior: Spectrographic and temporal characterizations in C57BL/6 mice

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Abstract

Early-life seizures are known to cause long-term deficits in social behavior, learning, and memory, however little is known regarding their acute impact. Ultrasonic vocalization (USV) recordings have been developed as a tool for investigating early communicative deficits in mice. Previous investigation from our lab found that postnatal day (PD) 10 seizures cause male-specific suppression of 50-kHz USVs on PD12 in 129 SvEvTac mouse pups. The present study extends these findings by spectrographic characterization of USVs following neonatal seizures.

On PD10, male C57BL6 pups were administered intraperitoneal injections of kainic acid, or physiological saline. On PD12, isolation-induced recordings were captured using a broad-spectrum ultrasonic microphone. Status epilepticus significantly suppressed USV quantity ($p = 0.001$) and total duration ($p < 0.05$). Seizure pups also utilized fewer complex calls than controls ($p < 0.05$). There were no changes in call latency or inter-call intervals. Spectrographic analysis revealed increased peak amplitude for complex, downward, short, two-syllable, and upward calls, as well as reduced mean duration for short and two-syllable calls in seizure mice. This investigation provides the first known spectrographic characterization of USVs following early-life seizures. These findings also enhance evidence for USVs as an indicator of select communicative impairment.

Graphical Abstract

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Conflict of interest

The authors declare that there are no conflicts of interest.

Keywords

Developmental disorder; ultrasonic; communication; vocalization; USV; comorbidity

1. INTRODUCTION

According to recent estimates, the prevalence rate of childhood epilepsy in the United States is 1%, affecting nearly 700,000 children [1]. Epilepsy is broadly defined as a having more than one spontaneous seizure occurring greater than 24 hours apart [2]. The incidence of seizures is significantly higher during the first year of life compared to any other time point [3, 4]. While many children only experience a single isolated seizure episode, this is sufficient to cause long-term changes in psychomotor and cognitive development [5]. Previous research recapitulates this effect in the rat model, with exposure to a single seizure episode leading to future cognitive impairments [6]. There is also evidence that these seizures lead to impaired sociability and autistic-like behavior later in life [7].

Ultrasonic vocalizations (USVs) are a pre-lingual communicative behavior analogous to human infant crying, which is highly conserved among several species [8]. This behavior is broadly defined in mice as ultrasonic whistle-like sounds occurring between 30 and 90 kHz, in response to isolation from the dam [9]. Spectrographic analyses of this behavior may serve as a useful tool for analyzing the acute behavioral impact of seizures. Indeed, empirical investigations in humans have shown that children with a history of epilepsy show a variety of changes in language development and production, and the likelihood of atypical language representation is directly related to the age of seizure onset [10]. Previous investigation from our lab found that one instance of status epilepticus (SE) induced on postnatal day 10 significantly impact quantitative aspects of mouse vocalizations on postnatal day 12 [11]. This was the first study to be published concerning the impact of seizures on vocalization behavior in neonatal mice.

It has shown that spectrographic characterization of neonatal USVs is particularly useful for understanding qualitative features of vocalization behavior [12]. Previous studies utilizing this paradigm have shown significant changes in spectrographic and temporal features of USVs in models of neurodevelopmental disorder, including FXS [13] and Autism Spectrum

Disorder (ASD) [14]. Although it is known that epilepsy shares high comorbidity with many neurodevelopmental disorders [15], it is unknown whether similar spectrographic changes occur. Therefore, the present study aims to explore potential qualitative changes in USVs via spectrographic characterization following kainic acid-induced SE in C57BL/6 mice. We hypothesize that neonatal seizures will significantly impact qualitative aspects of vocalization behavior.

2. METHODS

2.1. Animals

Male C57BL/6 pups (n = 26) were generated at Baylor University for this experiment. All pups were housed in individual cages with parents and littermates. The environment was controlled at an ambient temperature of 22°C with 14-h light/10-h dark diurnal cycles and *ad libitum* access to food and water. All procedures were conducted in compliance with Baylor University Institutional Animal Care and Use Committee and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Seizure Induction

On PD10, all pups were randomly assorted into either treatment (n=14) or control groups (n=12) and administered intraperitoneal (IP) injections of either 0.5% kainate (1.5mg/kg) or an equivalent dose of 0.9% physiological saline. We used approximately half of the males for any particular litter for our study. Each litter consisted of 2–5 males. The subjects were sampled across 8 litters in order to reduce the impact of any one particular litter. All pups were then placed into individual containers with clean bedding, warmed with a heating pad to approximately 35°C. Approximately 20–30 minutes following kainate injection mice were observed entering status epilepticus (SE), characterized by continuous tonic-clonic seizures for 1–2 hours. The SE period continued until natural cessation of seizure activity. Control group pups were simultaneously monitored during this time. Upon cessation of SE, both groups were returned to their home cages.

2.3. Ultrasonic Vocalization Recording

In order to assess changes in USV behavior following early life status epilepticus, we examined isolation-induced USVs in seizure and control groups on PD12. Prior to the recording phase, all pups were transferred into a housing pan with clean bedding that was warmed with a heating pad set to approximate ambient nesting temperature (~35°C). During the recording phase, one pup at a time would be placed into another housing pan, then placed within an acrylic sound-attenuating chamber. The USVs were recorded for 2 minutes for each pup. The recording apparatus consisted of a condenser microphone (CM16/CMPA, Avisoft Bioacoustics, Germany) connected to an ultrasound-recording interface (UltraSoundGate 116Hb, Avisoft Bioacoustics). This apparatus allowed us to record all USVs on a continuous spectrum from 0–125 kHz. Following completion of the recording phase, the pup would then be returned to the warmed housing pan. This procedure was repeated in sequentially until all pups had completed the recording phase. All pups were then returned to their home cage. Testing consisted of no more than 4 mice per litter, so time

separated from the mother was 20 minutes or less. In total, 718 calls ($n_{\text{control}} = 626$, $n_{\text{seizure}} = 92$) were obtained from recordings.

2.4. Ultrasonic Vocalization Analyses

Avisoft SASLab Pro software (Avisoft Bioacoustics, Germany) was used to convert all USV files into spectrograms via Fast Fourier transformation (FFT), with parameters set as follows: FFT-length = 1024 points, time window overlap = 75% (100% Frame, Hamming Window), frequency resolution = 488 Hz, and a time resolution = 1ms. All calls were manually identified using previously described classification scheme [14]. Duration, peak frequency, peak amplitude, and fundamental frequency were collected for each USV type.

2.5. Statistical Analyses

Quantitative changes were analyzed using a multivariate analysis of variance (MANOVA) with the independent factors of treatment and call-type, and dependent factors of USV quantity and total USV duration. Call-type distributions were analyzed using a Pearson Chi-Square test, and post-hoc z -tests with Bonferroni correction for multiple comparisons. Latency to begin vocalizing and inter-call intervals (ICIs) were analyzed using independent t -tests. For the purpose of this study, ICI is defined as the duration of time between successive vocal emissions. Spectrographic changes were analyzed using MANOVA with the independent factors of treatment and call-type, and dependent factors of peak amplitude, peak frequency, fundamental frequency, and mean call duration. Significant interactions were followed by independent t -tests with Bonferroni correction for multiple comparisons. For all statistical tests, significance was defined by having a p -value ≤ 0.05 . All data were analyzed using either GraphPad Prism 6 software (La Jolla, CA) or SPSS 20.0 (IBM, USA). Values are shown as mean $\pm$ standard error of the mean (S.E.M.) for each group.

3. RESULTS

3.1. Reduction in total number of calls and amount of time spent vocalizing after status epilepticus

The first branch of our analysis focused on the effect of early-life status epilepticus on quantitative aspects of mouse vocalization behavior. To do this, we first examined whether seizures elicited changes in the number of calls emitted and total time spent vocalizing by mice across all call types. In our evaluation, multivariate tests revealed significant effects of treatment $F_{2, 159} = 5.726$, $p < 0.01$ and call type $F_{18, 320} = 2.921$, $p < 0.001$ on USV quantity and total duration. However, we did not observe an interaction of these treatment and call-type effects $F_{18, 320} = 0.769$, $p = 0.737$. We performed subsequent ANOVAs to determine the specific direction of the statistically significant effects and found a statistically significant reduction of USV quantity $F_{9, 160} = 4.52$, $p < 0.001$ and total duration $F_{9, 160} = 1.92$, $p = 0.05$ across call types after SE (Fig. 1A–B). There were also significant differences in and across call types. These results suggest that early life seizures suppress neonatal vocalizations across all call types.

3.2. Select alterations in the frequency distribution of call types utilized in vocal behavior after status epilepticus

We next examined whether SE altered the frequency with which mice used each call type in order to determine the differences in call type that each group used. Using a Pearson's Chi-Square test, we found showed significant changes in the call type frequency distribution after seizures ($p < 0.05$). Subsequent z-tests revealed that seizure mice produce significantly fewer complex calls ($p < 0.005$), as compared to controls (Fig. 2). These results suggest that SE may impact how mice utilize select call types during neonatal vocal behavior.

3.3. Status epilepticus had no effect on latency to begin calling, nor inter-call intervals

Finally, we next examined whether SE impacted the latency to begin vocalizing when placed in isolation, as well as the inter-call interval (ICI). In our evaluation, we did not find statistically significant differences in latency to begin vocalizing ($t_{1,11} = 0.93$, $p = 0.37$) between control (12.57 ± 9.02 seconds) and seizure group mice (29.07 ± 17.51 seconds). There was also no statistically significant difference in ICIs between control (1.41 ± 0.17 seconds) and seizure group mice (2.42 ± 0.69 seconds) $t_{1,104.11} = 1.41$, $p = 0.16$.

3.4. Early-life status epilepticus led to changes in spectrographic & temporal properties of select call types

The second branch of our analysis focused on the effect early-life status epilepticus on qualitative aspects of vocalization behavior, using spectrographic and temporal characterization. To do this, we first examined whether seizures elicited changes in the peak amplitude, peak frequency, fundamental frequency, and mean call duration of USV emissions all call types. In our evaluation, multivariate tests revealed significant effects of treatment $F_{4,699} = 39.9$, $p < 0.001$ and call type $F_{32,2808} = 9.31$, $p < 0.001$ across all dependent variables. Further, we also observed a significant interaction of treatment and call-type effects across all dependent variables $F_{24,2808} = 3.23$, $p < 0.001$. Subsequent analysis of between-subject effects identified significantly higher peak amplitudes in mice after seizures $F_{1,702} = 137.26$, $p < 0.001$. We also observed a marginal reduction in fundamental frequency in mice after seizures $F_{1,702} = 3.822$, $p = 0.051$, but this did not quite reach statistical significance. There were no differences in peak frequency $F_{1,702} = 0.45$, $p = 0.50$ or mean call duration $F_{1,702} = 3.04$, $p = 0.08$. Evaluation of call-type effects revealed significant differences in mean duration $F_{8,702} = 38.37$, $p < 0.001$, peak frequency $F_{8,702} = 2.348$, $p < 0.05$, peak amplitude $F_{8,702} = 2.718$, $p < 0.01$, and fundamental frequency $F_{8,702} = 2.450$, $p < 0.05$ across all call types.

Further, we also observed a significant interaction of treatment and call-type effects for mean call duration $F_{6,702} = 5.954$, $p < 0.001$, peak frequency $F_{6,702} = 2.22$, $p < 0.05$, and fundamental frequency $F_{6,702} = 2.45$, $p < 0.05$, but not for peak amplitude $F_{6,702} = 1.70$, $p = 0.12$. Subsequent multiple comparisons revealed that SE mice displayed increased peak amplitudes for complex, downward, short, two-syllable, and upward calls. These mice also displayed reduced mean durations for short and two-syllable calls (Table 1). These results suggest that SE may impact spectrographic & temporal properties of select call types.

4. Discussion

Although significant efforts have been made to characterize neonatal vocalizations in mouse models of neurodevelopmental disorders, there has been little investigation into the effect of early-life status epilepticus (SE). The objective of the present study was to extend previous findings from our lab by conducting spectrographic and temporal characterization of USVs following kainic acid-induced seizures. Our results demonstrate that PD10 SE led to decreased utilization of complex calls on PD12. It was further shown that these mice display call-type specific reduction of call duration and increased peak amplitude.

The results of the present study are in line with a previous investigation from our lab showing suppressed USV quantity and duration in male pups on PD12 [11]. Our quantitative findings are also largely in agreement with one previous study, which investigated isolation-induced USVs following pilocarpine-mediated SE in neonatal rats [16]. Following separation from the nest, rat pups emit USVs restricted to the 40kHz frequency [17]. On PD15, these authors found a reduction in total time spent vocalizing and a reduction in total number of USVs [16]. In contrast to the present study, these authors observed reduced latency to begin vocalizing. One difference in this study is that they monitored USVs at 40kHz, whereas we recorded USVs at 30–90 kHz. It is possible that the rats had produced an earlier vocalization at a lower or higher frequency, which was not detected. An additional possibility is the postnatal day selected for USV recordings, which was PD15 for the Lopez-Meraz, 2014 study, as compared to PD12 in our study. It is unlikely that this difference is attributable to kainate versus pilocarpine-induced SE, as both compounds are known to produce seizures of equivalent severity [18]. The previous and present studies from our lab, as well as the Lopez-Meraz et al. [16] study, are contrasted by another study that found PD12 male pups display a greater number of USVs following febrile convulsions on PD7 [19]. The conflicting findings between this study and previous studies may be attributable to differences in seizure-induction method or PD time chosen. While kainic acid and pilocarpine mediate epileptogenesis by targeting agonism of kainate [20] and muscarinic receptors [21], respectively, fevers produce seizures via much more broad, non-specific effects on the brain.

Spectrographically, the present study found call-specific suppression of mean USV duration and enhancement of peak amplitude. When a sound is received by the auditory apparatus, its physical amplitude is subjectively interpreted as loudness [22]. The physiological basis of this altered vocal phenotype following early life SE is currently unclear. In one previous study, Cox et al (2012) recorded the USV behavior of Sprague-Dawley rats at several developmental time points following chronic prenatal exposure to cocaine and saline. On PD21, males from both groups were shown to have increased amplitude of loudest call and total USV duration, as compared to controls. When correlated with limbic immunofluorescent staining, call amplitude and total USV duration were directly correlated with increased neuronal density in the ventromedial hypothalamus (VMH) [23]. Given that both groups displayed equivalent VMH pathology, these authors attributed their findings to differences in early environmental stressors and stress mechanisms. One previous study corroborates this hypothesis, demonstrating similar VMH pathology in rats on PD21 following just 24 hours of maternal deprivation on PD3 [24] Interestingly, call peak

amplitude has also been linked to maternal attentiveness towards pups. In an mouse embryo-transfer study, Wohr et al (2008) found that high peak amplitude to be a key determinant of how much time mothers spent attending to pups [25]. The increased time spent with pups emitting high peak amplitude calls was also independent of maternal preference for her own pups. Interestingly, changes in peak amplitude were attributed solely to pup genotype, and were not influenced by the phenotype of its maternal caregiver.

Taken together, these studies suggest that early environmental stress contributes to specific neurogenic changes in the neonatal limbic system, altering both the development of vocal behavior and communication with caregivers. Given that our spectrographic findings are in line with those aforementioned, it is possible that early-life SE may be sufficiently traumatic to manifest USV changes by similar mechanisms. Future studies should consider utilizing similar paradigms to investigate how early-life SE affect neural substrates of vocal behaviors.

Similar to rodents, albeit more complex, human infants are known to modulate the physical characteristics of vocalizations to convey affective meaning [26–28]. One particular aspect of infant-caregiver communication that has been investigated is the averseness of cry sounds. For both parent and nonparent caregivers, the degree of perceived cry averseness signals an infants' current level of distress [29]. Consistent with this notion, previous studies find a positive association between perceived averseness and both latency and likelihood of responding to an infant cry [30]. Aversive sounding cries typically exhibit longer duration, dysphonation [31], and higher fundamental frequencies [32]. Interestingly, atypical infant crying patterns may be influenced by underlying neurological disorders. In a study of 6-month old infants at risk for ASD, researchers found they emitted cries with significantly higher fundamental frequencies than low risk counterparts [33]. Previous studies have also identified abnormal acoustic features in infants with other medical conditions, including chromosomal abnormalities, metabolic disorders, prenatal toxin exposure, and certain viral infections [34]. Therefore, further characterization of crying behavior, in conjunction with predictive modeling, may be useful for early identification of various pathological conditions in the pediatric population.

5. Conclusion

Taken together, the results of the present study extend previous findings by showing call-specific changes in the spectrographic and temporal features of USV behavior. These findings support a growing body of evidence that USV behavior may be an early behavioral marker for neurodevelopmental disorders. Currently, there are few tools available for practitioners to assess the depth of damage following an epileptic event in infancy. Given that early intervention can improve long-term quality of life outcomes in epilepsy [35], new diagnostic markers are urgently needed. Future studies may investigate whether seizure-induced USV changes are predictive of long-term behavioral deficits and brain damage. Such early detection through analysis of neonatal vocalization behavior could lead to translational methods for improved treatment outcomes in patients.

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ABBREVIATIONS

ASD	Autism Spectrum Disorder
USV	Ultrasonic Vocalization
IP	Intraperitoneal
IEG	Immediate Early Gene
PD	Postnatal Day
SE	Status Epilepticus
FFT	Fast Fourier transformation
MANOVA	Multivariate Analysis of Variance
SEM	Standard Error of the Mean
ICI	Inter-call Interval
VMH	Ventromedial Hypothalamus

References

- [1]. Russ SA, Larson K, Halfon N. A National Profile of Childhood Epilepsy and Seizure Disorder. *Pediatrics* 2012;129: 256–264. [PubMed: 22271699]
- [2]. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J Jr., Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Mathern GW, Moshe SL, Perucca E, Scheffer IE, Tomson T, Watanabe M, Wiebe S. ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* 2014;55: 475–82. [PubMed: 24730690]
- [3]. Kramer U Epilepsy in the first year of life: a review. *J Child Neurol* 1999;14: 485–9. [PubMed: 10456756]
- [4]. Hauser WA. The prevalence and incidence of convulsive disorders in children. *Epilepsia* 1994;35 Suppl 2: S1–6.
- [5]. Roy H, Lippé S, Lussier F, Sauerwein HC, Lortie A, Lacroix J, Lassonde M. Developmental outcome after a single episode of status epilepticus. *Epilepsy & Behavior* 2011;21: 430–436. [PubMed: 21705280]
- [6]. Cornejo BJ, Mesches MH, Benke TA. A single early-life seizure impairs short-term memory but does not alter spatial learning, recognition memory, or anxiety. *Epilepsy Behav* 2008;13: 585–92. [PubMed: 18678283]
- [7]. Lugo JN, Swann JW, Anderson AE. Early-life seizures result in deficits in social behavior and learning. *Exp Neurol* 2014;256: 74–80. [PubMed: 24685665]
- [8]. Lummaa V, Vuorisalo T, Barr RG, Lehtonen L. Why Cry? Adaptive Significance of Intensive Crying in Human Infants. *Evolution and Human Behavior* 1998;19: 193–202.

- [9]. Branchi I, Santucci D, Alleva E. Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. *Behav Brain Res* 2001;125: 49–56. [PubMed: 11682093]
- [10]. Saltzman J, Smith ML, Scott K. The impact of age at seizure onset on the likelihood of atypical language representation in children with intractable epilepsy. *Brain Cogn* 2002;48: 517–20. [PubMed: 12030499]
- [11]. Reynolds CD, Smith G, Jefferson T, Lugo JN. The effect of early life status epilepticus on ultrasonic vocalizations in mice. *Epilepsia* 2016;57: 1377–85. [PubMed: 27378279]
- [12]. Branchi I, Santucci D, Vitale A, Alleva E. Ultrasonic vocalizations by infant laboratory mice: A preliminary spectrographic characterization under different conditions. *Developmental Psychobiology* 1998;33: 249–256. [PubMed: 9810475]
- [13]. Roy S, Watkins N, Heck D. Comprehensive analysis of ultrasonic vocalizations in a mouse model of fragile X syndrome reveals limited, call type specific deficits. *PLoS One* 2012;7: e44816. [PubMed: 22984567]
- [14]. Scattoni ML, Gandhi SU, Ricceri L, Crawley JN. Unusual Repertoire of Vocalizations in the BTBR T+tf/J Mouse Model of Autism. *PLoS ONE* 2008;3: e3067. [PubMed: 18728777]
- [15]. Persad V, Thompson MD, Percy ME. Epilepsy and developmental disability part I: developmental disorders in which epilepsy may be comorbid. *Journal on Developmental Disabilities* 2003;10: 123–152.
- [16]. López-Meraz M-L, Medel-Matus J-S, Morgado-Valle C, Beltrán-Parrázal L, Pérez-Estudillo C, Manzo J. Effect of lithium-pilocarpine-induced status epilepticus on ultrasonic vocalizations in the infant rat pup. *Epilepsy & Behavior* 2014;31: 263–266. [PubMed: 24230988]
- [17]. Portfors CV. Types and functions of ultrasonic vocalizations in laboratory rats and mice. *Journal of the American Association for Laboratory Animal Science : JAALAS* 2007;46: 28–34. [PubMed: 17203913]
- [18]. Inostroza M, Cid E, Brotons-Mas J, Gal B, Aivar P, Uzcategui YG, Sandi C, Menendez de la Prida L. Hippocampal-Dependent Spatial Memory in the Water Maze is Preserved in an Experimental Model of Temporal Lobe Epilepsy in Rats. *PLOS ONE* 2011;6: e22372. [PubMed: 21829459]
- [19]. Keller A, Saucier D, Sheerin A, Yager J. Febrile convulsions affect ultrasonic vocalizations in the rat pup. *Epilepsy & Behavior* 2004;5: 649–654. [PubMed: 15380115]
- [20]. Lévesque M, Avoli M. The kainic acid model of temporal lobe epilepsy. *Neuroscience & Biobehavioral Reviews* 2013;37: 2887–2899. [PubMed: 24184743]
- [21]. Curia G, Longo D, Biagini G, Jones RSG, Avoli M. The pilocarpine model of temporal lobe epilepsy. *Journal of Neuroscience Methods* 2008;172: 143–157. [PubMed: 18550176]
- [22]. Phillips DP, Carr MM. Disturbances of loudness perception. *Journal of the American Academy of Audiology* 1998;9: 371–379. [PubMed: 9806411]
- [23]. Cox ET, Hodge CW, Sheikh MJ, Abramowitz AC, Jones GF, Jamieson-Drake AW, Makam PR, Zeskind PS, Johns JM. Delayed developmental changes in neonatal vocalizations correlates with variations in ventral medial hypothalamus and central amygdala development in the rodent infant: effects of prenatal cocaine. *Behavioural brain research* 2012;235: 166–175. [PubMed: 22867871]
- [24]. Oomen CA, Girardi CEN, Cahyadi R, Verbeek EC, Krugers H, Joëls M, Lucassen PJ. Opposite Effects of Early Maternal Deprivation on Neurogenesis in Male versus Female Rats. *PLOS ONE* 2009;4: e3675. [PubMed: 19180242]
- [25]. Wöhr M, Dahloff M, Wolf E, Holsboer F, Schwarting RKW, Wotjak CT. Effects of Genetic Background, Gender, and Early Environmental Factors on Isolation-Induced Ultrasonic Calling in Mouse Pups: An Embryo-Transfer Study. *Behavior Genetics* 2008;38: 579–595. [PubMed: 18712592]
- [26]. Perception of Infant Cry as an Interpersonal Event. New York: Plenum Press; 1985.
- [27]. Young KS, Parsons CE, Stein A, Kringelbach ML. Interpreting Infant Vocal Distress: The Ameliorative Effect of Musical Training in Depression. *Emotion (Washington, D.C.)* 2012;12: 1200–1205.

- [28]. Daga RP, Panditrao AM. Acoustical Analysis of Pain Cries' in Neonates: Fundamental Frequency. *International Journal of Computer Applications* 2011: 18–21.
- [29]. LaGasse LL, Neal AR, Lester BM. Assessment of infant cry: Acoustic cry analysis and parental perception. *Mental Retardation & Developmental Disabilities Research Reviews* 2005;11: 83–93. [PubMed: 15856439]
- [30]. Wood RM, Gustafson GE. Infant Crying and Adults' Anticipated Caregiving Responses: Acoustic and Contextual Influences. *Child Development* 2001;72: 1287–1300. [PubMed: 11699671]
- [31]. Gustafson GE, Green JA. On the Importance of Fundamental Frequency and Other Acoustic Features in Cry Perception and Infant Development. *Child Development* 1989;60: 772–780. [PubMed: 2758875]
- [32]. Zeskind PS, Marshall TR. The Relation between Variations in Pitch and Maternal Perceptions of Infant Crying. *Child Development* 1988;59: 193–196.
- [33]. Sheinkopf SJ, Iverson JM, Rinaldi ML, Lester BM. Atypical Cry Acoustics in 6-Month-Old Infants at Risk for Autism Spectrum Disorder. *Autism Research* 2012;5: 331–339. [PubMed: 22890558]
- [34]. Soltis J The signal functions of early infant crying. *The Behavioral and Brain Sciences* 2004;27: 443–58. [PubMed: 15773426]
- [35]. Eikeseth S, Klintwall L, Jahr E, Karlsson P. Outcome for children with autism receiving early and intensive behavioral intervention in mainstream preschool and kindergarten settings. *Research in Autism Spectrum Disorders* 2012;6: 829–835.

HIGHLIGHTS

- Seizures suppressed quantity and total duration of USVs
- Seizures altered the frequency distribution of call types utilized
- Seizures increased peak amplitude for five call types
- Seizures reduced mean call duration for short and two-syllable calls
- There were no changes in latency to first vocalization or inter-call intervals

Figure 1. Comparison of quantitative features of ultrasonic vocalizations (USVs) in mouse pups after early-life status epilepticus.

(A) Seizure group pups displayed a significant reduction in total number of USVs across all call types [$F(1, 160) = 11.350$, $p = 0.001$], (B) as well as total time spent vocalizing across all call types [$F(1, 160) = 5.237$, $p < 0.05$], compared to control pups. Bars represent the mean and the error bars represent the standard error of the mean.

Distribution of USV Call Types

Figure 2. Call type profiles.

Pie graphs show the percentages of the different call categories between control and seizure group. The call types utilized by status epilepticus and control group pups differed significantly [X^2 (8, N= 718) = 17.709, $p < 0.05$]. Subsequent post-hoc z-tests revealed seizure mice to produce significantly fewer complex calls ($p < 0.005$), as compared to controls.

Table 1.

Spectrographic & temporal features of neonatal mouse USVs after early-life status epilepticus. All values represent mean $\pm$ S.E.M. of the indicated measurement variable. If significant difference was detected between groups, Bonferroni corrected probabilities are shown with asterisks and the direction of effect is shown with arrows.

Call Type	Control	Seizure	Change	Sig.
<i>Peak Amplitude (dB)</i>				
Chevron	53.17 $\pm$ 6.97	62.040 $\pm$ 4.70	□	□
Complex	54.31 $\pm$ 5.64	64.069 $\pm$ 4.85	↑	***
Downward	56.10 $\pm$ 5.53	62.526 $\pm$ 1.85	↑	***
Flat	56.12 $\pm$ 9.32	N/A	□	□
Frequency Steps	50.48 $\pm$ 7.93	63.9600 $\pm$ 5.13	□	□
Harmonics	49.30 $\pm$ 9.87	N/A	□	□
Short	55.51 $\pm$ 5.32	66.875 $\pm$ 3.70	↑	***
Two-Syllable	52.69 $\pm$ 6.56	66.573 $\pm$ 5.94	↑	***
Upward	51.90 $\pm$ 6.58	62.986 $\pm$ 5.75	↑	***
<i>Peak Frequency (Hz)</i>				
Chevron	83285.41 $\pm$ 8905.89	87966.66 $\pm$ 2650.15	□	□
Complex	83747.53 $\pm$ 7737.93	80536.36 $\pm$ 7981.26	□	□
Downward	84824.13 $\pm$ 8865.53	82490.90 $\pm$ 6083.98	□	□
Flat	65566.66 $\pm$ 15157.94	N/A	□	□
Frequency Steps	73052.63 $\pm$ 9778.91	81866.66 $\pm$ 3401.96	□	□
Harmonics	73262.50 $\pm$ 13570.86	N/A	□	□
Short	83156.37 $\pm$ 11801.62	81421.42 $\pm$ 15118.67	□	□
Two-Syllable	76072.72 $\pm$ 10420.39	83973.33 $\pm$ 13506.84	□	□
Upward	87416.66 $\pm$ 11424.56	81171.42 $\pm$ 7863.35	□	□
<i>Fundamental Frequency (Hz)</i>				
Chevron	51404.16 $\pm$ 21447.83	28866.66 $\pm$ 30259.10	□	□
Complex	47289.50 $\pm$ 19731.62	53227.27 $\pm$ 18437.08	□	□
Downward	45884.42 $\pm$ 21107.46	25436.36 $\pm$ 20090.36	□	□
Hat	49266.66 $\pm$ 26747.39	N/A	□	□
Frequency Steps	43610.52 $\pm$ 10622.14	50633.33 $\pm$ 7735.84	□	□
Harmonics	43050.00 $\pm$ 13246.34	N/A	□	□
Short	45025.92 $\pm$ 22846.90	36533.33 $\pm$ 26530.67	□	□
Two-Syllable	43274.54 $\pm$ 17444.22	27726.67 $\pm$ 27181.15	□	□
Upward	47663.33 $\pm$ 20392.19	55385.71 $\pm$ 17348.62	□	□
<i>Call Duration (sec)</i>				
Chevron	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	□	□
Complex	0.04 $\pm$ 0.02	0.05 $\pm$ 0.03	□	□
Downward	0.022 $\pm$ 0.01	0.027 $\pm$ 0.01	□	□
Flat	0.027 $\pm$ 0.01	N/A	□	□
Frequency Steps	0.057 $\pm$ 0.03	0.04 $\pm$ 0.02	□	□

Call Type	Control	Seizure	Change	Sig.
Harmonics	0.048 ± 0.03	N/A	□	□
Short	0.01 ± 0.01	0.01 ± 0.01	↓	***
Two-Syllable	0.03 ± 0.19	0.01 ± 0.01	↓	***
Upward	0.01 ± 0.01	0.02 ± 0.00	□	□

Note:

p < 0.001