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The Effect of Early-life Status Epilepticus on Ultrasonic Vocalizations in Mice

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Summary

Objective—Infant crying is a series of innate vocal patterns intended to elicit the attention of adult caregivers for fulfillment of specific needs, such as pain, hunger, or hypostimulation. It is one of the earliest forms of observable communication. In neonatal rodents, this behavior has recently been investigated as a potential early behavioral marker of neural deficits in neurodevelopmental disorders. However, few studies have examined the effects of seizures on vocalization behavior during the neonatal period. The purpose of this study is to investigate the effect of a single kainate-induced early life seizure on vocalization behavior in mice. This study also investigates the subsequent effect of seizures on two pathways critical for early neural development and epileptogenesis: the PI3K-Akt-mTOR and Canonical Wnt intracellular signaling pathways.

Methods—On postnatal day 10, male and female 129SvEvTac mice received a single intraperitoneal injection of kainic acid (2.5 mg/kg) or vehicle injection. The kainate administration resulted in 1–2 hours of status epilepticus. On postnatal days 11 and 12, the quantity and duration of isolation-induced ultrasonic vocalizations were recorded. Western blotting analyses were performed using male and female pups on postnatal day 12.

Results—There was significant, male-specific suppression in the quantity and total duration of 50-kHz calls on postnatal day 12 following seizures. The hippocampi of males on this postnatal day also revealed male specific changes in the PI3K-Akt-mTOR intracellular signaling pathway, as well as changes in phosphorylated fragile × mental retardation protein.

Significance—These findings demonstrate that early life seizures can disrupt communication behavior in neonatal mice.

Keywords

PI3K; mTOR; neurodevelopmental disorder; epilepsy; isolation-induced vocalizations; USV

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None of the authors has any conflict of interest to disclose.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Introduction

The prevalence rate of neurodevelopmental disorders in the United States increased 17.1% in children ages 3–17 from 1997 to 2008, and is projected to continue rising¹. Early intervention has been shown to improve the long-term behavioral outcomes for children with Autism Spectrum Disorder², and may provide therapeutic benefit to those with other neurodevelopmental disorders. However, early diagnoses are often missed by newborn disease screenings that are limited to conditions with known and reliable interventions. The U.S. Department of Health and Human services has recently instituted new recommendations that expand the scope of newborn disease screening to include several heritable disorders, but these still only address a small fraction of the affected population³. Novel diagnostic methods are needed for earlier recognition of neurodevelopmental disorders.

Social communication deficits are commonly identified in children with neurodevelopmental disorders. One form of prelinguistic communication in humans may be neonatal vocalization behavior. This behavior is characterized as a series of innate calling patterns emitted by neonates, which are intended to orient parental attention towards the fulfillment of specific needs required for optimal growth and survival⁴. Neonatal vocalizations are also highly conserved among many non-human species, including birds, rodents, and primates⁵. Given that neonatal vocalizations are readily observable, acoustic analysis of these calls may have significant potential in determining early behavioral deficits after seizures.

Due to the strong genetic components involved in neurodevelopmental disorders, many studies have utilized mouse models to investigate whether deletion of certain genes can result in altered neonatal vocalization behavior^{6–8}. Neonatal mice vocalizations are whistle-like sounds emitted at ultrasonic frequencies between 30kHz–90kHz⁹. Previous studies have shown abnormal ultrasonic vocalizations (USVs) in mouse models of neurodevelopmental syndromes such as Fragile × Syndrome⁸, Rett Syndrome⁷, and Down Syndrome⁶. Though significant progress has been made in understanding USVs, very few studies have investigated the impact of early life seizures on this behavior^{10; 11}.

In mice, seizures during early development result in long-term deficits in social behavior and in learning and memory^{12–14}. In humans, the rate of epilepsy affects approximately 1% of the population according to the CDC (2012)¹⁵, and shares high comorbidities with other neurodevelopmental disorders¹⁶. Therefore, understanding the relationship between early life seizures and long-term comorbidities has potential to improve the lives of many children. The first aim of this study was to investigate the effect of a single kainate-induced seizure on neonatal vocalization behavior in mice. We hypothesize that early life seizures can result in altered ultrasonic vocalizations.

This study also examined the influence of early life seizures on two intracellular signaling pathways implicated in early neural development and epileptogenesis. The first pathway was the phosphoinositide 3-kinase|serine/threonine kinase|mammalian target of rapamycin (PI3K-Akt-mTOR) intracellular signaling pathway, which has been well characterized.

During early life, this pathway supports the developing central nervous system by mediating neuronal survival, as it preserves appropriately wired synaptic connections and prunes aberrant ones¹⁷. In adulthood this pathway is correlated with cell proliferation, survival, neural plasticity, learning, and memory¹⁸. The second pathway was the Canonical Wnt intracellular signaling pathway, which is primarily active during pre- and postnatal development. During early development, this pathway supports neurogenesis by mediating embryonic cell proliferation, differentiation, anteroposterior/dorsoventral axis formation and body patterning¹⁹. In adulthood, the activity of this pathway persists primarily in the neural stem cell microdomains, such as the subventricular zone of the lateral ventricle and subgranular zone of the hippocampal dentate gyrus. In these regions, Canonical Wnt signaling maintains the pluripotency of neural stem cells required for adult neurogenesis²⁰.

Neonatal seizures are known to cause both acute pathological changes in the hippocampus, including extensive cell loss in the CA1 and CA3 subfields and hilus region²¹. These are also associated with long-term impairments in hippocampal plasticity and working memory²². Previous studies indicate that the PI3K-Akt-mTOR and Canonical Wnt intracellular signaling pathways are significantly affected in the hippocampus by acute seizures in adult rodents^{23; 24}, but few similar studies have examined the impact of acute seizures on these pathways during early development¹³. Given the importance of the hippocampus in both neurodevelopment and epileptogenesis, we selected this structure as the focus for investigating molecular signaling changes. We hypothesized that changes in USV behavior may be associated with subsequent changes in one or both of these pathways.

Methods

Subjects

Male and female 129 SvEvTac mice were generated at Baylor University for ultrasonic vocalization recording (n=62) and western blotting analyses (n=36) in this study. All mice were subsequently housed with parents and littermates at an ambient temperature of 22°C, with a 14-hour light and 10-hour dark diurnal cycle. Mice were given *ad libitum* access to food and water. All procedures involving mice were conducted in compliance with Baylor University Institutional Animal Care and Use Committee and the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Seizure Induction

On postnatal day 10 (PD10), pups were randomly assigned to seizure and control groups. Pups were then weighed and administered intraperitoneal injections of either 2.5mg/kg kainic acid (0.5 mg/ml) (Tocris, USA) or equivalent doses of 0.9% physiological saline. Pups from both treatment groups were placed into individual containers with clean bedding, which was kept warm with a heating pad set at $\approx 35^{\circ}\text{C}$ throughout the seizure induction period. The seizure group entered status epilepticus within 30 minutes of kainic acid administration, which was characterized by continuous tonic-clonic seizures persisting for approximately 1–2 hours. This group was then monitored until cessation of all seizure activity and the saline group was monitored for a similar period of time. Upon full recovery, pups from both treatment groups were returned to their home cages.

Ultrasonic Vocalizations

It has previously been shown that pups emit USVs upon separation from their home cages in order to elicit parental signal retrieval⁹. To determine whether mice develop deficits in USV behavior following early life seizures, we examined isolation-induced USVs in seizure and control group pups on PD11 and PD12. Prior to testing mice were placed in a housing pan with clean bedding that was warmed with a heating pad to ambient nesting temperature ($\approx 35^{\circ}\text{C}$). During testing all mice were placed individually into an acrylic, sound-attenuating chamber with USV detectors mounted in each upper corner and set to 50, 60, 70, and 80 kHz (Mini-3 Detector, Ultra Sound Advice, United Kingdom). These frequencies were selected because they collectively span the critical mass of vocalizations emitted by mice. Quantity and total duration of USV emissions at each frequency were measured over a 5-minute period by automatic detection software (Ultravox Software by Noldus, Netherlands). Following testing, mice were placed back into the warm housing pan and then returned to their home cage upon completion of testing. No more than 6 mice were tested at a time, so time separated from the mother was 35 minutes or less. All groups were tested approximately 24 and 48 hours after receiving injections.

Western Blot Analysis

A separate cohort of mice was used for the western blotting experiments to avoid any effects due to repeated behavioral testing. Mice were sacrificed on PD12 by rapid decapitation with surgical scissors. The brain was removed by dissection and rinsed in cold 1X phosphate buffer solution. The hippocampi were then dissected by a hippocampus dissection tools, placed on dry ice, and stored at -80°C until used. Both hippocampi were homogenized in ice-cold homogenization buffer (0.32M sucrose, 1mM EDTA, 5mM Hepes) containing protease inhibitor cocktail (P3840, Sigma, USA) and processed for western blotting as previously described²⁵. Through this procedure we produced crude synaptosomes and total homogenate samples. Total homogenate samples were used for total Akt, S473-phosphorylated Akt, T308-phosphorylated Akt, total S6, S235/236-phosphorylated S6, S240/244-phosphorylated S6, p70S6k, and FMRP. The crude synaptosomes were used for FMRP, S499-phosphorylated FMRP, GSK3 α , GSK3 β , Axin1, Dvl2, Dvl3, and Naked2. Equivalent protein concentrations were confirmed using the Bradford Protein Assay (Bio Rad, Hercules, CA, USA) and diluted in Laemmli loading buffer (4X: 0.25M Tris, pH 6.8, 6% SDS, 40% Glycerol, 0.04% Bromophenol Blue, 200mM Dithiothreitol). Following SDS-PAGE, proteins were transferred to Hybond-P polyvinyl difluoride membranes (GE Healthcare, Piscataway, NJ, USA). Membranes were then incubated for 1 hour at room temperature in blocking solution [5% non-fat milk diluted in 1X Tris Buffered Saline (50mM Tris-HCl, pH 7.4, 150mM NaCl) with 0.1% Tween (1X TBS-T) and 1mM Na₃VO₄]. The membranes were then incubated overnight at 4°C with the following primary antibodies in 5% milk in TTBS: Akt, S473-phosphorylated Akt, S6, S235/236-phosphorylated S6, S240/244-phosphorylated S6, p70S6k, FMRP, S499-phosphorylated FMRP, GSK3 α , GSK3 β , Axin1, Dvl2, Dvl3, Naked2, and actin (See Table 1 in the Supplemental section for further antibody details). After the incubation period membranes were washed in 1X TTBS ($3 \times 5\text{min}$). Membranes were then incubated with horseradish peroxidase labeled secondary antibody, anti-rabbit IgG. Following a final wash cycle in 1X TBS-T, membranes were then incubated with GE ECL Prime (GE Healthcare, Piscataway,

NJ, USA). Immunoreactive bands were captured by a ProteinSimple western blot imaging system (ProteinSimple, Santa Clara, CA, USA).

ProteinSimple AlphaView software was used to measure the optical density of resulting immunoreactive bands. Measurements obtained from all bands of interest were normalized to each subject's actin level. All experimental points represent a single mouse ($n=1$). All groups were normalized to the average of the control group per blot. All values from control mice represent biological replicates and were collected from control littermates.

Statistical Analysis

All data were analyzed by using SPSS 21.0 for PC (SPSS, Chicago, IL) or by Graphpad Prism 6 for PC (San Diego, CA). For all comparisons, the level of significance was set at $p < 0.05$. We used a two-way repeated-measures design for the ultrasonic vocalization PD11 and PD12 data, as well as examined main effects and interactions. If we found an interaction, we then performed an independent samples t-test on each day or a repeated measures t-test per group across days. In order to keep the results section manageable, we only included significant statistical effects.

Results

Suppression of ultrasonic vocalization behavior after seizures

We first examined whether seizures elicited changes in the quantity of USVs at each frequency. In our evaluation we did not find a main group effect for change in the quantity of 50-kHz calls from PD11 to 12 between groups $F(1,58) = 0.187$, $p = 0.67$ (Figure S1A and 1B). However, there was a significant interaction between both time and group $F(1, 58) = 4.1$, $p < 0.05$, as well as time $\times$ group $\times$ sex for the quantity of 50-kHz calls $F(1,58) = 6.5$, $p < 0.05$. An independent samples t-test on PD 11 found no significant differences between the groups $t(1,60) = 0.9$, $p = 0.37$. However, comparisons on PD12 revealed a marginal suppression of 50-kHz calls in the seizure group $t(1,60) = 2.0$, $p = 0.054$, with seizure pups vocalizing fewer times than control pups. We found no other significant main effects or interactions at any other call frequency between the seizure and control groups.

We then examined whether seizures elicited changes in the mean and total duration of USVs at each frequency. We did not find a main effect of group or sex from PD11 to PD12. However, there was a significant interaction of time and group $F(1,58) = 5.1$, $p < 0.05$ for the total duration of 50-kHz calls (Figure S2D). Upon separating the postnatal days to investigate this interaction, we found no main effect of seizure-induction on the mean or total duration of USVs at any frequency on PD11 (Figure S2A–B). However, comparisons on PD12 revealed a significant difference between groups on $t(1,60) = 2.26$, $p < 0.05$, with seizure pups spending less total time vocalizing than control pups (Figure S2D). There were no other significant main effects or interactions at any other call frequency between the seizure and control groups (Figure S2C–D). These results suggest that early life seizures suppress neonatal vocalizations, with the strongest behavioral effect observable in 50-kHz calls on PD12.

Sex-specific suppression of ultrasonic vocalization behavior after seizures

In addition, we determined whether the seizure-induced USV suppression was equivalent between sexes. Since we initially found a significant group $\times$ time $\times$ sex interaction, we analyzed the total quantity of USVs emitted at each frequency by sex. There was a significant interaction between time and group only in males $F(1,28) = 8.3$, $p < 0.01$ (Figure 1C). Upon separating the postnatal days to investigate this interaction we found significant suppression in the quantity of 50-kHz calls in seizure group males PD12 $t(1,28) = 2.4$, $p < 0.05$, with male seizure pups vocalizing fewer times than male control pups. A repeated measures t-test also revealed a significant decrease in the quantity of 50-kHz calls from PD11 to PD12 in seizure group males $t(1,17) = 2.6$, $p < 0.05$. There were no significant changes in USV quantity for male seizure or control animals at any other call frequency. There were also no significant changes in USV quantity for female seizure or control animals at any call frequency.

There was no main effect of seizure-induction on the mean or total duration of USVs for male or female pups at any frequency on PD11 (Figure 2A–D). On PD12 there was no main effect of seizure-induction on the mean USV duration for male or female pups at any frequency (Figure 2E–F). However, comparisons did reveal a suppression in the total duration of 50-kHz calls only in male pups $t(1,28) = 2.4$, $p < 0.05$, with seizure pups spending less total time vocalizing than control pups (Figure 2G). There were no significant changes in total USV duration for male seizure or control animals at any other frequency. There were also no significant changes in total USV duration for female seizure or control animals at any frequency on PD12 (Figure 2H). These results suggest that males display an increased sensitivity to seizure-induced suppression of neonatal vocalization behavior.

Seizure-induced suppression of neonatal vocalization behavior is associated with sex-specific changes in PI3K-Akt-mTOR signaling

As a result of primary behavioral effects being observed only on PD12, this timepoint was selected for subsequent western blot analysis. In our evaluation of the PI3K-Akt-mTOR pathway, we found no differences in total Akt, the ratio of S473-phosphorylated Akt/ total Akt, or T308-phosphorylated Akt/ total Akt in either sex (Table 1 & 2). We also found no differences in total S6, the ratio of S240/244-phosphorylated S6/ total S6 in either sex (Table 1 & 2). However, there was a significant increase in the ratio of S235/236-phosphorylated S6/total S6 $t(1,16) = 2.367$, $p < 0.05$ in males, with seizure pups expressing a much larger ratio than control pups (Table 1 and S3A). The increase in S235/236-phosphorylated S6/total S6 was not reflected in female pups (Table 2). We also found no difference in total FMRP in either sex (Table 1 & 2), but observed a male-specific suppression of the ratio of S499-phosphorylated FMRP/total FMRP ratio $t(1,16) = 2.18$, $p < 0.05$ in seizure mice compared to control mice (Table 1 and S3B). The decrease in S499-phosphorylated FMRP/total FMRP was not reflected in female pups (Table 2).

In our evaluation of the Canonical Wnt pathway in PD12 mice, we found no changes in Axin1, Dvl2, Dvl3, GSK3a, GSK3b, total LRP6, the ratio of S1490-phosphorylated LRP6/ total LRP6, Wnt5a/b, or Naked2 in either sex (Table 1 & 2).

Discussion

Although there has been significant progress in understanding neonatal USVs in the context of neurodevelopmental disorders, there has been little investigation into whether early life seizures alter USVs. The objective of the present study was to determine the effect of one episode of kainate-induced status epilepticus on ultrasonic vocalization behavior in neonatal 129 SvEvTac mice. Further, given that the effect of neonatal seizures on neurodevelopmental protein signaling is poorly understood, we sought to characterize changes in several components of the PI3K-Akt-mTOR and Canonical Wnt signaling pathways. Through a series of experiments, we demonstrated that seizures on PD10 led to male-specific suppression of neonatal vocalization behavior at the 50-kHz frequency. This behavioral effect was associated with male-specific changes in several components of the PI3K-Akt-mTOR pathway, but not the Canonical Wnt pathway.

Few studies have previously examined neonatal USV behavior following seizures. One existing study by López-Meraz et al (2014)¹¹ used pilocarpine-induced status epilepticus on PD14 Wistar rats. Although pilocarpine and kainic acid differ in their mechanisms of eliciting status epilepticus in the brain^{26; 27}, previous studies indicate that they produce seizures of similar severity²⁸. In isolation-induced USVs on PD15, these authors observed a similar suppression of USV duration and a decrease in latency to vocalize at the 40 kHz frequency. This study used the same UV detectors as we used in our study; however, they only examined the USVs at the 40 kHz range. Interestingly, they also found decreased USV latency after seizures only in male rats. Similar sex-specific effects were found in a separate study where investigators induced febrile seizures on PD7 and reported an increase in USVs in male rats on PD12¹⁰. The authors of this study used a Petterson model D100 Ultrasound Detector and set the frequency range to 20–30 kHz. Even though the seizure induction methods were different across all three studies, they all found that male USVs are altered following early life seizures. However, our study is the only study that examined USVs across a broad range of frequencies.

The cause for differential effects of seizures between sexes in these three studies is unclear. The results of these studies are in line with several population-based epidemiological studies, which suggest the incidence of seizures is greater in males²⁹. It has been proposed that sex hormones may play a key role in the incidence and outcome of seizures; however, the exact mechanisms have yet to be elucidated. Future clinical studies in children with epilepsy could further expand on the sex-differences in this neonatal behavior.

The functional significance of 50-kHz calls has not been extensively investigated in mice, or other rodents during the neonatal periods. However, these calls are well characterized in studies using rat models during adolescence and adulthood. The 50-kHz calls are associated with the anticipation or presence of pleasurable stimuli, as well as a variety of social contexts^{30–33}. In a previous study, Wöhr et al (2008)³¹ recorded the ultrasonic vocalization behavior of 12 week old Wistar rats. These animals selectively emitted 50-kHz calls when isolated from cage mates into a separate housing pan, as well as during open field and elevated plus maze testing. Given that rats produced 50-kHz calls exclusively when separated from cage mates, these authors posit that such calls must serve a social-

coordinating function. In support of this hypothesis, another study by Wohr et al. (2007)³² demonstrated that playback of 50-kHz calls induce behavioral activation and increased exploratory behavior in the radial arm maze test. These animals also emitted 50-kHz calls during intervals between playbacks, even further suggesting their role in social coordination behavior. Although the functional significance of 50-kHz calls during the neonatal period has not yet been established in mice, it may be reasonable to conclude that calls at this frequency are essential for inducing parental-orienting behavior. In this case, 50-kHz calls would be essential in communicative function neonatal vocalization behavior.

In our study, we induced seizures on PD10 and investigated USV behavior on PD11 and PD12. In order to achieve a more robust characterization of this behavior, it may be useful to induce seizures at various time points and analyze subsequent changes in USV behavior. It may also be beneficial to observe vocalization behavior for additional days following seizures. In a previous investigation by Picker et al (2006)⁷, these authors characterized the developmental trajectory of USV behavior across PD3–18 in *MeCP2* deficient mice. Future studies would examine other physiological measures associated with development during the neonatal period. One previous investigation by Holtzman et al. (1996)³⁴ evaluated USV behavior along with several somatic measures, including ear/eyelid opening, incisor eruption, body length, and tail length, in a mouse model of Down Syndrome.

Previous studies indicate that early life seizures lead to long-term deficits in social behavior^{12; 13; 23}. The present study found that seizures on PD10 result in sex-specific suppression of neonatal vocalization behavior. It is possible that these results reflect an early detection of acute impairments in social behavior. Future studies may seek to correlate seizure-induced suppression of neonatal vocalizations with long-term changes in social behavior.

The present study found changes in PI3K-Akt-mTOR signaling to be associated with male-specific suppression of USV behavior after PD10 seizures, namely S499-phosphorylated FMRP and S235/236-phosphorylated S6. S499-phosphorylated FMRP is a phospho-specific protein known to antagonize ribosomal function by degrading target mRNA transcripts³⁵. Several of these targets are those known to be associated with Autism Spectrum Disorder and Down Syndrome^{36; 37}. Bernard et al. (2013)¹⁴ found that early life seizures are associated with long-term suppression of S499-phosphorylated FMRP, enhanced LTD, and autistic-like social behaviors in adult rats. Therefore, understanding the role of FMRP in neonatal vocalization behavior may provide further insights into altered calling patterns observed in models of neurodevelopmental disorders.

The S6 ribosomal protein is a component of the small 40S ribosomal subunit. In conjunction with the large 60S subunit, these compose the intracellular ribosome machinery necessary for all protein synthesis³⁸. The function of the small 40S subunit is highly regulated by the phosphorylation status of the S6 ribosomal protein. When phosphorylated, this protein influences the generation of proteins to regulate cell cycle progression, as well as new ribosomal proteins and elongation factors to aid in transcription³⁹. Although we did not find changes in overall levels of total or phosphorylated S6, there was a significantly elevated ratio of S235/236-phosphorylated S6/ total S6. This would seem to indicate a qualitative,

rather than quantitative, change in overall ribosomal function. Given that seizures are known to cause widespread damage across multiple brain areas^{26; 27}, we hypothesize that enhanced ribosomal function and suppression of S499-phosphorylated FMRP may be suggestive of compensatory neuroregenerative mechanisms.

We did not observe changes in Canonical Wnt signaling following neonatal seizures. This finding is in contrast with a previous study by Busceti et al. (2007)⁴⁰, which found acute kainic acid-induced seizures in Sprague-Dawley rats to cause elevation of Dkk-1 in the hippocampus, an inhibitor of the Canonical Wnt signaling pathway. The divergence in results obtained between this study and ours may be due to seizure induction at different time periods. Busceti et al. (2007) used rats that were 150–170 g. In addition, they found the Dkk-1 increase only in the hippocampus of rats that had neuronal damage. They did not find a change in Dkk-1 in “low” responders, which were rats that had received kainate but did not have neuronal damage. Unfortunately, few studies exist that examine the effect of early life seizure-inductions on the Canonical Wnt signaling pathway. Future studies could include induction of seizures at multiple developmental time points, as well as utilization of different chemoconvulsants.

Taken together, the results of this study confirm the hypothesis that early life seizures cause sex-specific suppression of USV behavior in neonatal mice, and suggest this effect may be secondary to changes in total and phosphorylated FMRP proteins. These findings support the growing body of evidence that USV behavior may serve as measure of early behavioral deficits for neurodevelopmental disorders. Future directions for neonatal USV behavior will involve much more detailed qualitative analyses of acoustic parameters, using spectrographic techniques to determine specific call-type deficits. Several recent studies conducting spectrographic analyses of neonatal USVs have observed altered calling patterns in models of neurodevelopmental disorders^{8; 41}. However, none of these studies have correlated their results with subsequent long-term behavioral analyses. Such studies could identify whether specific acoustic parameters of neonatal vocalization behavior are reliable, early predictors of neurodevelopmental disorder onset. This technology would enhance existing newborn disease screening protocols, and aid clinicians in earlier diagnoses for the growing population of children afflicted with these disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

- Neonatal USV behavior was selectively suppressed at the 50 kHz frequency in male pups following neonatal seizures.
- There were no changes in neonatal USV behavior in female pups following neonatal seizures.
- A decrease in phosphorylated fragile × mental retardation protein occurs following neonatal seizures.
- Neonatal changes in male USVs following neonatal seizures were associated with changes in the PI3K-Akt-mTOR pathway.

Figure 1.

The sex-specific effect of status epilepticus on ultrasonic vocalization quantity on postnatal days 11 and 12. (A-B) Seizures on PD10 do not affect the USV quantity in males or females on PD11. (C) However, there was large suppression of USVs quantity at the 50kHz frequency in males on PD12. (D) USV quantity was not affected in females on PD12. * = $p < 0.05$. Bars represent the mean and the error bars represent the standard error of the mean. (Sample size males: control = 12, seizure = 18; Sample size females: control = 19, seizure = 13)

Figure 2.

(A–D) The sex specific effect of status epilepticus on ultrasonic vocalization mean duration and total duration on postnatal days 11 and 12. Seizures on PD10 do not affect the mean or total duration of USVs in males or females on PD11, (E–F) nor affect mean duration of USVs in males or females on PD12. (G) However, there was a large suppression of total duration of USVs at the 50kHz frequency in males on PD12. (H) Total duration of USVs were not affected in females on PD12. * = $p < 0.05$. Bars represent the mean and the error

bars represent the standard error of the mean. (Sample size males: control = 12, seizure = 18; Sample size females: control = 19, seizure = 13).

Table 1

Western Blotting Summary for Males

Protein	Control	Seizure	Change	Significance
Total AKT	100.0 ± 3.146, n=9	96.28 ± 4.916, n=9	-	-
% Total Phospho-AKT (S473)	99.50 ± 3.554, n=9	104.1 ± 5.331, n=9	-	-
Total AKT	100.0 ± 4.053, n=9	100.0 ± 4.305, n=9	-	-
% Total Phospho-AKT (T308)	100.4 ± 12.50, n=9	92.29 ± 8.006, n=9	-	-
Total S6	100.0 ± 3.225, n=9	96.64 ± 2.386, n=9	-	-
% Total Phospho-S6 (S235/236)	100.1 ± 1.674, n=9	106.1 ± 1.886, n=9	↑	*
Total S6	100.0 ± 3.883, n=9	98.38 ± 1.681, n=9	-	-
% Total Phospho-S6 (S240/244)	100.1 ± 0.525, n=9	100.2 ± 0.8164, n=9	-	-
P70 S6 kinase	100.0 ± 15.94, n=9	80.63 ± 13.41, n=9	-	-
GFAP	100.0 ± 2.371, n=9	103.5 ± 4.328, n=9	-	-
FMRP	100.0 ± 10.98, n=9	80.50 ± 4.724, n=9	-	-
% Total Phospho-FMRP (S499)	100.4 ± 4.350, n=9	89.56 ± 2.393, n=9	↓	*
GSK3α	100.0 ± 24.03, n=9	104.1 ± 37.28, n=9	-	-
GSK3β	100.0 ± 28.75, n=9	95.33 ± 38.64, n=9	-	-
Axin1	100.0 ± 17.43, n=9	98.24 ± 21.48, n=9	-	-
Dvl2	100.0 ± 4.389, n=9	100.4 ± 8.847, n=9	-	-
Dvl3	100.0 ± 4.733, n=9	100.7 ± 6.201, n=9	-	-
Naked2	100.0 ± 10.37, n=9	106.1 ± 10.81, n=9	-	-
LRP6	100.0 ± 20.76, n=9	109.3 ± 19.95, n=9	-	-
% Total Phospho-LRP6 (S1490)	214.7 ± 84.29, n=9	107.5 ± 21.01, n=9	-	-
Wnt5a/b	100.0 ± 4.630, n=9	98.58 ± 10.13, n=9	-	-

*
= p < 0.05

Table 2

Western Blotting Summary for Females

Protein	Control	Seizure	Change	Significance
Total AKT	100.0 ± 5.646, n=9	108.8 ± 8.535, n=9	-	-
% Total Phospho-AKT (S473)	102.3 ± 7.722, n=9	100.2 ± 8.807, n=9	-	-
Total AKT	100.0 ± 7.406, n=9	105.0 ± 18.99, n=9	-	-
% Total Phospho-AKT (T308)	102.5 ± 37.29, n=9	87.18 ± 23.03, n=9	-	-
Total S6	100.0 ± 3.485, n=9	100.7 ± 4.994, n=9	-	-
% Total Phospho-S6 (S235/236)	101.4 ± 6.052, n=9	98.77 ± 5.632, n=9	-	-
Total S6	100.0 ± 3.485, n=9	100.7 ± 4.994, n=9	-	-
% Total Phospho-S6 (S240/244)	100.9 ± 3.367, n=9	103.4 ± 6.665, n=9	-	-
P70 S6 kinase	100.0 ± 9.935, n=9	113.0 ± 12.04, n=9	-	-
GFAP	100.0 ± 6.518, n=9	100.8 ± 6.688, n=9	-	-
FMRP	100.0 ± 13.18, n=9	88.82 ± 15.80, n=9	-	-
% Total Phospho-FMRP (S499)	105.3 ± 7.169, n=9	160.8 ± 39.61, n=9	-	-
GSK3 α	100.0 ± 6.486, n=9	93.16 ± 7.322, n=9	-	-
GSK3 β	100.0 ± 7.836, n=9	95.66 ± 8.770, n=9	-	-
Axin1	100.0 ± 6.683, n=9	92.40 ± 8.053, n=9	-	-
Dvl2	100.0 ± 9.099, n=9	100.7 ± 7.136, n=9	-	-
Dvl3	100.0 ± 8.009, n=9	85.93 ± 7.202, n=9	-	-
Naked2	100.0 ± 12.22, n=9	81.88 ± 8.164, n=9	-	-
LRP6	100.0 ± 20.76, n=9	109.3 ± 19.95, n=9	-	-
% Total Phospho-LRP6 (S1490)	99.67 ± 6.788, n=9	98.62 ± 6.244, n=9	-	-
Wnt5a/b	100.0 ± 26.27, n=9	100.3 ± 30.42, n=9	-	-