Behavioural Brain Research 259 (2014) 119–130

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Temporal and spectral differences in the ultrasonic vocalizations of fragile X knock out mice during postnatal development

Jonathan K.Y. Lai[a], Monica Sobala-Drozdowski[b], Linghong Zhou[a], Laurie C. Doering[c], Paul A. Faure[b], Jane A. Foster[a,*]

[a] Department of Psychiatry and Behavioural Neuroscience, McMaster University, Canada
[b] Department of Psychology, Neuroscience & Behaviour, McMaster University, Canada
[c] Department of Pathology and Molecular Medicine, McMaster University, Canada

HIGHLIGHTS

- USV call number and duration differed in *fmr1KO* compared to FVB wild type pups.
- *Fmr1KO* pups showed an increased number of USVs at P7.
- Spectral analysis showed an increase specifically in frequency jump calls.
- A developmental shift in the temporal distribution of calls showed distinct bout patterns at P10.
- FMRP plays a minor role in the development of USVs compared to other autism-related genes.

ARTICLE INFO

Article history:
Received 9 August 2013
Received in revised form 25 October 2013
Accepted 30 October 2013
Available online 7 November 2013

Keywords:
Ultrasonic vocalizations
Postnatal development
Spectral analysis
Fragile X
Autism spectrum disorders

ABSTRACT

The *fmr1* knock out (KO) mouse has been a useful animal model to understand pathology and treatment of FXS, both anatomically and behaviorally. Ultrasonic vocalizations (USVs) are a behavioral tool to assess early life communication deficits in mice. Here, we report on the temporal and spectral features of USVs emitted after maternal separation in wild type (FVB/N) and *fmr1 KO* pups at postnatal days (P) P4, P7 and P10. The results show changes in the number and duration of calls in *fmr1 KO* pups and wild type pups were dependent on age and call type. *Fmr1 KO* pups showed an increased number of USVs at P7 but not at P4 or P10. This increase was specific to Frequency Jump calls. In addition, *fmr1* KO mice showed a developmental shift in the temporal distribution of calls, with P10 mice calling in distinct bout patterns. Overall, these findings provide evidence that changes in USV outcomes were specific to certain call types and ages in *fmr1 KO* mice. Because early postnatal life is a window during which multiple neural systems activate and become established, behavioral measures such as using USVs as a measure of communication, may be useful as a predictor of brain changes and later developmental behavioral changes. Work is needed to better understand the functional outcomes of altered development of USVs and how these changes contribute to later emergence of autistic-like behaviors in animal models of autism.

1. Introduction

Fragile X Syndrome (FXS) is a human neurodevelopmental disorder affecting 1 in 4000 males and 1 in 8000 females [1–4]. To date, this disorder is the most common single gene cause of autism [5,6]. Between 40 and 60% of patients with FXS meet DSM-IV criteria for autism spectrum disorders (ASDs); 21% have autism, and up to 90% present with autistic symptoms [7–10]. The overlapping diagnoses lie in the shared behavioral traits of impairments in social communication and cognitive deficits. Specific initial symptoms of children with FXS are similar to autism and include poor eye contact, hand flapping, and social deficits [11–13]; shared symptoms that manifest late in life include shyness, social avoidance, anxiety, hyperactivity, inattention, impulsivity, tactile defensiveness, self-injurious behavior, aggression, irritability, and inflexible decision making processes [8,9,14]. Fragile X mental retardation 1 knockout mice (*fmr1 KO*) are used to investigate mechanisms of disease associated with loss of the fragile X mental retardation protein (FMRP) [15–17]. Behavioral and phenotypic manifestations of FXS in humans are well represented [18–23]. *Fmr1* KO mice demonstrate transient synaptic changes during postnatal development

* Corresponding author at: Department of Psychiatry and Behavioural Neurosciences, McMaster University, Brain-Body Institute, St. Joseph's Healthcare, 50 Charlton Ave. E, T3308, Hamilton, ON L8N 4A6, Canada. Tel.: +1 905 522 1155x35993; fax: +1 905 540 6593.
E-mail address: jfoster@mcmaster.ca (J.A. Foster).

0166-4328/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2013.10.049

[20,24–26]. Functional and morphological changes occur in various brain regions, in different neurons and/or neural compartments over different time windows [26–31]. For example, in the cortex, there is increased plasticity of thalamic afferents to cortical layer IV pyramidal neurons at P7, the typical end of a critical period of plasticity at this synapse, but not at P14 and P21 [29]. The projections from layer IV to layer II/III, however, have increased plasticity at P14, but not P21 [27]. In addition, the lack of FMRP modulates inhibitory projections from layer IV specifically to fast-spiking interneurons at P14 but not P28 [28]. Transient changes in cortical spine morphology reflect this laminar nature as well. Spines in layer II/III are thinner and less mature only at P14 but not P7 or P21 [25,26]. In layer V, spines are changed at P7 and P14 [30], normal at P28 [30,31] and changed again at P75 [31]. Therefore, *fmr1 KO* mice are useful in understanding the effects of FMR1 gene silencing on development.

Behavioral outcomes in mouse models are useful to examine neurodevelopmental disorders ([32–35]; reviewed in [36,37]). Ultrasonic vocalizations (USVs) are communication sounds emitted by mouse pups in the first two weeks of life in response to maternal separation [38–41]. Changes in USV production are a key communication-related health outcome measure in early life [42–44], and differences have been detected in multiple mouse models of neurodevelopmental disorders (see Table 1). Several studies have examined USVs across multiple time points during postnatal development [45–54] by comparing temporal characteristics such as call number and duration [55] and the pattern of call production. For example, compared to single calls, bouts of calling are more frequent within a 2 min test in the first postnatal week of development [56]. By the end of the second week, pups emit very few USVs. Strain differences in the number of calls emitted have also been reported [57]. The most common outcome measures include call number and call duration. Frequency (spectral) differences have also been reported [46–48,51,58–65] and in a small number of reports calls have been categorized based on their spectral pattern [51,59,64,66,67].

In this study, we recorded USVs emitted after maternal separation in wild type (WT- FVB/N) and *fmr1* KO pups at postnatal (P) days 4, 7 and 10 and conducted a detailed temporal and spectral analysis to characterize how USV production changes in *fmr1 KO* and WT mice during development.

2. Methods

2.1. Animals and housing

Wild type FVB/N (WT) and FVB/N/*fmr1* (*fmr1 KO*) mice were housed and bred in McMaster University's Central Animal Facility. All procedures were in accordance with the guide to the care and use of experimental animals by the Canadian Council on Animal Care, and were approved by the Animal Research Ethics Board of McMaster University.

2.2. Experimental setup and recording procedure

Sounds emitted by P4, P7, and P10 pups were recorded during 3 min of maternal separation between 0800 and 1130 h in a procedure room whose temperature (21 °C) was similar to that of the mouse holding room. For each litter, the dam was removed from the home cage 10 min prior to the start of pup recordings. The home cage with the isolated litter rested on a heating pad set to 37 °C throughout testing. Individual pups were placed into the center of a 34 cm × 29 cm × 15 cm polypropylene chamber whose walls were lined with 5 cm thick acoustic foam (Sonex COC-2, Acoustical Solutions, Inc.). A towel was placed on the bottom of the chamber to reduce the amplitude of scratching noises produced during pup movement. The towel was changed between recordings of different litters to prevent odor transfer between pups.

Pup USVs were recorded with a CM 16 condensor microphone connected to a UltraSoundGate 116 digitizer (Avisoft Bioacoustics, Berlin, Germany) and monitored with a laptop computer running Avisoft Recorder. The microphone was clamped to a retort stand and situated 17.5 cm above the center of the recording chamber.

2.3. Data analysis

Recordings were analyzed with Avisoft Sound Analysis and Synthesis Laboratory Professional software (SASLab Pro v 5.1.20). For each comparison group, the calls from up to five pups per litter were analyzed. In the WT group, there were 15 pups from 3 litters at P4, 21 pups from 5 litters at P7, and 18 pups from 4 litters at P10. In the *fmr1 KO* group, there were 23 pups from 5 litters at P4, 17 pups from 5 litters at P7, and 8 pups from 2 litters at P10. Calls were identified and classified by an individual observer whose was blind to treatment group. The onset and offset of each call was labeled automatically, although manual labels were added when needed. Call duration was calculated as the difference between the onset and offset times, whereas the intercall interval (ICI) was calculated as the difference in time between the onset of consecutive calls.

In addition to the above temporal outcome measures, USVs were classified spectrally into six categories of subtypes first described by Branchi et al. [68] (see Table 2 for a comparison with other research groups). These USV subtypes were defined by variation in frequency or bandwidth, and by their frequency modulation patterns. The subtypes we recorded included: composite (C), quasi-constant (QC) frequency, frequency jump (FJ), frequency modulated (FM), frequency jump plus composite (FJ + C), and short (S) call (Fig. 1).

Bouts of calling were identified based on an age-dependent minimum value in the distribution of the natural log of the ICI [67]. This minimum ICI value was considered a threshold, with bouts of calling to the left of this threshold and single USV calls to the right.

2.4. Statistics

Data analyses were performed in GraphPad Prism (v5b, GraphPad Software, San Diego, CA). Differences between treatment groups were detected with a two-way analysis of variance (ANOVA) with genotype and age as main factors followed by Bonferroni's post hoc tests. A p-value of <0.05 was considered significant. All data are expressed as the mean ± standard error of the mean (SEM).

3. Results

3.1. Total call number and call duration

We used a detailed temporal and spectral analysis to characterize the USVs emitted by WT and *fmr1 KO* mice at P4, P7 and P10. First, we first calculated the total number of calls, average call duration and average ICI per pup. There was a significant effect of age on the mean number of calls per pup ($F[2,98] = 4.48$, $p = 0.014$), and post hoc testing revealed an increase at P7 compared to P4 and P10 in *fmr1 KO* pups (Fig. 2A). While there was no main effect of genotype ($p > 0.05$), there was a significant interaction between age and genotype on number of calls emitted ($F[2,98] = 3.26$, $p = 0.04$), and post hoc tests revealed that the number of calls emitted by *fmr1 KO* mice on P7 was higher than by WT mice (Fig. 2A). There was no effect of genotype on call duration ($p > 0.05$); however, there was a significant effect of age ($F[2,94] = 16.6$, $p < 0.0001$) and post hoc analyses revealed an increase in call duration at P7 compared to P4