Behavioural Brain Research 235 (2012) 166–175

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

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

E.T. Cox[a,c,e,*], C.W. Hodge[a,b,c], M.J. Sheikh[b], A.C. Abramowitz[b], G.F. Jones[f], A.W. Jamieson-Drake[c], P.R. Makam[b], P.S. Zeskind[d], J.M. Johns[a,b,c]

[a] University of North Carolina, Curriculum in Neurobiology, USA
[b] University of North Carolina, Department of Psychology, USA
[c] University of North Carolina, Department of Psychiatry, USA
[d] Levine Children's Hospital at Carolinas Medical Center, Charlotte, NC, USA
[e] University of Utah, Center for Integrated Neuroscience and Human Behavior, The Brain Institute, USA
[f] University of North Carolina, Department of Biology, USA

HIGHLIGHTS

- Effects of prenatal cocaine on vocalizations were assessed in offspring.
- Prenatal cocaine is associated with age- and sex-dependent vocalization changes.
- Prenatal cocaine is associated with age- and sex-dependent limbic region changes.
- Developmental differences in limbic regions correlate with altered USV acoustics.

ARTICLE INFO

Article history:
Received 27 March 2012
Received in revised form 3 July 2012
Accepted 27 July 2012
Available online 4 August 2012

Keywords:
Neonate
Vocalization
Development
Limbic
Prenatal
Cocaine

ABSTRACT

While variations in neonatal distress vocalizations have long been shown to reflect the integrity of nervous system development following a wide range of prenatal and perinatal insults, a paucity of research has explored the neurobiological basis of these variations. To address this, virgin Sprague-Dawley rats were bred and divided into three groups: [1] untreated, [2] chronic-cocaine treated (30 mg/kg/day, gestation days (GDs) 1–20); or [3] chronic saline treated (2 mg/kg/day, GDs 1–20). Pregnant dams were injected with Bromodeoxyuridine (10 mg/kg) on GDs 13–15 to label proliferating cells in limbic regions of interest. Ultrasonic vocalizations (USVs) were recorded on postnatal days (PNDs) 1, 14, and 21, from one male and female pup per litter. Variations in acoustic properties of USVs following cocaine-exposure were age and sex-dependent including measures of total number, total duration and amplitude of USVs, and percent of USVs with at least one harmonic. Following USV testing brains were stained with standard fluorescent immunohistochemistry protocols and examined for variations in neuronal development and if variations were associated with acoustic characteristics. Limbic region developmental differences following cocaine-exposure were sex- and age-dependent with variations in the ventral medial hypothalamus and central amygdala correlating with variations in vocalizations on PND 14 and 21. Results suggest maturation of the ventral medial hypothalamus and central amygdala may provide the basis for variations in the sound and production of USVs. As vocalizations may serve as a neurobehavioral marker for nervous system integrity, understanding the neurobiological basis of neonatal vocalizations may provide the basis for early intervention strategies in high-risk infant populations.

1. Introduction

Variations in the acoustic parameters of neonatal crying have been consistently shown to reflect the integrity of central nervous system development in the human infant. Infants with such prenatal and perinatal insults as brain damage, prenatal malnutrition, and prenatal drug exposure, have been differentiated by a wide range of measures of infant crying, including a higher

* Corresponding author at: The University of North Utah, Center for Integrated Neuroscience and Human Behavior, The Brain Institute, Department of Pediatrics, Sorenson Molecular Biotechnology Building, SMBB, 36 South Wasatch, Salt Lake City, UT 84112-5001, USA. Tel.: +1 801 587 1423; fax: +1 801 587 8285.
E-mail address: elizabeth.cox@utah.edu (E.T. Cox).

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.07.035

fundamental frequency (F_0), longer latency to cry, and shorter overall durations of crying [48,99,100]. As such, these measures of infant crying have been used in the identification of infants at risk for poor neurobehavioral, social and cognitive development [55,99]. While physioacoustic models of infant crying have described the role of coordinated activity among brainstem, midbrain and limbic systems in the production of these and other variations in human infant cry characteristics [31,54,69,76], a paucity of research has explored their neurobiological bases. The analysis of rat pup ultrasonic vocalizations (USVs) may provide a valuable animal model by which the neurobiological basis of variations in the cry sound can be studied. Recent translational analyses suggest that the cry sounds of human infants may have comparable measures in the distress vocalizations of several mammalian species, including the USVs of rat pups [101]. Analyses of rodent vocalizations mirror clinical studies which show human infant cries are similarly age- and context-dependent [8,82,84,85]. Neonatal rodent offspring in isolation emit infant 40 kHz distress calls, whereas older rodents emit 22 kHz calls in similar aversive conditions [10,12]. Variations in rodent infant vocalizations are associated with different contextual conditions, such as size of the litter [35], suggested to be related to variations in maternal care individual pups receive in a small litter versus a large litter. It has also been shown that juvenile stress alters adult rodent vocalizations, including potentiation of 22 kHz USVs [98].

While studies of adult rodents indicate that specific brain regions are involved in the control, elicitation, and complexity of experience-dependent vocalizations [16,22,32,34], the neurobiology underlying variations in vocalizations during early developmenthas not been explored in a high-risk rodent model. Preclinical animal studies support the hypothesis that maturation of specific brain regions involved in adult vocalizing behavior, i.e. the amygdala [53,62], ventral medial hypothalamus (VMH) [11], and periaqueductal gray (PAG) [15,17,42,61] are associated with typical developmental changes in offspring behavior, including emergence of fear-like behavior [67,93] and behavioral sex-differences [52]. Whether differential development of these regions contributes to variations in neonatal vocalizations is unknown. One way to address this issue is to examine the effects of prenatal cocaine exposure (PCE) and/or other prenatal stressors on the acoustic characteristics of rat pup USVs, and their relationship to development of specific neural regions. PCE and other stressors have been associated with variations in human infant cry sounds including decreased number of cry expirations and increased amplitude, duration, and fundamental frequency of expirations [19,56,57]. However, mixed results following PCE have been reported in several studies [25,45] potentially related to age at time of cry elicitation, amount or time of drug exposure during pregnancy, and/or other risk factors.

The present study investigated whether PCE is associated with normal developmental changes in USVs in male and female rat offspring at three different age groups corresponding to neonate (designated as postnatal day (PND) 1), infant (PND 14), and early juvenile (PND 21) periods. Neuronal development in the dorsal (dPAG) and ventral PAG (vPAG) on PND 1 (before emotional control of USVs start), and additionally in the central (CeA) and basal lateral (BLA) amygdala and also the VMH on PNDs 14 and 21 (to correlate with emergence of "emotional-dependent" USVs) were measured and correlated with variations in vocalizations. We hypothesized that delayed PAG maturation would be associated with decreased number of USVs on PND 1 while delayed CeA and VMH maturation would coincide with increased number of USVs and a lower percentage of 22 kHz USVs on PND 21 in cocaine-exposed offspring.

2. Materials and methods

2.1. Animals

Following a one-week habituation period, virgin female (200–240 g) Sprague-Dawley rats (Charles River, Raleigh, NC) were placed with males on a breeding rack until a sperm plug was found, which was designated as gestation day (GD) zero. Subjects were randomly assigned to one of the three treatment or control groups and singly housed and maintained on a reversed 12:12 reverse light cycle (lights off at 09:00 h) for 7 days. They were then transferred to a room with a regular light cycle (lights on at 07:00 h) for the remainder of the experiment, a procedure that generally results in the majority of dams delivering their litters during daylight hours [64]. All procedures were conducted under federal and institutional animal care and use committee guidelines for humane treatment of laboratory subjects.

2.2. Treatment

Treatment groups included: chronic cocaine (CC), and two control groups, chronic saline (CS), and untreated (UN) dams. CC and CS dams received subcutaneous (SC) injections on alternating flanks of 15 mg/kg cocaine HCL (dose calculated as the free base, Sigma Chemical Company, St. Louis, MO) dissolved in 0.9% normal saline (total volume 2 ml/kg), or the same volume of normal saline (0.9%), respectively. Injections were delivered twice daily (at approximately 08:00 and 16:00 h) throughout gestation beginning on GD 1 and continuing until the day before delivery (GD 1–20) with the CS dams serving as controls for injection and nutritional stress. UN dams were weighed and handled daily, but received no drug treatment. CC and UN dams had free access to water and food (rat chow), while CS-treated dams were yoke-fed over the first week to match maximum consumption rates of CC dams to control for the anorectic effects of cocaine, as previously described [38,40]. To investigate neuronal postnatal development in offspring, pregnant dams from all three treatment groups received an injection of Bromodeoxyuridine (BrdU) (10 mg/kg) between 08:00 and 09:00 h, before any other injections or handling, for three consecutive days (GDs 13–15). These three consecutive days were chosen based on prior research showing this developmental window is the peak period of neurogenesis for brain regions of interest [6,7,79,80] and pilot staining showing positive BrdU staining in all regions of interest following BrdU administration on these respective days. A small cohort of animals were tested in a pilot study to examine the effects of BrdU on neonatal vocalizations, maternal behavior, and weight gain in all three treatment groups and findings suggested no BrdU-related differences at the dose employed here (data not shown). While BrdU has been found to have adverse effects on development when administered gestationally [47], the dose we employed is lower than that previously found to have no effect on cellular kinetics [59] and therefore caused no problems in the present study.

2.3. USV testing procedures

Offspring were left undisturbed with their biological dams for 3 h following delivery (designated as PND 1) and then brought to the test room and allowed to habituate to the room for 15 min. One male and female offspring were removed from the litter, weighed, and placed together in a plastic holding cage on top of a heating pad for 5 min. At the end of 5 min, skin temperature on the rear flank of each offspring was recorded with a laser thermometer (Fischer Scientific, Model 15-077-966). The male and female offspring from each litter were simultaneously placed onto two separate individual cold scales in two Med Associates sound attenuated boxes, each with a Med Associates Ultrasonic Vocalization Detector (model number ANL-937-1) attached to a unidirectional microphone and powered by SG-501 power supply. Med Associate USV detectors scan ultrasonic frequencies every 30 milliseconds and record the amplitude of sound at each frequency between 20 kHz and 100 kHz. Detectors are connected to a laptop computer and data acquired and analyzed through ANL-937-1 MED USV Application Software (SOF-937-1) and the "MED-USV.xls" macro for Microsoft Excel. USV testing lasted for 5 min, and after testing, offspring skin temperature was again recorded. The temperature of the cold scale was measured before and after testing to control for confounding environmental temperature differences using a laser thermometer. Following testing, both offspring had their temperature assessed and were sacrificed and their brains collected for immunohistochemical analysis. The offspring's biological dam and littermates were returned to the animal facility and left undisturbed until the next assigned test days. On PNDs 14 and 21, litters were brought to the testing room and the same PND 1 testing procedure was carried out on another male and female offspring pair.

2.4. USV analysis

Following vocalization testing with ANL-937-1 MED USV Application Software (SOF-937-1), data were loaded and analyzed with the "MED-USV.xls" macro for Microsoft Excel. Data output included the amplitude of sound at each frequency between 20 and 100 kHz for every instance that sound exceeded the manually set threshold level of 25 dB. Data output was examined by generated 3-D area graphs, where the *x*-axis was frequency (kHz), *y*-axis was amplitude (dB), and *z*-axis was