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Effects of prenatal ethanol exposure on acoustic characteristics of ultrasonic vocalizations in rat pups

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ABSTRACT

Rat pups produce ultrasonic vocalizations (USVs) on isolation from their dam. Ultrasonic vocalization is a sensitive tool for evaluating social behavior between pups and their dam. Prenatal ethanol-exposure leads to a reduction in USVs and have the potential of inducing difficulties in social behavior between pups and their dam. However, effects of prenatal ethanol-exposure on the acoustic characteristics of USVs remain unclear. In this study, we recorded USVs produced by rat pups that were prenatally exposed to ethanol and examined their acoustic characteristics. Ethanol was administered to 13 pregnant rats in three stages by gradually increasing concentrations between gestational days (GDs) 8–20. From GDs 14–20, ethanol-containing tap water at concentrations of 30% and 15% (v/v) was administered to the high- and low-ethanol groups, respectively. Tap water without ethanol was given to the control group. On postnatal days (PNDs) 4, 8, 12, and 16, individual newly-born pups were isolated from their dam and littermates and USVs produced by them were recorded for 5 min. The number of USVs in the high-ethanol group was greater than that in both low-ethanol and control groups on PND 12. The mean, minimum, and maximum fundamental frequencies of USVs were elevated in the high-ethanol group compared with that in both low-ethanol and control groups. Higher amplitudes of USVs were produced by male pups in the high-ethanol group than in those in both low-ethanol and control groups on PND 12. These results suggest that prenatal ethanol exposure changed emotionality and accordingly, the high-ethanol group produced more USVs as distress calls.

1. Introduction

Alcohol is a popular beverage worldwide. Alcohol intake can elicit feelings of euphoria but can also lead to various health problems, such as damages to internal organs, alcohol-dependency, and Korsakoff's syndrome. Fetal alcohol syndrome is the most important disorder associated with maternal alcohol consumption during pregnancy. Fetal alcohol syndrome is a permanent birth defect characterized by prenatal and postnatal growth deficiency, central nervous system damages/dysfunctions, and a unique cluster of facial anomalies (Jones et al., 1973; Astley and Clarren, 2000; Bertrand et al., 2005; Chudley et al., 2005; Hoyme et al., 2005). Although any amount of alcohol ingestion during the prenatal period can adversely affect infant development, high or moderate levels of alcohol ingestion can lead to long-term impacts on cognitive and social domains of brain functions (Streissguth et al., 1991; Mattson et al., 1999; Green et al., 2009). Atypical attachment behavior and impairments in state regulation are observed in infants that are prenatally exposed to alcohol (Kelly et al., 2000). In accordance with these findings, prenatal alcohol exposure is considered as one of the most common causes of developmental insults (Thomas et al., 1998; Day et al., 2002; Green et al., 2009).

The literatures on the most severe human form of prenatal alcohol exposure report that offspring of mothers who consume alcohol during pregnancy are known to suffer from developmental delays and/or numerous behavioral changes in a dose-dependent manner. Alcohol consumption during pregnancy, regardless of the amount, has been associated with an increase in infantile anomalies, attachment insecurity, distinctive patterns of crying, attention deficit hyperactivity disorder, slight intellectual deficiency, difficulties in initiating attention and encoding information and so on (Lester et al., 2002; O'Connor et al., 2002; Kable and Coles, 2004; O'Callaghan et al., 2007; Ornoy and Ergaz, 2010; Chen, 2012). Consequences of consuming alcohol have the potential to adversely affect attachment in infants if proper maternal care is not received during critical perinatal periods (Kim et al., 2006; Gershon et al., 2013). Infants with heavy prenatal alcohol-exposure demonstrated more negative emotionality, such as whining, shouting, crying, and gesturing, than those exposed to lower levels of alcohol (O'Connor, 2001). Moreover, these developmental and behavioral

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difficulties persist into adolescence (Streissguth et al., 1999). Therefore, studying effects of prenatal alcohol ingestion on infant–maternal interactions is critically important.

However, human studies are often complicated by co-ingestion of substances such as tobacco, marijuana, or psychotropic drugs, and other uncontrollable factors, like nutritional status, postnatal care, socio-economic status, and genetic variability. Animal model studies, therefore, are important and effective means to examine effects of prenatal ethanol ingestion on pup–dam interactions during developmental periods, allowing strict control of factors such as genetic variation, nutritional status, and multidrug use.

In animal model studies, disruption of the behavior in the pup–dam interaction is known to have a critical impact on the socio-emotional behavior of the pup (Meaney, 2001; Champagne and Meaney, 2007). Alterations caused by ethanol-exposure in rodents are dependent on the timing of exposure, age, and sex of the animal tested. In rats, prenatal ethanol-exposure alters aggression, social interaction, social recognition and communication, maternal behavior, and sexual behavior (Kelly et al., 2000). Prenatal ethanol-exposure can also result in a longer latency for pups breastfeeding and shorter durations to breastfeed (Rockwood and Riley, 1990; Barron et al., 1992; Subramanian, 1992). Another example of social interactions between pups and their dam is that the pups prenatally exposed to ethanol are unable to elicit retrieval behavior from their dam as quickly as the control pups (Ness and Franchina, 1990). Similar to results of reports on human studies, ethanol-exposure during development disturbs sleep and feeding rhythms (Hilakivi, 1986; Hilakivi et al., 1987; Subramanian, 1992; Stone et al., 1996) and the development of abilities to regulate body temperature (Zimmerberg et al., 1987, 1993) in rat pups by disrupting the process of psychobiological attunement that may underlie attachment (Field, 1996). Thus, ethanol-induced alterations in early pup–dam interactions suggest the likelihood of cascade-like effects (Ness and Franchina, 1990) such that deficits in pups could result in altered dam behavior, which in turn could result in even more aberrant behavior in the pups (Kelly et al., 2000).

It is well known that rodents produce ultrasonic vocalizations (USVs) and use them as a communication tool in social context (Brudzynski, 2010). For example, rat pups emit USVs with an approximate average frequency of 40 kHz when they are isolated from their dam. This signals the dam to locate her pups and retrieve them back to the nest (Portfors, 2007; Ise and Ohta, 2009; Schwarting and Wohr, 2012). USVs with an approximate average frequency of 40 kHz have important roles in the pup–dam communications.

Ethanol affects the production of USVs in rat pups. Prenatal exposure to ethanol has reduced the number of USVs (Kehoe and Shoemaker, 1991; Wellmann et al., 2015) and postnatal exposure has also decreased USVs (Barron et al., 2000). If ethanol-exposure reduces pups' USVs, these signals will not be noticeable to their dam and this may negatively impact the survival of pups in wild life where the pups are unable to receive necessary maternal care.

Apart from USV productions, USV acoustic characteristics, such as durations and frequencies, have crucial roles in pup–dam interactions because this information is used by the dam for identifying each pup (Hahn et al., 2000). Nevertheless, effects of ethanol on acoustic characteristics of USVs have not been ascertained. In this study, we recorded USVs emitted by rat pups prenatally exposed to ethanol and examined the acoustic characteristics, such as the number, duration, frequency, and amplitude. We predicted that there would be acoustic alterations in USVs in pups exposed to ethanol.

2. Materials & methods

2.1. Subjects

Thirteen pregnant Wistar rats, at gestational day (GD) 7, were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were randomly assigned into three groups: high-ethanol (n = 4), low-ethanol (n = 5), and control (n = 4) groups. They were housed in individual cages and were provided the certified rat chow MF (Oriental Yeast Ltd., Sapporo, Japan) and tap water ad libitum. Laboratory ethanol (purity = 99.5%; Kanto Chemical Co., Inc., Tokyo, Japan) was dissolved in tap water and administered to the animals as drinking water from GDs 8–20. Ethanol-containing water was administered to the animals in three stages by gradually increasing the concentration between GDs 8–20. From GDs 8–10, 10% and 5%, from GDs 11–13, 20% and 10%, and from GDs 14–20, 30% and 15% ethanol-containing water (v/v) was administered to the high- and low-ethanol groups, respectively. Blood ethanol levels from GDs 8–20 were estimated to reach at 250–350 mg/dl and 100–150 mg/dl for the high- and low-ethanol groups, respectively (Ness and Franchina, 1990; Marino et al., 2002; Lugo et al., 2003; Lawrence et al., 2008). Tap water without added ethanol was given to the control group throughout the study period. Before and after the ethanol-exposure periods, ordinary tap water was given to all groups as drinking water.

We administered ethanol to the animals via drinking water (Nio et al., 1991; Salami et al., 2004) because this was a simple and stress-free method. The idea of gradually increasing ethanol-concentrations was adopted from studies by several investigators (Jones et al., 1981; Sandberg et al., 1982; Ludena et al., 1983; Marquis et al., 1984; Testar et al., 1986; Raul et al., 1987; Macieira et al., 1997; Garcia-Moreno and Cimadevilla, 2012; Wellmann et al., 2015).

The day of birth of the pups was designated as postnatal day (PND) 0. On PND 4, each litter was culled to four male and four female pups to ensure uniformity in the rate of growth of the pups through the availability of breast milk in litters. Four pups (two males and two females) were randomly sampled from each litter as subjects. The remaining pups were used as subjects in subsequent studies during young adulthood. Thus, eight male and eight female pups from the high-ethanol and control groups and another 10 male and 10 female pups from the low-ethanol group, were sampled. These pups were repeatedly monitored for USV recording on PNDs 4, 8, 12, and 16.

The temperature of the breeding room was maintained at 22 °C ± 2 °C with relative humidity of 50% ± 10%. Animals were subjected to a 12-h light/dark cycle (light: 20:00–08:00 h and dark: 08:00–20:00 h). This research design was approved by the animal ethics committee of Hokkaido University, and all experimental conditions were compliant with guidelines for the Care and Use of Laboratory Animals, Hokkaido University.

2.2. Apparatus

An ultrasonic microphone and the Sonotrack system version 2.4.0 (Metris, Hoofddorp, The Netherlands) were used to record and analyze USVs of the pups. Sonotrack software was installed on a personal computer and was run on MS Windows 7 Ultimate. To avoid interference from external sound and light, the ultrasonic microphone was placed in a sound-proof box.

2.3. Recording of USVs

USVs were recorded on PNDs 4, 8, 12, and 16. Each pup was individually isolated from the dam and littermates in the breeding room and placed in a translucent cup with a 13-cm bottom diameter, 15-cm top diameter, and 15-cm height, and taken to the experiment room to record USVs. The pup was left alone in the sound-proof box. The first 5 min was the period of habituation for the pup, followed by another 5 min of USV recording. The ultrasonic microphone was positioned at a height of 20 cm from the bottom of the translucent cup. After recording, the body weight of the pup was measured, and the pup was returned to the dam and littermates. Thus, the pup was isolated from the dam and littermates for 10 min on each day of the experiment.

The temperature of the experimental room was maintained at