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Increased ultrasonic vocalizations and risk-taking in rat pups of sleep-deprived dams

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HIGHLIGHTS

- Increased crying in pups born to dams on acute sleep deprivation during pregnancy
- Sleep loss during pregnancy increases risk-taking behavior in pre-adolescent pups.
- USVs during ontogeny provide early signals to understand mother–child bonding.
- Maternal sleep during pregnancy influences the emotional development of babies.

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ABSTRACT

Ultrasonic vocalizations (USVs) in rodent pups are analogous to cries in human babies. There is reduction in USVs in pups after experimental deprivation of rapid eye movement sleep of dams during pregnancy. However, the effects of total sleep deprivation on the USVs of newborns and their emotional development are not documented. Male pups born to the rats that underwent total sleep deprivation for 5 h during the third trimester made higher vocalizations, when tested on early postnatal days (pnds) in an isolation-paradigm. Their anxiety-related behaviors during pnds 25–28, were tested using elevated plus maze (EPM). In comparison to the control pups, weanlings of sleep-deprived dams made increased entries into the open arms and higher mobility in the EPM. Enhanced distress calls during early pnds and reduction in risk assessment in weanlings indicate a link between the two behaviors. The USVs during ontogeny may provide early signals about altered emotional development.

1. Introduction

Cries of babies, immediately after birth and during early postnatal period, remain an enigma both in terms of cause and nature. This expression of distress by neonates is now recognized as a valid component in the developmental milestone of babies [1,2]. Neonates utilize the cry as a principal means to express their pervasive discomfort to isolation-induced stress [1,3]. Ultrasonic vocalizations (USVs) in rodent pups, which are analogous to cries in human babies, possibly signal stress/anxiety for an imperative retrieval by the dams [4–6]. It is also considered as a reflection of their anxiety traits or affective states [7–9]. Though recent reports indicated that decreased vocalization in neonates is associated with despair and desolation during early development [10–12], the significance of the increased USVs needs further investigation.

Maternal stress during pregnancy is emerging as a major concern due to increased reports of anxiety disorders and cognitive deficits in the offspring [13–16]. There are altered emotion and decline in cognition, vigilance, attention, memory, and risk-taking behavior after sleep restriction in women [17–21]. Sleep loss is a modern life style dependent stress across all age-groups [22]. Recently, it was demonstrated that a reduction in rapid eye movement (REM) sleep during the last trimester of pregnancy in rats adversely affected the rate and quality of vocalization in their pups [12]. Since sleep consists of REM and non-REM (NREM) components that are regulated by different mechanisms in the brain [23,24], it is likely that the total sleep loss might have different effects on the early development and vocalizations. There are practical difficulties and ethical issues, on studying the probable association between total sleep loss in pregnant women and its consequences on the newborn. To address this issue, the present study was designed in the rodent model to investigate the effects of acute maternal total sleep deprivation (TSD) of 5 h (TSDX5h) during the third trimester, on the USV profiles of pups from birth to weaning, using an isolation paradigm. This was followed by assessing their anxiety in the early adolescence in the elevated plus maze (EPM) test.

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2. Materials and methods

2.1. Subjects

The study was carried out on rat pups born to mothers that were sleep-deprived during the third trimester. Prior to mating, adult nulliparous female Wistar rats (body weight 220–240 g), were implanted with EEG and EMG electrodes under anesthesia for monitoring sleep onset [25]. Their sleep onset was assessed on the basis of electrophysiological parameters. The electrodes were soldered to an IC socket and covered with dental cement over the head as described elsewhere [25]. After a post-operative recovery period of 10 days, these females were kept with males of similar age for mating. Every day they were examined for vaginal plug formation in the morning. After confirmation of pregnancy, females were housed individually in polystyrene cages at controlled temperature (26 ± 1 °C) and light–dark schedule of 12 h (lights on at 06:00 h). Food and water were provided ad libitum throughout the experiment.

The pregnant rats (n = 10) were randomly distributed into two groups. In the first group of rats (n = 5) TSD for 5 h (9 am to 2 pm) was achieved by gentle manual handling, starting from gestational day 14 until day 19. They were sleep-deprived during their third trimester of pregnancy by gentle manual handling [26]. Electrophysiological signals from the pregnant rats, during 5 h of study, were monitored in a BIOPAC system (MP 150 system) using connecting leads [27]. After completion of 5 h sleep deprivation protocol, the recording leads were disconnected and the rats were left undisturbed in their home cages. These rats were monitored till their parturition in their home cages. The second group which did not undergo sleep deprivation was taken as control and monitored till their parturition. On day 1 of parturition, the litter size was brought down to 6 by culling the remaining pups in both the groups to maintain uniformity in maternal care. Mothers and pups were only very minimally disturbed during the experiment.

2.2. Ultrasonic vocalization measurement procedure

Male pups of control group (n = 13) and experimental group (n = 27), obtained from ten pregnant rats, were monitored for their USVs. Their USVs were recorded on brief isolation from their mothers, as described previously [12]. The control group pups provided data about the natural course of development of vocalizations on different postnatal days. These were compared with USVs of pups of TSDX5h group mothers. The USVs generated by individual pups on isolation were recorded on various pnds 1, 5, 9, 11, 15, and 21 for a period of 2 min at room temperature of 28 ± 1 °C. The home cage with the dam and the pups was carried to the testing room. Pups were placed inside a glass beaker (without any bedding), kept inside a sound attenuated chamber, to record USVs. The USVs were recorded using a microphone (CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) placed 10 cm above the pups. The microphone was connected to a pre-amplifier (Avisoft UltrasoundGate 416H, Avisoft Bioacoustics) and digitized sonograms were stored in a computer. The ambient temperature within the glass beaker, during the USV recording, was monitored using FLUKE True-rms digital multimeter (model 287/289; USA) with K type thermocouple containing chromel–alumel probe. It took 8 to 10 s to isolate the pups, one at a time, from the mother to a glass beaker. It was ensured that the USVs produced by the isolated pups did not reach the home cage, kept 2 m away, where the other pups remained with the mother. After USV recordings, body weights of the pups were taken.

2.3. USV signal analysis

The USVs were analyzed quantitatively and qualitatively using Avisoft SASLab Pro software (version 5.1). The Fast Fourier Transform (FFT) was done to generate the spectrogram (FFT length 256 points, frame size of 100% in flat top window with temporal resolution of 75%). The spectrogram was produced at frequency resolution of 977 Hz and a time resolution of 0.25 ms. On each test day, the parameters analyzed included (1) calling rates i.e. number of calls/min, (2) call types, (3) duration of calls, (4) total time spent in calling, (5) carrier or fundamental (F_0) and peak frequency of calls, (6) loudness and (7) temporal profile in call numbers (distribution of calls during first and second minute).

2.4. Anxiety testing using elevated plus maze

The weanlings were tested for anxiety on pnds 25–28 after weaning (early adolescence) in the EPM using ANY-maze video-tracking system (version 4.82) from Stoelting Co. (USA). The plexiglass EPM having two open arms (50 × 10 cm) and two closed arms (50 × 10 × 50 cm), arranged in plus shape, was kept at a height of 45 cm. The arms of same types faced each other and were connected through an open central zone (10 × 10 cm). At the beginning of the experiment, weanlings were placed in the central zone, facing one of the closed arms and test was conducted for 5 min. The parameters taken were time spent in the open and closed arms and the central zone, total distance traveled in 5 min, total distance traveled in different zones, number of line crossings in each zone, total mobile time and ethologically derived measures like grooming, rearing and head dipping (number of presses).

2.5. Statistics

One way analysis of variance (ANOVA) with repeated measures, and post-hoc comparison with Bonferroni correction were done to compare the natural developmental profiles of the USV number (calling rate) and duration (of call, F_0) in the control and TSDX5h groups over different postnatal days. Non-parametric analysis (Mann Whitney-U test) and Student's *t* test were used to analyze differences in parameters between two groups. The level of significance was set at $p < 0.05$ for all comparisons. Chi square test was performed to find the changes in call type distribution between the groups over the postnatal days.

2.6. Ethics

The study was approved and performed in accordance with the guidelines laid down by the Institutional Animal Ethics Committee of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala.

3. Results

Gentle handling of the pregnant rats in the experimental group, at the onset of their sleep resulted in a reduction of sleep by 97.41 ± 0.01% as compared to the control group of rats.

3.1. Quantitative changes in the USVs in pups during pnds

A total of 26,927 ultrasonic calls were obtained from the pups on various days. The total numbers of USVs per min were averaged for each developmental day studied in male pups (n = 13 in control and 27 in experimental group) obtained from 10 litters. The intra and inter group multiple comparisons were made for all days for pups in control and TSDX5h groups. In control pups, the calling rates were low during initial pnds 1–5, but significantly increased on pnd 9 and they reached the peak calling on day 11 (Fig. 1; one way ANOVA, $F_{5,67} = 29.55$, $p < 0.0001$). Thereafter, the calling rate was reduced, reaching to negligible values on day 21. In pups of TSDX5h group, similar developmental increases in vocalization were observed from pnds 1–9 (one way ANOVA, $F_{5,108} = 14.3$, $p < 0.0001$), but calling did not increase further on pnd 11.

In comparison to control pups, calling rate in the pups born to TSDX5h mothers were significantly higher during initial pnds 1–9 (Fig. 1). Thereafter, USVs made by pups born to TSDX5h mothers were