Effects of prenatal alcohol exposure on respiratory regulation during sleep in the early postnatal period

Emily N. Soltis and Kevin J. Cummings
Department of Biomedical Sciences, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA

Abstract:
The risk of the Sudden Infant Death Syndrome (SIDS), a leading cause of infant death, is elevated by prenatal alcohol exposure, but the mechanism for this is unknown. SIDS victims display respiratory instability during sleep, and increased active sleep (AS; similar to REM sleep), a state characterized by respiratory instability. We hypothesized that prenatal maternal alcohol exposure increases SIDS risk by increasing the duration of AS and destabilizing breathing during sleep. To address this hypothesis we studied rat pups at postnatal day 7-8, born from dams that were fed 10% ethanol (EtOH) or pure water as their sole source of liquid during pregnancy. We used whole-body plethysmography to record the respiratory pattern, and measured respiratory variables, including the co-efficients of variation of the respiratory period (CVT%), tidal volume (CVV%), and indices of instability. We also determined duration of AS episodes using behavioral criteria that were confirmed with nuchal electromyography. Our data demonstrate that prenatal exposure to EtOH increased respiratory instability, reflected by increased CVT% (Control: 29.1 ± 4.9 %; EtOH: 36.0 ± 2.2 %; p=0.027) and increased CVV% (Control:13.7 ± 2.2 %; EtOH: 16.3 ± 0.8 %; p=0.001). In addition, prenatal EtOH exposure significantly increased the duration of AS episodes (EtOH: 100.5 ± 4.9 sec; control: 80.85 ± 1.64 sec; p=0.001). These results suggest that prenatal EtOH exposure may increase SIDS risk by destabilizing breathing in infancy and increasing the time spent in AS, a “risky” sleep state with respect to cardiorespiratory control. As SIDS is highly associated with brainstem serotonin (5-HT) defects, future studies will address the hypothesis that prenatal EtOH destabilizes breathing by reducing brainstem 5-HT.

Introduction

- Sudden Infant Death Syndrome (SIDS) occurs during sleep and is the leading cause of infant death between 1 month and 1 year of age.
- Maternal alcohol consumption is a strong risk factor for SIDS, but the underlying reasons are unknown (Iyassu et al., 2003).
- SIDS victims display respiratory dysfunction in the days and weeks prior to death, including decreased respiratory stability (e.g., sleep apnea) (Kato et al., 2001).
- SIDS cases also display more active sleep (AS) (Schechtman et al., 1992), a state associated with cardiorespiratory instability.
- Maternal alcohol consumption during pregnancy could increase SIDS risk by destabilizing breathing and lengthening AS episodes, both of which would increase the risk for hypoxia and sudden death.

Hypothesis

We hypothesized that prenatal alcohol increases SIDS risk by destabilizing breathing during sleep and prolonging episodes of active sleep.

Methods

Animals and Groups:
Prior to mating, rat dams were provided either water (control group) or 10% ethanol (EtOH group) as their sole source of drinking water during pregnancy. All pups were born by Caesarian section at day 20 of pregnancy. At birth, EtOH dams were switched back to water. Sleep and respiration were recorded in unanesthetized, postnatal (PND 7) pups (n=6 control, n=12 EtOH).

Surgery:
For initial experiments (n=3 control and 7 EtOH pups), we confirmed the presence of AS using nuchal electromyography. Under 2% isoflurane, an electrode was implanted under the nuchal muscles and another that served as ground was implanted into the back muscles.

Experimental Protocol:
Pups were given a 20-30 min settling period in a closed surgical suite (pup was present) in a warmed (31°C) chamber with constant (300 ml/min) flow of air (P027). Sleep and respiration were recorded in unanesthetized, postnatal (PND 7) pups (n=6 control, n=12 EtOH).

Measurements:
Respiration was determined using whole body EMG and behavioral observation (n=3 control, 7 treated), or behavioral observation (Control, 4 treated). Breathing was monitored with whole-body plethysmography. Normoxic respiratory variables were determined in all pups (null control). EtOH-exposed; frequency of breathing, T tidal volume, VV, and ventilation, Vl. The co-efficients of variation of the respiratory period (CVT%) and tidal volume (CVV%, CVV%) were used as indices for instability. Duration of AS episodes were measured.

Statistical Analyses:
Students two-tailed t-tests were used to assess significant effects of prenatal EtOH on the duration of AS episodes and on respiratory variables during AS. Significant effects were observed at p<0.05.

Conclusion

- Prenatal EtOH exposure had no influence on respiratory frequency, tidal volume or overall ventilation.
- EtOH-exposed rats had an increased fraction of active sleep, a “risky” sleep state that is normally characterized by decreased respiratory stability.
- Thus, prenatal EtOH exposure may put infants at risk for SIDS because it destabilizes breathing, potentially increasing their exposure to hypoxia.

Future Directions

- The respiratory phenotypes of rat pups exposed to EtOH prenatal are similar to the phenotypes of rodent pups deficient in central serotonin (5-HT) (Hodges et al., 2002; Cummings et al., 2019).
- As most SIDS victims have 5-HT neuron abnormalities, including reduced 5-HT (Paterson et al., 2006), we address this hypothesis that prenatal EtOH exposure destabilizes breathing in offspring by reducing brainstem 5-HT levels.
- We will test this hypothesis using immunohistochemistry and high performance liquid chromatography to quantify how prenatal EtOH exposure affects the number of 5-HT neurons as well as the overall brainstem 5-HT content, respectively.