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

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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 (CV-P%), and tidal volume (CV-V_T%) as indices of stability. We also determined duration of AS episodes using behavioral criteria that were confirmed with nuchal electromyography. Our data demonstrate that prenatal exposure to EtOH decreased respiratory stability, reflected by increased CV-P% (Control: 29.1 ± 4.9 %; EtOH: 36.0 ± 2.2%; p=0.027) and increased CV–V_T% (Control:13.7 ± 0.7%; EtOH: 16.3 ± 0.8%; p<0.001). In addition, prenatal EtOH significantly increased the duration of AS episodes (EtOH: 100.0 ± 4.09 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 1m – 1yr of age.
- Maternal alcohol consumption is a strong risk factor for SIDS, but the underlying reasons are unknown *(Iyasu et al., 2002)*.
- 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. Dams were kept on their respective liquid diets throughout pregnancy. At birth, EtOH dams were switched back to water. Sleep and breathing were monitored in unanesthetized, postnatal (P)7-8 pups (n=9 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:

Following surgery, pups were given a 20-30 min settling period in a warmed (31°C) chamber with constant (300mL/min) flow of air (Fig.1a). Sleep and breathing were then recorded for 2 hrs.

Measurements:

Sleep state was determined using nuchal EMG and behavioral observation (n=3 control, 7 treated), or behavioral observation alone (n=6 control, 4 treated). Breathing was monitored with whole-body plethysmography. Normoxic respiratory variables were determined in all pups (n=9 control, 11 EtOH-exposed): frequency of breathing, f_B; tidal volume, V_T; and ventilation, V_E. The co-efficients of variation of the respiratory period (CV-P (%)) and V_T (CV-V_T (%)) were used as indices for respiratory stability. Duration of AS episodes were measured. **__Note:__ variables were only measured in AS as pups displayed little quiet sleep.

***Statistical* Analyses:**

Students two-sided 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 determined at p<0.05

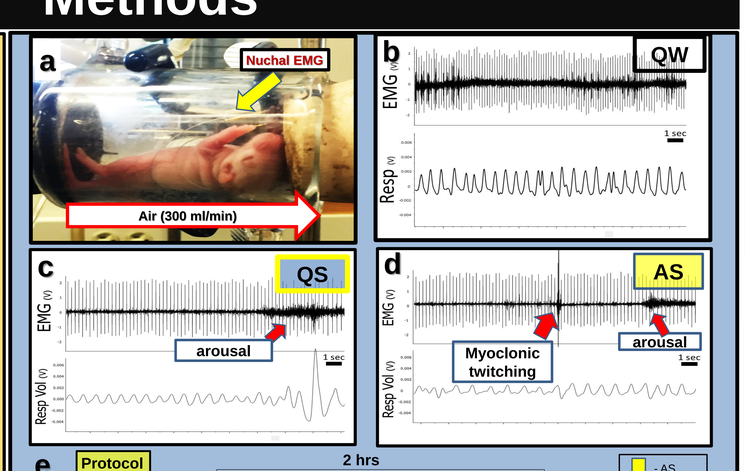

Figure 1. ***(a)*** Experiments were done using whole-body plethysmography to monitor breathing and nuchal EMG and behavior to determine sleep state, ***(b)*** EMG and breathing pattern during quiet wakefulness (QW). ***(c)*** Arousal from quiet sleep (QS). ***(d)*** Arousal from active sleep (AS); note the presence of myoclonic twitching which helps distinguishes AS from QS.

Results

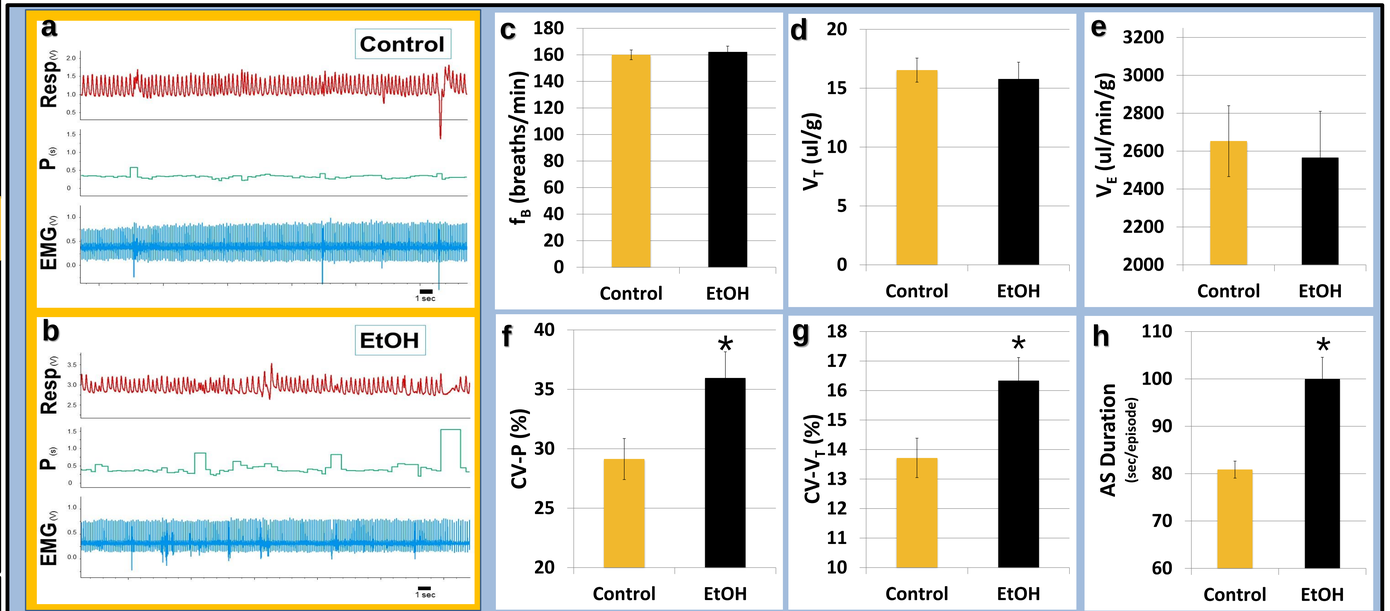

Figure 2. Prenatal EtOH exposure destabilizes breathing and increases the duration of active sleep (AS) episodes. Example traces of the raw respiratory pattern (Resp), calculated respiratory period (P), and nuchal electromyogram (EMG) of a control pup ***(a)*** and pup exposed to EtOH prenatally ***(b).*** Maternal EtOH consumption had no influence over average respiratory frequency (f_B; ***c)***, tidal volume (V_T; ***d)*** or overall pulmonary ventilation (V_E; ***e***). However, the stability of the respiratory pattern was reduced by prenatal EtOH. EtOH-exposed pups had significantly greater variation in both the respiratory period (CV-P %) (p=0.027; ***f)*** as well as V_T (CV-V_T %) (p<0.001; ***g).*** Finally, prenatal EtOH exposure increased the duration of AS episodes (p=0.001; ***h)***

Conclusions

- Prenatal EtOH exposure had no influence on respiratory frequency, tidal volume or overall ventilation.
- EtOH-exposed rat pups had more erratic breathing, characterized by significantly greater variation in both the timing and the volume of each breath.
- EtOH-exposed rat pups have significantly longer episodes 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 prenatally are similar to the phenotypes of rodent pups deficient in central serotonin (5-HT) *(Hodges et al., 2008; Cummings et al., 2010).*
- As most SIDS victims have 5-HT neuron abnormalities, including reduced 5-HT *(Paterson et al., 2006)*, we will address the 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.

References

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