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Neonatal sensitization to ethanol-induced breathing disruptions as a function of late prenatal exposure to the drug in the rat: Modulatory effects of ethanol's chemosensory cues

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HIGHLIGHTS

- Ethanol depresses respiration rates in newborn rats.
- Late gestational alcohol exposure sensitizes ethanol-induced breathing depression.
- Ethanol odor potentiates early ethanol-related disruptions upon respiration.

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ABSTRACT

Preclinical and clinical studies have systematically demonstrated abrupt changes in fetal respiratory patterns when the unborn organism is exposed to the effects of maternal ethanol intoxication. In subprimates, chronic exposure to this drug during gestation and infancy results in marked alterations of the plasticity of the respiratory network. These alterations are manifested in terms of an early incapability to overcome deleterious effects of hypoxic events as well as in terms of sensitization to ethanol's depressant effects upon breathing patterns. It has also been demonstrated that near term rat fetuses process ethanol's chemosensory cues when the drug contaminates the amniotic fluid and that associative learning processes occur due to the temporal contiguity existing between these cues and different ethanol-related physiological effects. In the present study during the course of late gestation (gestational days 17–20), pregnant rats were intragastrically administered with either 0.0 or 2.0 g/kg ethanol. Seven-day-old pups derived of these dams were evaluated in terms of respiration rates (breaths/min) and apneas when subjected to different experimental conditions. These conditions were defined by postnatal exposure to the drug (intragastric administrations of either 0.0, 0.5, 1.0 or 2.0 g/kg ethanol), postadministration time of evaluation (5–10 or 30–35 min) and olfactory context at test (no explicit ambient odor or ethanol ambient odor). The results, obtained via whole body plethysmography, indicated that brief prenatal experience with the drug sensitized the organisms to ethanol's depressant effects particularly when employing the higher ethanol doses. In turn, presence of ethanol odor at test potentiated the above mentioned respiratory alterations. Prenatal treatment with ethanol was not found to alter pharmacokinetic profiles resulting from postnatal exposure to the drug or to affect different morphometric parameters related with lung development. These results indicate that even brief exposure to the drug during late gestation is sufficient to sensitize the organism to later disruptive effects of the drug upon breathing responsiveness. These deficits are potentiated through the re-exposure to the olfactory context perceived in utero which is known to be associated with ethanol's unconditioned effects. As a function of these observations it is possible to suggest a critical role of fetal sensory and learning capabilities in terms of modulating later ethanol-related breathing disruptions.

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1 . Introduction

The teratological effects of ethanol, involving craneofacial anomalies, neurobehavioral alterations and growth retardation, do not cover all the possible consequences of early exposure to the drug. Beyond Fetal Alcohol Syndrome, intrauterine experiences with ethanol promote short and long term effects related with alcohol seeking, intake and preference patterns [1,2]. Preclinical and epidemiological research has indicated that these effects not only occur when utilizing high ethanol doses or chronic exposure to the drug that are known to result in gross morphological abnormalities [3].

The olfactory systems (principal, accessory and trigeminal) of different mammals, including humans, allow fetal detection of volatile substances present in the amniotic fluid [4–6]. Mere fetal familiarization with odorants changes subsequent detection and preference patterns of the stimuli perceived in utero. This non-associative learning process affects highly structured behaviors such as nipple attachment and lactation and even complex social patterns implying non-nutritive interactions with the mother or other conspecifics [7–10].

Fetuses exposed to subthreshold ethanol doses relative to its gross teratological properties, form memories relative to the chemosensory components of the drug. Rats that sense alcohol in the amniotic fluid will later react, behaviorally and autonomically, to the presence of this odorant [11,12]. When the drug is administered to the rat dam during the last four gestational days (blood and amniotic fluid ethanol levels ranging between 40–120 mg%) pups will later prefer the odor and they behaviorally react to this stimulus as alcohol-naïve pups respond to a biological odorant such as the amniotic fluid [13,14]. Analogous findings have been observed in healthy human babies delivered by mothers exhibiting moderate drinking patterns during pregnancy [15].

Fetal alcohol-related memories can also be established via associative learning processes. Numerous studies indicate that the near term fetus associates different chemosensory cues with ethanol's interoceptive or unconditioned effects [16,17]. From a correlational perspective the magnitude of ethanol-induced physiological disruptions (hypothermia) in the womb are highly predictive of neonatal responsiveness to the drug's chemosensory cues [18]. In neonates, and probably modulated by acetaldehyde central production and the involvement of the opiate system, ethanol exerts appetitive reinforcing effects [19–24]. Intrauterine pavlovian conditioning using ethanol as an unconditioned stimulus results in preferences to conditioned cues that signal ethanol intoxication [16,17]. In addition, it has been reported that prenatal ethanol exposure sensitizes the organism to the positive reinforcing effects of the drug [25]. Besides, it would be incorrect to state that relatively low doses of ethanol administered during short periods of time, do not exert short or long-lasting alterations in the developing organism [26].

Exposure to alcohol during prenatal development has also been associated with significantly reduced amniotic fluid volume and shortened umbilical cord length [27,28]. Umbilical cord length is a good indicator of fetal movement and provides direct evidence that maternal alcohol ingestion affects spontaneous fetal activity while suppressing breathing movements [29]. Fetal breathing movements (FBMs) are known to be present approximately 30% of the time in the near term human fetus and they represent a critical factor for normal development in different mammalian species [30,31]. Maternal human consumption of only two glasses of wine during late gestation disrupts fetal organization of behavioral states (particularly active sleep) and exerts a dramatic suppression of breathing activity [32–34]. The depressant effects of the drug upon FBMs have been systematically reported in humans and ewes [34–38]. The fact that fetal alcohol exposure is a risk factor for Sudden Infant Death Syndrome [39,40], has stimulated research focusing on the deleterious effects of the drug upon the respiratory system and its plasticity. In rats, chronic ethanol exposure (starting before mating and continuing throughout gestation and lactation) reduces brainstem-dependent respiratory rhythmic activity in the progeny and sensitizes juveniles to the depressant effects of acute ethanol upon phrenic and hypoglossal nerve activity [41]. Chronic prenatal ethanol exposure also affects neonatal rats in terms of compensatory respiratory processes that occur following hypoxia. Rather than observing long term facilitation of breathing after low oxygen exposure, neonates with a prenatal alcohol history exhibit long term depression of respiratory activity [42]. In the neonate rat, in vitro studies indicate significant depression in the respiratory-related hypoglossal nerve output caused by ethanol [43].

As mentioned, near term rat fetuses rapidly sensitize to the drug's chemosensory properties and its pharmacological effects and they are capable of acquiring associative memories comprising these factors. Under the consideration of these processes and the systematic reports of ethanol's effects upon FBMs, the present study (particularly Experiment 1) was guided by the following questions: i) will relatively short lasting near term fetal experiences with ethanol alter subsequent neonatal breathing frequencies? ii) is this type of exposure sufficient to generate either tolerance or sensitization to the drug's effects upon neonatal breathing or to disruptions such as apnea? and iii) is it possible that neonatal re-exposure to ambient ethanol odor modulates neonatal breathing patterns under the state of sobriety or intoxication? As can be observed the second question implies two opposite possibilities: development of tolerance or sensitization. As stated above, sensitization has been observed in juvenile rats after chronic ethanol exposure during gestation and lactation [41]. Yet, in near term sheep, development of tolerance to ethanol-induced suppression of FBMs occurs following short-term maternal administration of a moderate Ethanol dose (1 g/kg) [44].

Experiment 2 was conducted to determine if prenatal treatment with ethanol affected pharmacokinetic profiles in pups subjected to similar doses and postadministration times as those examined in Experiment 1. A third experiment examined whether prenatal exposure to the drug altered morphometric characteristics of the lungs of the neonates under consideration.

2. Material and methods

2.1.1. Subjects

Animals employed in this study were Wistar-derived rats born and reared at the vivarium of the Instituto Ferreyra (INIMEC-CONICET-UNC, Argentina). The animal colony was kept at 22–24 °C and under artificial lighting conditions (lights on: 08:00–20:00 h). Maternal lab chow (Cargill, Argentina) and water were available ad libitum. Vaginal smears of adult female rats were microscopically analyzed on a daily basis. On the day of proestrus, females (body weights: 200–300 g) were housed overnight with males. Vaginal smears were checked the following morning and the day of sperm detection was considered as gestational day 0 (GD 0). Pregnant females were individually placed in maternity cages partially filled with wood shavings. Day of parturition was considered as postnatal day 0 (PD 0). At PD 1, litters were culled to 10 pups (5 males and 5 females whenever possible). Animals used in this study were maintained and treated according to the guidelines for animal care established by the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

2.1.2. Drug treatment during gestation

From GDs 17 to 20, females were intragastrically intubated on a daily basis with either 0.0 (tap water) or 2.0 g/kg ethanol. The ethanol dose was achieved by administering 0.015 ml/g of a 16.8% v/v ethanol solution. Ethanol dosage and days of administration were selected based on prior studies showing fetal learning derived from the drug's sensory, physiological and behavioral effects and lack of deleterious effects of this treatment upon infantile gross morphological brain and body parameters, neuronal migration processes and sensory or perceptual and learning capabilities [3,13,14,45,46]. Intragastric intubations were