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Ethanol exposure during the early first trimester equivalent impairs reflexive motor activity and heightens fearfulness in an avian model

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Abstract

Prenatal alcohol exposure is a leading cause of childhood neurodevelopmental disability. The adverse behavioral effects of alcohol exposure during the second and third trimester are well documented; less clear is whether early first trimester-equivalent exposures also alter behavior. We investigated this question using an established chick model of alcohol exposure. In ovo embryos experienced a single, acute ethanol exposure that spanned gastrulation through neuroectoderm induction and early brain patterning (19–22 h incubation). At 7 days posthatch, the chicks were evaluated for reflexive motor function (wingflap extension, righting reflex), fearfulness (tonic immobility [TI]), and fear/social reinstatement (open-field behavior). Chicks exposed to a peak ethanol level of 0.23–0.28% were compared against untreated and saline-treated controls. Birds receiving early ethanol exposure had a normal righting reflex and a significantly reduced wingflap extension in response to a sudden descent. The ethanol-treated chicks also displayed heightened fearfulness, reflected in increased frequency of TI, and they required significantly fewer trials for its induction. In an open-field test, ethanol treatment did not affect latency to move, steps taken, vocalizations, defecations, or escape attempts. The current findings demonstrate that early ethanol exposure can increase fearfulness and impair aspects of motor function. Importantly, the observed dysfunctions resulted from an acute ethanol exposure during the period when the major brain components are induced and patterned. The equivalent period in human development is 3–4 weeks postconception. The current findings emphasize that ethanol exposure during the early first trimester equivalent can produce neurodevelopmental disability in the offspring.

Keywords: Fetal alcohol spectrum disorders; Chick embryo; Tonic immobility; Motor coordination; Fearfulness; Neurobehavior

Introduction

Prenatal alcohol exposure (PAE) can cause Fetal alcohol spectrum disorders (FASDs), a major cause of neurodevelopmental disability that affects 2–5% of live births and costs ~$3.6 billion annually (Centers for Disease Control and Prevention, 2009; Lupton et al., 2004; May et al., 2009). FASD can include somatic growth deficits and distinctive craniofacial and other physical anomalies. However, the major challenges confronting affected individuals are lifelong impairments in learning, attention, impulse control, memory, and motor skills (Jones and Smith, 1973; Streissguth and O'Malley, 2000). Alcohol exposure during all three trimesters causes significant behavioral and learning deficits, and these outcomes are improved if alcohol consumption is stopped or reduced during the first or second trimester (Autti-Rämö et al., 1992; Coles et al., 1985, 1987). It is less clear whether PAE causes behavioral deficits following binge exposure during the first month of pregnancy, a period when the condition often is unrecognized. Ethanol exposure during the early first trimester period causes craniofacial, cardiac, and brainstem deficits (Debelak and Smith, 2000; Dunty et al., 2001; Maier et al., 1997). The potential of PAE to adversely affect neurobehavior during the early developmental period is an important question because 10.8–13.7% of nonpregnant women aged 18–44 years report binge-drinking behavior (Denny et al., 2009).

We addressed this question in a well-characterized chick embryo model that displays craniofacial deficits similar to those of FASD (Debelak and Smith, 2000). Embryos receive an acute ethanol challenge at the onset of gastrulation (19 h incubation) via yolk injection. Embryonic ethanol levels peak 0.5–2 h later at 50–60 mM (0.23–0.28%) and then rapidly dissipate as the ethanol equilibrates between embryo, white, and yolk to a final level of ~9 mM (0.04%). During the next week, ~50% of the ethanol leaves the egg via gas

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diffusion through the shell (Pennington et al., 1983). Detectable alcohol dehydrogenase activity is first detected at day 9 of incubation and rapidly removes the remaining ethanol (Pennington et al., 1983). Thus, the model is largely one of acute binge ethanol exposure, but may also have influences from low-level chronic exposure during the organogenesis period.

The ethanol exposure protocol described here causes significant apoptosis within cranial neural crest populations and within progenitor neurons that reside in the presumptive forebrain, midbrain, and hindbrain (Debelak and Smith 2000; Su et al., 2001). To test whether the early exposure model also caused neurobehavioral deficits, we challenged in ovo embryos with a single ethanol dose at gastrulation and evaluated multiple behaviors posthatch. We report here that a single, acute ethanol exposure at gastrulation caused significant and selective motor impairments and enhanced the fearfulness of the hatched offspring.

Methods

Animals

Fertile white leghorn eggs were incubated at 37.5°C to gastrulation stage (19 h incubation, stage 4; Hamburger and Hamilton, 1951). Eggs were injected into the yolk core through the blunt end (avoiding the embryo) with saline or 0.43 mmol ethanol in isotonic saline in a final volume of 250 μL; some eggs were uninjected and served as untreated controls. Eggs were sealed, reincubated, and hatched using standard husbandry. Hatched chicks were housed in a communal heated brooder with free access to food and water. Protocols were approved by the UW-Madison Research Animal Care Committee and experiments conducted according to the NIH Guide for the Care and Use of Laboratory Animals. Separate sets of chicks were hatched for measures of body weight and for the pilot open-field test.

Behavioral testing

Behavioral testing was performed on a single day on randomly selected individual birds in this order, open field, righting reflex/tonic immobility (TI), and wingflap response, to minimize the effects of handling (Montevecchi et al., 1973). We chose tests that are well validated in domestic poultry. Note that, because the chicken is domesticated animal, the motivations (e.g., fearfulness, anxiety, social reinstatement) underlying these behaviors can differ from those of laboratory rodents (Forkman et al., 2007).

Novel open-field testing

At day 7 posthatch, randomly selected chicks were individually transferred in a closed heated container to a quiet room. After acclimating for 30 min, the chick was placed in the center of a 60 × 60 cm novel arena with 30 cm high white sides and padded floor cover (Eddy and Gallup, 1990; Gallup and Suarez, 1980). Behavior was monitored for 5 min by treatment-blinded experimenters seated quietly approximately 0.5 m distant; observer positions and tasks did not vary during the study. The following behaviors were recorded: number of soft (peep) and loud (distress cry) vocalizations, latency to first step, number of steps, hops and stumbles, number of escape attempts, latency to defecation, and number of defecations. The arena was cleaned between trials.

Righting reflex and TI

At the conclusion of the open-field testing, the bird was placed supine on a padded table top under gentle restraint. The bird was released when its head relaxed; this seldom took longer than 3 s. The time taken for the bird to right itself and stand after restraint removal was recorded; only those trials when the bird tried to right itself were used to calculate the median righting reflex time for the bird and trials that involved TI were excluded from that calculation. If a bird made no attempt to right itself after 10 s, the bird was stood on its feet. A trial in which the bird made no attempt to right itself was considered an instance of TI, or the temporary loss of the righting reflex and severe motor inhibition (Gallup et al., 1971). Each bird was tested in a single session of 10 trials. The median righting reflex time and the number of instances of TI during the session were recorded.

Wingflap response

After a 2-min rest period, the chick's balance and reflexes were tested using a standard protocol (Rager and Gallup, 1986). The chick was perched on the index finger of an extended hand; the experimenter's fingers securely anchored the feet so the bird did not fall. The hand was quickly and evenly lowered ~3 ft in 2–3 s (overhead to waist), stimulating chicks to extend wings and maintain balance. The degree of wing extension during the descent was visually scored by two observers using the criterion: 2—wings fully extended, energetic flapping; 1.5—wings extended but not quite fully or one wing extended and one not; 1—partial wing extension; 0—no wing extension. The trial was repeated 10 times with a 5-s interval between trials, and the median response for each trial and the overall trial was calculated.

Statistics

Data are presented as either mean ± standard error or median ± range as indicated. Data were first evaluated using the Shapiro–Wilk Normality test (SigmaStat, Jandal Scientific Software). Data not normally distributed were analyzed as indicated using either the Mann–Whitney *U* Statistic or Kruskal–Wallis one-way analysis of variance by ranks followed-up with the nonparametric Dunn test on rank sums. Frequency data were analyzed using either the Chi-square or Fisher Exact test as indicated.