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Attention, locomotor activity and developmental milestones in rats prenatally exposed to ethanol

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ABSTRACT

Rationale: Decline of attentional performance as a function of time engaged on a task and hyperactivity are features shared by children and adults with fetal alcohol syndrome or attentional deficit and hyperactivity disorders.
Objective: To investigate the effects of prenatal exposure to two doses of ethanol on developmental milestones, locomotor activity and attention.
Methods: Wistar rats born from dams exposed to one of four maternal treatments during pregnancy were used: A35 – liquid diet with 35% ethanol-derived calories; A10 – liquid diet with 10% ethanol-derived calories; control – ethanol-free liquid diet; chow – laboratory chow and water.
Results: A35 performed worse in grip strength than control and chow (postnatal day – 14, $p < 0.05$) but not in negative geotaxis (postnatal days 7–10); A35 also showed more locomotor activity than control and A10 ($p < 0.05$). Regarding attention, acquisition of the five choice reaction time task was similar between groups, but, the percentage of omission errors from A35 group was greater than other groups at baseline parameters, at shorter (2 s) and longer (7 s) inter-trial intervals and at a shorter stimulus duration (0.5 s) ($p < 0.05$). The percentage of omissions was larger in A35 as the blocks progressed in sessions with either longer or shorter inter-trial intervals (group × block $p < 0.05$). Animals from A10 group did not show any impairment in the tasks performed.
Conclusions: Our study demonstrates that as well as developmental impairments, prenatal ethanol can produce deficits associated with an increase in attentional demand in rodents, analogous to those observed in fetal alcohol syndrome and attentional deficit and hyperactivity disorders.

1. Introduction

Children and adults with fetal alcohol spectrum disorder (FASD) have increased propensity for learning problems (Streissguth et al., 1995), deficits in attention and memory (Streissguth et al., 1994), increased incidence of conduct disorders (Disney et al., 2008) and attention-deficit hyperactivity disorder during school years (Mick et al., 2002). Fetal ethanol exposure results in longer reaction times in both simple reaction time, choice reaction time (CRT) tests and in the continuous performance task (CPT), suggesting reduced speed of information processing and delays in responding (Simmons et al., 2002). Coles et al. (1997) also found that prenatal exposure to ethanol increased omission errors and decreased correct responses in a CPT.

Notably, some of these characteristics are also usually present in children diagnosed with attention deficit hyperactivity disorder – ADHD (Nanson and Hiscock, 1990). Children with ADHD and FASD showed declining performance of the CPT as a function of time, with increased latency to respond, more variability in latency and more omission errors during the final part of the task (Coles et al., 2002; Kooistra et al., 2009). Indeed, the ingestion of ethanol during pregnancy is a risk factor for the diagnosis of ADHD during childhood (Brown et al., 1991; Mick et al., 2002; Streissguth et al., 1994).

Attempts to verify the effects of prenatal alcohol exposure on attention in animal models are scarce. The first two studies that evaluated the effects of prenatal exposure to ethanol on attention in rats investigated the heartbeat orienting response to novel auditory (Caul et al., 1983) or olfactory (Hayne et al., 1992) stimuli. Neither study found evidence for any attentional abnormalities. Hausknecht et al. (2005) examined attention in rats prenatally exposed to ethanol using the choice reaction time task, an adaptation of the human CPT for animals. The performance of rats treated with ethanol was characterized by a more variable reaction time

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distribution and more false alarms. Recently, Kim et al. (2013) showed that prenatal exposure to ethanol results in attention deficit in mice and rats at post natal day 28. In a Y maze, animals showed decreased spontaneous alternation behavior, measured by the percentage of consecutive entries into three different arms.

In addition to a need for more studies directly measuring attention in animal models of FASD and ADHD, another contentious point relates to the route of administration and the minimum dose of ethanol needed to induce structural or behavioral consequences. Perhaps due to the difficulty in adequately controlling for factors such as dose, genetics and bias associated with the often-used method of self-report (Huizink, 2009), the literature is inconsistent with respect to the effects of small doses of ethanol (equivalent to one to two drinks a week) on the offspring (Abate et al., 2008; Henderson et al., 2007; Huizink, 2009; Kelly et al., 2008; Polygenis et al., 1998; Sood et al., 2001).

In comparison with injection, putting alcohol in drinking water or intragastric gavage, the use of liquid diets (nutritional preparations containing differing amounts of ethanol) allows for more stable levels of blood alcohol concentration – BAC (Driscoll et al., 1990; Uzbay and Bizarro, 2010). The liquid diets design includes two control groups: one is pair-fed with the ethanol group and receives the same liquid diet but with a carbohydrate substituted for the ethanol. The second control group receives chow and water ad libitum in order to control the effects of the liquid diet procedure (Driscoll et al., 1990).

Using liquid diets with methodology adapted from Riley et al. (1979), our goal was to investigate further the role of dose of ethanol during pregnancy in relation to possible developmental and attentional impairments in the offspring of rats. We compared the effects of a standard (35% ethanol-derived calories – EDC) and a lower dose (10% EDC) of prenatal ethanol on developmental milestones – weight, negative geotaxis, grip strength (Whishaw and Kolb, 2005) – locomotor activity and attention in the offspring. To the best of our knowledge, this is the first study to use the five-choice serial reaction time task (5-CSRTT) a robust method to evaluate attention in rodents, analogous to CPT used with humans to evaluate impulsivity, selective and sustained attention.

2. Methods

2.1. Animals

The subjects were Wistar rats born from 49 dams exposed to one of four maternal treatments during pregnancy: (1) A35 – liquid diet with 35% EDC, (2) A10 – liquid diet with 10% EDC (3) control – liquid diet without ethanol and (4) chow – free access to laboratory chow and water. Animal housing was maintained at a temperature of 22–24 °C and a 12 h light/dark cycle was in operation (lights on from 0700 h to 1900 h).

All pups (155 males and 153 females) were tested for body weight, negative geotaxis and grip strength. Females were culled on weaning. In the locomotor activity test only two randomly selected male pups from each litter were used. For the 5-CSRTT, one male was chosen at random from each litter in the chow ($n = 12$), control ($n = 12$) and A10 ($n = 13$) groups. In the A35 group, two or three animals from each litter (5 l) were used. Some of the remaining animals were subjects in another experiment (Pupe et al., 2011). National and institutional guidelines for animal welfare were followed and all procedures were approved by the ethics committee of the Hospital de Clínicas de Porto Alegre.

2.2. Liquid diet

The liquid diet was a commercially available formulation for pregnant rats (Rodent liquid diet pregnant/Weanling, Bio-Serv, Frenchtown, NJ, USA), with a nutritional composition based on the work of Lieber and DeCarli (1989), with ethanol (95%, Fmaia, São Paulo, Brazil). Diets offered to groups A10 (10% EDC, or 1.8%, v/v) and control were supplemented with maltodextrine (Sports Nutrition, São Paulo, Brazil), in order to match calorically the diet fed to the group A35 (35% EDC, or 6.7%, v/v). Females from each group were matched by weight at the beginning of the study. The A35 group had access to 150 ml of liquid diet each day; the amount consumed by each dam at a given gestational day was the limit provided to its weight-matched counterparts in the A10 and control groups on the same gestational day.

2.3. Pregnancy

Wistar rats (80 females and 40 males, purchased from Universidade Federal de Pelotas – Rio Grande do Sul, Brazil) were singly housed at the Unit of experimentation animal at Hospital de Clínicas de Porto Alegre. Dams were assigned to one of the four weight-matched groups: (1) A35, (2) A10, (3) control and (4) chow. A week before initiating the breeding procedures, the female animals in the A35 group were habituated to the liquid diet by progressively increasing the ethanol concentration up to the final concentration that delivered 35% EDC. The animals in groups A10 and control received liquid diet of the same composition as during pregnancy (10% and 0% EDC, respectively). During mating, all animals had free access to water and chow. The presence of sperm in the vaginal smear in the morning following mating was considered a confirmation of pregnancy, and the treatment with diets restarted.

Pregnant rats were weighed three times a week. The liquid diet was delivered in graduated feeding tubes and was replenished daily between 1400 h and 1600 h; the amounts consumed were recorded. Immediately after giving birth, the groups fed with liquid diet were switched to water and chow ad libitum. BAC was determined for 4 non-pregnant dams from each ethanol-treated group by gas chromatography in a commercial laboratory (Toxilab, Porto Alegre, RS, Brazil). After three weeks of liquid diet treatment, dams were food- and water-deprived during the dark phase of the diurnal cycle and given access to liquid diet for the last 3 h of the light phase. Animals were killed by CO_2 inhalation and the blood was collected from the heart in heparinized plastic tubes.

Whenever possible the litters were culled to 8 pups (4 males, 4 females). All behavioral tests were conducted during the second half of the light phase of the cycle. The pups were weighed at postnatal days (PND) 1, 7, 14, 21 and 40.

2.4. Developmental milestones

2.4.1. Negative geotaxis

On PND 7–10, each pup was placed face-downward on a plain wooden board with a 30° slant. Latency to rotate 180° was measured, with a maximum of 30 s allowed per trial, with two trials per day. If the pup completed only one trial within the allotted time, the data from it was its score for the day. If, however, a pup was able to complete the rotation within the allotted time in both trials, the mean latency of these two trials was used for analysis.

2.4.2. Grip strength

On PND 14 and 17, each pup was positioned vertically a few centimeters above the floor until its paws grasped a steel rod (diameter 0.2 cm) fixed at a height of approximately 20 cm and parallel with the floor. When the pup grasped the rod, it was released, and the time it held on to the rod before falling was recorded, with a maximum of 20 s per trial. There were two trials per day and the mean latency for both trials was analyzed.