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EFFECTS OF PRENATAL AND POSTNATAL MATERNAL ETHANOL ON OFFSPRING RESPONSE TO ALCOHOL AND PSYCHOSTIMULANTS IN LONG EVANS RATS

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Abstract—An important factor that may influence addiction liability is exposure during the early life period. Exposure to ethanol, early in life, can have long-lasting implications on brain function and drugs of abuse response later in life. In the present study we investigated the behavioral responses to ethanol and to psychostimulants in Long Evans rats that have been exposed to pre- and postnatal ethanol. Since a relationship between heightened drug intake and susceptibility to drug-induced locomotor activity/sensitization has been demonstrated, we tested these behavioral responses, in control and early life ethanol-exposed animals. The young adult male and female progeny were tested for locomotor response to alcohol, cocaine and *d*-amphetamine. Sedative, rewarding effects of alcohol and alcohol consumption were measured. Our results show that early life ethanol exposure behaviorally sensitized animals to subsequent ethanol and psychostimulants exposure. Ethanol-exposed animals were also more sensitive to the hyperlocomotor effects of all drugs of abuse tested and to those of the dopamine receptor agonist apomorphine. Locomotor sensitization to repeated injections of cocaine was facilitated in ethanol-exposed animals. Ethanol-induced conditioned place preference was also facilitated in ethanol-exposed animals. Ethanol consumption and preference were increased after early life ethanol exposure and this was associated with decreased sensitivity to the sedative effects of ethanol. The altered behavioral responses to drugs of abuse were associated with decreased striatal dopamine transporter and hippocampal NMDAR binding. Our results outline an increased vulnerability to rewarding and stimulant effects of ethanol and psychostimulants and support the epidemiological and clinical data that suggested that early chronic exposure to ethanol may increase the propensity for later self-administration of ethanol or other substances.

Key words: prenatal ethanol, addiction, vulnerability, sensitization, reward.

Ethanol use during pregnancy is one of the most common known causes of preventable birth defects, and can result in long-term deficits in physical and cognitive growth and development. The combined incidence of fetal alcohol syndrome and its partial forms, fetal alcohol effects and alcohol-related neurodevelopmental disorder, has been estimated to 9.1 per 1000 (Sampson et al., 1997). Brain imaging studies have identified structural changes in various brain regions of children exposed to prenatal ethanol including basal ganglia, corpus callosum, cerebellum and hippocampus that may account for the cognitive deficits (Mattson et al., 2001). Clinical studies reported that fetal ethanol exposure may increase risk of later ethanol and other drug dependence (Famy et al., 1998; Yates et al., 1998; Baer et al., 1998, 2003; Alati et al., 2006).

This epidemiological evidence has also been suggested in preclinical studies. Numerous neurobehavioral effects have been detected in animals following pre- and/or postnatal ethanol exposure and among them, early ethanol exposure can yield later enhancement of ethanol intake in rodents (Spear and Molina, 2005; Chotro et al., 2007). Earlier studies demonstrated altered response to the hypothermic effects of different drugs of abuse such as ethanol (Abel et al., 1981; Taylor et al., 1981) and morphine (Nelson et al., 1986), after prenatal ethanol exposure.

The long-term effects of early life ethanol exposure on drugs of abuse vulnerability and the neurobiological substrates involved in this vulnerability remain to be elucidated, even though numerous studies identified some mechanisms involved in CNS dysfunctions observed after *in utero* ethanol exposure (see for review Guerri, 2002). In addition to ethanol, we also analyzed the effects of both amphetamine and cocaine in order to test if early life ethanol exposure affects only the future response to ethanol or also alters the response to other drugs of abuse or dopaminergic agents. In this regard, previous studies (Xu and Shen, 2001; Choong and Shen, 2004) have shown that psychostimulants can restore the alterations of the neuronal activity of the ventral tegmental area (a critical brain area involved in addiction) by prenatal ethanol exposure, thus suggesting that animals exposed to prenatal ethanol may respond differently to the effects of psychostimulants. It is noteworthy, that a previous report has shown an increase in ethanol responding after prenatal exposure to cocaine in mice (Kelley and Middaugh, 1996), suggesting a potential heterosensitization.

In order to understand the mechanisms underlying the increased vulnerability to drugs of abuse disorders induced by early life ethanol exposure, we used a pre- and post-

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Abbreviations: ANOVA, analysis of variance; BEL, blood ethanol level; CPA, conditioned place aversion; CPP, conditioned place preference; DAT, dopamine transporter; LORR, loss of righting reflex; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate receptor; RM, repeated measures.

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natal ethanol exposure paradigm in rat (Naassila and Daoust, 2002; Othman et al., 2002; Dubois et al., 2006, 2008; Kervern et al., 2009) to investigate the long-term alterations on both behavioral responses to drugs of abuse and neurotransmission systems.

EXPERIMENTAL PROCEDURES

Drugs

All chemicals were obtained from Sigma Chemicals (Paris, France). [^{3}H]MK-801, [^{3}H]raclopride, [^{3}H]mazindol, [^{3}H]muscimol and [^{3}H]SCH23390 were obtained from PerkinElmer (Courtaboeuf, France). Cocaine hydrochloride, *d*-amphetamine, quinpirole, sulpiride and SCH23390 were obtained from Sigma Chemicals (Paris, France). Ethanol (95% v/v) was obtained from Carlo Erba réactifs (Val de Reuil, France). Ethanol was diluted to 20% v/v in physiological saline prior to i.p. injection. Cocaine and *d*-amphetamine injections were made in volumes of 1 ml/100 g and ethanol injections were made in volumes of 1.25 ml/100 g. Saline injections were made in volumes equal to that of the corresponding drug for each animal.

Animals and prenatal ethanol exposure

Adult male and virgin female Long Evans rats (200–300 g) were obtained from Elevage Janvier (Le Genest-Saint-Isle, France), maintained on a 12-h light/dark cycle (light on between 7 a.m. and 7 p.m.) and were used at least after 1 week of habituation in our facilities. The procedures described comply with ethical principles and guidelines for care and use of laboratory animals adopted by the European Community, Law 86/609/EEC and were approved by the Animal Care and Use Committee responsible for our institution. One hundred forty females were randomly assigned to two groups. The ethanol-treated group received as sole drinking fluid a 10% v/v ethanol solution, prepared from 95% ethanol, for 4 weeks before mating and had unlimited access to standard rat chow (UAR, France; standard diet). To avoid any dehydration, the forced ethanol consumption group had limited access to water every day for 2 h in the morning (during the light phase). Importantly, there was no difference in the daily total fluid consumption between the two groups (see Table 1). The *ad libitum* control group had unlimited access to standard rat chow and water. After successful mating, the ethanol-treated group was maintained on 10% ethanol solution until after delivery and also throughout gestation and lactation periods (Othman et al., 2002; Naassila and Daoust, 2002; Dubois et al., 2006, 2008; Kervern et al., 2009). This ethanol exposure is associated with an ethanol intake of 7–9 g/kg body weight/day in the dams before and during gestation and with an ethanol intake of 16–20 g/kg body weight/day during the two last weeks of lactation and peak BELs reached an average of 100 mg/dl (see Table 1) (Naassila and Daoust, 2002).

This procedure of forced oral ethanol (10%–18% in drinking water) consumption by dams has been used in numerous other studies (Jänicke and Coper, 1993; Othman et al., 2002; Naassila and Daoust, 2002; Carneiro et al., 2005; Dubois et al., 2006, 2008; Nowak et al., 2006; Servais et al., 2007; Kervern et al., 2009). The exposure to ethanol during the early postnatal period (i.e. the three weeks of lactation) was maintained since part of this period corresponds to the third trimester in human neural development (Andersen, 2003). Naive rats were used in each experiment and a particular animal was used only in a single behavioral test. One thousand eighty-four offspring born from 140 dams were used in the present study. A maximum of two siblings per litter (one male and one female) was randomly assigned to each of the experimental groups. All behavioral experiments have been conducted with two-month-old offspring and only the experiments on behavioral sensitization to cocaine have been conducted in both two and three-month-old offspring. Females have been tested for their basal locomotor activity, the hypnotic effects of ethanol and the locomotor effects of drugs of abuse. Hippocampal and striatal brain regions were dissected from two-month-old male pups. The animals used in

Table 1. Ethanol consumption, total fluid consumption, dam weight gain, litter size, pup weight at birth and at postnatal day 60 and BELs measured in offspring and dams during the 2nd week of gestation and the 3rd week of lactation

Measure	Ethanol-exposed group	Control group
Daily ethanol consumption (g ethanol/kg body weight) (n=20)		
Before gestation (4 weeks)	6.88±0.34	—
Gestation	8.62±0.42	—
Lactation week 1	13.19±0.93	—
Lactation week 2	16.12±0.56	—
Lactation week 3	20.35±1.17	—
Daily total fluid consumption (ml/dam)	(n=20)	(n=20)
Before gestation (4 weeks)	40.1±4.05	37.9±2.10
Gestation	46.2±6.52	47.3±3.32
Lactation week 1	62.3±7.94	65.8±6.30
Lactation week 2	78.6±5.56	83.6±8.09
Lactation week 3	75.10±6.64	78.8±7.40
Mean weight gain (g)	62±6.9 (n=20)	55.82±16.25 (n=20)
Litter size	9.46±0.69 (n=213)	9.02±0.43 (n=201)
Mean pup birth weight	6.5±0.5 (n=213)	7.7±0.7 (n=201)
Body weight at PND 60	201.7±3.5 (n=213)	206.1±4.2 (n=201)
Mean BELs in suckling offspring (mg/dl)	19.1±5.5 (n=10)	—
Maximum BELs in pregnant dams (mg/dl)	124.8 (n=10)	—
Minimum BELs in pregnant dams (mg/dl)	8.7 (n=10)	—
Maximum BELs in lactating dams (mg/dl)	161.6 (n=10)	—
Minimum BELs in lactating dams (mg/dl)	10.0 (n=10)	—

Mean BELs were determined from the blood samples collected at 08:00 h. BELs in dams are reported as minimum and maximum values obtained at that time of blood sampling. Values represent the means±SEM. —, Not applicable.