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Prenatal ethanol increases sucrose reinforcement, an effect strengthened by postnatal association of ethanol and sucrose

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ABSTRACT

Late prenatal exposure to ethanol recruits sensory processing of the drug and of its motivational properties, an experience that leads to heightened ethanol affinity. Recent studies indicate common sensory and neurobiological substrates between this drug and sweet tastants. Using a recently developed operant conditioning technique for infant rats, we examined the effects of prenatal ethanol history upon sucrose self-administration (postnatal days, PDs 14–17). Prior to the last conditioning session, a low (0.5 g/kg) or a high (2.5 g/kg) ethanol dose were paired with sucrose. The intention was to determine if ethanol would inflate or devalue the reinforcing capability of the tastant and if these effects are dependent upon prenatal ethanol history. Male and female pups prenatally exposed to ethanol (2.0 g/kg) responded more when reinforced with sucrose than pups lacking this antenatal experience. Independently of prenatal status, a low ethanol dose (0.5 g/kg) enhanced the reinforcing capability of sucrose while the highest dose (2.5 g/kg) seemed to ameliorate the motivational properties of the tastant. During extinction (PD 18), two factors were critical in determining persistence of responding despite reinforcement omission. Pups prenatally exposed to ethanol that subsequently experienced the low ethanol dose paired with sucrose, showed higher resistance to extinction. The effects here reported were not associated with differential blood alcohol levels across prenatal treatments. These results indicate that fetal ethanol experience promotes affinity for a natural sweet reinforcer and that low doses of ethanol are also capable of enhancing the positive motivational consequences of sucrose when ethanol and sucrose are paired during infancy.

Introduction

Newborn and infant rats share many characteristics in terms of ethanol affinity with those observed in genetically selected alcohol-preferring rats. Pups self-administer highly concentrated alcohol solutions (15–30% v/v) without the need of initiation procedures (Spear & Molina, 2005). Ethanol has also been found to exert rapid positive and negative (anti-anxiety) reinforcing effects in newborns (Abate, Pueta, Spear, & Molina, 2008; Abate, Varlinskaya, Cheslock, Spear, & Molina, 2002; Chotro, Arias, & Laviola, 2007; Pautassi, Sanders, Miller, Spear, & Molina, 2006; Petrov, Varlinskaya, & Spear, 2001, 2003). Motor stimulating effects of ethanol, effects which seem to share neurobiological mechanisms with positive motivational properties of the drug, have been detected early in development (Arias, Molina, Mlewski, Pautassi, & Spear, 2008). The preclinical and epidemiological research indicate that the effects of early ethanol experiences persist, and strongly predict alcohol abuse in adolescents and adults (Abate et al., 2008; Bannoura, Kraebel, Spear, & Spear, 1998; Domínguez, López, Chotro, & Molina, 1996; Faden, 2006; Grant & Dawson, 1997; Molina, Domínguez, López, Pepino, & Faas, 1999; Spear & Molina, 2005; Windle, 2003).

The near-term rat fetus acquires and retains ethanol-related information. The organism senses the drug's chemosensory cues present in the amniotic fluid while low to moderate maternal ethanol administrations act as appetitive unconditioned stimuli (Abate et al., 2008; Chotro, Córdoba, & Molina, 1991; Chotro & Molina, 1990, 1992; Dominguez et al., 1996). Exposure to subthreshold levels of ethanol, in terms of teratogenic properties, sensitize the organism to the drug's positive reinforcing effects (Nizhnikov, Molina, Varlinskaya, & Spear, 2006). Fetal ethanol exposure affects later alcohol affinity and strengthens the predisposition to abuse other addictive agents, probably because of common neurobiological mechanisms (Arias & Chotro, 2005a,

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2005b; Bachmanov et al., 2003; Chotro & Arias, 2003; Scher, Richardson, Coble, Day, & Stoffer, 1988). When considering natural reinforcers such as sweet tastants, there are also common neurobiological processes underlying the positive consequences of ethanol and such tastants. Positive correlations have been observed between sweet preference and ethanol affinity in animals and humans with a biological predisposition toward alcoholism (Kampov-Polevoy, Eick, Boland, Khalitov, & Crews, 2004; Kampov-Polevoy, Garbutt, & Janowsky, 1999; Kampov-Polevoy, Tsoi, Zvartau, Neznanov, & Khalitov, 2001; Kampov-Polevoy et al., 2003; Lange, Kampov-Polevoy, & Garbutt, et al., 2010). Alcohol-preferring rats consume higher levels of sucrose and accept more highly concentrated solutions than alcohol-avoiding animals (Fortuna, 2010). The reinforcing effects of sweet tastants and of alcohol partially converge in terms of common mechanisms, implying central release of endogenous opiates and dopamine. When focusing on ethanol reinforcement, opiate antagonism during early life inhibits subsequent alcohol preference. Similarly, opiate antagonism in newborn rats blocks sucrose preference as well as its negative reinforcing effects (Cleary, Weldon, O'Hare, Billington, & Levine, 1996; Garbutt et al., 2009; Philopena, Greenberg, & Smith, 1996).

To our knowledge, there have been no systematic advances in the analysis of how early ethanol experience influences reinforcement by sweet substances. There are only two studies in which the effects of fetal or infantile ethanol exposure were evaluated through sucrose consumption tests. In both studies no clear conclusions were evident due to ceiling effects of sucrose consumption across groups (López & Molina, 1999; Molina et al., 1996).

The present study takes advantage of recently developed learning procedures for the infant rat. Utilizing exploratory patterns (e.g. nose-poking) in infants, we have developed operant conditioning procedures that require minimal amounts of training (Bordner, Molina, & Spear, 2008; Domínguez, Bocco, Chotro, Spear, & Molina, 1993; March, Abate, Spear, & Molina, 2009; Miranda-Morales, Molina, Spear, & Abate, 2012; Pautassi, Truxell, Molina, & Spear, 2008; Ponce, Pautassi, Spear, & Molina, 2006, 2008). In the present study, goals relevant to understanding effects of early ethanol exposure upon subsequent sucrose reinforcement capability were subjected to experimental analysis based on operant associative learning. Given the effects of late prenatal ethanol exposure upon the predisposition to use and abuse this drug, it was decided to expose rat pups to ethanol during the stage of nursing to evaluate: a) the effect of this exposure on the reinforcing capabilities of sucrose, b) whether these capabilities are modified through subsequent revaluation procedures in which the sweetened solution is associated with a low or high ethanol dose, and c) seeking behavior for sucrose as a function of prenatal and revaluation treatments through the use of an extinction procedure. For item "b" it is relevant that recent studies show that during commencement of a state of acute intoxication, a relatively low ethanol dose (0.5 g/kg) exerts profound positive reinforcing effects. In the case of utilizing a higher dose (2.5 g/kg), we have observed minor reinforcing effects (Molina, Ponce, Truxell, & Spear, 2006; Molina, Pautassi, Truxell, & Spear, 2007; Pautassi, Nizhnikov, & Spear, 2009).

It is well known that the representation of an unconditioned stimulus (US) in memory may undergo revaluation if subsequently paired with another stimulus with clear aversive or appetitive unconditioned effects. Infants show significant decrements in aversive responsiveness (US: citric acid) to a given conditioned stimulus (CS), when after conditioning, ethanol's anti-anxiety effects are paired with the original US (Pautassi et al., 2006). In the present experiment the revaluation procedure was meant to determine whether ethanol is capable of revaluating the appetitive consequences of sucrose and if this revaluation is dependent upon prenatal ethanol experience. The study was conducted through 4 sequential phases: i) prenatal vehicle or ethanol exposure during gestational days (GDs) 17–20, ii) operant conditioning using sucrose as a reinforcer during PDs 14–16, iii) a sucrose revaluation procedure where ethanol or vehicle were paired with the sweetened solution, and a subsequent operant session reutilizing sucrose as a reinforcer (PD 17), and iv) an operant extinction session in which sucrose was omitted (PD 18).

Material and methods

Subjects

Animals employed in this study were Wistar-derived rats born and reared at the vivarium of the Instituto Ferreyra (INIMEC-CONICET), Argentina. The animal colony was kept at 22–24 °C and under artificial lighting conditions. Maternal lab chow and water were available *ad libitum*. Vaginal smears of adult females 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 designated GD 0. Day of parturition was designated PD 0.

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).

Drug treatments during gestation

Twenty dams were utilized. From GDs 17–20, females were intragastrically intubated once each day with either 2.0 g/kg ethanol or water (vehicle). 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 motivational effects and a lack of deleterious effects of this dose of ethanol upon infantile morphological and behavioral parameters (Domínguez et al., 1996; Domínguez, López, & Molina, 1998; Molina, Chotro, & Domínguez, 1995; Pueta, Abate, Spear, & Molina, 2005).

Infantile intraoral cannulation procedures

On each experimental day (postnatal days, PDs 14–18), male and female pups were removed from their maternal cages and intraorally implanted with a polyethylene cannula to allow the intraoral infusion of liquid reinforcers. This procedure is minimally stressful in younger preweanlings (PD 4) than those here utilized, as operationalized through the release of corticosterone or growth hormone (Spear, Kucharski, & Miller, 1989). We cannot discard certain responsiveness to this apparent mild stressor that, from a procedural perspective, is consistent across groups. We have employed this procedure in a variety of studies and it appears that the cannulation procedure does not seem to overshadow basic sensory and learning capabilities (Dominguez et al., 1993, 1996; Hunt, Kraebel, Rabine, Spear, & Spear, 1993; Pepino, Kraebel, López, Spear, & Molina, 1998; Pepino, López, Spear, & Molina, 1999; Pueta et al., 2005).

The procedure is performed in only 20 s. The location of the cannula varied each conditioning day. In other words, we never cannulated the same cheek on 2 consecutive days. Cannulas were made from 7-cm sections of PE 10 polyethylene tubing (Clay-Adams, Parsippany, NJ). A small flange was created in one end of these devices. The unflanged end was attached to a curved 27-G ½