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Ontogenetic differences in ethanol's motivational properties during infancy

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ABSTRACT

Pairing a conditioned stimulus (CS) with ethanol generally produces aversion for that CS in adult rodents. However, infant rats (PD1-PD3) exposed to ethanol demonstrate appetitive reinforcement to ethanol (Nizhnikov, Varlinskaya, Petrov, & Spear, 2006; Petrov, Varlinskaya, & Spear, 2003). This sensitivity to the appetitive properties of ethanol during infancy may be transient, as during the second postnatal week rat pups tend to exhibit conditioned aversions to flavors paired with ethanol. The present study examined changes in the motivation properties of ethanol through ontogeny and the neurobiology underlying these changes. Rat pups were exposed to a taste conditioning procedure on PD4 or PD12. Rat pups were intraorally infused with 2.5% of their body weight of saccharin solution (0.1%) and immediately after injected intraperitoneolly (i.p.) with one of six doses of ethanol (0.0–2.0 g/kg). A day later pups were given saccharine infusions and percent body weight gain was used as an index of ethanol's reinforcing effects. PD4 pups expressed appetitive reinforcement to ethanol, as indicated by greater saccharin intake, as compared to control counterparts and to the older PD12 pups. Subsequent experiments revealed that PD4 pups were less sensitive to the aversive properties of the drug than PD12 pups. The older pups found high doses of ethanol aversive while PD4 rat pups did not condition aversions to this dose of ethanol after a single trial. A similar pattern of results was observed between the low doses of ethanol and the highest doses of a kappa opioid agonist. The PD12 animals did not condition to the kappa opioid agonist, while the younger rats expressed an appetitive response. These results illustrate an ontogenetic change in the motivational properties of ethanol, with sensitivity to its appetitive properties declining and responsiveness to the aversive properties increasing with age during early infancy.

Introduction

Experience with alcohol either prenatally or during early infancy may be a contributing factor for exacerbated alcohol use during adolescence. This may in turn enhance the likelihood of alcohol-related problems in adulthood (i.e., the "alcoholism generator"; Miller & Spear, 2006). Evidence that supports this hypothesis, however, is still scarce (but see Spear, 2002). Exposure to ethanol during infancy in humans is, surprisingly, not as rare as one might think (for review see Spear & Molina, 2005) and can occur through breast-feeding by a drinking mother or when the drug is applied to the infant for medicinal purposes (e.g., as a sedative-hypnotic or anesthetic, Mennella & Beauchamp, 1991, 1993). Recent epidemiological studies confirm that humans exposed prenatally to moderate amounts of ethanol are at risk for alcohol abuse as adolescents, and subsequently as adults (Alati et al., 2008; Baer, Bar, Bookstein, Sampson, & Streissguth, 1998; Baer, Sampson, Barr, Conner, & Streissguth, 2003; Yates, Cadoret, Troughton, Stewart, & Giunta, 1998). This phenomenon has been experimentally confirmed through the use of preclinical animal models (Spear & Molina, 2005). For example, it has been shown that rats exposed to ethanol during late gestation (1.0 or 2.0 g/kg on gestational days 17–20 GD 17–20) exhibit enhanced ethanol intake at mid infancy and adolescence (e.g., Arias & Chotro, 2005; Chotro & Arias, 2007; Chotro, Arias, & Laviola, 2007; Molina, Dominguez, Lopez, Pepino, & Fas, 1999). This effect is also evident when ethanol exposure occurs during the first two-weeks of life (Hayashi & Tadokoro, 1985; Lopez & Molina, 1999), even when pups are exposed to the drug through nursing with an intoxicated dam (e.g., Pepino, Abate, Spear, & Molina, 2004; Ponce, Pautassi, Spear, & Molina, 2004, 2011).

Very young animals (postnatal day 1–3: PD1 – PD3) easily condition appetitive responses to ethanol in first order conditioning paradigms (Cheslock et al., 2001; Nizhnikov, Molina, & Spear, 2007; Nizhnikov, Molina, Varlinskaya, & Spear, 2006; Nizhnikov, Varlinskaya, et al., 2006; Petrov et al., 2003). On the other hand

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rats 1–2 weeks of age, although readily ingesting ethanol, tend to exhibit aversion to conditioned stimuli (CS) paired with ethanol (Dominguez, Lopez, & Molina, 1998; Hunt, Spear, & Spear, 1991; Molina et al., 1996; Pautassi, Godoy, Spear, & Molina, 2002). One especially clear example of this effect can be seen in experiments conducted by Arias and Chotro (2006). When 7–8 day-old rat pups were exposed to ethanol (3.0 g/kg) they exhibited a preference for ethanol 3 days later at test. On the other hand if this exposure occurred on days 10–11 an aversion was exhibited at test. This discontinuity of conditioning suggests an ontogenetic shift in the sensitivity of the infant rat to the motivational properties of ethanol. Recent studies by Nizhnikov, Pautassi, Truxell, and Spear (2009) and Pautassi, Nizhnikov, Acevedo, and Spear (2011) have found clear conditioning of place preference to ethanol (1.0 g/kg) in infant rats 13–15 days old. It seems that even though older infants are capable of responding to the positive reinforcing properties of ethanol, aversions to stimuli paired with its effects are more readily expressed.

Taking into account the established appetitive reinforcing effects of ethanol at these young ages it is plausible that the experience of appetitive conditioning with ethanol during infancy may lead to increases in drinking during adolescence or adulthood. Some results indicative of this can be found in studies by Ponce, Pautassi, Spear, and Molina (2008). In these Experiments infant rats (PD12 – PD16) readily learned to nose poke for ethanol and consume considerable amounts of the drug in this procedure. When tested 2 weeks later (P30) on ethanol intake in a free choice two bottle test, rats that had been conditioned with ethanol as reinforcer exhibited significantly greater alcohol intake than their yoked controls. Furthermore, a control group given only water or noncontingent ethanol exposure during infancy always drank more water than ethanol during the two bottle test at P30, while ethanol conditioned subjects drank more alcohol than water (Ponce et al., 2008). These results suggest that learning or conditioning associated with early ethanol experience might be an important determinant of future ethanol intake.

Several lines of evidence have implicated endogenous opioid systems in ethanol intake, as well as in its reinforcing properties (Herz, 1997; Ulm, Volpicelli, & Volpicelli, 1995). For example, animal studies have demonstrated that non-selective opioid receptor antagonists (Myers, Borg, & Mossberg, 1986; Myers & Lankford, 1996; Reid & Hunter, 1984; Samson & Doyle, 1985; Stromberg, Volpicelli, & O'Brien, 1998), as well as selective mu (Hyytiä & Kiianmaa, 2001; Krishnan-Sarin et al., 1998; Stromberg et al., 1998) and delta antagonists (Hyytiä & Kiianmaa, 2001; June et al., 1999; Krishnan-Sarin et al., 1995; Krishnan-Sarin, Portoghese, Li, & Froehlich, 1995), reduce ethanol intake in adult animals. This effect is seen across a variety of species and selected lines, as well as under a variety of experimental conditions.

Ethanol has been shown to stimulate the release of endogenous ligands for mu and delta opioid receptors (beta-endorphin, enkephalins) in distinct brain regions associated with reward and reinforcement (De Waele & Gianoulakis, 1993; Olive, Koenig, Nannini, & Hodge, 2001; Rasmussen et al., 1998). Ethanol-induced release of beta-endorphin in the hypothalamus, nucleus accumbens, and ventral tegmental area (De Waele & Gianoulakis, 1993; Olive et al., 2001; Rasmussen et al., 1998) and interaction of this endogenous ligand with mu opioid receptors in the mesolimbic reward system seem critical for the euphoric, positively reinforcing effects of ethanol. Furthermore, findings from clinical trials demonstrate that non-selective opioid antagonists are effective in reducing ethanol consumption in alcoholics (see Oswald & Wand, 2004 for references and review).

In contrast to mu opioid receptors and their endogenous ligands, the dynorphin/kappa opioid receptor system has been implicated in mediating ethanol's aversive properties in adulthood. Several studies have investigated the effects of kappa pharmacological manipulations on ethanol intake and reinforcement in adult animals. In general, selective kappa agonists have been shown to attenuate ethanol intake in adult rats while antagonists increase it (Lindholm, Werme, Brene, & Franck, 2001; Mitchell, Liang, & Fields, 2005, but also see Nestby et al., 1999). Dynorphin, the endogenous ligand for kappa opioid receptors (Chavkin, James, & Goldstein, 1982), reduces ethanol preference in adults, and a selective kappa receptor agonist, U50, 488H, effectively attenuated ethanol-induced place preference (Matsuzawa, Suzuki, Misawa, & Nagase, 1999; Sandi, Borrell, & Guaza, 1988). Another important point to consider is that enhanced ethanol-induced dopamine (DA) response in the nucleus accumbens has been reported following pharmacological blockade or genetic deletion of kappa opioid receptors (Zapata & Shippenberg, 2006). This indicates that endogenous activity at kappa opioid receptors counteracts activation of the mesolimbic DA system induced by ethanol and thereby diminishes the reinforcing effects of acute ethanol in adults (Shippenberg, Zapata, & Chefer, 2007).

Unlike adult responding, however, newborns seem to require activity at kappa opioid receptors in order to find ethanol rewarding (Nizhnikov, Molina, et al., 2006; Nizhnikov, Varlinskaya, et al., 2006). The effects of pharmacological blockade of these receptors on ethanol reinforcement were assessed using a surrogate nipple technique in 3-hr-old newborn rat pups. Blockade of kappa opioid receptors by a selective antagonist, nor-binaltorphimine, completely eliminated the reinforcing effects of ethanol (Nizhnikov, Molina, et al., 2006; Nizhnikov, Varlinskaya, et al., 2006) without affecting conditioning to an aversive stimulus. This finding indicates that the kappa opioid system is critical for mediating ethanol's appetitive reinforcing properties very early in ontogeny.

Taken together the data indicate an ontogenetic shift in the motivational properties of the kappa opioid system and may be one reason for the disparity in responding to alcohol and other reinforcers across ontogeny. One of our goals in this set of studies was to test the positive or aversive motivational properties of kappa opioid agonists at the two different ages employed in this set of studies. More specifically, we wanted to know whether the effects of kappa opioid agonists on ethanol reinforcement later in ontogeny are similar to those during very early infancy.

Another possible explanation for the ontogenetic difference in responding to ethanol could be differential metabolism of the drug across these young ages. For example, Kelly, Bonthius, and West (1987) found that adolescent rats eliminate ethanol from the blood at a much faster rate than infants. Silveri and Spear (2000) also found that 16 day-old rats metabolize ethanol at a much slower rate than older subjects (PD26 – PD56). In more general terms, older animals metabolize ethanol faster than their young counterparts (Hollstedt, Olsson, & Rydberg, 1980; Hollstedt & Rydberg, 1970; Walker & Ehlers, 2009). Therefore, any examination of differences in the reinforcing properties of ethanol across age must take this into account.

The goal of the current experiments was to assess ontogenetic differences in responding to ethanol during infancy. Experiment 1 examined differences in conditioned taste aversion across age when several high doses of ethanol were employed as the US. Conversely, Experiment 2 tested differences in responding to lower doses of ethanol across early infancy. Experiment 3 replicated the results obtained from Experiment 2 since they were so unexpected. Changes in responding to activation of the kappa opioid system across early infancy were tested in Experiment 4. Finally, age-related differences in blood ethanol content after i.p. injections of 2.0 and 0.25 g/kg ethanol were explored in Experiment 5.