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NEONATAL EXPOSURE TO AMPHETAMINE ALTERS SOCIAL AFFILIATION AND CENTRAL DOPAMINE ACTIVITY IN ADULT MALE PRAIRIE VOLES

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Abstract—The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent species that forms pair bonds after mating. Recent data have shown that amphetamine (AMPH) is rewarding to prairie voles as it induces conditioned place preferences. Further, repeated treatment with AMPH impairs social bonding in adult prairie voles through a central dopamine (DA)-dependent mechanism. The present study examined the effects of neonatal exposure to AMPH on behavior and central DA activity in adult male prairie voles. Our data show that neonatal exposure to AMPH makes voles less social in an affiliation test during adulthood, but does not affect animals' locomotor activity and anxiety-like behavior. Neonatal exposure to AMPH also increases the levels of tyrosine hydroxylase (TH) and DA transporter (DAT) mRNA expression in the ventral tegmental area (VTA) in the brain, indicating an increase in central DA activity. As DA has been implicated in AMPH effects on behavioral and cognitive functions, altered DA activity in the vole brain may contribute to the observed changes in social behavior.

Key words: affiliation, amphetamine, dopamine, nucleus accumbens, elevated plus maze, open field.

INTRODUCTION

The third trimester during gestation is a critical period for the formation of important areas and connections in the human brain that are involved in the regulation of many cognitive and behavioral functions. Prenatal exposure to amphetamine (AMPH) has become a concern due to the increased usage by women of childbearing age (Kuczkowski, 2007; Substance Abuse and Mental Health Services Administration, 2009) and by women with confirmed pregnancy (Terplan et al., 2009). Indeed, nearly 24% of pregnant women seeking treatment for drug addiction in 2006 reported abuse of AMPH or methamphetamine as compared to 8% in 1994 (Terplan et al., 2009). Although considerable information is available for the effects of AMPH on the adult brain (Berman et al., 2008; Panenka et al., 2013), the long-term effects of AMPH on a human fetus are complex and not yet understood. Some studies suggest that exposure to AMPH during pregnancy affects birth weight, growth rate, physiological stress, cognitive performance and social behaviors in children, and these effects may last into adolescence (Eriksson et al., 2000; Smith et al., 2006, 2008; Kwiatkowski et al., 2014).

In terms of brain development, the neonatal period in rats is considered equivalent to the second to third trimester of pregnancy in humans (Bayer et al., 1993; Rice and Barone, 2000; Clancy et al., 2007a,b). Exposure to AMPH or methamphetamine during the neonatal period has been found to induce multiple developmental and behavioral deficits in rodents. For example, neonatal exposure to methamphetamine induces a decrease in body weight (Williams et al., 2004b), a sustained increase in corticosterone (CORT) and adrenocorticotropin hormone (ACTH) (Williams et al., 2000; Grace et al., 2008), and a reduction in dopaminergic (DA-ergic) and serotonergic markers in rats (Crawford et al., 2003; Schaefer et al., 2008). All of these are followed by developmental long-term impairments in spatial learning and memory, and changes in neuronal morphology in several brain regions, including the hippocampus and nucleus accumbens (NAcc) (Williams et al., 2004a; Vorhees et al., 2007). Interestingly, a critical period for AMPH or methamphetamine effects has even been identified within the neonatal period in rats. Previous studies have found evidence of cognitive deficits in learning and memory in rats exposed to substituted AMPH such as methamphetamine or ecstasy (3,4-methylenedioxymethampheta mine) during postnatal days (PNDs) 11–20 (Vorhees et al., 1994; Broening et al., 2001; Williams et al., 2003; Vorhees et al., 2007, 2009), but not during PND 1–10 (Vorhees et al., 1994, 2000; Broening et al., 2001). In fact, PND 11–20 are important for the development of the hippocampus and the reward pathways that control social behavior and drug addiction (Levitt, 1998; Wise, 1998).

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Abbreviations: ACTH, adrenocorticotropin hormone; AF, affiliation; AMPH, amphetamine; ANOVA, analysis of variance; CORT, corticosterone; CP, caudate putamen; D1R, D1 receptor; D2R, D2 receptor; DA, dopamine; DA-ergic, dopaminergic; DAT, dopamine transporter; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EPM, elevated plus maze; mPFC, medial prefrontal cortex; NAcc, nucleus accumbens; OF, open field; PND, postnatal day; SNK, Student Newman–Keuls; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

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The central dopamine (DA) system has been shown to play an important role in both natural and drug reward (Wise, 1998; Willuhn et al., 2010; Baik, 2013). In particular, the mesolimbic DA-ergic pathway, originating from the ventral tegmental area (VTA) and projecting to the NAcc and the medial prefrontal cortex (mPFC), has been implicated in mediating the rewarding effects of AMPH (Mukda et al., 2009). AMPH induces DA release in the NAcc and inhibits DA reuptake by acting on the DA transporter (DAT) (Heikkila et al., 1975; Seiden et al., 1993; Rothman and Baumann, 2003). Further, the rewarding effects of AMPH can be attenuated or inhibited by selective DA receptor antagonists (Wise, 1998).

The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent species that forms long-lasting pair bonds following 24 h of mating (Williams et al., 1992; Carter et al., 1995; Insel et al., 1995). Therefore, this species has been used as an excellent model for the study of the neurobiology of pair bonding (Wang and Aragona, 2004; Young and Wang, 2004; Young et al., 2011a). Recent data have demonstrated the role of central DA, particularly the mesolimbic DA pathway, in pair bond formation and maintenance (Young et al., 2011a). Interestingly, AMPH has also been shown to be rewarding to prairie voles as it induced conditioned place preferences in both males and females (Aragona et al., 2007; Young et al., 2011c), and this effect is mediated by mesocorticolimbic DA (Liu et al., 2010; Young et al., 2011, 2014). Recent evidence indicates an interaction between drug and social reward in prairie voles. Social bonding prevented AMPH-induced conditioned place preference in male prairie voles through a D1 receptor (D1R)-mediated mechanism (Liu et al., 2011). However, experience with AMPH impaired mating-induced pair bonding in male and female prairie voles through a mechanism dependent on DA and/or its interaction with oxytocin neurotransmission within the brain reward circuitry (Liu et al., 2010; Young et al., 2014). Together, these findings suggest that mesolimbic DA may play an important role in the regulation of social behavior and its interaction with drug rewards. In the present study, we tested the hypothesis that exposure to AMPH during a critical period in early development induces long-lasting changes in social or other related behaviors, as well as in mesolimbic DA activity in male prairie voles.

EXPERIMENTAL PROCEDURES

Subjects

Subjects were male prairie voles (*M. ochrogaster*) that were the offspring of a laboratory breeding colony. We focused on male voles because AMPH reward, its interaction with social bonding, and the underlying NAcc DA mechanism have been better demonstrated in adult male prairie voles (Liu et al., 2010, 2011). It has also been shown that male rats are more prone to methamphetamine-induced neuronal effects on DA-ergic markers (Crawford et al., 2003). Neonate voles were housed with both parents in plastic cages (20 × 25 × 45 cm) while male subjects received AMPH treatment (see below). Subjects were weaned at PND 21 and then were housed in same sex pairs. All cages contained cedar chip bedding. Water and food were provided *ad libitum*. All animals were maintained on a 14L:10D photoperiod with lights on at 0700, and the temperature was controlled (21 ± 1 °C). All the animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 and were approved by the Institutional Animal Care and Use Committee at the Florida State University. All efforts were made to minimize the number of animals used and their suffering.

AMPH treatment

From PND 13 to PND 15, subjects were assigned into one of three treatment groups and received injections of 25 μl saline containing AMPH at the concentration of 0.0 (n = 8 from 8 litters), 0.5 (n = 16 from 12 litters), or 3.0 (n = 12 from 10 litters) mg/kg body weight. The injection was given subcutaneously (s.c.) once per day for three days. Injection sites were rotated to minimize irritation and discomfort. The doses of AMPH and the administration protocol were determined based on previous studies in adult voles (Aragona et al., 2007; Liu et al., 2010) and other animals (Tzschentke, 2007). After injections, subjects were put back with their parents and female siblings without further disturbance. Subjects were marked by toe-clips for identification. A split litter design was used and thus no more than two pups within each treatment group were from the same parents.

Body weights

Body weights were obtained before each injection (saline or AMPH) on PND 13–PND 15. Weaning weights were obtained on PND 21 and adult weights were obtained on PND 86, after the behavioral tests and before euthanasia.

Social affiliation (AF) test

Subjects were tested for affiliation on PND 80. The testing apparatus consists of two chambers (13 × 18 × 29 (H) cm) that were connected by a hollow tube (7.5 × 16 cm), as previously described (Sun et al., 2014). A stimulus male prairie vole, which was about the same age as the subject, was tethered in one of the two chambers. Thereafter, the subject was released into the remaining chamber and allowed to move freely throughout the apparatus. A customized computer program using a series of light beams across the connecting tube was used to monitor the subject's movement between cages. The frequency of the subject's cage crossings and the time spent in each cage were recorded on the computer. The social affiliation test lasted for 3 h. Thereafter, subjects were put back into their original cages.

Open-field (OF) test

The OF test was conducted on PND 82 to evaluate locomotor activity and anxiety-like behaviors (Lieberwirth et al., 2013). The apparatus was made of plastic