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

Daniela F. Fukushiro, Anlys Olivera, Yan Liu, and Zuoxin Wang*

Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL 32306, USA

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.

Keywords

affiliation; amphetamine; dopamine; nucleus accumbens; elevated plus maze; open field

1. 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 & 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

*Correspondence to: Zuoxin Wang, Ph.D., Department of Psychology, Florida State University, Tallahassee, FL 32306-1270 Phone: 850-645-5615, Fax: 850-644-7739, zwang@neuro.fsu.edu.

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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; Smith et al., 2008; Kwiatkowski et al., 2014).

In terms of brain development, the neonatal period in rats is considered equivalent to the second to third trimester pregnancy in humans (Bayer et al., 1993; Rice and Barone, 2000; Clancy et al., 2007a; Clancy et al., 2007b). 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-methylenedioxymethamphetamine) during postnatal days (PND) 11–20 (Vorhees et al., 1994; Broening et al., 2001; Williams et al., 2003; Vorhees et al., 2007; Vorhees et al., 2009), but not during PND 1–10 (Vorhees et al., 1994; Vorhees et al., 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).

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 hrs 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., 2011c; Young et al., 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.

2. Experimental Procedures

2.1 Subjects

Subjects were male prairie voles (*Microtus 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; Liu et al., 2011). It has also been shown that male rats are more prone to methamphetamine-induced neuronal effects on DA 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 Institute 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.

2.2 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 (Tzschenke, 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 2 pups within each treatment group were from the same parents.

2.3 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.

2.4 Social affiliation test

Subjects were tested for affiliation on PND 80. The testing apparatus consists of two chambers ($13 \times 18 \times 29$ (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 hrs. Thereafter, subjects were put back into their original cages.

2.5 Open-field test

The open-field test was conducted on PND 82 to evaluate locomotor activity and anxiety-like behaviors (Lieberwirth et al., 2013). The apparatus was made of plastic ($56 \times 56 \times 20$ (H) cm) and its floor was divided into 16 squares, each measuring 14×14 cm. Each subject was placed into the center of the arena, and the time each subject spent in the center squares (a measure of anxiety-like behavior) and the frequency of line crossings (i.e., locomotor activity) were quantified for 10 mins. The apparatus was cleaned thoroughly with soapy water between animals.

2.6 Elevated plus maze test

To further examine animal's anxiety, an elevated plus maze (EPM) test was performed when subjects reached PND 85. This test was employed and validated also in our previous studies in voles (Lieberwirth et al., 2013, Liu et al., 2014). Briefly, the EPM (Columbus Instruments, Columbus, OH) is comprised of two open arms (35×6.5 cm) and two closed arms ($35 \times 6.5 \times 15$ (H) cm) that cross in the middle, and is elevated 45 cm off the ground. Experiments began by placing a single vole on the central platform facing an open arm. The number of entries into each arm (defined as an animal placing all four paws onto an arm) and time spent in open or closed arms were recorded for 5 mins. Anxiety-like behavior was evaluated by the percent of time spent and the percent of entries in the open arms of the apparatus. The maze was cleaned thoroughly with soapy water between animals.

2.7 In situ hybridization for TH, DAT, D1R and D2R mRNA labeling

One day after the EPM test, subjects were decapitated and labeling of the DA-ergic marker mRNAs was performed. Briefly, after decapitation subjects' brains were rapidly harvested, frozen on dry ice, and subsequently cut on a cryostat ($14 \mu\text{m}$ thickness). Sections were thaw-mounted on slides. Four sets of slide-mounted sections at $98 \mu\text{m}$ intervals were processed,

respectively, for the *in situ* hybridization labeling of tyrosine hydroxylase (TH), DAT, D1R and D2 receptor (D2R) mRNAs.

Antisense riboprobes were used for TH, DAT, D1R and D2R mRNA labeling in the VTA and NAcc, using an established method validated in our previous study (Young et al., 2011c). Probes were labeled in 1× transcription buffer containing 1 µg linearized plasmid antisense cDNA, 500 µM each of ATP, GTP, and UTP, 10 mM of dithiothreitol (DTT), 250 µCi [³⁵S]-CTP, 20 U of RNase inhibitor, and 20U of transcription enzyme at 37°C for 90 mins. The probes were then purified by Bio-Rad Micro Bio Spin Chromatography Columns (Bio-Rad Hercules, CA). The probes were diluted in hybridization buffer containing 50% formamide, 10% dextran sulfate, 3× saline sodium citrate (SSC), 10 mM sodium phosphate buffer (pH 7.4), 1× Denhardt's solution, 0.2 mg/ml yeast tRNA and 10 mM DTT to yield 5× 10⁶ cpm/ml.

The brain sections were fixed in 4% paraformaldehyde for 30 mins, followed by 3 washes in 2× SSC. The sections were then incubated in 0.1 M triethanolamine (TEA, pH 8.0) containing 0.25% acetic anhydride for 10 mins, rinsed in distilled water and dehydrated through 50, 75, 95, and 100% ethanol. After air-drying, the sections were coverslipped with 100 µl hybridization buffer containing either the TH, DAT, D1R, or D2R ³⁵S-labeled cRNA probe and placed in a moist chamber at 55 °C overnight. Following hybridization, the coverslips were removed and sections were rinsed twice in 2× SSC for 5 mins each, and then incubated in 25 µg/ml RNase A in a buffered solution (8 mM Tris-HCl, 0.8 mM EDTA, and 0.4 M NaCl, pH 8.0) at 37°C for 1 hr. The sections were washed in 2×, 1×, and 0.5× SSC for 5 mins each, followed by incubation in 0.1× SSC at 65 °C for 1 hr. After rinsing in distilled water, the sections were dehydrated through graded concentrations of ethanol, air-dried and exposed to a BioMax MR film (Kodak, Rochester, NY) for 1–3 days to generate autoradiograms for visualization of the mRNA labeling. Control sections were hybridized with the ³⁵S-labeled cRNA sense probes, which did not generate any specific labeling (Young et al., 2011c).

2.8 Data quantification and analysis

Body weights were analyzed by one-way analysis of variance (ANOVA) for each time point.

For the social affiliation test, time that subject spent either with the stimulus male or alone and frequencies of cage crossings (index of locomotor activity) among treatment groups were analyzed by one-way analysis of variance (ANOVA), followed by the Student Newman-Keuls (SNK) *posthoc* test. For the open-field test, the time in the center of the apparatus and the total number of line crossings were compared among treatment groups by one-way ANOVA to evaluate anxiety-like behavior and locomotor activity, respectively. For the EPM test, the percent of time and the percent of entries in the open arms were analyzed by one-way ANOVA.

The optical densities of TH mRNA and DAT mRNA labeling in the VTA and of D1R and D2R mRNA labeling in the NAcc from the autoradiograms were quantified, using a computerized image program (NIH IMAGE 1.64). Sections on the autoradiograms were

visually inspected and anatomically matched between subjects. For each brain area, the optical density of the mRNA labeling was measured bilaterally from 3 sections per subject and the mean was used for data analysis. Data were analyzed by one-way ANOVA followed by the SNK *posthoc* test.

3. Results

3.1 AMPH treatment alters affiliative behavior

For the social affiliation test, group differences were found in the duration of social contact with the stimulus male ($F_{(2, 33)} = 3.43, p < 0.05$). The *posthoc* test indicates that AMPH treatment at either a low (0.5 mg/kg) or a high (3.0 mg/kg) dose during PND 13–15 significantly decreased social affiliation, compared to the saline-treated controls (Figure 1A). Further, AMPH treatment at both doses significantly increased the time that subjects stayed alone, compared to the control animals ($F_{(2, 33)} = 3.49, p < 0.05$) (Figure 1A). No group differences were found in the frequency of cage crossings (Figure 1B). AMPH treatment did not affect subject's time spent in the central squares and the total number of line crossings in the open-field test (Figure 1C & D) as well as the percentages of open arms duration and entries in the EPM test (Figure 1E & F), indicating that the effect of AMPH treatment on social affiliation is behavior-specific.

In addition, no group differences were found in the body weight throughout the AMPH treatment, at weaning and following the behavioral tests (Table 1).

3.2 AMPH treatment influences DA-ergic marker mRNA expression

Dense clusters of TH mRNA and DAT mRNA labeling were clearly detected in the VTA of the prairie vole brain (Figure 2). In addition, labeling for D1R mRNA and D2R mRNA was found in the vole NAcc and caudate putamen (CP) (Figure 3). AMPH treatment during PND 13–15 had significant, dose-dependent effects on TH mRNA ($F_{(2, 13)} = 13.96, p < 0.001$) and DAT mRNA ($F_{(2, 17)} = 9.46, p < 0.01$) labeling in the VTA in male prairie voles (Figures 2 & 4). The *posthoc* test indicates that treatment with 0.5 mg/kg AMPH significantly elevated the level of TH mRNA labeling in the vole VTA. Treatment with 3.0 mg/kg AMPH also increased the TH mRNA labeling to a level higher than the saline controls but lower than the animals treated with 0.5 mg/kg AMPH. Furthermore, treatment with 3.0 mg/kg AMPH significantly increased the level of DAT mRNA labeling in the VTA of male voles but this effect was not found when a lower dose of AMPH (0.5 mg/kg) was injected. Neonatal AMPH exposure did not alter D1R mRNA or D2R mRNA labeling in the NAcc in male prairie voles (Figures 3 & 4).

4. Discussion

Exposure to AMPH during early development has been shown to influence animal's behavioral and cognitive functions (Vorhees et al., 1994; Broening et al., 2001; Williams et al., 2003; Vorhees et al., 2007; Vorhees et al., 2009). In the present study, we found that neonatal exposure to AMPH had long-lasting effects on social affiliation of male prairie voles during adulthood. Specifically, such AMPH exposure impaired social affiliation and facilitated stay-alone behavior. Importantly, no group differences were found in locomotor

activities and anxiety-like behaviors in any of the behavioral tests conducted, suggesting that the AMPH effects on social affiliation was behavior-specific and was unlikely due to alterations in locomotion, anxiety, and/or novelty seeking.

This long-lasting effect on social deficits induced by neonatal AMPH exposure in male voles is in agreement with studies in the literature on the effects of early development exposure to other psychostimulants such as cocaine. In rats, for example, prenatal cocaine exposure was associated with less social interaction during infancy and adulthood (Wood et al., 1994; Wood et al., 1995; Overstreet et al., 2000). Rats prenatally exposed to cocaine exhibited less social play as juveniles (Wood et al., 1994; Wood et al., 1995) and interacted less with conspecifics when tested as adults (Overstreet et al., 2000). It should be noted that in adult prairie voles, repeated administration of these same doses of AMPH to males and females disrupted their social bonding induced by mating (Liu et al., 2010; Young et al., 2014). Therefore, exposure to AMPH either during early development or in adulthood can lead to the impairment of social affiliation and bonding behaviors in prairie voles. AMPH abuse during early development or in adulthood has also been known to have profound consequences on other types of social behaviors including maternal, sexual and aggression in a variety of species (Young et al., 2011b).

In our study, neonatal exposure to AMPH increased TH mRNA expression in the VTA, indicating an increased DA synthesis, and possibly an increased DA release in projection areas, such as the NAcc, in male prairie voles. It is possible that such AMPH exposure during early development induced an increase in DA synthesis and release, and subsequently, through the regulation of gene expression by epigenetic mechanisms, such increased DA activity remained in adulthood. In fact, it has been demonstrated that repeated exposure to AMPH induces histone modifications and DNA methylation within the brain reward circuitry in adult rodents and that these epigenetic modifications may regulate gene expression and contribute to the maintenance of AMPH addiction (Godino et al., 2015). In agreement with our data, Bubenikova-Valesova et al. (2009) demonstrated that prenatal exposure to methamphetamine increased the basal levels of DA and its metabolites in the NAcc of rats. However, it has also been shown that in other rodent species neonatal AMPH exposure induced a permanent decrease in DA-ergic markers including DA content, fibers, receptors, and TH levels (Crawford et al., 2003; Mukda et al., 2009). Therefore, it is also possible that the increase in DA synthesis and release in adult voles may serve as a compensatory mechanism in response to social and environmental stimuli. In line with this notion, prenatally methamphetamine-exposed rats showed higher responses in the mesolimbic DA system to the methamphetamine challenge when compared to prenatally saline-treated rats (Bubenikova-Valesova et al., 2009). Finally, neonatal exposure to AMPH may induce a sustained increase in circulating CORT levels (Williams et al., 2000) which, in turn, can up-regulate DAT expression (Lucas et al., 2007). An increase in DAT mRNA under a high dose of AMPH may play a role in the prevention of stimuli habituation or over-excitation due to a prolonged increase in DA synthesis and release. Such a positive correlation between TH and DAT expression has also been shown previously in mice, indicating a highly controlled mechanism of DA synthesis and inactivation (Filipenko et al., 2001).

One could argue that the observed effects on behavior and DA-ergic markers were not specific to neonatal AMPH exposure, but to temporary malnutrition and delayed body development caused by repeated treatment with this psychostimulant. Indeed, some authors have reported temporary decrease in body weights of rats during neonatal AMPH exposure (Williams et al., 2003; Vorhees et al., 2007; Grace et al., 2012). However, our data in prairie voles revealed that there were no significant differences among treatment groups in the body weights during neonatal AMPH treatment, at weaning or following the behavioral tests, suggesting that the AMPH effects on social affiliation and DA-ergic markers mRNA expression in the VTA were probably not due to secondary effects of AMPH on delayed and interfered body development. Another concern refers to the fact that the molecular measurements were performed following the behavioral tests. This opens the possibility that the increased DA-ergic activity observed in the VTA may be the result of the neonatal AMPH treatment, the alterations in behavior, or both. Further studies are needed to a better understanding of this issue.

Another interesting phenomenon in the present study is that the effects of neonatal exposure to AMPH seem to be presynaptic, as no group differences were found in the density of either D1R or D2R mRNA labeling in the NAcc. These data contrast from the data in adult prairie voles: in addition to the induced DA release within the NAcc (Curtis and Wang, 2007; Young et al., 2014), AMPH treatment altered DA receptor mRNA expression in the NAcc in adult males and females (Young et al., 2007; Liu et al., 2010; Young et al., 2011c), but did not change TH and DAT mRNA expression in the VTA of adult males (Liu et al., 2010). Although the regulating mechanism is still unknown, together, these data may indicate different mechanisms involved in the AMPH-induced cellular activation at different stages during development and in adulthood.

4.1 Conclusions

AMPH exposure has been shown to induce enduring changes in synaptic and protein expression in the brain which, in turn, may underlie behavioral impairment (Robinson and Kolb, 1997; Williams et al., 2004a; Vorhees et al., 2007). Central DA has been implicated in social bonding (Young and Wang, 2004; Young et al., 2011a), AMPH reward (Liu et al., 2010; Young et al., 2011c; Young et al., 2014), and the interaction between the two (Liu et al., 2010; Liu et al., 2011; Young et al., 2014) in prairie voles. As both social- and drug-reward are mediated by the same central DA system, experience with one type of reward may affect the DA system, which, in turn, can alter an animal's response to another type of reward (Panksepp et al., 2002). Indeed, studies have shown that pair bonding prevents AMPH reward, and repeated AMPH treatment disrupts social bonding through mesolimbic DA-mediated mechanisms in adult prairie voles (Liu et al., 2010; Liu et al., 2011; Young et al., 2014). Our data from the present study demonstrate that exposure to AMPH during a critical period in early development has long-lasting effects on the impairment of social behavior associated with changes in mesolimbic DA activity. Therefore, we speculate that the altered DA activity in the vole brain by neonatal AMPH exposure may contribute to the observed impairment in affiliative behavior. Needless to say, such speculation should be examined in future experiments.

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Abbreviations

ACTH	Adrenocorticotropin hormone
AF	affiliation
AMPH	Amphetamine
ANOVA	analysis of variance
CORT	corticosterone
DA	dopamine
CP	caudate putamen
DAT	dopamine transporter
DA-ergic	dopaminergic
D1R	D1 receptor
D2R	D2 receptor
EPM	elevated plus maze
OF	open field
mPFC	medial prefrontal cortex
PND	postnatal day
NAcc	nucleus accumbens
SNK	Student Newman-Keuls
TH	tyrosine hydroxylase
VTA	ventral tegmental area

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- Neonatal amphetamine disrupts social affiliation in adult male prairie voles
- Locomotion and anxiety-like behaviors were not affected by neonatal amphetamine
- VTA dopaminergic markers are increased in amphetamine-exposed voles
- Disrupted social affiliation may be related to increased central dopamine activity

Figure 1.

The effects of neonatal amphetamine (AMPH) exposure on the behavior of male prairie voles. Males were injected with saline containing 0.0, 0.5, or 3.0 mg/kg AMPH once per day for three days during PND 13–15. They then went through the social affiliation (AF), open field (OF) and elevated plus maze (EPM) tests when they reached PND 80–85. (A): During the AF test, saline-injected controls spent more time with a stimulus male than males injected with AMPH at either dose during PND 13–15. Neonatal AMPH exposure at either dose also increased the time animals spent alone during the AF test as compared to the saline controls. No group differences were found in the total number of cage crossings during the AF test (B), in the time spent in the center of the OF (C), in the total number of line crossings in the OF (D), or in the percent of time (E) and in the percent of entries (F) in the open arms of the EPM. Alphabetic letters indicate group differences following a SNK

posthoc test. Groups with different letters differ significantly from each other. Bar = mean $\pm$ SEM.

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Figure 2.

Photomicrographs displaying *in situ* hybridization labeling for tyrosine hydroxylase (TH) mRNA and dopamine transporter (DAT) mRNA in the ventral tegmental area (VTA) in the brain of male prairie voles. In comparison to the saline-injected controls, injections of AMPH at 0.5 mg/kg or 3.0 mg/kg doses for 3 days during PND 13–15 increased the level of TH mRNA labeling in the VTA. Injections of AMPH at a 3.0 mg/kg dose also increased DAT mRNA labeling in the VTA. Scale bar = 500 μ m.

Figure 3.

Photomicrographs displaying *in situ* hybridization labeling for D1 receptor (D1R) and D2 receptor (D2R) mRNA in the nucleus accumbens (NAcc) and caudate putamen (CP) in the brain of male prairie voles. Treatment with AMPH during PND 13–15 did not alter dopamine receptor mRNA expression in the NAcc. Scale bar = 500 μ m.

Figure 4.

The effects of amphetamine (AMPH) exposure during PND 13–15 on the expression of tyrosine hydroxylase (TH), dopamine transporter (DAT), D1 receptor (D1R) and D2 receptor (D2R) mRNAs in the ventral tegmental area (VTA) and nucleus accumbens (NAcc) in the brain of male prairie voles. Neonatal exposure to AMPH at 0.5 mg/kg or 3.0 mg/kg doses significantly increased the level of TH mRNA labeling in the VTA. Such AMPH exposure at a 3.0 mg/kg dose also elevated the level of DAT mRNA labeling in the VTA. No group differences were found in D1R and D2R mRNA labeling in the NAcc. Alphabetic letters indicate group differences following a SNK *posthoc* test. Groups with different letters differ significantly from each other. Bar = mean $\pm$ SEM.

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Table 1

Subjects' body weight (g)

Group	PND13	PND14	PND15	PND21	PND86
Saline-Control	9.20±0.59	9.83±0.63	11.08±0.72	17.41±0.80	44.54±2.57
0.5mg AMPH	8.91±0.29	9.49±0.33	10.31±0.38	17.30±0.42	44.85±1.47
3.0mg AMPH	9.11±0.34	9.73±0.38	10.68±0.41	17.69±0.53	46.22±1.54