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Place preferences associated with pups or cocaine change the expression of D2R, V1aR and OTR in the NAcc and MeA and the levels of plasma AVP, OT, T and E2 in mandarin vole fathers

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ABSTRACT

Drug abuse often has negative impacts on parenting behavior. The dopamine (DA), arginine vasopressin (AVP) and oxytocin (OT) systems are involved in paternal behavior and drug-induced behaviors. Mandarin voles (*Microtus mandarinus*) are socially monogamous rodents with high levels of paternal behavior. The aims of this study were to examine the protein expression levels of the DA 2-type receptor (D2R), AVP receptor 1A(V1aR) and OT receptor (OTR) in the nucleus accumbens (NAcc) and medial amygdala (MeA) as well as the plasma hormone responses after mandarin vole fathers were conditioned with their pups or cocaine. Our experimental models are based on the conditioned place preference (CPP) paradigm. We observed CPP in response to either pup- or cocaine-associated cues in the mandarin vole fathers. Fathers that were conditioned to either pups or cocaine had a lower expression of D2R and V1aR in the NAcc than did controls. Fathers that were conditioned to pups had higher levels of OTR expression in the MeA and higher plasma levels of AVP, OT, estradiol (E2), and lower plasma levels of testosterone (T) than did controls. Fathers that were conditioned to cocaine exhibited lower levels of plasma AVP and T. These results indicate that the reward effects of pup and cocaine are both mediated by D2R, V1aR and OTR in the NAcc and MeA and that there are subtle differences between the pup and cocaine reward mechanisms that are associated with altered plasma AVP, OT, T and E2.

1. Introduction

Drug abuse influences parental behavior in both human and rodents. Cocaine impairs the parenting ability of male and female rats regardless of previous parenting experience (Zimmerberg and Gray, 1992). In humans, fathers that engage in repeated drug-use show reduced responsiveness and care-giving and increased familial neglect (McMahon et al., 2007; Gerra et al., 2009), while cocaine-abusing mothers experience less enthusiasm for and enjoyment from interacting with their infants and are less sensitive to the child's cues and needs (Burns et al., 1991; Barabach et al., 1992). A previous study has suggested that there are more fathers than mothers entering drug abuse treatment (McMahon et al., 2005). However, the studies on drug reward and parents-offspring bonding in rodents primarily focus on dams (Mattson et al., 2001; Mattson and Morrell, 2005; Seip and Morrell, 2007; Wansaw et al., 2008) with few studies investigating father-offspring bonding and drug reward, partly because paternal care is rare in mammals.

Pups provide natural rewards that have some intrinsic value. In rats, the rewarding value of pup stimuli is dependent upon full interaction between the mother and her pups (Fleming et al., 1994; Mattson et al., 2001), and pups could become reinforcing to a dam throughout the entire postpartum period (Mattson et al., 2001). Males of some monogamous rodent species engage in pre-weaning parental care and form closer social bonds with their offspring compared to polygamous males (Young et al., 1998; Campbell et al., 2009). Furthermore, pre-weaning pups can elicit rewarding effects on monogamous mandarin vole fathers (*Microtus mandarinus*) (Wang et al., 2012a). A growing body of literature suggests that dopamine (DA) projections from the ventral midbrain to nucleus accumbens (NAcc) and prefrontal cortex and connections with the amygdala play key roles in drug reward and conditioning (Wise, 2002; See et al., 2003; Koob and Kreek, 2007). The NAcc is a key component of the mesolimbic DA pathway and is thought to be involved in the reinforcing or rewarding effects of natural stimuli and drugs of abuse (McBride et al., 1999). The medial amygdala (MeA) is a

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steroid-concentrating region that receives dopaminergic transmission from the ventral tegmental area (VTA) (Holder et al., 2010) and is involved in the effect of drug reward (Covington and Miczek, 2005). Additionally, the MeA plays an important role in the regulation of parental behaviors in several rodent species (Kirkpatrick et al., 1994a,b; Numan and Insel, 2003). For example, lesions of the MeA decreased paternal behavior in prairie voles (*Microtus ochrogaster*) (Kirkpatrick et al., 1994a). One ongoing assumption is that the use of drugs activates neural pathways underlying natural rewards (Nestler, 2002; Pitchers et al., 2014). Pups and cocaine activate a common neural substrate (Ferris et al., 2005). However, whether differences in pup and cocaine reward mechanisms exist in the NAcc and MeA and how they might be displayed are unknown.

DA in the mesolimbic brain regions is implicated in the mediation of natural and drug rewards (Spanagel and Weiss, 1999; Wise, 2006, 2008), including parent-offspring bonding (Champagne et al., 2004; Numan et al., 2005). For example, a childhood experience of neglect and poor parent-child attachment may partially contribute to complex neurobiological abnormalities including DA system dysfunctions, which play a crucial role in susceptibility to addictive and affective disorders (Gerra et al., 2009). The effects of DA in the central nervous system are mediated through activation of DA receptors (DARs), and DARs are involved in mediating paternal behavior (Lonstein, 2002; Wang et al., 2015). The neuropeptides, arginine vasopressin (AVP) and oxytocin (OT), can facilitate close social attachment to enhance the reward value by co-activating the dopaminergic circuits that are involved in motivation and reward, which leads to social-bond formation (Liu and Wang, 2003; Delgado, 2007; Skuse and Gallagher, 2009; Wang et al., 2017). Both AVP and OT have been shown to influence paternal behavior (Insel and Shapiro, 1992; Wang et al., 1994; Wang et al., 2000; Parker et al., 2001; Bales et al., 2004). In the socially monogamous prairie vole, male affiliation and parental care are influenced by AVP, and parental behavior such as licking and grooming of pups is modulated by AVP receptor 1A (V1aR) (Wang et al., 1994; Ophir et al., 2008). OT and its receptor (OTR) are found in key areas within the mesocorticolimbic system, and stimulation of OT/OTR in the mesocorticolimbic system has been implicated in mediating paternal behavior (Carter, 1998; Bales et al., 2004; Wang et al., 2015). Blocking the activity of both V1aR and OTR by icv injection of antagonists affects paternal behavior in a dose-dependent manner (Bales et al., 2004). Interestingly, DARs, AVP, V1aR, OT and OTR are also involved in drug-relevant behaviors as well (Johns et al., 2004; Rodríguez-Borrero et al., 2010; Sarnyai, 2011; Wood et al., 2015).

The androgen and estrogen modulate levels of AVP and OT and their receptors (although there are species specific differences) and thereby influence sexually dimorphic social behaviors (De Vries and Simerly, 2002; Ferris, 2005; Carter, 2007). Furthermore, gonadal hormones are important determinants of drug effects by affecting neuronal activity and brain plasticity (Chin et al., 2002; Segarra et al., 2010). Plasma testosterone (T) and estradiol (E2) have also been implicated in the regulation of paternal behavior (Berg and Wynne-Edwards, 2001; Nunes et al., 2001; Trainor and Marler, 2002; Trainor et al., 2003) and cocaine abuse (Long et al., 1994; Martinez-Sanchis et al., 2002; Hu and Becker, 2003).

The mandarin vole is a monogamous rodent species with high levels of paternal care (Tai et al., 2001; Tai and Wang, 2001; Smorkatcheva, 2003), and they display reinforcing effects in response to cocaine (Wang et al., 2012a, 2012b). Thus, this species provides an ideal model to investigate the neural mechanisms underlying pup and drug reward processes in paternal males. Based on the associations between AVP, OT and DA reward systems in the formation of paternal behavior and drug abuse, we predict that there are subtle differences in the DARs-, V1aR- and OTR-mediated mechanisms in the reinforcing nature of pups and cocaine. To test our predictions, we sought to establish the mandarin vole as a model for investigating the differences in the protein expression levels of DA 2-type receptor (D2R), V1aR and OTR in the NAcc and MeA in conditioned responses to pup and cocaine. We also assayed plasma AVP, OT, T and E2 levels to further understand the role of these substances in response to the reinforcing effects of pups and cocaine.

2. Materials and methods

2.1. Animals

Mandarin voles were laboratory-reared F 3 generation animals derived from wild populations in Henan, China. The animals were housed in plastic cages (l × w × h, 44 cm × 22 cm × 16 cm) in a temperature and humidity-controlled environment under a 14:10 h light/dark cycle (lights on 20:00 h). Each cage contained bedding of cotton and wood shavings, and the animals were allowed free access to food (carrots and rabbit chow) and water. After weaning at the age of 21 days, pups were separated from their parents and housed with the same sex peers. At 70–80 days of age, virgin female and male mandarin voles were paired, and all of the females were checked daily from day 20 after pairing for signs of labor. The day of birth was considered postnatal day (PND) zero (day of birth = day 0).

2.2. Conditioned place preference (CPP) test

The animals were conditioned in a three-chamber apparatus consisting of two larger chambers (l × w × h, 34 cm × 25 cm × 32 cm) separated by a smaller middle chambers (l × w × h, 11 cm × 25 cm × 32 cm). The two larger conditioning chambers contained different visual cues: one chamber had gray walls (gray chamber), and the other chamber had white and black striped walls (striped chamber). The middle chamber served as the acclimation chamber and had gray walls with two 7 × 9 cm doors at the center of the base.

2.2.1. Pretest

The purpose of the pretest was to determine whether the voles had an inherent preference for one of the two conditioning chambers prior to conditioning. The mandarin vole fathers ($n = 18$) were individually placed in the acclimation chamber with the guillotine doors removed. The animals were allowed to freely explore the entire apparatus for a 5-min acclimation period, then a 15-min pretest. The amount of time spent in each chamber was recorded by a digital video camera and was later scored by a blind rater using Noldus Observe 9.0 (Noldus Information Technology, Wageningen, Netherlands). The apparatus was cleaned with 70% ethanol and allowed to dry between the tests. We determined that the voles spent more time in the striped chamber than in the gray chamber.

A CPP is defined by a change in the duration of time spent in the drug-paired chamber before and after conditioning (Bardo et al., 1995; Aragona et al., 2007). Since the pilot tests suggested that the vole fathers preferred the striped chamber, we aimed to reduce this preference by using the non-preferred chamber (gray chamber) as the cocaine or pups paired environment in the subsequent conditioning and posttest.

2.2.2. Conditioning with cocaine and posttest

Cocaine hydrochloride (Northwest Pharmaceutical, Sinopharm, China) was dissolved in sterile 0.9% buffered saline. The mandarin vole fathers (n = 6) were conditioned during postpartum days 5–8 using both cocaine and saline that were administered on the same day for four consecutive days. In the morning, the voles were injected with saline and immediately placed in the striped chamber