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Prenatal cocaine exposure alters progenitor cell markers in the subventricular zone of the adult rat brain

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ABSTRACT

Long-term consequences of early developmental exposure to drugs of abuse may have deleterious effects on the proliferative plasticity of the brain. The purpose of this study was to examine the long-term effects of prenatal exposure to cocaine, using the IV route of administration and doses that mimic the peak arterial levels of cocaine use in humans, on the proliferative cell types of the subventricular zones (SVZ) in the adult (180 days-old) rat brain. Employing immunocytochemistry, the expression of GFAP$^+$ (type B cells) and nestin$^+$(GFAP$^-$) (type C and A cells) staining was quantified in the subcallosal area of the SVZ. GFAP$^+$ expression was significantly different between the prenatal cocaine treated group and the vehicle (saline) control group. The prenatal cocaine treated group possessed significantly lower GFAP$^+$ expression relative to the vehicle control group, suggesting that prenatal cocaine exposure significantly reduced the expression of type B neural stem cells of the SVZ. In addition, there was a significant sex difference in nestin$^+$ expression with females showing approximately 8–13% higher nestin$^+$ expression compared to the males. More importantly, a significant prenatal treatment condition (prenatal cocaine, control) by sex interaction in nestin$^+$ expression was confirmed, indicating different effects of cocaine based on sex of the animal. Specifically, prenatal cocaine exposure eliminated the basal difference between the sexes. Collectively, the present findings suggest that prenatal exposure to cocaine, when delivered via a protocol designed to capture prominent features of recreational usage, can selectively alter the major proliferative cell types in the subcallosal area of the SVZ in an adult rat brain, and does so differently for males and females.

1. Introduction

A high rate of drug use in the US is consistently found among our youth; for 8th, 10th, and 12th grade students, lifetime usage figures of any illicit drug in the 12 months preceding the survey were 19%, 36%, and 47% (Johnston et al., 2010). In other words, just under half of American recent secondary school students have tried an illicit drug by the time they near high school graduation. In 2009, an estimated 21.8 million Americans aged 12 or older were current illicit drug users, meaning they had used an illicit drug during the month prior to the survey interview (SAMHSA, 2010). This estimate represents 8.7% of the population aged 12 years-old or older. The rate of current illicit drug use among persons aged 12 or older in 2007 (8.7%) was higher than the rate in 2008 (8.0%). The population of young female drug users of childbearing age is of particular note. Among pregnant women aged 15–44 years, 4.5% used illicit drugs in the past month (SAMHSA, 2010).

Additional troublesome data on the specific abuse of cocaine in the US is also available. Today, about one in seven young adults (14% in 2009) have tried cocaine, and 6% have tried it by their senior year of high school (i.e., by age 17 or 18). More than 1 in every 42 twelfth graders (2.4%) has tried crack. In the young adult sample, 1 in 28 (3.6%) has tried crack by age 29–30 (Johnston et al., 2010). Presently, there are 1.6 million current cocaine users aged 12 or older, comprising 0.7% of the population. These estimates were similar to the number and rate in 2008 (1.9 million or 0.7%). Clearly, cocaine/crack use among adult and young women of childbearing age remains a significant societal concern, placing future generations at risk. Furthermore, the recent review of countries around the world has suggested high lifetime prevalence use of cocaine in Argentina (7.9%), Italy (6.6%), United Kingdom (6.5%), Chile (5.9%), and Ireland 5.3% (Degenhardt et al., 2011); the implication of cocaine abuse for future generations appears to be far more than a US health issue. Thus, comprehending the effects of prenatal drug exposure, such as cocaine/crack, on the development

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of the fetal brain remains extremely important; understanding the long-term effects of prenatal cocaine exposure that are detectable in adulthood is even more essential.

Knowledge of the mechanisms through which cocaine may cause adverse effects on the developing brain has been, and remains, an important quest. It is of interest that measures of head circumference have been reported as correlated with behavioral and psychological impairments associated with *in utero* cocaine exposure (Chiriboga et al., 2009; Bateman and Chiriboga, 2000; Singer et al., 2008; Eyler et al., 2009). However, even when prenatal cocaine effects on physical growth including head circumference are reported, they may not persist beyond infancy (Lumeng et al., 2007). Multiple comprehensive reviews covering several decades of research have failed to definitively identify any unique pathogenic consequence(s) of prenatal cocaine. For example, among children aged 6 years or younger, there is no convincing evidence that prenatal cocaine exposure is associated with developmental toxic effects that are different in severity, scope, or kind from the sequelae of multiple other risk factors (Frank et al., 2001). A similar recent reaffirmation noted that studies through 6 years have shown no long-term direct effects of prenatal cocaine exposure on children's physical growth, developmental test scores, or language outcomes (Ackerman et al., 2010). Many findings once thought to reflect specific effects of *in utero* cocaine exposure are correlated with other factors, including prenatal exposure to tobacco, marijuana, or alcohol, and/or a host of social/environmental factors, such as poverty, caregiver education, placement stability, and quality of child–caregiver relationships that are known to affect a child's development.

Most recent data employing state-of-the-art neuroimaging techniques do suggest subtle differences between prenatal cocaine-exposed and nonexposed children on structural measures of the brain (Roussotte et al., 2010). For example, volumetric MRI suggested reductions in cortical gray matter and total parenchymal volume (and smaller head circumference); however, the decreases were no longer statistically significant after controlling for polydrug exposure to alcohol, cigarettes, and marijuana (Rivkin et al., 2008). In an earlier symposium report, which suggested gray matter reductions in occipital and parietal lobes, the volume decreases remained significant after controlling for polydrug exposure, but the reductions were not dose-dependent (Singer et al., 2006).

Preclinical studies with various strains of animals have shown prenatal cocaine exposure may cause abnormalities in the developing cerebral cortex. In sub-human primates, administration of cocaine at the time of neocortical neurogenesis (the second trimester) reduced neocortex volume, disturbed lamination, altered positioning of cerebral cortical neurons, and reduced density and number of cortical neurons (Lidow, 1995; Lidow and Song, 2001). In the mouse, cocaine administered during corticogenesis disrupted horizontal lamination, impaired establishment of vertical columns, and markedly decreased the number of radially organized axonal-dendritic bundles (Gressens et al., 1992). Despite these demonstrations of the capability of prenatal cocaine to disturb corticogenesis, it is important to note that both of those models used very high drug doses; i.e., altered corticogenesis in the sub-human primate was found after maternal doses of cocaine sufficient to reduce the entire brain volume of the offspring by >20% (Lidow, 1995). Similarly, cocaine-induced disturbances of corticogenesis in the developing mouse brain are reported under conditions in which the entire brain size is reduced by about 25% (Gressens et al., 1992). The translation of such findings to the clinical situation is challenging; what can we infer from such findings for the recreational usage of cocaine?

Several studies using a preclinical intravenous injection model, to more closely mimic the route and pharmacokinetics of the smoking of crack cocaine, have nevertheless discovered a more subtle disruption of cortical development (disruption of the organization of the anterior cingulate cortex in 20–25 day-old rabbits, Stanwood et al., 2001; irregularities in the formation of cortical minicolumns in weanling rats, Buxhoeveden et al., 2006). An inhibition of progenitor cell proliferation by acute cocaine treatment, as shown in cell culture and rat fetuses, affords one potential mechanism for such cortical architectural disruptions (Lee et al., 2008). Given the unambiguous demonstration that neurogenesis continues throughout life into adulthood (McKay, 1997; Temple and Alvarez-Buylla, 1999; Weiss et al., 1996), it is important to establish whether adult brain plasticity can be altered by prenatal cocaine exposure.

The subventricular zone (SVZ) harbors the largest population of proliferating cells in the adult brain (Altman, 1969; Lois and Alvarez-Buylla, 1994; including in humans, Sanai et al., 2004; Curtis et al., 2007); the focus of the present study was specifically on an examination of that brain region. The prevailing view (Petreanu and Alvarez-Buylla, 2002; Nissant and Pallotto, 2011) holds that neural stem cells (NSCs) of the SVZ are quiescent GFAP$^+$ cells that share properties of astrocytes (referred to as type B cells). Type B cells give rise to transient amplifying type C cells that are GFAP$^-$. The intermediate progenitor cells give rise to neuroblasts (referred to as type A cells) that migrate a significant distance via the rostral migratory stream (RMS) to the olfactory bulb where they differentiate and durably integrate (~50% of these nascent cells) into the existing neural circuitry. Thus, the most important function of NSCs is to generate neurons (but see elaboration of their functions, Zhao et al., 2008); however, adult NSCs of the SVZ are also involved in gliogenesis generating oligodendrocytes (Jackson et al., 2006).

Thus, prenatal cocaine exposure was hypothesized to have long-term consequences for proliferative plasticity in the adult brain. Using immunocytochemistry, we determined whether or not prenatal cocaine treatment altered glial fibrillary acidic protein (GFAP$^+$) (type B cells) and nestin$^+$/GFAP$^-$ (type A and type C cells) expression in the offspring at 6 months of age. Potential sex differences were also examined.

2. Materials and methods

2.1. Animals

Nulliparous female Long-Evans rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) at approximately 10–12 weeks of age (225–249 g), placed into quarantine for one week, and subsequently moved to the animal vivarium. The animals were maintained according to NIH guidelines in AAALAC accredited facilities. Food (Pro-Lab Rat, Mouse, Hamster Chow No. 3000) and water were available *ad libitum*. The animal facility was maintained at 21 ± 2 °C, 50 ± 10% relative humidity and had a 12 h light:12 h dark cycle with lights on at 07:00 h (EST). The protocols for the use of rats in this research were approved by the IACUC of the University of South Carolina.

2.2. Chemicals

Cocaine HCl (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile isotonic saline based on the weight of the salt. Immediately prior to use, the drug solution was prepared in a volume of 1 ml/kg.

2.3. Experimental design

Half of the adult female animals were randomly selected and surgically implanted with vascular catheters (as described below); the remaining half served as surrogate dams and received neither drug treatment nor the daily handling associated with drug. On GD8, pregnant catheterized females were randomly assigned to either a cocaine (3.0 mg/kg) or saline vehicle group and treated on GD8-21, as described below. At six months of age, one male and one female from each litter were sacrificed for use in the present study.

2.4. Surgery

Catheterization was performed as previously described (Mactutus et al., 1994). Briefly, the animals were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg/ml) and xylazine (3.3 mg/kg/ml) and a sterile Intracath IV catheter