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Prenatal exposure to psychostimulants increases impulsivity, compulsivity, and motivation for rewards in adult mice

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HIGHLIGHTS

- Prenatal psychostimulant exposure alters executive functions in adult mice.
- 5CSRTT analysis was used to assess adult alteration in executive functions.
- Prenatal methamphetamine increases impulsivity, compulsivity, and motivation.
- Prenatal methylphenidate increases impulsivity, compulsivity, and motivation.
- Prenatal psychostimulant exposures produce sex-specific effects in adult mice.

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ABSTRACT

Given the widespread use and misuse of methamphetamine (METH) and methylphenidate (MPD), especially in relation to women of childbearing age, it is important to consider the long-lasting effects of these drugs on the brain of the developing fetus. Male and female C57Bl/6J mice were prenatally exposed to METH (5 mg/kg), MPD (10 mg/kg), or saline. Following a 3-month washout, behavioral analysis using the 5-Choice Serial Reaction Time Task (5CSRTT) was performed on adult mice. After reaching training criteria, performance on a pseudo-random intertrial interval test session revealed decrements in 5CSRTT behavior. Prenatally-treated METH and MPD mice demonstrated significant increases in impulsivity, compulsivity, and motivation for reward compared to their saline controls. There were sex by drug interactions indicating a possible sexually dimorphic response to these prenatal drug exposures. Of particular clinical interest, we find that mice prenatally exposed to METH or MPD express characteristics of both inhibitory control decrements and heightened motivation for rewards, which represent core symptoms of addiction and other impulse control disorders.

1. Introduction

Methamphetamine (METH) and Methylphenidate (MPD) are commonly used and abused psychostimulant drugs. METH is a potent psychostimulant and indirect agonist of monoamines, which works through multiple presynaptic and synaptic mechanisms of action. The resultant increases in dopamine (DA), norepinephrine (NE), and serotonin contribute to its reinforcing properties as well as other central and peripheral effects [1]. Methylphenidate (marketed as Ritalin™ and Concerta™) is a psychostimulant drug commonly prescribed for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) and less commonly prescribed for the treatment of narcolepsy, depression, and other psychiatric conditions. Although MPD also blocks reuptake at the NE transporter, its primary target is the DA transporter (DAT), where it has a similar, yet more potent, effect as cocaine [2].

Prenatal psychostimulant exposure is a major societal issue in the US. In 2010, there were an estimated 2.2 million people who reported previous illicit stimulant use (1.3 million of which were METH users) and 7 million people who used prescription-type psychotherapeutic drugs non-medically in the past month. Many of these cases involved women of childbearing age. An estimated 4.4% of all pregnant women 15–44 years of age reported current illicit drug use with much higher rates for the younger age groups (i.e., 16.2% for 15–17 year olds and 7.4% for 18–25 year olds) [3]. In 2009, 6.7% of people seeking treatment for METH abuse were pregnant [4] and estimates place the prevalence rate of prenatal METH exposures to 5% in certain parts of

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the US [5]. While the data on MPD use and abuse is less clear, there are recent reports of large increases in MPD misuse, diversion, and abuse in people of childbearing age, which coincide with clinical trends in ADHD overdiagnosis and misdiagnosis [6–9].

Given the apparent widespread use and misuse of these Schedule II, Class C compounds, especially in relation to women of childbearing age, it is important to consider the teratological effects of METH and MPD on the brain of the developing fetus. DA, NE, and their rate limiting enzymes are detected during early mouse gestation (E9.5, E10.5, and E8.5, respectively) and peak neonatally [10,11]. A large body of literature supports the role of these neurotransmitter systems in key neurodevelopmental processes, including neuronal and glial proliferation, differentiation, migration, survival, and connectivity. Prenatal exposures to drugs that target monoaminergic systems produce a complicated array of neurobehavioral phenotypes likely stemming from the disruption of the tropic and trophic functions they serve [1,12]. Since there are also sex differences in the time course of development and connectivity of monoaminergic systems, which appear to be regulated by sex hormones [13,14], it is possible that male and female offspring may experience differential effects of these suspected teratogens. Sexual dimorphism is documented using preclinical indicators of neuroanatomical, neurochemical, and neurobehavioral deficits after prenatal exposures to cocaine [15,16] and methamphetamine [17–20]. Taken together, these studies suggest that prenatal psychostimulant exposures are capable of producing long-term, yet, potentially, sexually dimorphic, effects on the developing nervous system.

The clinical literature provides some support for these preclinical conclusions. When tested later in life, children with *in utero* METH exposure demonstrate ADHD-associated behaviors (e.g., impulsivity) as well as other maladaptive executive functions (i.e., attention, working memory, goal setting, flexibility, and emotional control) [21–24]. Similar effects have been noted in children exposed prenatally to cocaine [25]. While many of these reports use well-established clinical methodologies, there are a number of potential confounds. For example, some of the noted neurobehavioral deficits reported in these studies can be explained by environmental and maternal effects on neural development and behavior as well as other implicated social risk factors [25]. In addition, the children in many of the clinical cohorts describing neurobehavioral teratogenic effects of METH are still relatively young. Recent reports suggest that at least some developmental deficits arising from prenatal METH exposure resolve over time [26]. Finally, there is a paucity of data on the long-term effects of developmental exposures to MPD, which is both a heavily prescribed and heavily abused psychostimulant with similar mechanisms of action of, yet more potency than, cocaine [2].

There is a need for preclinical studies that are capable of controlling for confounding variables and are sensitive enough to measure subtle neurobehavioral deficits of offspring exposed to these drugs over a protracted period of time. To address these issues, this study employed a murine model of prenatal exposure to METH and MPD and tested executive functions in adulthood using the 5-Choice Serial Reaction Time Task (5CSRTT) paradigm. The 5CSRTT is a well-documented behavioral model that uses conditioned responses to visuospatial cues to elucidate potential alterations in executive functions [27]. Since the cues are delivered in an unpredictable location and time, response errors on the 5CSRTT uncover executive function/attention deficits related to impulsivity, compulsivity, motivation, and sensory and motor processing [28,29]. The major findings of this study are that prenatal exposure to METH and MPD in a murine model produce clear deficits in adult executive functions, including alterations in impulsivity, compulsivity, and motivation, that persist three months after the animals were exposed. Furthermore, a few sex by drug interaction effects were uncovered, which suggest a potential difference between long-term alterations in male and female sexes.

2. Materials and methods

2.1. Animals

Pregnant adult (2–3 months of age) C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME) were randomly assigned to a treatment group and given injections of saline, METH, or MPD. Strain differences support the use of the C57Bl/6J in the 5CSRTT paradigm [29], especially given their impulsivity–hyperactivity phenotype [30] and sensitivity to motivational drives [31]. Breeding female mice were checked daily (0800–0900) for the presence of a vaginal plug signifying embryonic day (E)0.5. Daily monitoring of weight gain on E0.5-E8.5 was used to confirm pregnancy. Twenty dams received once daily i.p. injections of 5 mg/kg (+)-methamphetamine hydrochloride (Sigma-Aldrich, St. Louis, MO) in a volume of 0.5 mg/ml sterile saline, 10 mg/kg methylphenidate hydrochloride (Sigma-Aldrich, St. Louis, MO) in a volume of 1.0 mg/ml sterile saline, or an equal volume of sterile saline from E8.5 until parturition. The drug doses and timing were chosen based on existing pharmacological data [2,32–35] and because E8.5 to birth constitutes the major window of DAergic and NEergic development in the murine brain [10–12]. Dams from different treatment conditions were housed together ($n = 3–5$ per cage). On E21, the dams were singly housed into maternity cages. Prenatal MPD or METH exposures did not alter gestational length, sex composition, litter size, or fetal mortality. Eighty-three resultant pups were raised in a drug-free environment and were weaned on postnatal day 21. Mice from each treatment, by sex, were group housed ($n = 4–6$) by random assignment into standard caging (Micro-Vent Caging System, Allentown Inc., Allentown, NJ) with free access to food and water (except during training) and 12-hour light:12-hour dark cycle with lights on at 0600 in a temperature (20–22 °C) and humidity (55–60%) controlled colony room. All procedures were conducted according to the *Guide for the Care and Use of Laboratory Animals*. Training on the 5CSRTT started when the mice reached 3–4 months of age (Fig. 1). Of the 83 animals used in the study, 37 were exposed prenatally to METH (female, $n = 22$; male, $n = 15$), 27 were exposed prenatally to MPD (female, $n = 15$; male, $n = 12$), and 19 were exposed prenatally to saline (female, $n = 9$; male, $n = 10$). Each animal was trained only once per day on a five-day per week schedule, with water availability restricted to two hours immediately following training [36].

2.2. Behavioral apparatus

A 22″ × 15″ × 16″ operant chamber with a grid floor and a five-hole curved wall on one side of the chamber was used (Med Associates, Inc., St. Albans, VT). The opposite wall of the chamber contained a liquid dipper that delivered a 10% condensed milk reinforcer. The dipper receptacle and each of the five response holes were equipped with a cue light and infrared sensor. A house illumination light was mounted in the center of the chamber, which was used as positive punishment during training sessions. The operant chamber was kept in a dark, ventilated, and sound-attenuating box. Control of the operant chamber and data collection were performed using the MED-PC IV software (Med Associates, Inc., St. Albans, VT).

2.3. 5CSRTT habituation and training procedure

All habituation, 5CSRTT training, and pseudo-random test sessions were performed according to protocols detailed elsewhere [36,37], but a brief account follows. Habituation to the operant chamber and 10% condensed milk reinforcer, acclimation to being handled, and water restrictions were performed over a two-week period. During the initial training phases, the nose-poke holes were blocked to prevent superstitious learning. Magazine training ensued whereby pairings between the magazine light and the delivery of a reinforcer were learned. This was followed by training sessions that paired a psuedo-randomly delivered