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Research report

Prenatal oxycodone exposure impairs spatial learning and/or memory in rats

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ABSTRACT

Recent changes in demographic patterns of drug use have resulted in the increased non-medical use of prescription opiates. These users are younger and more likely to be female, which has the potential for increasing rates of *in utero* exposure. Therefore, we developed a rat model that simulates a prescription opiate-dependent woman who becomes pregnant. Adult female Sprague–Dawley rats were treated for 30 days via oral gavage with ascending doses of oxycodone HCl up to a final dose of 15 mg/kg/day, which was maintained during breeding and gestation. Controls were treated with water. The adult male offspring of these treated dams were tested on the radial arm maze, the Morris water maze (with a short and a long intertrial interval), and a spatial T-maze. Prenatal oxycodone exposure led to a deficit in the radial arm maze characterized by a greater number of reference memory errors, especially in the beginning of testing. In contrast, in the T-maze, prenatal oxycodone-exposed rats learned the task as well as well as the prenatal water controls. However, they had a modest deficit in retention of the task when assessed 5 days after acquisition training ended. For the Morris water maze, the intertrial interval affected the pattern of learning. While there was no deficit when the training had a short intertrial interval, when there was a long intertrial interval, prenatal oxycodone-exposed rats had poorer acquisition. The spatial learning deficit was characterized by and increased latency to find and a greater distance traveled to the platform in the prenatal oxycodone-exposed rats. These data were corroborated by analysis of the behavioral search strategy, which showed a decreased use of spatial strategies and an increase in non-spatial strategies, especially wall-hugging, in prenatal oxycodone-exposed rats as compared to prenatal water control rats on day 2 of acquisition. These results indicate that prenatal oxycodone exposure consistently impairs learning and memory in a battery of spatial tasks.

1. Introduction

Oxycodone, a powerful opiate analgesic used for moderate to severe pain, can result in dependence, tolerance, and addiction [9]. While most illicit drug use is decreasing, recreational use of prescription drugs has increased in young adults age 18–25 and prescription drugs are used as often as marijuana as a drug of initiation in youths aged 12–17 [14]. The same report shows that women aged 15–17 who were pregnant had a higher rate of drug use (22.6%) than those who were not pregnant (13.3%). These data together demonstrate that there is now a growing risk for exposure to prescription opiates *in utero*.

There have been surprisingly few comprehensive studies examining the effects of prenatal opiate exposure on cognitive development in humans, and none to date with prescription opiates. Olofsson et al. [23] investigated children born to opiate-dependent mothers (majority were methadone dependent) 1–10 years after birth. Over half of the children displayed maladaptive behaviors including hyperactivity, aggression, and lack of concentration, and over 20% had moderate or severe delays in psycho-motor development. However, the authors noted that many of the children were reared in unstable households and had multiple shifts in their primary caregiver, so it was difficult to assess the degree to which early drug exposure vs. a chaotic environment contributed to the behavioral effects. A decade later Van Baar and de Graaf [34] examined cognitive measures in children exposed to opiates (heroin and methadone, alone or in combination with other illicit drugs), most of whom underwent neonatal withdrawal after birth. In comparison to a non-drug-exposed reference group, non-verbal intelligence deficits were noted in children aged three to four and language and general intelligence deficits were notes in children aged four to six. The researchers attempted to control for background characteristics, but study attrition led to a lower incidence of being reared by both parents, a lower level of education of the mother, and a less stable home environment in the drug-exposed group. And more recently a study of heroin and methadone dependent mothers in a residential treatment facility

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in Switzerland reported lower performance IQs in the offspring at approximately 5 years compared to population norms [32]. Studies looking at poly-drug abuse including opiate exposure have noted similar cognitive deficits (e.g., [24]). As noted above, it has been difficult to control for the effects of environmental influence in these studies, which strengthens the need for controlled animal studies. Thus, to better understand the deleterious effects on cognition later in life that accompany opiate exposure in the womb, we have developed a model of prenatal oxycodone exposure in rats.

Prenatal opiate exposure has been shown previously to have adverse effects on cognition when embryos are exposed *in utero* to classically studied opiates like heroin, morphine, and the methadone analogue, *l*-α-acetylmethadol (LAAM). For example, radial arm maze [31] and Morris water maze [40] deficits were observed after exposure to prenatal heroin. Prenatal morphine increased latency in the radial arm maze [30] and caused a deficit in long-, but not intermediate-term memory in the one-trial passive avoidance task paradigm in the chick [8]. However, no prenatal exposure studies on learning and memory have been performed using prescription opiates.

Further, some of the studies with the above-mentioned opiates have been performed with exposure beginning at some time during gestation: prenatal heroin during embryonic days 9–18 in rodents [31,40], prenatal morphine during embryonic days 11–18 in rats [30], and embryonic days 12–16 in the chick [8]. Late gestation is a time frame critical for hippocampus and cortical development and these studies are important in showing that drug-induced deficits can occur during such exposure [3]. However, they do not model a woman taking an opiate chronically who becomes pregnant. Therefore, we developed a model in which prenatal opiates are administered before and during the entire gestational period. Previous work in our laboratory using this approach with prenatal exposure to LAAM found poor performance in acquisition of the radial arm maze, a spatial memory task [28]. Prenatal LAAM-exposed rats had more reference and working memory errors, but were able to acquire the task after five days of training. In this study, prenatal LAAM resulted in a decrease in the synaptic expression of brain-derived neurotrophic factor (BDNF), which has been implicated in playing a role in synaptic architecture in development [6], in plasticity associated with drug exposure and addiction [5], and in synaptic plasticity and learning and memory in the adult [4,15]. While studies like the ones described above have provided a foundation for the effect of prenatal opiates on learning and memory, these studies have not addressed whether prescription opiates have similar effects. Additionally, few reports have described the effect of the same prenatal opiate on multiple spatial memory tasks.

Many tasks have been used to assess spatial memory. Some of the most common include the Morris water maze, radial arm maze, and T-maze. The advantages of the radial arm maze and T-maze are that rats use natural foraging capabilities, as well as their exploratory instinct [16]. The disadvantages are that the rats must be food-restricted and this could lead to confounding differences in motivation, metabolism, or energy level that may affect the question to be studied. The rats must also learn the procedure of moving through a relatively non-natural maze and turning specific directions to find food, which involves procedural memory. Some of these disadvantages are overcome in the Morris water maze, which has been used extensively to study spatial memory [10,19]. Rats are placed in a large circular tank of water with a platform submerged just beneath the surface of the water. They must use spatial cues within the room to find the location of the platform. Unlike the radial arm maze and T-maze, the rats do not have to be food-restricted and therefore are not metabolically challenged. However, the rats may undergo thermoregulatory stress in the water. The procedural learning component of the task is presumably less, because rats can naturally swim; however, procedural memory is still present. Therefore, multiple spatial memory tasks should ideally be used to determine differences potentially caused by drug treatment. Our hypothesis is that prenatal oxycodone will produce selective deficits in spatial learning and/or memory in three different spatial memory tasks, radial arm maze, T-maze, and the Morris water maze.

2. Materials and methods

2.1. Subjects

The subjects assessed were adult male offspring of dams treated with oxycodone prior to and during pregnancy (treatment and breeding described below). Testing was done in male offspring so that the behavioral parameters were not confounded by fluctuations in hormones from estrous cycling. To prevent littermate effects from confounding data interpretation, only one male per litter was assigned to each of the testing groups. The rats were 4–6 months of age at the time testing was initiated. The experimental subjects were group-housed in standard flat bottom plastic cages containing hardwood bedding. Temperature (22 ± 1 °C), humidity (40–50%), and 12 h light:dark cycle remained constant throughout the experimentation. Food (Teklad, Harlan) and water were available *ad libitum* for all rats except as noted in the radial arm maze and T-maze procedures. The experimental protocol and animal husbandry procedures were approved by the *Institutional Animal Care and Use Committee* and comply with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (publication number 85-23, revised 1985).

2.2. Prenatal drug treatment

The development of the prenatal oxycodone treatment paradigm is described in detail in [28]. Nulliparous female (64–70 days of age) Sprague–Dawley rats (Harlan, Indianapolis, IN) were treated with oxycodone HCl (Mallinckrodt, St. Louis, MO) or the water vehicle. Drug or vehicle was administered via a 7.6 cm 18-gauge oral gavage needle (Popper and Sons, New Hyde Park, New York) in a volume of 1 ml/kg. An ascending dosing procedure was used wherein doses of 10 mg/kg/day oxycodone were orally gavaged for 5 days. The dose was escalated by 0.5 mg/kg/day for 10 days to a final dose of 15 mg/kg/day, which was maintained for 15 days. After 28 days of treatment, the females were harem bred to proven breeder males (3 females: 1 male), with the males rotated daily. Treatment continued through breeding and gestation until parturition. Because the half-life of oxycodone is relatively short and drug distribution can be altered by pregnancy, throughout gestation dams were monitored for signs of opiate withdrawal. The presence of opiate withdrawal can confound data interpretation. Thus, withdrawal signs such as weight loss, diarrhea, and irritability were monitored daily. Pregnant dams were individually housed from gestational day 17 until parturition, when litters were culled to 10 pups. Pups were reared by their biological mothers, weaned at postnatal day 21, and housed with like-treated male subjects. Three separate breedings were conducted to generate sufficient subjects for the behavioral studies described below. The exposed offspring remain undisturbed until testing commenced.

2.3. Food restriction for radial arm maze and T-maze

The rats for the radial arm maze and T-maze studies were food-restricted to 85% of their free-feeding body weight. There was no difference in the initial adult body weights between prenatal water and prenatal oxycodone-exposed rats, nor was their a difference in the amount of time that was needed to reach 85% of the free-feeding weight. Shortly before testing, rats were supplemented by approximately 0.5 g of a Maypo (Parsippany, NJ), a sweetened maple-flavored oatmeal cereal that served as the food reinforcer for the radial arm maze and T-maze tasks. During all phases of the radial arm maze and T-maze training, rats were maintained at 85–90% of their pre-testing body weight.

2.4. Radial arm maze

The radial arm maze was constructed of black painted wood finished with a polyurethane coating. The center platform was 58 cm in diameter with 8 arms (15 cm wide × 80 cm long) extending from the center. A 2.5 cm diameter hole at the end of each arm held a plastic disposable cup that contained the food reinforcer, a small drop of Maypo mash (approximately 0.01–0.025 mg).

Radial arm maze testing was performed in three phases: shaping, acquisition, and retention. Shaping was done with all of the arms baited with the food reinforcer. It was initially done in groups and then individually. During the social shaping, 3 rats were allowed to explore the baited maze simultaneously for 10 min to become acclimated to the apparatus. There were at least 2 days of social shaping. Food rewards eaten were recorded and used as criterion for exclusion. Rats were excluded if they did not eat at least one food reward in the social shaping trials (one prenatal water and two prenatal oxycodone rats failed to meet these criteria). For the individual shaping, each rat was allowed 3 min to explore the maze with all eight arms baited, such that the rats received experience in gaining a food reinforcement by completely traversing an arm.