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Methamphetamine exposure during pregnancy at pharmacological doses produces neurodevelopmental and behavioural effects in rat offspring

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ABSTRACT

In recent years methamphetamine (MA) use has become more prevalent, and of particular concern is its growing popularity of MA among women of childbearing age. However, to date, studies examining MA effects on the developing offspring in laboratory animals are limited. Thus, the aim of this study was to determine if *in utero* MA exposure in rats at pharmacological doses can have a negative impact on neonatal neurodevelopment and behaviour. Pregnant Sprague-Dawley dams ($n = 10$ dams/group) received MA (0, 0.625, 1.25, 2.5 mg/kg) once daily *via* oral gavage from gestational day 7 to 21. Maternal body weight, food and water consumption were recorded daily. A range of standard neurodevelopment parameters was examined in the offspring during the neonatal period. There were no neurodevelopmental deficits observed with offspring exposed to 0.625 mg/kg MA, in fact, there were enhancements of neurodevelopment in some parameters at this low dose. However, exposure to the 1.25 mg/kg MA dose resulted in significant impairments in surface righting reflex and forelimb grip in both sexes. Exposure to the 2.5 mg/kg MA dose resulted in a significant reduction in ano-genital distance in males, and in both sexes resulted in delayed fur appearance and eye opening, impairments in surface righting reflex and negative geotaxis, and a reduction in body length. In conclusion, this study demonstrates that pharmacologically relevant doses of MA can have profound dose-related effects on neonatal outcome. If extrapolated to the clinical scenario this will give cause for concern regarding the risks associated with this drug of abuse at relatively low doses.

1. Introduction

The use of illicit drugs has shown a steady increase in prevalence in young people and this is particularly evident in the last five years (Substance Abuse and Mental Health Services Administration, 2013). Between 2008 and 2012, reports showed that there was an increase in illicit drug use in persons aged between 18 and 25 from 19.7% to 21.3% (Substance Abuse and Mental Health Services Administration, 2013). Substance abuse in women typically occurs during the childbearing age (Anderson and Choonara, 2007) and thus the prevalence of substance abuse during pregnancy is increasing, which in turn means that the amount of drug-exposed babies is increasing. There are many potential health risks and adverse effects associated with substance abuse during pregnancy for not only the foetus but for the mother as well (Schempf and Strobino, 2008; Good et al., 2010).

The amphetamine-like stimulants (ALS) include amphetamine (AMP), methamphetamine (MA) and 3,4-methylenedioxymethamphetamine (MDMA) and these are appealing to pregnant females due to their low cost, ease of use and availability. Early human studies found that prenatal MA-exposed infants exhibited poor alertness, poor feeding and lethargy (Dixon, 1989). Adverse effects previously reported include low birth weights (Smith et al., 2006) and a higher incidence of cleft lip, ambiguous genitalia and anencephaly (Good et al., 2010). Similar studies also showed altered neurocognitive performance in that MA-exposed children had lower verbal memory, long-term spatial memory, sustained attention and visual motor integration (Chang et al., 2004). Although each of these studies looks at prenatal MA exposure, the patterns of use of MA and doses of MA used can differ greatly between pregnant females. In 2010, a study from the US in areas with high reports of MA use showed that in the first trimester 14.7% of females in this study took MA 1–2 times during pregnancy whereas 23.6% of the females took MA almost every day. Again this pattern of use

Abbreviation: MA, methamphetamine.

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changed as females progressed through the pregnancy and moved into the second and third trimester with 29.3% of females staying on a consistently high frequency of use and 35.6% of females actually decreasing their frequency of use of MA during this period (Della Grotta et al., 2010).

The use of animal models has been essential in helping to understand the neurodevelopmental and behavioural consequences of drug exposure during pregnancy and breastfeeding (Thompson et al., 2009). An advantage of using an animal model is being able to explore any consequences of this drug exposure while eliminating the aforementioned limitations that are common in clinical studies. Many early animal studies aimed to elucidate the short and long-term effects of prenatal MA exposure. Adverse effects that have been reported with prenatal MA include cleft palate (Yamamoto et al., 1992), retinal eye defects (Acuff-Smith et al., 1992) and delayed physical growth and motor development (Cho et al., 1991). Behavioural consequences have been seen in early adulthood of rat offspring prenatally exposed to MA including a higher pain score in the formalin test suggesting a long lasting hypersensitivity to pain (Chen et al., 2010).

However to date, the current literature with regards to prenatal MA exposure has failed to reflect an accurate clinical experience in aspects such as route of administration, dose of MA and time and duration of MA exposure. For example, the most common route of administration in these animal studies is subcutaneous (s.c.) injection with 88% of the existing studies using this route (Vorhees et al., 2009; Grace et al., 2010; Slamberova et al., 2011). Intraperitoneal (i.p.) injection has also been used in some studies (Inoue et al., 2004; Wong et al., 2008; Siegel et al., 2010) and this shows the same advantages and disadvantages as the s.c. route although the drug is absorbed much quicker. In contrast in humans, MA is most commonly taken orally where the drug is ingested in powder or tablet form, or by inhalation where the drug is smoked (US Department of Health and Human Services, 2006). In preclinical studies, in order to accomplish an oral route of administration, MA has previously been administered *via* the drinking water (Tonge, 1973). However, as animals are sensitive to potent smells, they may reject the drug in this form.

Among the different dosing regimens, throughout the entire gestation is the most commonly used duration of exposure (Bubenikova-Valesova et al., 2009; Schutova et al., 2009; Yamamotova et al., 2011). This dosing regimen relates to the clinical situation where the mother is abusing MA everyday throughout pregnancy. This is consistent with a study by Della Grotta et al. (2010) that showed 55% of pregnant MA users did not change their pattern of use over their pregnancy. It is unknown if the women that decreased their use during pregnancy were able to maintain this or relapsed and returned to their original patterns of use. For this reason, many studies have looked at the postnatal period as a potential time of exposure for the offspring. Unfortunately, some previous studies have administered MA directly to the offspring during the postnatal period (Gomes-Da-Silva et al., 2004; Williams et al., 2004; Schaefer et al., 2008). These studies are trying to target a certain point of development in the offspring, which correlates to the third trimester of pregnancy in humans. However, in the clinical situation we know that the offspring are exposed indirectly to the drug through breast milk and not by direct exposure *via* injection and so, the same should be performed in preclinical studies.

Methamphetamine abusers generally use a dose starting at 20 mg with a common MA dose being 30 mg (Golub et al., 2005). When extrapolating this dose back to a preclinical model, the use of an allometric scale (Reagan-Shaw et al., 2008) takes into account the body weight and the body surface area of the human and the animal. Therefore, the MA dose to use in a preclinical model (rat) would be 2.5 mg/kg. In order to accurately model the clinical scenario, then doses within this range would be most appropriate and this should be administered directly to the mother *via* oral gavage during the gestation period.

The aim of this study was to use a clinically relevant animal model of MA abuse during pregnancy to elucidate the consequences of exposure at a variety of doses. The hypothesis is that prenatal MA exposure at pharmacological doses can have a negative influence on the neurodevelopment of the rat offspring.

2. Materials and methods

2.1. Animal housing

Adult male (275–300 g) and female (250–275 g, approx. 3.5 months old) Sprague-Dawley rats were used for this study. All females were bred in house and housed in groups of three from the beginning of the study. All males came from Charles River (Kent, UK) and from the day of arrival, males were housed singly. All animals were housed in plastic bottom cages with appropriate bedding material and were handled daily. All female rats were housed singly after mating occurred and additional nesting materials were also given. All animals were maintained under standard laboratory conditions under artificial 12-h light/dark cycle (lights on from 08:00 h) and temperature was maintained at 21–24 °C with relative humidity at 35–60%. Animals had free access to food and water throughout (food and water consumption were measured once daily at 14.00 h). Following littering, the rat pups were all housed with their biological mothers until post-natal day (PND) 21 when the pups were weaned. The pups stayed with their siblings for 7 days until PND 28, at which point they were then separated by sex; littermates of the same sex remained together throughout adulthood. All experiments were approved by the Animal Care and Research Ethics Committee, National University of Ireland, Galway (12/NOV/07) and in compliance with the European Communities Council directive 86/609.

2.2. Mating

For this research project, 72 female rats and 24 male rats were acquired to ensure adequate numbers of females became pregnant and that there was a male:female ratio of 1:3 for mating. The animals were left undisturbed and allowed to habituate for one week before the study began. Each female was allowed to habituate to the 2 other female cagemates and then each cage of female rats (3) were housed overnight with one sexually mature male rat ensuring that the same females remained together. At the beginning of the light phase the following morning, vaginal smears were taken from all females to check for the presence of sperm. All smears were examined under a light microscope. Gestation Day (GD) 1 was deemed to be the day that sperm was present in the smear, at which point the female were then removed and singly housed.

2.3. Gestation period and deliveries

The females were checked daily prior to the start of the dosing period (GD 7-birth) and the females were weighed daily. The expected day of delivery (birth) is GD 21–22 (Daston et al., 2004). Deliveries that occurred before 17.00 h were considered to have their and PND 1 on this day and deliveries that occurred after 17.00 h were considered to have their PND 1 on the following day. Offspring in each litter were checked and counted for the week after delivery to monitor for pup mortalities. The pups were randomly culled to 10 per litter on PND 1 with a litter ratio of 50:50, males to females whenever possible. Two males and two females were selected for neonatal testing from each litter in order to avoid litter effects. These four pups represented the average weight of the litter. Each of these pups was injected intradermally with black India ink in the footpad for unique identification purposes in a litter.

2.4. Drug treatment

The first dosing day corresponded with GD 7; this timeline corresponds to the second trimester of human pregnancy and to the human prenatal development of the CNS (Daston et al., 2004). Methamphetamine HCl was purchased from Sigma–Aldrich (Wicklow, Ireland; M8750). Rats were assigned randomly to control or MA treated groups by way of latin square based on body weights (weight gained since before mating and since GD 1) and likelihood of pregnancy to ensure even distribution across all groups. The doses of MA given used were 0.625, 1.25, 2.5 mg/kg at a volume of 1 ml/kg and controls received the vehicle alone *i.e.* 1 ml/kg distilled H_2O (dH_2O). These doses were chosen as it relates to clinically relevant doses in the human scenario and the use of an allometric scale takes into account the body weight and body surface area of human *vs.* rat (Reagan-Shaw et al., 2008) therefore maintaining the viability of the project and its comparison to clinical situations. For MA or control treatments, the animals were dosed *via* oral gavage once daily at 14.00 h until dams gave birth. Oral gavage was used as this represents the most common route of MA administration in humans, but has before been overlooked in preclinical investigations.