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Impacts of Prenatal Nanomaterial Exposure on Male Adult Sprague Dawley Rat Behavior and Cognition

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Abstract

It is generally accepted that gestational xenobiotic exposures result in systemic consequences in the adult F1 generation. However, data on detailed behavioral and cognitive consequences remain limited. Using our whole body nanoparticle inhalation facility, pregnant Sprague Dawley rats (gestational day 7) were exposed 4 days per week to either filtered air (control) or nanotitanium dioxide aerosols (nano-TiO_2; count median aerodynamic diameter of 170.9±6.4 nm, 10.4±0.4 mg/m^3, 5 hr/day) for 7.8±0.5 days of the remaining gestational period. All rats received their final exposure on GD 20 prior to delivery. The calculated daily maternal deposition was 13.9±0.5 μg. Subsequently, at 5 months of age, behavior and cognitive functions of these pups were evaluated employing a standard battery of locomotion, learning, and anxiety tests. These assessments revealed significant working impairments, especially under maximal mnemonic challenge, and possible deficits in initial motivation in male F1 adults. Evidence indicates that maternal engineered nanomaterial exposure during gestation produces psychological deficits that persist into adulthood in male rats.

INTRODUCTION

Engineered nanomaterials (ENM), anthropogenic materials <100 nm in diameter, represent a class of compounds with high potential for human exposure given their diverse range of applications that span occupational settings through the consumer market (Zhao and Castranova, 2011; Cupaioli *et al.* 2014; Weir *et al.* 2012; Oberdoerster et al, 2015). Thus, the potential for unintentional and/or intentional ENM exposures exists across the population independent of health and/or developmental stage.

It is generally accepted that ENM exposures may exert detrimental impacts on various tissues including kidney (Blum *et al.* 2015) and central nervous system (CNS) function due to their physiochemical properties including size, charge, solubility and conductance and the time-course of the physiological responses (Cupaioli *et al.* 2014). Inhalation of ultrafine and nano-sized particles were found to translocate toward the CNS via the olfactory bulb (Kao *et al.* 2012; Oberdorster *et al.* 2004) affecting cognition and neuronal health (Li *et al.* 2010). Given the evidence that ENM are capable of crossing numerous barriers in the adult, it is reasonable to expect that these compounds may penetrate into the placental and fetal compartments during gestation, possibly in a size-dependent manner that begins in the maternal lung (Semmler-Behnke *et al.* 2014; Blum et al, 2015).

Data on adult outcomes associated with *in utero* ENM exposure remains limited (Hougaard *et al.* 2015). Initial studies provide evidence that exposure to xenobiotic material during

gestation may affect the health of future generations (Hougaard *et al.* 2015; Stapleton 2015; Blum et al, 2015); including neurological outcomes and susceptibilities in learning and memory of young adult male offspring (Yokota *et al.* 2015). Due to inconsistencies between animal species, offspring age, xenobiotic materials, maternal exposure routes, and behavioral/cognitive testing, studies of neurofunction after exposures have yielded variable behavioral and cognitive impairment results (Cui *et al.* 2014; Hougaard *et al.* 2010; Jackson *et al.* 2011; Mohammadipour *et al.* 2014). Therefore, the aim of this investigation was to develop an experimental design to characterize the behavioral and cognitive abilities of adult rat offspring. The hypothesis that alterations in the gestational environment and/or fetal development induced by prenatal nano-TiO_2 exposure might lead to cognitive disruptions in adulthood was tested.

METHODOLOGY

All breeding and inhalation exposures were carefully monitored as previously described (Stapleton *et al.* 2015). All procedures were approved by the West Virginia University Institutional Animal Care and Use Committee.

Male (300-325 g) and female (250-275 g) Sprague-Dawley rats (Hilltop Laboratories, Scottsdale, PA) were housed at West Virginia University with *ad libitum* access to food and water and acclimated for at least 72 hours prior to breeding as previously described (Stapleton *et al.* 2015b). Pregnant rats were placed within the inhalation chamber and exposed to either filtered air (n=4; Control) or nano-TiO_2 (n=4; Prenatal nano-TiO_2) four days per week for 5 hr/day from gestational day 7 up to and including day 20. Nano-TiO_2 P25 powder was placed in our nanoparticle aerosol generator (U.S. Patent #8,881,897) developed specifically for rodent nanoparticle inhalation exposures and monitored in real time (170.9±6.4 nm; Electrical Low Pressure Impactor, Dekati, Tampere, Finland) which affirmed a consistent final mass concentration (10.4±0.4 mg/m^3) for each exposure (Stapleton *et al.* 2015; Yi *et al.* 2013). Calculated total deposition was normalized based upon previous methodologies to achieve a daily calculated deposition of 43.3 ± 1.6 μg nano-TiO_2 or 13.9 ± 0.5 μg after clearance (Stapleton *et al.* 2015). The schedule within the experimental design ensured the last exposure took place on gestational day 20. Male progeny were aged to adulthood (20±1 weeks) to undergo behavioral assessments, while the female progeny were used in a parallel study (Stapleton *et al.* 2015).

Eleven male rats, taken from 4-control or 4-exposed litters, were pair- or triple-housed during development to reduce animal stress and anxiety (Sharp *et al.* 2002) and were randomly selected from each dam to undergo a behavioral test battery. The behavioral tests (provided in Table 1) assessed a full range of affective, locomotor, and cognitive abilities. These tests also identified and controlled for potential motor, visual, or motivational alterations effected by nano-TiO_2 exposure (Cryan *et al.* 2002; Engler-Chiurazzi *et al.* 2012). Between trials, each apparatus was cleaned with disinfectant and paper towels to remove fecal debris and olfactory cues which may be distracting to subsequent test animals (Rosenfeld and Ferguson, 2014; Huynh *et al.* 2011).

All data are expressed as mean ± standard error and analyzed using two-tailed repeated measures ANOVA or Student's t-tests, $p<0.05$ was used to identify significance. All statistical analyses were completed using GraphPad Prism 5 (San Diego, CA).

RESULTS

Nano-TiO_2 exposure exerted no marked impact on maternal weight, implantation site number, or pup number per litter. With respect to behavioral analyses, gestational nano-TiO_2 exposure via maternal inhalation did not significantly affect locomotor, balance, affective, anxiety-like, or depressive-like behavior in the male adult exposed as a fetus. In addition reference memory learning, retention, or perseveration in any phase of the Morris water maze testing were not markedly altered.

Gestational nano-TiO_2 exposure significantly altered visible platform performance. Exposed rats took significantly longer to reach the visible platform (Figure 1a), an average latency of 17.32±2.82 sec to reach the visible platform compared to controls (8.52±1.0 sec). However, group comparisons on the final trial were not significant (Figure 1b). Therefore, the effects of prenatal nano-TiO_2 exposure are likely due to motivational differences in the initial challenge posed by the water maze and not locomotor or visual differences.

Working Memory Correct errors (repeat entries into arms that once contained a water-escape platform) were also significantly different between groups. Controls committed an average of 1.22±0.8 errors vs. exposed rats 1.49±0.91 errors (Figure 1c). Because trial 4 represents the trial with the highest memory demand, when assessed alone control rats committed 2.08±0.17 errors vs. 2.62 ± 0.20 errors in the exposure group (Figure 1c inset), providing evidence of short term memory impairments. There were no significant effects for either Working Memory Incorrect (Figure 1d) or Reference Memory (Figure 1e) errors.

DISCUSSION

This study investigated the influence of prenatal exposure (via maternal inhalation) to nano-TiO_2 on cognitive behaviors in adult male offspring. Prenatal nano-TiO_2 exposure induced significant working or short term memory impairments and initial motivation. These findings demonstrated that prenatal ENM exposure imparts significant alterations in cognitive behaviors detectable in adulthood. To extrapolate these findings from the lab to the environment, these impairments may increase risk of predation; while the motivational delay demonstrates a lag in decision-making when faced with novel settings. These deficits in initiation were shown to dissipate with repetition, evidence of acclimation.

Behavioral alterations following prenatal ENM exposure were previously reported (Hougaard *et al.* 2010); however, specific studies of neurofunction yielded inconsistent behavioral and cognitive results (Cui *et al.* 2014; Jackson *et al.* 2011; Mohammadipour *et al.* 2014). Therefore, converging findings from this and other studies highlight the importance of optimal prenatal health for the proper development of the CNS and the behaviors this system modulates. If true, future studies in young healthy males may reveal cognitive dysfunction similar to the decline seen in aging animals. It is reasonable to speculate that these impairments are present at birth and/or remain through development at some intensity.

The prenatal period represents a crucial developmental phase when organisms are highly sensitive to changes in development and/or the gestational experience (Makri et al, 2004). Xenobiotic compounds were shown to alter the gestational environment and/or prenatal development and result in long-lasting physiological disruptions (Hougaard *et al.* 2015; Stapleton and Nurkiewicz 2014). ENM exposure is known to impact the function of cardiovascular (LeBlanc et al, 2009; Stapleton *et al.* 2013, 2015), pulmonary (Hougaard *et al.* 2010), renal (Blum et al, 2015) and reproductive systems (Yoshida *et al.* 2010) among dams and their offspring exposed during pregnancy. In this case, male progeny of animals exposed to nano-TiO_2 during gestation displayed impaired working memory indicating that prenatal exposure to ENM via maternal inhalation might attribute to cognitive decrements into and through adulthood.

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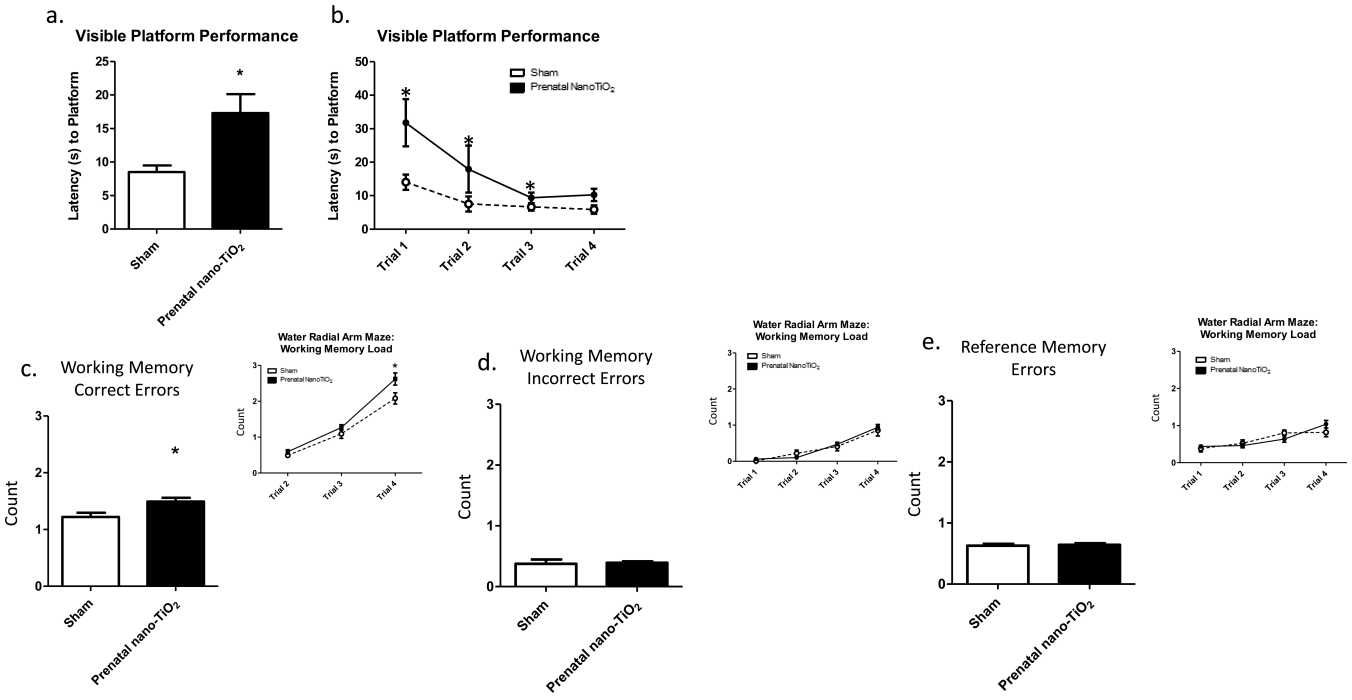

Figure.

Behavioral and Cognitive Testing Outcomes (Mean ± SEM). a) Average time (sec) to Visible Platform. Animals exposed to nano-TiO_2 during their prenatal development had longer latencies to the visible platform than unexposed controls (* indicates $p<0.05$). b) Time (s) to Visible Platform across trials. On the final test trial, the effect was not significant. c) Working Memory Correct Errors within the Water Radial Arm Maze (WRAM). Animals exposed to prenatal nano-TiO_2 committed more working memory correct errors than unexposed controls (* indicates $p<0.05$), especially on the trial of the highest working memory load (* indicates $p<0.05$; insert). d) WRAM Working Memory Incorrect Errors. There were no group differences. e) WRAM Reference Memory Errors. There were no group differences.

Table 1

Behavior Test Battery

Behavioral Test	Functional Outcome of Interest	Dependent Variable	Translational Outcome(s)	Reference
Open Field	Locomotion and Anxiety-like behavior	Distance moved (cm) in each zone (arena, outer, corners, center)	Stress and Anxiety	(Huynh *et al.* 2011)
Elevated Plus Maze	Anxiety-like behavior	Number of entries into each arm pair	Stress and Anxiety	(Huynh *et al.* 2011)
Accelerating Rotarod	Locomotor coordination and balance	Latency (s) to fall	Physical Condition	(Hamm *et al.* 1994)
Modified Forced Swim Test	Depressive-like behavioral despair	Time (s) spent immobile (day 2)	Stress, Depression, and Anxiety	(Cryan *et al.* 2002)
Morris Water Maze: Acquisition	Hippocampal-dependent spatial reference memory	Distance moved (cm) and Velocity (cm/s)	Learning and Memory	(Morris 1984)
Morris Water Maze: Probe Performance	Verification of spatial strategy use	Percent distance moved (cm) in target versus opposite quadrant	Reference Memory	(Engler-Chiurazzi *et al.* 2011) (Engler-Chiurazzi *et al.* 2012)
Morris Water Maze: Reversal	Striatal-dependent perseveration	Distance moved (cm); Velocity (cm/s); and Percent distance moved (cm) in target vs. opposite quadrant	Memory Impairment	(D'Hooge and De Deyn 2001)
Visible Platform	Locomotor capacity Visual capacity	Latency (s) to platform	Physical and Visual Condition	(Engler-Chiurazzi *et al.* 2012)
Radial Arm Water Maze (4/8 platformed arms)	Hippocampal-dependent spatial working and reference memory	- Working Memory Correct Errors - Working Memory Incorrect Errors - Reference Memory Errors	Reference and Working Memory	(Engler-Chiurazzi *et al.* 2011) (Jarrard *et al.* 1984)