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Effect of prenatal exposure to diesel exhaust on dopaminergic system in mice

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ABSTRACT

Diesel exhaust (DE) is composed of particles and gaseous compounds. It has been reported that DE causes pulmonary and cardiovascular disease. We have previously reported that fetal exposure to DE had deleterious effects to the reproductive system of mice offspring. However, there is still little known about the effects of prenatal exposure to DE to the central nervous system (CNS). In the present study, we found that prenatal exposure to DE induced reduction of locomotion, furthermore, dopamine (DA) turnover was significantly decreased in the striatum and nucleus accumbens. These results suggest that prenatal exposure to DE has an effect on the CNS. Hypolocomotion could be due to a decrease in DA turnover associated with DA nervous system abnormality. The present study provides the possibility that maternally inhaled DE might influence the development of central dopaminergic system and result in behavior disorder.

There is growing international concern regarding the adverse health effects of air pollution. Diesel exhaust (DE), one of the more serious air-pollutants, is generated by the motor vehicles. DE is comprised of a complex mixture of hundreds of constituents in either a gas or particle phases. Gaseous components of DE include carbon dioxide, oxygen, nitrogen, water vapor, carbon monoxide, nitrogen compounds, sulfur compounds, and low-molecular-weight hydrocarbons. The particles in DE (i.e., diesel exhaust particles; DEP) are composed of elemental carbon, adsorbed organic compounds, and small amounts of sulfate, nitrate, metals, and other trace elements. DEP consists of fine and ultrafine particles, which are highly respirable and have a very large surface area that adsorbed lots of inorganic and organic compounds [19,21,24]. The most toxicologically relevant organic compounds that are adsorbed into the particles include polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs, and oxidized PAH derivatives. PAHs and their derivatives comprise about 1% or less of the DEP mass.

Recently, it has been reported that DE has various detrimental health effects, including lung cancer [6,14], and asthma-like disease [18,23] and cardiovascular disease [5,15]. We first reported that DE-exposed adult male mice showed remarkable damages to spermatogenesis [26].

Generally, sensitivity to chemicals is considered to be higher in fetuses than in adults. We found that mRNA expression of steroidogenic factor-1 (Ad4BP/SF-1) and of mällerian inhibiting substance, which play essential roles in male gonadal differentiation, were significantly decreased by maternal exposure to DE [27]. Furthermore, Ono et al. reported that prenatal exposure to DE induced spermatogenetic arrest and alterations in serum testosterone levels. In addition, partial vacuolation of the seminiferous tubules was found in mice exposed to DE during the fetal period [17]. These findings suggest directly or indirectly, that maternally inhaled DE can lead to a reduction of the reproductive system. We have also reported that placentas exposed to DE may promote an inflammatory reaction in the placenta. For example, inflammatory cytokines IL-2, IL-5, IL12 alpha, IL12 beta, and GM-CSF mRNA levels increased in placentas exposed to DE [4]. It is possible that expression of mRNA in the placenta affects fetal development.

Although prenatal exposure to DE or DEP may have the potential to exaggerate the effect of maternal exposure to DE in the central

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Table 1
Concentrations of DE constituents.

	Concentrations
DE particle mass (mg/m^3)	1.0
CO (ppm)	2.67
NO_2 (ppm)	0.23
SO_2 (ppm)	<0.01

nervous system (CNS), this was not well understood in vivo. The present study was designed to investigate the impact on the CNS (e.g., motor function) in DE exposed mice using a behavioral test and measuring the levels of dopamine and its metabolites in the striatum and nucleus accumbens.

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Tokyo University of Science adopted by the Committee on Animal Research of Tokyo University of Science. All efforts were made to minimize the number of animals used and their suffering. All experiments were conducted using pregnant ICR mice obtained from SLC Co. (Shizuoka, Japan). Pregnant mice were exposed to DE for 8 h, 5 days per week (from Monday to Friday, 9:00–17:00) in an inhalation chamber (Japan Anti-Tuberculosis Association, Kiyose, Tokyo) from gestational day (GD) 2 to GD 17. After DE exposure, mothers and male pups were maintained in a clean room. Control animals were kept in a clean room. Each pup was weaned on postnatal day 21, after which male mice were transported to Tokyo University of Science. Animals were acclimated for 2 weeks and were exposed to a 12-h light/dark cycle (lights on from 8:00 to 20:00). Food and water were provided ad libitum. All experimental mice were handled in accordance with institutional and national guidance for the care and use of laboratory animals.

A 2369-cc diesel engine (Isuzu Motors, Ltd., Tokyo, Japan) was operated at a speed of 1050 rpm and 80% load, using a commercial light oil. Exhaust was introduced into a stainless steel dilution tunnel (218-mm diameter × 5.55 m). In the tunnel, the exhaust was mixed with clean air, and average concentrations of exhaust constituents were maintained at 1.0 mg/m^3 for particles, 2.67 ppm for CO, 0.23 ppm for NO_2, and less than 0.01 ppm for SO_2 (Table 1).

To investigate DE-induced behavioral changes, we used an activity monitor with an infrared ray sensor (NS-AS01; Neuroscience Inc., Tokyo, Japan) to measure spontaneous motor activity (SMA) by the release of temperature-associated infrared rays. SMA was counted at 10 min intervals for 2 days. Data were automatically analyzed with a computerized system (multidigital 32-port counter system; Neuroscience Inc.).

Following the behavioral test, mice were killed by decapitation and brains were immediately collected as samples. In the daytime, the brain was dissected into striatum and nucleus accumbens, immediately frozen in liquid nitrogen, and stored at −80 °C until neurochemical analysis.

Frozen brain tissue was homogenized in ice-cold 0.2 M perchloric acid (Nakalai Tesque, Inc., Kyoto, Japan) containing 100 μM EDTA 2Na (Dojindo Laboratories, Kumamoto, Japan) and 1 ng/mL isoproterenol as an internal standard (Sigma–Aldrich Co., St. Louis, MO). Homogenates were placed on ice for 30 min and centrifuged at 20,000 × *g* at 0 °C for 15 min. The pellets were used for protein assay. Supernatants were mixed with 1 M sodium acetate to adjust the pH to 3.0 (Kanto Chemical Co., Inc., Tokyo, Japan) and were immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

10 μL of the final supernatant was injected with a microsyringe (702 SNR; Hamilton, Co., Reno, NV) into a high-performance liquid chromatography (HPLC) system equipped with an electrochemical detector (HTEC-500MAB; EICOM, Kyoto, Japan). The standard solution contained dopamine (DA; Sigma–Aldrich Co.) and its metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC; Wako Pure Chemical Industries, Ltd., Osaka, Japan), and homovanillic acid (HVA; Sigma–Aldrich Co.). Separation of DA and its metabolites was performed on a C18 reverse-phase column (Eicompak SC-5ODS; Ø 3.0 mm × 150 mm; Eicom), maintained at 25 °C with an electrochemical detector (EPC-500, Eicom). The mobile phase was 0.1 M acetic acid/citric acid buffer (pH 3.5) containing EDTA 2Na (5 mg/L), octanesulfonic acid (190 mg/L; Nakalai Tesque), and methanol (15%, v/v; Kanto Chemical Co., Inc.). The flow rate was maintained at 0.5 mL/min. Data were collected and analyzed with PowerChrom version 2.3 chromatography software (eDAQ Pty, Ltd., New South Wales, Australia), with the use of area ratios to determine sample concentrations.

Pellets were resuspended in 100 mM Tris–HCl for protein determination by high-sensitivity Bradford method with a commercial reagent (ADV-01; Cytoskelton, Inc., Deriver, CO) and measurements were performed according to the manufacturer's protocol. Absorbance was read at 595 nm on a 96-well microplate reader (Model 550; BioRad, Hercules, CA) and protein concentration was calculated from a standard curve generated with the use of bovine gamma globulin (Pre-Diluted Protein Assay Standards; Bovine Gamma Globulin Set; Biotechnology, Inc., Rockford, IL).

Concentrations of DA and its metabolites are expressed as nanograms per milligram of protein, while the catabolism rate is expressed as the ratio of metabolites to DA and indicated by the index of catabolism rate (i.e., DOPAC/DA, HVA/DA, DOPAC + HVA/DA). Indices were calculated from individual tissue samples.

The results were analyzed by two-way ANOVA with replication and by unpaired *t*-test: the Mann–Whitney *U*-test was used for comparison between the two groups. All data are reported as means ± S.E.M.

There have been increasing reports that neurodegenerative disorders may begin in the early stages of development and environmental factor may be a key role [3]. In the present study, prenatal exposure to DE in male mice decreased spontaneous motor activity (Fig. 1A). According to environmental standard value in Tokyo metropolitan, suspended particles matter (SPM) concentration in the environment is 0.1 mg/m^3. DEP is included approximately 40% in SPM. Considering the duration of exposure, the DEP concentration in this study was 6-hold greater than that of environment. It has been reported that the mice exposed to DE during adult or neonatal periods showed less spontaneous locomotor activity than that of controls in standard running wheel cages [11]. The DE concentration in that study was 6 mg DEP/m^3, approximately 6-fold greater than that in the present study. Our result indicates that the lower concentration of DE could affect offspring locomotion in mice. Interestingly, we also showed that DE exposed mice exhibited decreased locomotor activity during the light phase in comparison to the control mice (Fig. 1B). This result might suggest that the regulation of the sleep-arousal cycle of maternally DE exposed mice is affected. It is well known that noradrenaline (NA) in locus ceruleus and serotonin (5-HT) in raphe nucleus activities changes with the sleep-arousal cycle [20]. Our findings suggest the possibility that prenatal exposure to DE may affect the persistent changes in the NA and the 5-HT system. On the other hand, dopaminergic mechanisms within the striatum and nucleus accumbens play an important role in the control of locomotor activity, and a change in DA turnover depends essentially on a change in impulse flow in the dopamine neurons [9]. Isoflurane, for example, has been shown to induce hyperlocomotion during emergence and may be associated with increased DA turnover in the striatum and nucleus accumbens [7]. Moreover benzo[*a*]pyrene, included in DE suppressed DA in the striatum and DA turnover in the nucleus accumbens [10]. To inves-