Table 3 Dopamine turnover in each part of the brain.

Brain region	Group	Turnover			
		DOPAC/dopamine	3-MT/dopamine	HVA/dopamine	(DOPAC+HVA)/dopamine
Striatum	Control	0.076 ± 0.004	0.077 ± 0.004	0.121 ± 0.004	0.197 ± 0.006
	Exposed	0.064 ± 0.002*	0.071 ± 0.003	0.098 ± 0.004*	0.162 ± 0.005**
Hippocampus	Control	–	0.60 ± 0.14	24.66 ± 12.22	–
	Exposed	–	0.51 ± 0.06	5.15 ± 0.64	–
Midbrain	Control	0.61 ± 0.05	0.20 ± 0.03	0.99 ± 0.13	1.601 ± 0.165
	Exposed	0.55 ± 0.03	0.17 ± 0.01	0.86 ± 0.06	1.404 ± 0.079
Cerebellum	Control	–	–	17.22 ± 5.55	–
	Exposed	–	–	39.49 ± 13.83	–
Brainstem	Control	3.22 ± 0.13	–	0.16 ± 0.01	3.38 ± 0.13
	Exposed	3.25 ± 0.09	–	0.35 ± 0.04	3.60 ± 0.07

Data are presented as mean ± SEM (n = 10 per group). The statistical significance is shown as *P < 0.05, **P < 0.01; ND, not detectable. Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine.

Table 4 Noradrenaline turnover in each part of the brain.

Brain region	Group	Turnover	
		NM/noradrenaline	MHPG/noradrenaline
Striatum	Control	–	20.62 ± 3.14
	Exposed	–	14.44 ± 2.15
Hippocampus	Control	0.069 ± 0.006	4.59 ± 0.35
	Exposed	0.074 ± 0.007	2.97 ± 0.24**
Midbrain	Control	0.034 ± 0.002	1.84 ± 0.11
	Exposed	0.028 ± 0.003	1.12 ± 0.06**
Cerebellum	Control	0.145 ± 0.011	10.82 ± 1.30
	Exposed	0.131 ± 0.013	6.87 ± 0.36*
Brainstem	Control	0.0568 ± 0.003	0.415 ± 0.015
	Exposed	0.0487 ± 0.003	0.380 ± 0.030

Data are presented as mean ± SEM (n = 10 per group). The statistical significance is shown as *P < 0.05, **P < 0.01; ND, not detectable. Abbreviations: MHPG, 4-hydroxy-3-methoxyphenylglycol; NM, normetanephrine.

the cerebrum of newborns in the exposure group as well as mRNA for *CYP1A1* and *HO-1*. The results indicate that prenatal exposure to diesel exhaust during the critical period of sexual differentiation of the brain may affect endocrine function. Estrogen has many important roles in the brain, including brain development, neuroprotection such as inhibition of apoptosis and synaptogenesis, and functions of the monoaminergic systems [25], the effects of perinatal exposure to diesel exhaust on CNS have been focused on. There is a report that shows serum level of estradiol is increased in male rats exposed to diesel exhaust after birth [26]. However, whether the serum estradiol level is affected by prenatal exposure to diesel exhaust remain unknown. Further investigation is required to clarify the mechanisms of the effects of diesel exhaust exposure on the levels of estradiol and CNS of offspring.

When pregnant mice were exposed to diesel exhaust, cytoplasmic granules of granular perithelial cells contained ultrafine DEP-like particles and the apoptosis of endothelial cells and stenosis of some capillaries were observed [16]. Furthermore, caspase-3 positive cells were observed in the cerebral cortex and in the hippocampus of the newborn [27]. These observations suggest that exposure of pregnant mice to diesel exhaust might carry a risk of cellular atrophy and might affect development of the fetal brain. A review by Herlenius and Lagercrantz [28] indicated that stimulation or insult at critical phases of development of the nervous system could result in long-term changes in organismal structure and function. Perinatal exposure to environmental contaminants, including DEPs, that have hormone-like activity [20-23] and can generate reactive oxygen species [29], might be able to disturb the timetable of expression of neurotransmitters and neuromodulators and their receptors, evoking permanent changes in cellular proliferation and differentiation and in growth, leading to behavioural and neurophysiologic abnormalities. The details of the mechanism how DEPs affect fetus and damage CNS of offspring remain unclear. However, when gold nanoparticle (1.4 - 18 nm) is intravenously injected into female rats, inversely size-dependent uptake in the placenta and translocation into the fetus were found [30]. Nanosized fraction of DEPs may majorly contribute to adverse effects on CNS of offspring. The toxicity of DEP for dopamine neurons was shown in an *in vitro* study [12] and the toxicity for CNS *in vivo* should be further investigated. The present study showed that daily SLA decreased in the exposure group compared to that in the control group when the mice were put into a new environment. This alteration is similar to that of ovariectomized mice treated with estradiol [31,32], suggesting that it may be caused by

estrogenic activity of diesel exhaust. The dopamine and noradrenaline systems in the PFC have an important role in the control of locomotor activity. Destruction of mesocortical and dopamine projections in rats results in increased motor activity [33-36], suggesting that one of the roles of dopamine in the PFC is to suppress locomotor activity. In the present study, the data showed that dopamine and its metabolites were increased in the PFC of the exposure group and the basal stress level was not altered by diesel exhaust exposure because the levels of serum corticosterone were not different between groups. The dopamine and noradrenaline systems in the PFC are responsive to various stressors [37,38]. The response of the dopamine system is independent of the pituitary-adrenocortical axis [39]. The cause of decreased SLA in mice exposed prenatally to diesel exhaust may be a transiently facilitated release of dopamine as a result of exposure to novel stimuli rather than alteration of the level of basal stress. Our unpublished data showed that the effects of diesel exhaust exposure on CNS of offspring are reduced by removing the DEPs from the exhaust with a filter. It suggests that particulate component of diesel exhaust contribute to the effects of maternal exposure to diesel exhaust on CNS.

Conclusions

The exposure to low concentrations of diesel exhaust *in utero* decreased spontaneous locomotor activity and altered monoaminergic neurochemistry in several regions of the brain in male mice. However, the mechanism connecting the behavioural and neurochemical alterations remains unclear. We cannot rule out an indirect effect of diesel exhaust exposure via the mother's behaviour toward the pups and how this in turn altered SLA and monoamine metabolism in the offspring since this was not investigated. Further investigations are needed to clarify the critical factor for the effects on offspring. Since current observations are done at a particle mass concentration of diesel exhaust close to the environmental quality standard of daily-averaged level of suspended particulate matter (SPM) in Japan, these finding warrant revisiting of present air quality standards for particulate matter.

List of abbreviations used

CNS: central nervous system; DEP: diesel exhaust particle; DOPAC: 3,4-dihydroxyphenylacetic acid; GD: gestational day; HPLC: High-performance liquid chromatography; HVA: homovanillic acid; MHPG: 4-hydroxy-3-methoxyphenylglycol hemipiperazinium; 3-MT: 3-methoxytyramine hydrochloride; NM: normetanephrine hydrochloride; PFC: prefrontal cortex; PM: particulate matter; PND: postnatal day; SLA: spontaneous locomotor activity; SPM: suspended particulate matter.

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Author details

[1]Department of Hygiene Chemistry, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda-city, Chiba 278-8510, Japan. [2]Department of Hygiene Chemistry, School of Pharmaceutical Sciences, Ohu University, 31-3 Misumido, Tomita-cho, Koriyama-city, Fukishima 963-8611, Japan. [3]Department of Molecular Pathology, Research Institute of Tuberculosis, 3-1-24 Matsuyama, Kiyose-city, Tokyo 204-8533, Japan. [4]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency), 4-1-8 Hon-cho, Kawaguchi-city, Saitama 332-0012, Japan. [5]School of Medicine and Medical Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba-city, Ibaraki 305-8577, Japan.

Authors' contributions

TS, SO, MI, HS and TO were substantially involved in conducting the experiments. TS and MU was involved in data analyses and in drafting the manuscript. TU and IS conducted and controlled the diesel exhaust exposure. KT is the main project leader and conceived the overall research idea. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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