

TRAFFIC RELATED POLLUTION REDUCES THE LEVELS OF MATRIX METALLOPROTEINASES IN HIPPOCAMPUS AND STRIATUM AFTER CEREBRAL ISCHEMIA IN MICE.

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Background and aims

Stroke is the second leading cause of death globally (WHO), affecting 15 million people worldwide.

One risk factor that is now gaining interest, is the exposure to **particulate matter** (PM) derived from diesel combustion (Fig. 1) (Bathmanabhan, S 2010), which is the main particulate matter pollutant in urban areas where over 600 million people worldwide live in.

The most harmful component of the PM is the smallest fraction: particles sized 2.5µm (PM_{2.5}) and below (UFPM), which can access systemic circulation after reaching the lungs or even translocate to the brain directly by diffusion processes (Claderón-Garcidueñas, L et al. 2010) (Figs. 1 and 2).

However, the underlying mechanisms of their damaging actions in the brain are not well understood.

The aim is to investigate the influence of Diesel exhaust particles (DEP) exposure in a mouse model of cerebral ischemia.

In vivo Methodology

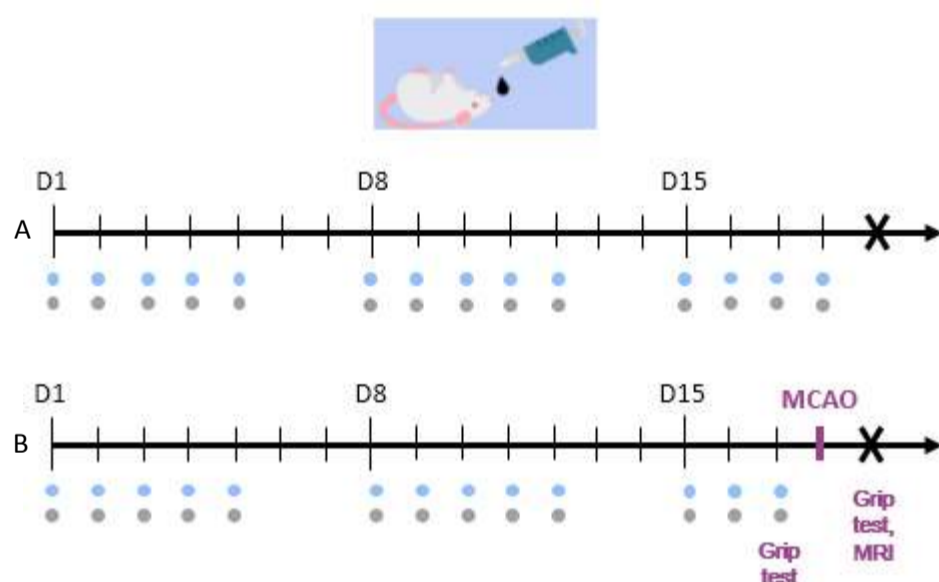


Figure 4: Experimental procedure: Balb/c mice received either PBS or DEP (150µg/20µl) sonicated for 10'. A: Non-Ischemic animals were instilled 14 days and sacrificed 24 hours later. B: Ischemic animals were instilled 13 days, and evaluated via grip test pre and post MCAo surgery. MCAo surgery (distal occlusion of the MCA by electrocoagulation) was performed the day 18th. 24 hours later, animals were imaged by MRI and euthanized (X). Brains were then studied.

Results: Ischemic Brains

Ischemic brains, after DEP instillation, change the ipsilateral pro-inflammatory response of MMPs to a bilateral overexpression compared to naïve brains.

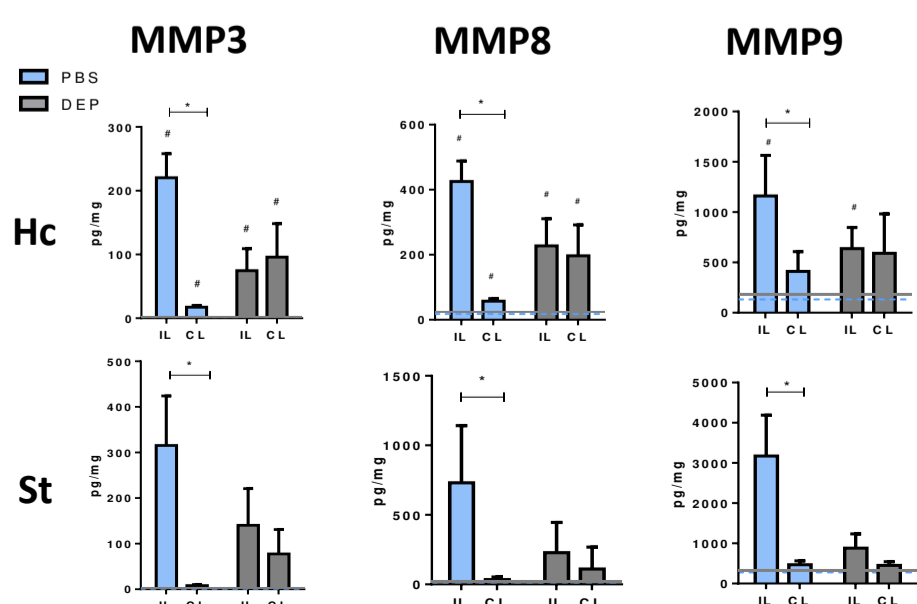


Figure 7: Amount of Matrix Metalloproteinases (MMP) 3, 8 and 9 in the Hippocampus (Hc) and the Striatum (St) of ischemic mice. MMP levels increase after MCAo surgery (represented as bars) especially in the IL side, both in PBS and DEP instilled animals, when compared to baseline levels (bottom lines). However, DEP-exposed mice after MCAo surgery show a similar response between IL and CL sides, when PBS-exposed show a higher response of the IL side. [Data expressed as \pm SEM, non-ischemic mice n= 6-7, ischemic mice n= 3-5, * p-value <0.05 in comparisons between hemispheres (IL: ipsilateral, CL: contralateral), # p-value <0.05 comparing IL or CL levels of MMPs with basal levels of non ischemic animals].

Figure 1: Standard Reference Material (SRM 2975) of Diesel Particulate Matter, from an industrial forklift.

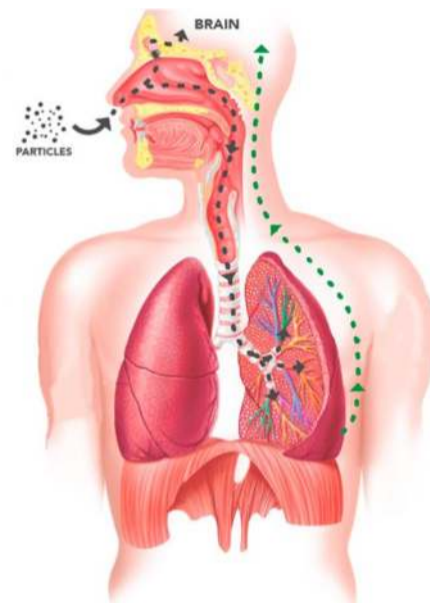


Figure 2: translocation of inhaled particulate matter (PM) to CNS. There are two main pathways: (1) Inhaled particles reach the respiratory airways, the smallest fraction reaches the alveoli, then the blood stream and finally the brain. (2) Particles access the brain directly through the nasal olfactory mucosa, then the olfactory nerve and finally the olfactory bulb.

Results: Naïve Brains

DEP-exposure increases vasculature in caudal cortex but not in whole cortex

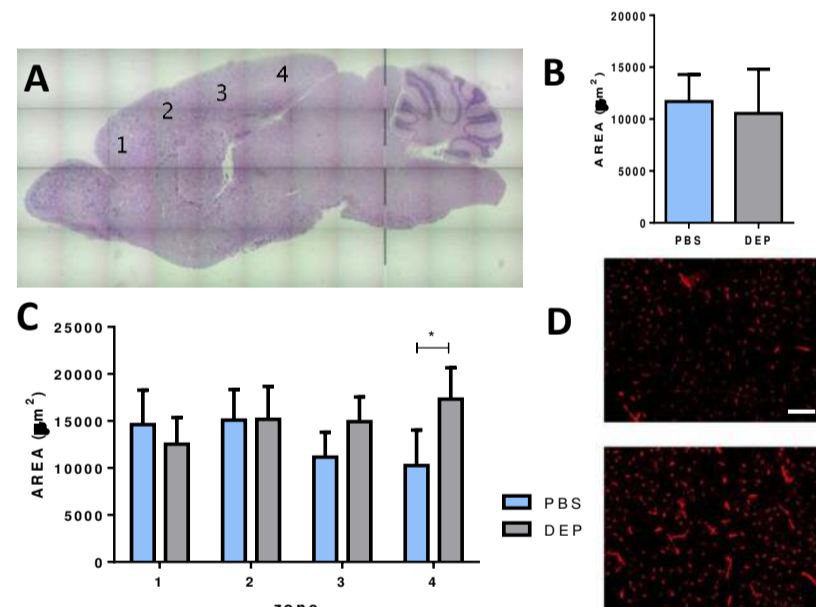


Figure 5: A: Sagittal brain slice with H/E staining showing the main structures. Cortex was divided in 4 different regions from more rostral to more caudal (1 to 4). B: No difference was observed in the quantification of Lectin+ immunofluorescence (µm²) in the whole cortical area. C: quantification of Lectin+ area in the 4 cortical regions (µm²). Caudal cortex (4) presented largest Lectin+ area in DEP-treated animals when compared to controls. D: Representative images of fluorescent vessels (Lectin+) in the most caudal cortical area (Scale: 100µm). (Data expressed as mean \pm SEM, n=5/group, *p-value <0.05).

Characterization of DEP working suspension

Best sonication time was 10 minutes when the highest percentage of the smallest particles was obtained in the shortest time.

Sonication time (min)	SRM 2975 Particles' diameter (µm)		SRM 2975 Particles' size percentage (%)		
	50th percentile (µm)	90th percentile (µm)	PM ₁₀ (%)	PM _{2.5} (%)	UFPM (%)
0	7,03	28,12	65,96	12,38	0
10	0,42	3,56	88,63	74,08	14,46
30	0,53	3,99	91,95	80,03	11,48
60	0,52	5,56	-	-	-

Table 1: Median values of the particles' size (µm) in percentiles. DEPs (Standard Reference Material 2975, Fig. 1) were suspended in PBS (0,5 mg/ml). Working suspensions (n=4) were sonicated (0, 10, 30 or 60 minutes) and analyzed by Mastersizer 2000. In bold the sizes at 10 minutes sonication (the selected working time). Median values of PM₁₀, PM_{2.5} and UFPM of the total DEP suspension (100%). In bold, the percentage of PM_{2.5} after 10 minutes-sonication.

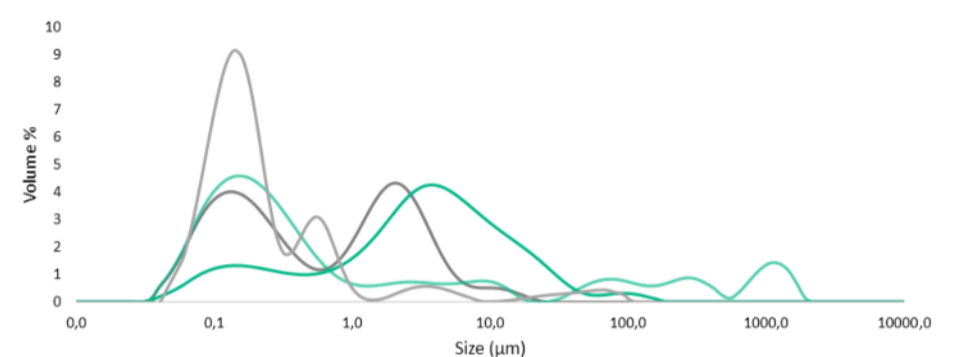


Figure 3: Particle size distribution of the working DEP suspension analyzed by Mastersizer 2000 after 10 minutes sonication (n=4).

DEP-exposure reduces migrating Neuroblasts (DCX+) of the Rostral Migratory Stream

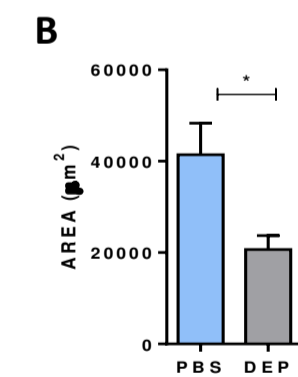
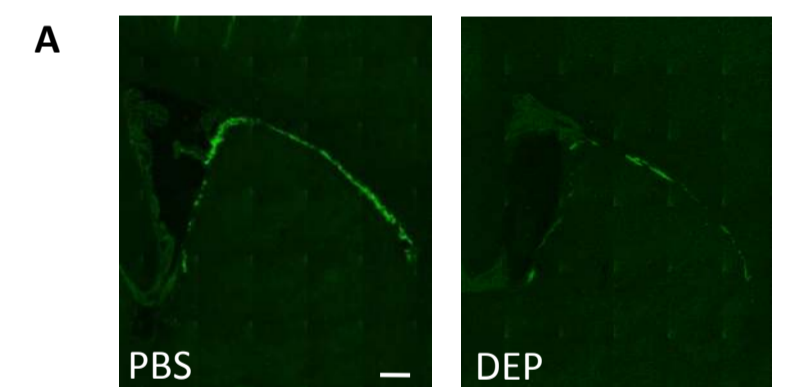


Figure 6: A: Representative images of Migrating Neuroblasts (DCX+) in the Rostral Migratory Stream (RMS) of vehicle and DEP-exposed mice (scale 200 µm) B: Immunofluorescent area quantification (µm²). DEP-exposed animals showed a significant decrease of DCX+ area. (Data expressed as mean \pm SEM, n=5/group, * p-value <0.05).

Infarct volume was not increased in DEP-exposed animals.

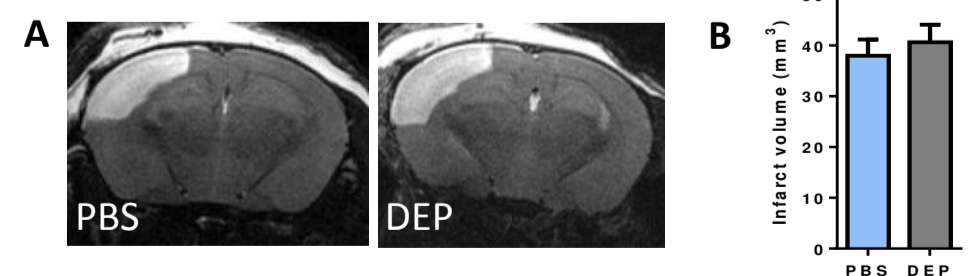


Figure 8: A: MRI showing the cortical lesions 24 hours after stroke. B: quantification of the lesion size. (Data expressed as mean \pm SEM, PBS n=3, DEP n=4, * p-value <0.05).

Take home messages

- Diesel particulate matter, reduces migratory Neuroblasts in the Rostral Migratory Stream and modulates vessel density in the cortex of non-ischemic animals.
- Only in the context of cerebral ischemia Diesel exposure, alters the inflammatory response.