

Supplemental Materials

Sex differences in sensitivity to prenatal and early childhood manganese exposure on neuromotor function in adolescents

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1. Classifications of Occupations

Occupations were grouped in three categories, according to a hypothetical ordinal scale that considered criteria of the International Classification, socioeconomic situation of Italy (and in particular of Lombardy Region) and variables like decision latitude and job demand, developed by Cesana et al. (Cesana et al. 1995).

The three categories were: low (housewife, skilled/unskilled worker, hospital ancillaries,...) middle (clerical workers, teachers, educators, nurses, shop assistant,...) and high (engineer, entrepreneur, tradesman, craftsman,...).

Since the Cesana model was created in the 90's, we updated the three levels according to the current Italian socio-economic, cultural and work conditions. Criteria followed indications from ISTAT (The Italian National Institute for Statistics) and an agreement between five independent researchers (Table S1).

Table S1. Professions level after internal agreement (H=high, M=medium, L=low);

Profession	Level	Profession	Level
ACCOUNTANT	M	DOMESTIC WORKER	L
ANCILLARY	L	DRIVER	M
ARCHITECT	H	DRIVING INSTRUCTOR	M
ARTISAN	M	EDUCATOR	M
ARTIST	L	ELECTRICIAN	M
BARMAN	L	EMPLOYEE	M
BIOLOGIST	M	ENGINEER	H
BRICKLAYER	M	ENTREPRENEUR	H
BROKER	H	FACTORY WORKER	L
BUILDER ARTISAN	M	FARM ENTREPRENEUR	H
BUILDING SURVEYOR	M	FARM WORKER	L
BUSINESS CONSULTANT	H	FARMER	M
BUTCHER	M	FINANCIAL ADVISOR	M
CAR DRIVER	M	FIREMAN	L
CAR WORKER	M	FOREST TECHNICIAN	M
CARETAKER	L	FREELANCER	M
CARETAKER SCHOOL	L	GARDENER	L
CARPENTER	M	GOLDSMITH	H
CONSULTANT	M	HELP COOK	L
COOK	M	HOUSEWIFE	L
DENTAL TECHNICIAN	M	JOURNALIST	M
DENTIST	H	LABORATORY TECHNICIAN	M
DIETICIAN	M	LAND SURVEYOR	M
DOCTOR	H	LAWYER	H
LORRY DRIVER	M	SHOP MANAGER	M
MANAGER	H	SMITH	M
MECHANIC DESIGNER	M	SOCIAL OPERATOR	L

MERCHANDISER	M	SOLDIER	M
NURSE	M	STOREKEEPER	M
PATTERN MAKER	L	SURVEYOR	M
PAYMASTER	M	TAILOR	M
PHARMACIST	H	TEACHER	M
PHYSICIAN	H	TECHNICAL DESIGNER	M
PHYSIOTHERAPIST	M	SALESMAN	M
POLICEMAN	M	THERAPIST	M
POSTMAN	L	TOURIST AGENT	M
POULTRY FARMER	M	TRADER	M
PREVENTION TECHNICIAN	M	TRAIN CONDUCTOR	M
PRODUCT MANAGER	H	UNEMPLOYED	L
PROGRAMMER	M	VETERINARY	H
RADIOLOGY TECHNICIAN	M	WAITER	L
RAILWAY WORKER	M	WORKER	L
RESTORER	M	SECRETARY	M
RETIRED	L	SERVICEMAN	L
SALES REPRESENTATIVE	M	SHOP ASSISTANT	L

2. Socioeconomic Status (SES) Index

The combination of education and occupation levels was then used to obtain three levels of the socioeconomic (SES) index: low, medium, high (Table S2).

To obtain the final SES index, we combined higher level of education and occupation between mother and father. When mother or father are not economically present (unemployed or deceased) we calculated her/his status as LOW.

Table S2. SES index as a combination of education and occupation levels

<i>SES INDEX</i>		Occupation		
		High	Medium	Low
Education	High	<i>HIGH</i>	<i>HIGH</i>	<i>MEDIUM</i>
	Medium	<i>HIGH</i>	<i>MEDIUM</i>	<i>LOW</i>
	Low	<i>MEDIUM</i>	<i>MEDIUM</i>	<i>LOW</i>

3. Sensitivity analyses additionally including Mn levels in the prior time points

It is possible that for the model examining postnatal Mn exposure and neuromotor outcomes, the prenatal Mn level might be a potential confounder as it is associated with both postnatal Mn and outcome. Similarly, for the model examining effects of childhood cumulative Mn, prenatal Mn and early postnatal Mn might be potential confounders. Of note, as mentioned in the first paragraph of the Results section in the main manuscript, overall there were no correlations among Mn levels in different time periods (i.e., prenatal, early postnatal, cumulative childhood tooth Mn, and concurrent blood Mn at adolescence), except that prenatal Mn was marginally correlated with early postnatal Mn ($r=0.17$, $p=0.02$), indicating that confounding by Mn at other time points is less likely. Nonetheless, we conducted sensitivity analyses additionally including Mn levels from prior time points in the same model, for early postnatal and cumulative childhood models. These results are shown in Table S3 below, which generally yielded similar findings in terms of directions and patterns as seen in the main analyses.

Table S3. Adjusted associations between perinatal Mn exposure and opened-eye body sway: sensitivity analysis additionally adjusting for Mn levels at prior time period(s)

Opened-eye Sway indices ^a	Boys			Girls			<i>p</i> for interaction ^b
	β	s.e.	p-value	β	s.e.	p-value	
<i>Early postnatal Mn^c</i>							
Mean sway	-0.44	0.50	0.39	-0.38	0.44	0.39	0.61
Transversal sway	-0.16	0.29	0.58	-0.30	0.26	0.25	0.78
Sagittal sway	-0.39	0.43	0.37	-0.16	0.39	0.68	0.53
Sway area	-0.05	0.18	0.76	-0.18	0.15	0.24	0.90
Sway velocity	-0.57	0.73	0.44	-0.45	0.66	0.50	0.59
Sway intensity	-0.17	0.37	0.65	-0.29	0.30	0.34	0.78
<i>Childhood cumulative Mn^d</i>							
Mean sway	-0.72	0.47	0.13	0.37	0.33	0.27	0.07
Transversal sway	-0.27	0.28	0.33	-0.10	0.20	0.63	0.74
Sagittal sway	-0.62	0.41	0.13	0.53	0.29	0.07	0.02
Sway area	-0.14	0.15	0.37	0.07	0.11	0.52	0.33
Sway velocity	-0.35	0.67	0.61	0.48	0.46	0.30	0.33
Sway intensity	-0.49	0.35	0.17	0.05	0.21	0.80	0.24

^a Sway area was ln-transformed to reduce heteroskedasticity

^b p-value for Mn \times sex interaction term in the interaction models.

^c Adjusted for children's age, SES index, tooth attrition, and prenatal Mn level; children's sex was also adjusted in the interaction models

^d Adjusted for children's age, SES index, tooth attrition, prenatal Mn level, and early postnatal Mn level; children's sex was also adjusted in the interaction models

4. Concurrent blood Mn levels and neuromotor function

Table S4 below shows the results of regression analyses examining the associations between blood Mn levels (measured around the same time as neuromotor function tests) and neuromotor outcomes in adolescents in the PHIME cohort. Overall, we did not find statistically significant associations between blood Mn levels measured concurrently and neuromotor function, and also did not find sex differences on these associations.

Table S4. Adjusted^a associations between concurrent blood Mn levels and neuromotor outcomes: sex-specific associations

Neuromotor Outcomes	Boys			Girls			<i>p</i> for interaction ^c
	β	s.e.	p-value	β	s.e.	p-value	
Whole body postural balance							
<i>Body sway (opened-eye)</i>							
Mean sway	-0.07	0.09	0.45	-0.02	0.05	0.73	0.55
Transversal sway	0.02	0.05	0.72	-0.01	0.03	0.74	0.71
Sagittal sway	-0.09	0.08	0.23	-0.02	0.05	0.69	0.36
Sway area ^b	0.01	0.03	0.72	0.001	0.02	0.96	0.84
Sway velocity	0.10	0.13	0.42	0.06	0.07	0.42	0.78
Sway intensity	0.05	0.07	0.40	0.05	0.03	0.16	0.99
Hand-related motor outcomes							
<i>Pursuit Aiming Test</i>							
total correct dots	-0.93	1.14	0.42	0.89	0.79	0.27	0.21
total error dots	1.59	1.19	0.19	0.90	1.22	0.46	0.65
<i>Luria-Nebraska Motor Scale</i>							
sum score of 5 subtasks	0.24	0.38	0.54	-0.19	0.35	0.59	0.43
mean score of 5 subtasks	0.05	0.08	0.54	-0.04	0.07	0.54	0.40
<i>Hand Resting Tremor</i>							
Tremor intensity (R) ^b	-0.01	0.01	0.45	0.00	0.01	0.77	0.62
Tremor intensity (L) ^b	0.01	0.01	0.37	0.01	0.01	0.56	0.93

^a Multivariable linear regressions adjusted for children's age, SES index, tooth attrition; children's sex was also adjusted in the interaction models.

^b Sway area and tremor intensity were ln-transformed to reduce heteroskedasticity

^c p-value for blood Mn \times sex interaction term in the interaction models.

REFERENCES:

Cesana GC, Ferrario M, De Vito G, Segà R, Grieco A. 1995. Evaluation of the socioeconomic status in epidemiological surveys: hypotheses of research in the Brianza area MONICA project (in Italian). *Med Lav* 86:16-26.