Prenatal and Postnatal Exposure to NO₂ and Child Attentional Function at 4-5 Years of Age

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41 **Short running title:** Air Pollution and Child Attentional Function

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44 cognition; neuropsychological test; environmental pollution; air pollution

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70 **Conflict of interest:**

71 All of the authors declare that they have no conflicts of interest.

72

73 **Abbreviations:**

74 NO_2 = Nitrogen dioxide; INMA = INfancia y Medio Ambiente (Environment and

75 Childhood); HRT(SE)= standard error of the hit reaction time; CI = Confidence

76 Interval; PM = particulate matter; PM2.5= particulate matter of diameter less than 2.5

77   µm; ADHD= Attention deficit and hyperactivity disorder; HRT= hit reaction time;

78   IQ=intelligence quotient.

79

80   **Abstract**

81   **Background:** Prenatal and postnatal exposure to air pollution has been linked to

82   cognitive impairment in children, but very few studies have assessed its association with

83   attentional function.

84   **Objectives:** To evaluate the association between prenatal and postnatal exposure to

85   nitrogen dioxide (NO_2) and attentional function in children at 4-5 years of age.

86   **Methods:** We used data from four regions of the Spanish INMA—Environment and

87   Childhood—Project, a population-based birth cohort. Using land-use regression models

88   (LUR), we estimated prenatal and postnatal NO_2 levels in all of these regions at the

89   participants' residential addresses. We assessed attentional function using the Kiddie-

90   Conners Continuous Performance Test (K-CPT). We combined the region-specific

91   adjusted effect estimates using random-effects meta-analysis.

92   **Results:** We included 1,298 children with complete data. Prenatal exposure to NO_2 was

93   associated with an impaired standard error of the hit reaction time (HRT(SE)) (increase

94   of 1.12 ms [95%CI; 0.22 a 2.02] per $10\mu g/m^3$ increase in prenatal NO_2) and increased

95   omission errors (6% [95%CI; 1.01 to 1.11] per $10\mu g/m^3$ increase in prenatal NO_2).

96   Postnatal exposure to NO_2 resulted in a similar but borderline significant increase of

97   omission errors (5% [95%CI;=0.99 to 1.11] per $10\mu g/m^3$ increase in postnatal NO_2).

98   These associations did not vary markedly between regions and were mainly observed in

99   girls. Commission errors and lower detectability were associated with prenatal and

100   postnatal exposure to NO_2 only in some regions.

101   **Conclusions:** This study indicates that higher exposure to ambient NO_2, mainly during

102   pregnancy and to a lesser extend postnatally, is associated with impaired attentional

103   function in children at 4-5 years of age.

1. Introduction

Air pollutants, and especially particulate matter (PM) and its soluble components, can become deposited in the alveolar region of the lungs, thereby causing oxidative stress and local-systemic inflammation (Block et al. 2012). They can also translocate into the systemic circulation, thereby reaching other organs, such as the brain, where they can activate microglia (Block et al. 2012). Microglia induce an inflammatory cascade, and when this activation is chronic and excessive, it can result in neurotoxicity and can initiate and/or amplify neuronal damage (Block et al. 2012). Fetal life and infancy is characterized by periods of rapid growth, cell differentiation, organogenesis, and development of the brain's neural network (Rice and Barone 2000), so this is a key window of vulnerability to the harmful effects of exposure to air pollution. Air pollution exposure during pregnancy can impair brain development through oxidative stress and systemic inflammation (Romieu et al. 2008). It can also harm the fetus' general health, resulting in smaller size for gestational age and lower birth weight (Heinonen et al. 2010), or decreased placental–fetal exchange of oxygen and nutrients by binding PM to receptors for placental growth factors (Kannan et al. 2006). Prenatal exposure to particulate matter of diameter <2.5 µm (PM2.5) has also been associated with reduced expression of two genes implicated in normal neurodevelopmental trajectories (Saenen et al. 2015), and we showed that exposure to air pollution impairs certain neuropsychological functions in both humans and animals (Guxens and Sunyer 2012; Suades-González et al. 2015).

Attention deficit and hyperactivity disorder (ADHD) is the most common mental disorder in children (CDC 2016). Children with ADHD generally have difficulty in paying attention and controlling impulsive behaviors (i.e. acting without thinking about the results of their actions), and they can be over-active (CDC 2016). Studies of the

129   impact of prenatal and postnatal air pollution levels on ADHD diagnoses and symptoms

130   have had conflicting results (Abid et al. 2014; Forns et al. 2015; Gong et al. 2014;

131   Newman et al. 2013; Perera et al. 2006, 2011, 2012, 2014; Siddique et al. 2011).

132   However, only one study assessed prenatal air pollution levels (Chiu et al 2016), and

133   only a few studies have examined how postnatal air pollution levels could affect

134   attentional function (Chiu et al. 2013; Kicinski et al. 2015; Sunyer et al. 2015; van

135   Kempen et al. 2012; Wang et al. 2009). Attentional function is a complex cognitive

136   function that encompasses several processes, including capacity to focus on a stimulus

137   over a period of time while ignoring other perceivable information, to absorb this

138   stimulus, and then report it back immediately (White et al. 2009). Assessing attentional

139   function in a more objective and precise way could hence provide a better

140   understanding of its potential relationship with air pollution exposure.

141   The aim of the present study was, therefore, to assess whether prenatal and

142   postnatal exposure to nitrogen dioxide (NO_2) could be associated with altered

143   attentional function in children at 4-5 years of age.

144

145   ## 2. Methods

146   ### 2.1. Study design and participants

147   The INMA Project is a prospective population-based birth cohort established in seven

148   regions of Spain following a common protocol (Guxens et al. 2012b). In the current

149   study, we used data from the INMA regions of Valencia, Sabadell (Catalonia), Asturias,

150   and Gipuzkoa (Basque-Country), where the cohorts were established between 2003 and

151   2008. Pregnant women who met the inclusion criteria (age ≥16years; intention to

152   deliver at the reference hospital; no communication problems; singleton pregnancy; no

153   assisted conception) were enrolled at public primary health care centers or public

154   hospitals during the 1st trimester of their pregnancies Ultimately, 56% of the women

155   who were invited to participate agreed to do so, and were followed up throughout their

156   pregnancies, until delivery. Their children were followed up from birth until 4-5 years

157   of age. A total of 2,764 women were enrolled, and 2,157 (78%) children participated in

158   the 4-5 years follow-up (see Figure 1). We included 1,298 children for whom data on

159   the exposure and outcome variables were available. Most losses to follow-up were due

160   to the late inclusion of the attentional function assessment at the 4-5 years follow-up, at

161   which time some children had already been visited. All participants gave informed

162   written consent, and the study was approved by the ethics committees of the hospitals in

163   each study region.

164

165   **2.2. Assessment of the exposure to NO$_2$**

166   NO$_2$ was measured using passive samplers distributed throughout the study areas

167   according to geographic criteria (see Supplemental Material, Table S1) (Estarlich et al.

168   2011). The samplers remained exposed during several 7-day sampling periods within a

169   year. NO$_2$ levels were measured during pregnancy for all cohorts, and at 4 years in the

170   Gipuzkoa and Valencia cohorts. We used land-use regression models (LUR) models to

171   estimate NO$_2$ levels at each participant's residential address during pregnancy and from

172   birth until the neuropsychological assessment using a geographic information system

173   for each considered exposure period.

174   NO$_2$ estimates obtained from LUR models were translated to individual

175   estimates for specific periods (prenatal and postnatal) by combining one or two maps,

176   depending of the cohort and the period, by the mean daily levels from monitoring

177   network stations. Measurements from different periods were combined as arithmetic

178   averages, thus providing the annual average concentration for each site. For all

179   estimations and for each period, changes in home addresses were taken into account.

180

181   **2.3. Children's attentional function**

182   Attentional function was measured using the 2nd edition of the Conners Kiddie

183   Continuous Performance Test (K-CPT) (MHS 2006). The K-CPT is a 7.5-minute

184   computerized test that evaluates inattention, impulsivity, sustained attention, and

185   vigilance in children aged 4-7 years. Children were individually tested in a quiet room

186   by trained investigators.Children are instructed to push the space bar as quickly as

187   possible when they see an image on the computer screen (the target), except if the image

188   portrayed a ball (the non-target). The primary outcomes of interest were: the number of

189   omission errors (i.e. the number of targets to which the test subject failed to respond);

190   the number of commission errors (i.e. the number of times that the individual

191   responded erroneously to a non-target); the hit reaction time (HRT, the mean response

192   time -expressed in milliseconds- for all correct target hits during the entire test); the

193   standard error of the hit reaction time (HRT(SE), the standard error of the reaction time

194   for responses to target hits), and the detectability or attentiveness (d', a measure of the

195   difference between the proportion of correct target hits out of the total number of targets

196   and the proportion of individual failures to respond to a non-target out of the total

197   number of non-targets) (MHS 2006). Omission errors reflect poorer orientation and a

198   slower response. When combined with commission errors, a fast HRT reflects

199   impulsivity; while, when combined with omissions and/or commission errors, a slow

200   HRT is indicative of inattention. HRT(SE) indicates the consistency of the response

201   time, such that high values indicate erraticness and inattention. Detectability reflects the

202 subject's perceptual sensitivity to targets, or how well they discriminate between targets

203 and non-targets.

204

205 **2.4. Potential confounding variables**

206 To decide which potential cofounding variables should be included in our models, we

207 drew a direct acyclic graph (DAG) (Hernán et al. 2002) based on up-to-date published

208 evidence (Supplemental Material, Figure S1). Questionnaires were administered during

209 pregnancy to obtain information on: maternal and paternal educational level; maternal

210 and paternal age; parents' social class defined by their occupation during the pregnancy

211 according to the Spanish adaptation of ISCO88; maternal and paternal countries of

212 birth; maternal height and pre-pregnancy weight; paternal body mass index; maternal

213 smoking and exposure to second hand smoke; maternal alcohol use; maternal

214 consumption of fish, fruit, vegetables, vitamin D, and folic acid; maternal noise

215 annoyance; and household gas appliances (cooking stove and heating. Maternal

216 circulating blood levels of vitamin D (25-hydroxyvitamin D3 [25 (OH) D]) were

217 measured during the first trimester. The child's sex was recorded according to the

218 clinical records. When the children were 4-5 year old, a questionnaire was administered

219 to collect information on the number of siblings; maternal mental health (assessed using

220 the Symptom Checklist-90-R questionnaire, with higher scores indicating poorer mental

221 health status); and maternal verbal intelligence quotient (IQ) (using the Wechsler Adult

222 Intelligence Scale, third edition, with higher scores indicating higher verbal IQ). All

223 questionnaires were administered in person by trained interviewers. The child's age at

224 the time of the attentional function test was recorded in days. Lastly, we recorded

225 urbanicity at the place of residence during pregnancy and during the postnatal period

226 (e.g. urban versus rural).

227

2.5. Statistical analyses

In children for whom we had data on exposure and outcome variables (n=1,298), we performed multiple imputation of missing values for the potential confounding variables using chained equations: we generated 25 complete datasets and analysed them using standard combination rules for multiple imputation (Spratt et al. 2010; Sterne et al. 2009). The distributions of the imputed data were similar to those of the directly collected data (data not shown).

Of the children recruited initially, those with available exposure and outcome data (n=1,298) were more likely to have mothers from a higher socioeconomic position than those without available exposure and outcome data (n=1,466) (see Supplemental Material, Table S2). We used inverse probability weighting to correct for loss to follow-up, i.e. to account for potential selection bias that results from using participants with data in the last visit as opposed to the full cohort with data during pregnancy (Weisskopf et al. 2015; Weuve et al. 2012). Briefly, we used information for all participants at recruitment to predict the probability of participating in the study, and used the inverse of these probabilities to weight the analysis so that results would be representative of the initial cohort.

We used linear regression models to test for association between prenatal and postnatal NO_2 exposure and HRT, HRT(SE), and detectability, and binomial negative regression models to test for association between prenatal and postnatal NO_2 exposure and omission and commission errors. We could not include both prenatal and postnatal NO_2 levels in the models since they were highly correlated (0.86 in Valencia, 0.71 in Sabadell, 0.92 in Asturias, and 0.58 in Gipuzkoa). First, the models were adjusted for the child's sex and age at the time of the attentional function test (minimally adjusted

252 models). Second, the models were further adjusted for all potential confounding

253 variables described above (fully-adjusted models). We used generalized additive models

254 to assess the linearity of the relationship between prenatal and postnatal NO_2 levels and

255 each attentional function outcome, using fully-adjusted models by graphical

256 examination and deviance comparison.

257 We used a two-stage approach: i) we analyzed associations separately for each

258 region and ii) we used random-effects meta-analysis to combine region-specific effect

259 estimates from regression models. We assessed heterogeneity in the estimates using the

260 Q test and the I^2 statistic.

261 We performed additional meta-analyses stratified by the sex of the child.

262 Statistical hypothesis tests were two-tailed, with significance set at 0.05. All analyses

263 were performed using STATA (version 13; Stata Corporation, College Station, TX,

264 USA).

265

3. Results

267 Table 1 shows the characteristics of the mothers recruited in each region. Women

268 exposed to higher levels of NO_2 during pregnancy were more likely to be younger, less

269 well-educated, to consume more alcohol during the pregnancy, and to live in urban

270 areas, compared to women exposed to lower levels of NO_2 (Supplemental Material,

271 Table S3).

272 Table 2 shows the results for NO_2 exposure and attention function in each

273 region. The mean prenatal NO_2 level was 31.1 μg/m^3 (ranging from 18.4 to 37.9 μg/m^3

274 between regions), while the mean postnatal NO_2 level was 25.6 μg/m^3 (ranging from

275 19.5 to 35.2 μg/m^3 between regions). Children from the Valencia region were older and

276 had lower HRT and HRT(SE), and fewer omission errors (Table 2). Impaired

277   attentional function, revealed mainly by HRT(SE), was associated with lower

278   socioeconomic status (data not shown).

279   In the fully adjusted models, higher exposure to prenatal levels of NO_2 was

280   associated with a 1.12 ms increase in HRT(SE) (95% confidence interval [CI] =0.22 to

281   2.02) and a 6% increase in the number of omission errors (95%CI=1.01 to 1.11) per

282    $10\mu g/m^3$ increase in prenatal NO_2 (Table 3). For postnatal NO_2 levels, we found a

283   similar but borderline significant association with the number of omission errors (5%

284   more omission errors (95%CI=0.99 to 1.11) per $10\mu g/m^3$ increase in postnatal NO_2

285   levels). These associations did not vary markedly between regions (Figure 2). In

286   contrast, we only found an association between pre- and postnatal NO_2 levels and a

287   higher number of commission errors in the regions of Gipuzkoa and Asturias regions;

288   and lower detectability in the Gipuzkoa region (p for interaction = 0.02) (Figure 2).

289   Stratifying the analysis by sex, the observed association between higher exposure to

290   pre- and postnatal NO_2 levels and a higher number of omission errors only persisted in

291   girls (6% (95%CI=0.99 to 1.14) and 8% (95%CI=1.00 to 1.17) per $10\mu g/m3$ increase in

292   prenatal and postnatal NO_2 levels, respectively) (Table 3). The minimally-adjusted

293   analysis showed similar associations overall (Table S4 and Supplementary Figure S2).

294

295   **4. Discussion**

296   In this study we assessed the association between prenatal and postnatal exposure to

297   residential NO_2 air pollution and attentional function in children at 4-5 years of age. We

298   found that prenatal NO_2 levels impair two attentional function outcomes, namely

299   HRT(SE) and omission errors, which are both indicative of inattentiveness (Egeland

300   and Kovalik-Gran 2010). We found a similar association between postnatal NO_2 levels

301   and omission errors, and that these associations between pre- and postnatal NO_2

302 exposure and omission errors were predominantly observed in girls. In two regions,

303 Gipuzkoa and Asturias, higher pre- and postnatal NO_2 levels were also associated with

304 impairment of two further attentional function outcomes, detectability and commission

305 errors.

306   The majors strengths of this study are the use of standardized air pollution

307 assessment in all regions; the prospective design; and exposure assessment during two

308 crucial periods, namely pregnancy and early life. Additional strengths are the

309 assessment of attentional function using a computerized test, which provides an

310 objective measure that allows us to detect small changes in vigilance/attention control

311 and inhibition responses in young children. Another strength is the fact that we adjusted

312 for many socioeconomic and lifestyle variables that are known to confound the

313 association between prenatal and postnatal air pollution exposure and attentional

314 function in children.

315   A limitation of our study is that we had no data on air pollution exposure at the

316 children's daycare centers and schools, which could have an important influence on the

317 children's neuropsychological development given that they spend a lot of time at these

318 sites on weekdays (Sunyer et al. 2015). Another limitation of the study was that we only

319 had data on self-reported noise annoyance during pregnancy, as a proxy for exposure to

320 noise pollution. Air and noise pollution mainly come from motor vehicle traffic, and

321 some studies have reported an association between road traffic noise and cognition in

322 children (Clark and Stansfeld 2007). We adjusted for noise annoyance in our study, but

323 we cannot fully rule out a possible residual confounding by noise exposure.

324   To our knowledge only one previous study has assessed the association of

325 prenatal exposure to air pollution, assessed during each week of pregnancy, and

326 attentional function in children aged 6-7 years (Chiu et al. 2016a). They found that

327 higher PM2.5 levels at the end of pregnancy was associated with poorer attentional

328 function in boys and poorer memory in girls (Chiu et al. 2016). In contrast, we observed

329 in the current study that NO_2 was associated with poorer attentional function

330 predominantly in girls, although we assessed this association in younger children. The

331 stronger effect in girls may be due to some biological mechanism, such as sex

332 hormones, which could modulate visual temporal attention in girls (Kranczioch et al.

333 2016) or module their response to pro-inflammatory exposures such as air pollution

334 (Melcangi et al. 2008). Moreover, female foetuses have been reported to be more

335 vulnerable to certain suboptimal intrauterine environments, and to be particularly

336 susceptible to attention difficulties in childhood (Murray et al. 2015). Further studies

337 with larger samples are needed to better understand the sex-differences in the

338 association between air pollution exposure and child neuropsychological development.

339 This is especially important given the marked differences between girls and boys in

340 terms of attention, brain development, and the effects of air pollution (Chiu 2016).

341   Regarding postnatal exposure to air pollution, exposure to traffic pollution have

342 been shown to affect sustained attention in adolescents aged 14-16 years (Kicinski et al.

343 2015), and balck carbon to impair attentional function in 7- to 14-year-old boys (Chiu et

344 al. 2013). A study in 8- to 10-year-old children also found that exposure to higher levels

345 of NO_2 was associated with impaired attentional function (Wang et al. 2009). We

346 recently reported similar findings, namely that exposure to traffic-related air pollutants

347 at school (i.e. elemental carbon, NO_2, and ultrafine particles) is associated with

348 inattentiveness in 7- to 10-year-olds (Sunyer et al. 2015). In contrast, a previous study

349 found no association between NO_2 levels at school and attentional function in 9- to 11-

350 year-olds (van Kempen et al. 2012).

351   In general, we interpret NO_2 as a marker of traffic, rather than as just a toxicant.

352 Note that attentional function at this age is crucial for achieving proper

353 neuropsychological development (Garon et al. 2008). In relation to prenatal exposure,

354 our results, and those of Chiu et al. (2016a), show that the association with prenatal

355 exposure is stronger and more homogeneous than for postnatal exposure, so prenatal

356 exposure to air pollution appears to be the main driver of impaired attentional function.

357 However, we cannot completely dissociate prenatal and postnatal air pollution

358 exposures because they are strongly correlated. Future improvements in our air

359 pollution models, combining land use variables and satellite remote sensing data, will

360 allow us to better estimate the differencial effects of shorter time windows of exposure

361 (Chiu et al. 2016b). There may be various biological mechanisms through which

362 prenatal, as opposed to postnatal, exposure to air pollution impairs brain development in

363 children. Prenatal brain development may be impaired by damage to the organ itself as a

364 result of oxidative stress and systemic inflammation (Romieu et al. 2008), or by

365 inhibited fetal growth, among other mechanisms (Heinonen et al. 2010). Regarding

366 postnatal exposure, in addition to systemic inflammation (Romieu et al. 2008),

367 pollutants may reach the brain directly through the nose and olfactory bulb (Minn et al.

368 2002), thereby directly damaging the prefrontal cortex.

369   We found that NO_2 levels were associated with a higher number of commission

370 errors and impaired detectability, specifically in the Gipuzkoa and Asturias regions.

371 These regions are situated on the Atlantic coast, while the other two regions, Valencia

372 and Sabadell, are situated on the Mediterranean coast. Precipitation maps show much

373 higher rainfall on the Atlantic coast than the Mediterranean coast, which may explain

374 the regional variation in NO_2 levels observed in our study, given the known effects of

375 rainfall on air pollution parameters (Guo et al. 2016). Also, rainfall can regulate

376 nitrification inhibitors used in agriculture, leading to lower NO_2 emissions under

377 semiarid Mediterranean conditions (Abalos et al. 2017). Despite this, we observed

378 lower NO_2 levels in the Atlantic areas than in the Mediterranean areas, probably

379 because of lower traffic congestion in the former. In a previous study of the same

380 regions (Guxens et al. 2012a), we also observed that the association between prenatal

381 exposure to residential air pollution and mental development at a younger age was only

382 observed in the Atlantic regions.

383   In this study, we assessed attentional function using a standardized,

384 computerized neuropsychological test. In contrast, other studies have assessed the

385 relationship between air pollution exposure and ADHD symptoms or diagnosis using

386 information reported mainly by the parents, and have yielded inconsistent results (Abid

387 et al. 2014; Forns et al. 2015; Gong et al. 2014; Newman et al. 2013; Perera et al. 2006,

388 2011, 2012, 2014; Siddique et al. 2011). The late age-onset of the inattentive subtype of

389 ADHD in children (Applegate et al. 1997) could partly explain these contrasting results

390 for ADHD, while it has been reported that the performance of the computerized test

391 used in our study improves during childhood (Mani et al. 2005). A remaining question

392 is whether exposure to air pollution is simply related with impaired attentional function,

393 as we observed in our study (with all the negative consequences that this can entail), or

394 whether it may also drive the development of inattention and hyperactivity symptoms,

395 or the appearance of ADHD.

396

5. Conslusions

398   In conclusion, this study shows that higher exposure to NO_2 during pregnancy is

399 associated with impaired attentional function, especially increased inattentiveness, in

400 children aged 4-5 years. Our data suggest that postnatal exposure to NO_2 is also

401 associated increased inattentiveness, although we could not completely dissociated the

402 effects of pre- and postnatal exposure because they are highly correlated. These

403 associations were more pronounced in girls. In this sample, for each 1-month increase in

404 the children's age, we observe a general decrease in HRT(SE) of 0.76 ms,

405 corresponding to their improving attentional function. In comparison, we observed a

406 1.12 ms increase in HRT(SE) for each $10\mu g/m^3$ increase in prenatal NO_2 exposure,

407 which represents an almost 2-month delay in the development of attentional function.

408 The clinical impact of this increase in HRT(SE) is unknown, but it could have

409 implications for the whole population, especially because of the ubiquity of the

410 exposure. Reduced attentional function in the population could lead to poorer

411 educational indicators, thus affecting the population's productivity at subsequent stages

412 (Das et al. 2012; Plamondon and Martinussen 2015).

416

417   **Appendix A. Supplemental Material**

418   Supplemental Material to this article can be found online.

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584 **Figure Legend**
585

Figure 1. Flowchart illustrating the main stages of the study
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590 **Figure 2. Forest plots of fully adjusted association between NO_2 exposure during**

591 **pregnancy and attentional function outcomes. Region-specific and summary risk**

592 **estimates from random effects analysis (coefficient/IRR and 95% CI) for: HRT**

593 **(A), HRT(SE) (B), Detectability (C), Omission errors (D), and Commission errors**

594 **(E) for each 10 μg/m³ increase in NO_2 level**

595 P-value from Heterogeneity test (p-heter)/I^2 index to quantify the degree of

596 heterogeneity in a meta-analysis (I^2(%)) for   prenatal NO_2 exposure (overall) to

597 HRT=0.92/0,   HRT(SE)=0.92/0,   Detectability=0.02/69.5,   Omissions=0.65/0,

598 Commissions=0.02/69.7. Models were adjusted for several cofounding variables (see

599 fully adjusted model in methods section).

Table 1. Maternal characteristics of the study population by region.

	Valencia (n=524)	Sabadell (n=274)	Asturias (n=241)	Gipuzkoa (n=259)
Educational level during pregnancy				
University	28.4	36.2	38.6	50.6
Secondary	43.9	38.8	45.2	38.6
Primary	27.7	25.0	16.2	10.8
Social Class during pregnancy				
I/II managers/technicians	19.1	23.2	24.1	32.0
III skilled manual/non-manual	26.7	31.9	23.2	28.6
IV/V semiskilled/unskilled	54.2	44.9	52.7	39.4
Country of birth (Spain vs. elsewhere)	92.2	90.9	97.9	97.3
Residence urbanicity during prengancy (Rural vs. urban)	15.3	0.0	7.0	47.9
Age at child's birth (years)	30.5 (4.1)	30.5 (4.2)	31.9 (4.1)	31.3 (3.3)
Pre-pregnancy body mass index				
Normal weight/underweight	71.0	78.3	70.1	79.9
Overweight	19.8	15.2	23.2	15.4
Obese	9.2	6.5	6.6	4.6
Smoking during pregnancy (yes vs. no)	38.2	27.4	27.0	20.5
Alcohol consumption during pregnancy				
No consumption	73.8	74.3	90.9	81.1
Below the median[a]	15.3	14.1	2.1	12.0
Above the median[b]	10.9	11.6	7.0	6.9

Values are percentages for categorical variables and mean ± SD for continuous variables

[a]0.01/0.938 servings/week

[b]>0.939/maximum servings/week

Table 2. Distribution of NO$_2$ levels and attentional function scores by region.

	Valencia (n=524)	Sabadell (n=274)	Asturias (n=241)	Gipuzkoa (n=259)
NO$_2$ levels (μg/m³)				
Prenatal	37.9 ± 11.8	37.7 ± 10.3	22.7 ± 7.5	18.4 ± 6.2
Postnatal	31.0 ± 9.9	35.2 ± 8.0	21.0 ± 6.6	19.5 ± 5.7
Attentional Function Scores				
Hit reaction time (milliseconds)	676.3 ± 103.9	734.3 ± 121.7	775.2 ± 161.1	763.2 ± 145.4
Hit reaction time standard error (milliseconds)	24.9 ± 12.5	35.5 ± 13.3	34.5 ± 15.6	33.9 ± 15.8
Detectability (no units)	0.6 ± 0.4	0.6 ± 0.4	0.7 ± 0.5	0.6 ± 0.5
Omission errors (number)	15 (13, 82)	27 (17, 15)	27 (20.81)	34 (22, 12)
Commission errors (number)	22 (9, 51)	23 (10, 98)	20 (13, 32)	19 (10, 63)
Age at attentional function assessment (years)	5.77 ± 0.16	4.53 ± 0.17	4.43 ± 0.23	4.47 ± 0.10

Values are mean ± SD for normally distributed continuous variables and median (interquartile range) for non-normally distributed continuous variables.

Table 3. Fully-adjusted combined association between prenatal and postnatal NO$_2$ (overall and by sex) and attentional function[a].

Prenatal NO$_2$ (per Δ 10 μg/m³)	N	β	HRT (95% CI)	β	HRT(SE) (95% CI)	β	Detectability (95% CI)	IRR	Omissions (95% CI)	IRR	Commissions (95% CI)
Overall	1,298	-1.12	(-9.00; 6.75)	1.12	(0.22; 2.02)	-0.03	(-0.09; 0.03)	1.06	(1.01; 1.11)	1.04	(0.97; 1.12)
By sex											
Girls	648	2.07	(-9.63; 13.77)	0.47	(-0.51; 1.45)	-0.02	(-0.08; 0.04)	1.06	(0.99; 1.14)	1.00	(0.96; 1.05)
Boys	650	-0.85	(-12.06; 10.37)	1.23	(-0.22; 2.68)	-0.00	(-0.07; 0.07)	0.99	(0.89; 1.11)	1.01	(0.93; 1.10)
Postnatal NO$_2$ (per Δ 10 μg/m³)											
Overall	1,298	-4.70	(-13.80; 4.39)	0.81	(-0.82; 2.43)	-0.03	(-0.09; 0.03)	1.05	(0.99; 1.11)	1.04	(0.96; 1.13)
By sex											
Girls	648	1.97	(-11.53; 15.46)	0.34	(-0.80; 1.49)	0.01	(-0.03; 0.05)	1.08	(1.00; 1.17)	0.99	(0.94; 1.04)
Boys	650	-7.82	(-21.01; 5.38)	0.09	(-2.38; 2.55)	-0.01	(-0.08; 0.05)	0.96	(0.85; 1.10)	1.03	(0.94; 1.14)

β = beta coefficient. 95% CI= 95% confidence interval. IRR= Incidence-rate ratio. HRT = Hit reaction time (ms). HRT(SE) = Hit reaction time standard error (ms). Omissions = Omission errors (n). Commissions = Commission errors (n). NO$_2$ = Nitrogen dioxide. Δ = per each increase of 10 μg/m³.

P-value from Heterogeneity test (p-heter)/ I² index to quantify the degree of heterogeneity in a meta-analysis (I²(%)) for prenatal NO$_2$ exposure (overall) to HRT =0.92/0, HRT(SE) =0.92/0, Detectability= 0.02/69.5, Omissions =0.65/0, Commissions = 0.02/69.7.

p-heter/I²(%) for prenatal NO$_2$ exposure in girls to HRT =0.62/0, HRT(SE) =0.82/0, Detectability= 0.22/30.6, Omissions =0.81/0, Commissions = 0.44/0.

p-heter/I²(%) for prenatal NO$_2$ exposure in boys to HRT =0.71/0, HRT(SE) =0.72/0, Detectability= 0.09/53.49, Omissions =0.12/49.03, Commissions = 0.03/66.9.

p-heter/I²(%) for postnatal NO$_2$ exposure (overall) to HRT =0.54/0, HRT(SE) = 0.15/43.8, Detectability=0.05/62.8, Omissions =0.78/0, Commissions = 0.01/71.1.

p-heter/I²(%) for postnatal NO$_2$ exposure in girls to HRT =0.43/0, HRT(SE) =0.95/0, Detectability= 0.43/0, Omissions =0.76/0, Commissions = 0.45/0.

p-heter/I²(%) for postnatal NO$_2$ exposure in boys to HRT =0.69/0, HRT(SE) =0.17/39.6, Detectability= 0.22/32.3, Omissions =0.11/49.6, Commissions = 0.05/61.2.

[a]Estimated associations by random-effects meta-analysis by region. Models were adjusted for several cofounding variables (see fully adjusted model in methods section).
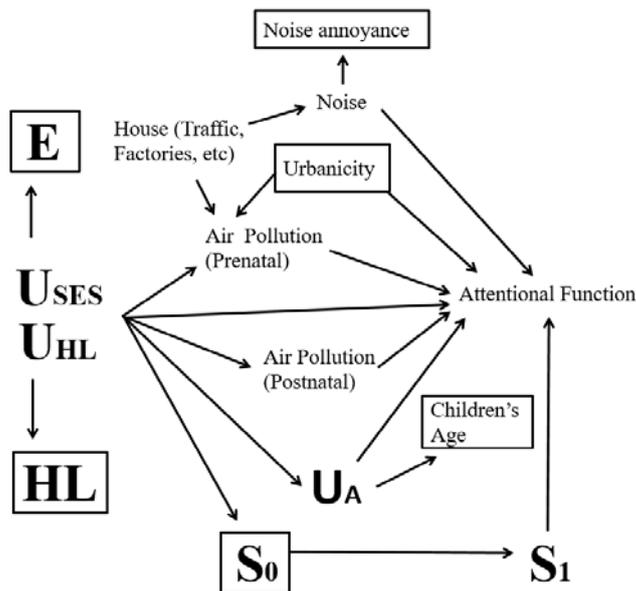
Supplementary data

Prenatal and Postnatal Exposure to NO$_2$ and Child Attentional Function at 4-5 Years of Age

Alexis Sentís, Jordi Sunyer, Albert Dalmau-Bueno, Ainara Andiarena, Ferran Ballester, Marta Cirach, Marisa Estarlich, Ana Fernández-Somoano, Jesús Ibarluzea, Carmen Íñiguez, Aitana Lertxundi, Adonina Tardón, Mark Nieuwenhuijsen, Martine Vrijheid, and Mònica Guxens on behalf of the INMA Project

Table of contents

Figure S1. Direct acyclic graph (DAG)

E: proxy variables for the unmeasured socioeconomic status of the parents (i.e. parental educational level, parental social class, parental country of birth, parental age, maternal intelligence coefficient, maternal mental health, child's number of siblings)

HL: proxy variables for unmeasured maternal and paternal health and life-style (i.e. maternal fish, fruits, and vegetables consumption during pregnancy, maternal pre-pregnancy body mass index, paternal body mass index, maternal folic acid and vitamin D levels during pregnancy, maternal smoking and alcohol consumption during pregnancy, maternal second hand smoking during pregnancy, and housesold gas appliances (cooking stove and heating) during pregnancy)

S_0: selection into the cohort

S_1: selection into the analysis

U_A: unmeasured variables related to the time the child comes to the attentional function assessment (i.e. child's sex and child's age at attentional function assessment)

U_{UHL}: unmeasured variables related to maternal and paternal health and life-style

U_{SES}: unmeasured variables related to socioeconomic status

Models were adjusted or conditioned by the boxed variables

Figure S2. **Forest plots of minimally-adjusted association between postnatal NO$_2$ exposure and attentional function outcomes. Region-especific and summary risk estimates from random effects analysis (coefficient/IRR and 95% CI) for: HRT (A), HRT(SE) (B), Detectability (C), Omission errors (D), and Commission errors (E) for each 10 µg/m³ increase in postnatal NO$_2$ level**

A. HRT
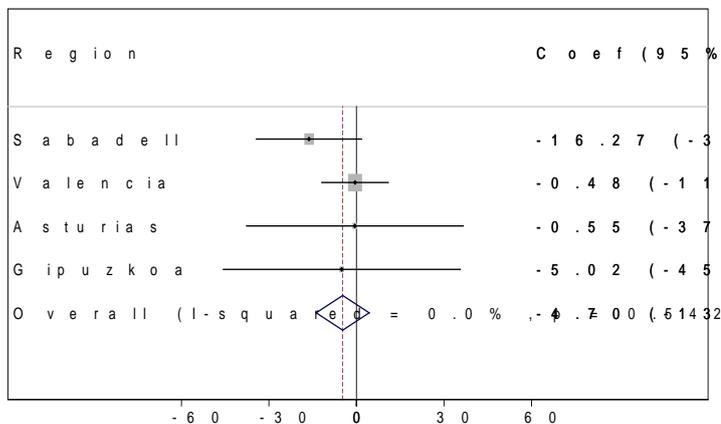
B. HRT (SE)
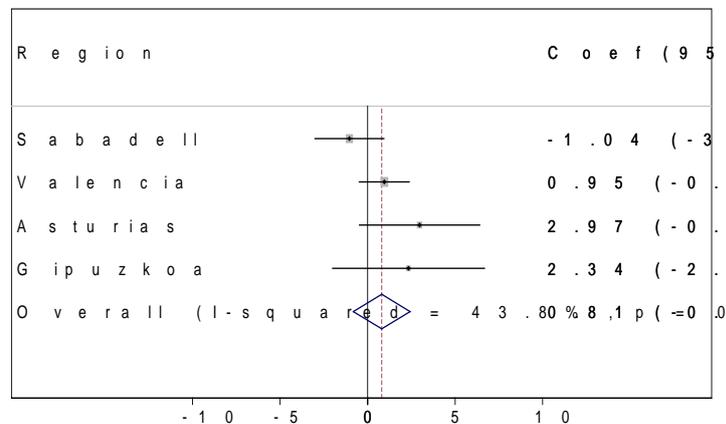
C. Detectability
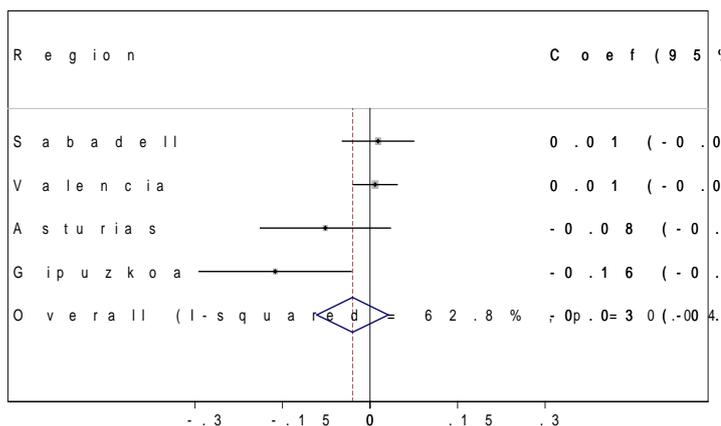
D. Omissions errors
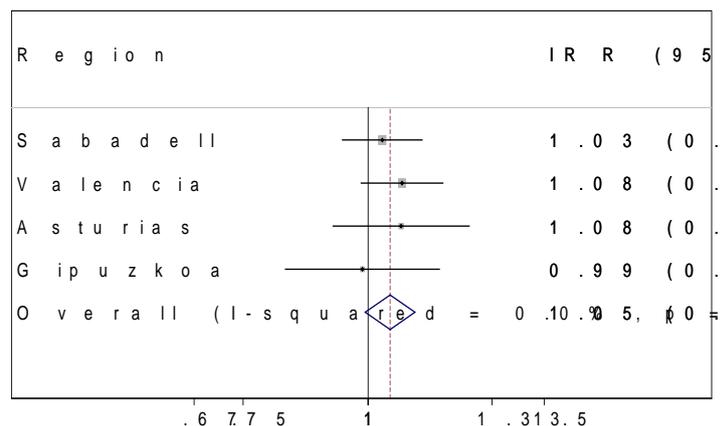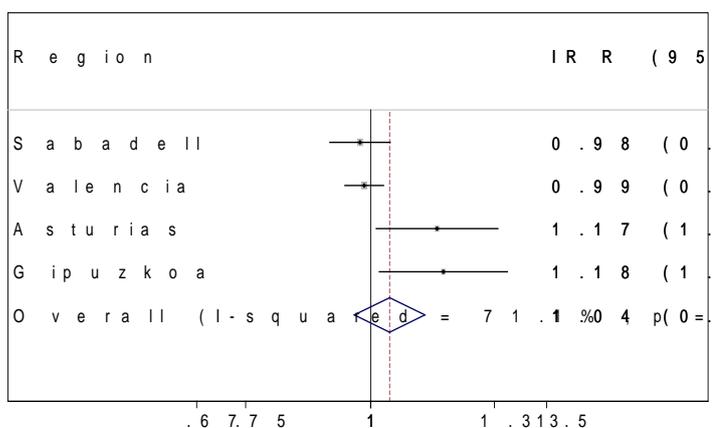

E. Commission errors

P-value from Heterogeneity test (p-heter)/I² index to quantify the degree of heterogeneity in a meta-analysis (I²(%)) for postnatal NO$_2$ exposure (overall) to HRT=0.54/0, HRT(SE)=0.15/43.8, Detectability=0.05/62.8, Omissions=0.78/0, Commissions= 0.01/71.1. Models were adjusted for child's sex and child's age at the time of the attentional function assessment.

Table S1. Description of the region-specific LUR models for NO$_2$

Region	Period	no. passive samplers	Date campaigns	Predictor variables	R^2 model	Number of monitoring stations	Pollutant used to correct for seasonality
Asturias	During pregnancy	67	June 05 November 05	Altitude Distance to nearest major road Agricultural or forest land cover (300 m-buffer)	0.521	4	NO$_2$
Gipuzkoa	During pregnancy	79	February 07 June 07	Altitude (3 categories) Valley factor Distance to nearest major road (MDI>20000) Urban land cover (100 m-buffer) Industrial land cover (300 m-buffer)	0.509	3	NO$_2$
	At 4-5 years	70	November 2012	Valley factor Distance to nearest major road (MDI>20000) Urban land cover (100 m-buffer) Industrial land cover (300 m-buffer)	0.570	3	NO$_2$
Sabadell	During pregnancy	57	April 05 June 05 October 05 March 06	Altitude Urban or industrial land cover (500 m-buffer) Road tipe	0.750	1	NO$_2$
Valencia	During pregnancy	93	April 04 June 04 November 04 February 05	Kriging[a] Industrial or urban land cover (500 m-buffer) Distance to nearest major road (MDI=10000)	0.730	4	NO$_2$
	At 4-5 years	98	February 09 April 09 July 09	Universal kriging with covariates: Major road length of all roads in a buffer of 300m Industrial land use in a buffer of 100m High density residential land use in a buffer of 1000m	0.790	4	NO$_2$

MDI: Mean daily traffic count
[a]Mean of estimated NO$_2$ from kriging among campaigns

Table S2. Comparison of maternal, paternal, and child characteristics of included subjects during pregnancy to those of non-included subjects, out of 2,764 eligible individuals

	Subjects included (n=1,298)	Subjects not included (n=1,466)	P-value of the difference
Maternal characteristics			
Educational level during pregnancy			<0.001
University	36.3	30.8	
Secondary	42.1	41.2	
Primary	21.6	28.0	
Social class during pregnancy			<0.001
I/II managers/technicians	23.5	19.2	
III skilled manual/non-manual	27.5	23.9	
IV/V semiskilled/unskilled	49.0	56.9	
Country of birth (Spain vs. elsewhere)	94.0	87.8	<0.001
Residence urbanicity during pregnancy (rural vs. urban)	17.0	19.1	0.167
Age at child's birth (years)	30.9 (4.0)	30.2 (4.8)	<0.001
Pre-pregnancy body mass index			0.275
Normal weight/underweight	74.2	72.2	
Overweight	18.6	18.9	
Obese	7.2	8.9	
Smoking during pregnancy (yes vs. no)	30.3	32.8	0.169
Alcohol consumption during the pregnancy			0.315
No consumption	78.6	80.8	
Below the median[a]	11.9	11.2	
Above the median[b]	9.5	8.0	
Paternal characteristics			
Educational level during pregnancy			0.726
University	21.2	20.7	
Secondary	43.6	42.6	
Primary	35.2	36.7	
Social class during pregnancy			0.068
I/II managers/technicians	20.3	18.7	
III skilled manual/non-manual	18.5	15.8	
IV/V semiskilled/unskilled	61.2	65.5	
Country of birth (Spain vs. elsewhere)	94.9	86.1	<0.001
Age at child's birth (years)	32.9 (4.8)	32.6 (5.3)	0.203
Child characteristics			
Sex (female vs. male)	49.9	46.9	0.121
Number of siblings at attentional function assessment			0.604
0	34.1	31.9	
1	57.3	59.2	
≥ 2	8.6	8.9	

Values are percentages for categorical variables and mean (SD) for continuous variables
[a]0.01/0.938 servings/week
[b]>0.939/maximum servings/week

Table S3. Association between prenatal NO$_2$ levels and potential confounding variables

	Prenatal NO$_2$ levels[a]			P-difference	P-trend
	Low	**Medium**	**High**		
Maternal characteristics					
Educational level during pregnancy				<0.001	<0.001
University	42.6	35.1	31.4		
Secondary	41.7	42.5	41.8		
Primary	15.7	22.4	26.8		
Social class during pregnancy				0.080	0.030
I/II managers/technicians	26.7	24.2	19.4		
III skilled manual/non-manual	27.7	25.2	29.8		
IV/V semiskilled/unskilled	45.6	50.6	50.8		
Country of birth (Spain vs. elsewhere)	97.0	94.5	90.5	<0.001	<0.001
Residence urbanicity during pregnancy (rural vs. urban)	39.4	9.0	2.5	<0.001	<0.001
Age at child's birth (years)	31.2 (3.7)	30.9 (4.1)	30.6 (4.3)	0.012	0.030
Pre-pregnancy body mass index				0.140	0.160
Normal weight/underweight	76.0	72.5	73.9		
Overweight	19.4	18.7	17.8		
Obese	4.6	8.8	8.3		
Smoking during pregnancy (yes vs. no)	27.2	31.2	33.3	0.143	0.072
Alcohol consumption during the pregnancy				0.009	<0.001
No consumption	82.0	80.8	72.8		
Below the median[b]	10.4	10.2	15.2		
Above the median[c]	7.6	9.0	12.0		
Paternal characteristics					
Educational level during pregnancy				<0.001	<0.001
University	24.0	21.2	18.0		
Secondary	48.6	42.5	39.7		
Primary	27.4	36.3	42.3		
Social class during pregnancy				0.642	0.060
I/II managers/technicians	22.4	20.1	18.5		
III skilled manual/non-manual	18.0	19.7	18.2		
IV/V semiskilled/unskilled	59.7	60.2	63.3		
Country of birth (Spain vs. elsewhere)	97.0	94.7	87.0	<0.001	<0.001
Age at child's birth (years)	33.6 (4.8)	32.8 (4.7)	32.2 (4.7)	0.780	<0.001

Values are percentages for categorical variables and mean (SD) for continuous variables
[a]Low levels = 3.8-24.5 μg/m³; medium levels = 24.5-36.8 μg/m³; and high levels = 36.8-74.2 μg/m³
[b]0.01/0.938 servings/week
[c]>0.939/maximum servings/week

Table S4. Minimally-adjusted combined associations between prenatal and postnatal NO$_2$ (overall and by sex) and attentional function[a]

Prenatal NO$_2$ (per Δ 10 µg/m³)	N	HRT β	(95% CI)	HRT(SE) β	(95% CI)	Detectability β	(95% CI)	Omissions IRR	(95% CI)	Commissions IRR	(95% CI)
Overall	1298	2.67	(-3.47; 8.82)	1.02	(0.31; 1.74)	-0.03	(-0.08; 0.02)	1.06	(1.02; 1.11)	1.03	(0.97; 1.08)
By sex											
Girls	648	5.91	(-3.36; 15.19)	0.50	(-0.63; 1.63)	-0.05	(-0.12; 0.03)	1.05	(0.99; 1.11)	1.04	(0.96; 1.13)
Boys	650	0.28	(-7.91; 8.46)	1.43	(0.36; 2.51)	-0.01	(-0.06; 0.04)	1.07	(1.00; 1.14)	1.00	(0.95; 1.07)
Postnatal NO$_2$ (per Δ 10 µg/m³)											
Overall	1298	0.87	(-6.27; 8.02)	0.73	(-0.12; 1.58)	-0.03	(-0.08; 0.02)	1.05	(1.00; 1.11)	1.04	(0.97; 1.12)
By sex											
Girls	648	5.80	(-4.94; 16.54)	0.18	(-0.78; 1.14)	-0.01	(-0.07; 0.05)	1.05	(0.98; 1.13)	1.04	(0.94; 1.15)
Boys	650	-2.82	(-12.47; 6.84)	0.93	(-0.94; 2.80)	-0.03	(-0.09; 0.03)	1.05	(0.97; 1.14)	1.02	(0.95; 1.10)

β = beta coefficient. 95%CI = 95% confidence interval. IRR= Incidence-rate ratio. HRT = Hit reaction time (ms). HRT (SE) = Hit reaction time standard error (ms). Omissions = Omission errors (n).
Commissions = Commission errors (n). NO$_2$ = Nitrogen dioxide.
p-value from Heterogeneity test (p-heter)/ I² index to quantify the degree of heterogeneity in a meta-analysis(%)(I²(%)) for prenatal NO$_2$ exposure (overall) to HRT = 1.00/0, HRT (SE) = 0.85/0, Detectability = 0.03/67.54, Omissions = 0.68/0, Commissions 0.07/56.74.
p-heter/I²(%) for prenatal NO$_2$ exposure in girls to HRT =0.87/0, HRT(SE) =0.32/14.07, Detectability= 0.03/65.87, Omissions =0.95/0, Commissions = 0.06/58.82.
p-heter/I²(%) for prenatal NO$_2$ exposure in boys to HRT =0.81/0, HRT(SE) =0.82/0, Detectability= 0.12/48.83, Omissions =0.33/13.16, Commissions = 0.12/49.16.
p-heter/I²(%) for postnatal NO$_2$ exposure (overall) to HRT = 0.72/0, HRT (SE) = 0.47/0, Detectability = 0.04/65.22, Omissions = 0.37/4.55, Commissions = 0.01/72.26.
p-heter/I²(%) for postnatal NO$_2$ exposure in girls to HRT =0.59/0, HRT(SE) =0.49/0, Detectability= 0.19/37.25, Omissions =0.56/0, Commissions = 0.03/65.72.
p-heter/I²(%) for postnatal NO$_2$ exposure in boys to HRT =0.99/0, HRT(SE) =0.15/43.65, Detectability= 0.13/46.92, Omissions =0.32/13.65, Commissions = 0.10/52.28.
[a]Estimated associations by random-effects meta-analysis by region. Models were adjusted for child's sex and child's age at the time of the attentional function assessment.