STATISTICAL ANALYSIS PLAN

Effect of Air Pollution on the Cognitive Function of Adolescents (ATENC!Ó)

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Introduction

Background and Rationale

Previous observational studies have reported an association between higher air pollution exposure and lower attention in children. With this project, the investigators aim to confirm this association in adolescents using an experimental design. In addition, the study will assess the relationship between air pollution exposure and individual decision-making with respect to risk, time and social considerations. High school students in 3rd grade (ESO, 14-15 years of age) in different high schools in the Barcelona province (Spain) will be invited to participate. For each class in each high school, participating students will be randomly split into two equal-sized groups. Each group will be assigned to a different classroom where each participant will complete several activities during two hours, including an attention test (Flanker task) and a reduced version of the Global Preferences Survey. One of the classrooms will have an air purifier that will clean the air. The other classroom will have the same device but without the filters, so it will only re-circulate the air without cleaning it. Students will be masked to intervention allocation. The investigators hypothesize that students assigned to the clean air classroom will have better scores in the attention test, and that decision-making will also present differences between the two classrooms.

Objectives

The objectives of the study are to identify if cleaning the air of a classroom has an impact on:
1) The attention of high-school students
2) The individual preferences with respect to risk, time and social considerations

Study Methods

Trial Design and Randomization

This is an interventional, randomized, 2-arm trial with equal allocation. Allocation will be done completely at random. Briefly, for each class in each high school, participating students will be randomly split into two equal-sized groups, and each group will go to a different classroom to perform the same activity for two hours. The two classrooms need to be as similar as possible in terms of size, orientation and windows. The two classrooms will have an identical air purifier (Pure Airbox, Zonair 3D) that will be running from 30 minutes before the session until the end of the 2-hour session. The purifier in one room will have the filters installed, and therefore will clean the air, while the purifier in the other classroom will not have the filters installed (sham device that will simply recirculate the air without filtering it). Participants will be masked to the intervention. Assignment of purifier to classroom will be done at random.

During the whole time, we will have measure continuously in both classrooms the concentrations of black carbon with Microaeth (AethLabs, USA), the concentration of $PM_{2.5}$ with DustTrak (TSI, USA), the levels of CO_2 and values of temperature and humidity (Extech, USA).

Once students are in the classroom they have been assigned to, they will complete several activities, including several validated tests and tasks. All of them will be administered though a

laptop (each student will be provided one laptop with the tests and questionnaires). The tests and questionnaires will be completed using headphones to block the noise.
The chronology of the activities will be the following:

- Assignment of groups to classrooms, initial explanation (10 minutes).
- Completing the baseline Attention Network Task (ANT, adult version, Flanker task) (15 minutes).
- Completing the PMA-R (Primary Mental Aptitudes) test (10 minutes).
- Watch several videos related to Science and Environment (50 minutes).
- Completing a questionnaire about the videos (5 minutes).
- Completing the post Attention Network Task (10 minutes).
- Completing a questionnaire on factors that can affect attention, designed by the students (10 minutes).
- Completing a short version of the Global Preferences Survey (10 minutes).

Sample size Calculation

A previous study (Sunyer et al., 2017) detected a mean reduction of 5 ms in the hit reaction time standard error for correct answers (HRT-SE) calculated from the ANT test, which we will take as our main outcome, associated with a 37% increase in the concentration of NO_2, a marker of exposure to traffic pollution. With the air purifier it is expected to achieve a 80% reduction in the concentration of fine particles, which is why we expect to find a difference of 10 ms in HRT-SE between the group that goes to the purified classroom and the ones that go to the regular classroom. Bearing in mind that the standard deviation of HRT-SE is 90 ms (Sunyer et al., 2017), to have a statistical power of 80% with a type I error of 5% in the comparison of the two groups, it is necessary to include 2,500 students. Assuming a participation of 25 of the 30 children for each participating class, the participation of 100 classes is required. By recruiting high schools with 2 or more lines, it is expected to have to recruit about 35 high schools.

Statistical analyses will be conducted after all information for all participants has been collected.

Statistical Principles

Statistical significance will be considered at the 5% level. Results will be provided with 95% confidence intervals.

Adherence to protocol will be defined as staying in the classroom during the 2 hours, or in exceptional cases, leaving it for less than 5 minutes. Adherence will be described as percentage of students being adherent. Non-adherent students will be excluded from statistical analyses.

Trial Population

Eligibility criteria: all students in the 3rd grade of ESO in participating high schools and with informed consent signed by them and by the parents will be eligible for the analysis. There are no exclusion criteria.
The CONSORT flow diagram will include the number eligible, the number that did not participate, the number allocated in each arm, the number not adherent (with reasons), and the excluded from the analyses (with reasons). Anticipated reasons for exclusion from analyses

include: missing data in an outcome (the subject will be excluded from the analyses of that outcome) or low accuracy tests. For ANT, a test will be considered of low accuracy if the number of correct responses is less than 70%. Reaction times (RT) below 100 ms will be excluded from RT calculations because of physiological implausibility implying that such a response is perseverative or anticipatory, although this does not imply the exclusion of the subject (other RT are available).

Baseline characteristics will be summarized by providing, for each arm, percentages for categorical variables and medians and 1^{st} and 3^{rd} quartiles for continuous variables. The following baseline variables will be summarized: sex, age, PMA-R results, and baseline ANT measures.

Analysis

Analysis on Attention

Outcomes

Attention outcomes are all derived from the Attention Network Task-Flanker Task (ANT). It is a computerized test that takes approximately 9 minutes to complete. A row of five arrows appears either above or below a fixation point. Participants have to use the arrow keys from the keyboard to indicate as quickly as possible if the central arrow is pointing to the left or to the right. They have to ignore the flanker arrows, which point in either the same (congruent) or opposite (incongruent) direction than the middle arrow. The target can be preceded by no cue; a center cue or a double cue, which inform about the upcoming of the target but not on its location; or an orienting cue that alerts about the upcoming of the target as well as its location (orienting cue). The task is divided into four experimental blocks of 32 trials each (a total of 128 trials).

The primary outcome regarding attention will be the response speed consistency throughout the post Attention Network Task-Flanker Task (ANT). It will be calculated as hit reaction time standard error (in milliseconds) for correct responses (HRT-SE). A higher HRT-SE indicates highly variable reactions related to inattentiveness.

Secondary outcomes include the following, derived from the post ANT:

- Impulsivity, calculated as the number of incorrect responses (responses made in the opposite direction to the direction of the target arrow).
- Selective attention, calculated as the number of omission errors (failure to respond to the stimulus).
- Alerting score, computed subtracting the median reaction time (RT) in milliseconds for double cue from median RT for the no cue condition (calculations performed after removing the incongruent trials).
- Orienting score, computed subtracting the median RT in milliseconds for spatial cue from the RT for central cue (calculations performed after removing the incongruent trials).
- Conflict score (executive attention), calculated as the median RT in milliseconds for each flanker condition (across cue conditions) and subtracting the congruent from the incongruent RTs.

Analysis Methods

Analyses will be conducted using conditional linear regression using class (the group of 30 students from the same class in the same high school that were randomly split into each arm and that undertook the experiment on the same day and time) as strata. This way, the analysis will be automatically matched by high school, class, day and time of the day. Intervention arm will be the explanatory variable of interest in the model. The coefficient associated with arm will then be interpreted as the mean difference in the outcome when comparing the two arms. Models will be adjusted for year of birth; sex; average temperature during the experimental session; average relative humidity during the experimental session; and average CO_2 concentration during the experimental session; as these conditions can differ in the two classrooms.

Analysis of residuals will be used to detect influential observations using DFBeta. Analyses will be repeated by excluding subjects with DFBetas clearly separated from the rest.

Sensitivity analyses will include:

- Additionally adjusting the models for the same outcome measured in the baseline session.
- Additionally adjusting the models for the results of the PMA-R test (number of correct responses).
- Replacing the arm indicator by the average values of black carbon from the beginning of the experiment to the end of the post ANT test. In this case, the effect of black carbon on the outcome will be reported by an interquartile range increase. This analysis will allow taking into account the difference in black carbon levels in the two classrooms, i.e. it will not estimate a common change in the outcome for all strata. Linearity of the association will be examined using generalized additive models (GAM). If visual inspection of the resulting exposure-response relationship suggests a non-linear association, we will try to transform variables to achieve linearity. If transforming variables does not work, we will report the curve estimated by the GAM models.
- Same as the previous point, but using $PM_{2.5}$ levels instead of black carbon.

Subgroup analyses will be conducted by sex using the same methodology described above.

Missing Data

Missing data are only expected in the outcome variables if the participant did not complete the test, in which case it will be excluded. Other potential cases of missing data include an error in the monitors that precludes proper adjustment for covariates. If there are missing values for CO_2, temperature or humidity in one of the classrooms, we will replace them by the values from the other classroom, provided that this approach is reasonable according to the data obtained in the other classrooms. Otherwise, the classroom will be excluded from the adjusted analyses, but an unadjusted analysis including all classrooms will also be carried out to compare the results. For the analyses using black carbon or $PM_{2.5}$ concentrations, missing values on these two variables will be imputed using the baseline value in the other classroom and the rate of reduction over time estimated in all other classrooms with sham or real air purifier, as appropriate.

Statistical Software

Analyses will be conducted in the Stata and R softwares. Stata comman xtreg with the fixed effects option will be used to fit conditional linear regression models, which can also be fitted using regular linear regression commands in Stata (regress) or R (lm) by including a strata factor as adjustment variable. GAM models will be fitted using the mgcv package in the R software. The tlm package in R will be used to interpret models with transformed variables (Barrera-Gómez and Basagaña 2015).

Analysis on Decision-making with respect to Risk, Time and Social Considerations

Outcomes

The primary outcomes of analysis will be the (1) risk taking scores, (2) time preference scores, (3) the positive reciprocity scores, (4) the altruism scores, and (5) the self-assessment of trust. All the scores will be measured from the responses to the questionnaire, which is a subset of the questionnaire in Spanish used in Falk et al (2018). For each of the traits (risk preferences, time preferences, positive reciprocity, altruism, and trust), an individual-level index will be computed that aggregates responses across different survey items following the methodology in Falk et al. (2018).

Analysis Methods

The analysis will follow the basic analysis of Falk et al (2018), where groups, instead of being countries, will be our arms. In addition, we will follow the empirical strategy described above for the analysis on attention. That is, we will perform regression analysis, controlling for the relevant observable variables such as gender, age, cognitive ability, attention outcomes, and other physical variables measured at the time of the experiments such as temperature, relative humidity, or average CO_2 concentrations. We will also control for outliers, and will study heterogeneous treatment effects.

Missing Data

In terms of missing values in the questionnaire on preferences, we will follow the methodology of Falk et al (2018, Section I.H): “If one (or more) survey items for a given preference were missing, then the missing items will be predicted using the responses to the available items:
– Suppose the preference was measured using two items, call them a and b. For those observations with missing information on a, the procedure will be to predict its value based on the answer to b and its relationship to a, which was estimated by regressing b on a for the sub-sample of subjects who had nonmissing information on both, a and b (on the world sample).
– For the unfolding-brackets time and risk items, the imputation procedure will be similar, but making additional use of the informational content of the responses of participants who started but did not finish the sequence of the five questions. Again suppose that the preference is measured using two items and suppose that a (the staircase measure) is missing. If the respondent did not even start the staircase procedure, then imputation will be done

using the methodology described above. On the other hand, if the respondent answered between one and four of the staircase questions, a will be predicted using a different procedure. Suppose the respondent answered four items such that his final staircase outcome would have to be either x or y. A probit was run of the "x vs. y" decision on b, and the corresponding coefficients were used to predict the decision for all missing values (note that this constitutes a predicted probability). The expected staircase outcome will be then obtained by applying the predicted probabilities to the respective staircase endpoints, i.e., in this case x and y. If the respondent answered three (or less) questions, the same procedure was applied, the only difference being that in this case the obtained predicted probabilities were applied to the expected values of the staircase outcome conditional on reaching the respective node. Put differently, the procedure outlined above was applied recursively by working backwards through the "tree" logic of the staircase procedure, resulting in an expected value for the outcome node.
– If all survey items for a given preference were missing, then no imputation will take place"

Statistical Software

Analyses will be conducted in Stata and Matlab.

References

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