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Protocol of the 24 months' follow-up of the BISC project

- Version 1 -

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Summary

Title	Protocol of the 24 months' follow-up of the BISC project - Version 1 -
Description of the study	In the 24 months' follow-up we will conduct an EEG study to investigate the effect of pre and postnatal exposure to environmental exposures, particularly air pollution, on cognitive control function.
Objectives	The project will prospectively test the relationship between various pre-natal and post-natal exposures, on the one hand, and brain function and cognitive control, on the other, at 24 months after birth by means of EEG.
Design	Birth cohort (longitudinal study)
Inclusion and exclusion criteria	24-months old babies from the BISC cohort will be recruited. Infants with previous MRI and eye-tracking measures will be prioritized
Treatments or interventions	Does not apply
Variables	EEG, questionnaires.
Study population	100 babies and their parents
Participant centers	ISGlobal, three major hospitals, Sant Joan de Déu (SJdD), Maternitat-Clínic and Santa Creu i Sant Pau, and the Bellvitge Biomedical Research Institute (IDIBELL)
Duration	Three years
Duration of the study for the study participant	75 minutes (a pilot study will be conducted to evaluate whether the length of the study is suitable for the participants or if efforts to reduce the time are necessary).

Figure 1. Calendar of the study

	2022				2023			
	T1	T2	T3	T4	T1	T2	T3	T4
Recruitment	X	X	X	X	X	X		
EEG acquisition	X	X	X	X	X	X	X	
Questionnaires	X	X	X	X	X	X	X	
Data Management	X	X	X	X	X	X	X	X
Data analysis & Publications					X	X	X	X

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RECORD OF VERSIONS

Version	Date	Description of the change	Brief justification
V1 (current version)	02/11/2021	First version	First version

1. Justification

More than 80% of the world's population lives in urban areas where air pollution levels exceed the limits set by the World Health Organization (WHO). Air pollution thus constitutes one of the greatest environmental risks in these areas, with a great impact on the development of respiratory and cardiovascular diseases. But the effect of pollution is not limited to these diseases. The fine and ultrafine particles present in air pollution also access the nervous system, where they can provoke neuro-inflammatory responses triggering neurological problems. Specifically, exposure to air pollution (EAP) has been associated with adverse effects on neurodevelopment, deficits in cognitive functions such as attention and learning, and a higher prevalence of attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASD) in early ages (1,2).

In humans, the studies that have evaluated the impact of EAP on brain function are mostly cross-sectional and used structural neuroimaging techniques. These studies have shown an association between EAP and anatomical changes in the frontal lobe and striatum, as well as deficits in communication between both regions (3). These structures play a key role in attentional and learning processes, and are generally found dysfunctional in individuals with ADHD and ASD (4,5). Furthermore, in the striatum, neuronal activity is mainly modulated by dopamine, a key neurotransmitter in attentional, motivational, and learning processes. This neurotransmitter is prone to oxidation and its metabolism generates reactive oxygen species (6). The compensatory antioxidant systems of the striatum could potentially saturate when dealing with any additional oxidative challenges, such as the oxidation generated by EAP (7). In this sense, occupational studies that have investigated the impact of exposure to pollutants present in foundries and welding fumes, such as copper or manganese, have observed that these pollutants have a particularly neurotoxic effect on the striatum (8,9). Thus, this region may be particularly susceptible to air pollutants.

Although most studies have focused on the impact of EAP on different stages of life, a period that can be particularly vulnerable and has received little attention is the prenatal period. This period is especially critical because it is when brain structures are forming and environmental factors can cause permanent brain damage (10). The only human study that has investigated the effects of EAP during fetal life on brain changes was conducted in school-age children. The study was based on retrospective estimates of EAP during the prenatal period, and only

investigated the integrity of cortical structures (11). Using this method, the authors showed that the estimated EAP during fetal life was associated with structural changes in frontal regions at the age of 6 years, and these structural changes, in turn, partially mediated the association between EAP and deficiencies in cognitive control.

Thus, the evidence for the effects of EAP on prenatal human brain function is scarce and presents several methodological problems. On the one hand, no study has investigated the impact of EAP during the prenatal period on brain function in babies. In addition, EAP levels during fetal life are generally estimated based on residential levels following geographic models and ignoring the variation in the air pollutant mix and composition, and therefore the toxicity of the various pollutants in different environments. In this regard, having more direct measures of EAP (by the personal sampling of EAP in different settings) is essential to provide a more detailed assessment of the impact of EAP on neurodevelopment and establish dose-response relationships. Moreover, the development of mobile air pollution monitors has provided an unprecedented opportunity to assess personal exposure and/or indoor and outdoor air pollution levels in homes and workplaces in large samples.

In addition to the limitations described above, it should be noted that most neuroimaging studies that have investigated the impact of EAP on the brain, in either children, adolescents, or adults, have assessed structural changes of the brain. Because structural neuroimaging shows a static image of the brain anatomy, these studies have associated brain structural changes with cognitive and behavioral aspects through correlations with independent psychometric scales and neuropsychological tests. Although this procedure has provided relevant information on the neural mechanisms underlying EAP-induced cognitive deficits in humans, it does not allow us to investigate whether brain function is actually affected *during* the performance of cognitive tasks, or drawing conclusions about the specific processes that may be affected. For example, cognitive deficits, such as memory impairments, observed by psychometric or neuropsychological tests may be due to difficulties in encoding (poor neural representation of the elements), learning (poor ability to establish relationships between the different elements), or attention (poor capacity to orient attention towards relevant elements), among others. Thus, it is essential to evaluate brain function in real-time while performing cognitive tasks to confirm that brain function associated with cognitive control is indeed affected and to determine the specific processes that are altered.

In this context, electroencephalography (EEG) is a powerful tool for investigating the

functioning of the human brain in vivo. When the ongoing EEG is averaged, after the presentation of the same stimulus or response, the resulting waves are called evoked-related potentials (ERPs). ERPs allow us to visualize cognitive processing as it develops. They provide high temporal resolution, from milliseconds to seconds, of the various stages of information processing, and provide useful information about the nature and timing of neural events that underlie sensory, perceptual, and cognitive processes. ERPs can be easily used in infants and have been very valuable for studying a wide variety of cognitive processes in this population, since they can be registered in stimuli relevant or irrelevant to the task, and passively or actively. Thus, ERPs provide complementary information and a more precise measure of neurodevelopment than the neuropsychological tests that have been used more commonly (12). Of great relevance for the present project is the fact that several ERP components represent great markers of the functioning of frontal areas and the integrity of the striatum (particularly affected by EAP) as well as potential early markers of ADHD, ASD, or affective disorders.

One of the most well-known ERPs for identifying cognitive impairment in various disorders and with great potential as a risk indicator is the MisMatch Negativity (MMN) (13). The MMN, generated by frontotemporal circuits, reflects an automatic process of detecting irregularities that triggers a change in the focus of attention (14). This automatic processing fulfills two critical functions: 1) alerting the system when new or unknown stimuli are presented that require attentional processing, and 2) freeing up attentional resources by processing routine and familiar stimuli. Previous studies have shown atypical responses in MMN in children with ASD (15), ADHD (16), or dyslexia (17), thus becoming a valuable tool to detect deficits in neurodevelopment.

Besides, different pathologies associated with striatal and dopaminergic dysfunctions have been related to atypical responses in three other ERPs in learning tasks [Stimulus-Preceding Negativity (SPN), Feedback-Related Negativity (FRN), and P300], resulting in useful markers to investigate the integrity of the frontostriatal and dopaminergic circuits (18,19). Specifically, the SPN is a slow negative component with a frontal distribution that progressively increases in amplitude before the presentation of an informational event or feedback. That is, the SPN is observed in "waiting periods", reflecting attentional and motivational processes in anticipation of relevant or informative events (20). As an individual learns to associate two elements, the SPN increases its amplitude in response to the first element, reflecting a greater predictive

capacity (21). When the result or feedback is presented, two more components emerge: the FRN and the P300. The FRN, with a fronto-central distribution, reflects whether a result is different than expected (22), while the P300, appearing later and with a parietal distribution, reflects the salience of the stimulus (23). In general, violations of learned rules are associated with a higher FRN and P300, reflecting a higher predictive capacity (more surprise) and a higher orientation of attention, respectively. Disorders associated with the integrity of the striatal dopaminergic system (18,19), individual differences in baseline dopaminergic levels (24,25), and interventions with dopaminergic drugs (26,27) have been related to aberrant responses in these three ERPs components, becoming potential indicators of risk of suffering alterations in striatal circuits.

Despite ERPs is a predominant technique in cognitive neuroscience to investigate cognitive function in adults and neurodevelopment in infants, no epidemiological study has taken advantage of this technique to assess the impact of EAP on brain function, particularly in early ages. In this context, the objective of the present study is to investigate the impact of EAP during the prenatal and postnatal period in various ERPs associated with the functioning of frontal and striatal circuits, and involved in attentional and learning processes in a large sample of babies at 24 months of age from the BiSC birth cohort. Unlike other cohorts, in BiSC, air pollutants are measured by personal sampling in different settings and stages of pregnancy and also during the first 18 months of the baby's life, providing a unique opportunity to assess the impact of EAP at different stages (pregnancy and postnatal life) in brain function (see the BiSC protocol from birth until the age of 18 months).

Studying the effects of EAP during fetal and early postnatal life on brain function is vital, given the potentially permanent nature of these effects in these time windows and the greater possibility of implementing preventive strategies during these periods. To date, interventions to reduce air pollution have generally been modest, and in fact, air pollution continues to grow globally. Thus, demonstrating its impact on infant brain function could create a momentum to definitively implement policy interventions that genuinely protect the health of citizens in urban areas globally, as has previously happened with other pollutants, such as lead in gasoline. Moreover, the current protocol aims to include the analysis of the effects of other exposures, including noise, green spaces, etc (see the BiSC protocol from birth until the age of 18 months).

2. Hypothesis

Our hypothesis is that air pollution during the prenatal period has a negative impact on the neural processing of tasks requiring attention and learning processes, and specifically in ERPs sensitive to the functioning of frontal areas and the striatum.

3. Objectives

In this study, we intend to investigate the impact of air pollution during the fetal and early postnatal stages in babies of 24 months, through personal measurements of air pollution in different stages of pregnancy (first and third trimesters) and postnatally (6 and 18 months) and using ERPs as a tool to assess the impact of EAP on brain function. This will allow us to investigate whether neurodevelopmental deficiencies associated with air pollution are mediated by deficiencies in neural processing in different aspects of cognitive processing and the integrity of different circuits. In addition, we will also be able to investigate the impact of the different components present in air pollution, as well as other exposures (e.g. noise, green spaces) on the brain response of babies.

4. Materials & Methods

4.1 Study population

In order to achieve the proposed objectives, we intend to study 24-month-old babies from the BiSC cohort. The cohort includes 1100 babies. In August, the first babies of the cohort will become 24 months old, and over the next two years, the 1100 babies will reach this age. From all the participants, during pregnancy, sociodemographic data, lifestyle, place of residence, medical history, clinical data are obtained, the parent's mental health and cognitive abilities of the parents are evaluated, and, currently, information is requested about COVID19 infection. In addition, during the first and third trimesters of pregnancy, air pollution exposure samples are taken both personally (with portable meters) and at home (with stations at home) (see the BiSC protocol from birth until the age of 18 months for further details).

During the first two years of the baby's life, various neuropsychological tests and tests are performed to assess the baby's development at a cognitive, language, motor, and emotional level at 6, 8, and 18 months using a diversity if tests, such as the Bayley test, the Developmental Profile 3 or the MacArthur-Bates Communicative Development Inventories, and eye-tracking, among others (see the BiSC protocol from birth until the age of 18 months for further details). The set of all these measures will allow us to determine individually the degree of exposure to air pollutants as well as other potentially confusing factors, and the development of the baby at the time of the current study.

Out of the 1100 babies of the BiSC project, we aim to include a sub-sample of 100 babies at 24 months of age.

4.2 Inclusion & exclusion criteria

In the subsample to be included in the 24 month's follow-up (N=100) we will prioritize infants with MRI data (acquired 27 days after birth, N=132) and eye-tracking measures (measured at 6 and 18 months, expected N=200) to relate those to babies' outcome at 24 months. Among infants without MRI or eye-tracking data, we will prioritize those with better accurate exposure assessment to air pollution and with complete information on potential confounders.

4.3 EEG procedure

Invited families will be scheduled to come to a laboratory specially adapted for the present study. After explaining the procedure to the parents, a helmet with active electrodes will be put on the baby's head. These electrodes allow for a quick and easy setup without causing discomfort to the baby. At all times, the baby will sit on the lap of one of the parents and in front of a screen and two speakers that will be used to present the stimuli. The EEG setup will take 10-20 minutes. Throughout the session, infants will be presented with various auditory and visual stimuli across different tasks to assess their cognitive abilities and ERPs associated with frontal areas and basal ganglia function. Details on the consent form are provided in section 5 (Ethical consideration) and in annexes (section 7). The visit will last 75 minutes, but a pilot study will be conducted to evaluate whether the length of the study is suitable for the participants or if efforts to reduce the time are necessary. The task that babies will perform are:

4.3.1 Auditory oddball task.

In this auditory paradigm, a sequence of sounds is presented passively. There is one "standard" sound that occurs frequently (in most cases) following the same pattern, while another, "deviated" (which differs in pitch, duration, intensity, and other acoustic properties from the standard), occurs randomly and infrequently. Previous studies have shown that the presentation of the "deviated" sound generates a ERP component known as the "mismatch negativity" (MMN). This component reflects the surprise of the "deviated" sound, has a negative polarity, and reaches its maximum amplitude around 160 to 220 ms after the start of the stimulus. It has been suggested that MMN acts at the pre-attention level, since it can occur in comatose individuals (13). Thus, the MMN is generally recorded while the participants' attention is focused on some other activity (for example, reading a book or watching a video). Therefore, while the sounds are presented by the loudspeakers, a children's film will be presented on the screen to divert the baby's attention. This task will last 14 minutes.

4.3.2 Music listening task.

To investigate individuals' abilities to identify more complex auditory patterns, infants will also passively listen to 12 melodic pattern (40 sec duration each) that will differ in their degree of complexity and surprise. MMN amplitude in response to musical surprises will be used as a measure of babies' abilities to learn high-order patterns of auditory stimulation. As in the oddball task, sounds will be presented by the loudspeakers and a children's film will be presented on the screen to divert the baby's attention. This task will last for about 8 minutes.

4.3.3 Associative learning task

Babies will carry out the task adapted by (28) to study their ability to direct attention and learn simple associations. The stimuli will be presented on a computer screen. For this task, an eye tracker will be used to record the babies' eye movements while they perform the task and thus assess when they are looking at the screen and measure their learning and attention throughout the task. At the beginning of each trial, either a rabbit or a turtle will be presented in the center of the screen, together with a specific sound for each of the images (to catch the baby's attention), for two seconds. After these two seconds, a cross will appear in the center of the screen. This cross will remain on the screen for two or four seconds depending on whether the

previous image was a rabbit, or a turtle, respectively. After that time, either an apple or a banana will be presented, also depending on whether the initial image was a rabbit or a turtle, respectively, for a period of two seconds. At the end of the test, a cross will appear again for one second, after which a new trial will begin. These relationships, both identity (rabbit-apple, turtle-banana) and temporal (rabbit-two seconds, turtle-four seconds), will be maintained in 70% of the trials (out of a total of 75 trials). However, in 15% there will be a temporal violation (the corresponding fruit will be presented sooner or later than it would be expected) and in the other 15% there will be an identity violation (an apple will be presented when it should be a banana and vice versa). Finally, the animal-time-fruit associations will be counterbalanced across participants. Combining this paradigm with ERPs will allow us to investigate different components related to attentional processes and reinforcement learning. The evolution through learning of either the SPN during the "waiting period" before the feedback (fruit presentation), or the FRN and the P300 in response to feedback (and particularly to violations) will allow us to assess babies' predictive abilities. This task will last 10 minutes.

4.3.4 Statistical learning task

Babies will carry out the task adapted by (29) to investigate statistical learning. They will be presented with a series of images on the screen. The stimuli will be colorful, fractal-like images. Images will appear every 1 sec, looming in size from 2.4° of visual angle at onset to 14.6° at offset. Each block will contain 36 images presented sequentially one at a time in a unique order, followed by 6 s of rest with the screen blank. Blocks will alternate between Structured and Random conditions. The condition will appear first will be randomly assigned. In the Structured condition, six fractals (A-F) will be organized into three pairs (AB, CD, EF). The sequence of each block will be generated by randomly inserting six repetitions of each pair. The first member of a pair (A, C, E) will be always followed by the second (B, D, F, respectively) resulting in a transition probability of 1.0. After the second member of a pair, another pair will begin, resulting in a transition probability of 0.33 on average. In the Random condition, six different fractals (G-L) will be presented individually. The sequence of each block will be generated by randomly inserting six repetitions of each fractal, avoiding back-to-back repetitions of the same fractal. This will result in a uniform transition probability of 0.20 on average. The six fractals in each condition will be consistent across all blocks of that condition. Participants will complete up to 12 blocks. ERPs to structured and random blocks will be used as an index of individuals' statistical learning capabilities. The task will last 8

minutes.

4.4 Questionnaires

Parents will be also asked to answer two questionnaires related to individual differences in reward sensitivity in relation to primary and high-order pleasure to assess its contribution to babies' abilities to learn by reinforcement. Specifically, parents will answer the Barcelona Music Reward Questionnaire (BMRQ, 30), and the Physical Anhedonia Scale (PAS, 31). Parents will fill these questionnaires via an online test that will be sent to their emails. See Annexes, section 7. Total time of completion of both questionnaires is 5-10 minutes.

4.5 Data management & analysis

Data collection files: the data collection files (address, telephone information, ID number) are kept in separate, password protected database on a secure server at ISGlobal and are only accessible to study staff with the appropriate training and clearance to access personal health information. All data acquired during this project are only labeled with this study ID and no other identifying information. Data linking subject names with study IDs are kept in a separate, password-protected database on a secure server controlled by Dr. Sunyer and the BiSC data manager. Participants and staff are informed of the confidentiality of information and assured that the data will only be used for statistical purposes and group analyses in which individuals cannot be identified. Data will only be presented in tabular form, and no information on individual identification will be revealed in any published reports. No data beyond what has been consented will be obtained without authorization from the subject.

Data management and cleaning: BiSC data management team will be the responsible to clean the data (with the help of key investigators from each area) and store it in Comma Separated Values (csv) files, which can be linked via the unique BISC id. The repository data includes codebooks and Standard Operation Procedures.

Internal requests: All internal requests for data must be submitted in writing for documentation, and addressed to the BiSC Publication Committee (PC). The request will include: Title, Lead author(s) (junior and senior), Rationale and background, Research hypotheses/objectives, Analysis plan, including exposures, outcomes and main covariates considered, and statistical analysis protocol, Key references, Foreseen timeline and target

submission date, and Foreseen co-authorship. The PC will review the proposal to verify that the proposal format has been followed and to determine if there is potential overlap with any other papers or abstracts, proposed or in progress. The PC will then work with the authors to reduce or remove overlap. Accepted manuscript proposals will be available on the BISC website intranet to help investigators determine available topics in advance. When the above requirements have been satisfied, the BiSC data management team will prepare a comma separated value (csv) file. The use of this text format will ensure compatibility with any statistical software. Internal researchers will have to destroy the data once the analysis has been completed. Authorship will follow BiSC regulation to ensure that appropriate credit is given to BiSC researchers and all public and private funding supporting BiSC will be acknowledged in the published work. Authors will be notified of the intent to publish and will be provided with an advance copy of any submitted manuscript that relies on the requested data.

External use: Data obtained as part of this project will be made available to external investigators to this proposal through a formal Data Transfer Agreement (DTA). In no case will explicit identifiers (e.g. contact information, medical record numbers) be included in analytic files for either internal use or sharing with external collaborators.

Data transfer: To ensure secure transfer of data and to facilitate access of authorized collaborators to these increasingly large data sets, ISGlobal has implemented a secure data transfer system (private cloud - Namek), which is mounted as a Virtual File System to the main ISGlobal servers, that guarantees the security and integrity of all data from Information System, and also complies with the General Data Protection Regulation (GDPR).

Data analyses: We will estimate the effects of air pollutants, as well as other exposures, on child brain development. We will also explore whether this effect is direct or indirect through the mediation of factors such as placental impairment, and will consider potentially more vulnerable subgroups based on sex or contextual variables such as noise, greenness or SES (i.e. interaction). To do so, we will combine classical epidemiological analysis with novel methods based on the counterfactual framework for causal inference when analyzing mediation.

We will use general linear models, distributed lag models and b-splines to test for association between total maternal exposure to air pollution and dose during different time windows of

pregnancy and during the first 18 months on brain development, as reflected by the ERPs. A similar approach will be followed for other exposures. We will construct a conceptual model that accounts for effects and correlations between exposures at different time points by creating an exposure lag space. We will explore the most relevant window(s) of exposure to air pollution or other pollutants using distributed lag models.

Based on up-to-date published evidence, we will draw a direct acyclic graph (DAG) to decide which of the potential confounding variables selected *a-priori* should be included in the statistical models, including: contextual socioeconomic indicators at residential address and individual data for total personal exposure and dose; and temporal variables such as temperature and humidity for the spatial-temporal variables. To test the independent and interaction effects between the various exposures, after controlling for collinearity, we will apply penalized regression methods.

For the study of the Event-Related Potentials, single trials evoked by the desired stimuli will be selected (-100 to 1 second after the stimuli) and each electrode will be reference to a baseline -100 ms to 0 ms respect the event. Independent Component Analysis (ICA) will be then applied to the data to discard the electrical activity due to the eyes movements (blinks and horizontal eye movements) and trials presenting activities >100 microVolts in any electrode will be discarded from further analysis. Trials from the same condition will be averaged to obtain individuals' ERPs.

5. Ethical considerations

Ethical issues within the BiSC project are related to:

a) the recruitment of participants
b) the administration of questionnaires to the parents
c) the acquisition of brain EEG signals in babies
d) personal data collection and storage

Research studies in Spain are regulated by both international and national legal and ethical rules. The Principal Investigator and the research team are aware and will conform to the International, European and National legislations in all the various aspects of the research as detailed below.

The candidate project is aware of further relevant guidance and codes, including:

- The Nuremberg Code (1946) addressing volunteer consent and proper acting;
- The Revised Declaration of Helsinki in its last version of 2013
- The convention for the protection of human rights and dignity of human being with regard to the application of biology and medicine called the "Convention on Human Rights and Biomedicine" (Council of Europe, 1997) and its additional protocol on biomedical research (2005)
- The Recommendation Rec (2006)4 of the Committee of Ministers to member states on research on biological materials of human origin (Council of Europe) are the main international guidelines for medical research.
- Convention of the Council of Europe on Human Rights and Biomedicine signed in Oviedo on 4 April 1997, and the Additional Protocol on the Prohibition of Cloning Human Beings signed in Paris 12 January 1998;
- The Spanish Law on Biomedical Research (14/2007, of 3rd July) which regulates biomedical research in Spain
- The charter of Fundamental rights of the EU Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to processing of personal data and on the free movement of such data;
- UN Convention on the Rights of the Child (1990);
- The Royal Decree that establishes the basic requirements for the authorisation and functioning of biobanks with biomedical research purpose and for the processing of human samples and regulating the functioning and organisation of the National Register of Biobanks for Biomedical Research (1716/2011, of 18th November).

We have reviewed the guidance available on the web (http://ec.europa.eu/research/participants/docs/h2020-funding-guide/cross-cutting-issues/ethics_en.htm; http://ec.europa.eu/research/swafs/index.cfm?pg=policy&lib=ethics).

ISGlobal- Campus Mar is bond to the PS-Mar Ethics Committee (Clinical Research Ethics Committee of the Municipal Health Care Service, created and accredited for the first time on November 11th, 1993 by the General Direction of Health Resources of the Department of

Health of the Government of Catalonia, in accordance with the Order of 26 October 1992). The PS-Mar CEIC evaluates all research protocols in humans conducted by ISGlobal-Campus Mar researchers. According to Spanish regulations, our local Ethic Committee will follow the implementation of the study by giving its approval to every protocol (including Participant Information Sheet and Consent Form) that will be developed through the study. All ISGlobal researchers are self-regulated by the Code of Good Scientific Practice (http://www.prbb.org/system/uploads/attachment/file/3/en/eng_a4.pdf).

General procedures included in the research protocol to safeguard the privacy of study subjects:

- Written consent will be obtained from all the participants in the study to use their personal data.
- All material obtained in the framework of the project (questionnaires, EEG, etc) will be identified through a code, the name and/or other personal data that could allow the identification of the participant will never be indicated. This unique identifier will link all basic data required for the study. The master key file linking the centre's study numbers with personal identifiers will be maintained in a password protected file with limited access.
- All files containing personal data will be stored in encrypted and password-locked files. Access to these files will be limited to authorized project personnel;
- Only researchers linked to the project will have access to personal data.
- Personal data will not be transferred, except in the cases considered by law.
- Reported study results will pertain to analyses of aggregate data. No individual's name will be associated with any published or unpublished report of this study.
- All project personnel will be trained in the importance of confidentiality of individual records and required to sign a confidentiality agreement.

We provide detailed information regarding:

- Informed consent: the protocol includes a consent form to collect information from the babies (EEG) and from the parents (reward sensitivity questionnaires) (see section 4.4 and Annexes for details), another for parents authorizing babies' participation. The consent form will be provided to the participants before starting the experimental session.

- Informed consent procedures, including details on the procedures used to ensure that there is no coercion on participants

Prior to the enrolment into the follow-up of the 24 months' of the baby, the BISC personnel will contact parents from the BiSC cohort and shall provide detailed information on the study aims and objectives and the tasks that participants are expected to accomplish. Moreover, they shall highlight the volunteer nature of the participation and the ability of the participants to leave the study whenever they wish together with assurances that not accepting to participate or leaving the study by no means would affect the healthcare that they would receive. See annexes, section 7, for details on the consent form. In all cases one of the parents or a legal tutor will have to sign the consent form for babies' enrollment. Parents will receive a copy of the consent (see section 4.4 and Annexes for more details).

- Procedures to ensure welfare

All investigators involved in the study will be appropriately trained and well qualified to conduct research with humans and to perform all study procedures.

The protocol will include details about which measures will be taken to ensure welfare of participants in all study procedures. These are summarized below:

Questionnaires

- Questionnaires will be administered to the parents via email (see Annexes). Two questionnaires will be administrated, the Barcelona Music Reward Questionnaire (BMRQ) and the Physical anhedonia skill (PAS) (see section 4.4 and Annexes for more detail)
- The volunteers will be free to not respond to any of the questions if they don't feel comfortable with.

EEG testing

EEG acquisitions will be performed at ISGlobal facilities. The babies's scalp area will be cleaned with alcohol and an EEG cap will be positioned on their head. Electrodes will be filled with a conductive gel to maintain electrode impedances below 20 kΩ. Particular care will be taken to protect babies' welfare during the setup to minimize their discomfort.

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7. Annexes

The following annexes are attached:

- Consent form (Consent_form_24m.pdf)
- Questionnaires (Parents_Questionnaires.pdf)