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ID-RCB N°: **2023-A00523-42**

Clinical Study Protocol N°: 4BIO1

Physiological brain EEG responses of healthy subjects during an attentional task and an auditory stimulation.

Investigational Medicinal Product Code: Not applicable

Investigator:

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Version: 1.0 Date: 02 MAR 2023

Protocol Approval Form

The protocol entitled " Physiological brain EEG responses of healthy subjects during an attentional task and an auditory stimulation ", version 1.0 dated 02 MAR 2023 has been approved for submission to the French Ethics Committee (CPP) by:

Sponsor's Representative:

Date: 03-mars-2023 | 11:47 A

DocuSigned by:
Philippe L'Hostis
Nom du signataire : Philippe l'Hostis
Motif de la signature : J'approuve ce document
Heure de signature : 03-mars-2023 | 11:47 AM CET
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Philippe L'HOSTIS

Investigator's Approval

I, the undersigned, have examined this protocol and agree to conduct this trial according to this protocol, to comply with its requirements, subject to ethical and safety considerations, as set out in this protocol, the Declaration of Helsinki 1964 (latest revision Fortaleza 2013) and all other laws and regulations on the use of investigational medicinal products.

Investigator:

DocuSigned by:
Valérie Bertaina-Anglade
Signer Name: Valérie Bertaina-Anglade
Signing Reason: I approve this document
Signing Time: 03-Mar-2023 | 11:41 AM CET
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Valerie BERTAINA-ANGLADE, PhD

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1. SYNOPSIS

Name of Company:

BIOTRIAL

7-9 rue Jean-Louis Bertrand

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FRANCE

Title of Study:

Physiological brain EEG responses of healthy subjects during an attentional task and an auditory stimulation.

Principal Investigator:

Valérie BERTAINA-ANGLADE, PhD

Study centres:

Phase I type clinical site: Biotrial, 7-9 rue Jean-Louis Bertrand, CS 34246, 35042 Rennes CEDEX, France.

Publication (reference):

Olvet DM, Hajcak G. The error-related negativity (ERN) and psychopathology: toward an endophenotype. Clin Psychol Rev. 2008 Dec;28(8):1343-54

Clinical Phase:

Interventional study with low constraints in healthy volunteers.

Rationale:

This study will be conducted in compliance with Good Clinical Practice (GCP).

The *#EEGManyLabs* initiative, a large-scale international collaborative effort, aims at investigate the replicability of previously published influential electroencephalogram (EEG) experiments. The present study will be part of this international initiative and aims at replicate the results of the Olvet and Hajcak study (2008), showing that an EEG biomarker (ERN) may predict the magnitude of defensive reactivity after errors.

Prior to enrolment, participants will be informed about the objectives, methods and potential risks of the study. There will be no benefit of the study to individual subjects (no treatment, only assessments performed in the study).

Objectives and associated endpoints:

	Objectives	**Endpoints**
Primary	• To obtain brain Event-Related Potential (ERP) responses during the attentional task after errors or correct responses. • To measure the defensive reactivity intensity during the attentional task after errors or correct responses.	• ERN • Startle response (blink magnitude from the obicularis oculi in response to a 105 dB white noise sound) in error and correct trials

Design:

This study will involve female and male participants and will be performed in a phase I clinical facility. Subjects will be enrolled in the study after being informed about the study and after having signed an informed consent form.

First, at screening, subject's demographic information will be gathered and subject will be informed on the study procedures (Informed Consent Form) and on the inclusion/exclusion criteria.

Then, after screening and on the same day, subject will be enrolled and prepared for the main phase of the study (set-up of the EEG cap). Once equipped, the subject will first perform a short training phase before the full procedure of behavioural testing/EEG and electromyogram (EMG) recordings.

Details of the timing of the assessment visit, and the assessments to be performed are summarised in the Schedule Of Activities (SOA).

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Number of subjects:

A total of 20 neurotypical male / female participants will be included. The ratio of female participants should be approximately 50%, to the extent possible.

Rationale for number of subjects:

The sample size in this study has been arbitrarily defined based on the minimum sample size for meaningful results.

Number of study centres:

One = Biotrial, Rennes, France

Duration of study:

The duration of study participation by a subject will be approximately 0.5 day at the most. Participation will include a screening evaluation before admission to the facility for a single evaluation visit, which will last approximately 3 hours.

Diagnosis and main criteria for inclusion / exclusion:

Inclusion:

Participants must satisfy all of the following inclusion criteria before being allowed to enter the study:

1. A signed and dated informed consent form before any study-specific screening procedure is performed.
2. Male and female subjects aged 18-45 years (inclusive).
3. Able to undergo study assessments and willingness to comply with study procedures.
4. All participants: typical-to-corrected vision needed in order to properly complete the behavioral task
5. All participants: no known hearing problems.

Exclusion:

If any of the following exclusion criteria apply, the participant must not enter the study:

1. Participation in an investigational drug or device study within 4 weeks prior to the clinic visit. The participant must not take part in any other trial during the study.
2. Pregnant women.
3. CNS-active medications within 4 weeks prior to the study.

Prior/Concomitant medications and study restrictions:

No CNS-active medications are allowed during and 4 weeks prior to the study.
The subjects will be requested to abstain from consuming alcohol for 48 hours before admission and during the inclusion visit. Participants will also be instructed to abstain from smoking for 1 hour before admission and during the inclusion visit.

Study Schedule:

Screening:

Subjects will be screened for eligibility before admission to the clinic for the inclusion visit.

Written informed consent will be obtained before any study procedure is performed. The screening for subjects will consist of gathering the subject's demographic information including age (birth year, age at screening) and gender, number of years of education, handedness and review of the inclusion/exclusion criteria.

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Assessment visit: Eligible subjects will be admitted to the research facilities on the day of testing. The subjects will then be prepared for the recording of physiological responses including facial electromyography (EMG), electrooculography (EOG) and electroencephalogram (EEG). At H1.5 the subjects will be asked to perform the behavioural task, starting with a short training part before the full procedure that will include EMG, EOG and EEG recordings. Once the task ended, the subjects will be discharged from the study.	
Criteria for evaluation:	
Pharmacodynamic and Biomarker Assessments	The following parameters will be evaluated: • EEG parameter : ERN as difference between error and correct trials. • EOG parameters : startle response magnitude and latency for each trial type
Statistical methods	Startle response magnitudes and latencies and ERN were statistically analysed using an analysis of variance (ANOVA) with the Greenhouse–Geisser correction applied to p values associated with multiple df repeated-measures comparisons. All data will be provided to the #EEG manylabs Core Team in order to perform a meta-analysis and compare them to data from Olvet and Hajcak (2008).

2. SCHEDULE OF ACTIVITIES (SOA)

Screening	**Test Day (and relative time)**			
	0	**0.5**	**1.5**	**3.0**
	Admission			**Discharge**
Information on study by the Investigator				
Signature of informed consent form		Subject preparation for EEG, EMG and EOG recording		
Demographics			Flanker task EEG, EMG recordings	End of test

LIST OF ABBREVIATIONS

CNS	Central Nervous System
CPP	Comité de Protection des Personnes (independent ethics committee)
CRF	Case report form
EEG	Electroencephalogram
EMG	Electromyography
EOG	Electrooculography
ERN	Error-Related Negativity
ERP	Event-Related Potential
EU	European Union
FAIR	Findable, Accessible, Interoperable and Re-usable
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
ICF	Informed consent form
ICH	International Council on Harmonisation
ICJME	International Committee of Medical Journal Editors
IEC	Independent ethics committee
MedDRA	Medical Dictionary for Regulatory Activities
NIOSH	National Institute for Occupational Safety and Health
ULN	Upper limit of normal
OSHA	Occupational Safety and Health Administration
WMA	World Medical Association

3. INTRODUCTION

3.1. Study Rationale

This study will be conducted in compliance with Good Clinical Practice (GCP).

This study is part of a large scale international initiative named *#EEGManyLabs* which aims at replicating influencal EEG published results in order to assess their robustness (Pavlov et al, 2021). A total of 27 studies were selected by the group for replication (studies shown in Figure 1).

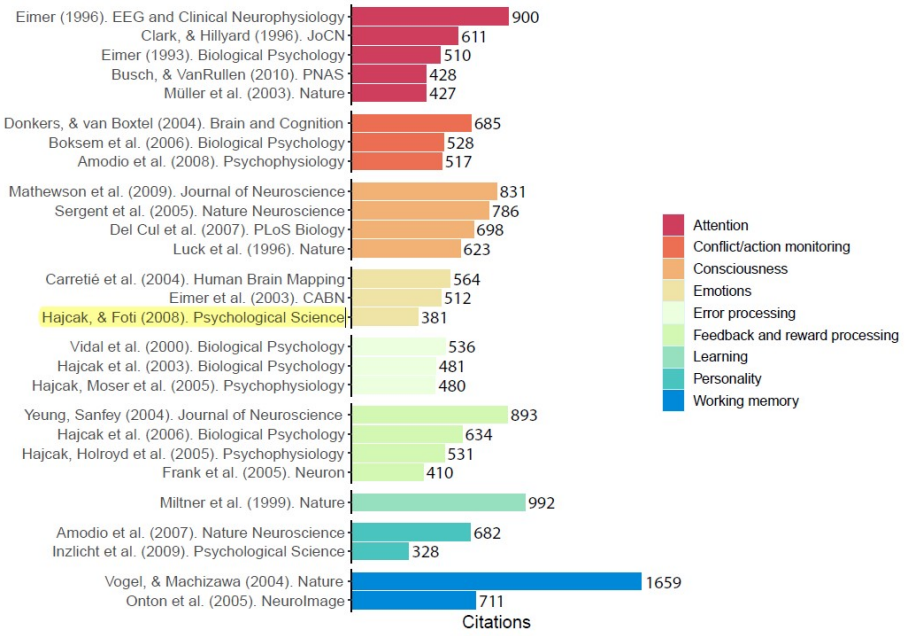

Figure 1: Selected studies for *#EEGManyLabs* initiative with associated number of citations per study (Google Scholar as for 01.10.2020) groupes by broaded defined topics. Colors indicates the domain of the study. Yellow = present study.

For each selected study, several international replicating labs (2 to 3) will be involved and will collect data and generate analyses, using metholodologies and procedures as close as possible to the original publications.

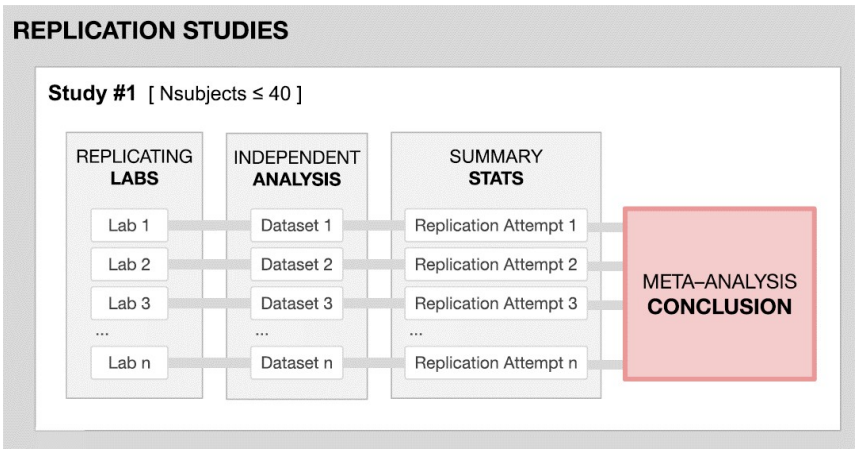

Figure 2: Modes of participation of the Replicating labs.

For each selected study, a Lead replicating lab will be defined and will conduct meta-analysis, the replication success being operationnaly defined as a statistically significant random-effects meta-analytic estimate (at $p<0.02$) combining the results of the different laboratories, in the same direction as the original study.

The present study, as part of the *#EEGManyLabs* initiative, aims at replicating results obtained in healthy participants by Olvet and Hajcak (2008).

Prior to enrolment in the study, participants will be informed about the objectives, methods and potential risks of the study. There will be no benefit of the study to individual subjects (no treatment, only assessments performed in the study).

3.2. Introduction

People tend to avoid mistakes. In a broad sense, errors are maladaptive responses that may place an organism in danger and threaten its safety. Without the ability to rapidly detect errors, it would not be possible to remedy them, or to adaptively regulate behaviours in changing environment (Falkestein et al, 2000). Research on error processing accelerated in the early 1990s and became a new largely shared focus in the Event-Related Potential (ERP) field of research through the discovery of Error-Related Negativity (ERN).

When recorded by electrophysiology tools (mainly EEG), errors are found to elicit a robust and sharp negative deflection in the brain ERP that peaks approximately 50 ms after the unintended response (Falkestein et al, 1991, 2000). From a brain anatomical perspective, when subjects make mistakes, the response-locked ERP is mainly observed at fronto-central recording sites. Studies using source localization method suggest that ERN is generated in the medial frontal cortex, most likely the anterior cingulate cortex, a region involved in cognitive and emotional processing (Deheane at al, 1994; Holroyd et al 1998; Miltner et al, 1997).

Because the ERN has been observed across different stimuli and response modalities, it is thought to reflect the activity of a generic response monitoring system (Bernstein et al, 1995; Deheane at al, 1994, Falkestein et al, 1991, 2000), acting as a kind of early alarm following error commission in order to increase cognitive control and make behavioural adjustments (Botwinick et al, 2001). However, as affective and motivational variables influence the magnitude of ERN, the authors hypothesized that ERN may relate to emotional and motivational aspects of error detection and may predict the magnitude of defensive reactivity (evaluated by startle in response to auditory stimuli). In other words, Olvet and Hajcak have shown in their study that :

1. The defensive startle responses were larger when participants made errors than when they responded correctly to the task
2. The impact of startle predictability was assessed by introducing both predictable and unpredictable startle stimuli in correct trials : predictability did not change the startle magnitude
3. The magnitude of the ERN predicted the degree of startle potentiation following errors: participants with larger ERNs demonstrated greater startle potentiation following errors.

The present clinical study, as part of the *#EEGManyLabs* replication effort, will attempt to replicate these results, using procedures as close as possible to the ones of the initial study:

- same behavioural task, the Flanker task, with identical settings (number of trials, inter-trial intervals, stimulus presentation time for the task)
- the EEG settings and the data processing pipeline will be similar to those of the initial study
- for startle responses, the auditory stimulus, the recording electrodes positioning and the data processing will be performed as described in the publication.

The present study will include healthy participants with no hearing problems and typical-to-corrected vision as test population.

This specific project proposed to provide new insight into the cognitive affective effects of making mistakes by measuring muscular and brain wave responses. Since the magnitude of muscular responses is increased when people find something to be aversive in some way (e.g., if they are irritated), such a correlation would provide evidence that making errors is aversive for participants. The degree of this correlation may mark individuals who are more or less sensitive to making mistakes which could be an objective marker of emotions such as anxiety.

3.3. Benefit/Risk Assessment

This is an interventional study with low risks and low constraints on human subjects.

No benefit will result for individual subjects enrolled in this study, except for participating to an innovative research project that will help to improve scientific knowledge on EEG biomarker ERN. The study's findings may benefit the broader society by helping to better understand the replicability of influential neurophysiology data, which could help researchers and the public assess the current state of the field. The findings may also help to understand the cognitive affective effects of making mistakes, which could improve understanding of negative affects such as anxiety and help to improve treatments for excessive anxiety and depression.

Areas of perceivable physical risk of possible concern are judged to be minor: hearing discomfort from exposure to bursts of white noise ("startle probes") used to elicit startle reflex, and minor skin irritation from either the physiological sensor gel or the sensor application process.

Noise exposure :

At a maximum of 105dB, the intensity of this sound is safely below levels at which there might be any risk of pain or physical damage as established by OSHA (Occupational Safety and Health Administration) and NIOSH (National Institute for Occupational Safety and Health) guidelines. Specifically, risk associated with noise exposure is reduced in the current experiment by limiting noise intensity to 105dB, limiting total noise exposure time to no

more than 5s, and using broadspectrum noise (i.e., white noise). The portion of the experiment in which white noise bursts will be delivered to participants will last approximately 10 minutes. During this period, participants will be exposed to no more than 100, 50-millisecond bursts of white noise for a total of 5 seconds of exposure. The noises will be delivered via headphones. The OSHA recommended limit for noise exposure at 105 dB is no more than 1 hour/day (OSHA section 191095).

According to the more conservative NIOSH guidelines (http://www.cdc.gov/niosh/98126.html), workers should not be exposed to 105 dB for more than 4 min and 43 sec per 8 hour day. As such, the noise exposure of the participants of the current trial is far under the more conservative limit recommended by NIOSH. By comparison, noise levels in small music venues (e.g., bars) often exceed 105 dB, and noise levels at rock and roll shows often exceed 115 dB. Students are occasionally in these environments for more than 1 hour. An additional safety factor is that "white noise" is being employed. White noise is full-spectrum, so there is no single frequency with concentrated energy. This acts as further protection to the subject by preventing the focus of energy to a limited area of the basilar membrane in the subject's cochlea.

Sensor application:

In addition to warning the participants of the risks, clinical staff will be fully trained by EEG internal specialists to practice EEG cap application to the subject and sensor (electrodes) placement prior to being cleared to attach sensors to research participants. They will also ask the participants to tell them if they feel any discomfort at all so they can adjust or stop the procedure. Additionally, the alcohol swabs used to clean electrode locations on the scalp are the same as those used in hospitals or other health facilities. The sensor gel used is a water based solution and does not cause any risk for the subject.

4. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To obtain brain ERP responses during the attentional task after errors or correct responses	• ERN as difference between error and correct trials
• To measure the defensive reactivity intensity during the attentional task after errors or correct responses	• Blink magnitude from the obicularis oculi in response to a 105 dB white noise sound in error and correct trials

5. STUDY DESIGN

5.1. Overall Design

This is an interventional study with low risks and constraints, in which the participants will be enrolled in a single study visit after being informed and after having signed an ICF.

The study to be replicated here involves participants making quick decisions during a Flanker (attentional) task about the direction of specific central arrows on screen. These arrows are surrounded by other arrows that face different directions. During the task and the participant's decision time window, brain waves and muscular responses will be simultaneously recorded. The goal is to observe a significant correlation between participants' brain wave response to making errors and the magnitude of their muscular response when making errors.

Details of the timing of the clinical visit, and the assessments to be perform are summarized in the schedules of activities (SOAs) in Section 2.

Participants will be prepared for the recording of physiological responses including facial electromyography (EMG) and electroencephalogram (EEG).

First, the EEG cap will be positioned on the participant's head and each EEG electrode will be filled with a conductive gel. Four additional EOG facial electrodes will be placed on the orbicularis oculi and two electrodes will be placed under the left eye to record eyeblink startle response.

Once all sensors will be placed, participants will complete a training session of the main task (shorten version) where acoustic white noise probes of 105dB will be presented through earphones at random points during the procedure (see the section 3.3 on risks for further details about noise exposure). These probes are used to elicit a startle reflex, which is used to index baseline emotional response. In addition, we will measure neuronal ERPs (via the scalp sensors). The training session will last approximately 3 minutes.

During the main task, participants will view a series of arrows on the computer monitor. Participants will perform a reaction time task using a standard button box. This involves participants making quick decisions about the direction of specific arrows shown on screen, arrows surrounded by other arrows that face different directions. In the meantime, participants' brain activity and muscular responses to the acoustic startle probe given in the task will be measured. Response to these probes will be used to index participants' affective responses. The main task will last approximately 10 minutes.

5.2. Number of Subjects

A total of twenty (20) neurotypical male / female participants will be included. The ratio of female participants should be approximately 50%, to the extent possible.

5.3. Number of Study Centres

The study will be performed at one (1) investigational site in Rennes (France).

5.4. End of Study Definition and Duration of Participation

The end of the study is defined as the date of the last visit of the last subject in the study.

The maximum duration of the study for a participant will be approximately 0.5 day at the most between screening and the assessment visit on D1.

5.5. Scientific Rationale for Study Design

The sample size in this pilot study has been arbitrarily defined based on the minimum sample size for meaningful results.

5.6. Identification of Source Data

The source data will consist of the data generated on-site by the EEG, the Flanker task, the EMG and EOG systems and the data collected in the Investigator's source data book.

6. STUDY POPULATION

Each volunteer must participate in the informed consent process and sign and date the informed consent form (ICF) before any procedure specified in this protocol is performed.

6.1. Inclusion Criteria

Participants will be required to fulfil all of the following inclusion criteria:

1. A signed and dated ICF before any study-specific screening procedure is performed.
2. Male and female subjects aged 18-45 years (inclusive).
3. Able to undergo study assessments and willingness to comply with study procedures.
4. All Subjects: typical-to-corrected vision needed in order to properly complete the behavioural task
5. All subjects: no known hearing problems.

6.2. Exclusion Criteria

If any of the following exclusion criteria apply, the participant must not enter/continue in the study:

1. Participation in an investigational drug or device study within 4 weeks prior to the clinic visit. The participant must not take part in any other trial during the study.
2. Pregnant women.
3. CNS-active medications within 4 weeks prior to the study.

6.3. Lifestyle Restrictions

The participants will be requested to abstain from consuming alcohol for 48 hours before admission and during the assessment visit. Participants will also be instructed to abstain from smoking from 1 hour before admission and during the assessment visit.

6.4. Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently included, because they fail to comply with inclusion and exclusion criteria.

Individuals who do not meet the criteria for participation in this study (screen failure) may not be rescreened.

6.5. Subject identification number

Subjects will be assigned an identification number (S followed by 3 digits) in chronological order at admission to the assessment visit, with the lowest number being S001.

Subjects withdrawn from the study retain their subject number, if already given. New subjects must always be allotted a new subject number: once assigned, subject numbers are never reused. Note that subjects will be identified by their subject number throughout the entire course of the study.

7. PRIOR AND CONCOMITTANT TREATMENTS

7.1. Prior Treatments

The use of CNS-active medications within 4 weeks before the assessment visit is prohibited.

7.2. Concomitant Treatments

No CNS-active medications are allowed during and 4 weeks prior to the study. Participants will be instructed to refrain from strenuous physical activity one day before and on the day of the assessment visit.

8. DISCONTINUATION CRITERIA AND RELATED PROCEDURES

8.1. Withdrawal/Discontinued Criteria

Any subject may be withdrawn from the study at the discretion of the investigator.

The participant is also free to terminate his/her participation at any time.

Participants withdrawn or discontinued from the study must not be reincluded.

8.2. Replacement of study participants

The investigator and the sponsor will decide to replace subjects who withdraw from the study for any reason other than safety.

Participant that will not complete the assessment visit will be replaced.

9. PROCEDURES

9.1. Staff training

Within the month prior to the start of the study, Biotrial EEG Corelab will organize a training session for the study team members that will be performing the experimental procedures.

Each staff member involved in the study will perform a dummy run as operator. The output of the dummy run will be assessed by the Corelab referent in terms of compliance with the instructions.

9.2. Investigational Schedule

The schedule of assessments is described in Section 2, Schedule Of Activities (SOA).

9.3. Screening visit

Demographic information will be collected at screening, including age (birth year and age at screening), gender, number of years of education, handedness.

9.4. Assessments

9.4.1. Assessment Methods and Experimental Schedule

All experimental procedures will be described in details in the study *User manual* written by the Biotrial study team.

The test session during the assessment visit will always start for the participant by a short training session (approximately 3 min) before the full-length test session (approximately 10 min).

9.4.2. Flanker task

The Flanker task assesses cognitive performance in domains of attention, speed of processing and executive functions.

In the present version of the Flanker task (Hajcak et al, 2005), for each trial, stimuli (arrow heads) will be presented on the computer screen in one of the following configurations (< < < < <, > > > > > , < < > < < , > > < > >) and participants will be required to focus on the center arrow and to signal whether they see the center arrow pointed in the same (compatible trials, < < < < < or > > > > >) or in the opposite direction (incompatible trials, < < > < < or > > < > >) versus the others by pressing a button of a response box (left button for compatible trials, right button for incompatible trials). A total of 8 blocks (with 30 trials per block) will be presented to the participant (total of 240 trials) with an equal frequency (50%) between compatible and incompatible trials. All stimuli will be presented during 200 ms, with an inter-trial interval that varies randomly from 500 to 1000 ms. At the end of each block, participants will receive performance feedback designed to encourage fast and accurate reponding:

- ✓ When performance is 75% correct or lower, the feedback will be “Please try to be more accurate”

- ✓ When performance is above 90% correct, the feedback will be "Please try to respond faster"
- ✓ When performance is between these levels, the feedback will be "you're doing a great job".

All participants will start by a practise block of 30 trials lasting around 3 min. Full test duration is 10 minutes.

9.4.3. EEG recordings

The electroencephalography (EEG) signal will be collected using an EEG cap with 32 channels, a set of individual electrodes located according to the 10-20 system rules. Contact between electrodes and scalp will be insured by a gel. The cap will be connected to an EEG amplifier and an acquisition computer. In addition, startle probe delivery will be performed through a headset (worn by the subject during the test) connected to a stimulation computer (to generate the auditory stimulus) and a response box (to record the subject's response).

The electroocculogram (EOG) signal used for artefact rejection and generated by blinks and eye movements will be recorded using 4 facial electrodes: ear linked references as well as two bipolar eye movement channels (VEOG, HEOG) placed on the left and right mastoids. The electrodes POz and GND will be used for reference.

Further details of the equipment and procedures are described in the *User Manual.*

9.4.4. Startle responses (EMG)

Recordings

The participant startle response will be measured using standard procedures and recommendations for assessing human startle eyeblink reactivity (Blumenthal et al, 2005).

Startle eyeblink participants responses will be measured using electromyographic (EMG) surface recording electrodes placed under the left eye, in the lower orbital portion of the orbiculairs oculi muscle.

Startle will be elicited using a 105 dB burst of white-noise of 50-ms duration and an instantenous rise time. Each time, startle probes will be delivered 300 ms after the participant' response.

Startle probes will be presented in 3 different situations described in Table 1.

Type of trial	Frequency	Situation
Error trial	50% of the error trials	Trial in which the participant made an incorrect response
Predictable correct trial	50% of correct trials that	Trial in which the participant

	follow error trials	made a correct response, preceded by a trial in which the participant made an incorrect response
Unpredictable correct trial	4% of the remaining correct trials (following a correct trial)	Randomly selected remaining correct trials

Table 1 : type of trials with starle probe delivery.

9.5. Outcomes measurements

Data metrics may include but are not limited to the followings.

9.5.1. Outcomes from the Flanker task

No behavioural endpoints will be derived from the Flanker task.

9.5.2. EEG/ERP outcomes

The primary EEG outcome will be :

- ✓ ERN (Error-Related Negativity).

ERN is defined as the difference between ERPs from error trials and ERPs from correct trials Δ(error-correct) in the window from 0 to 100 ms following response onset.

ERN will be primarly calculated on Fz and Cz channels (maximal response in FCz according to Hajcak et al, 2008).

For each EEG channel and in order to calculate ERN, ERPs from each trial type will be calculated and the following endpoints will be extracted from the difference between these ERPs :

- mean amplitude on the [0;100ms] period
- maximal amplitude on the [0;100ms] period
- peak latency on the [0;100ms] period.

9.5.3. Startle response outcomes

The startle endpoints will be :

- ✓ Startle response magnitude
- ✓ Startle response latency.

These outcomes will be quantified in terms of the peak in the window from 20 to 120 ms following startle probe onset.

10. DATA MANAGEMENT AND STATISTICS

10.1. Data Entry and Management

10.1.1. Data Collection

All the results from evaluations conducted during the study will be recorded in source data for each participant. Any personal information, including the study participant's name, must be removed or rendered illegible to preserve individual confidentiality.

All of the data from this study will be anonymised in order to meet EU regulatory requirements [General Data Protection Regulation (GDPR, 2016/679 EU)].

All the documents must be archived for a minimum of 25 years or according to the Sponsor's procedures, whichever is longer.

10.1.2. Data Sharing

All study results will be shared among study replicating labs, complying with FAIR principles, making the material Findable, Accessible, Interoperable, and Reusable (Wilkinson et al., 2016).

10.2. Statistical Considerations

10.2.1. Sample Size

The sample size in this pilot study has been defined based on the minimum sample size for meaningful results.

10.2.2. Statistical Methods

Startle response magnitudes and latencies and ERN will be statistically analyzed using an analysis of variance (ANOVA) with the Greenhouse–Geisser correction applied to p values associated with multiple df repeated-measures comparisons.

All data will be provided to the #EEG manylabs Core Team in order to perform a meta-analysis and compare them to data from Olvet and Hajcak (2008).

10.2.3. Protocol Deviations

A summary table of protocol deviations and the corresponding listing will be prepared.

11. REFERENCES

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12. APPENDICES

Appendix 1: Regulatory and Legal Considerations

RIGHT OF ACCESS TO DATA AND SOURCE DOCUMENTS

Monitoring

The investigator will allow the representative of the sponsor and the study monitor:

- To check the site, the facilities and the materials used for the study.
- To meet all members of the team involved in the study.
- To consult all the documents relevant to the study.
- To check that the CRFs have been correctly completed.
- To have direct access to source documents for comparison of data therein with the data in the CRFs.
- To check that AEs have been documented.
- To verify that the study is carried out in compliance with the protocol.

This study will be monitored at regular intervals, by mutual agreement of the investigator and monitor.

All information dealt with during these visits will be treated as strictly confidential.

The investigator will provide the sponsor with the following:

- Progress reports at regular intervals.
- Adequately completed CRFs.

Audit- Inspection

The investigator will be informed that an audit will be carried out, at the request of the sponsor, before, during or after the study.

The investigator will be informed that the regulatory authorities may also carry out an inspection. In this case, the Investigator must inform the sponsor as soon as he receives the notification of inspection.

The investigator must allow the representatives of the regulatory authorities and persons responsible for the audit to:

- Inspect the site, facilities and material used for the study.
- Meet all members of his team involved in the study.
- Have direct access to study data and source documents.
- Consult all the documents relevant to the study.

QUALITY CONTROL AND QUALITY ASSURANCE

Written procedures describing data flow, data entry or electronic capture, data cleaning and processing, and required quality control must be utilized.

The entered/electronically transferred data will be systematically checked by Biotrial staff, either by using error messages printed from validation programs or by manual checks carried out based on database listings.

After the completion of the data cleaning and data review process and full resolution of the data queries, the database will be locked. Any changes to the database after that time may only be made by joint written agreement between the Clinical Pharmacologist and the investigator.

The Investigator must guarantee the safety of the study data in the medical files by implementing security measures to prevent unauthorized access to the data and to the computer system.

STUDY SUSPENSION, TERMINATION, AND COMPLETION

The sponsor may suspend or terminate the study or any part of the study at any time for any reason.

If the investigator suspends or terminates the study, the investigator will promptly inform the sponsor and the regulatory authorities and provide them with a detailed written explanation.

Upon study completion, the Investigator will provide the sponsor and IEC with final reports and summaries as required by regulations.

ETHICS AND REGULATORY ASPECTS

Current texts

The study will be carried out in accordance with:

- The most recent recommendations of the World Medical Association (WMA).
- The ICH recommendations: Good Clinical Practice [E6 (R2)], (CPMP/ICH/135/95), 2016.
- French law n°78-17 of 6th January 1978 relative to Data processing, Data files and individual liberties, modified by law n°2016-41 of 26 JAN 2016, ruling n°2016-800 of 16 JUN 2016 and updated by deliberation n°2016-262.
- GDPR (EU n° 20/6/679).
- French law n° 2012-300 of 5 MAR 2012 and decree n°2016-1537 of 16 NOV 2016.
- Decree of 12 APR 2018 with regards to the type of research involving human persons described in 2° of article L1121-1 of Code de la Santé Publique.

Subject Information and Consent

An unconditional prerequisite for a subject's participation in the trial is their written informed consent. The volunteer's written informed consent to participate in the trial must be given before any trial-related activities are carried out.

The subjects will be verbally informed by an investigator of all pertinent aspects of the trial: the nature of the study, its aim, its possible risks and restrictions, its duration and for the healthy volunteers the fee that they will receive. The protocol will be explained during a meeting prior to the study and each subject must be informed that participation in the study is voluntary and that they may withdraw from the study at any time. At this meeting, an information sheet will be given to each subject. The language used in doing so must be chosen so that the information can be fully and readily understood by lay persons.

The subject should carefully read before signing and dating the informed consent form. They can ask all necessary questions to the investigator. The informed consent form must be signed and personally dated by both the subject and the investigator. A copy of the signed document should be given to the subject and kept by the investigator for 25 years.

Whenever important new information becomes available that may be relevant to the subject's consent, the written participant information sheet and any other written information provided to the subjects will be revised by the sponsor and be submitted again to the IEC for review and favourable opinion/authorisation. The agreed, revised information will be provided to each subject in the trial for signing and dating. The investigator will explain the changes from the previous version.

Submission to the authorities

Ethics Committee (CPP)

It is the responsibility of the sponsor to seek and obtain the favourable opinion of the CPP (Comité de Protection des Personnes, i.e. IEC). This activity is delegated to Biotrial.

The present biomedical trial will not be initiated until this favourable opinion is obtained.

ANSM authorization

No ANSM authorisation is required as no investigational medicinal product will be used. However, the summary of the study and the opinion of the CPP will be sent to the ANSM.

Protocol Amendments

Any significant change (substantial modification) in the study requires a protocol amendment for CCP approval. Concerning a protocol amendment sent to the CPP for approval, an investigator must not make any changes to the study without approval of the CPP and the sponsor except when necessary to eliminate apparent immediate hazards to the subjects. A protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, but the change must then be documented in an amendment, reported to the IEC within 5 working days, and submitted in the required time frame. All protocol amendments must be reviewed and approved following the same process as the original protocol.

DATA PROCESSING AND ARCHIVING OF DOCUMENTS AND DATA RELATIVE TO THE RESARCH

After the study, the investigator will keep all information relevant to the study for 25 years.

CONFIDENTIALITY

Before starting the study, the investigator must confirm receipt of adequate documentation from the sponsor so as to be able to decide whether or not to perform the study.

All documents and information given to the investigator by the sponsor with respect to the study are strictly confidential.

The investigator and his colleagues agree to use them only with the framework of this study, in order to carry out the protocol. This agreement is binding as long as the confidential information has not been disclosed to the public by the sponsor.

The Investigator may use the technical protocol to obtain the informed consent of study subjects. It must not be disclosed to other parties without the written authorisation of the sponsor.

The investigator keeps a confidential participant identification list for the study. The investigator must maintain source documents for each participant in the study.

Data on the subjects collected in CRFs during the study will be documented in an anonymous fashion. All information in the CRFs must be traceable to these source documents.

DATA AVAILABILITY

Data will be shared with researchers within the #EEGManylabs project. These data will be shared outside the group e.g. for integration with other international efforts, including the use of platforms for data integration that allow federation and open access.

Access may require recipients to consent to a data sharing agreement (particularly in light of GDPR, and other local regulations as relevant).

REPORT AND PUBLICATION

Study Report

Upon completion of the study, data will be globally analysed with those issued from the #EEGManylabs project Core Team for scientific publication. No Clinical Study Report will be prepared by Biotrial.

Data Ownership

The data collected in this study are the property of Biotrial.

Data sharing

Biotrial will provide study data to the #EEGManylabs project Core Team that will follow the provided Data Management Plan.

Publications

The results of this study will be submitted for publication at the discretion of the #EEGManylabs project Core team.