Neurovegetative decoupling in the visceral-brain axis and cognitive-emotional vulnerability in somatoform disorders: Interest of vagal biofeedback

BIOFEE-SOMATO

Monocentric study

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STUDY PROTOCOL & STATISTICAL ANALYSIS PLAN

I : Study protocol

The study requires 3 sessions (T1 / T2 / T3) with at least 24 days between each. The 3 sessions are identical in term of experimental design.

The first session (T1) will start by asking to the participants to answer to 8 self-reported questionnaires (STAI Y-A, PANAS, CES-D, PSS, CFS, VAS, SWLS and ICQ-18) aiming to evaluate the emotional state, the life satisfaction and the acceptance level of pathology.
It will be followed by an examination of the autonomic nervous system [ANS] functioning and the ANS - central nervous system [CNS] – enteric nervous system [ENS] electrophysiological coupling by using different electrophysiological tools (electrogastrogram [ECG], galvanic skin responses [GSR], photoplesthysmography [PPG], breathing belt, electrogastrogram [EGG], electroencephalogram [EEG]). All the electrophysiological recordings will be synchronized and an acclimatization period will be respected. The recordings will be done seated in a quiet room. The physiological measures will ever be recorded at rest (30 minutes, eyes-closed), during a reactivity emotional task (reactivity period) and in the recovery period following the task (30 minutes, eyes-closed). Electrophysiological data will be collected by the acquisition systems BIOPAC® (MP 150, CEROM, France) and BioSemi®. The ECG data will be recorded using 3 single use and adhesive electrodes placed on the inner side of the right wrist, on the right shoulder and on the left side in accordance with the DII standard position (Einthoven). Physiological data recorded are related to the heart rate variability [HRV]. The breathing effects on the HRV will be controlled by means of the breathing rate recording using a breathing belt. The GSR data will be recorded using 2 skin sensors placed on the third phalanx of the forefinger and of the middle finger of the left hand. Physiological data recorded are related to the cholinergic sympathetic activity (tonic GSR / phasic GSR). The PPG data will be recorded using a finger sensor. Physiological data recorded are related to the adrenergic sympathetic tone and allowing a record of the blood pulse waves associated with the heart rate. The EGG data will be recorded using 6 single use and adhesive electrodes placed on the skin of the abdomen. Physiological data recorded are related to the gastric electrical activity. The EEG data will be recorded using a EEG headsets including 64 electrodes. The EEG is related to the brain activity generated by the neural functioning. The emotional reactivity task will be explained carefully.

The emotional reactivity computerized task was developed specifically for our protocol with OpenSesame® software. The task looks like a type of stroop task. Instructions are to identify as soon as possible the ink color of the word on the screen matches the meaning of the word, in doing as less as possible mistake. We ask to the participants to press a specific button on the keyboard in the case of the ink color of the word matches the meaning of the word and to press on another button on the keyboard if they don't. We developed the task in 3 similarly but not identical versions with the aim of randomly testing the 3 versions on each participant. Each version lasts 12 minutes and has some particularities such as the colors of the words. We generated 3 versions of the task in the aim to limit the classical habituation effect in participants. Each version is made of 6 blocs in 20 trials that is aiming at progressively increasing the stress level because of an increase of the mental load. Bloc 1[1] and bloc 2 are associated with a low stress, bloc 4 and bloc 5 are associated with a moderate stress and bloc 5 and bloc 6 are associated with a high stress. The increase of the mental load is permitted by slowly decreasing the word presentation time, by putting feed-back, instruction switches, time constraints and lastly, by the necessity to do a dual task.

STAI Y-A / PANAS / CES-D / PSS / CFS / SWLS / ICQ-18	ECG/EGG/EEG/ GSR/PPG/Emwave/ respiratory belt positioning	Resting period (30 minutes, seated, eyes closed)	Reactivity period (during the task, seated)	VAS	Recovery period (30 minutes, seated, eyes closed)

3h30

The period (24 days) between the first session and the second session (T1-T2) will be considered as the control period. The larger BFB effects than the unspecific effects related to the spontaneous physiological and emotional variations will be verified through the control period. We will measure the parameters of interest before (T1) and at the end of the control period (T2). During the period, the participants will have to answer to online questionnaires (SSQ6, MAIA-2, STAI Y-B, Brief-Cope, BFI-N, CTQ) aiming to examine the psychological profile (social support, interceptive sensitivity, trait anxiety, style of coping, early life events).

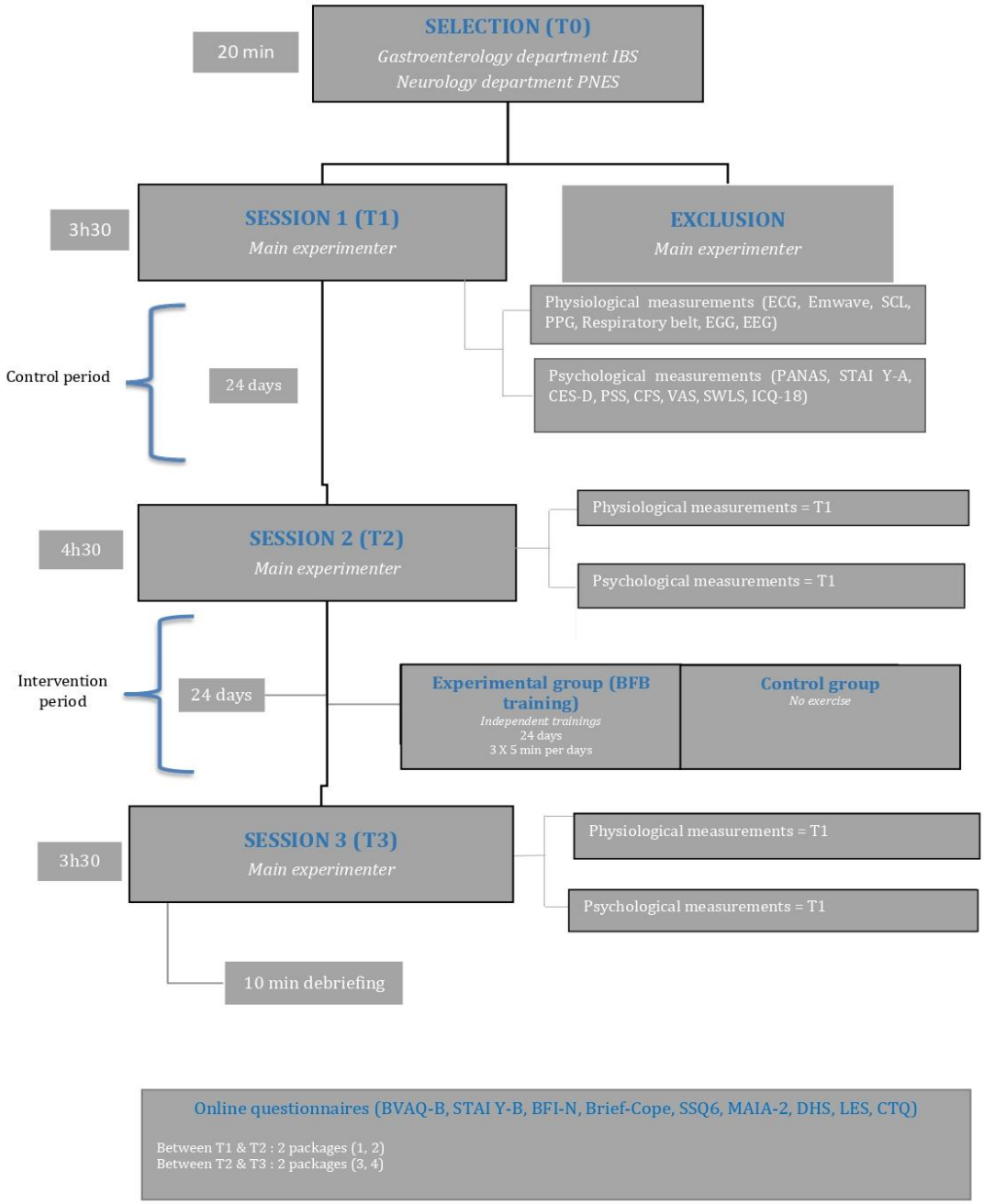
The second session (T2), T1 + 24 days, will be similar of the first session. We will use the same questionnaires and will record the same electrophysiological parameters. At the end of the

[1] The bloc 1 will not be included in the final statistical analysis because is considered as a training bloc. This choice in accordance to the potential biases related to the increase of the sympathetic activity at the beginning of the task caused by a simple moving to action following the resting period.

second session, a short training session of the biofeedback [BFB] technique will be done and the software using for the biofeedback training will be supplied to each participant included in the experimental group to allowed to us to practice BFB by their self at home. The installation of the program and the explanations needed for using it, will be done during the second session (T2). The BFB software (Emwave Pro®) includes a photoplethysmography sensor that can be positioned on the earlobe. The participants will have to practice the biofeedback technique the next 24 days (3X5 min/day).

The period (24 days) between the second session and the third session (T2-T3) will be considered as the intervention period. At the end of the second session (T2), the participants will be separated into two inter-subject groups: an experimental group performing BFB technique (3X5 min per day) in the intervention period (T2-T3) and a control group not performing a specific exercise in the intervention period (T2-T3). The first 40 participants (20 IBS / 20 PNES) will be included into the experimental group (BFB training) whereas the last 10 participants (5 IBS / 5 PNES) will be included into the control group (no training). The participants won't be informed of the condition to which they belong. We will measure the parameters of interest before (T2) and at the end of the intervention period (T3). During the intervention period, all the participants will have to answer to online questionnaires (BVAQ-B, LES, DHS) aiming to examine the psychological profile (alexithymia, traumatic events).

The third session (T3), T2 + 24 days, will be similar of the two previous sessions. We will use the same questionnaires and will record the same electrophysiological parameters. Finally, a debriefing will be done at the end of the session for each participant. The session will be considered as the end of the study visit.



Concerning the online questionnaires: the package 1 is constituted by the SSQ6, the MAIA-2 and the STAI Y-B. The package 2 is constituted by the Brief-Cope, the BFI-N and the CTQ. The package 3 is constituted by the DHS. The package 4 is constituted by the BVAQ-B and the LE

II : Statistical analysis plan

Independent variables

Analysis A:

The participants will be separated into two inter-subject groups:

- An experimental group performing BFB technique in the intervention phase (T2-T3)
- A control group no performing an exercise in the intervention phase (T2-T3)

The participants won't be informed of the condition to which they belong.

Independent variables

- Measurement time (T1 / T2 / T3) intra-subject
- Group (experimental group / control group) inter-subject

Analysis B:

The participants will be separated into two inter-subject groups performing by a cluster analysis (HF or RMSSD) in the first session (T1):

- A dysfunctional neurovegetative profile (low vagal tone + low vagal flexibility) group
- A functional neurovegetative profile (high vagal tone + high vagal flexibility) group

The participants won't be informed about their neurovegetative profile. We will include all the participants.

Independent variable

- Neurovegetative profile (dysfunctional profile / functional profile) inter-subject

Analysis C:

The participants will be separated into two inter-subject groups performing by a cluster analysis (HF or RMSSD) in the first session (T1):

- A dysfunctional neurovegetative profile (low vagal tone + low vagal flexibility) group
- A functional neurovegetative profile (high vagal tone + high vagal flexibility) group

The participants won't be informed about their neurovegetative profile. We will include only the experimental group performing the BFB technique.

Independent variables

- Neurovegetative profile (dysfunctional profile / functional profile) inter-subject
- Measurement time (T2 / T3) intra-subject

Dependent variables

Physiological variables:

ECG –HRV:

- HF (0.15-0.40 Hz – high frequency) frequency-domain parameter
- LF (0.04-0.15 Hz – low frequency) frequency-domain parameter
- LF-0.1 Hertz (0.075-0.108Hz - spectral power of the low-frequency 0.1Hz band) frequency-domain parameter
- LF/HF (ratio of LF to HF power) frequency-domain parameter
- Total power (total power of the 0-0.40 Hertz band) frequency-domain parameter
- RMSSD (root mean square of successive RR interval differences) temporal-domain parameter
- SDNN (standard deviation of all NN intervals) temporal-domain parameter
- EDR (ECG-derived-respiration)

GSR (tonic GSR / phasic GSR):

- SCR frequency: number of the spontaneous galvanic skin responses by periods
- SCR amplitude: amplitude of the spontaneous galvanic skin responses by periods
- Integrated skin conductance responses [ISCR]: area of the galvanic skin responses identified on the signal

PPG:

- Pulsatility index variation (PI): Transit time flow

Breathing belt:

- Breathing rate by cycles per minute

EGG:

- Dominant power
- Total power [0-0.15 Hertz]
- Slow-waves frequency per minute

EEG:

- Spontaneous brain rhythms: spectral power of the 0-30 Hertz band

Physiological adaptation to the aversive situation (emotional reactivity task):
HRV, GSR and PPG differences will be calculated to measure the physiological changes causing by the task compared to the resting and the recovery periods. The differences will be calculated as follows:

- Physiological reactivity during the task compared to the resting period (Δ = reactivity HRV – resting HRV / Δ = reactivity GSR – resting GSR / Δ = reactivity PPG – resting PPG)
- Physiological recovery following the task (Δ = recovery HRV – reactivity HRV / Δ = recovery GSR – reactivity GSR / Δ = recovery PPG – reactivity PPG)

- Physiological recovery following the task compared to the resting period (Δ = recovery HRV – resting HRV / Δ = recovery GSR – resting GSR / Δ = recovery PPG – resting PPG)

Psychological variables:

- Alexithymia score (BVAQ-B)
- Neuroticism score (BFI-N)
- Trait anxiety score (STAI Y-B)
- Style of coping (Brief Cope)
- Positive affectivity score (PANAS)
- State anxiety score (STAI Y-A)
- Depressive symptoms score (CES-D)
- Perceived-stress level (PSS)
- Coping flexibility + metacoping scores (CFS + VAS)
- Acceptance score (ICQ-18)
- Social support score (SSQ6)
- Interceptive sensitivity score (MAIA-2)
- Life satisfaction score (SWLS)
- Negative impact scores (LES)
- Frequency, severity and intensity scores (DHS)
- Abuse scores (CTQ)

Statistical analyses

All statistical analyses will be performed using IBM SPSS Statistics®, R software® and JASP®. The alpha value for statistical significance will set at $p<0.05$.

Analyses of the scores collected will be done in several stages:

- Highlighting outlier participants to exclude from our statistical analysis.
- All the conditions for application of statistical tests will be verified.
- In the case of a failure to respect the conditions for parametric test application, we would make a data transformation to jointly improve normality and homogeneity residues. If the normality is failed and the homogeneity is respected, we would execute non-parametric tests.
- Analysis will be done on the subjects included. Analysis concerning the reactivity period (during the task) will be performed on the all blocs excepted the bloc 1[2].
- Main and secondary judgment criterions analysis will be performed by parametric tests or by non-parametric tests depending on the conditions for application:

  - *Analyse A:*
    - An ANOVA to repeated measurement with an intra factor (measurement time: T1 / T2 / T3) and an inter factor (group: experimental / control) on the physiological and psychological data collected.
    - Student's test for independent samples will be performed on the psychological scores collected in the second session (T2) to account for the initial level of psychological states between the 2 groups.

[2] The bloc 1 will not be included in the final statistical analysis because is considered as a training bloc. This choice in accordance to the potential biases related to the increase of the sympathetic activity at the beginning of the task caused by a simple moving to action following the resting period.

- Contrasts will then be made by group on the scores in order to specify the effect of the condition on the physiological and psychological data.

· *Analyse B:*
- K means non-hierarchical clustering will be performed with K = 2. To categorise participants depending on their resting neurovegetative profile (low vagal tone / high vagal tone) and on their dynamical neurovegetative profile (low vagal flexibility / high vagal flexibility), two K means non-hierarchical clustering will be performed with K = 2. One with HF or RMSSD and one with Δ HF or Δ RMSSD.
- Student's test for independent samples (dysfunctional neurovegetative profile / functional neurovegetative profile) will be performed on the psychological vulnerability (trait scores) collected.

· *Analyse C:*
- An ANOVA to repeated measurement with an intra factor (measurement time: T2 / T3) and an inter factor (neurovegetative profile: dysfunctional profile / functional profile) on the physiological and psychological data collected only into the experimental group performing the BFB technique.

· *Correlational analysis:*
- Regressions or the Pearson correlations will be performed in order to measure the inter-signal correlation (ECG-EGG-EEG)
- Regressions or the Pearson correlations will be performed in order to test our hypothesis about the HF/RMSSD related to the "low vagal tone" profile will be more correlated to Δ HF/RMSSD related to the "low vagal flexibility" profile than to Δ HF/RMSSD related to the "high vagal flexibility" profile. As the same as HF/RMSSD related to the "high vagal tone" profile will be more correlated to Δ HF/RMSSD related to the "high vagal flexibility" profile than to Δ HF/RMSSD related to the "low vagal flexibility" profile

Additional analyses
- We will control the age effect and the gender effect.
- Some additional statistical analyses will be exploratory: stability of the resting and the dynamical neurovegetative endophenotypes before the control period (T1) and at the end of the control period (T2).
- Correlation between the LF 0.1Hz and the clinical outcomes.
- To confirm the physiological effects of the BFB (BFB *vs* pseudo-BFB) on the flexibility of the electrophysiological coupling concerning the brain-visceral axis.

Sample
Power analysis (G * Power; Faul et al., 2007) was performed to determine a priori the sample size needed for this study. The power analysis, for an ANOVA (repeated measures, within-between factors), was performed for an effect size (η_p^2) of .12 with a power of .95 on the main judgment criterion (HRV – vagal tone HF) and reported a necessary sample of at least 22 participants (Kudo et al., 2014; Paul & Garg, 2012; Wells et al., 2012). We decided to include at least 25 IBS patients SII (20 test & 5 control) and 22 PNES patients (20 test & 5 control) in accordance with the possible inclusion rate and the duration of the study.