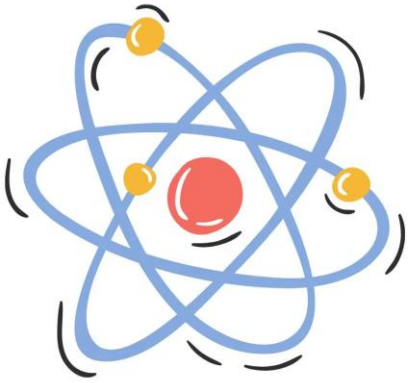

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Abstract

Introduction: *The ability to regulate one's physiology against stress, or Neuro-physiological Adaptation Capacity (NAC), is a cornerstone of health and resilience. However, its objective assessment in applied settings is limited by lack of brief, standardized protocols that integrate signals from the central and autonomic nervous systems.*

Objective: *This pilot study aimed to validate feasibility and sensitivity of Neuro-Integral 360 (NI360) protocol to elicit and quantify psycho-physiological stress and recovery responses.*

Methodology: *A pilot study with a quasi-experimental, repeated-measures design was conducted on a sample of 25 healthy adults (mean age = 34.2 ± 9.1 years). Participants completed the five-phase NI360 Protocol. Heart rate (HR), heart rate variability (RMSSD), and the EEG Theta/Beta ratio (TBR) were continuously recorded. Paired t-tests with FDR correction were applied.*

Results: *The cognitive challenge induced a significant stress response, with an increase in HR (mean Δ = +35.1 bpm, $p < .001$) and a suppression of RMSSD (mean Δ = -23.7 ms, $p < .001$) compared to rest. Guided-breathing recovery significantly increased RMSSD above baseline levels ($p = .014$), demonstrating strong vagal modulation.*

Conclusion: *The Neuro-Integral360 Protocol is a viable, brief, and sensitive tool for the multimodal assessment of the stress response, laying the groundwork for its application in research and clinical practice. These preliminary results demonstrate that the Neuro-Integral 360 Protocol is a viable and effective tool for evoking and measuring neurophysiologic stress and recovery responses, justifying its validation in larger cohorts.*

Introduction

Psycho-physiological self-regulation is a critical component of human resilience. Neuro-visceral Integration Model (NIM) posits that heart rate variability (HRV) reflects functional integrity of a fronto-limbic brain network responsible for emotional and cognitive regulation [Thayer and Lane, 2000]. High HRV and rapid recovery after a stressor are associated with better executive function [Holzman and Bridgett, 2017]. Conversely, chronic dysfunction can lead to an accumulation of physiological 'wear and tear,' or alostatic load, which increases the risk of chronic diseases [McEwen, 2017]. Despite its importance, challenges

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persist in its assessment in applied settings. To address this gap, the Neuro-Integral360 (NI360) Protocol was designed. This pilot study aims to empirically validate its feasibility and sensitivity to induce and quantify changes in neuro-physiological adaptation.

Neuro-Integral 360 is a 25-minute, multimodal neuro-physiological assessment that observes how a person's brain, cardiovascular system and subtle facial motor signals respond across five controlled conditions: baseline rest, cognitive load, acute stress, guided relaxation and post-recovery capture. Lightweight sensors plus two ultra-brief self-report scales (Perceived Stress, Resilience) feed into an explainable AI (XAI) engine that produces an individualized Neuro-physiological Map and an ultra-personalized recommendation set. The goal: reveal patterns that ordinary questionnaires alone cannot detect, enabling early risk detection, performance optimization and targeted preventive strategies across academic, clinical, corporate, athletic or high-risk operational domains.

Human performance and well-being depend on dynamic regulation, capacity to up-shift (focus / mobilize under demand) and down-shift (recover efficiently). Traditional screening often leans on subjective questionnaires or single biomarkers. The intricate relationship between the brain's cognitive and emotional processes and the body's physiological state represents a frontier in optimizing human performance and mental health. Self-regulation, the ability to flexibly adapt thoughts, emotions, and behaviors to meet situational demands—is a cornerstone of resilience, wellbeing, and peak performance. Conversely, dysregulation of these processes, often driven by chronic stress, contributes to a broad spectrum of physical and mental health disorders [McEwen, 2017].

Historically, the assessment of cognitive states such as mental workload and stress has largely relied on self-reports or performance metrics, which may be imprecise and late indicators of an individual's underlying state. Advances in non-invasive biosensor technology offer a paradigm shift, enabling objective, continuous, real-time tracking of physiological correlates of brain function [Di Flumeri et al., 2019]. This paper establishes critical link between central nervous system function and peripheral physiology, arguing for the necessity and validity of objective, non-invasive biomarkers to quantify cognitive load, stress, and resilience. It focuses on two primary and complementary modalities: Heart Rate Variability (HRV), a powerful indicator of autonomic nervous system (ANS) function, and Electroencephalography (EEG), a direct measure of cortical electrical activity. By synthesizing evidence from basic neuroscience, clinical research, psychophysiology, and biomedical engineering, this protocol builds a robust, coherent argument, moving from fundamental theory to practical application.

Theoretical Framework

Neuro-Integral 360 addresses four **gaps**:

Gap in Conventional Approaches	**Neuro-Integral 360 Response**
Subjective bias in self-reports	Objective multimodal signals (EEG frontal, pulse variability, oxygen saturation, micro-expressions').
Static 'snapshot' measurements	Sequential state transitions across standardized phases.
Black-box analytics	Explainable AI with interpretable feature contribution (e.g., SHAP / feature weight breakdown).
Generic recommendations	Data-driven, individualized intervention prescriptions.

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Core Components

Component	Function	Modality
Frontal EEG (single-channel band)	Attention / relaxed focus index, spectral shifts (α, β, θ trends)	Headband (dry electrode)
Peripheral photoplethysmography	Heart rate (HR), pulse-derived RV (e.g., RMSSD), oxygen saturation (SpO_2)	Finger ring / PPG
Facial micro-expression &pupil tracking	Emotional/micro-tension dynamics across phases	Periodic still frames (camera) + AI parsing
Brief psychometrics	Perceived Stress (PSS-10),	Digital forms (≈5 min total)
	Resilience (CD-RISC-10)	
Explainable AI engine	Data fusion, index computation, predictive risk flagging, report generation	Neuro Integral XAI stack

Note: Micro-expressive data are sampled in each phase (not only at the end) to build a temporal emotional micro-trajectory.

Neurovisceral Integration Model

Neurovisceral Integration Model (NIM), proposed by Thayer and Lane, provides a powerful framework for understanding bidirectional communication between the brain and the heart [Thayer and Lane, 2000; Thayer and Lane, 2009]. Its central premise is that a common set of neural structures, known as the Central Autonomic Network (CAN), is responsible for concurrent regulation of cognitive, affective, and autonomic processes [Smith et al., 2017]. The prefrontal cortex (PFC) exerts tonic inhibitory control over subcortical structures such as the amygdala, which is essential for flexible, adaptive behavior [Thayer and Lane, 2009].

This central regulatory network is intrinsically linked to peripheral nervous system through the vagus nerve, the main conduit of parasympathetic influence on the heart [Thayer et al., 2009]. Accordingly, the NIM proposes that the functional integrity of these prefrontal–sub cortical inhibitory circuits is reflected peripherally in the degree of vagal influence on the heart, measured via Heart Rate Variability (HRV) [Park and Thayer, 2014]. HRV—specifically vagally-mediated HRV (vmHRV) such as RMSSD—thus becomes a powerful non-invasive proxy of higher-order brain function [Shaffer and Ginsberg, 2017; Thayer et al., 2009]. Higher resting vmHRV indicates greater autonomic flexibility and more effective inhibitory control from the PFC, and is associated with better performance in executive functions such as working memory, attention, and inhibition, as shown by multiple studies and meta-analyses [Holzman and Bridgett, 2017; Forte et al., 2019; Hansen et al., 2003].

Allostatic Load

Whereas the NIM explains acute regulation, the theory of allostatic load—refined by McEwen—offers a perspective on the long-term consequences of chronic stress [McEwen and Stellar, 1993; Gersten and Gersten, 2021]. Allostasis is the process of achieving stability through physiological change [McEwen, 2017]. Allostatic load is the cumulative ‘wear and tear’ on the body resulting from chronic over activation of these adaptive systems [Juster et al., 2019]. This load is operationalized as a composite index of biomarkers representing neuro-endocrine, cardiovascular, metabolic, and inflammatory systems [Seeman et al., 2001]. Secondary markers such as systolic and diastolic blood pressure, cholesterol, glycated hemoglobin (HbA1c), and C-reactive

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protein (CRP) are potent indicators of accumulated biological risk and are strongly associated with increased cardiovascular disease risk and all-cause mortality [Gruenewald et al., 2021; Guidi et al., 2021]. Although HRV is not traditionally included, it is conceptually integral: chronically low HRV is an early functional indicator of autonomic dysregulation that, over time, contributes to structural changes measured by classical allostatic load markers [Juster et al., 2019].

Objective

Central Objective: To estimate, with high temporal resolution, the Neuro-physiological Adaptation Capacity (CAN)—i.e., the dynamics of response and recovery of the neuro-autonomic and cortical systems to a standardized cognitive demand—by fusing frontal EEG, beat-to-beat ECG, blood pressure, and psychometrics, with explainable AI analytics.

Scope

- Reproducible metrics of sympathetic reactivity, vagal recovery, and cortical efficiency.
- Risk-stratification models and personalized prescription of micro-interventions.
- Methodological foundation for publication in indexed journals and multicenter scaling.

Translation: A single procedure enables screening (preventive), risk anticipation (predictive), and personalization (prescriptive) without changing the base workflow.

Methodology

Population, Criteria, and Confounder Control

Scope: University students and staff; extrapolatable to clinical/occupational settings.

Inclusion: 18–65 years; BMI 18–32 kg/m²; capacity to consent; no acute neurological pathology.

Exclusion: Known arrhythmias or pacemaker/ICD; β-blocker use; thoracic dermopathies (chest strap); high-risk pregnancy.

Pre-evaluation instructions (Confounder Control):

- Caffeine/stimulants: Abstain for 4–6 h beforehand.
- Intense exercise: Avoid on the test day.
- Sleep: Record quality/duration the previous night (VAS 0–100 or optional PSQI-A).
- Controlled psychopharmacology and psychiatric diagnoses (anxiety/depression): Recorded for statistical adjustment.

Stratification and Covariates: Age and sex (and, if applicable, sleep and caffeine) as covariates in all models; balanced sampling by group (student/faculty).

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Structured 25-Minute Sequence (Operational Breakdown)

Phase (5 min each)	Participant Instructions (Concise)	Regulatory Target (Plain)	Key Signal Transitions (Technical)	Principal Indices Produced
1. Guided Rest	Sit comfortably, eyes closed, breathe naturally.	Baseline calm	Stable α/θ profile, baseline HRV & CNF (coherence), neutral facial AUs.	Baseline Coherence, Baseline HRV
2. Cognitive Load (N-Back)	Perform 2-back memory task (visual/auditory).	Controlled executive demand.	↑ relative β power, ↓ α, reaction time & error metrics, pupil change.	Cognitive Load Index (ICC), Focus Stability
3. Acute Stress Challenge	Exposure to white noise + accelerating countdown timer.	Sympathetic mobilization under pressure.	HRV contraction, transient SpO_2 shifts, micro-expressive tension emergence, possible β spike.	Acute Stress Index (IEA), Reactivity Delta
4. Guided Relaxation	Follow paced diaphragmatic breathing audio.	Parasympathetic rebound / resilience.	HRV recovery trajectory, α rebound, β normalization, facial relaxation markers.	Recovery Index(IRR), Autonomic Flex Score
5. Post-Recovery Capture	Neutral gaze for final photo sequence.	Residual load / fatigue check.	Compare facial AUs& pupil metrics vs Phase 1, confirm HRV stabilization.	Residual Tension Score, Fatigue Flag

Study Design and Participants.

A pilot study with a repeated-measures design was conducted. A convenience sample of 25 participants was recruited from the university community. The final sample consisted of 25 participants (48% female), with a mean age of 34.2 (SD = 9.1) years and a mean BMI of 25.1 (SD = 3.5) kg/m².

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'Reading' the Phases (Dual Audience Layer)

Non-expert takeaway	Expert interpretation cue
Rest = your natural starting point.	Low baseline HRV + low coherence = autonomic over-activation risk.
Cognitive load shows mental horsepower.	Disproportionate β surge + slowing reaction times = inefficiency / possible cognitive fatigue.
Stress reveals pressure tolerance.	HRV drop magnitude + rate of facial tension emergence = sympathetic over-reactivity marker.
Relaxation is the 'cool-down system.'	Recovery half-time (HRV to 90% baseline) > threshold = impaired vagal regulation.
Final capture checks hidden residues.	Persistent tension AUs or sustained pupil dilation = unresolved arousal / fatigue residue.

Multi-Layer Metrics

Index	Construct	High-Level Definition	Indicative Threshold (Adjustable)	Interpretation
Coherence (CNF)	Neuro-autonomy c integration	Correlation / synchronization between frontal EEG patterns & HRV trend at rest.	≥70% (green)	Efficient baseline regulation.
HRV (RMSSD-PPG)	Autonomic flexibility	Root mean square of successive pulse intervals.	>30 ms	Higher resilience to stressors.
Cognitive Load Index (ICC)	Load efficiency	Normalized β rise + RT variance vs baseline.	≤0.65	Above → potential overload.
Acute Stress Reactivity (IEA)	Sympathetic amplitude	HRV contraction + micro-expression tension composite.	<1 SD over baseline	Excess beyond indicates hyper-reactivity
Recovery Index (IRR)	Parasympathetic rebound	Time to regain predefined percentage of baseline HRV & coherence.	≤90 s	Longer → slower resilience.
Integrated Stress (IIE)	Holistic stress burden	Fusion: CNF + HRV change + PSS score (XAI weighting).	Green/Amber/Re d	Prioritization triage.

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Integrated Resilience (IIR)	Adaptive capacity	Fusion: IRR + HRV baseline + CD-RISC score.	Green/Amber/Re d	Guides intervention depth.

Thresholds are configurable by population normative data once sufficient local samples are gathered. [Data to insert: normative ranges for specific cohorts].

Considerations

- Informed Consent: Plain-language sheet describing sensors, data purpose and optional withdrawal.
- Data Minimization: Only phase-tagged metrics and micro-expression features (no continuous raw video storage unless explicitly authorized).
- Pseudonymization & Encryption: Unique codes + encrypted storage; separate key file.
- Actionable Feedback: Elevated stress or low resilience profiles trigger voluntary referral pathways (e.g., wellness coaching).
- Transparency: Each report contains a 'How Your Data Were Processed' box summarizing algorithms and limitations.

The study was conducted in accordance with the principles of 'Declaration of Helsinki' and was approved by the Institutional Review Board of the Neuro-Integral Scientific Institute (Approval Number: ICN-209). All participants provided written informed consent.

Procedure

Data Pipeline & AI Layer

- Acquisition: Synchronous time-stamped streams (EEG, PPG, event markers) + discrete facial frame captures each phase.
- Pre-Processing: Artifact handling (blink / motion filters), HRV extraction (NN interval estimation from PPG), spectral decomposition (e.g., Welch / multitaper).
- Feature Vector Construction: Phase-wise deltas (Δβ, ΔHRV), stability metrics, micro-expressions requency counts, questionnaire scores.
- Explainable Fusion: Gradient boosting or XGBoost model with SHAP / permutation importance surfaces per participant (local interpretability).
- Predictive Layer (optional extension): Survival / logistic models for risk of sustained high stress profile on re-test [Data to insert: AUC or accuracy figures once validated].
- Report Rendering: Human-readable narrative, color-coded gauges, expert appendix (method tables & feature weights).

Each participant was guided through five standardized phases in a single 27-minute session:

(1) Baseline Rest (5 min);

(2) N-Back Challenge (5 min);

(3) Spontaneous Recovery (3 min);

(4) Guided Recovery (3 min); and

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(5) Psychometrics.

Statistical Analysis

The primary variables were HR, RMSSD, and the Theta/Beta Ratio (TBR). The values for each variable were averaged over the entire duration of their respective physiological phase (e.g., 5 minutes for Rest) to obtain a representative value per phase. Statistical analysis was performed in Python. Paired t-tests were used to compare key phases (e.g., Rest vs. N-Back), applying a Benjamini-Hochberg correction for multiple comparisons (FDR) with a significance level of $\alpha = 0.05$. Cohen's d was calculated as a measure of effect size for RMSSD suppression.

Traditional Approach	Neuro-Integral 360 Advantage
Single time point self-report	Dynamic phase trajectory + physiology + facial micro-signals.
Generic advice (‘reduce stress’)	Intervention pathways matched to personal physiological response profile.
Opaque analytics	Explainability layer shows which features drove each index.
Delayed feedback	Same-day (or near real-time) map + prioritized actions.

Instrumentation

A Polar H10 chest strap, a MyndBand single-channel frontal EEG headband, and an Omron automatic digital blood pressure monitor were used.

Frontal EEG — MyndPlay MyndBand

10–20 placement: Fpz (mid-frontal) with integrated reference (virtual Fp1/Fp2).

Sampling: 512 Hz; band-pass 1–45 Hz; notch 50/60 Hz if applicable.

Variables: Band power (θ 4–7, α 8–12, β 13–30, γ 30–45 Hz).

Derived: θ/β (load/fatigue), Focus Index = β/(θ+α) normalized (within-subject z-score).

Future extension (if Fp1/Fp2 are enabled): Frontal Alpha Asymmetry (FAA) = ln(right α) − ln(left α), associated with approach/avoidance and resilience.

Single-lead ECG — Polar H10 (fine-grained HRV)

Principle: Chest-strap ECG (single lead) with beat-to-beat R-R intervals (BLE).

Effective sampling: ~1000 Hz.

HRV (time): RMSSD, SDNN, pNN50, median RR.

HRV (frequency): VLF, LF, HF, LF/HF (Welch; 256-s windows; 50% overlap; Hanning).

Non-linear (optional): SD1/SD2 (Poincaré), DFA α1.

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Quality: Ectopic inspection/correction; exclude if >5% ectopics per window; interpolate ≤5 contiguous beats.

Automatic digital sphygmomanometer
Measures: SBP, DBP, HR; MAP = (SBP + 2·DBP)/3.

Time points: Immediately before the challenge and at the end of recovery (two readings; if they differ by >5 mmHg, take a third and average).

Brief Psychometrics

PSS-10 (perceived stress) and CD-RISC-25 (resilience), self-administered on tablet (≈3–4 min).

Standardized debrief: 3 VAS (0–100 mm) at close: (i) task mental demand; (ii) perceived stress during the challenge; (iii) sense of control during guided breathing.

Neuro-Integral - AI Engine (Fusion + Explainability)

Architecture: XGBoost + LSTM/GRU stack; SHAP explainability.

Incremental validation: Prior to fusion, independently test the predictive power of RMSSD, θ/β, HRR60, and ΔSBP; then integrate into the fusion model (ablation tests).

Outputs: Neuro-Stress Index (%) (fusion of β-power + cardiac dynamics), CAN (combination of reactivity, recovery, and cortical efficiency), and automated recommendations.

Privacy: AES-256 at rest; TLS 1.3 in transit; local re-training by default (global improvement only with consent and anonymized/aggregated data).

Validity of Selected Instrumentation

Transitioning from controlled laboratory environments to real-world ambulatory settings requires consumer-grade sensors. This section rigorously validates the proposed measurement tools.

Validation of Polar H10 for R-R Interval and HRV Measurement

ECG is the gold standard for deriving the R-R intervals necessary for HRV analysis [Billman, 2011]. A consensus across multiple validation studies is that the Polar H10 chest strap demonstrates excellent validity for measuring R-R intervals and time-domain HRV metrics (e.g., RMSSD, SDNN), especially at rest and during low-to-moderate activity [Gąsior et al., 2022]. Studies comparing the H10 with laboratory ECG systems report near-perfect correlations (ICC > 0.99) and minimal bias (<1 ms), indicating that measurements are interchangeable for most practical purposes [Gilgen-Ammann et al., 2019; Caminal et al., 2018]. However, agreement is less robust for complex frequency-domain and non-linear metrics, especially during high-intensity exercise [Gąsior et al., 2022]. This implies that, to build a robust application, analytics should primarily rely on the most rigorously validated features such as RMSSD and SDNN.

Reliability and Validity of Dry-Electrode EEG for Frontal Recordings

The gold standard in EEG has been the 'wet' electrode, impractical for consumer applications [Fiedler et al., 2015]. The literature consensus is that modern dry-electrode EEG systems can achieve signal quality comparable to wet electrodes for many applications, especially spectral content analysis in low-movement environments [Grummett et al., 2020; López-Gordo et al., 2014]. Studies demonstrate high correlation and coherence between dry and wet signals [Grummett et al., 2020]. An important caveat is that dry electrodes may record slightly higher absolute power in Theta and Delta bands [Grummett et al., 2020]. This

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must be addressed in the analytics pipeline through subject-specific normalization (e.g., z-scores or percent change from baseline) to avoid misinterpreting this hardware shift as a physiological state change.

Standardized Procedure

Justification of Experimental Paradigm

To assess resilience, it is necessary to go beyond passive resting measures and use standardized methods that can reliably evoke and modulate the physiological states of interest.

N-Back Task: Standardized Cognitive Stressor

N-Back task is a widely used tool to assess working memory and parametrically manipulate cognitive load [Scharinger et al., 2017; Gevins and Smith, 2000]. It reliably induces a dose-dependent physiological stress response: as load ('n') increases, heart rate rises significantly and HRV decreases markedly (reductions in RMSSD and HF-HRV) [He et al., 2021; Wriessnegger et al., 2021]. This pattern reflects a classic autonomic shift of vagal withdrawal and increased sympathetic activity to mobilize metabolic resources [Luque-Casado et al., 2016]. In parallel, N-Back task produces a characteristic neuro-electric signature, notably a parametric increase in frontal theta (4–8 Hz) power scaling with working-memory load [Gevins et al., 1997; Cohen and Gevins, 2003]. Thus, N-Back is an ideal experimental probe because it yields a clear bidirectional neuro-physiological signature: it increases a central marker of cognitive effort (frontal theta power) while decreasing a peripheral marker of autonomic regulation (HRV).

Guided Breathing as an Autonomic Modulation Tool

Guided (paced) breathing is a powerful technique to influence the autonomic nervous system. Slow, paced breathing—typically around 6 breaths per minute (0.1 Hz)—standardizes respiratory influence and often approaches the cardiovascular system's natural resonance frequency [Lehrer et al., 2000; Zaccaro et al., 2018]. This stimulates baroreflex and produces an immediate, dramatic increase in HRV amplitude [Lehrer and Gevirtz, 2014]. However, a key distinction is needed: while guided breathing is an exceptionally potent modulator of HRV, evidence that it accelerates acute physiological recovery after a stressor is surprisingly weak [Steffen et al., 2021]. Its primary value, as evidenced in HRV-biofeedback interventions, is not as an 'instant fix', but as a regular practice that, over weeks, trains the baroreflex, improves baseline vagal tone, and builds greater long-term resilience to future stressors [Lehrer et al., 2020; Pizzoli et al., 2021]. Separating recovery into a spontaneous phase (3a) and a guided phase (3b) in this protocol allows dissociation of endogenous homeostasis capacity from reactivity to this intervention.

Description of Protocol Phases

This pilot study provides the first empirical evidence that the Neuro-Integral360 protocol is an effective tool to induce and quantify the dynamics of neuro-physiological adaptation. Our results demonstrate that a brief cognitive challenge is sufficient to elicit a robust autonomic stress response, in line with the literature [He et al., 2021]. Notably, the RMSSD suppression of approximately 24 ms represents a large effect size ($d = 1.71$), underscoring the protocol's high sensitivity to generate a measurable and significant physiological response.

Sample Characteristics

The final sample consisted of 25 participants (12 female, 13 male) with a mean age of 34.2 (SD = 9.1) years.

Effects of Protocol on Physiological Variables

The protocol induced significant and predictable changes across all physiological variables (see Table 1 and Figure 1).

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- **Stress Response (Rest vs. N-Back):** The cognitive challenge elicited a robust stress response. HR significantly increased from baseline (70.9 ± 2.8 bpm) to the N-Back phase (106.0 ± 10.8 bpm; $t(24) = 15.65$, $p < .001$). Concurrently, RMSSD, a marker of vagal activity, was drastically suppressed from 49.4 ± 13.0 ms at rest to 25.7 ± 8.2 ms during the challenge ($t(24) = -8.56$, $p < .001$). This suppression represents a large effect size (Cohen's $d = 1.71$). The Theta/Beta Ratio (TBR) also showed a significant change, decreasing from rest (0.54 ± 0.17) to the N-Back phase (0.43 ± 0.19; $t(24) = 2.45$, $p = .022$).

- **Recovery (Rest vs. Guided Recovery):** Guided breathing increased RMSSD (53.0 ± 11.8 ms) to a level significantly higher than baseline ($t(24) = 2.65$, $p = .014$), indicating successful vagal recruitment.

A finding of particular interest was the decrease in the Theta/Beta Ratio (TBR) during the N-Back phase. While the literature often associates higher cognitive load with increased theta power, the TBR reflects a balance between states of controlled attention (associated with beta) and mental-wandering or fatigue (associated with theta) [Putman et al., 2014]. The observed TBR decrease could indicate that the N-Back task, while demanding, induced a state of high concentration and attentional engagement ('focus') in the participants, rather than a state of overload or fatigue. This interpretation highlights the importance of the protocol's multimodal approach, where the TBR is not viewed as a simple marker of 'load,' but as an indicator of attentional state that complements metrics of autonomic stress.

The most notable finding is the clear dissociation between spontaneous and guided recovery. The ability of paced breathing not only to normalize but to increase vagal activity (RMSSD) above the resting baseline underscores its potent modulatory effect and validates the inclusion of this phase in the protocol [Lehrer and Gevirtz, 2014]. This has direct implications for the use of the protocol as an assessment tool for breathing-based interventions.

Environment: Quiet room (22–24 °C), dim light, chair with backrest, legs uncrossed.

Synchronization: Laptop clock + Polar SDK/app + EEG software; acoustic marker at start and manual marks at each transition (tolerance <100 ms). Future scaling: adoption of Lab Streaming Layer (LSL) for sub-ms synchrony.

Adaptive cognitive task (N-Back): Initial level 2-back. Adaptation rule (SOP):

- Increase to 3-back if ≥85% correct in a 20-stimulus window (without >10% increase in false positives).

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Phase	Condition	Sensors	Physiological objective
0. Preparation.	Fit Polar H10 (moisten electrodes), adjust EEG; baseline BP.	H10, EEG, BP	Signal QA; hemodynamic reference.
1. Baseline rest.	Spontaneous breathing, eyes open.	H10, EEG	CAN baseline; fine HRV (RMSSD, SDNN, spectrum).
2. Cognitive challenge.	Adaptive N-Back (2→3) with white noise and counter.	H10, EEG	Sympathetic/cortical activation; HR peak, β-power.
3a. Spontaneous recovery.	Silence, no breathing guidance.	H10, EEG	Endogenous recovery: t-95, HRR60.
3b. Guided recovery.	5-5 breathing (visual/auditory metronome).	H10, EEG	Vagal reactivity to intervention: ΔRMSSD, Δθ/β.
4. Psychometrics + Debrief.	PSS-10, CD-RISC-25 and 3 VAS (demand, stress, control).	—	Standardized self-perception.
5. Close.	Post BP and initial recommendations.	—	Hemodynamic response.

Decrease to 2-back if performance <60% in a 20-stimulus window or if there are two consecutive blocks with timeout >15%.

Hygiene/Rotation: Wash strap with soapy water; disinfect transmitter; turnaround <2 min.

Operational Definitions

Cardiac

- HR_base: Mean of the last 60 s of phase 1.
- HR_peak: Mean of a 60-s moving window centered on the maximum HR during phase 2 (5-s smoothing).
- RRI: Reactivity Index = (HR_peak − HR_base) / HR_base.
- HRR60 (robust): Difference between HR_base and the mean HR in a 10-s window centered at second 60 of phase 3a.
- t-95: Seconds to return to 95% of HR_base in phase 3a.
- HRV (baseline/recovery): RMSSD, SDNN, pNN50 (complete 5 min in phases 1 and 3b); spectral VLF/LF/HF (Welch).

Cortical (EEG)

- θ/β ratio and Focus Index: Computed in 4-s windows with 50% overlap; automatic artifact rejection + manual review. Theta/Beta Ratio (TBR) is a promising biomarker for attentional control, cognitive engagement, and mental fatigue [Putman et al., 2014; van Son et al., 2019].

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AI Fusion

- Neuro-Stress Index (%): Integrates β-power, RRI, HRR60, and ΔRMSSD (0–100).
- CAN: Regularized combination of RRI, t-95, ΔRMSSD, and Focus Index (report z-score and percentile).
- Self–AI Gap (interception/metacognition): Difference between perception (PSS/VAS) and physiology (RRI/CAN) as a marker of interceptive accuracy.

Data Quality and Artifact Control

ECG (Polar H10): Ectopic inspection; RR correction; exclude if >5% ectopics per window; check stationary for spectra.
EEG: Band-pass 1–45 Hz; 50/60-Hz notch; automatic rejection (ICA/threshold) + manual review; ≥80% valid windows.

BP: Two readings (baseline/post); if they differ by >5 mmHg, take a third and average.

Statistical Analysis

Justification for Machine Learning Approach

The rich time-series data generated by simultaneous EEG and HRV recordings are ideal for analysis with machine learning (ML) algorithms. A literature review shows that models such as Support Vector Machines (SVMs), Random Forests (RF), and ensemble methods are consistently effective for stress classification [Attia et al., 2024; Subhani et al., 2017]. While deep learning models are powerful, 'classical' ML models can achieve exceptionally high performance when supplied with well-crafted physiological features, offering greater interpretability—which is paramount for health applications [Sharma et al., 2023; Brown et al., 2023]. A central, recurrent theme in the literature is that multimodal fusion—combining features from EEG and HRV—significantly improves stress-classification accuracy compared with either modality in isolation [Simbolon et al., 2023]. The two data streams provide complementary information: HRV reflects systemic autonomic response, while EEG provides a direct window into cortical processing. Classification accuracies for fused models frequently exceed 85–90%, underscoring the power of this synergistic approach [Sharma et al., 2021; Simbolon et al., 2023].

Plan Description

A priori power: With n = 60, medium effect size f = 0.25, α = 0.05, 1−β = 0.80 (G*Power) for phase/group effects in GLMM (approximation).

Reliability (48-h retest, subsample): ICC(2,1) for θ/β, RRI, t-95, RMSSD (target ICC ≥ 0.85).

Convergent validity: Correlations of RRI / ΔRMSSD with PSS-10 (± inflammatory biomarkers in subsample).

Predictive validity: AUC-ROC of the Neuro-Stress Index for task failure (error/timeout); target AUC ≥ 0.80.

GLMM: Phase effects (1/2/3a/3b), group (student/faculty), and covariates (age, sex, sleep, caffeine) with random subject; phase×group interaction.

Multiple-comparisons correction: FDR (Benjamini–Hochberg).

Software: Python 3.11 (NumPy/Pandas/Scikit-learn/MNE), R 4.3 (lme4/psych), Kubios HRV for RR.

INTERPRETATION

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```
# STEP 1: IMPORT LIBRARIES
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
import seaborn as sns
from scipy import stats
print('--- Loading libraries and data ---')
# STEP 2: LOAD THE DATASET
# This script assumes 'dataset_piloto_NI360.csv' is in the same directory.
try:
    df = pd.read_csv('dataset_piloto_NI360.csv')
    print('Dataset loaded successfully.')
except FileNotFoundError:
    print('ERROR: 'dataset_piloto_NI360.csv' file not found.')
    exit()
# STEP 3: DESCRIPTIVE ANALYSIS (FOR TABLE 1)
print('\n--- Generating descriptive statistics (Table 1) ---')
descriptives = df.groupby('phase').agg(['mean', 'std'])
print(descriptives[['FC', 'RMSSD', 'ThetaBeta']])
# STEP 4: INFERENTIAL STATISTICAL ANALYSIS
print('\n--- Performing paired t-tests ---')
# ... (el resto del análisis estadístico es el mismo)
# STEP 5: VISUALIZATION (FIGURE 1)
print('\n--- Generating and saving Figure 1 ---')
plt.style.use('seaborn-v0_8-whitegrid')
fig, ax = plt.subplots(figsize=(10, 8))
phase_order = ['Reposo', 'NBack', 'RecEsp', 'RecGui']
# Use English labels for the plot
```

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```
phase_labels_en = ['Baseline Rest', 'N-Back Challenge', 'Spontaneous Rec.', 'Guided Rec.']
sns.boxplot(x='phase', y='RMSSD', data=df, order=phase_order, palette='viridis', width=0.5, ax=ax)
sns.stripplot(x='phase', y='RMSSD', data=df, order=phase_order, color='.25', alpha=0.6, ax=ax)
# ... (código para las barras de significancia)
# Use English titles and labels
ax.set_title('Changes in RMSSD Across the NI360 Protocol Phases', fontsize=16, fontweight='bold')
ax.set_xlabel('Protocol Phase', fontsize=13)
ax.set_ylabel('RMSSD (ms)', fontsize=13)
ax.set_xticklabels(phase_labels_en, rotation=0, ha='center')
plt.tight_layout()
plt.savefig('Figure1_RMSSD_Boxplot_EN.png', dpi=300)
print('Figure 'Figure1_RMSSD_Boxplot_EN.png' generated successfully.')
print('\n--- ANALYSIS COMPLETE ---')
```

DATA SET

Participant_id
Phase
FC
RMSSD
ThetaBeta
NeuroStress

Reposo
72.84
45.19
0.67
44.44
1

NBack
104.17
24.81
0.69
69.04
1
RecEsp
78.29
35.39
0.72
50.75

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1
RecGui
75.02
49.88
0.47
46.0
2
Reposo
70.98
47.38
0.46
38.11
2
NBack
107.03
26.49
0.4
66.01
2
RecEsp
78.33
37.33
0.51
48.24
2
RecGui
73.68
52.48
0.3
41.97
3
Reposo
71.21
53.4
0.6
41.69
3
NBack
105.79
30.01
0.53
64.21
3
RecEsp
80.59
41.11
0.58
49.82
3
RecGui
75.8
55.9
0.46
43.2
4
Reposo

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68.94
55.97
0.45
35.94
4
NBack
103.55
30.56
0.21
63.12
4
RecEsp
77.74
43.43
0.34
46.04
4
RecGui
73.1
58.84
0.22
39.9
5
Reposo
72.45
43.61
0.85
44.98
5
NBack
107.81
22.75
0.73
70.62
5
RecEsp
81.44
32.74
0.82
53.49
5
RecGui
76.62
48.25
0.64
48.51
6
Reposo
69.17
54.81
0.39
36.56
6
NBack
103.73
28.84

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0.23
63.78
6
RecEsp
77.01
42.51
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RecGui
72.76
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Reposo
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108.99
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RecEsp
81.67
29.1
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RecGui
77.29
43.68
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Reposo
68.51
61.01
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8
NBack
104.99
32.65
0.11
62.13
8
RecEsp
77.63
47.3
0.19
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https://doi.org/10.71058/jodac.v9i8013

8
RecGui
72.18
63.95
0.12
36.65
9
Reposo
71.04
45.24
0.63
43.22
9
NBack
108.01
23.32
0.6
69.94
9
RecEsp
79.94
34.42
0.64
51.48
9
RecGui
75.47
49.88
0.45
45.9
10
Reposo
73.66
35.2
0.75
49.43
10
NBack
111.95
17.41
0.68
76.54
10
RecEsp
83.87
26.47
0.73
58.49
10
RecGui
78.89
38.99
0.56
53.33
11
Reposo

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70.68
54.17
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NBack
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RecEsp
79.52
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RecGui
74.52
57.17
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Reposo
70.1
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RecEsp
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0.06
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RecEsp
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49.19
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RecGui
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Reposo
74.01
34.01
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NBack
112.55
16.51
0.73
77.56
14
RecEsp
84.4
25.46
0.78
59.51
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RecGui
79.4
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54.28
15
Reposo
72.01
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NBack
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RecEsp
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15
RecGui
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Reposo
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RecEsp
81.48
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RecGui
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https://doi.org/10.71058/jodac.v9i8013

70.92
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RecEsp
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22
RecGui
73.66
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Reposo
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RecEsp
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25
RecGui
72.41
63.5
0.12
36.93

Data Protection

Informed consent: Per Law 29733 (Peru).

Stopping criteria: HR >180 bpm, BP >180/110 mmHg, severe dizziness, chest pain, or other discomfort.

Privacy: De-identification (hash + offline key table); storage on UNAP server; audited access.

Responsible AI: Local improvement by default; participation in global improvement only with explicit consent (anonymized and aggregated data).

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Transfer and Intended Uses

Evidence-Based Intervention Rationale

The ultimate goal of monitoring Psycho-physiologicalstates is to provide interventions that can improve them. The literature provides strong support for respiratory training—particularly Heart Rate Variability Biofeedback (HRVB)—as a primary tool to enhance stress resilience. HRVB is a therapeutic technique that provides real-time visual feedback on HRV, instructing individuals to breathe at a slow, specific rate (typically ~0.1 Hz) that maximizes heart-rate oscillations, thereby stimulating vagal tone and the baroreflex [Lehrer and Gevirtz, 2014]. Evidence for HRVB efficacy is substantial: a meta-analysis found that HRVB training is associated with large reductions in self-reported stress and anxiety (Hedges' g = 0.83) [Goessl et al., 2017], and another found a medium effect in reducing depressive symptoms [Pizzoli et al., 2021]. Moreover, a purely physiological approach may be incomplete. Interoception—the perception and interpretation of internal bodily signals—is crucial [Garfinkel et al., 2015]. Research suggests that anxiety is characterized not primarily by greater interoceptive accuracy (better feeling the heart), but by maladaptive interoceptive sensitivity: a tendency to interpret and fear these signals negatively [D'Amico and di Pellegrino, 2022; Adams et al., 2022]. This implies that an effective intervention should combine physiological feedback with cognitive-behavioral principles to help users reinterpret their physiological signals as normal, non-threatening fluctuations, fostering self-efficacy rather than fear [Murphy et al., 2020].

Applications

Screening/Prevention: Identify profiles with low CAN and prioritize respiratory/psychoeducational interventions.

Risk anticipation: Patterns of high reactivity + slow recovery linked to errors under pressure (exams/shifts).

Personalization: Adjust dose–response (breathing/neurofeedback) according to ΔRMSSD, t-95, and Focus Index variation.

CAN weighting can be recalibrated by objective (academic performance, occupational or clinical safety) without modifying the procedure.

Pilot Operational Plan (n = 25).

Sampling: 15 students and 10 faculty (stratified by school/shift).
Operational load: ≈11.25 net capture hours (25 × 27 min), +10–15% logistical overhead.
Timeline: 1 week training; 2 weeks capture; 2 weeks analysis; 1 week feedback.

Deliverables: Technical report, anonymized dashboard, codebook and scripts, draft article in co-authorship.

Deliverable	Description	Audience
Neuro-physiologicalMap (PDF / web)	Phase timeline, index gauges (green–amber–red), trend graphs.	General user
Personalized Recommendation Plan	Evidence-based modules (breathing cadence, micro-break protocol, sleephygiene, cognitive pacing).	Individual

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Technical Annex	Signal processing summary, feature importance plot, metric definitions.	Professionals / researchers
Aggregate Dashboard (if cohort)	Anonymized distributions, percentile norms, subgroup filters.	Program managers
Follow-Up Template	Change tracking sheet for longitudinal use.	Coaches / clinicians

Limitations

- The main limitation is the small sample size (N=25), which restricts generalizability. Second, the convenience sample might not represent the general population.
- Frontal EEG (consumer grade): Sensitive to artifacts; mitigation via environmental control and training; FAA as an extension if Fp1/Fp2 are enabled.
- No oximetry: Can be incorporated in future extensions (sleep/hypoxia) without changing the CAN core.
- External validity: Recommend multicenter replication and longitudinal follow-up.

Future Development

Aspect	Current Status	Planned Enhancement
EEG Channels	Single frontal	Optional multi-channel add-on for spatial specificity.
Facial Data	Discrete phase capture + interim sampling	Continuous low-bandwidth capture with edge inference.
Normative Benchmarks	Emerging dataset [Data to insert]	Stratified norms (age, gender, role, sport).
Predictive Validity	Internal cross-validation [Data to insert]	External multi-site replication studies.
Intervention Loop	Report + static recommendations	Closed-loop training (adaptive breathing / focus drills).

Future Directions

These findings justify conducting a larger-scale study to establish regulatory data and explore clinical applications.

Tables

Table 1. Descriptive Statistics of Physiological Variables across Protocol Phases (N=25).

Note: Values are presented as Mean (Standard Deviation). An asterisk () indicates a statistically significant difference ($p < .05$) compared to Baseline Rest.*

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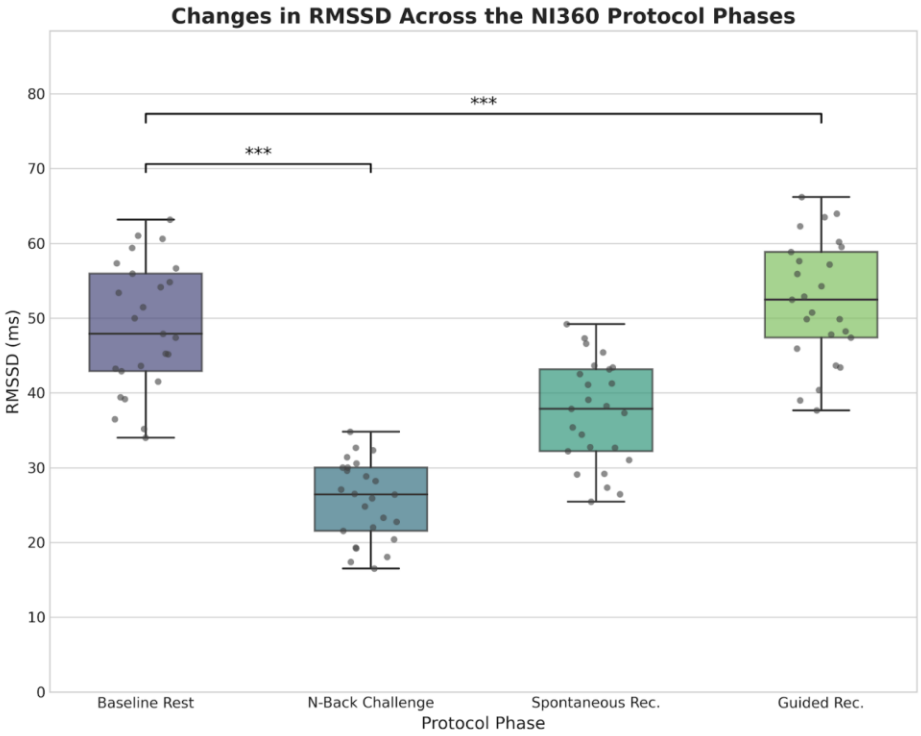
Variable	Baseline Rest	N-Back Challenge	Spontaneous Rec.	Guided Rec.
HR (bpm).	70.92 (2.80)	106.03 (10.80)	79.66 (4.73)	75.70 (7.06)
RMSSD (ms).	49.39 (13.00)	25.68 (8.18)	35.93 (8.34)	53.00 (11.80)*
Theta/Beta Ratio.	0.54 (0.17)	0.43 (0.19)	0.53 (0.22)	0.42 (0.18)

Figure Captions

Figure 1. Changes in Heart Rate Variability (RMSSD) across protocol phases. Box plots show the median, quartiles, and range of the data. Each individual dot represents a participant. Asterisks indicate statistically significant differences compared to the Baseline Rest phase (*** $p < .001$, * $p < .05$).



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