Supplementary Material

Altered interoceptive predictive coding in Functional Neurological Disorders

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Clinical Details of Population

Table S1. Clinical Details and Comorbidities

Variable	Overall N = 78	HC N = 42	FND N = 36	*p-value*
Intake of Pain Medication [yes]	13 (17%)	2 (5%)	11 (31%)	*0.005*
Intake of Psychotropic Medication [yes]	16 (21%)	2 (5%)	14 (39%)	*<0.001*
Intake of Sleep Medication [yes]	7 (9%)	0 (0%)	7 (19%)	*0.001*
Intake of Other Medication [yes]	18 (23%)	6 (14%)	12 (33%)	*0.065*
Comorbidity of ADS or AD(H)D [yes]	5 (6%)	1 (2%)	4 (11%)	*0.2*
Comorbidity of Psychiatric Disorder [yes]	11 (14%)	2 (5%)	9 (25%)	*0.010*
Comorbidity of Hormonal Dysfunction [yes]	3 (4%)	1 (2%)	2 (6%)	*0.6*
Comorbidity of other Diagnosis [yes]	10 (13%)	3 (7%)	7 (19%)	*0.10*

Note: statistical test between the two groups, portraying count (%) with Fisher test

Table S2. FND symptom subtypes

Variable	Overall N = 36	Female N = 27	Male N = 9
Functional Dissociative Seizures [yes]	6 (17%)	6 (22%)	0 (0%)
motor + symptoms [yes]	13 (36%)	9 (33%)	4 (44%)
motor - symptoms [yes]	26 (72%)	18 (67%)	8 (89%)
sensory symptoms [yes]	19 (53%)	15 (56%)	4 (44%)
dizziness (PPPD) [yes]	2 (6%)	2 (7%)	**0 (0%)**
cognitive symptoms [yes]	3 (8%)	3 (11%)	0 (0%)

Note: FND symptom frequency where one patient might also report more than one symptom subtype. Numbers indicate count (%).

Description of the real-time R-peak detection algorithm

Real-time R-peak detection from the continuous ECG was performed using a custom script based on the methodology of Pfeiffer and De Lucia (Pfeiffer & De Lucia, 2017). It was programmed directedly in EEG/EMG acquisition tool ActiView version 903 (Biosemi, Amsterdam, Netherlands) based on LabView 2022 Q3 software (National Instruments, Austin, USA). For each packet of data received (on average every 2-4ms), the software calculated the variance over the last 50ms of the ECG signal. If this variance reached the participant's individual threshold (see below for its calculation), a marker was added to the recording (R-peak detected) and the experimental stimulation (either a tone or an omission) was triggered in the synchronous condition. After a hit, there is a cooldown period of 200ms where no tests were performed to avoid over detection. We observed a delay of 5.02ms (SD=2.48ms) between real-time and offline R-peak detection. In addition, we measured a sound emission latency of 38.48ms (SD=3.02ms) using a Blackbox Toolkit V2 (The Black Box Toolkit Limited, Sheffield, UK). In summary, the average delay between an R-peak and sound perception was 43.5ms (SD=5.5ms).

An individual R-peak detection threshold was calculated based on the 15-minute ECG signal recorded prior to the main experiment, during which participants were instructed to direct their attention to the heartbeat or an external sound while seated to measure their attentional HEP, similar to the set-up of Petzschner et al (2019) (Petzschner et al., 2019). The script to compute the individual threshold was created on MATLAB R2021b (MathWorks Inc., Massachusetts, USA). First, R-peaks of this recording were detected offline using the script by Sergey Chernenko, available on the website http://www.librow.com/articles/article-13 website (Cherneko, 2007). Its reliability on our data was checked and confirmed by visual inspection. A copy of this detection was recorded to define the participant’s cardiac pattern, which was then used in the asynchronous condition of the CAS paradigm. Then, the mean variance over 50ms before the R-peaks, present in the first half of this ECG signal, was then calculated to obtain the first version of the threshold. To optimize this value, different versions of the threshold were used to simulate real-time R-peak detection (30, 40, ..., 100% of the original value), using the same method described in the previous paragraph, over the second half of the ECG signal. The chosen threshold value was the one that produced an average peak position closest to that obtained offline (i.e. the more reliable method). Only the best of the two available ECG channels (left or right electrodes at the bottom of the rib cage versus right or left electrodes on the clavicles) was used. This was determined automatically by the channel with the largest mean amplitude of R-peaks.

For comparability of the asynchronous condition to the synchronous condition, we also tested whether the heartrate and heartrate variability would be different during the more active HEP task and the more passive CAS task. Using a paired Wilcoxon or t-test, separated per group, we did not identify a significant difference in these cardiac measures between the two tasks ($p > 0.454$.

Further Assessment of Interoceptive Variables

In the heartbeat-counting task (HCT), participants were instructed to count only the heartbeats they would perceive in three different time epochs (25, 35, 45s in randomized order). This was repeated once in an arousal condition, where participants were instructed to hold their breath for as long as they could tolerate. The mean of the six trials per person was used as an interoceptive HCT accuracy score.

Also, participants completed the respiratory resistance sensitivity task (RRST), a respiratory task using the techniques of signal detection theory in psychophysics to reliably measure individual sensitivity. After 50 trials participants would show an individual threshold for the detection of obstruction in breathing, which is considered the respiratory sensitivity score. For more details see previous publication (Stoffel et al., 2025).

Inverse Solution of Interaction (time intervals significant for the GFP analysis)

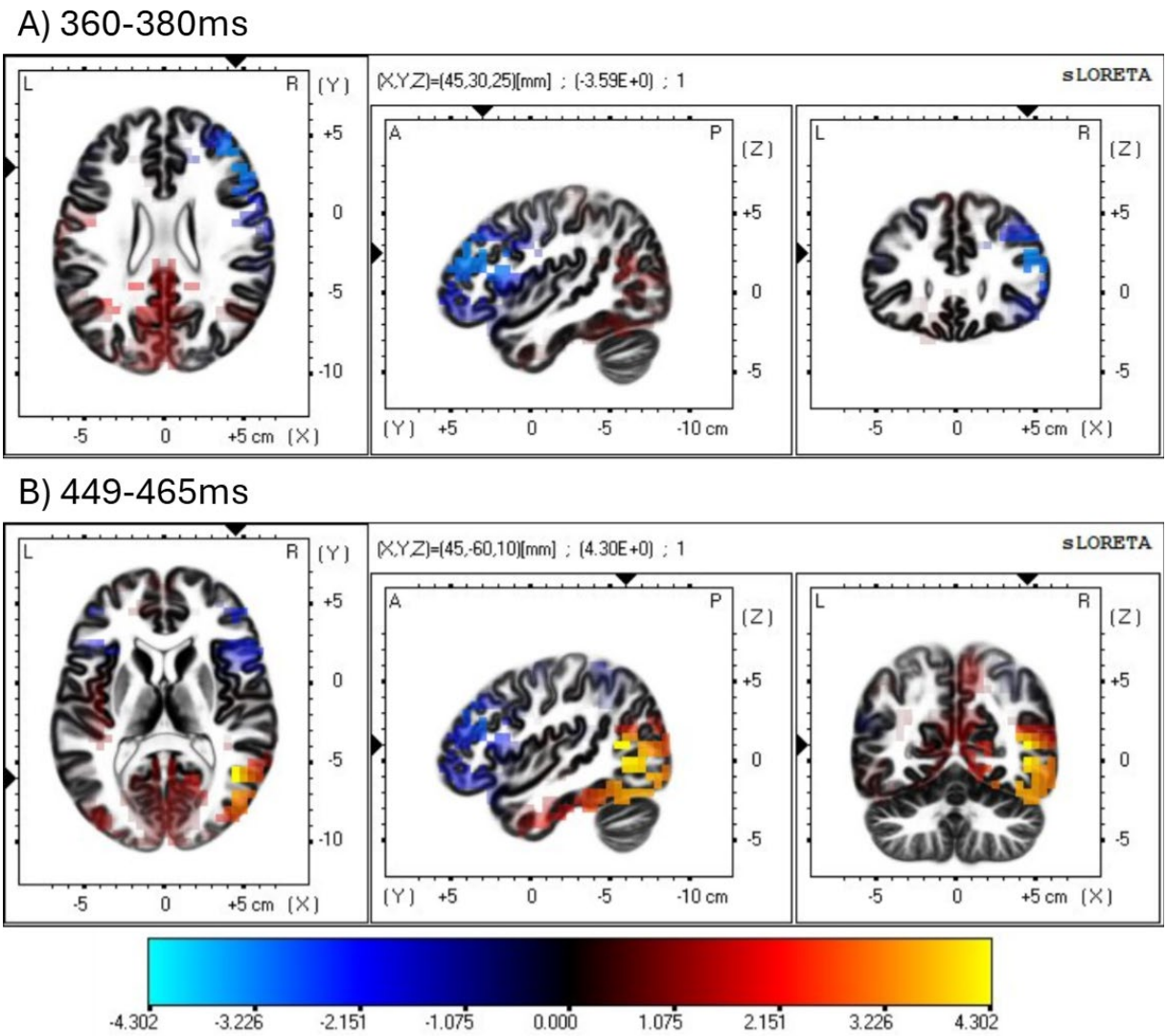

Figure S1. Visualization of origin for GFP differences from Interaction-Effects, using the epochs of both conditions and group and calculating an independent t-test with dependent conditions in sLORETA (FNDsynch-FNDasynch vs HCsynch-HCasynch). Thus, the identified regions for the source localization shows the activation for FND compared to HC for this contrast of synch-asynch with a peak minima at Brodmann area 46, the Middle Frontal Gyrus and the Frontal Lobe for A) at 360-380ms following the R-peak, and a peak maxima at Brodmann area 39, the Middle Temporal Gyrus and the Temporal Lobe at B) for 449-465ms following the R-peak

Control Analysis on the ECG trace

For a control analysis, whether the detected EEG differences were related to cardiac differences detectable in the ECG trace, the same analysis as for results presented in section 3.2.1 and 3.2.2 were conducted on the ECG trace itself. The ECG signal was filtered between 0.5 and 40 Hz to correct for signal drift and high-frequency noise (Rangayyan, 2001), and the trials were averaged using epochs of the same length as the HEP (-100 to 500ms).

No significant differences were found between the synchronous and asynchronous conditions for either FND or HC ($p > 0.05$ with FDR correction over the number of time points tested), and the comparison between groups for the synch-asynch difference waves did also not show any significant difference, Figure S4. These statistics were computed with using the Statistics module of EEGpal (https://github.com/DePrettoM/EEGpal) which relies on the Statistics and Machine Learning Toolbox from MATLAB and the script *fdr_bh* of David Groppe for the FDR correction (https://www.mathworks.com/matlabcentral/fileexchange/27418-fdr_bh).

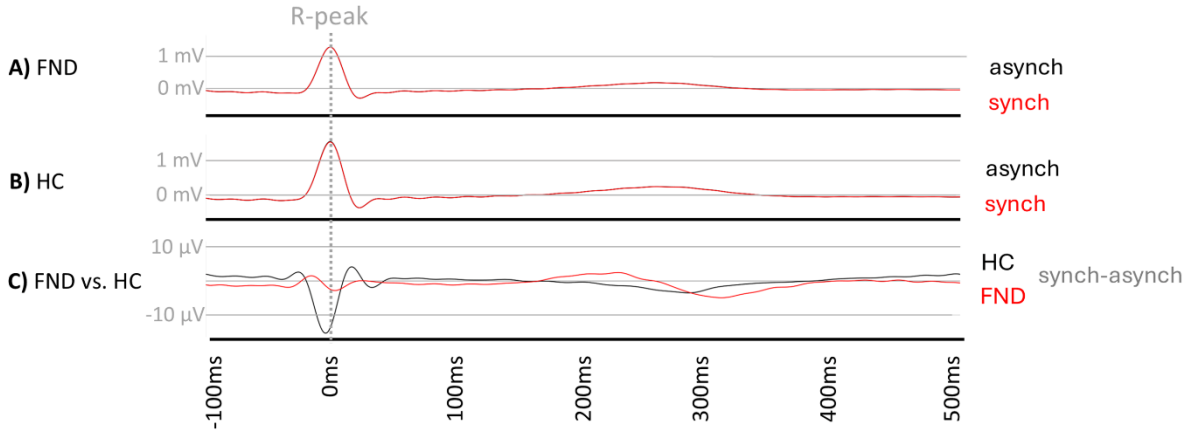

Figure S2. Differences of the ECG during omission trials between conditions and groups (same analysis as in Figure 1 of main manuscript). A) FND = patients with functional neurological disorders and **B**) HC = healthy controls comparison comparing synchronous (red) and asynchronous (black) using a paired t-test. **C**) Same analysis using the difference wave of synch-asynch traces of the HEP during omissions of sound to compare FND and HC ECG traces. No significance detected.

Subgroup analysis of non-medicated participants

The control analysis for the medication effect was done by excluding all participants who reported an intake of psychotropic, antiepileptic, opioid, or benzodiazepine medication. Thus, the sub-analysis consisted of N = 59 participants, wherefrom N = 19 (32.2 %) were patients with FND. Neither sex nor age where different in between the groups in this sub-analysis. By excluding patients with an intake of medication, we did arguably exclude also those with high psychiatric comorbidity and thus did not have a significant difference between the groups in state or trait anxiety in this subsample (State: p = 0.057, Trait: p = 0.084). **Table S3**.

Table S3: Demographics for subpopulation after exclusion of medicated participants

	Overall N = 59	HC N = 40	FND N = 19	p-value
Sex (female)	43.0 (72.9)	30.0 (75.0)	13.0 (68.4)	*0.8* [a]
Age (mean)	37.0 (12.3)	36.9 (12.6)	37.3 (11.9)	*>0.9* [b]
Body Mass Index (median)	23.4 (3.7)	22.7 (3.4)	25.6 (4.6)	*0.016* [b]
Heart Variability: RMSSD (median)	38 (30)	40 (37)	32 (17)	*0.2* [b]
RR-Interval (mean)	887 (124)	913 (125)	830 (104)	*0.010* [b]
Depression: BDI-II (median)	6 (10)	3 (6)	11 (12)	*<0.001* [b]
State Anxiety: STAI-S (median)	31.0 (10.0)	29.5 (9.3)	32.0 (11.0)	*0.057* [b]
Trait Anxiety: STAI-T (mean)	38.9 (10.3)	37.4 (10.4)	42.3 (9.6)	*0.084* [b]

Note: [a] = Chisq-test (count, %), [b] = t-test (mean, sd)

On the level of trait sensibility, the group differences in the MAIA and the IAS would not remain as in the full population (p > 0.5976).

For the cluster analysis, the comparison between synch and asynch for HC showed a similar cluster to the main results (cluster 130–324ms, mainly centered over the FC1, C1, C2, CP1 and CPz electrodes), as well as an additional cluster occurring later (359–500ms, mainly centered over the CP3, CP1, CPz, P3, P1 and Pz electrodes). In the FND group, there was no significant difference compared to the main result. Crucially, when analyzing the condition contrast between group, there is no longer a significant effect. Consequently, this change in results might indicate a medication effect as a driver for the FND effect, or that this is simply due to the lower sample size (as this subsample analysis was done on half of the patients with FND while almost no HC had to be excluded due to the intake of medication).

Also on a global level, there are some differences in results when excluding medicated participants with an overview illustrated in **Table S4**.

Table S4

	TANOVA sig. Time intervals		GFP sig. Time intervals	
Sample	full sample (N = 78)	exclusion of medicated participants (N = 59)	full sample (N = 78)	exclusion of medicated participants (N = 59)
Group Effect (FND vs HC)	463-480ms	-	10-20ms	-
Effect of Condition (synch vs asynch)	**56-72ms** **144-167ms** 382-410ms	**59-72ms** **145-158ms** -	**31-55ms** **77-96ms** 247-261ms **476-488ms**	(-67) -(-50)ms **28-55ms** **70-106ms** 128-145ms 160-181ms **458-489ms**
Interaction Effect (Group*Condition)	(-17)-(-5)ms **184-197ms** 215-241ms 309-322ms	72-87ms **182-197ms** 390-408ms	**360-380** **449-464**	**364-381ms** **452-464ms**

Note: Overview of significant time intervals when running the global statistics with the 2x2 factor model, in the full sample (N = 78) or in the subsample (N = 59), with excluding participants that report an intake of medication (psychotropic, antiepileptic, opioid or benzodiazepine medication). Reported time intervals indicate a significant interval after R-peak in the omission period, with for $p < 0.05$ and a minimal duration of 10ms. Time periods that were robust for the medication effect are shown on the same line and are printed in bold font.

For the effect of condition, we replicated most of the time intervals identified in the full population also in the non-medicated participants. As apparent in Table S4, the interaction effects between condition and group of the full population were partially replicated for the TANOVA because only the result between 184-197ms was similar. The other periods did not match between the two analyses, even though they can be considered as similar time intervals. In the case of GFP analysis, the same interaction results were observed. Again, we must consider that our identified group effect in the FND population might be driven by the medication, even though we cannot exclude that the disappearance of these results here might also be a power-issue, due to only having half of the previous sample size to calculate with.

References

Cherneko, S. (2007). *ECG processing. R-peaks detection*. Librow: Kyiv. http://www.librow.com/articles/article-13

Garfinkel, S. N., Seth, A. K., Barrett, A. B., Suzuki, K., & Critchley, H. D. (2015). Knowing your own heart: Distinguishing interoceptive accuracy from interoceptive awareness. *Biological Psychology*, *104*, 65–74. https://doi.org/10.1016/j.biopsycho.2014.11.004

Petzschner, F. H., Weber, L. A., Wellstein, K. V., Paolini, G., Do, C. T., & Stephan, K. E. (2019). Focus of attention modulates the heartbeat evoked potential. *NeuroImage*, *186*, 595–606. https://doi.org/10.1016/j.neuroimage.2018.11.037

Pfeiffer, C., & De Lucia, M. (2017). Cardio-audio synchronization drives neural surprise response. *Scientific Reports*, *7*(1), Artikel 1. https://doi.org/10.1038/s41598-017-13861-8

Rangayyan, R. M. (2001). *Biomedical Signal Analysis*. IEEE. https://doi.org/10.1109/9780470544204

Stoffel, N., Sojka, P., Gninenko, N., Mouthon, M., von der Weid, L., Serranová, T., & Aybek, S. (2025). Respiratory sensitivity is reduced in functional neurological disorder and associated with higher somatoform dissociation. *Brain Communications*, fcaf283. https://doi.org/10.1093/braincomms/fcaf283