# Biomedical Signals Lab

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# Introduction

The analysis of **Event-Related Potentials (ERPs)** is a cornerstone of non-invasive neurophysiology, utilizing time-locked Electroencephalography (EEG) activity to capture neural processes associated with sensory, cognitive, and motor events. These potentials reflect the summed, synchronous activity of large populations of cortical neurons, providing a millisecond-by-millisecond record of brain function.

The critical challenge in ERP analysis lies in overcoming the low signal-to-noise ratio, as the ERP is often obscured by higher-amplitude background EEG noise. To extract the signal, we employ the technique of **synchronized averaging**, relying on the premise that random noise cancels out over repeated trials, enhancing the time-locked ERP. This laboratory session focuses on key ERP components elicited within choice reaction time tasks:

- **Stimulus-Locked Potentials:** We analyze the **P3 (P300)** component, a marker of attention and working memory updating, typically studied here in the context of the **Eriksen Flanker task**.
- **Response-Locked Potentials:** We evaluate the **Error-Related Negativity (ERN)**, a component time-locked to an incorrect response, which serves as a physiological correlate of performance monitoring and error detection.

The principal objectives of this practical work are structured as follows:

1. Quantify the effect of **synchronized averaging** by examining how the **number of trials** influences the stability of key ERP features (amplitude and latency).
2. Assess the impact of poor event synchronization by simulating and analyzing the effect of **misalignment** (time jitter) on the resulting average ERP7.
3. Localize the **P3** and **ERN** generators by mapping their peak amplitude distributions across the scalp using **topography**.
4. Apply these techniques to a different dataset by analyzing the **P300** from EEG recorded during a **Visual Short Term Memory** task, complemented by a basic behavioral assessment.

# 1 Analysis of the First Five ERP Epochs

## Introduction

The purpose of this first exercise is to examine the morphology and variability of the initial five EEG epochs recorded in both stimulus-locked and response-locked conditions. Event-Related Potentials (ERPs) are time-locked neural responses that reflect perceptual, cognitive, and motor processes. However, ERPs are embedded within the background EEG, which exhibits large stochastic fluctuations. Therefore, single-trial ERPs often do not resemble the canonical components typically observed after averaging many repetitions.

This section evaluates how five individual epochs behave across the midline electrodes Fz, Cz, and Pz, and demonstrates why averaging is essential for recovering P2, N2, P3, and ERN components.

## 1.1 Stimulus-Locked Epochs (P2, N2, P3)

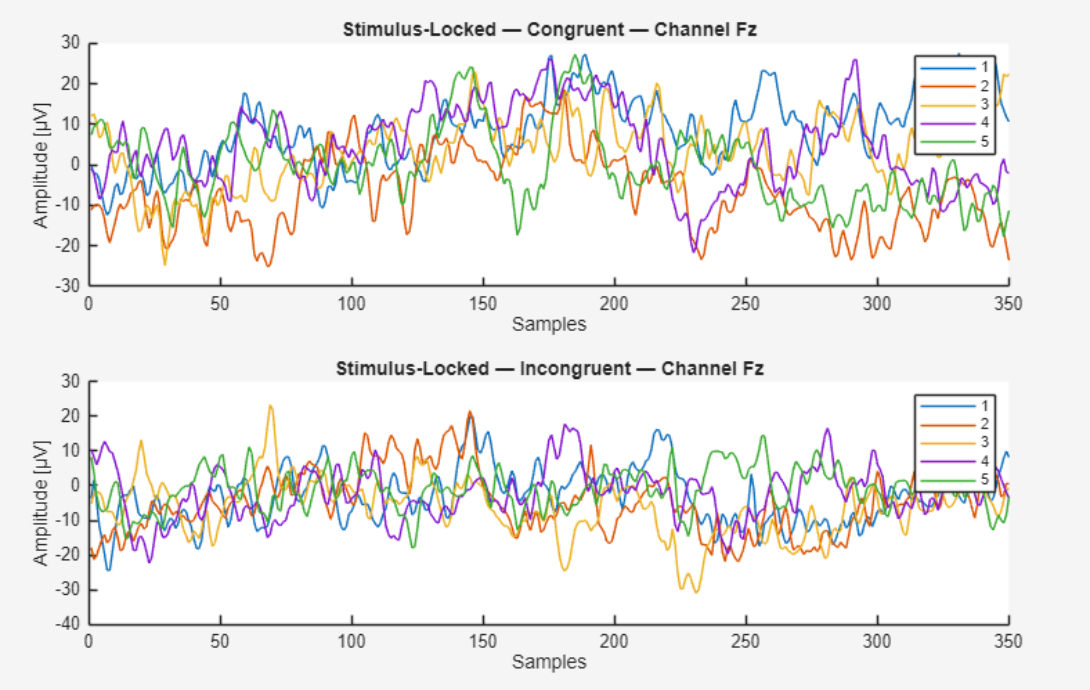


Figure 1: Stimulus-locked single-trial epochs (first 5 trials) — Channel Fz. Large fluctuations dominate the waveform, preventing identification of early or late ERP components.

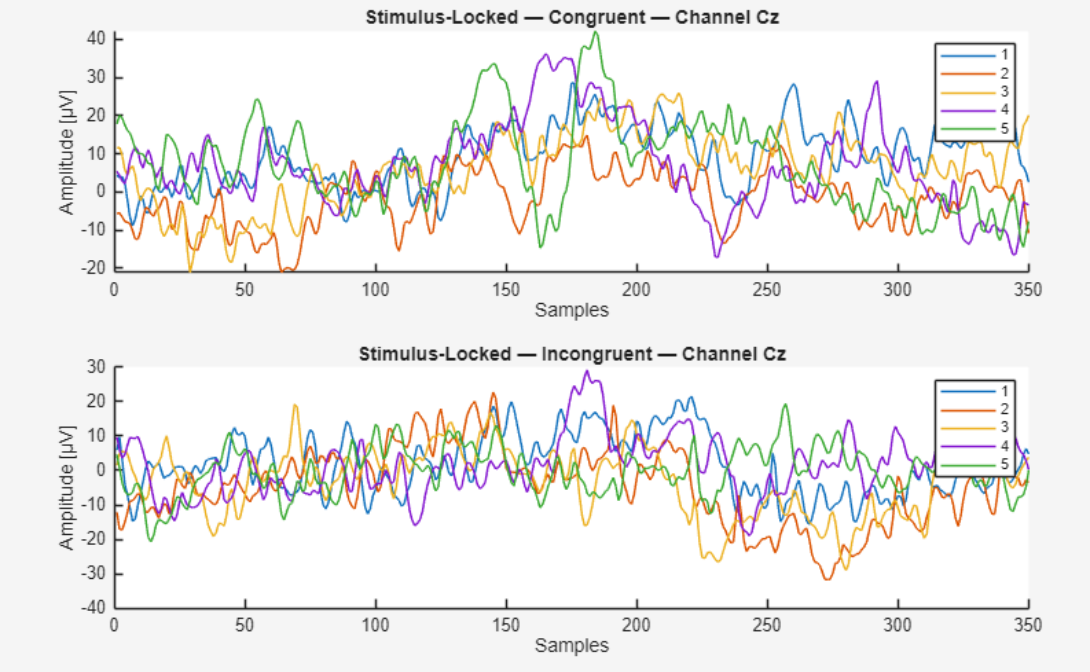


Figure 2: Stimulus-locked single-trial epochs (first 5 trials) — Channel Cz. Midline activity remains highly variable, masking morphology typically associated with P2, N2, or P3.

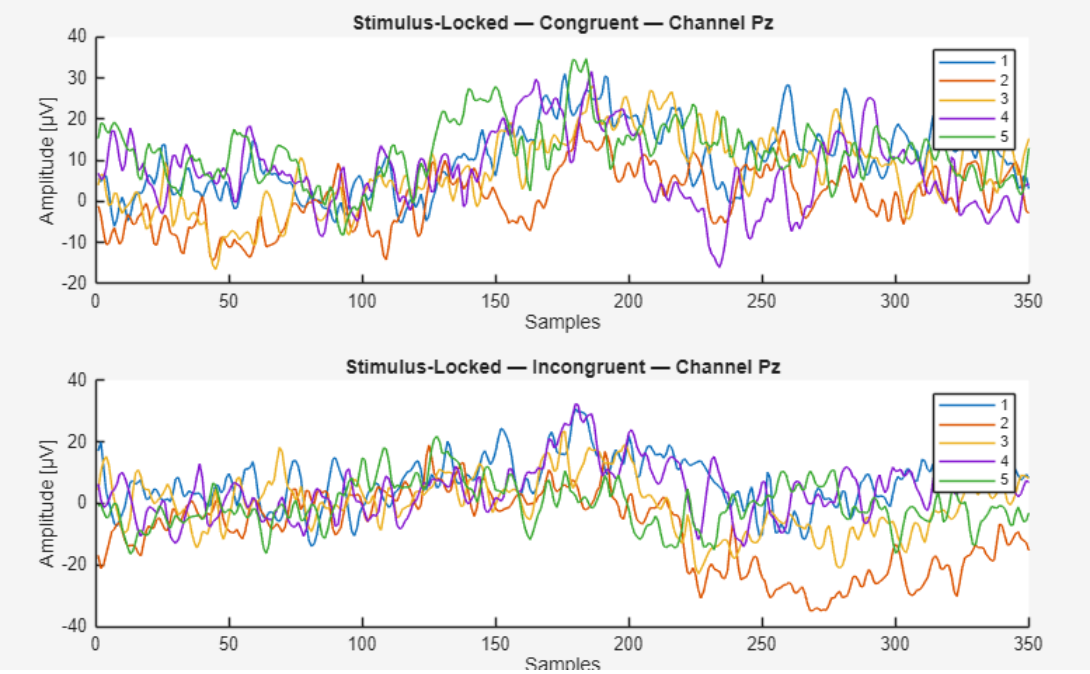


Figure 3: Stimulus-locked single-trial epochs (first 5 trials) — Channel Pz. Parietal oscillations, including strong alpha rhythms, obscure stimulus-locked components.

### Interpretation of Stimulus-Locked Epochs

Across the three midline electrodes (Fz, Cz, Pz), the first five stimulus-locked epochs exhibit substantial amplitude variability (commonly $\pm 30$–$40\,\mu$V), typical of raw EEG. Such variability masks the underlying ERP components:

- **P2 (150–250 ms)** cannot be isolated due to the dominance of high-frequency oscillations.
- **N2 (around 200 ms)** does not appear consistently across trials.
- **P3 (250–600 ms)** is not visually identifiable in any channel.

There are also no observable differences between congruent and incongruent conditions, which is expected because cognitive contrasts only emerge reliably after substantial averaging.That is going to be done in the next excercice.

**Topographically:**

- **Fz** shows slow frontal drifts consistent with prefrontal sources.
- **Cz** presents fluctuating midline activity but no stable ERP features.
- **Pz** displays rhythmic parietal oscillatory activity, predominantly alpha, which masks potential late ERP components.

This confirms that single-trial recordings are insufficient to extract stimulus-locked ERP morphology.

## 1.2 Response-Locked Epochs (ERN)

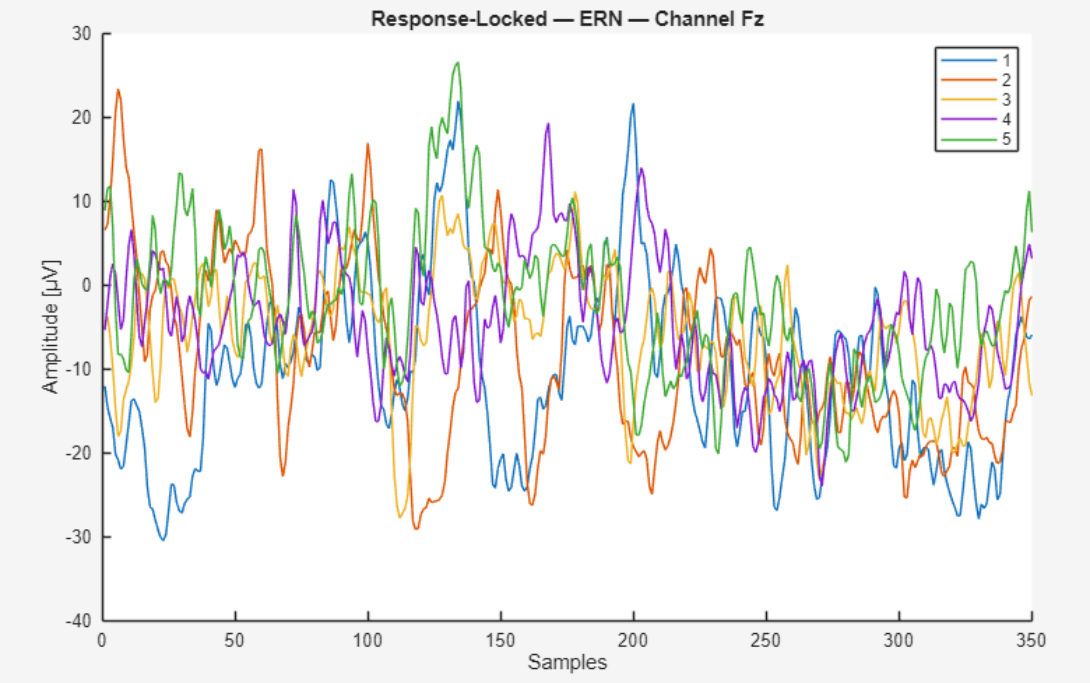


Figure 4: Response-locked single-trial epochs (first 5 trials) — Channel Fz. Although ERN typically peaks maximally at fronto-central sites, no identifiable ERN component is visible at the single-trial level.

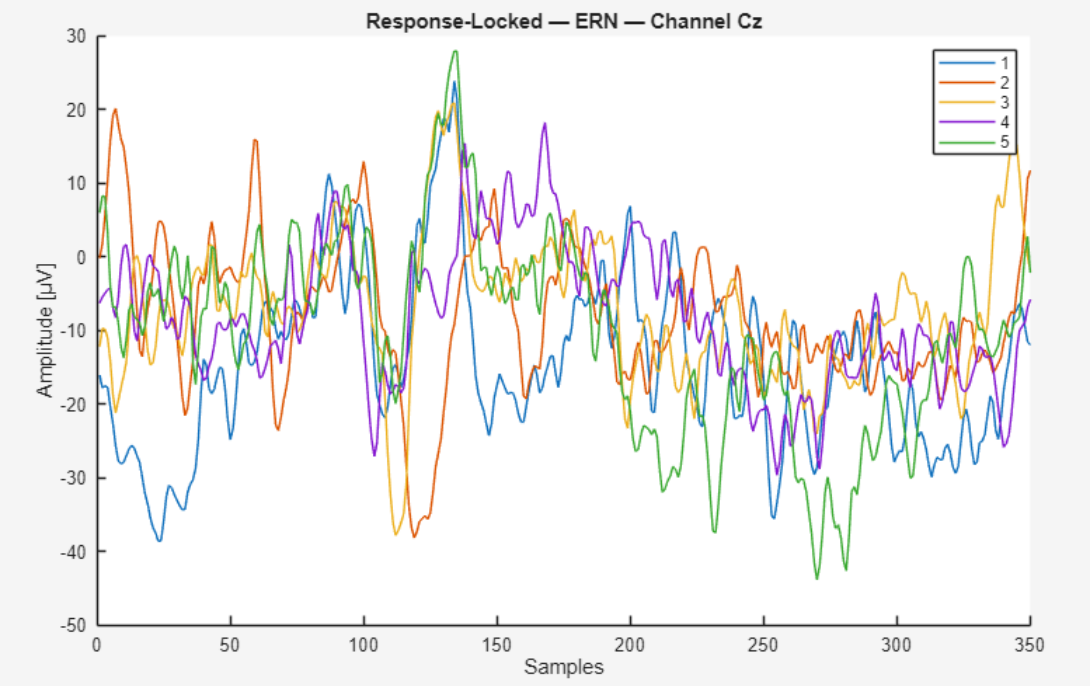


Figure 5: Response-locked single-trial epochs (first 5 trials) — Channel Cz. Strong fluctuations obscure the expected ERN (0–100 ms post-response).

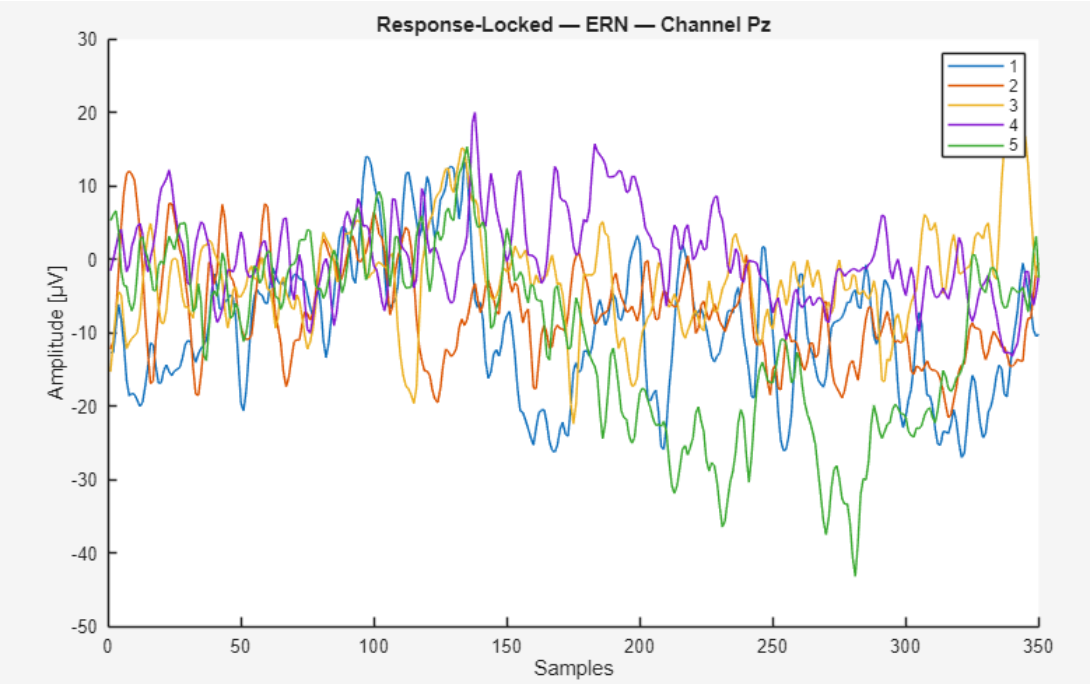


Figure 6: Response-locked single-trial epochs (first 5 trials) — Channel Pz. Parietal areas exhibit weaker error-related activity and pronounced noise.

### Interpretation of Response-Locked Epochs

The response-locked windows show similar behaviour to the stimulus-locked case. The ERN is a small negative deflection occurring 0–100 ms after an erroneous response, typically ranging from $-5$ to $-15\,\mu$V. Because of its low amplitude, it is completely masked by single-trial noise:

- No detectable ERN is visible in the first five trials in any channel.
- Fz and Cz, where ERN should be maximal, show considerable variability and inconsistent polarity.
- Pz exhibits even weaker error-related signals, as expected given its distance from the anterior cingulate cortex (ACC), the ERN generator.

These observations highlight that the ERN cannot be interpreted without averaging a sufficiently large number of error trials.

## 1.3 Interpretation

Taken together, the stimulus-locked and response-locked analyses show that single-trial EEG is dominated by spontaneous cortical activity, eye blinks, muscle artifacts, and non-phase-locked noise. As a consequence:

- **ERP components are not visible** with only 5 trials.
- **Congruency effects are completely obscured**.
- **Topographic specializations** (frontal ERN, parietal P3) do not emerge.
- **Single-trial polarity is inconsistent**, making interpretation impossible.

From a neurophysiological standpoint, the absence of identifiable components in single epochs directly illustrates the necessity of synchronized averaging in ERP research. ERP components represent phase-locked neural activity, whereas noise is stochastic; therefore, only averaging cancels out uncorrelated fluctuations and reveals consistent time-locked responses.

# 2 Effect of Trials on ERP Averaging

Synchronized averaging is essential for recovering ERP components from noisy EEG. While the ERP waveform is deterministic across repetitions, the background EEG activity is stochastic. Therefore, averaging $N$ trials increases the signal-to-noise ratio (SNR), causing the ERP to emerge progressively from the noise. The noise variance decreases proportionally to $1/\sqrt{N}$, which explains why larger numbers of trials yield clearer and more stable waveforms.

## 2(a). Stimulus-Locked Averages (P2, N2, P3)

For both congruent and incongruent stimuli, progressive averages were computed using the first 10, 20, 30, and 40 trials, followed by the full average across all available epochs.

The averaged ERP responses for each channel are shown below.

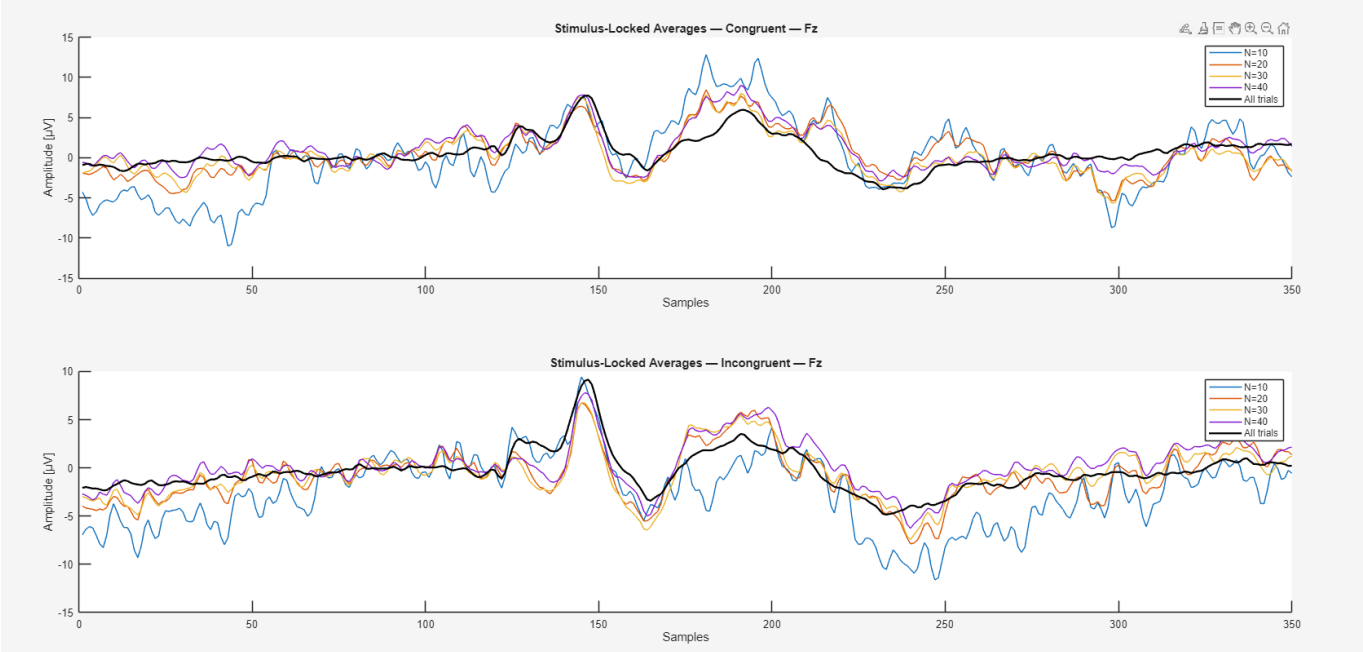


Figure 7: Averaged stimulus-locked ERP — Channel Fz. Clear emergence of P2, N2, and P3 components as the number of averaged trials increases.

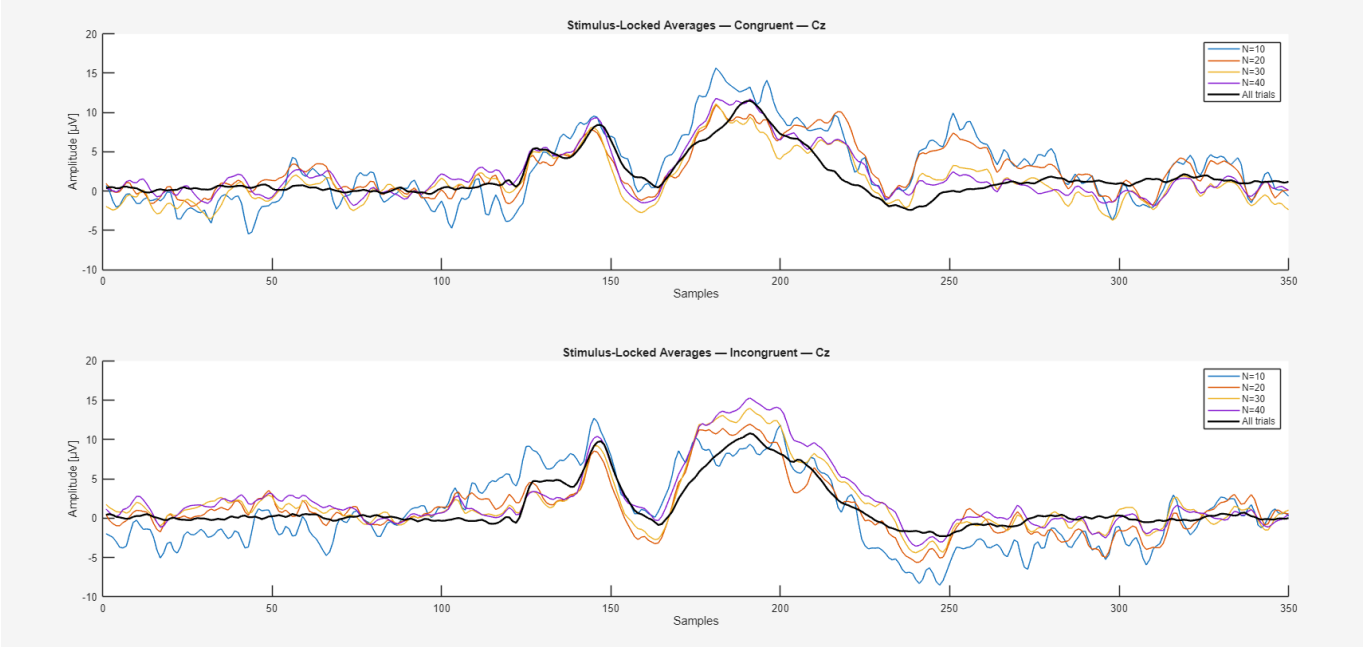


Figure 8: Averaged stimulus-locked ERP — Channel Cz. ERP morphology becomes progressively stable, especially for the P3 component.

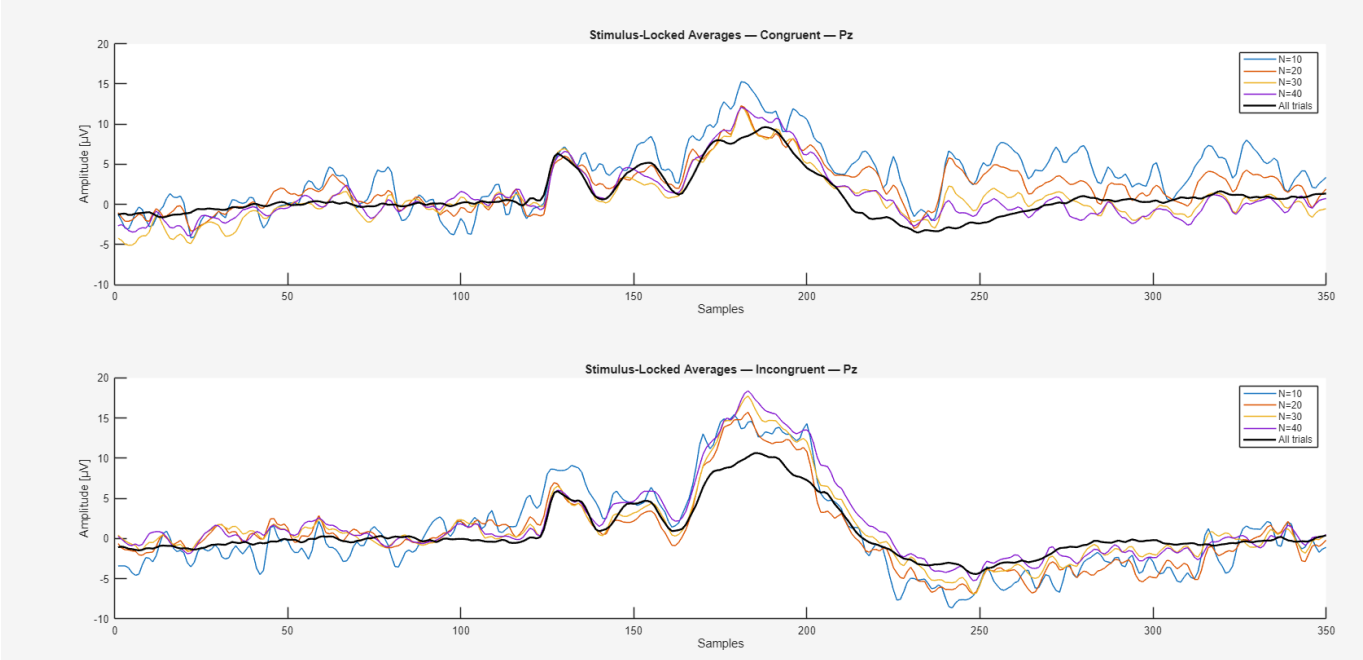


Figure 9: Averaged stimulus-locked ERP — Channel Pz. Parietal sites show the strongest P3 amplitude, consistent with classical ERP topography.

Figures for channels Fz, Cz, and Pz show that:

- With only 10 trials, the waveform is dominated by noise and ERP components are difficult to identify.
- With 20-30 trials, the P2, N2, and P3 begin to emerge with consistent polarity and latency.

- With 40 trials and the full average, the morphology stabilizes and the ERP peaks appear clearly defined.

This behaviour is expected from ERP theory: components such as P3 (250–600 ms) are highly sensitive to averaging because of their low amplitude relative to background EEG.

## 2(b). Response-Locked Averages (ERN)

A parallel analysis was performed for response-locked epochs. The averaged ERN waveforms for each channel are shown below.

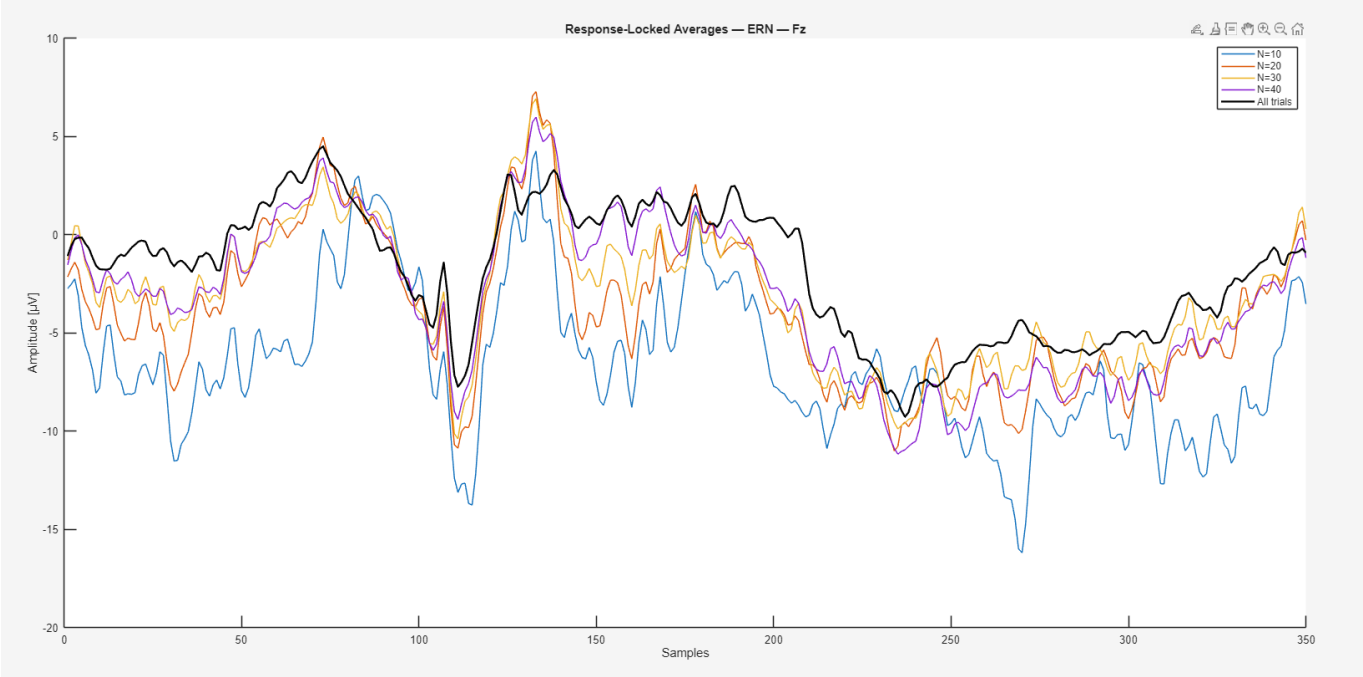


Figure 10: Averaged response-locked ERN — Channel Fz. Clear frontal negative peak emerging as the number of trials increases.

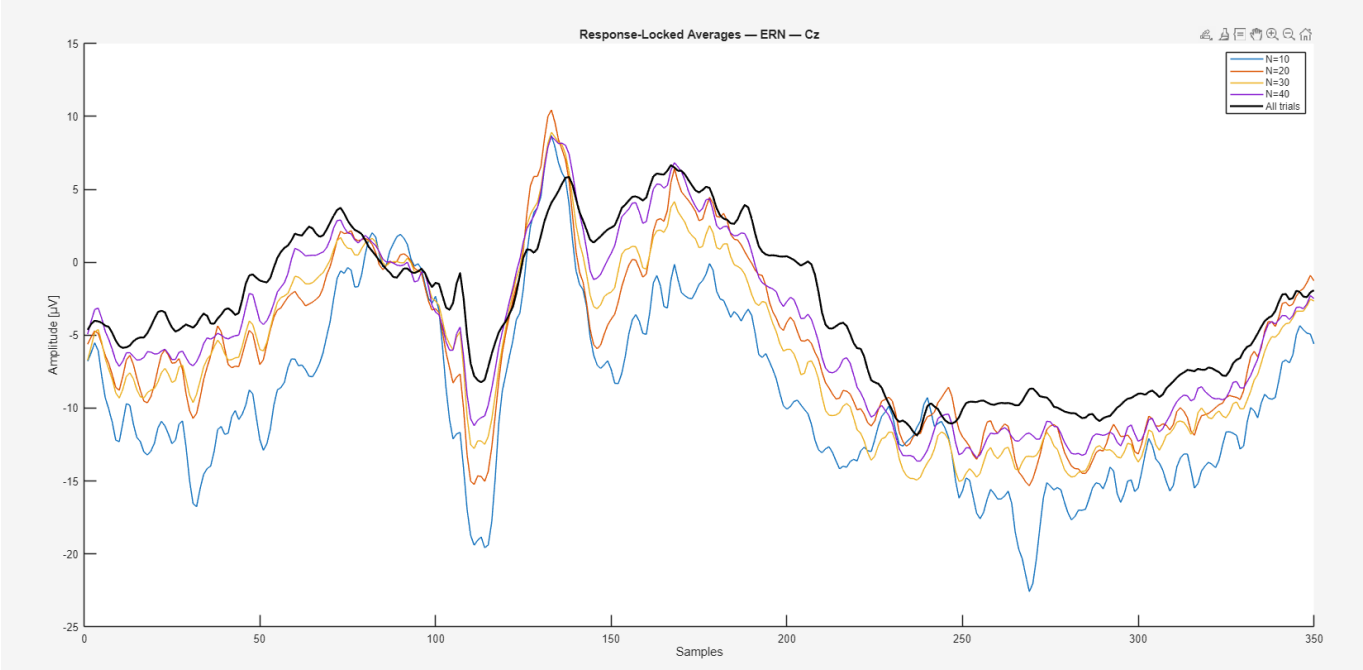


Figure 11: Averaged response-locked ERN — Channel Cz. The ERN becomes more defined around 30–40 trials, showing the expected fronto-central distribution.

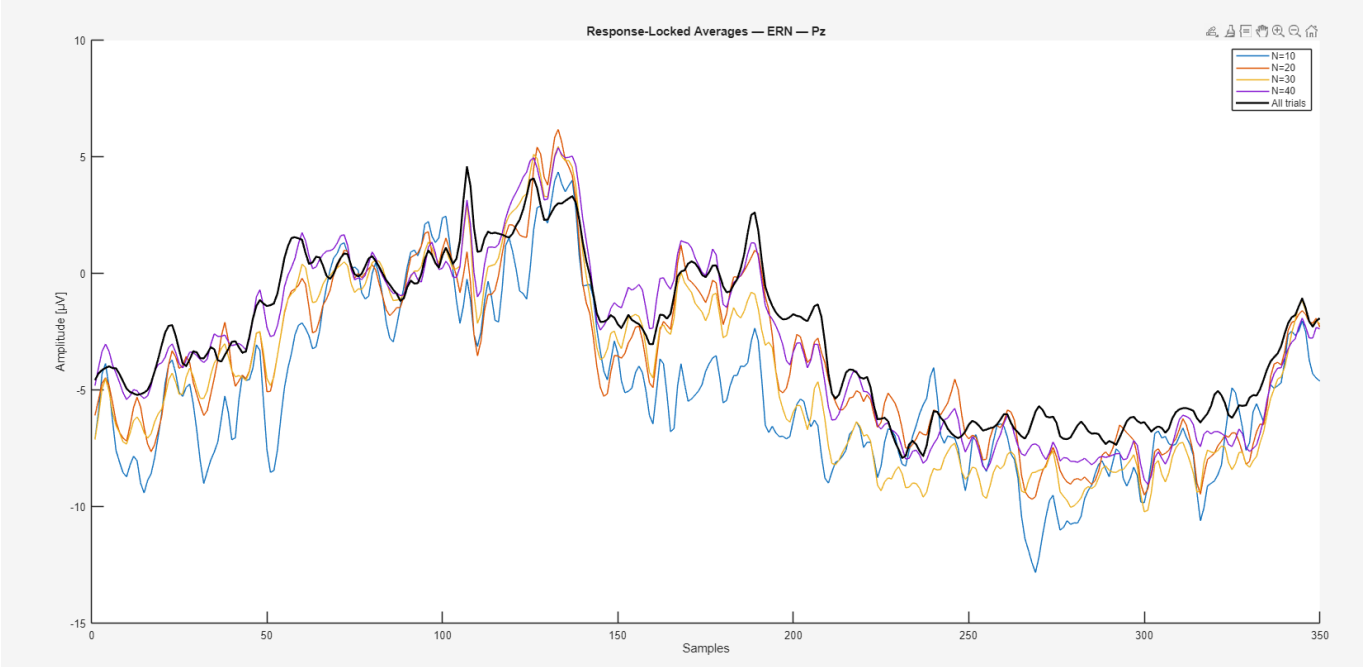


Figure 12: Averaged response-locked ERN — Channel Pz. Parietal regions show weaker ERN amplitude, consistent with its typical topography.

The ERN (0–100 ms after the response) shows:

- High variability for small $N$, with several trials showing opposite polarity relative to the expected ERN.
- Clear negative deflection as $N$ increases, stabilizing around 30–40 trials.

- The full average produces a well-defined ERN morphology with the characteristic frontocentral distribution.

## Interpretation

Across both stimulus-locked and response-locked analyses:

- Increasing the number of trials significantly improves ERP SNR.
- The morphology, amplitude, and latency of P2, N2, P3, and ERN stabilize as $N$ increases.
- Beyond approximately 40 trials, improvements become marginal, indicating convergence toward a stable ERP estimate.

Thus, this exercise confirms the fundamental importance of synchronized averaging for recovering reliable ERPs from noisy EEG recordings.

# 3 Effect of Trials on ERP Feature Stability

In this section, we quantify how the number of averaged trials influences the stability of the Event-Related Potential (ERP) features. Specifically, we analyse two canonical components:

- The **P3** (stimulus-locked), measured from incongruent trials.
- The **Error-Related Negativity (ERN)** (response-locked), measured from corrected responses.

Both amplitude and latency were computed for increasing numbers of averaged epochs, ranging from $N = 5$ to $N = 200$ in steps of five.

The analysis was performed on three representative electrodes:

$$\text{Fz (frontal)}, \quad \text{Cz (central)}, \quad \text{Pz (parietal)}.$$

This selection captures the expected topographies of both components: P3 maximally expressed parietally, and ERN maximally expressed fronto-centrally.

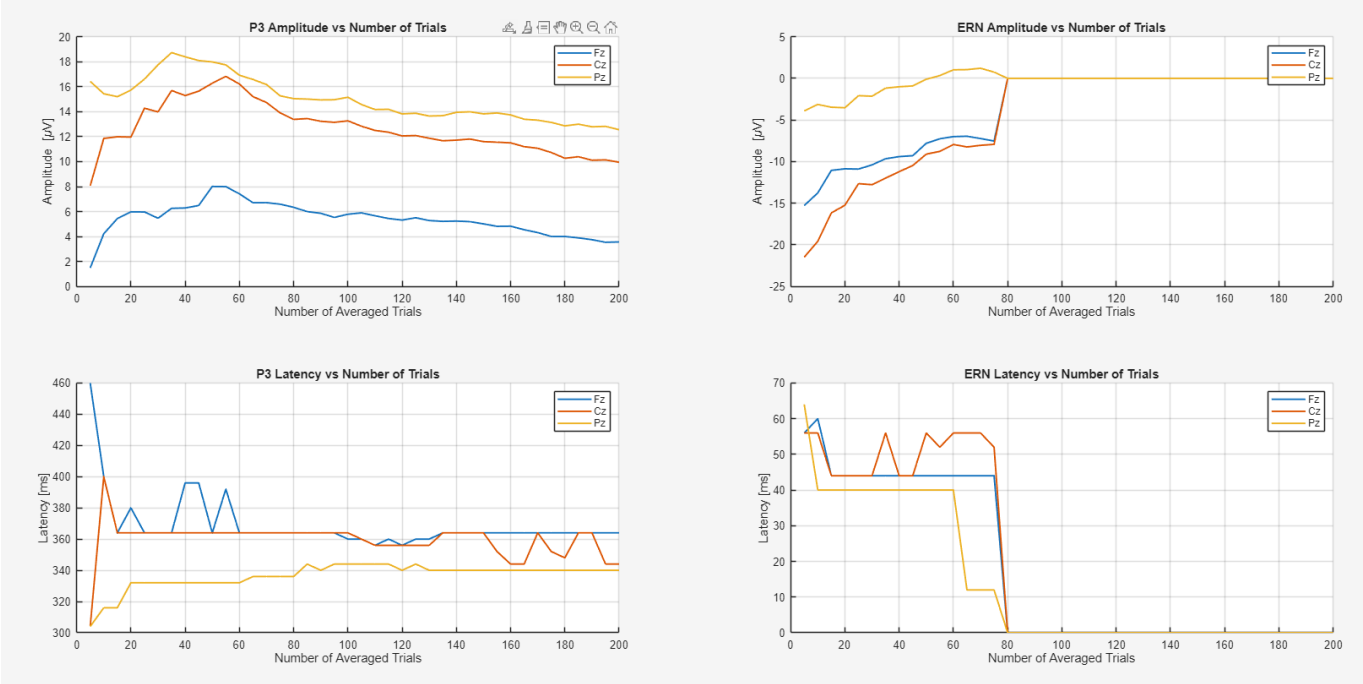


Figure 13: Evolution of ERP feature extraction as a function of the number of averaged trials for channels Fz, Cz and Pz. Top-left: P3 amplitude. Top-right: ERN amplitude. Bottom-left: P3 latency. Bottom-right: ERN latency.

## 3.1 P3 Component (Stimulus-Locked)

### Amplitude

A clear stabilisation pattern emerges (Fig. 13): with small numbers of trials ($N < 20$), the extracted amplitude fluctuates substantially. This reflects the low signal-to-noise ratio typical of early-stage averaging.

As $N$ increases:

- Fz shows the smallest P3 amplitude, consistent with its frontal topography.

- Cz increases moderately and becomes stable around $N \approx$ 60-80.
- Pz shows the expected maximal amplitude with a robust peak between $N =$ 20-40.

This behaviour aligns with well-known characteristics of the P3b component, which peaks over parietal regions and requires sufficient averaging to overcome background EEG noise.

#### Latency

P3 latency converges faster than amplitude. Although early averages ($N < 20$) produce unstable peak detection, all channels stabilise at physiologically plausible latencies (approx. 350-380 ms) for $N \geq 40$.

Notably, Pz again displays the most consistent values, reflecting its higher signal strength.

### 3.2 ERN Component (Response-Locked)

#### Amplitude

The ERN amplitude behaves similarly: initially unstable when few trials are averaged, but progressively converging to a characteristic negative deflection ($-10$ to $-15\,\mu$V) as $N$ increases.

Topographically, the ERN is maximal at Fz and Cz — a hallmark of its medial frontal generators. Pz exhibits a notably reduced amplitude, as expected.

Stability is largely reached with $N \geq$ 40-60 trials.

#### Latency

ERN latency stabilizes around 30 - 70 ms, consistent with the classical timing of early error-monitoring processes originating from the anterior cingulate cortex.

The transition from unstable measurements ($N < 20$) to stable and reproducible latencies occurs again around $N \approx$ 40-50.

### 3.3 Minimum Number of Trials Required for Stable ERP Features

The results strongly support the notion that ERP components require adequate averaging to overcome spontaneous EEG variability.

Across all channels and features:

- The stabilisation of amplitude and latency consistently occurs between **40 and 60 averaged trials**.
- This threshold is remarkably similar for both the P3 and ERN components.
- Components with stronger topographical expression (P3 at Pz, ERN at Fz/Cz) achieve stability slightly faster.
- With fewer than 20 trials, amplitudes and latencies exhibit large fluctuations, making the measurements unreliable for scientific or clinical interpretation.

## 3.4 Interpretation

The convergence analyses demonstrate that averaging is essential to recover clean, interpretable ERP features. Both P3 and ERN follow classic neurophysiological patterns in amplitude and latency, and require a minimum of 40-60 repetitions to produce robust and stable metrics. If we have to give a final number we would agree on 50 as a good threshold.

This finding is consistent with the literature on EEG signal processing and highlights the trade-off between experiment duration and ERP feature reliability.

## 3.6 Effect of Temporal Misalignment on ERP Averaging

The purpose of this exercise is to evaluate how temporal jitter in the extraction of epochs affects the averaged Event-Related Potentials (ERPs). Two modified functions, `promedioStimulusLockedv2.m` and `promedioResponseLockedv2.m`, introduce a random temporal perturbation in the alignment point following a Gaussian distribution with zero mean and standard deviation $\sigma$ (in samples). Since the EEG sampling frequency is 250 Hz, the two tested misalignments ($\sigma = 10$ and $\sigma = 20$) correspond to temporal uncertainties of 40 ms and 80 ms, respectively.

Three averaged epochs were computed for each condition:

$$\sigma = 0 \text{ (perfect alignment)}, \qquad \sigma = 10, \qquad \sigma = 20.$$

For the Stimulus-locked ERPs, congruent and incongruent trials were analysed separately. For the Response-locked ERN, all corrected error trials were combined.

The following figures show the three averaged ERPs for the Fz, Cz, and Pz channels.

### Stimulus-Locked ERPs

#### Channel Fz

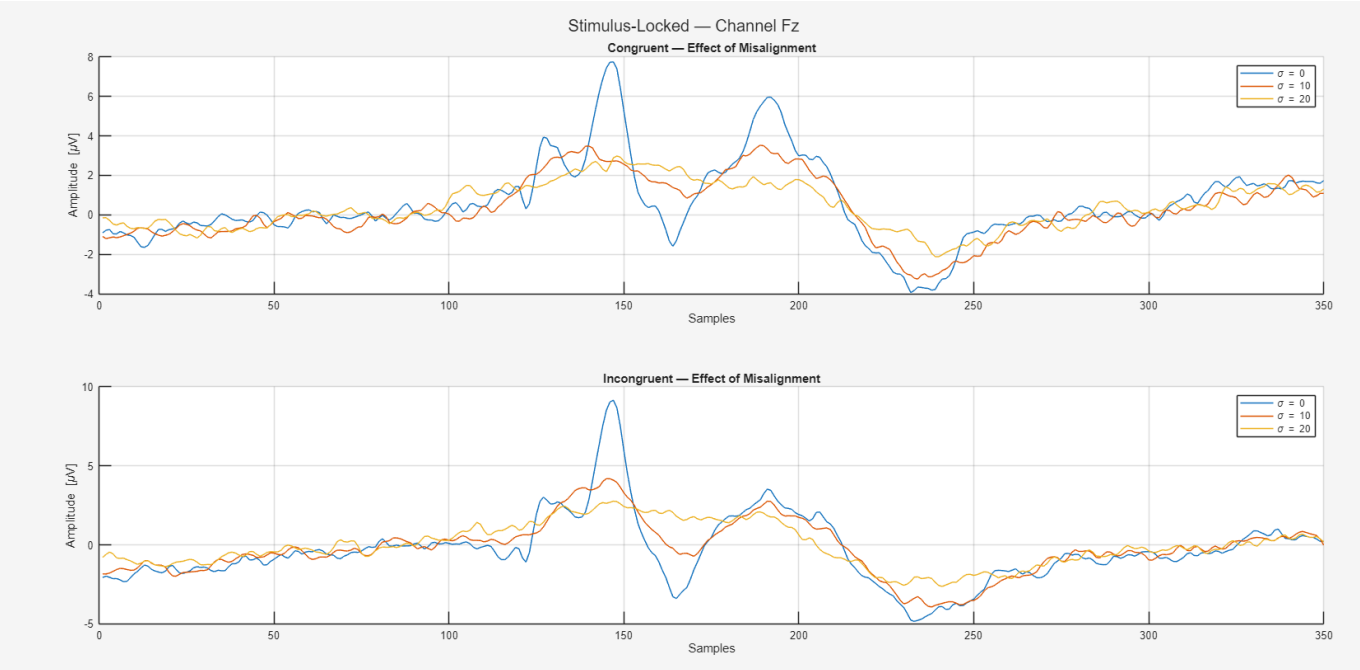


Figure 14: Stimulus-locked ERPs at Fz: effect of misalignment for congruent (top) and incongruent (bottom) trials.

### Channel Cz

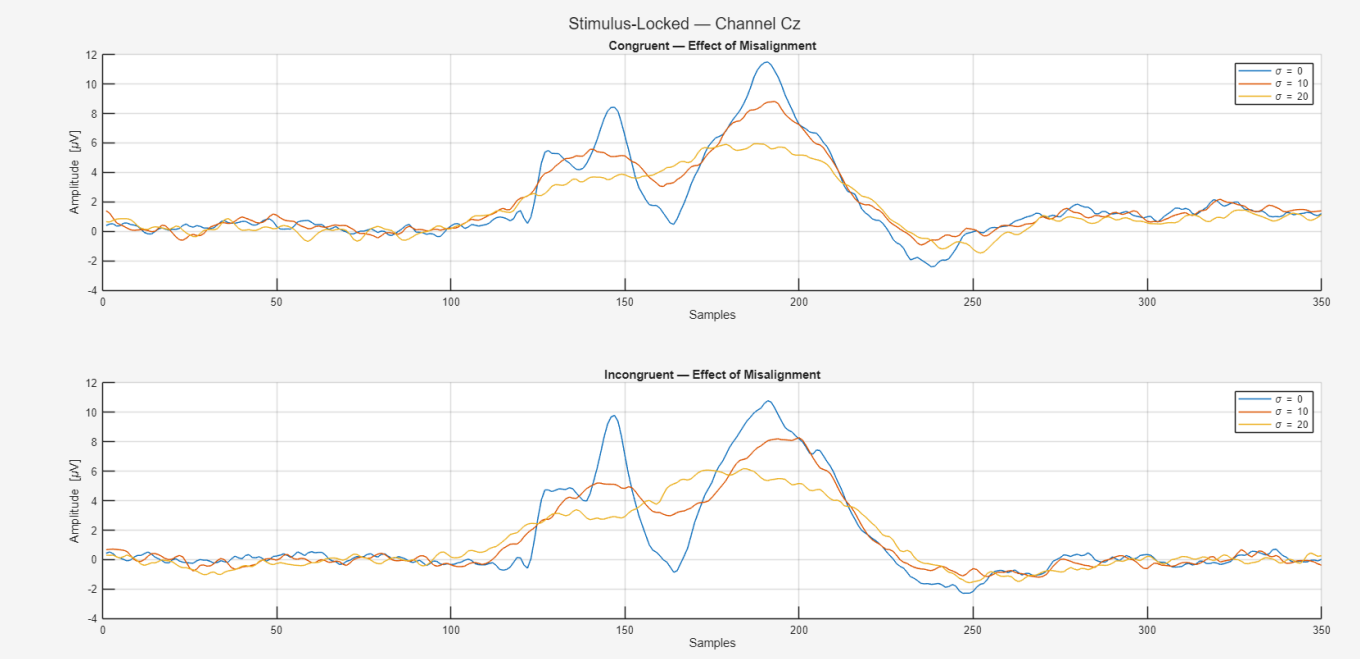


Figure 15: Stimulus-locked ERPs at Cz under perfect alignment and temporal jitter ($\sigma = 0, 10, 20$).

### Channel Pz

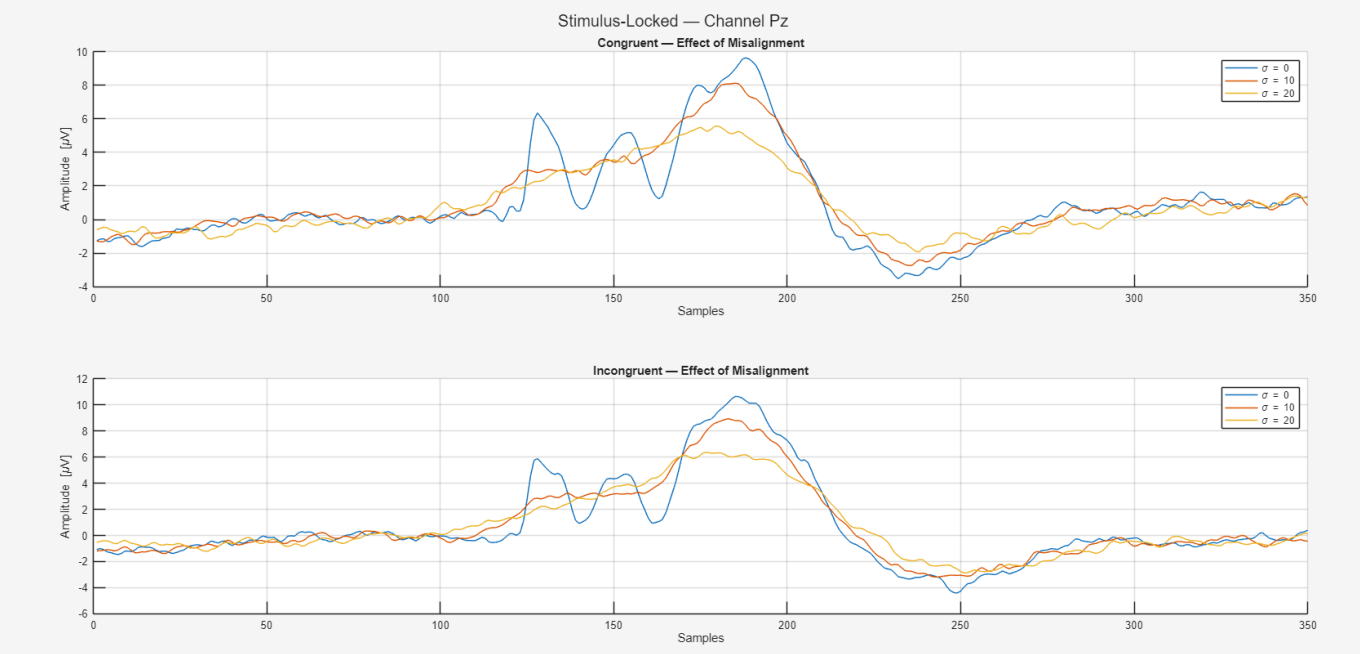


Figure 16: Stimulus-locked ERPs at Pz for congruent and incongruent conditions. Misalignment reduces peak sharpness.

## Response-Locked ERN

### Channel Fz

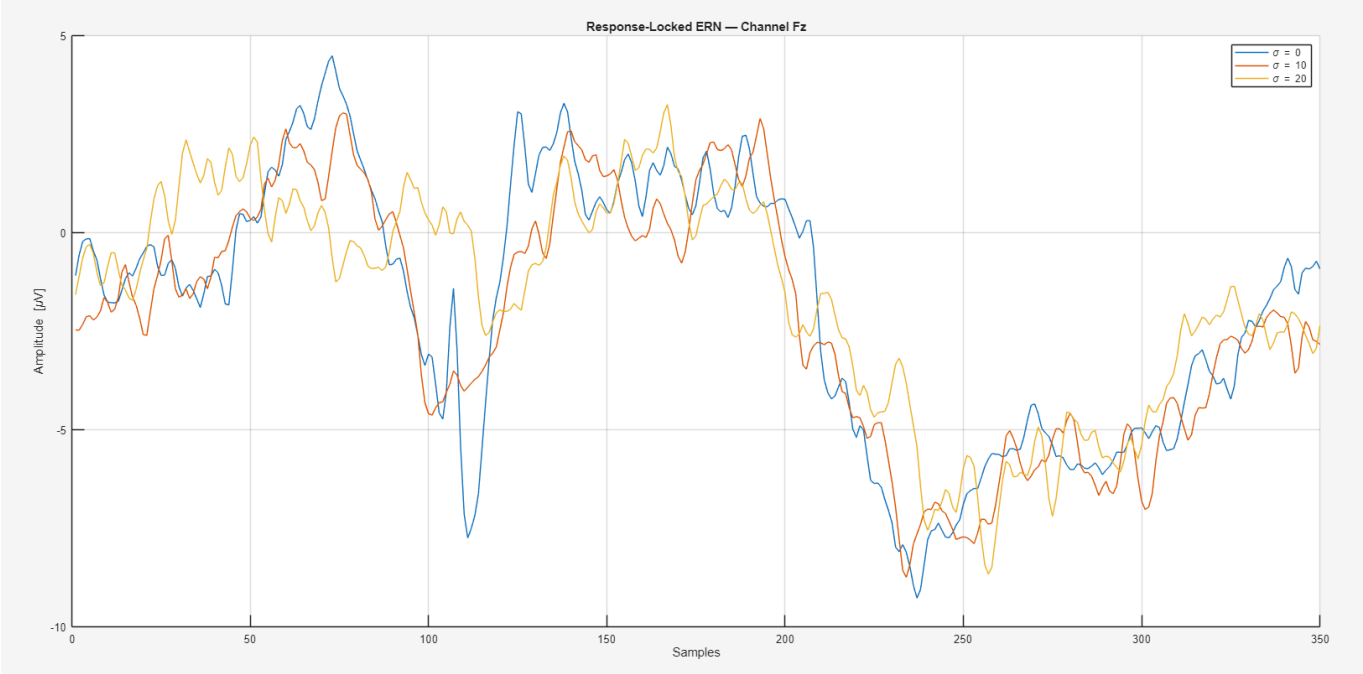


Figure 17: Response-locked ERN at Fz. Increasing temporal jitter reduces the ERN amplitude.

### Channel Cz

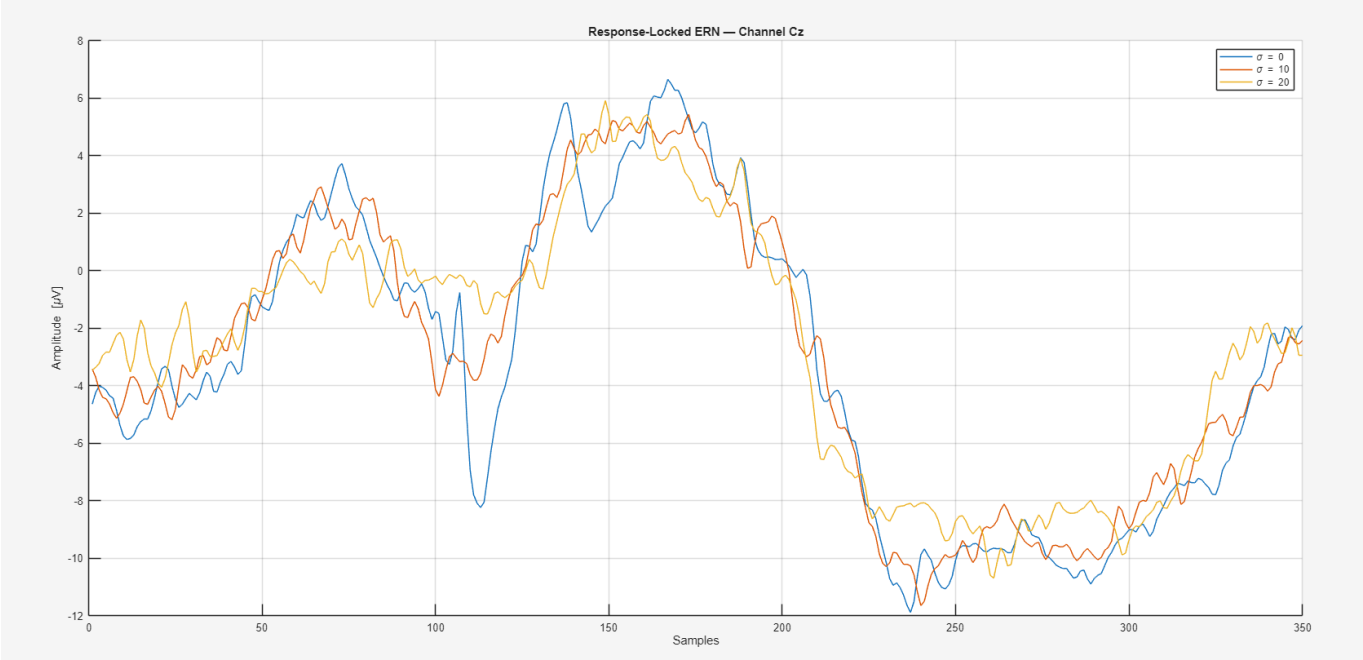


Figure 18: Response-locked ERN at Cz for $\sigma = 0$, 10, and 20 samples. Misalignment broadens the negative peak.

### Channel Pz

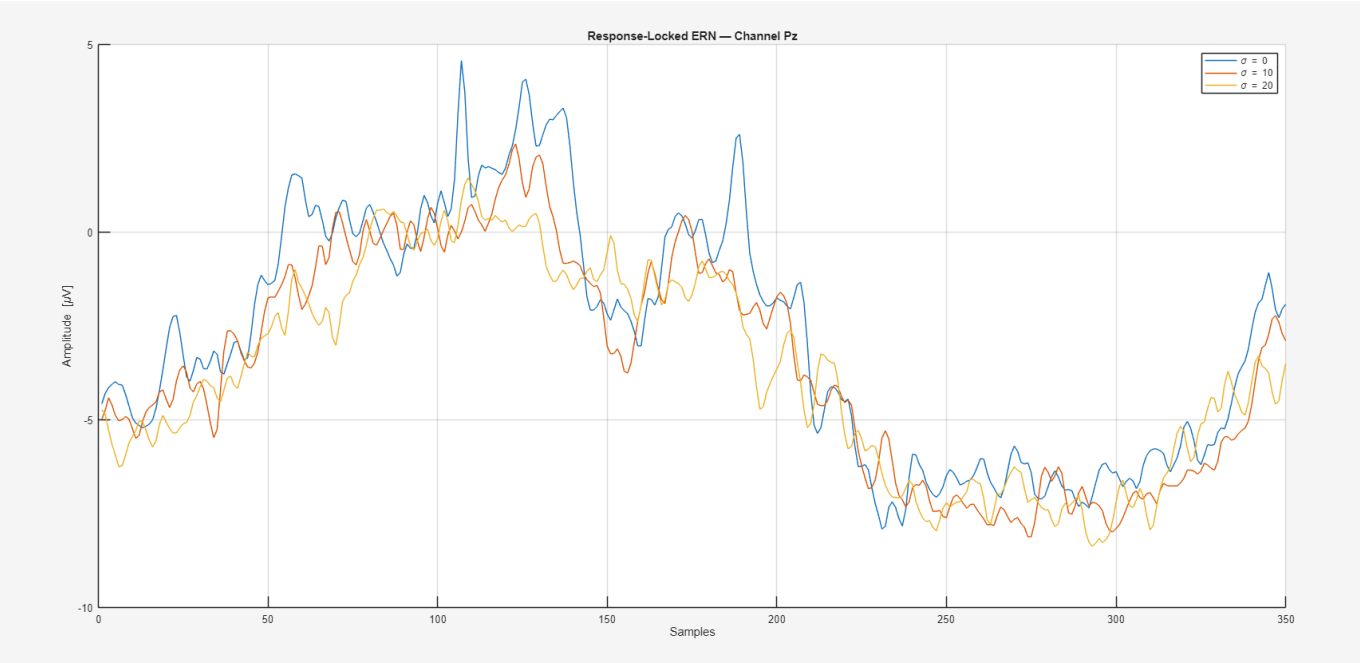


Figure 19: Response-locked ERN at Pz. Increasing misalignment reduces temporal precision of the component.

## Analysis and Interpretation

The results confirm that temporal misalignment has a clear impact on the morphology of both the P3 (stimulus-locked) and ERN (response-locked) components:

- **Peak amplitude decreases with misalignment:**. It causes individual peaks to occur at slightly different timings across trials. When averaging, these peaks no longer sum constructively, resulting in reduced amplitude. This effect is particularly evident at Fz and Cz.
- **Waveforms become smoother and less sharp:** Temporal smearing leads to broader, flatter peaks. The P3, originally sharp around 300–350 ms, becomes more rounded as $\sigma$ increases.
- **Latency becomes less reliable:** With greater temporal jitter, the position of the maximum (P3) or minimum (ERN) becomes harder to localize. This is reflected in small shifts and increased variability in the peak location.
- **Effects are stronger for larger $\sigma$:** The comparison between $\sigma = 10$ and $\sigma = 20$ shows an almost linear degradation of waveform clarity and peak amplitude.
- **ERN is more sensitive than P3:** Because ERN is a very early and narrow component (within 0–100 ms), even small misalignments cause strong distortion. This matches the observed larger morphological changes in ERN plots compared with P3.

## Conclusion

Temporal misalignment introduces destructive averaging effects that attenuate peak amplitude, broaden ERP components, and decrease latency precision. Given that even $\sigma = 10$ samples (40 ms)

produces noticeable distortions, maintaining accurate temporal alignment during preprocessing is essential. These findings highlight the importance of precise event marking and justify the common practice of artifact rejection and event correction before ERP averaging.

## 3.7 Topographic Distribution of P3 and ERN

To localize the sources of the ERP components on the scalp, we analysed the spatial distribution of the peak amplitudes across 19 standard electrode positions. The P3 amplitude was calculated as the maximum positive deflection (250-600 ms) for both congruent and incongruent stimuli. The ERN was quantified as the maximum absolute negative deflection (0-100 ms) in the response-locked averages.

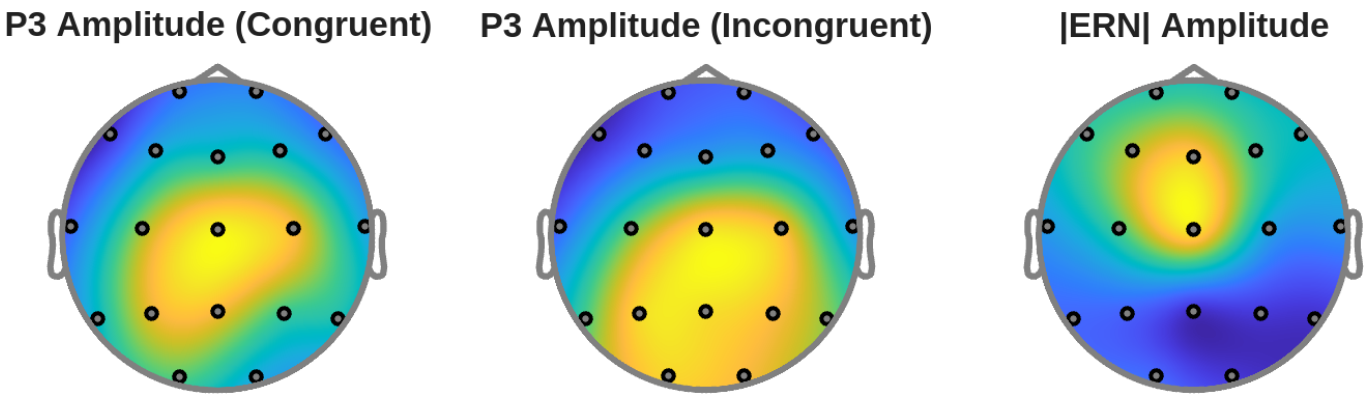


Figure 20: Topographic distribution of ERP peaks. Left: P3 Congruent. Center: P3 Incongruent. Right: ERN (absolute amplitude).

### Interpretation

- **P3 Distribution:** As shown in the left and center topograms, the P3 component is maximally distributed over the **parietal and centro-parietal regions** (around Pz and Cz). This is consistent with the classic "P3b" topography associated with task-relevant stimulus processing. The amplitude appears slightly higher at the posterior area for incongruent stimuli (center) compared to congruent ones (left).
- **ERN Distribution:** The ERN (right map) shows a distinct **fronto-central distribution** (maximal around Fz and FCz). This topography is distinct from the P3 and aligns perfectly with the anatomical location of the Anterior Cingulate Cortex (ACC), which is considered the neural generator of error monitoring signals.

# 4 Exercise 2: Visual Short Term Memory (VSTM) Task

In this section, we analyse EEG data recorded from a different cognitive paradigm: a Visual Short Term Memory task (Student 2 data). We extracted the stimulus-locked P300 component elicited by the second image of the pair, considering only trials with correct responses. The signals were low-pass filtered (7 Hz) to smooth the waveforms.

## 4.1 P300 Waveforms and Topography (Student 2)

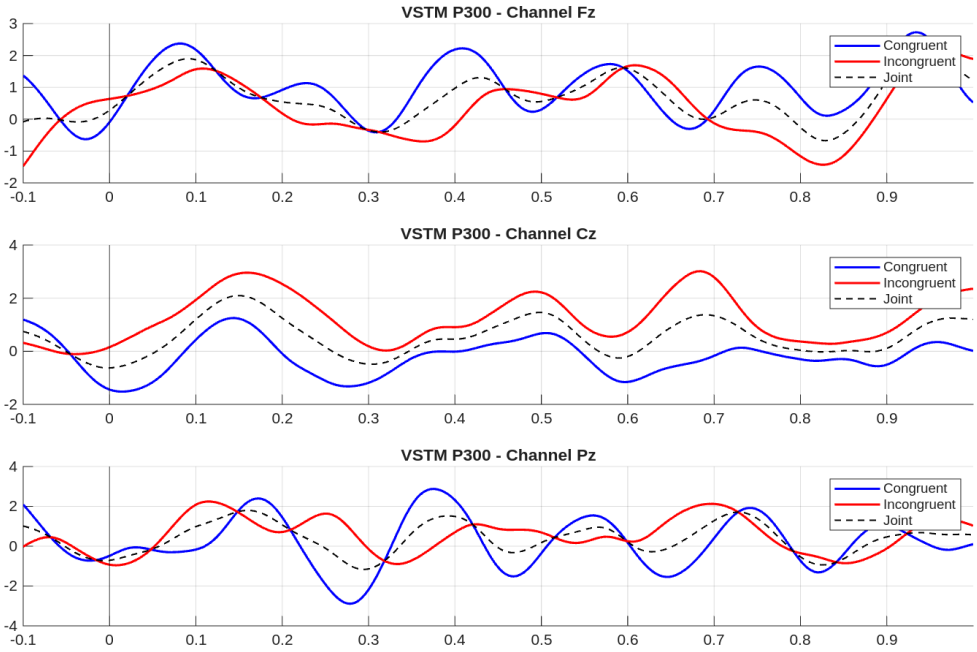


Figure 21: Stimulus-locked P300 for Student 2 at Fz, Cz, and Pz. Blue: Congruent. Red: Incongruent. Dashed: Joint Average. Note the high variability and oscillatory nature of the signals.

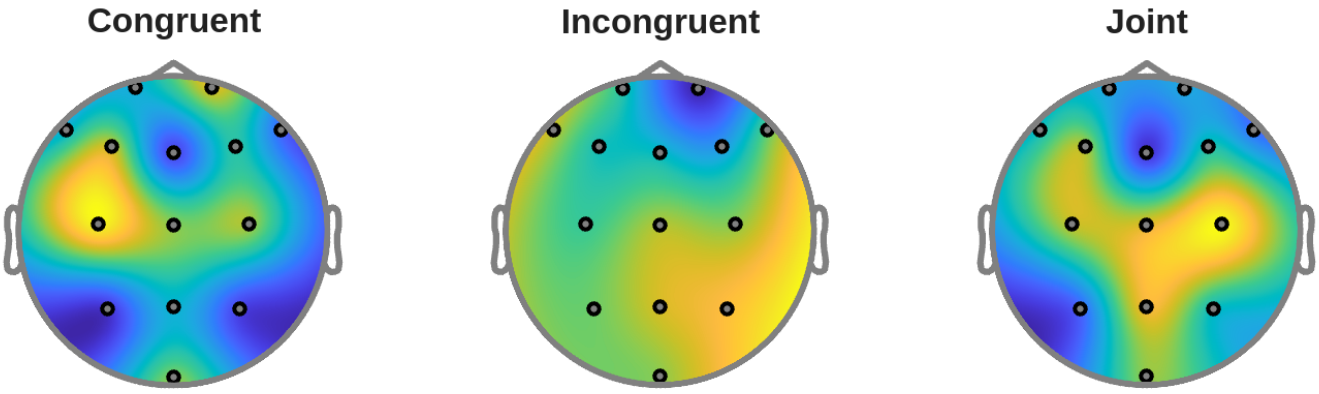


Figure 22: Topographic distribution of the P300 peak (250-400 ms) for the VSTM task. The distribution is atypical, showing strong lateralization.

**Interpretation**

The waveforms in Figure 21 display considerable variability compared to the standard Grand Averages seen in Exercise 1.

- **Waveforms:** At **Pz** and **Fz**, the Congruent condition (blue) shows a distinct positive peak around 350–400 ms, consistent with P300 timing. However, the Incongruent condition (red) shows a different morphology, with a delayed or broader positivity particularly visible at **Cz** around 450 ms.
- **Topography:** The topographic maps (Figure 22) deviate from the classic midline centro-parietal distribution. The **Congruent** condition shows a focal positivity over the left central/-parietal region, while the **Incongruent** condition displays positivity concentrated in the right posterior/occipital region. The **Joint** average shows a bilateral posterior distribution.

These irregular distributions likely reflect the lower signal-to-noise ratio of a single-subject recording (Student 2) compared to the grand average of 9 subjects used in the previous exercise.

## 4.2 Behavioral Analysis

We calculated the response time (RT) and accuracy for the congruent (matching images) and incongruent (non-matching images) conditions.

| **Condition** | **Response Time (Mean ± STD)** | **Accuracy (%)** |
|---|---|---|
| Congruent | $0.147 \pm 0.033$ s | 96% |
| Incongruent | $0.145 \pm 0.034$ s | 100% |

Table 1: Behavioral performance for Student 2.

The behavioral results for this specific student are notably fast ($\approx$ 145 ms). Contrary to the standard "Stroop-like" interference effect—where incongruent stimuli typically cause slower reactions and more errors—this subject performed **equally fast (or slightly faster)** on incongruent trials and achieved **100% accuracy**. This suggests the subject did not experience the expected cognitive conflict, or the task difficulty was low enough to allow ceiling performance.

# 5 Conclusion

This laboratory session allowed us to practically verify the fundamental principles of Event-Related Potential (ERP) analysis, moving from theoretical concepts to the processing of real EEG data. Our results highlight the critical necessity of signal processing techniques to extract meaningful neural correlates from background noise.

The key findings from our analysis can be summarized as follows:

- **Necessity of Synchronized Averaging:** Our analysis of single-trial epochs demonstrated that the high amplitude of spontaneous background EEG completely obscures ERP components. Neither the P3 nor the ERN were identifiable in individual trials, confirming that synchronized averaging is indispensable for cancelling out stochastic noise and revealing time-locked neural responses.
- **Minimum Trials for Stability:** By systematically increasing the number of averaged epochs, we determined that a minimum of **40 to 50 trials** is required to obtain stable and reliable ERP features. Below this threshold, both amplitude and latency measurements fluctuated significantly, making them unreliable for clinical or experimental interpretation.
- **Topographic Specificity:** We successfully replicated the known topographic distributions of the studied components. The **P3** component showed a clear parietal dominance (maximal at Pz), consistent with attention and memory processing. In contrast, the **ERN** exhibited a fronto-central distribution (maximal at Fz and Cz), aligning with its generation in the Anterior Cingulate Cortex.
- **Impact of Temporal Precision:** The misalignment simulation revealed that precise event marking is as critical as trial count. Introducing a temporal jitter of just $\sigma = 10$ samples (40 ms) caused destructive interference, significantly reducing peak amplitude and smoothing the waveform morphology.
- **Individual Variability (VSTM Task):** Applying these methods to the Visual Short Term Memory task highlighted the challenges of analyzing single-subject data. Unlike the clean "Grand Mean" averages, Student 2's data exhibited "messier" waveforms and atypical lateralization. Furthermore, the unexpected behavioral result-where the subject was faster and perfectly accurate on incongruent trials-suggests that the cognitive load was insufficient to induce the standard interference effect for this individual.

In conclusion, while group-level averaging reveals clear and robust neurophysiological trends, this session demonstrated that individual EEG analysis requires careful attention to signal quality, precise synchronization, and an adequate number of repetitions to draw valid conclusions.