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# Methodological Challenges in EEG Research with 11-Month-Old Infants - A Pilot Study

Moira C. Schummer[a,*] · Robin Nehler[b] · Sarah Jessen[b,*]

[a]Student of Psychology - Cognitive Systems, Universität zu Lübeck, Lübeck, Germany
[b]Institute of Medical Psychology, Center of Brain, Behaviour and Metabolism, Universität zu Lübeck, Lübeck Germany
[*]Corresponding author, email: moira.schummer@student.uni-luebeck.de; sarah.jessen@uni-luebeck.de

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**Abstract**
Conducting experiments with infants poses unique challenges. During the investigation of brain responses on social perception of infants, specific difficulties, such as increased mobility, arose regarding 11-month-olds. To address these, we modified the experimental setup and conducted a pilot study with 11-month-old infants (N = 8), recording electroencephalographic (*EEG*) signals using two different EEG preprocessing pipelines (an open-science vs. a custom pipeline). We analyzed the *Nc* (Negative central) response to familiar vs. unfamiliar faces and to happy vs. fearful expressions. While no significant differences were found, possibly due to the small sample size, the setup changes proved effective, with standard retention rates for valid and clean trials. No significant differences in data between pipelines indicate the effectiveness of both, highlighting the reliability of open-science tools in infant EEG research. However, the custom pipeline's small amplitudes in Nc responses emphasize the need for further validation in a larger sample.

## I. Introduction

Information about the social world is essential for humans and especially newborns whose survival depends on their social environment. A key mechanism is the preference for faces and the ability to interpret facial expressions, providing insight into a person's age, race, gender or emotional state. By 6 to 8 months, infants can distinguish emotional expressions and use caregiver's facial cues for social referencing [1], [2], [3]. As attention develops, infants initially focus on familiar faces, particularly those of primary caregivers. By 6 to 7 months, this bias gradually shifts to novel stimuli with infants displaying an attentional preference for fearful expressions and strangers' faces. However, for 11-month-old infants, no specific preferences have been found [4], [5]. These behavioral changes are linked to neural substrates, such as the *Nc* (*Negative central*), an *event related potential* (*ERP*) that reflects attention allocation. In 7-month- old infants, the Nc amplitude increases in response to a stranger's compared to a mother's face, as well as to fearful compared to happy expressions. These preferences remain unclear for 11-month-olds [2].

To investigate brain responses linked to social perception, Nehler et al. (2024, ongoing) are conducting a longitudinal study with 4-, 7-, and 11-month-old infants. By 11 months, infants show significant developmental changes, such as improved mobility and increased resistance to disliked stimuli like an EEG cap. A pilot study was conducted to adjust the experimental setup to achieve a more age appropriate environment, minimizing visual distractions. Furthermore, videos were incorporated to regain infant attention. With discontinuation rates of 25 % to 75 %, pilot testing infant experiments is essential to improve data collection and maximize the acquisition of usable, valid data [6].

This study addresses two key objectives. We aim to validate an experimental setup that is suitable for 11-month-old infants to study brain responses. Furthermore, we compare the results of two EEG preprocessing pipelines, an open-source (*HAPPE*) and a custom approach, to assess their effectiveness as well as to validate open-source methods and accessibility in research settings. Therefore, we tested the following hypotheses: We expect (i) smaller Nc amplitudes for their mother's vs. a stranger's face, (ii) smaller Nc amplitudes for happy faces compared to fearful ones, regardless of whether the face presented is from their own mother or a stranger, (iii) no significant differences in Nc amplitudes between infants shown an engaging vs. a calming video during breaks, (iv) no correlation between the number of breaks and EEG data quality, measured in valid trials, and (v) no significant differences in EEG data analysis results between an open-source and a custom pipeline.

# II. Methods and Materials

## II.I. Participants

The pilot sample included 8 healthy infants (mean age: 336.5 ± 9.77 days; range: 324–351 days), recruited via the maternity ward of the local University Hospital (*Universitätsklinikum Schleswig-Holstein*). They were randomly assigned to one of two equal-sized groups (4 each). In the open-source pipeline, 3 participants, and in the custom pipeline, 2 participants were excluded from further analysis ($N_{\text{HAPPE}} = 5$, $N_{\text{Custom}} = 6$), as they did not meet the criterion of ten valid trials per stimulus category.

Parents provided written consent after being informed about the study's privacy requirements, methods, and potential risks. To ensure familiarization in the Baby Laboratory, all infants were given time to explore the room, play with toys, and interact with the experimenters. The room's lighting was dimmed to create a less sterile atmosphere, and the experiment took place behind a curtain open at the back, see Fig. 1. This setup minimized distractions while allowing parents to see their infant at all times.

## II.II. Experimental Setup

To measure social perception, we used emotional face stimuli consisting of two sets of colored photographs displaying happy and fearful facial expressions. One set featured the infants' mothers and the other featured an unfamiliar mother. The four stimuli were: *mother happy*, *mother fear*, *stranger happy*, *stranger fear*, see Fig. 1.

To record cerebral brain waves, we used an elastic cap (BrainCap, Easycap GmbH) with 27 AgAgCl electrodes attached according to the international 10-20 system. The infants were seated in a highchair, approximately 60 cm from a 24-inch screen in a darkened room with an isolated space, see Fig. 1. Parents were instructed to stay at least 1.5 meters behind the infants and refrain from interacting with them during the experiment. The EEG session included up to 200 trials with face stimuli presented for 800 ms and the intertrial interval lasted 800 ms to 1200 ms. Short animated videos were played to regain infants' attention if they became distracted. If attention could not be regained, longer videos were used. Infants were assigned to one of two groups: one watched an engaging video of wild baby animals to boost their alertness, while the other watched a calming video of animated lanterns with relaxing music to soothe them.

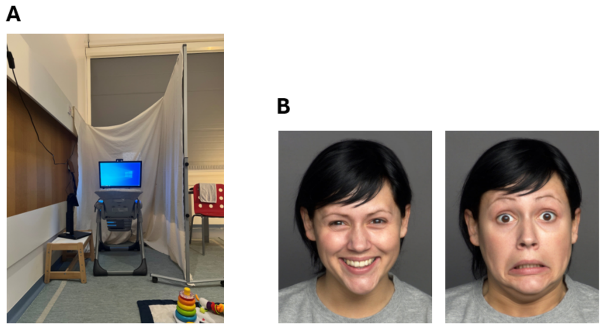


**Figure 1:** (A) Experimental setup; (B) Example of emotional face stimuli displaying happy and fearful expressions.

## II.III. EEG Preprocessing

We analyzed our EEG data using *MATLAB* (*R2024b*) and *EEGLAB*, applying two different preprocessing pipelines: the *Harvard Automated Processing Pipeline for Electroencephalography* (*HAPPE*) and a custom pipeline. Both use different approaches to clean and prepare raw EEG data by removing artifacts (e.g. eye movements, muscle activity) and filtering noise to enhance signal quality. The HAPPE pipeline applied a 0.2 Hz to 20 Hz bandpass filter. Artifact correction was performed using a wavelet-enhanced Independent Component Analysis and automated component rejection. Rejected channels were interpolated. Both pipelines re-referenced the data to the average of TP9 and TP10 (linked mastoids) [7]. In our custom pipeline, a 0.2 Hz to 20 Hz bandpass filter was used. For artifact correction, we interpolated channels exhibiting amplitude deviations that exceeded ±2 standard deviations for 50% of the time. For artifact subspace reconstruction, we used the *clean_rawdata* function in EEGLAB. Epochs −200 ms to 800 ms were extracted relative to stimulus onset. All trials were assessed via video recordings during the experiment and trials in which the infant did not watch the presented stimuli were discarded. To analyze the Nc response, the mean response was calculated across frontocentral electrodes (*F3, Fz, F4, C3, Cz, C4*).

## II.IV. ERP Analysis

For the ERP analysis we used *R Studio* (*RStudio 2024.12.0+467*). Due to the small sample size, Wilcoxon

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signed rank tests were conducted as post-hoc tests to assess group differences with effect sizes reported as $r$. To examine the connection between number of breaks (short and long videos) and data quality (number of valid trials), we computed a Spearman correlation. To compare preprocessing pipelines, a two-factor ANOVA was performed using the mean Nc-responses for the different stimulus categories (*mother happy, mother fear, stranger happy, stranger fear*) as a within-subject factor and preprocessing pipelines (HAPPE, Custom) as between-subject factor. To account for violations of sphericity the Greenhouse-Geisser correction was applied. We computed the effect size using partial Eta-squared ($\eta^2$).

# III. Results

**Hypothesis 1** - *No Significant Difference in Nc Responses to Mother's and Stranger's Faces:* In the dataset preprocessed by the HAPPE pipeline, no significant difference in the Nc amplitude in response to the mother's faces compared to the stranger's faces ($V = 27, p = 1, r = 0.3$) was found, suggesting that potential differences are minimal and due to random variation. Similarly, the dataset preprocessed by the custom pipeline showed no significant difference in Nc responses ($V = 48, p = 0.52, r = 0.3$).

**Hypothesis 2** - *No Significant Difference in Nc Responses to Happy versus Fearful Faces:* For the dataset preprocessed by the HAPPE pipeline, no significant difference in Nc amplitudes was found in response to happy or fearful facial expressions, regardless of the person expressing the emotion ($V = 25, p = 0.85, r = 0.3$). Similarly, the custom pipeline dataset showed no significant difference ($V = 38, p = 0.97, r = 0.04$), emphasizing the absence of notable effects.

**Hypothesis 3** - *No Influence of Video Groups on Data Quality:* The dataset processed by the HAPPE pipeline lacked variance, as only one infant in the engaging video group had more than 10 valid trials in each stimulus condition. Therefore, we were not able to analyze the difference between the video groups. The dataset preprocessed using the custom pipeline found no significant differences between the engaging and calming video group ($V = 6, p = 0.44$) with only a small effect size ($r = 0.317$).

**Hypothesis 4** - *Relationship of Breaks and Valid Trials:* A correlation analysis for the dataset processed by the HAPPE pipeline showed a moderate negative relationship between the number of breaks and the number of valid trials ($r = -0.41$). However, this finding was not significant ($p = 0.49$). Similarly, for the dataset preprocessed by our custom pipeline, we found no significant correlation ($r = -0.03, p = 0.96$).

**Hypothesis 5** - *Effect of Preprocessing Pipeline on Stimulus Category:* The analysis of datatsets preprocessed by the HAPPE and custom pipelines found no significant main effect of *preprocessing pipelines*, ($F(1,9) = 1.82, p = 0.210; \eta^2 = 0.118$) and no significant interaction between *stimulus category* and *preprocessing pipelines* ($F(1.43, 12.88) = 0.93, p = 0.388; \eta^2 = 0.0337$), see Fig. 2 and Fig. 3.

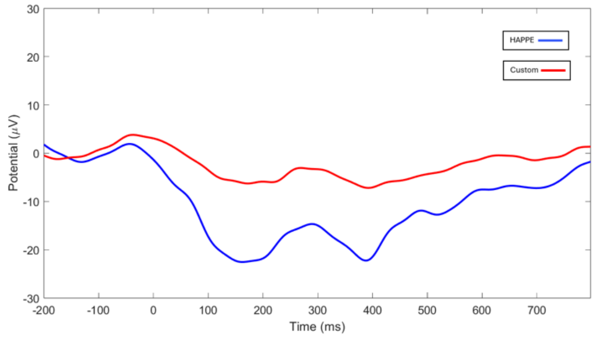


**Figure 2:** Mean Nc amplitudes averaged across all stimulus categories: Comparison between EEG preprocessing pipelines.

# IV. Discussion and Conclusion

The setup for 11-month-old infants proved to be effective, yielding 63% usable EEG data for the HAPPE pipeline and 75% for the custom pipeline, indicating expected retention rates and promising prospects for data acquisition in the longitudinal study [6], [8].

**Nc Response to Facial Stimuli:** We found no significant difference in Nc responses to mother's versus stranger's faces, and no difference in Nc responses between happy and fearful facial expressions. This may be due to quicker habituation to novel cues by 11 months, especially with repetitive stimuli, as used in our study. Individual variability in the need for novel cues may be an important factor [3], [4]. By 6 months, infants show an attentional bias toward fearful faces and caregiver's vocal and facial reactions to potential threats help regulate infant's actions. However, inconsistent findings at 11 months indicate variability in the ability to self-regulate emotion control, shifting attention away from distress-related stimuli. This could be linked to stronger connectivity between the prefrontal cortex and limbic structures [2], [5].

**Impact of Setup-Changes on Data Collection:** Collecting reliable EEG data from 11-month-old infants remained challenging as they required more frequent and longer breaks than younger infants. While engaging and calming videos showed no significant differences in effectiveness, both helped to regain the infant's attention. Longer breaks, during which mothers could calm their infants, including breastfeeding, proved to be effective for resuming the experiment. However, breastfeeding, as an interaction between mother and infant, introduced variability. The curtain setup partially reduced distractions by limiting infants' ability to seek their mothers instead of focusing on the presented stimuli. However, some measurements were aborted prematurely when

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infants became tired or fussy. Despite limited attention span and stamina, most infants provided enough clean trials to be included in the pilot dataset. Even few clean and valid trials early in the experiment can yield sufficient high-quality data for EEG data, demonstrating that high data loss does not equal low data quality [8].

**Comparing Preprocessing Pipelines: Challenges and Insights:** We observed no significant differences in Nc responses between datasets preprocessed with two different pipelines, indicating the effectiveness of both for EEG infant analysis. However the HAPPE dataset produced mean amplitudes of $-9.17\,\mu V$, reflecting expected neural responses, while the custom dataset showed unusually low amplitudes ($\mu = -2.47\,\mu V$), appearing very strict in smoothing the data, reducing amplitude fluctuations, and removing slow drifts or high-frequency artifacts, see Fig. 2. Infant research typically reports amplitudes up to $-20\,\mu V$ [2], [9].

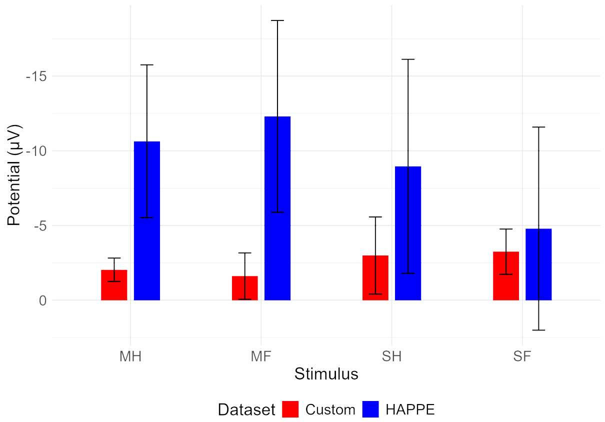


**Figure 3:** Mean Nc responses for mother happy (MH), mother fear (MF), stranger happy (SH) and stranger fear (SF), comparing between EEG preprocessing pipelines. Error bars represent standard deviations (SD).

Interestingly, the HAPPE pipeline excluded more participants (N = 5) than the custom pipeline (N = 6). This seems contradictory to the initial assumption that the custom pipeline, with stricter artifact removal, would exclude more trials and participants, as well as reduce the amount of usable data per individual. Considering the observed high variance in the HAPPE data (SD = 13.48) compared to the custom data (SD = 4.01) (Fig. 3.), these findings must be interpreted with caution. When balancing artifact removal and preservation of neural signals, the HAPPE pipeline remains a reliable open-source tool for data analysis in EEG infant research.

**Conclusion and Implications for Future Research:** For the longitudinal study, both video options and extended break times can be tailored to the infant's needs for maintaining engagement in 11-month-old infants. This highlights the importance of carefully designed interventions to ensure high data quality. In future studies, other strategies should be explored to further improve infant participation. We recognize the need to test our custom pipeline on a larger EEG dataset to refine its accuracy and reliability. The HAPPE pipeline seems to be a reliable alternative for analyzing our EEG data. We look forward to evaluating the data from the longitudinal study (N = 120) and expect significant results.

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## Author's statement

Conflict of Interest: Authors states no conflict of interest. Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee. AI assistance disclosure: AI-based tools , including DeepL and ChatGPT, were used to assist in language editing and text refinement. The final content was reviewed and approved by the authors.

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