

DOCUMENTATION

of

PSYCHOPHYSIOLOGY

METHODS

in

MIDUS 3

Neuroscience Project (P5)

University of Wisconsin ♦ Institute on Aging
February 2026

TABLE OF CONTENTS

| | |
|---|----------|
| INTRODUCTION | 2 |
| DIFFERENCES BETWEEN MIDUS WAVES | 2 |
| HEARING TEST | 2 |
| FACIAL ELECTROMYOGRAPHY (EMG)..... | 2 |
| EYEblink STARTLE REFLEX (EBR) | 3 |
| CORRUGATOR SUPERCILI (CORR) | 4 |
| ZYGOMATICUS MAJOR (ZYGO) | 4 |
| HEART RATE VARIABILITY (HRV)..... | 5 |
| ELECTROCARDIOGRAM (ECG) IN PSYCHOPHYSIOLOGY SESSION:..... | 5 |
| PHOTOPLETHYSMOGRAM (PPG) DURING fMRI SCAN SESSION: | 6 |
| REFERENCES | 7 |

INTRODUCTION

This document provides an overview of the MIDUS 3 Neuroscience Project's psychophysiology methods. Various psychophysiology data were collected during the Psychophysiology Emotional Response Task and during the Functional MRI Emotional Response Task and Resting State Scans. Variable names have been provided where appropriate. For more detailed information on the Psychophysiology Emotional Response Task paradigm, variable names and data collection procedures, please see:

[*M3_P5_DOCUMENTATION_OF_BEHAVIORAL_COGNITIVE_20260206*](#)

[*M3_P5_VARIABLE_NAMES_20260206*](#)

[*M3_P5_DOCUMENTATION_OF_PROCEDURES_20260206*](#)

During the Psychophysiology Emotional Response Task we collected facial electromyography, electrodermal activity, respiration, and electrocardiography data. During the fMRI Emotional Response Task we collected electrodermal activity, respiration, and pulse oximetry. For more information about raw psychophysiology data see [*M3_P5_RESTRICTED_ACCESS_20260106*](#).

Differences Between MIDUS Waves

MIDUS 2 vs MIDUS 3

The hearing test as well as the collection of electrocardiography and electromyography of the zygomaticus major were not started until after MIDUS 2. Therefore, measures from the hearing test, zygomaticus EMG and heart rate variability are only available in MIDUS Refresher 1 and MIDUS 3.

MIDUS Refresher 1 vs MIDUS 3

Part way through MIDUS 3, two additional frequencies (2000 and 4000Hz) that were not collected during MIDUS Refresher 1 were added to the hearing test.

For the MIDUS Refresher 1 sample, there was a variable timing delay (mean ~62 ms) between when the startle probe was intended to be presented and when it was actually presented due to computer software/hardware changes. Data processing procedures were adapted to account for this timing delay when processing EBR data from the MIDUS Refresher 1 sample. No such delay existed for data collected in the MIDUS 2 sample nor the MIDUS 3 sample.

Additionally, the summary measures of heart rate variability were calculated using different processing methods for MIDUS Refresher 1 and MIDUS 3. Please see below for more information on heart rate variability processing for MIDUS 3.

HEARING TEST

Hearing Test **[C5O]**: Tones of various frequencies (250, 500, 1000, 2000, 4000 Hz) were played for participants in one ear at a time. Participants indicated when they were able to hear a tone. Data represents the lowest decibel level at which participants were able to hear a tone at a particular frequency in each ear. NOTE: Frequencies 2000 and 4000 were added part way through the study so they are not available for all participants.

FACIAL ELECTROMYOGRAPHY (EMG)

An experimenter placed electrodes on the seated participant's face. Electrodes were placed on either the left or right side of the face for each EMG measure (eyeblink startle reflex, corrugator supercilii, and zygomaticus major) following a predetermined counterbalance system, and then the participant was escorted into an electrically shielded booth to complete the computerized Psychophysiology Emotional Response Task (for description of task please see

M3_P5_DOCUMENTATION_OF_BEHAVIORAL_COGNITIVE_20260206). Participants were instructed to remain seated and face forward with both feet flat on the floor while avoiding large body and head movements. All EMG related variables were calculated from physiological recordings during the Psychophysiology Emotional Response Task.

From April 2017 until the COVID-19 pandemic shutdown in March 2020, pairs of non-disposable Ag-AgCl 4mm Touchproof shielded electrodes were used. Starting in October 2020, pairs of disposable cloth facial electrodes Biopac EL513 with a 10 mm contact area on 2 cm x 2 cm backing were used. The electrode front had a standard snap for unshielded Biopac LEAD110 1m TP snap connection. The electrode back had conductive adhesive solid gel. Variables [**C5PDATE_YR**] and [**C5PDATE_MO**] can be used to differentiate when a participant had the disposable sensors applied.

Participants may have worn a disposable face mask depending on their comfort-level within the data collection booth. Face masks did not affect data quality. A computer located outside the booth recorded psychophysiological data. This portion of data collection took place in the Waisman Brain Imaging Core, located in the Waisman Center on the UW-Madison campus.

BIOPAC recording:

Raw EMG signals were amplified 5,000 times prior to digitization at 1000 Hz with 16-bit precision using Biopac AcqKnowledge software. The BIOPAC MP150 hardware system (ERS100C amplifiers) was used from April 2017-October 2017, then updated to the BIOPAC MP160 hardware system (EMG 100C amplifiers) in November 2017 (BIOPAC systems, Inc., Goleta, CA).

Eyeblink Startle Reflex (EBR)

A pair of electrodes were placed below one eye on the inferior orbicularis oculi muscle to measure blink magnitude in response to acoustic startle probes (50ms duration at 105 dB) presented at one of three possible timings: 2.9 seconds after picture onset, 0.4 s after picture offset or 1.9 s after picture offset (pictures are presented for 4 seconds). For an introduction to EBR methods please see Blumenthal and colleagues (2005) guidelines paper and/or "The Skeletomotor System: Surface Electromyography" chapter in the *Handbook of Psychophysiology* (Tassinary & Cacioppo, 2000; Tassinary et al., 2007). For research on emotion and attentional modulation of EBR see Bradley et al. (2006).

Startle processing/scoring:

Processing with Matlab included 30 Hz highpass filtering, rectification and integration with a time constant of 20 ms. Eyeblink reflex magnitudes (in microvolts) were calculated by subtracting the amount of integrated EMG at reflex onset from that at peak amplitude (maximum amount of integrated EMG between 20 and 120 ms following probe onset). Trials with no perceptible eyeblink reflex were assigned a magnitude of zero and included in analysis. Trials scored as "bad" (a blink happening earlier than 20ms or later than 120 ms, or deemed as just noise) are not included in analysis. Eyeblink reflex magnitudes were log-transformed to normalize the data, then z-scored to range-correct the data separately for each participant. Eyeblink reflex amplitudes were calculated similarly, except trials with no perceptible eyeblink reflex were excluded from the analysis. Summary variables are separated by trial type (valence type and startle probe time). Trial-by-trial data of all startle responses is available via restricted access (see *M3_P5_RESTRICTED_ACCESS_20260106*).

Data quality filter variable:

A filter variable is provided indicating the quality of the EBR measures [**C5BF**]. Poor-quality data may have included issues during acquisition, so it is recommended to exclude the poor-quality data if at all possible given the sample. Another variable is provided including the number of valid eyeblink responses across the entire session [**C5B**]. There were a total possible of 81 eyeblinks across the session (9 trials did not include a probe, or, 3 trials per valence). Therefore, it is recommended that those participants who did not provide at least 10 or more valid eyeblinks across the session be

dropped from analyses.

Corrugator supercilii (CORR)

A pair of electrodes were placed above one brow line on the corrugator supercilii muscle to measure “frowning of the brow” responses to positive, neutral, and negative pictures. For an introduction to emotion-modulation of CORR and CORR methods, please see Cacioppo et al. (1986), Larsen et al. (2003), and/or "The Skeletomotor System: Surface Electromyography" chapter in the *Handbook of Psychophysiology* (Tassinary & Cacioppo, 2000; Tassinary et al., 2007).

CORR processing:

After 60 Hz notch filtering, the data were visually inspected, and artifacts were removed from the corrugator data. A Fast Fourier Transform (FFT) was performed on all artifact-free 1s chunks of data (extracted through Hanning windows with 50% overlap) to derive estimates of spectral power density ($\mu\text{V}^2/\text{Hz}$) in the 30 – 200 Hz frequency band. These values were log-transformed to normalize the data. Corrugator activity was computed for 13 distinct epochs for each of the image valences (positive, neutral, negative). The first epoch covers a 1s pre-picture epoch that served as a baseline recording and was subtracted from corrugator activity in the subsequent 12 epochs. The baseline-corrected epoch data were then Z-scored within subject and averaged across 4-seconds creating 3 distinct blocks, in order to create a summary score of corrugator activity during the picture presentation (1-4 seconds~EARLY corrugator activity), immediately following picture offset (5-8 seconds~MIDDLE corrugator activity), and later after offset (9-12 seconds~LATE corrugator activity). Summary variables are separated by trial type (valence) and epoch timing (early, middle, late). Trial-by-trial data of all 1-second epochs per picture are available via restricted access. Some processing steps vary between summary and trial-by-trial data. The 4-second aggregates are highly correlated but may differ (see [M3_P5_RESTRICTED_ACCESS_20260106](#)).

Data quality filter variable:

A filter variable is provided indicating the quality of the corrugator measures **[C5C]**. Poor-quality corrugator data may include significant noise and artifact, so it is recommended to exclude the poor-quality data if at all possible given the sample.

Zygomaticus major (ZYGO)

A pair of electrodes were placed on one cheek along the zygomaticus major muscle to measure “smiling” responses to positive, neutral, and negative pictures. For an introduction to emotion modulation of ZYGO and ZYGO methods, please see Cacioppo et al. (1986), Larsen et al. (2003), and/or "The Skeletomotor System: Surface Electromyography" chapter in the *Handbook of Psychophysiology* (Tassinary & Cacioppo, 2000; Tassinary et al., 2007).

ZYGO processing:

After 60 Hz notch filtering, the data were visually inspected, and artifacts were removed from the zygomaticus data. A Fast Fourier Transform (FFT) was performed on all artifact-free 1s chunks of data (extracted through Hanning windows with 50% overlap) to derive estimates of spectral power density ($\mu\text{V}^2/\text{Hz}$) in the 30 – 200 Hz frequency band. These values were log-transformed to normalize the data. Zygomaticus activity was computed for 13 distinct epochs for each of the image valences (positive, neutral, negative). The first epoch covers a 1s pre-picture epoch that served as a baseline recording and was subtracted from zygomaticus activity in the subsequent 12 epochs. The baseline-corrected epoch data were then Z-scored within subject and averaged across 4-seconds creating 3 distinct blocks, in order to create a summary score of zygomaticus activity during the picture presentation (1-4 seconds~EARLY zygomaticus activity), immediately following picture offset (5-8 seconds~MIDDLE zygomaticus activity), and later after offset (9-12 seconds~LATE zygomaticus activity). Summary variables are separated by trial type (valence) and epoch timing (early, middle, late). Trial-by-trial data

of all 1-second epochs per picture are available via restricted access. Some processing steps vary between summary and trial-by-trial data. The 4-second aggregates are highly correlated but may differ (see *M3_P5_RESTRICTED_ACCESS_20260106*).

Data quality filter variable:

A filter variable is provided indicating the quality of the zygomaticus measures [**C5L**]. Poor-quality zygomaticus data may include significant noise and artifact, so it is recommended to exclude the poor-quality data if at all possible given the sample.

HEART RATE VARIABILITY (HRV)

The M3 Neuroscience Project HRV analysis outlined below for both ECG and PPG was done using NeuroKit2 (Makowski, 2021; Makowski et al., 2021), which differs from previously shared MR1 Neuroscience Project HRV analyses which was done using QRSTool and CMetX.

Electrocardiogram (ECG) in psychophysiology session:

An experimenter placed a pair of EL503 electrodes 2 inches below the right collarbone and between the hip and rib cage on the participant's left side. BIOPAC GEL100 was applied to the electrodes used to measure the electrical activity of the participant's heart. The participant was escorted into an electrically shielded booth to complete the computerized Psychophysiology Emotional Response Task. An initial five-minute baseline recording obtained prior to starting the task was used to compute ECG HRV measures. During the entire procedure participants were instructed to remain seated and face forward with both feet flat on the floor while avoiding large body and head movements. A computer located outside the booth recorded data. This portion of the study took place in the Waisman Brain Imaging Core, located in the Waisman Center on the UW-Madison campus.

BIOPAC recording:

Raw ECG signals were amplified 5,000 times prior to digitization at 1000 Hz with 16-bit precision using Biopac AcqKnowledge software. The BIOPAC MP150 hardware system (ERS100C amplifiers) was used from April 2017-October 2017, then updated to the BIOPAC MP160 hardware system (ECG100C amplifiers) in November 2017 (BIOPAC systems, Inc., Goleta, CA).

Processing:

Electrocardiogram data was processed using a customized pipeline in NeuroKit2, an open-source Python package (Makowski, 2021; Makowski et al., 2021). Data was passed through a 60 Hz notch filter, then passed through a second order Butterworth filter with a .5-40 Hz bandpass. R-peaks from the ECG signal were initially found via NeuroKit2's default method and then run through a peak correction using minimum (typically .5 seconds, 120 BPM) and maximum (typically 1.3 seconds, approx. 46 BPM) interbeat interval parameters check and correct for ectopic or skipped beats. All data was visually inspected and sections of poor-quality data were removed. Poor-quality data included non-physiological artifact or signal loss that lasted for greater than 5 consecutive seconds or that could not be corrected via interpolation. Ectopic beats that could not be corrected via interpolation were also removed.

Data quality filter variable:

A filter variable is provided indicating the quality of the ECG HRV measures [**C5V1Q**]. Data quality was deemed unusable if it included significant noise or equipment error during acquisition and should be excluded from analysis. Participants with large amounts of signal removed [**C5V1M**] should be interpreted carefully following guidelines from Society of Psychophysiological Research (Quigley et al., 2024). Participants who had beats removed [**C5V1B**] had their non-linear stats run on only the longest consecutive stretch of beats [**C5V1N**].

Analysis:

Timestamps of R-peaks were generated by NeuroKit2 (Makowski, 2021; Makowski et al., 2021) after cleaning the data and any beats that occurred within marked out artifact sections were not included in analysis. Frequency-domain **[C5V1F]** indices were extracted in the low-frequency (.04-.15) and high frequency (.15-.40) bands and analyzed using a Fast Fourier Transform method for spectral density estimation on data that had been interpolated at a rate of 10 Hz. Time **[C5V1T]** and nonlinear **[C5V1N]** domain indices used the original sampling rate of 1000 Hz. All stats were run using NeuroKit2 version 0.2.10 (Makowski, 2021; Makowski et al., 2021).

Photoplethysmogram (PPG) during fMRI scan session:

A BIOPAC TSD123A pulse oximeter transducer was placed on the participant's left index fingertip. This transducer connected to the BIOPAC OXY100C pulse oximeter module utilizing red and infrared light to measure the participant's pulse and blood oxygenation during the 8-minute resting state fMRI scan. Participants were instructed to remain still and relaxed while looking at a fixation cross. A computer located in a booth outside the scanner room recorded data. This portion of the study took place in the Waisman Brain Imaging Core, located in the Waisman Center on the UW-Madison campus.

BIOPAC recording:

The PPG light signals were transmitted and filtered through the OXY100C module set to "normal". The signals were then recorded using AcqKnowledge software and BIOPAC hardware MP150 system from April 2017-July 2021 and BIOPAC hardware MP160 system from August 2021-May 2022 (BIOPAC Systems, Inc., Goleta, CA).

Processing:

Photoplethysmography (PPG) data was processed using a customized pipeline in NeuroKit2 (version 0.2.10), an open-source Python package (Makowski, 2021; Makowski et al., 2021). Data was passed through a 60 Hz notch filter, then passed through a second order Butterworth filter with a .5-40 Hz bandpass. R-peaks from the PPG signal were initially found via NeuroKit2's default method, modeled after methods in Elgendi et al. (2013) and then run through a peak correction using minimum (typically .5 seconds, 120 BPM) and maximum (typically 1.3 seconds, approx. 46 BPM) interbeat interval parameters to check and correct for ectopic or skipped beats. All data was visually inspected and sections of poor-quality data were removed. Poor-quality data included non-physiological artifact or signal loss that lasted for greater than 5 consecutive seconds or that could not be corrected via interpolation. Ectopic beats that could not be corrected via interpolation were also removed.

Data quality filter variable:

A filter variable is provided indicating the quality of the PPG HRV measures **[C5V2Q]**. Data quality was deemed unusable if it included significant noise or equipment error during acquisition and should be excluded from analysis. Participants with large amounts of signal removed **[C5V2M]** should be interpreted carefully following guidelines from Society of Psychophysiological Research (Quigley et al., 2024). Participants who had beats removed **[C5V2B]** had their non-linear stats run on only the longest consecutive stretch of beats **[C5V2N]**.

Analysis:

Timestamps of PPG peaks were generated by NeuroKit2 (Makowski, 2021; Makowski et al., 2021) after cleaning the data and any beats that occurred within marked out artifact sections were not included in analysis. Frequency-domain **[C5V2F]** indices were extracted in the low-frequency (.04-.15) and high frequency (.15-.40) bands and analyzed using a Fast Fourier Transform method for spectral density estimation on data that had been interpolated at a rate of 10 Hz. Time **[C5V2T]** and nonlinear **[C5V2N]** domain indices used the original sampling rate of 1000 Hz. All stats were run using NeuroKit2 version 0.2.10 (Makowski, 2021; Makowski et al., 2021).

REFERENCES

- Blumenthal, T.D., Cuthbert, B.N., Filion, D.L., Hackley, S., Lipp, O.V., & Van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, 42, 1-15.
- Bradley, M. M., Codispoti, M., & Lang, P. J. (2006). A multi-process account of startle modulation during affective perception. *Psychophysiology*, 43(5), 486–497. doi: 10.1111/j.1469-8986.2006.00412.x
- Cacioppo, J.T., Petty, R.E., Losch, M.E., Kim, H.S. (1986). Electromyographic activity over facial muscle regions can differentiate the valence and intensity of affective reactions. *Journal of Personality and Social Psychology*, 50(2), 260–8.
- Elgendi, M., Norton, I., Brearley, M., Abbott, D., & Schuurmans, D. (2013). Systolic peak detection in acceleration photoplethysmograms measured from emergency responders in tropical conditions. *PloS one*, 8(10), e76585. <https://doi.org/10.1371/journal.pone.0076585>
- Larsen, J. T., Norris, C. J., & Cacioppo, J. T. (2003). Effects of positive and negative affect on electromyographic activity over zygomaticus major and corrugator supercilii. *Psychophysiology*, 40(5), 776–785. doi: 10.1111/1469-8986.00078
- Makowski, D. (2021). *Neurophysiological Data Analysis with NeuroKit2*. NeuroKit. <https://neuropsychology.github.io/NeuroKit/>
- Makowski, D., Pham, T., Lau, Z. J., Brammer, J. C., Lespinasse, F., Pham, H., Schölzel, C., & Chen, S. A. (2021). NeuroKit2: A Python toolbox for neurophysiological signal processing. *Behavior Research Methods*, 53(4), 1689-1696. <https://doi.org/10.3758/s13428-020-01516-y>
- Quigley, K. S., Gianaros, P. J., Norman, G. J., Jennings, J. R., Berntson, G. G., & Geus, E. J. C. de. (2024). Publication guidelines for human heart rate and heart rate variability studies in psychophysiology—Part 1: Physiological underpinnings and foundations of measurement. *Psychophysiology*, 61(9), e14604–e14604. doi: 10.1111/psyp.14604
- Tassinary, L. G., & Cacioppo, J. T. (2000). The Skeletomotor System: Surface Electromyography. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of Psychophysiology* (2nd ed., pp. 163–199). Cambridge University Press.
- Tassinary, L. G., Cacioppo, J. T., & Vanman, E. J. (2007). The Skeletomotor System: Surface Electromyography. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of Psychophysiology* (3rd ed., pp. 267–299). Cambridge University Press.