Methods: Electrophysiological, eye tracking, physiology

Dr. Caitlin Hudac University of South Carolina

PSYC 888– Affective (Cognitive) Neuroscience Spring 2023



Overview

- Course updates
 - Short essay open: due in 1
 week = 2/27
- Class next week @ IMB meet in large conference room 125
 - Front door will be open
 - Free parking!



Overview

- Emotion and arousal
- Physiological responses
 - Respiratory sinus arrhythmia (RSA; e.g., cardiac::breathing, vagal tone)
 - Electrodermal activity (EDA; e.g., skin conductance)
- Eye tracking
 - Pupillometry
 - Area of focus

Emotion and arousal



Emotion and arousal



https://www.youtube.com/watch?v=2xwalfxKl8E

Emotion and arousal



Emotion and arousal (and attention)





RSA: Heart rhythm altered based upon stimulation from the vagus nerve



Normal Breathing Pattern in Time





Cardiac signals (heart rate variability)

Time domainMean RRaverage of pulse peak intervalsSDRRStandard deviation of the pulse intervalsCVRRCoefficient of variance of the pulse peak intervals (ratio of the standard deviation and the mean of the intervals)

Frequency domain

HF	0.15 - 0.4 Hz: Respiratory sinus arrhythmia
\mathbf{LF}	0.04 - $0.15~\mathrm{Hz}:$ Oscillation of the baroreflex
LHratio	Ratio of low and high frequency

<u>828</u><u>845</u><u>754</u><u>742</u>

Statistical analysis

Kurto	Shapes of the probability distributions
Skew	Amount of asymmetry in a data set probability distribution
Entropy	Randomness of the data

Table 2Features to calculate from HRV for emotion classification [33].



- Respiratory sinus arrhythmia (RSA)
 - Capacity for flexible, regulated affective reactivity
 - Low baselines found in many clinical disorders



Fortunato et al. 2013

Methods: During the emotion induction task, cardiac measures were collected continuously at 500 Hz via the Biolab 2.4 acquisition system (Mindware, Westerville, OH). Three disposable, pre-gelled cardiac electrodes were placed over the child's distal right collar bone, lower left rib, and lower right rib. The ECG data were visually inspected and corrected as necessary by a trained research assistant. Because the validity of RSA may be compromised if participants' respiration frequency exceeds a normal range, respiration was estimated from the impedance wave (measured with an additional four cardiac electrodes) in order to verify that the respiratory frequency within each epoch was within the 0.12–0.40 Hz range established for RSA calculation (Allen, Chambers, & Towers, 2007; Berntson, Quigley, & Lozano, 2007). A peak respiration frequency outside this range resulted in elimination of that epoch from analysis (<1 % of available epochs were removed).</p>



Fortunato et al. 2013

- <u>Methods (cont)</u>. For all viable epochs, RSA scores were derived through **spectral analysis** (fast Fourier transform) of the interbeat interval time series obtained from the ECG following procedures specified by Berntson et al. (1997).
- RSA was calculated in **30-s epochs across the task**, resulting in a total of 27 epochs, including baseline and the four emotion-inducing conditions. When repeated across multiple epochs, 30 s is an appropriate length of time for valid RSA extraction, allowing for maximum sensitivity of the RSA calculation to dynamic changes across the task (Berntson et al., 1997). Epoch 1 reflects the initial (pretask) baseline, Epochs 2 through 9 reflect the fear condition, Epochs 10 through 14 reflect the sadness condition, Epochs 15 through 21 reflect the happiness condition, and Epochs 22 through 27 reflect the anger condition. Each emotion condition included the fixation baseline epoch that preceded it and the neutral film clip epoch that followed it.

Duration of the epoch



Fortunato et al. 2013

Presentation order of

<u>Analytic plan</u>. *Multiphase latent basis growth curves* (see Ram & Grimm, 2007) were used to model nonlinear changes in RSA over the course of the emotion induction task. In brief, this model provides an alternative representation of the change trajectories often modeled via polynomial models (e.g., quadratic or cubic) and is particularly useful for representing complex-shaped trajectories in a parsimonious manner (McArdle & Epstein, 1987; Meredith & Tisak, 1990). Specifically, the conditional five-factor latent-basis growth curve model shown in Fig. 1 was used to model systematic nonlinear changes in RSA across the 27 epochs that were associated with the baseline, fear, sadness, happiness, and anger conditions and how between-person differences in baseline levels and in the extents of change in RSA during the four emotion-inducing conditions were related to differences in internalizing and externalizing problems.



EDA: Arousal related to thermoregulation (e.g., sweat)

(B) Activity covarying with EDA

Right anterior insula / orbitofrontal cortex



Critchley, H. D. (2002). Electrodermal responses: what happens in the brain. The Neuroscientist, 8(2), 132-142.



- **Methods**: Skin conductance data were acquired using an MRI-compatible system system [^{17,20}], from **left hand digits II/III** with **Ag/AGCI electrodes** and 0.05 sodium chloride gel. Data did not require filtering or smoothing.
- The presence of a phasic skin conductance response to individual stimuli was determined by an unambiguous increase (>0.05 μS), **1–3 s post-stimulus**. Software based on a sigmoidexponent model [²¹] enabled quantification of frequency, peak amplitude and latency, rise time and recovery time [²¹]. χ² was used to analyse frequency, and ANOVA with paired t test contrasts, the four other parameters.



Williams et al. 2005, BOLD, sweat and fears: fMRI and skin conductance distinguish facial fear signals. Neuroreport, 16(1), 49-52.

Skin conductance results: Figure 1a,b depicts the skin conductance parameters for fear, anger and disgust. Frequency did not differ across emotions (X² 2.3.84, p.0.15), but there was a difference of borderline significance for amplitude (F(2,36).3.20, p.0.05), due to greater responses for fear compared to anger and disgust (p00.05; Fig. 1a). There was a highly significant effect for rise time (F(2,36).60.24, p00.0001); due to rise time being the fastest for anger and the slowest for disgust, with fear in between (Fig. 1a), and producing significant pair-wise contrasts (p00.0001). A significant difference in latency (F(2,36)¼39.71, p00.0001) was due to slower responses for disgust relative to fear and anger (p00.0001), whereas the significant effect for recovery time (F(2,36)¼9.167, p¼0.001) was due to prolonged recovery for anger compared to fear and disgust (p00.01; Fig. 1b).



Fig. I. Mean $(\pm s.e.)$ for SCR peak amplitude and rise time (a) and peak latency and recovery time (b) for perception of fear, anger and disgust.

Williams et al. 2005, BOLD, sweat and fears: fMRI and skin conductance distinguish facial fear signals. Neuroreport, 16(1), 49-52.

Questions? Chat?

ET: Measurement of eye movements and pupil shape change using infrared light





- (1) **Pupil**: Computer finds the pupil, tracks how its shape changes
- (2) Corneal reflection: Sends infrared light, tracks when it rebounds













Corneal Reflection



Α	Calibration	В	Calibratio	n				
	Accept Calibration		X	Accept Calibration				
	Left Eye Right Eye			Left Eye			Right Eye	
				N			· · · · · · · · · · · · · · · · · · ·	
	1 1						e	
					۰	•	•	
0 Points Marked for Recalibration Check Calibration				0 Points Marked for Recalibration				
							Check Calibration	
	Recalibrate Accept Cancel					Recalibrate	Accept Cancel	

Eye tracking – Endless DV options

- Fixations
 - Duration/dwell time
 - Location
 - Quantity (#Fix)
 - Time to first fix
 - #Visits
- Saccades
 - Amplitude
 - Latency
 - Velocity
 - Direction

- Area explored
- Visual trajectories



Eye tracking – Pupillometry

- Change pupil shape & size, due to changes in..
 - Luminance
 - Arousal
 - Cognitive or affective state
- Latency ~200 ms
- Fit stimuli within foveal vision









Eye

0

Mouth

Feature at central fixation

 Methods: Participants sat in a comfortable chair approximately 60 cm from a 23-inch computer screen (1920~ 1080 pixels). The height of the chair and screen were adjusted to ensure that participants' eyes were level with the center of the screen. Eye-tracking data were collected using a 30 Hz Tobii X2 eye tracker mounted below the screen. iMotions Biometric Research Platform (https ://imotions.com/) was used for stimuli presentation, data synchronization, and automatic calibration. Participants could freely observe presented stimuli. Before each experimental period, a fivepoint calibration procedure consisting of animated cartoon characters paired with an auditory cue was performed.

Distance, visual angle Sampling rate Set-up constraints (e.g., headrest) Calib, Validation?



Mutual gaze



Methods (cont): Standard region-of-interest (ROI) analysis techniques were adapted for the analysis of gaze patterns (Fig. 1). The examined ROIs included the shared Activity area, the Bodies, and Heads of the two actors in a video, and the remaining Background.





Mutual gaze



Kaliukhovich et al 2020

Overview

- EEG methodology
- Event-related potentials outcomes
- Spectral analysis outcomes

Advantages of EEG

- Correlation with cognitive & physiological events
- Time resolution (ms)
- Spatial resolution
- Portability
- No age limits
- Useful with or without behavioral response
- Cost



History of electroencephalography (EEG)







1950











Tra

The EEG signal

- The EEG signal: a summation of electrical fields generated by large neural populations
- Neuronal activity can be thought of as many small oscillators
- Activity from different frequencies and in different polarities will cancel out.



Decompose the EEG signal into different frequencies will reflect the synchronized activity in each frequency
Dipoles

- 1. Dipoles perpendicular to surface (since cortex folds, not necessarily perpendicular to scalp surface).
- 2. Reflects differences in soma and dendrite ion flow across cortical layers.
- 3. Activity at scalp not necessarily result of ion movements immediately below electrode.

Caution: Dipoles generated in one hemisphere may generate higher shifts in other hemisphere.



Dipole



EEG is inherently dynamic

- Signal will naturally fluctuate and evolve at every data point
- We can record at every millisecond at every channel
 - High-density nets (>32 channels)





Back of Head

ERP to speech syllable "ba" Neonate Adult



ERPs to CVC Words



Molfese & Erwin, 1981



Oscillations in frequency range 0.2



Advantage: Broad "feeling", "thinking"

0

-0.2

-0.4

-0.6

Baseline cond

Target cond

- Ongoing EEG signal; Focus on "overall" information
- Can be task related (e.g., eyes open, eyes closed, watching X kind of video)
- But not necessarily task-locked



Advantage: Discrete cognitive processes

- Portion of Ongoing EEG
- Time-locked to stimulus onset
- · Focus on temporal and spatial information
- Comparability across the lifespan

Event-Related Potential (ERP) Technique

Raw EEG

Mummun







ERPs are not everything...

Single trials



ERPs are not everything...

What we lose:

- Non event-related activity (e.g., gustatory)
- 8 Event-related activity that is not phase-locked
- 8 Averaging has a "smoothing" effect removes highfrequency information
- 8 ERPs are a summation of activity in all frequencies, and do not show modulation of individual frequencies

What info do we collect?



Time



- Person-specific characteristics
 - Demographics: Age, gender, SES, etc.
 - Constructs: Reading ability, temperament, emotion regulation, social responsiveness, IQ, etc.

Interpreting ERP *components*

- Time-locked to an evoking or eliciting event or stimulus.
- Sequence of serially activated "processes" (components) detected at the scalp (or some biological surface) as distinct positive-negative fluctuations.

Measures:

- (1) peak latency from evoking stimulus onset (ms)
- (2) peak amplitude in microvolts μV
- (3) polarity (deflection from baseline to + or -)

Interpreting ERP *components*

Event Related Potential (ERP)



Interpreting ERP *components*

- Peaks (positive or negative)
- Latency (post stimulus onset)
- Duration (e.g., slow wave)
- Scalp topography (maximal peak location)
- Source (location within the brain)

Remember that:

- Current flow across the scalp
- Produces latency shifts from one part of scalp to another
- Also produces amplitude shifts across scalp
- Signals sum across the scalp
 - large positive wave on scalp meeting large negative wave could sum to flat line!



P1 / P50 / P70

- Peak amplitude and latency decrease with age (disappear, even)
- P50: Auditory inhibition → Sensory gating: 2 clicks presented quickly. 1st amplitude < 2nd amplitude. Reduced suppression in schizophrenia, neurodegenerative diseases.
- P1: Use pattern reversal (e.g. flickering checkerboard) task. Largest over occipital regions. May relate to attention / arousal.



N1 / N100

- Selective attention to basic stimulus characteristics (necessary for later pattern recognition and discrimination processing).
- Auditory stimuli \rightarrow larger N1 with shorter latency than visual stimuli (Hugdahl, 1995).
- Amplitude is larger in discrimination tasks, but smaller (if it exists at all) if short interstimulus intervals (ISIs).



P2/ P200

- Low inter-individual variability and high replicability.
- Often occurs together with the N1, yet peaks can be dissociated.
- Can be double-peaked.
- Amplitude increases with complexity of stimuli.



N2/ N200

- Like Mismatch-negativity (e.g. Oddball paradigm), detection of deviation of subject's expectation but N2 – only if subject is paying attention to stimuli. MMN – requires no attention!
- Interpretations: Orienting response, stimulus discrimination, target selection, response inhibition (e.g. Go-NoGo)
- N2 smaller in amplitude and shorter in latency for shorter ISIs.

Time: Components



N170

- Human face-processing (face vs. natural or human-made objects)
- Turns out, not specific to faces but to expert object recognition! (Tanaka & Curran, 2001)

Time: Components



P3a/ P300a

- Orienting reflex
- Occurs when not required to actively respond to the targets
- Involuntary attention as well as inhibition (e.g. NoGo > Go)

P3b/ P300b

- Controlled processing
- Subject must pay attention and respond to stimuli. Also fewer targets → larger peak.
- Variability: Amplitude (attention, stimulus relevance). Latency: stimulus complexity

Time: Components

300

MSEC

400

500

N4 / N400

• Larger for unexpected, low probability (e.g. sentence endings):

200

• It was the first day at work.

0

• He spread the warm bread with socks.

100

- Amplitude: Incongruent > Congruent
- Modality: Earlier in visual than auditory in temporal & frontal electrode sites.



Late positive component (also: late slow wave LSW; late positive potential, LPP)

• Explicit and implicit recognition memory: (e.g. "Old/new" effect)

ERP methods & considerations

- Review the participant characterization per usual
- Review experimental design
 - What is the stimulus "time-lock"?
 - Length of the ERP epoch
 - Was a baseline period included?
 - ERP epoch length relative to stimulus duration
- EEG acquisition protocol device used:
 - Wet vs. dry net
 - Number of recording channels & number within ERP

ERP methods & considerations

Acquisition protocol

EEG was recorded from 32 AgAgCl active electrodes (BrainProducts GmbH, Gilching, Germany) placed into an elastic EEG cap at the following positions of the 10–20 system80: F5, F3, FT7, FC5, FC3, T7, C5, C3, CP3, CPP5H, P7, P5, P3, F4, F6, FC4, FC6, FT8, C4, C6, T8, CP4, CPP6H, P4, P6, P8, Fz, Pz, and Cz (cf. Figure 7). Vertical and horizontal electrooculogram were recorded above and next to the right eye with electrodes FP2 and F10. An electrode (TP9) at the left mastoid served as online reference, while an electrode at the right mastoid (TP10) was recorded for further re-referencing during offline analyses. Position AFz served as ground electrode. Electrode impedance was kept below 10 kΩ (actiCAP Control, Brain Products GmbH, Gilching, Germany). The EEG signal was measured by means of BrainVision Recorder (Brain Products GmbH, Gilching, Germany) software with a sampling frequency of **1000** Hz (amplified between 0.016–450 Hz) and filtered before digitalization by means of the analog/digital converter with an upper cut-off of 450 Hz (24 db/ oct) to prevent aliasing.

Reference electrode

Sampling rate

Steber et al., 2020



	ERP processing	EEG	ERP
Filter	Fix high/low frequencies	\bigcirc	\checkmark
Segmentation	Need to chop up EEG into epochs/segments/trials		\checkmark
Artifact detection & rejection	Fix blinks, muscle movements, etc.	\checkmark	\checkmark
Baseline correction	ERP signal "level" may vary for each segment	\mathbf{O}	\checkmark
Bad channel correction	Channels with poor signal (e.g impedance, bad electrode)	\checkmark	\checkmark
Averaging	Re-reference; combine trials	\bigcirc	\checkmark

ERP methods & considerations

• ERP processing

EEG data. EEG data was filtered offline with a **30 Hz low pass Butterworth zero phase filter** (high cutoff: 30 Hz; slope: 12 dB/oct). Data was then segmented from -200 ms to 1500 ms with 0 ms representing the time point of the pseudoword onset. An ocular correction based on the Gratton & Coles algorithm was applied to correct vertical eye movement artifacts. Overly contaminated channels were rejected manually from each segment by inspecting each segment visually for artifacts. Only subjects in whom at least 2/3 of all segments per condition (angry vs. happy vs. neutral) in at least 15 of all 29 scalp electrodes survived this procedure were included in the final analyses. This criterion applied to 48 of all 50 subjects. In the next steps, data was re-referenced to averaged mastoids (TP9, TP10) and a pre-stimulus baseline of 200 ms was applied. Event-related brain potentials (ERPs) were extracted by averaging the segments for each subject and each condition (angry prosody, happy prosody, neutral prosody). In addition, a 50-msanalysis was performed in order to select the time windows for final statistical analyses. This analysis included ANOVAs on each electrode in consecutive 50 ms steps between 100 and 1500 ms with the factor Condition for experimental halves separately. We decided to include the factor halves into all statistical analyses, as we wanted to control our results for potential habituation/repetition effects over the course of the experiment. Results from the 50-ms-analysis as well as visual inspection of the grand averages revealed 100-150 ms, 250-350 ms, 500-550 ms, 600-700 ms, and 700–900 ms to be the time windows indicating differences between conditions, which were therefore were selected to perform further statistical analyses on.

Time window	Electrodes for
for ERP	analysis

Steber et al., 2020

Properties of oscillations

Oscillation: Periodic modulation of voltage over time



Properties of oscillations



Power=Amplitude² [μν²]

Frequency decomposition

- The "raw" signal (EEG/ERP): Voltage modulations over time
- Assumption: The signal is a linear combination of activity in different frequencies
- The goal: To see how activity in different frequencies changes over time



Interpreting time-frequency plots

What does enhanced amplitude mean?

- Larger degree of synchronization within a given set of neurons
- Larger area of synchronized neurons





Integrating information in different senses, perceptual integration of different features,

Active concentration

Relaxed, inhibition of control, closing eyes decrease correlated with increase in attention

Active inhibition

Adult slow-wave sleep, continuous attention

Delta: 0-4 Hz

Ongoing EEG data -- Spectral components



"EEG" methods & considerations

- Review the participant characterization per usual
- Review experimental design
 - Length of the EEG epoch?
 - Epoch length relative to stimulus duration
- EEG acquisition protocol device used:
 - Wet vs. dry net
 - Number of recording channels & number within each epoch

Spectral methods & considerations

Acquisition protocol

Brain activities were recorded by an EEG (Mitsar Co., Ltd., Saint Petersburg, Russia). The device includes **19 main electrodes (Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, P3, P4, Pz, T3, T4, T7, T8, O1, and O2), two reference electrodes (A1 and A2)**, and a ground electrode (Fpz), according to the 10–20 system of electrode placement. The data were collected using a **sampling rate of 250 Hz** and filtered in WINEEG software with a frequency band of 1 to 25 Hz with a notch filter of 45–55 Hz. Linked Ear references were used with all EEG. The electrolytic gel was applied and each site gently abraded until impedances were below 10 kOhm. Eyes-closed and eyes open conditions were used for recording signals that were 3 min each in duration. During the eyes-closed condition, we instructed the participants to place their hands on their knees, half-open their mouths, and avoid blinking or opening the eyes. The eyes-open condition had similar instructions except that we requested them to additionally fixate their eyes on a central point.

Reference Sampling rate

Mosirian Farahi et al., 2019

"EEG" processing for spectral analysis EEG ERP

Filter	Fix high/low frequencies	
Segmentation	Need to chop up EEG into epochs/segments/trials	
Artifact detection & rejection	Fix blinks, muscle movements, etc.	\checkmark
Baseline correction	ERP signal "level" may vary for each segment	
Bad channel correction	Channels with poor signal (e.g impedance, bad electrode)	
Averaging	Re-reference; combine trials	
Spectral methods & considerations

EEG Protocol

After recording the signals, the data were saved in EDFC format in WINEEG and opened in Neuroguide software. The artifacts were rejected by automatic rejection method. The criteria of automatic rejection included drowsiness, eyemovement, and muscle with a high sensitivity. After that, a **1-s at a 250 sample rate, artifact-free epochs** with a Hanning window (50% overlapping) was extracted through Neuroguide software and submitted to the Fast Fourier Transform (FFT; the resolution was 1 Hz). To address the aims of the study, **frontal alpha (8–12 Hz) asymmetry** indices were calculated by computing asymmetry scores (**log [left]–log [right]**) for **mid-frontal (F3–F4**), frontal pole (Fp1–Fp2), and lateral frontal (F7–F8). Positive scores indicate greater alpha power at left compared to right frontal electrode sites, and therefore greater relative right-sided frontal activity. According to reliability of EEG recording, a split test was conducted. The split test showed that the reliability is 0.96 (SD: 0.02). The mean of alpha across the scalp in the eyes-closed condition was 64.52 (median: 61.30, SD: 31.72). To reduce artifacts in our data, eyes-closed condition was applied for analyzing data.

Frequency window E for spectral band

Electrodes for analysis

Mosirian Farahi et al., 2019

Questions?