Topographical Analysis of Somatosensory Evoked Potentials Recorded from Premature Infant Cortex in Response to Painful Stimulation

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Abstract

Recent advances, from both animal and human studies, have increased our understanding of the immediate effects of pain during infancy. To better understand these effects, the Fitzgerald group has extensively collected and analyzed EEG human infant data where the noxious stimulation was a brief, discrete event that occurred when the infant’s heel was lanced for the clinical purpose of blood extraction. The Fitzgerald pain group has shown that human infants are able to experience pain in the cerebral cortex and even demonstrate certain pain-specific behaviours.

In this project we are interested in whether lancing the right heel of the infant, a noxious stimulation, is producing a left-hemispheric response signal and vice versa. This kind of response is what would be expected for a human adult. We also consider whether there is a tendency for signal responses to be larger on one of the brain hemispheres. For this purpose, a statistical analysis was implemented and a mathematical model for the EEG traces is described.
## Contents

1. Introduction
   a. Project Aim................................................................................................................4
   b. Motivation ..................................................................................................................4
   c. Current Status of Pain Processing in Neonates ......................................................4
   d. The Fitzgerald Pain Research Group .......................................................................6
      i. NIRS studies .......................................................................................................6
      ii. EEG studies ......................................................................................................7
   e. Cortical Lateralization in Infants ...........................................................................7
   f. Data Analysed ...........................................................................................................7

2. Event-Related Potentials Analysis
   a. EEG Traces from Neonates ....................................................................................8
   b. Artifacts .....................................................................................................................8
   c. Signal Filtering .........................................................................................................9
   d. Analysis of the Fitzgerald Data .............................................................................9

3. Methods
   a. Scope .......................................................................................................................10
   b. Model .......................................................................................................................10
   c. Principal Component Analysis .............................................................................10
   d. Singular Value Decomposition ............................................................................13
   e. Implementation ........................................................................................................15
   f. Limitations ...............................................................................................................16

4. Analysis of The Infant Data
   a. 1st PCs Analysis .....................................................................................................16
   b. Eigenvector Analysis .............................................................................................17
   c. Lateralization Test .................................................................................................19

5. Results
   a. PCA Plots .................................................................................................................26
   b. PCA Centered Plots ...............................................................................................26
   c. Statistical Interpretation .........................................................................................27
   d. Lateralization Tests Results ..................................................................................28

6. Conclusions ................................................................................................................31

Bibliography ..................................................................................................................32

Appendix

A. PCA Analysis of Full Trace EEG ..............................................................................i
B. PCA Analysis of Centred Trace EEG .....................................................................xii
C. 1st PCs Comparison Across Infants .........................................................................xxiii
D. Infant Demographics of the Sample .........................................................................xxiv
1. **Introduction**

1.1 **Project Aim**

The aim of the project is to analyse the cortical activity, evoked by painful stimuli in human infants measured by electroencephalogram (EEG) electrodes. The EEG traces generated during a time-locked heel lance, a noxious stimulus required for clinical care, represent the evoked somatosensory pain potential in infants. A statistical analysis of the field of electrical activity is to be implemented in order to simply and improve the analysis of the data. This statistical analysis will be used to derive information with respect to topography, specifically the lateralization, of the response within the infant brain.

1.2 **Motivation**

There has been a recent and most important change in the field of pain assessment: the realization that infants can experience pain [1]. Recent advances, from both animal and human studies, have increased our understanding of the immediate effects of pain during infancy. Furthermore, the effects of early exposure to pain in developing rodents and other species have been shown to cause alterations in the fully developed adult nervous system [2].

The infants, however, can also suffer from adverse effects due to the high degree of uncertainty during analgesic administration. For an optimal utilization of analgesia, the risk to benefit ratio must be calculated for each individual. However there is a lack of a “gold standard” for measuring infant pain mainly because there are no known reliable and quantitative biological markers for assessing pain in infants to date. Personalized pain management is therefore extremely difficult to quantify. To practically assess if the risks and the cost are worth the benefits, it is important to understand and characterize how is it that the infant central nervous system processes pain.

1.3 **Current Status of Pain Processing in Neonates**

Because infants are unable to describe pain verbally, surrogate measures of pain processing are used in clinical settings. The current indicators of pain in human infants rely on behavioural and physiological cues, which may not correlate directly with the actual perceived pain. Especially for extremely premature infants, observed behavioural responses may not be directly proportional to the noxious sensory information that was actually transmitted to the cortex [3].

The Fitzgerald pain group has shown that human infants are able to experience pain in the cerebral cortex and even demonstrate certain pain-specific behaviours. While a noxious
stimulation in human infants causes a functional activation of the somatosensory cortex [4], it has been recently noted that abnormal or excessive neural activity due to pain during a critical developmental period may have long-term effects in the infant’s ability to process pain [5].

Nociceptive and antinociceptive neural pathways undergo substantial changes during the early neonatal period. The nervous system undergoes as well important alterations in both its structure and function in the postnatal period, depending on the underlying neural activity. This makes the young infants, especially preterm babies, vulnerable to the effects of noxious stimulations. To better understand these effects, the Fitzgerald group has extensively collected and analyzed EEG human infant data where the noxious stimulation was a brief, discrete event that occurred when the infant’s heel was lanced for the clinical purpose of blood extraction. The infant’s response in the brain area was recorded by 17 electrodes that described cortical activation as a direct response to the noxious stimulation. These responses have been distinguished by visual inspection from the rest of the diffuse sensory information with the aid of a time lock that establishes the time interval in which the response occurs. As an experimental consideration, the infant’s foot was not disturbed for a period of 30 seconds after the heel lance was administrated.

Recent reports indicate that infants receiving intensive care undergo approximately 14 painful procedures a day. The extremely premature infants that are admitted due to life threatening health problems may even go through more than 50 painful procedures in a day [6]. Even though these procedures are considered by health care specialists as painful, analgesia is provided in less than 35% of the procedures. It has also been noted that in the United Kingdom, more than 80% of neonatal units lack a pain assessment protocol. This is mainly due to a lack of information about pain in infants and its effective management. Regular methods of managing pain in neonates consist mostly of efforts aimed at comforting the infant such as swaddling, caressing, gentle touches and soft talking.

A physiological and behavioural pain assessment of neonates, the Premature Infant Pain Profile (PIPP), has tried to correlate facial expressions with a scoring of pain perceived. The PIPP score uses three facial actions based on the neonatal facial coding system (NFCS). The ability for a neonate, however, to mount a facial expression in response to a noxious stimulus requires that the motor neural activity is sufficiently mature and coordinated to produce such facial muscle contractions. The Fitzgerald group have noted that extremely premature infants may lack the ability to produce such a facial display, but still may be able to process pain in a higher cortex level [6]. This limitation of using facial expression as the only pain scoring parameter may not correlate appropriately with the magnitude of the actual cortical activation. In their study, the Fitzgerald group has found that a clear cortical response can be observed without any associated change in facial expression. Even if an infant has a zero score on the PIPP scale, he or she may still be processing pain at a cortical level.

A more careful characterization of the cortical responses in the infant’s immature brain could provide a more reliable parameter of pain assessment. This is especially important in light of the adverse effects that inappropriate pain management can cause. The use of morphine, for
example, dramatically reduces the heart rate and may cause a respiratory depression that may in turn lead to requiring artificial ventilation [7]. An inappropriate use of morphine would then lead to a retardation of the development of normal lung functions, and cause great damage to the developing infant.

1.4 The Fitzgerald Pain Research Group

The focus of the studies carried out by the Fitzgerald pain group is to determine how pain is processed at higher levels of the infant nervous system and whether neonate infants experience pain in the same way that adults do. They not only aim to improve pain measurement and treatment in this vulnerable patient population but they also wish to contribute to our basic understanding of the development of sensory processing in the human brain.

(i) NIRS Studies

While it was previously thought that in preterm infants the responses were mostly sub cortical (with functional maturation of higher brain centres being required to produce a true pain experience), the Fitzgerald group have found that, even in the youngest, pre-term infants, acute pain causes an activation of the sensory cortex. By employing Near-Infrared Spectroscopy (NIRS) techniques, the group provided evidence that noxious stimuli evoke a specific and localized hemodynamic response in the infant cortex from 25 weeks postmenstrual age [6].

Near Infrared Spectroscopy (NIRS) is a technique that enables the indirect measurement to blood and tissue oxygenation by directly measuring different changes in the concentration of oxygenated and deoxygenated haemoglobin. Because the absorption of near infrared light by a tissue is dependent on the amount of oxygenated and deoxygenated haemoglobin present, a change in intensity between emitted and absorbed light reflects the changes in tissue oxygenation. An increase in tissue oxygenation is associated with an increase in cerebral flow, which in turn describes an increase in neural activity. A near-infrared light source and a detector placed in an infant’s head allow the absolute hemodynamic parameters (blood flow, cerebral volume) to be calculated and provide an appropriate assessment of the functional activation of the brain [6].

(ii) EEG Studies

While hemodynamic measurements are helpful, they are inevitably dependent upon blood flow and oxygenation which could be problematic in the developing brain [8]. Nociceptive and antinociceptive neural pathways are known to undergo substantial changes during the early neonatal period; alterations in both the structure and function of connections take place in the postnatal period, depending on the underlying neural activity. To better understand the changing
neural activity in the infant cortex it is necessary to directly record cortical neuronal activity, and so the Fitzgerald group has recently extended its techniques to EEG recordings. EEG recordings allow the accurate measurement of event related cortical potentials in response to a time-locked noxious stimulus. Scalp EEG can measure the activity of post-synaptic currents, composed of multiple oscillations or spike waves.

A further advantage of using EEGs over NIRS is a higher temporal resolution. In EEGs changes in electrical activity can be recorded within milliseconds. Due however to poor spatial resolution, a considerable number of electrodes need to be placed on the infant’s scalp. The results of the EEGs however have yielded new valuable information. Recent analysis of this data have provided evidence that the infant cortex can discriminate between noxious and non-noxious stimulation, as the Event-Related Potentials (ERPs) recorded from the two stimuli are different in latency, amplitude and waveform profile [9].

### 1.5 Cortical Lateralization in Infants

Activation in different hemispheres of the brain in response to a stimulus is known as brain lateralization. Because neural pathways in human adults cross over to the contralateral side of the brain, the stimulus felt on one side of the body case hemispheric activation on the opposite side of the body. In human adults, a contralateral response can be observed for noxious stimuli.

Independent of the contralateral connections, these is also evidence that one side of the brain may preferentially process pain. There is evidence that in some cortical areas there may be some tendency for pain responses to be greater on the left side of the brain [10], whereas other reports claim no lateralization or right side dominance [11].

It is currently unknown whether lateralization is established before birth, or if the development of lateralized systems occurs only in postnatal life, although there is some evidence that complete lateralization is not yet accomplished by 38-49 PGA weeks [12]. Recent research suggests that cortical networks of the preterm infant are characterized by transient regional and functional organizational patterns that develop and change between 24 weeks and birth [13]. The data obtained by the Fitzgerald Pain group provides an excellent opportunity for the analysis of lateralization in infants across different ages. This analysis could provide a deeper understanding of the nature of lateralization.

### 1.6 Data Analyzed

To date the Fitzgerald group has extensively collected and analyzed EEG human infant data where the noxious stimulation was a brief, discrete event that occurred when the infant’s heel was lanced for clinical blood extraction. The infant’s cortical response was recorded by 17 electrodes that described cortical activation as a direct response to the noxious stimulation. These responses
have been distinguished by visual inspection from the rest of the diffuse sensory information with the aid of a time lock that establishes the time interval in which the response occurs. As an experimental consideration, the infant’s foot was not disturbed for a period of 30 seconds after the heel lance was administrated. The infants that have been studied by the group range between 24-42 weeks PMA (post menstrual age). It is this data which forms the basis of our statistical analysis.

2. Event-Related Potentials Analysis

2.1 EEG Traces from Neonates

An Event-Related Potential (ERP) is evoked by a particular event, and is observed as a change in the Electric potential that is clearly distinct form the background EEG activity. Traditional EEG analysis relies heavily on visual examination and quantitative description of the time series. A clinical physiologist’s visual examination of the data is carried out in a systematic manner, with the main characteristics of the EEG described mainly by the observed positive and negative peaks of the ERP waveform.

Because ERPs are composed of underlying signals that may overlap in time and space, ERP peak identification faces the difficulty of separating the source waveforms. There are an infinite number of possibilities for the combinations of source waveforms that could produce a given ERP [14]. This is known as the inverse problem. The electrode placement takes the form of a referential montage with a symmetrical distribution over the scalp, according to the International 10-20 system placement ([15]).

2.2 Artifacts

Undesirable signals in the EEG that are from non-cerebral origin are called artifacts. Artifacts that may contaminate a signal include eye blinks, eye movements, muscle and heart activity and even Alpha waves. In the case of infants, external artifacts such as the movement of the patient, the setting of the electrodes, poor grounding or even the presence of an IV drip and cause low voltage bursts. These artifacts are problem in the context of this project and present a challenge for neonatal data acquisition. Because the EEG traces display a response to a noxious stimulus, in this case a hell lance to extract blood from the infant, there are natural movements from the infant that in response to being lanced. These anxious movements make the data acquisition an even more delicate process and are a primary source for noise in the signal.
2.3 Signal Filtering

The acquired EEG signals from the infant study have been stored in a digital database with a sampling frequency of 2000 Hz. It is possible to convert a continuous analog signal into a set of discrete samples without losing any information. However, this can only be done if the rate of digitization is at least twice as high as the highest frequency in the signal that is to be digitized [14]. On the other hand, if very high frequencies are found in the original signal, those that are more than twice as high as the digitization rate, they will appear as low frequency artifacts due to the physical phenomena of aliasing.

For the purpose of noise reduction, the EEG traces are considered to consist of a signal with added noise. Some of this noise is sufficiently different in frequency from the signal that it can be suppressed by removing or attenuating such frequencies. However, as the frequency content of the noise becomes more similar to that of the signal, it becomes difficult to remove the noise without distorting the signal. Our high-pass filter of 0.5 Hz aims to remove the very slow voltage changes that have a non-cerebral origin: sweating, electrode drifts, head and body movements that are common in infant data recordings.

2.4 Analysis of the Fitzgerald Data

ERPs evoked in response to a noxious stimulation form different human infants were considered for statistical analysis. In order to determine a general structure of the response, as described by EEG traces, the following restrictions in the subject population were taken into account:

- No extremely premature infants were considered (>28 weeks PMA).
- Infants who had undergone surgery were not considered.
- Infants with administered analgesia were not considered.
- Data with trigger failure or manual artifact were not included.
- Included only subjects whose waveform was present in electrode CPz.
- Selected individuals whose maximum peak response could be centred in a ±0.35 ms interval.

Due to the nature of the data and its acquisition, all the infants had been admitted into intensive care for a particular reason. The restrictions imposed in the subject population have as an objective to derive a more homogeneous data set for a more reliable comparison across subjects. The 17 electrodes considered from the International 10-20 system placement, and their actual scalp distribution can be seen in figure (1).
3. Methods

3.1 Scope

Correlation based techniques are useful for indentifying latent ERP components. In ERP data, the variables are the DC microvolt readings across the infant’s head in a given time course. The major source of variance is considered to be the ERP components, which describe the characteristic features of the waveforms associated with a cognitive process. The waveforms are spread across multiple electrode channels, and consist of discrete measurements made across multiple time points.

Ideally, since Principal Component Analysis uses the correlation of the data set to define the set of principal components, an analysis of the statistical decomposition of the brain’s electrical patterns would correspond to a separate ERP component. In the PCA of EEG traces, different time points are considered as part of a single component as they tend to vary in a correlated manner, as would be expected for a common cognitive process.

3.2 Model

The project considers the analysis of several EEG time series measured at a number of locations in the infant’s scalp. We consider the observations to correspond to recordings at $J$ electrode locations, denoting a response per individual infant $p$ in time $t$ at location $j$ by...
\[ Y_t^{(p,j)} = Y_t^{(p,j)} + \sigma_p \epsilon_t^{(p,j)}, \quad p = 1, \ldots, P, \; j = 1, \ldots, J \]  

For the purpose of statistical analysis, we have subtracted the mean from each variable, the electrode channels. We can represent this model in the form the matrix

\[
Y(p) = \begin{pmatrix}
Y_{t1}^{(p1)} & \cdots & Y_{tn}^{(p1)} \\
\vdots & \ddots & \vdots \\
Y_{t1}^{(pj)} & \cdots & Y_{tn}^{(pj)}
\end{pmatrix},
\]

\[
Y^{(pj)} = (Y_{t1}^{(pj)} \cdots Y_{tn}^{(pj)})^T = y^{(pj)} + \sigma_p \epsilon^{(pj)}
\]

considering that the variances are not altered in the noise by the shift and mean subtraction.

### 3.3 Principal Component Analysis

Principal Component Analysis is a vector space transform helpful in reducing multidimensional data into lower dimensions to improve its analysis. It searches for linear combinations of the variables in the data that capture a maximum amount of variance. This statistical tool is useful for an exploratory analysis of data sets, and sometimes as a predictive model. The mathematical derivation involves finding the covariance matrix of the data set followed by the calculation of its Eigenvalue decomposition. The resulting scores and loadings are then used to explore the data set. Because the PCA is a non-parametric method of extracting relevant information of complex data sets, it is useful in revealing the, sometimes hidden, simplified structure of the data set.

If the first few uncorrelated PCs capture most of the variation of the original variables, and if those variables can in turn be interpreted, then the PCs offer an alternative, much simpler description of the data that the original variables [16].

PCA is mathematically defined as an orthogonal linear transform. The purpose of PCA is to transform a set of data into a new coordinate system in such a way that the greatest variance due to any projection of the data lies on the first coordinate, the first principal component. The second greatest variance lies then on the second orthogonal coordinate, generating the second principal
component, and so on. The optimum transform for the data set in calculated in terms of least squares. This ensures that the low order Principal Components retain the characteristics of the data which contribute the most to its variance.

The central concept of PCA is to take a linear combination of the original variables,

$$f_t = \sum_{k=1}^{K} \beta_t X_t^{(k)}, \ t = 1, \ldots, N,$$  \hspace{1cm} (4)

where $\beta_t$ is a weighting coefficient that is applied to the observed values $x_{ij}$ of the $j$th variable. It can then be re-expressed as:

$$f_t = \beta'X_t, \ t = 1, \ldots, N,$$  \hspace{1cm} (5)

where $\beta$ is the vector $(\beta_1, \ldots, \beta_p)'$ and $X_t$ is the vector $(X_{t1}, \ldots, X_{t1})'$. The weights are chosen in such a fashion as to represent types of variation that are present in the data. PCA defines a set of normalized weights that maximize the variation in the $f_t$'s. It finds a weighting vector $a_t = (a_{11}, \ldots, a_{kt})'$ such that the linear combination values:

$$f_t = \sum_{k} a_{tk}X_t^{(k)} = a_t'X_t^{(k)}$$  \hspace{1cm} (6)

have the largest possible mean square $N^{-1}\sum_t f_t^2$. To make the problem well defined, and avoid linear combination values that are arbitrarily large, we submit it to the constraint of a unit sum of squares, such that

$$\sum_j a_{j1}^2 = \|a_1\|^2 = 1$$  \hspace{1cm} (7)

This ensures that the mean square is maximized, and thus enables us to identify the strongest, and most important mode of variation in the variables [17]. This is then implemented the rest of the weights, defining them to be orthogonal to those indentified previously. The amount of variation will then decline on each step, and we will be interested only in those modes.
that can explain a high amount of the variation in the data. The values of the linear
combinations $f_{ij}$ are called the principal component scores, and will be used to help describe the
characteristics of the variables.

By identifying and subtracting the mean for each variable before doing the PCA we are
able to maximize the mean square of the principal component scores, which in turns corresponds
to maximizing their sample variance [17].

Under this analysis, the ERP components represented in the EEG traces are described then
in terms of components $k$, the loadings $a_{kj}^{(p)}$, and their corresponding eigenvector $X_t^{(p,k)}$ and
noise vector $\varepsilon_t^{(p,j)}$.

$$Y_t^{(p,j)} = \sum_{k=1}^{K} a_{kj}^{(p)} X_t^{(p,k)} + \sigma_p \varepsilon_t^{(p,j)} \quad (8)$$

We assume for simplicity that $a_{kj}^{(p)} X_t^{(p,k)}$ are fixed and deterministic for each infant, but could
also in a larger study characterize these as arising from a population.

### 3.4 Singular Value Decomposition

For the purpose of analysis, it is useful to consider also the singular value decomposition (SVD) of
the $(m \times n)$ data matrix $Y^{(p)}$. The SVD re-expresses the matrix $Y^{(p)}$ as the product of three
matrices

$$Y^{(p)} = U^{(p)} \Xi^{(p)} V^{(p)^T} \quad (9)$$

Where $U^{(p)}$ is a $(m \times q)$ matrix, and $V^{(p)}$ is an $(n \times q)$ matrix, for some integer $q \leq \min(m, n)$. $\Xi^{(p)}$ is a diagonal matrix with strictly positive elements on its diagonal, denoted $\xi_k$, where

$$\Xi^{(p)} = \begin{pmatrix} \xi_1 & 0 & 0 \\ 0 & \ldots & 0 \\ 0 & 0 & \xi_q \end{pmatrix} \quad (10)$$

The diagonal elements of $\Xi$ are called the singular values of $Y^{(p)}$ [17], which will be used to obtain
an estimate of the variance of the PCA loadings. Furthermore, the SVD of matrix $Y^{(p)}$ has the
properties $U'U = I$, and $V'V = I$.

### 3.5 Biplots

Because the main objective of PCA is to reduce the dimensionality of the data, in order to be able
to analyse the data appropriately, one must turn to graphical tools that enable a visual
representation of the data. An appropriate visualization of data is a difficulty in multivariate statistics due to the abundance of relationships between the many variables. Plotting the relationship between only two variables at a time for a multivariate analysis of a data set is highly restrictive regarding its information content [18]. Fortunately, in multivariate statistics, groups of variables may behave in a correlated fashion, and thus their overall behaviour can be visualized in a lower dimensionality. PCs may be employed to look at the data graphically [16].

A statistical graph, the Biplot, allows information obtained in PCA of both, samples and variables to be displayed graphically. In the Biplot, samples are displayed as points, and variables as vectors. The Axes in the Biplot space are generated by the orthogonal Principal Components. Variables poorly represented with respect of the axes of interest have relative short vectors. Short vectors will contribute little to the approximation whereas, in exact representations, the vectors are unit length. The fundamental assumption of the PCA Biplot analysis is that the contributions from the variables are independent [19].

The purpose of PCA, in the context of this project, is to find a good representation of the data in a smaller number of variable dimensions than originally contained in the data set. Since the First PCs extract the best-fitting structure that maximises the variance of the data set, we can consider these to give a good description of the overall behaviour of the data set. If the analysis is restricted to the first 2 PCs, we can obtain in principle the best possible two visualize the behaviour of the variables in the data [16].

By comparing the coefficients that each variable has with respect to their first and their second principal component, it is possible to construct a good visualization of the relationship between all the variables. Each variable is represented then by a vector, whose direction and length are a descriptive account of the contribution to the Principal Components in the plot. By representing the First Principal Component as the horizontal axis of the Biplot, the normalized score that each variable has with respect to the 1st PC can be visualized. The second principal component, represented by the vertical axis, can then produce a second coordinate for each variable in the Biplot, describing the score that each variable has with respect to the 2nd PC. Taken both axes together, 17 vectors, each representing one of the Electrode channels, are drawn from the origin to their corresponding scores. The 2 Principal components taken together then can distinguish between the contributions of the specially separated electrode channels to the represented data. The Biplot can be extended to include more than 2 PCs, but the graphical representation becomes cumbersome and difficult to interpret [16].

In this interpretation, the lengths of the variable vectors are proportional to the variance of the variables, and the cosines of the angles between the variables represent the correlation between them. The distances considered for this analysis are considered to be Euclidean.

A problem with this kind of analysis arises when the 2nd principal component is ill-identified. Some infants appear to have 3 non-noise related eigenvalues, whilst some have 2 or even one. In the first two cases, one would in general have to ensure that the correct 2nd and 3rd
components are compared. In a larger study, the number of components could groups the infants into different classes.

3.6 Implementation

The PCA analysis of EEG traces across electrode channels considers the data in the form of a matrix in which each row represents a different repetition of the experiment in time, and each column gives the result from a particular electrode channel. The analysis considers then an experimental setup consisting of 17 electrodes distributed across the infant’s head. Each electrode channel records the Electric field (DC) in that location through a period of time. A time series is generated for each electrode, and is stored as a Matrix in which the columns represent the electrode channels and the rows contain the DC time series recordings in µVolts. The time intervals considered are the ones which describe the response to a noxious stimulus.

The PCA analysis is carried out in MatLab with the following considerations:

1. The data is arranged such that the variables are the electrode locations (matrix columns), and the observations are the time points (matrix rows).
2. The Mean is subtracted from each of the data columns.
3. The Covariance Matrix is then calculated for the data.
4. The Eigenvectors and Eigenvalues of the Covariance Matrix are calculated.
5. The Eigenvectors are reordered forming a new feature vector: the factor loadings.
6. The data is projected into the factor loadings vector, deriving a new data set: The factor scores.

The factor loadings then describe the scalp topography and therefore are considered to be the same across the entire dataset. They represent the correlations between the variables and the factor scores. The factor scores represent the relationship between the factors and the observations, and are free to vary between conditions and across subjects. The factor score matrix contains the magnitude of the factors for each of the observations.

It is possible to get the original data set back, with the added option of retaining the higher principal components since the lower factors are most likely to contain an interpretable signal. By projecting the factor scores back into the factor loadings, the original data matrix is reproduced. The original variable means should then be restored.
3.7 Limitations

Some concerns have been raised with respect to the use of PCA analysis to describe ERP potentials. Because PCA does not provide a single unique set of underlying components, it has been suggested that additional assumptions need to be made in order to decide by means of the component’s wave shapes which ones to consider [20]. The greatest concern at this point is the lack of an accepted method to verify that the assumptions made are correct.

The extraction of the lower order principal components maximizes the variance of the initial factors by including variance from as many variables as possible. It has been noted that hybrid components may arise during the analysis [20]. If 2 different cognitive processes co-vary, they would be considered as part of a single component, even if they occur in different areas of the brain. On the other hand, if a component varies in latency across channels, PCA would consider this single component as multiple components. The components obtained through PCA are then subject to misallocation of variance across the data. With respect to the loadings, it is possible that components with similar scalp topographies may be difficult to differentiate, even when they have separate time dynamics.

4. Analysis of the Infant Data

4.1 1st PCs Analysis

It is of interest to explore the similarity of the Principal Components across subjects. By considering the 1st Principal Component as a vector, geometrical properties of lineal algebra can be applied. We consider the projection of one standardized vector, representing the 1st PC of one subject, onto another that represents the 1st PC of a comparison subject such that:

\[ \rho_{(p_1,p_2)} = \frac{\sum t X_t^{(p_1)} X_t^{(p_2)}}{\sqrt{\sum t X_t^{(p_1)}^2 X_t^{(p_2)}^2}} \]

(11)

Where

\[-1 \leq \rho \leq 1\]
By using the vector properties of linear algebra, if a standardized vector is projected into itself, ρ will yield unity. As the vectors differ in their structure, ρ decreases in value in such a way that ρ takes a value of zero for completely dissimilar vectors. On the other hand, ρ takes negative values for exact opposite structure.

4.2 Eigenvector Analysis

We have considered matrix $\mathbf{Y}^{(p)}$, containing all of the $j$ recordings during $t$ times, to be fixed and deterministic for each infant. A method of moments approach, obtaining the mean and the variance directly from the sample, would be feasible if all the infants had the same eigenvectors, but since $a_1$ was found to be different for all the infants, a different approach is required.

Following the approach of Olhede and Walden [21] we can employ a similar methodology, however, we note that $\hat{\sigma}^2_0$ is different; ours not being distributed by chi-square with the stated degrees of freedom. To estimate the noise, we use the non-zero eigenvalues corresponding to mode 3 and above. Since we assume that the noise is small compared to the data, we can adopt the expansion described in (B 26). From the Singular Value Decomposition of the data, we consider then the $(J \times J)$ symmetric matrix.

$$
S^{(p)} = [\mathbf{Y}^{(p)}]^T [\mathbf{Y}^{(p)}] \equiv \mathbf{Y}^{(p)} \Sigma^{(p)} \mathbf{Y}^{(p)^T}
$$

$$
= \sum_{j} [\mathbf{Y}^{(p)j}] [\mathbf{Y}^{(p)j}]^T
$$

$$
= \sum_{j} \left[ \mathbf{Y}^{(p)j} + \sigma_p \epsilon^{(pj)} \right] \left[ \mathbf{Y}^{(p)j} + \sigma_p \epsilon^{(pj)} \right]^T
$$

$$
= S^{(0)}_p + \sigma_p S^{(1)}_p + \sigma_p^2 S^{(2)}_p
$$

for a given infant $p$. We obtain through this decomposition a noise-free version $S^{(0)}_p$, noise term $S^{(1)}_p$ (the first error correction term), and an extra term $S^{(2)}_p$, that does higher order error correction.
In the context of this analysis, \( \hat{a}_1^{(p)} \) expresses the estimated noisy eigenvectors of the sample eigenvectors. The purpose of this expansion is to express the loadings as an eigenvector of the series, such that:

\[
\hat{a}_1^{(p)} = a_1^{(p)} + \delta a_1^{(p)}
\]

(16)

where the covariance of \( \delta a_1^{(p)} \) is specified by (B 14) in Olhede and Walden [21]. To investigate the effect of the fact that we are not obtaining the true loadings in the SVD, an expansion of the vector in the eigenvector & eigenvalue series can provide us with an insight on the properties of the noise-free version \( S^{(0)} \).

We adopt the mathematical derivation of Olhede and Walden with a change of notation from the original paper (left) [21], to that corresponding to our context (right), such that:

\[
p = J \quad \text{(number of electrodes)}
\]

\[
k = n \quad \text{(time points)}
\]

\[
q = J - 2 \quad \text{(eigenvalues that we expect to be zero)}
\]

We estimate the first loading \( a_1^{(p)} \) by considering \( l = m = 1 \) in (B 14) as described by Olhede and Walden [21]. We are interested in the first eigenvector, summing over the higher term. From the SVD, we consider only 2 of the singular vectors \( \xi_k \) to be meaningful. We estimate \( \xi_1 \) as the square of the first un-normalized singular value, \( \xi_2 \) as the square of the second un-normalized singular value, and all the others as zero. To estimate the noise, we sum the square of all the singular values 3 & above such that:

\[
\delta p^2 = \frac{1}{j-2} \sum_{k=3}^{J} \xi_k^2
\]

(17)

Through this series, we expect to obtain an estimate of the variance directly from the singular values. A difficulty with this approach is that we interpret the different loadings as coming from noise, but it need not be the case.
4.3 Lateralization Test

We have assumed a model for the Eigenvectors, such that:

$$E\{a_{ij}^{(p)}\} = \mu_{ij}^{(p)}$$  \hspace{1cm} (18)

$$\mu^{(r)} | \text{the infant's left foot was heel lanced}$$

$$\mu^{(l)} | \text{the infant's right foot was heel lanced}$$

We will consider the Null Hypothesis, $H_0$, stating that the eigenvectors are irrespective of which foot was lanced. This would imply that the amount of signal to the electrodes on the left and on the right hemispheres is equivalent.

$$\sum_{\text{left electrodes}} \left[ \hat{a}_{ij}^{(p)} \right]^2 - \sum_{\text{right electrodes}} \left[ \hat{a}_{ij}^{(p)} \right]^2 = 0$$  \hspace{1cm} (19)

We have considered the responses from the right hemisphere to be constituted by electrode signals proceeding from locations Fp2, F8, C4, T4, T6 and O2 (in blue), and the right hemisphere by electrode locations Fp1, F7, T3, C3, T5 and O1 (in red) as seen in figure (1). Considering the same number of electrodes on each hemisphere, we are interested in the distribution of $\hat{a}_{ij}^{(p)}$.

Our assumption is not that there is perfect replication across infants with respect to the eigenvectors. Our hypothesis states that we are only looking for differences between the response of the left and right hemisphere. We wish to determine the distribution of

$$T^{(p)} = \sum_{\text{left electrodes}} \left[ \hat{a}_{ij}^{(p)} \right]^2 - \sum_{\text{right electrodes}} \left[ \hat{a}_{ij}^{(p)} \right]^2$$  \hspace{1cm} (20)
In order to create an appropriate test statistic, we need to determine the mean and variance of $T^{(p)}$. Finding the covariance between the two vectors following (B 14) [21], we are interested in the first. Because we assume $T^{(p)}$ to be Gaussian, such that:

$$T^{(p)} \sim N \left( \mu^{(p)}, \xi_p^2 \right)$$

We already have derived an estimate of that, and now know that the sum

$$\bar{T} = \frac{1}{p} \sum_p T^{(p)} \sim N \left( \sum_p \mu^{(p)}, \frac{1}{p^2} \sum_p \xi_p^2 \right)$$

is also Gaussian. The sum of the means is still zero under the null hypothesis $H_0$, so we find the estimated variance for each of the singular vectors, such that:

$$T^{(p)} \mid H_0 \sim N \left( 0, \frac{1}{p^2} \sum_p \xi_p^2 \right)$$

From which we derive that

$$\frac{\bar{T}^{(p)}}{\sum_p \xi_p^2} \mid H_0 \sim N \left( 0, 1 \right)$$

Because the signal is strong and clearly distinguishable, a big fraction of it can be explained with the first and the second vector. There is on top of that, we suspect, a white noise signal.

$$\hat{\sigma}_p^2 \sim \frac{\sigma_p^2}{n(j-2)} X^2_{(j-2)(n-2)}$$

While the theoretical, true variance of $T^{(p)}$ is expressed as:

$$\text{var} \left( T^{(p)} \right) = \xi_p^2$$

$\hat{\sigma}_p^{(p)}$, however, is not equal across all the infants, so we need to find the variance for the vectors in a different fashion. Our estimate for the variance then becomes constituent of equation (10) and equation (17), such that
The variance of the loadings for a given infant is then determined by the product of the estimated variance, the 1/n data points and an expression that involves the singular values from the first and the second loadings squared, their expected values and the remaining eigenvectors, as adapted from (B 14) [21]. This should compare with the sample variance, suspecting it should be less. We can then investigate the distribution of the $T^{(p)}$ terms and compare them in a t test.

We will employ the assumption that $\zeta_p^2$ is identical over $p$. For our test, all we need is to consider $T^{(p)}$ across $p$ and divide it by the square root of their estimated variances, such that

$$
\frac{T^{(p)}}{\sqrt{\zeta_p^2}} \mid H_0 \sim t_{(j-2)(n-2)}
$$

(28)

We are interested in the variance of each $T$, such that:

$$
var(T^{(p)}) = var\left\{ \sum_{j \leftarrow t} \left[ \hat{a}^{(p)}_{ij} \right]^2 - \sum_{k \rightarrow r} \left[ \hat{a}^{(p)}_{1k} \right]^2 \right\}
$$

(29)

$$
= var \sum_{j \leftarrow t} \left[ \hat{a}^{(p)}_{ij} \right]^2 + var \sum_{k \rightarrow r} \left[ \hat{a}^{(p)}_{1k} \right]^2 - 2 cov \left\{ \sum_{j \leftarrow t} \left[ \hat{a}^{(p)}_{ij} \right]^2 , \sum_{k \rightarrow r} \left[ \hat{a}^{(p)}_{1k} \right]^2 \right\}
$$

(30)

With

$$
var\left\{ \left[ \hat{a}^{(p)}_{ij} \right]^2 \right\} \approx var\left\{ \left[ a_{ij}^{(p)} + \delta a_{ij}^{(p)} \right]^2 \right\}
$$

(31)
The last term of the RHS of eq (3), we use the loadings from the PCA such that:

$$
cov\left\{\sum_{j \leftarrow k \rightarrow} \left[\alpha_{1j}^{(p)}\right]^2, \sum_{k \leftarrow k \rightarrow} \left[\tilde{\alpha}_{1k}^{(p)}\right]^2\right\} = \sum_{j \leftarrow k \rightarrow} \sum_{k \leftarrow k \rightarrow} \text{cov}\left\{\left[\alpha_{1j}^{(p)}\right]^2, \left[\tilde{\alpha}_{1k}^{(p)}\right]^2\right\} \tag{32}
$$

$$
= \sum_{j \leftarrow k \rightarrow} \sum_{k \leftarrow k \rightarrow} 4 a_{1j}^{(p)} a_{1k}^{(p)} \Phi_{jk}^{(p)} \tag{33}
$$

Following the expansion of eq (32), the first term of the RHS of eq (30) can be expressed as:

$$
\text{var} \sum_{j \leftarrow k \rightarrow} \left[\tilde{\alpha}_{1j}^{(p)}\right]^2 = \sum_{j \leftarrow k \rightarrow} \text{var}\left\{\left[\tilde{\alpha}_{1j}^{(p)}\right]^2\right\} + \sum_{j \neq j'} \text{cov}\left\{\left[\tilde{\alpha}_{1j}^{(p)}\right]^2, \left[\tilde{\alpha}_{1j'}^{(p)}\right]^2\right\} \tag{34}
$$

$$
\approx \sum_{j \leftarrow k \rightarrow} \left[2\tilde{\alpha}_{1j}^{(p)}\right]^2 \text{var} \delta a_{1j}^{(p)} + \sum_{j \leftarrow k \rightarrow} \sum_{k \leftarrow k \rightarrow} 4 a_{1j}^{(p)} a_{1k}^{(p)} \Phi_{jk}^{(p)} \tag{35}
$$

With

$$
\text{var} \delta a_{1j}^{(p)} = \Phi_{jj}^{(p)} \tag{36}
$$

By doing this, we are taking the estimated variance of each of the eigenvector from each infant and then all of the noise structure can be estimated by investigating the zero eigenvalues. Because \( n \) is large, the sample and the population values become indistinguishable. We expect all of the errors to have been averaged out.

Finally, we find an estimate for the noise components. We could not use the sample variance because the deviation of the mean is not indicative of the noise. This calculation is repeated to obtain the variance of the sum over the right electrodes, the last second term of the RHS of eq (30).
Two tests are to be made:

a) Whether a given infant appears to have more energy with respect of a left or right heel lance.

We want to test the approximate distribution of $T^{(p)}$, such that:

\[
\frac{T^{(p)}}{\sqrt{c_p^2}} \mid H_0 \sim N(0, 1)
\]  

(37)

or

\[
\frac{T^{(p)}}{\sqrt{c_p^2}} \mid H_0 \sim t_{(j-2)(n-2)}
\]

(38)

b) If there is a distinction between lancing the left and right foot.

\[
\frac{\sum_{p \text{ left}} T^{(p)} - \sum_{p \text{ right}} T^{(p)}}{\sqrt{\sum_p c_p^2}} \sim t_{(j-2)(n-2)}
\]

(39)

We acknowledge that the critical values should really be adjusted to looking at 16 tests instead of one. Because we do not have the same number of infants who were lanced on the left foot as we have who have been lanced on the right foot, a further consideration needs to be made. Considering $p_l$ to be the number of infants that were hell lanced on the left foot, we rewrite eq (39) as:

\[
\frac{1}{p_l} \sum_{p \text{ left \ foot}} T^{(p)} - \frac{1}{p - p_l} \sum_{p \text{ right \ foot}} T^{(p)} \sim t_{(j-2)(n-2)}
\]

(40)

We assume that only 2 signal eigenvectors exist as we go throughout the calculations from Olhede and Walden [21], but it is possible that more eigenvectors are needed for consideration. It is also possible that we are susceptible to mode mixing. An unobserved components model could
be appropriate [22]. If the eigenvalues are repeated—which is not completely improbable with modes from eigenvectors 2 and 3, we could in fact re-parameterize by

\[ \hat{a}'_2 = \alpha_1 \hat{a}'_2 + (1 - \alpha^2)^{1/2} \hat{a}_3 \]  

\[ \hat{a}'_3 = (1 - \alpha^2)^{1/2} \hat{a}_2 - \alpha_1 \hat{a}'_3 \]  

(41)

(42)

where \( \hat{a}_2, \hat{a}_3 \) are the original eigenvectors. By choosing \( \hat{a}_2 \) to minimize \( \hat{a}_{2CPZ} \). This yields a unique choice. This may also increase the correlation of the second eigenvector across infants, and allow us to recognize the same second component, which is something to further investigate.

5. Results

We are interested in whether lancing the right heel of the infant, a noxious stimulation, is producing a left-hemispheric response signal and vice versa. This kind of response is what would be expected for a human adult. We also need to consider whether there is a tendency for signal responses to be larger on one of the brain hemispheres. However, this kind of analysis can only be done if we have enough stimuli on the right and the left foot for comparison.

5.1 PCA Plots

The Principal Components Analysis decomposed the data time series into a sequence of data points that reveal the underlying structure responsible for the maximum variability in the data. To understand the underlying context of the data points, and construct a more general model, we first turn to a graphical inspection of the 1st Principal Components (PCs).

Figure (2)- Butterfly plot of the simultaneous EEG traces (left), compared to the plot of the first 3 Principal Components (right) from infant 07590101.
The first 3 principal components explain a considerable amount of the total variability in the data, as evidenced by the analysis shown for each infant. Although the first PC has a similar geometry across infants, there is no visual consistent structure in the 2nd and 3rd principal components. It is therefore reasonable for us to focus on the first principal component in order to visualize the data.

The first PC of each infant, taking into account experimental and theoretical considerations, reveals a structure that can be identified as a latent ERP component of the entire data set.

In ERP data, the major source of covariance is assumed represent the characteristic features of the waveform that are spread across the brain across time [20]. Detected by 17 electrodes across the scalp, we assume these characteristic features to be the ERP components. Because of the mathematical constraints of the statistical analysis, each Principal Component would be uncorrelated and would thus correspond to a separate ERP component. Most of the PCs only account for small proportion of variance, which is identified with background noise.

We note that the spatio-temporal features of the waveforms obtained through PCA vary across subjects in the latency of the response. The factor loadings give a general description of the scalp topography, which is best visualized on a Biplot. The employed Matlab Biplot tool imposes a sign convention that forces the element with the largest magnitude of the loadings to be positive, altering the sign of the rest of the loadings. Because of this, we have inverted the axis of some plots to consider the vector representing electrode CPz, to be in the First quadrant (positive with respect to both axes). This enables a more direct a visual comparison of Biplots across individuals.
5.2 PCA Centered Plots

A histogram of the location of the maximum response across time detected on the electrode CPz shows high variability with respect of the latency of the response to the noxious stimulus across infants. Even though the signals are geometrically similar, as seen in Appendix A, due to these differences in latency it is not possible to compare them directly with each other. In order to carry out an appropriate comparison between the structures of the PCs across infants, a significant interval of the data is considered.

We aligned the signals from different infants by finding the maximum response across time detected on the electrode CPz. A significant interval of 700 data points (±0.35 ms) around the
maximum detected on electrode CPz was considered. We considered this centred significant interval to be the same for the rest of the electrodes. PCA was carried out for each infant on this interval, through which we obtain a structure that is comparable across infants, as seen on Appendix B.

To obtain a quantification of the similarity between the 1st PCs across infants, we compare the geometry of the loadings. Following eq (11), we generated a symmetric table describing the degree of similarity between 1st PCs across infants, as seen on table (C.1) on Appendix C.

**Statistical Interpretation**

The values for $\rho^{(P_1P_2)}$, obtained through the comparison of 1st PCs across infants, show a high degree of overall similarity. This is specially the case of the comparison of infants 07420201-07590101 and infants 07540101-07550101. The 1st PCs compared have similar features, and most follow a similar trajectory, as can be appreciated in figure (6). The individual loadings that make up the PCs, however, seem to be different for each individual.

We generated a plot of the loadings for each electrode, obtained through the PCA of the sample, as seen in figure (7). The plot illustrates the high variability of the amount of the electrode contributions towards the overall signal. The absolute value of the loadings was considered.
In order to account for the high variability of the loadings in the analysis, we turned to the methodology developed by Olhede and Walden [21], and Figueiredo [23], as described in the methods section.

**Laterization Tests Results**

From the assumption that

$$E\left\{ \tilde{a}^{(p)}_{ij} \right\} = E\left\{ \tilde{a}^{(p')}_{ij} \right\}$$  \hspace{1cm} (43)

The estimated variance of $T$, $\text{var}(T^{(p)})$, using the method of moments, was calculated to be $0.02$. This however is not the true variance of our sample. From the results obtained from the Biplot analysis, it is clear that

$$E\left\{ \tilde{a}^{(p)}_{ij} \right\} \neq E\left\{ \tilde{a}^{(p')}_{ij} \right\}$$  \hspace{1cm} (44)
Because the vector $\mathbf{a}_1^{(p)}$ is not equal across all the infants, we need to find the variance for the vectors in a different fashion. We proceeded with an eigenvector analysis, as described in the methods section.

We have determined the statistical properties for each infant lanced on the left, as seen in table (1), and for those lanced in the right foot, as seen in table (2).

<table>
<thead>
<tr>
<th>Infant</th>
<th>$\text{var}(T^{(p)})$</th>
<th>$\zeta_p$</th>
<th>$T^{(p)}$</th>
<th>$T^{(p)}/\sqrt{\zeta_p^2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;07260102&quot;</td>
<td>9.72E-06</td>
<td>3.12E-03</td>
<td>0.02</td>
<td>6.46E+00</td>
</tr>
<tr>
<td>&quot;07310101&quot;</td>
<td>1.31E-05</td>
<td>3.62E-03</td>
<td>0.23</td>
<td>6.41E+01</td>
</tr>
<tr>
<td>&quot;07420201&quot;</td>
<td>5.85E-06</td>
<td>2.42E-03</td>
<td>0.29</td>
<td>1.19E+02</td>
</tr>
<tr>
<td>&quot;07420202&quot;</td>
<td>6.29E-05</td>
<td>7.93E-03</td>
<td>0.26</td>
<td>3.24E+01</td>
</tr>
<tr>
<td>&quot;07540101&quot;</td>
<td>3.57E-05</td>
<td>5.98E-03</td>
<td>0.07</td>
<td>1.13E+01</td>
</tr>
<tr>
<td>&quot;07590101&quot;</td>
<td>1.11E-05</td>
<td>3.33E-03</td>
<td>-0.04</td>
<td>-1.16E+01</td>
</tr>
</tbody>
</table>

Table (1)- Estimated statistical properties from the eigenvector analysis for individual infants lanced on the left foot.

<table>
<thead>
<tr>
<th>Infant</th>
<th>$\text{var}(T^{(p)})$</th>
<th>$\zeta_p$</th>
<th>$T^{(p)}$</th>
<th>$T^{(p)}/\sqrt{\zeta_p^2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;07100101&quot;</td>
<td>9.43E-05</td>
<td>9.71E-03</td>
<td>0.22</td>
<td>2.26E+01</td>
</tr>
<tr>
<td>&quot;07100103&quot;</td>
<td>1.65E-05</td>
<td>4.06E-03</td>
<td>-0.20</td>
<td>-4.90E+01</td>
</tr>
<tr>
<td>&quot;07260103&quot;</td>
<td>2.06E-05</td>
<td>4.54E-03</td>
<td>0.10</td>
<td>2.12E+01</td>
</tr>
<tr>
<td>&quot;07330101&quot;</td>
<td>1.91E-05</td>
<td>4.37E-03</td>
<td>0.12</td>
<td>2.65E+01</td>
</tr>
<tr>
<td>&quot;07550101&quot;</td>
<td>2.03E-05</td>
<td>4.51E-03</td>
<td>0.19</td>
<td>4.22E+01</td>
</tr>
</tbody>
</table>

Table (2)- Estimated statistical properties from the eigenvector analysis for individual infants lanced on the right.

In order to test our hypothesis, we wish to compare the estimated values of

$$T^{(p)} / \sqrt{\zeta_p^2} \mid H_0 \sim t_{(J-2)(n-2)}$$

(45)
which include the theoretical (and hypothesized) mean of zero. Because we consider the degrees of freedom of our sample to be extremely large \((l - 2)(n - 2) = 20985\), we compare this to the critical value obtained with an infinite number of degrees of freedom,

\[
t_{\alpha} = 1.96
\]  

(46)

All the values obtained from the table surpass this value. The magnitude of the values obtained for eq(28), as seen in table (1) and table (2), however reveal a difficulty with the order of magnitude of the values to compare. We believe that this is due to a sub-population problem, and that a larger study is required to adequately model the population heterogeneity.

Although we cannot accept at this point the Null Hypothesis, we can make some inferences form the data analysis. From the analysis displayed on table (1) and table (2), it is apparent that the left and the right heel lances do not have the same brain response. We note that there is always a tendency to have more energy to the left, rather to the right. Because a heel lance administered in the left foot seems to cause a higher activation on the cerebral region, it is possible that the infant brain is more attuned to left-sided stimulus. This suggests that, if lateralization is found, there would be a tendency for signal responses to be larger on one of the brain hemispheres depending on which foot the infant was stimulated.

For the second test, we consider the possibility of lateralization. Our Null hypothesis states that the response of the brain is not differentiated with respect of which foot was lanced. To accept our Null Hypothesis, we shall compare it to the same critical value for a t test obtained with an infinite number of degrees of freedom. The calculation from eq (40) yields as a result

\[
\begin{align*}
&\frac{1}{p_l} \sum_{p \text{ left}} T^{(p)} - \frac{1}{p - p_l} \sum_{p \text{ right}} T^{(p)} \\
&\sqrt{\frac{1}{p_l} \sum_{p \text{ left}} \sigma_p^2 + \frac{1}{(p - p_l)^2} \sum_{p \text{ right}} \sigma_p^2} = 16.2 > 1.96
\end{align*}
\]

(47)

Again, we compare this value to the critical value obtained with an infinite number of degrees of freedom for a t test. The value obtained through eq (16) is one order of magnitude greater than the critical value. Even though the value calculated surpasses the value for the null hypothesis to be true, more considerations are needed. In principle, however, we cannot reject at this point the hypothesis that the response to a heel lance is lateralized.
Conclusions

The usefulness of this type of analysis in understanding the topography of these signals from the infant brain is mostly evident in the overall reduction do the dimensionality of the data. By employing well-known mathematical constraints to the data, we are able to abstract more general features of the EEG traces. By identifying the linear combination of variables that account for the maximum variance of the data to be the ERP components, we can begin to make a direct analysis on the brain’s response. The techniques employed during this project are still not fully developed, but have so far yielded promising results.

Through this kind of analysis we may be able, eventually, to understand the temporal sequence of analysis in different parts of the brain. The development of a mathematical model of the responses in the area of the cortex, the main source of the signal, is an important step. It allows the possibility of a description in mathematical terms of the developmental changes that occur from extreme prematurity (<28 weeks) to full term (40 weeks) and beyond. Such a model could then incorporate the effects of analgesic agents and other external factors to develop an individualised pain management assessment.

Although during this analysis the results obtained could not be conclusively compared with a value corresponding to a given statistical significance level, we have established a basic methodology for comparison. Or alternative hypothesis, the existence of lateralization in the infant brain, cannot be rejected at his point. Further considerations are needed.

We have found an apparent difference in the brain’s activation with respect of the heel which was lanced. There is a tendency within the sample to have more energy to the left, rather than to the right. This indicates that left foot stimuli may cause a greater cerebral activation.

There is still work to be done. It is of interest to further investigate whether we are losing some of the structure by filtering the signal, or if this has a positive effect on the overall analysis. On the other hand, due to the variability found in the PCA analysis, a stochastic model could also be considered. Refinement on the PCA should be implemented and SparsePCA would be considered as our choice for refinement [24].

We have seen recently huge advances in the field of medical science, which had led in turn to a higher survival rate of fragile neonates. While it was rare for neonates to have nociceptive experiences, now it is becoming more and more common. Surviving infants who are hospitalized, sometimes through lengthy periods, face daily repeated nociceptive stimulations [25]. The immediate and long-term consequences of early exposure to nociceptive experiences are becoming evident. It is therefore of uttermost importance for us to understand and quantify the pain-modulating systems of the neonates. As our knowledge and understanding of the mechanism of pain grows, and more and more evidence is put forward, the gap between research and practice should decrease. The final objective of this kind of analysis is to eventually be able to prevent and minimize pain and its consequences.
Bibliography


APPENDIX A

A.1) Full trace analysis of Infant 07100101

A. 1. a) Butterfly Plot of EEG Traces

A. 1. b) First 3 Principal Components of the Data

A. 1. c) Biplot of the PCA loadings

A.1. d) Variance Explained by each PC (in %)

A.1. e) Plot of the 1st Principal Component

A.1. f) Comparison of 1st PC and CPz trace
A.2) Full trace analysis of Infant 07100103

A.2. a) Butterfly Plot of EEG Traces

A.2. b) First 3 Principal Components of the Data

A.2. c) Biplot of the PCA loadings

A.2. d) Variance Explained by each PC (in %)

A.2. e) Plot of the 1st Principal Component

A.2. f) Comparison of 1st PC and CPz trace
A.3) Full trace analysis of Infant 07260102

A.3. a) Butterfly Plot of EEG Traces

A.3. b) First 3 Principal Components of the Data

A.3. c) Biplot of the PCA loadings

A.3. d) Variance Explained by each PC (in %)

A.3. e) Plot of the 1st Principal Component

A.3. f) Comparison of 1st PC and CPz trace
A.4) Full trace analysis of Infant 07260103

A. 4. a) Butterfly Plot of EEG Traces

A. 4. b) First 3 Principal Components of the Data

A. 4. c) Biplot of the PCA loadings

A.4. d) Variance Explained by each PC (in %)

A.4. e) Plot of the 1st Principal Component

A.4. f) Comparison of 1st PC and CPz trace
A.5) Full trace analysis of Infant 07310101

A.5. a) Butterfly Plot of EEG Traces

A.5. b) First 3 Principal Components of the Data

A.5. c) Biplot of the PCA loadings

A.5. d) Variance Explained by each PC (in %)

A.5. e) Plot of the 1st Principal Component

A.5. f) Comparison of 1st PC and CPz trace
A.6) Full trace analysis of Infant 07330101

A.6. a) Butterfly Plot of EEG Traces

A.6. b) First 3 Principal Components of the Data

A.6. c) Biplot of the PCA loadings

A.6. d) Variance Explained by each PC (in %)

A.6. e) Plot of the 1st Principal Component

A.6. f) Comparison of 1st PC and CPz trace
A.7) Full trace analysis of Infant 07420201

A.7. a) Butterfly Plot of EEG Traces

A.7. b) First 3 Principal Components of the Data

A.7. c) Biplot of the PCA loadings

A.7. d) Variance Explained by each PC (in %)

A.7. e) Plot of the 1st Principal Component

A.7. f) Comparison of 1st PC and CPz trace
A.8) Full trace analysis of Infant 07420202

A. 8. a) Butterfly Plot of EEG Traces

A. 8. b) First 3 Principal Components of the Data

A. 8. c) Biplot of the PCA loadings

A.8. d) Variance Explained by each PC (in %)

A.8. e) Plot of the 1st Principal Component

A.8. f) Comparison of 1st PC and CPz trace
A.9) Full trace analysis of Infant 07540101

A. 9. a) Butterfly Plot of EEG Traces

A. 9. b) First 3 Principal Components of the Data

A. 9. c) Biplot of the PCA loadings

A.9. d) Variance Explained by each PC (in %)

A.9. e) Plot of the 1st Principal Component

A.9. f) Comparison of 1st PC and CPz trace
A.10) Full trace analysis of Infant 07550101

A. 10. a) Butterfly Plot of EEG Traces

A. 10. b) First 3 Principal Components of the Data

A. 10. c) Biplot of the PCA loadings

A.10. d) Variance Explained by each PC (in %)

A.10. e) Plot of the 1st Principal Component

A.10. f) Comparison of 1st PC and CPz trace
A.11) Full trace analysis of Infant 07590101

A. 11. a) Butterfly Plot of EEG Traces

A. 11. b) First 3 Principal Components of the Data

A. 11. c) Biplot of the PCA loadings

A.11. d) Variance Explained by each PC (in %)

A.11. e) Plot of the 1st Principal Component

A.11. f) Comparison of 1st PC and CPz trace
APPENDIX B

B.1) Centered trace analysis of Infant 07100101

B. 1. a) Butterfly Plot of EEG Traces

B. 1. b) First 3 Principal Components of the Data

B. 1. c) Biplot of the PCA loadings

B. 1. d) Variance Explained by each PC (in %)

B.1. e) Plot of the 1st Principal Component

B.1. f) Comparison of 1st PC and CPz trace
B.2) Centered trace analysis of Infant 07100103

B.2. a) Butterfly Plot of EEG Traces

B.2. b) First 3 Principal Components of the Data

B.2. c) Biplot of the PCA loadings

B.2. d) Variance Explained by each PC (in %)

B.2. e) Plot of the 1st Principal Component

B.2. f) Comparison of 1st PC and CPz trace
B.3) Centered trace analysis of Infant 07260102

B.3. a) Butterfly Plot of EEG Traces

B.3. b) First 3 Principal Components of the Data

B.3. c) Biplot of the PCA loadings

B.3. d) Variance Explained by each PC (in %)

B.3. e) Plot of the 1st Principal Component

B.3. f) Comparison of 1st PC and CPz trace
B.4) Centered trace analysis of Infant 07260103

B. 4. a) Butterfly Plot of EEG Traces

B. 4. b) First 3 Principal Components of the Data

B. 4. c) Biplot of the PCA loadings

B.4. d) Variance Explained by each PC (in %)

B.4. e) Plot of the 1st Principal Component

B.4. f) Comparison of 1st PC and CPz trace
B.5) Centered trace analysis of Infant 07310101

B.5. a) Butterfly Plot of EEG Traces

B.5. b) First 3 Principal Components of the Data

B.5. c) Biplot of the PCA loadings

B.5. d) Variance Explained by each PC (in %)

B.5. e) Plot of the 1st Principal Component

B.5. f) Comparison of 1st PC and CPz trace
B.6) Centered trace analysis of Infant 07330101

B.6. a) Butterfly Plot of EEG Traces

B.6. b) First 3 Principal Components of the Data

B.6. c) Biplot of the PCA loadings

B.6. d) Variance Explained by each PC (in %)

B.6. e) Plot of the 1st Principal Component

B.6. f) Comparison of 1st PC and CPz trace
B.7) Centered trace analysis of Infant 07420201

B.7. a) Butterfly Plot of EEG Traces

B.7. b) First 3 Principal Components of the Data

B.7. c) Biplot of the PCA loadings

B.7. d) Variance Explained by each PC (in %)

B.7. e) Plot of the 1st Principal Component

B.7. f) Comparison of 1st PC and CPz trace
B.8) Centered trace analysis of Infant 07420202

B. 8. a) Butterfly Plot of EEG Traces

B. 8. b) First 3 Principal Components of the Data

B. 8. c) Biplot of the PCA loadings

B. 8. d) Variance Explained by each PC (in %)

B.8. e) Plot of the 1st Principal Component

B.8. f) Comparison of 1st PC and CPz trace
B.9) Centered trace analysis of Infant 07540101

B.9. a) Butterfly Plot of EEG Traces

B.9. b) First 3 Principal Components of the Data

B.9. c) Biplot of the PCA loadings

B.9. d) Variance Explained by each PC (in %)

B.9. e) Plot of the 1st Principal Component

B.9. f) Comparison of 1st PC and CPz trace
B.10) Centered trace analysis of Infant 07550101

B. 10. a) Butterfly Plot of EEG Traces

B. 10. b) First 3 Principal Components of the Data

B. 10. c) Biplot of the PCA loadings

B. 10. d) Variance Explained by each PC (in %)

B.10. e) Plot of the 1st Principal Component

B.10. f) Comparison of 1st PC and CPz trace
B.11) Centered trace analysis of Infant 07590101

B. 11. a) Butterfly Plot of EEG Traces

B. 11. b) First 3 Principal Components of the Data

B. 11. c) Biplot of the PCA loadings

B.11. d) Variance Explained by each PC (in %)

B.11. e) Plot of the 1st Principal Component

B.11. f) Comparison of 1st PC and CPz trace
APPENDIX C

Centered trace analysis of Infant 07100101

Table (C.1)- The values for $\rho^{(p_1,p_2)}$, obtained through the comparison of 1st PCs across infants, show a high degree of overall similarity. This is specially the case of infants 07420201-07590101 and infants 07540101-07550101.
## APPENDIX D

### Infant Demographics of the Sample

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<th>sleep state pre heel lance</th>
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<td>active awake</td>
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<tr>
<td>07100103</td>
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<td>active awake</td>
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<tr>
<td>07260102</td>
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<td>active asleep</td>
<td>left</td>
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<tr>
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<td>quiet asleep</td>
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