THE DETECTION OF THE MISMATCH NEGATIVITY (MMN) IN NEWBORNS USING PRINCIPAL COMPONENT ANALYSIS (PCA)

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Master's thesis
June 2001
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CONTENTS

JANI PIETIKÄISEN OSUUDESTA SINIKKA AUVINEN PRO GRADU-TYÖSSÄ JYVÄSKYLÄN YLIOPISTON PSYKOLOGIAN LAITOKSELLE

Olen avustanut Sinikka Auvisen pro gradu-tutkielman tilastollisen analyysin toteuttamisessa. Tilastolliseen analysointiin liittyvää osuutta käytän sopivasti muokattuna tilastotieteen opintoihini.

Jani Pietikäinen

ACKNOWLEDGEMENTS

This study is part of the Jyväskylä Lognitudinal Study of Dyslexia (JLD). The study was financially supported by The Academy of Finland. Contribution of Jani Pietikäinen to the statistical analysis in this study will be used in his studies in statistics. We would like to thank the families and their infants who participated in this study; Paavo H. T. Leppänen and Heikki Lyytinen for accepting to use the infant data; Tomi Guttorm and Heikki Lyytinen for all the instructions during our pro gradu study; and Asko Tolvanen for expert assistance in statistical analysis.

We are especially grateful to Tomi Guttorm for the instructions with EEG-recordings and data-analysis; and to Prof. Heikki Lyytinen (our supervisor) for many valuable comments.

ABSTRACT

This study focuses on investigating the mismatch negativity in event-related potential (ERP) waveforms in newborns. ERPs from 28 subjects were studied using principal component analysis (PCA). In the experiment rarely occurring deviant tones of 1100 Hz (probability 12%) were embedded among repeated standard tones of 1000 Hz (probability 88%) in an oddball-sequence with an interstimulus interval (ISI) 425 ms. In this condition the infrequent stimulus elicited in most newborns a slow positive deflection. The factors extracted by PCA indicated the appearance of a mismatch negativity-like response hidden by the positive response. Significant main effects were found in factors 1 and 2 using multivariate analysis of variance (MANOVA). In factor 1 an interaction between stimulus, electrode positions and hemisphere was found in a latency of 210-420 ms, also in factors 1 and 2 in the latency of 120-260 ms the stimulus main effect was found. These results indicate that a mismatch negativity-like (MMN) response exists in newborns in the latency of about 200-400 ms, in which MMN is elicited in adults in the previous studies.

Keywords: mismatch negativity, event-related potential, principal component analysis, time window, latency, amplitude, interstimulus interval

TIIVISTELMÄ

Tämä tutkimus keskittyy poikkeavuusnegatiivisuuden tutkimukseen vastasyntyneiden herätevasteissa. 28 koehenkilön herätevasteita tutkittiin pääkomponenttianalyysillä. Kokeessa harvoin esiintyviä 1100 Hz:n devianttiääniä (todennäköisyys 12%) esitettiin toistuvien 1000 Hz standardiäänien (todennäköisyys 88%) lomassa oddball-paradigmassa ISI:n ollessa 425 ms. Tässä asetelmassa harvinainen devianttiärsyke sai aikaan useimmilla vastasyntyneillä hitaan positiivisen poikkeaman. Faktorit, jotka saatiin pääkomponenttianalyysillä, osoittivat poikkeavuusnegatiivisuuden kaltaisen vasteen, joka peittyi positiivisen vasteen taakse. Merkitsevät päävaikutukset löydettiin faktoreilla 1 ja 2 MANOVA:lla. Faktorilla 1 interaktio stimuluksen, elektrodipositioiden ja aivopuoliskojen välillä löydettiin latenssilla 210-420 ms, myös faktoreilla 1 ja 2 latenssilla 120-260 ms löydettiin ärsykkeen päävaikutus. Nämä tulokset osoittavat, että poikkeavuusnegatiivisuuden kaltainen vaste esiintyy vastasyntyneillä latenssilla n. 200-400 ms. Poikkeavuusnegatiivisuus esiintyy 200-400 ms:n latenssilla aikuisten EEG käyrässä edeltävissä tutkimuksissa.

Avainsanat: poikkeavuusnegatiivisuus, herätevaste, pääkomponenttianalyysi, aikaikkuna, latenssi, amplitudi, stimulusten välinen intervalli

1. INTRODUCTION

In our study we investigate newborn electroencephalogram (EEG) data. EEG is the record of the potential differences between electrodes placed on the human scalp. EEG consists of periodic voltage fluctuations, which can be classified according to their frequency content. Four patterns of EEG activity are present in neonates: tracé alternant (TA), high-voltage slow (HVS), low-voltage irregular (LVI), and mixed (M). TA is characterized by an alternating backround EEG pattern with 2-4 s bursts of high voltage (amplitudes up to 200 μV) slow theta (4-7 Hz) and delta (slower) activity mixed with continued, low-voltage (amplitudes 20-40 μV) activity in alpha (8-13 Hz) and beta (>13 Hz) frequencies, as well as some isolated theta waves with the duration of 4 s (Stockard-Pope et al., 1992). The HSV pattern consist of fairly continuous, diffuse, and moderately rhythmic EEG activity. Dominant frequencies are in the theta band mixed with frequent delta, with amplitudes of 50-150 μV and frequency of 0.5-4 Hz, and some alpha and faster EEG rhythms. LVI is characterized by a low-voltage (20-50 μV) mixture of theta and beta activity with alpha ripples. It is quite similar in all scalp regions and shows little variability during an epoch. Frequencies vary between 1-8 Hz. The M pattern consists of both HVS and LVI components which are intermingled with little periodicity. The amplitude is usually lower (40 to 100 μV) than seen in the HVS pattern (Stockard-Pope et al., 1992).

EEG records can be classified into two categories, the background EEG and the event-related potentials (ERP). ERPs are discrete waveforms that are associated with an event. They are hidden in the background EEG, so that they cannot be directly detected. Basic difference between background EEG and ERPs is that the former doesn't have a temporal relation to the event and the latter does. Averaging of discrete time epochs will thus result in the visibility of the ERPs. ERPs can then be plotted so that they manifest the underlying ERP components, of which the total potential consists of. Subtraction methods (subtracting an ERP waveform elicited in one condition from that

of another condition) have often been successfully used in this connection to reveal the underlying component structure of the ERPs elicited. In addition, certain statistical methods, such as the principal-component analysis (Donchin & Heffley, 1978; Glaser & Ruchkin, 1976) have also been used to disentangle components of the recorded ERP waveform.

When studying ERPs, the first important distinction to be made is between a component, on the one hand, and a peak, deflection or waveform, on the other hand. Peaks are usually defined observationally using characteristics such as sequence, polarity and latency. In the studies of Donchin and Heffley the advantages and disadvantages of the peak analysis are discussed. A peak is defined a largest or smallest amplitude value in the latency of the component being measured. Peaks are expressed as the difference between successive peaks (e.g. N100-P300), or the difference between the peak and the average of several points (e.g. N100 – baseline). An essential issue to notice is that in peak analysis unlike in PCA, peaks may contain components that do not account for the peak. The advantages of the peak analysis are intuitiveness, the fact that peaks are easy to obtain from X-Y plots and an independence of differences in latencies of components across ERPs. The disadvantages of the peak analysis are the experimenter bias, difficulty of reference peak and component overlap (e.g slow wave) (Donchin and Heffley, 1998). Peak analysis may also lead to lack of support for visual inspection by statistical tests. This may occur for example when there are two positive deflections, e.g. at P450 and P750 (van Boxtel, p.89, 1998). The statistical analysis requires large time window to obtain results.

In our study we focus on detecting the mismatch negativity (MMN) from the newborn EEG. Crucial to any understanding of the MMN is some idea of what constitutes ´standard´ and ´deviant´. Two processes underlie the brain´s distinction between standars and deviants: parsing the incoming information into units and categorizising these units in terms of their probability of occurrence. The units may vary with the type of information being received and with the sensory ability of the receiver. Although the deviant is most typically a deviant stimulus, it may also be a deviant feature or, when

defined by multiple stimuli, a deviant event. However it is defined, the variant is less probable than the standard. In order for the MMN system to recognize that must be a memory of the standard (Picton et al., 2000). Näätänen (1992) considered the relevant memory to be sensory memory. However, two levels of representation seem to be involved: representations of the recent acoustic past and representations of regularities or invariances extracted from what is available in sensory memory (Schröger, 1997; Ritter et al., 1998; Winkler et al., 2001). Sensory memory provides the raw data from which invariant aspects of the stimuli are extracted. The extraction of the rules governing the stimuli might occur in sensory memory or in some other system to establish a representation of invariances remains to be determined. Once a representation of invariance has been establish a stimulus that violates that representation can elicit an MMN. Five kinds of invariances can be considered: simple, complex, hypercomplex, patterned or abstract (Picton et al., 2000). In our study simple invariance is studied. Simple invariance involves situation wherein the standard stimuli are all identical in every possible way (frequency, intensity, duration, location, etc.). Infrequent deviant stimuli that differ in any discriminable manner from the standard stimuli elicit an MMN, indicating that representations of invariance for all the acoustic parameters are established. The classic example is a simple oddball paradigm, such as was used in the discovery of the MMN (Näätänen et al., 1987). Although the standards may be simple, they can also be complicated in structure. For example, they may be phonemic stimuli composed of different formants (Näätänen et al., 1997). What is crucial is that all standards are identical (Picton et al., 2000).

The MMN is generated by an automatic comparison process in which new stimuli are compared with a neural memory trace formed by the repetitive, standard stimulus (Näätänen, 1992). In other words, The MMN probably reflects a cerebral mismatch process triggered by the deviant sensory input in an automatic comparison process with a neuronal memory trace left by the repetitive, standard stimulus (Näätänen, 1992). To define more presicely, MMN is not just a response generated by new, non-refractory afferent elements activated by an occasional infrequent stimulus. This statement by Näätänen and his colleagues (see e.g., Näätänen et al., 1978; Näätänen, 1992) is backed

up by several pieces of evidence: first, MMN is neither elicited by the first stimulus in a series (Cowan et al., 1993), nor it is obtained with the very long ISI or when the deviant stimuli are presented alone without intervening standard stimuli. (Näätänen, 1985; Näätänen et al., 1987; Lounasmaa et al., 1989; Mäntysalo & Näätänen, 1987; Sams et al., 1985b). Second, MMN can be elicited by the omission of an element of a compound stimulus or of the second of two paired stimuli if the ISI is short (Yabe et al., 1997). Third, the MMN latency and duration are relatively long for minor stimulus changes, which is atypical to the afferent responses (Näätänen et al., 1989).

During the last two decades, MMN has been one of the most intensively studied ERPs. However, despite numerous studies conducted in adults, investigations in children and especially in infants are still quite rare (Cheour, 1998). However, the few studies investigated infant MMN have shown the appearance of the infant MMN. An important issue to notice in the infant study is the fact that MMN do not demand subjects attention to the stimuli presented to them and are therefore ideal for studying children (Cheour, 1998). In the very first infant MMN study Alho et al., 1990 studied neonates in quiet sleep when frequent 1000 Hz and infrequent 1200 Hz tones were presented to them. The deviant tones elicited fronto-centrally largest MMN-like response that peaked at the latency of 200-400 ms. Importantly, when the deviant stimuli were presented alone, without intervening standard stimuli, no MMN-like response was elicited. This finding supports the idea that the negativity found in response to deviant tones occurring among standard tones was indeed MMN, which is generated by a comparison of a deviant input with a memory trace formed by a standard stimulus (Näätänen et al., 1989). For example, in the study of Cheour et al., 1997 an infrequent wovel (deviant) presented among frequent vowels (standard) elicits in awake 3-month-old infants a negativity in the auditory event-related potential resembling the mismatch negativity elicited by the same stimuli in previous studies in adults (Aaltonen, Niemi, Nyrke, & Tuhkanen, 1987) and newborns (Cheur-Luhtanen et al., 1995 and Cheour et al., 1997).

According to Courschesne (1990) the MMN response maturates quite early in terms of latency and amplitude as compared to the other components of ERPs (Courschesne,

1990). According to Cheour 1998, MMN maturates exceptionally early as compared to other components of ERPs, and, furthermore, that it is the ontogenetically earliest discriminative response ever recorded from the human brain (Cheour, 1996). MMN amplitude begins to grow soon after birth since in 3-month-old infants it tended to be larger than in neonates. Cheour et al., 1997 have found that the mismatch negativity amplitude for the deviant vowels was larger and the event-related potentials to standard stimuli were in general more positive in 3 month-old infants than in newborns, which might reflect increased maturation of their nervous system relative to that of newborns (Cheour et al., 1997). The MMN amplitude rapidly changes between ages of 6 months and 1 year. MMN is usually larger amplitude in children than in adults (Csépe et al., 1995; Kraus et al., 1992; 1993; Sharma et al., 1992). To be more precise the greatest difference between the MMN in adults and children seems to be that there is much larger inter-individual variation in the MMN amplitudes, and especially in the MMN latencies, in infants and even in school-age children than in adults (Cheour, 1998). Hence, like the amplitude of many other ERP components the MMN amplitude seems to follow an inverted U-shaped function as maturation proceeds (Cheour, 1998). According to Cheour 1998, the responses to standard stimuli became more positive the older the infants were (Cheour et al., 1994; Cheour et al.,1997; Cheour et al., 1996; Cheour et al.,1998). This result is in line with previous studies (Weitzman & Graziani, 1968 Kurtzberg et al., 1984; Novak et al., 1989) demonstrating that ERPs change from predominantly surface-negative to predominantly surface-positive response.

Pihko et al., (1999) studied how the brain's detection of speech sound duration change is reflected in ERPs at birth and how development affects these ERPs. Pihko et al. 1999 reported on data obtained from two age groups, partly comprising of the same infants, first at birth and then at the age of 6 months (Pihko et al., 1999). The slow positive standard /kaa/ and short deviant /ka/ stimuli elicited ERPs with slow positive deflection peaking about 300ms after the stimulus onset in the newborns. The response to the deviant stimulus differed significantly from that to the standard stimulus only at the F4 site, the deviant response being more positive at a range of 280-325ms, but only in the at-risk group ($ts > 2.3$, $ps > 0.05$). The ERPs of the 6 month-old infants had a clear

negative-positive-negative waveform at this age the groups differed between each other in their responses to the standard /kaa/ at C4 (405-525ms) and P4 (405-450ms); ts > 2.1, ps 0.05), the amplitude of the at-risk group being more negative. The responses to the long standard /kaa/ and short deviant /ka/ stimuli differed significantly from each other in both control and at-risk groups. The changes in the ERP waveforms in the study of Pihko et al. 1999 from a slow and wide spread positivity at around 300ms in newborns to a sharper negative-positive-negative waveform at 6 months show maturational changes in the ERPs and are in line with previous experiments with full-term neonates and young infants (Leppänen et al., 1997; Kurtzberg D. et al., 1984; Thomas DJ and Crow CD, 1994). That the general waveform changes seen between the ERPs of the newborns (in quiet sleep) and those of 6-month-olds (awake) is not due to different arousal states, is shown by the fact that newborn ERPs in these corresponding states resembled each other to a great extent (Pihko et al., 1999).

Even though MMN-like response has been recorded both in awake children (Csépe, Diekmann, Hoke, & Ross 1992; Korpilahti & Lang, 1994); Kraus et al., 1993; Kraus, McGee, Sharma, Carrell & Nicoll, 1992; Leppänen, Laukkonen, & Lyytinen, 1992) and in sleeping newborns (Alho, Sainio, Sajaniemi, Reinikainen, & Näätänen, 1990; Cheur-Luhtanen et al., 1995; Kurtzberg et al. (1995) in response to a frequency change of a tone as well as to a change in a repetitive phonetic stimulus (Cheour 1997), we only pay attention in the present study in quiet sleep state in newborns. Quiet sleep is characterized by behavioural quiescence with only occasional body movements, which may resemble startles, or episodes of mouth and clonic chin movements (De Weerd, 1985; Lombroso 1981; Stockard-Pope et al., 1992). Rapid eye movements are absent, except for rare single movements. Respiration and physiological activities are regular and the EMG is considerably increased (Cheour et al., 1998).

The detection of the MMN in newborn EEG is not unproblematic. According to Leppänen et al. (1997) the ERPs with a positive polarity most likely represent a typical normative response in full-term newborns to auditory stimuli (Leppänen et al., 1997). Such positive responses have earlier been reported to auditory stimuli presented with

rather long ISIs (e.g., Barnet et al., 1975). Due to the positivity of newborn EEG the MMN of the EEG waveform is rather difficult to extract. A way to approach this problem according to Leppänen et al., 1997 is to study the reduction of positivity in which the mismatch response is reflected (Leppänen et al., 1997). Some evidence was found for a possibility that the positive response would be overlapped by smaller negative component reflecting a mismatch process, which requires discriminable difference between tones, and that strong enough neural representation of one of these tones is developed (Cowan, Winkler, Teder, & Näätänen, 1993). MMN is, then, elicited by deviation or a change of stimulus in relation to this neural representation. Leppänen et al. (1997) suggest that such a mismatch process is reflected in a small negative peak of the difference wave in few participants in their study, and possibly in a reduction of the positive amplitude in most participants (Leppänen et al., 1997). Further Leppänen et al. (1997) found that this reduction occurs at the latency of the MMN-like response reported earlier by Alho, Sainio, et al. (1990). The study of Leppänen et al. (1997) is in line with the finding of Cheour-Luhtanen et al. (1995), in which the MMN-like negativity was smaller, when the deviation was of a smaller magnitude. Thus, in this view the MMN response of newborns would be enhanced with the increase of stimulus difference, which is, clearly, the case in adults (Sams, Hämäläinen, et al., 1985). At first sight, it may thus seem that a greater stimulus difference (30%) is required for the MMN response than for identifiable from the overlapping positive deflection occurring with a greater stimulus difference (Leppänen et el., 1997). In our study we assume that the MMN is overlapped by the positivity in difference waveform.

We use principal component analysis as a methodological tool to study newborn EEG. Our aim is to show and locate the variance of the data and an attempt to detect the MMN using PCA. The use of PCA as tool for the study of ERPs was advocated by Donchin (1966). The reason we decided to analyse our data using PCA is based on previous studies by Guttorm (1999, 2001). According to Guttorm (2001) the typical traditional analyses of ERPs examine stimulus specific or group differences searched from the latencies or time ranges based on the visual identification of the largest deflection on the basis of grand average waves. Grand averaging across individual data

may, however, confound important information, especially in infants, in whom there is greater inter-individual variability. The PCA procedure is an effective way to identify the initial variance in the data, and it may also provide an objective procedure to locate the response latencies of interest (Guttorm et al., 2001). On the basis of that study we assume PCA could be a useful method compared to averaging.

In general, PCA is one of the techniques under the general label of factor analysis. Common to all these techniques is that they intend to describe the complex relations between a large number of variables and the variables themselves in terms of lesser number of hypothetical, unobserved, latent variables. PCA differs from these other techniques in that the factors extracted are closely related to the grand mean of the original dependent variables. The time points are used as variables for the PCA. In PCA, each principal component is a weighted linear combination of all the original dependent variables. These components should be considered as theoretical constructs of the data. As many components may be extracted as there are dependent variables (van Boxtel, 1998). Furthermore, the principal components are extracted from the data set in hierarchical fashion: The first component accounts for the largest proportion of the variance in the data, and the successive components must be both orthogonal to the preceding ones and account for the largest portion of the residual variance. For typical ERP data, this percentage drops off rapidly after the first five or six components, which usually account for 90-95% of the variance in the data. Besides reducing the often huge amount of data that is usually collected in typical ERP measurements, the promise of PCA is that it gives insight into the unobserved, theoretical components from the observed measurements recorded at the scalp. The PCA procedure itself is blind to individual experimental conditions and generates the same solution regardless of the order on which the ERPs are entered. The strength of PCA is expected to be in the fact that it does not require interesting latencies before the analysis (van Boxtel, 1998).

As stated, we investigate newborn electroencephalogram (EEG) data and focus on detecting the mismatch negativity (MMN) from the newborn EEG. We only pay attention in the present study in quiet sleep state in newborns. We assume that the

MMN is overlapped by the positivity in difference waveform. Principal component analysis is used as a methodological tool to study newborn EEG. Our aim is to show and locate the variance of the data and an attempt to detect the MMN by PCA.

2. METHODS

2.1. Participants

This study is part of the larger lognitudinal JLD-project (Jyväskylä Lognitudinal Study of Dyslexia). The participants in this study are the same newborns as they are in the study reported by Leppänen, Eklund and Lyytinen (1997). In the experiment were twenty-eight healthy newborn participants (16 males, 12 females) included. Originally, the ERPs of 49 newborns were recorded, but data from 21 participants were excluded because either of no data or not enough artefact-free data (see following) were obtained during quiet sleep. The remaining 28 participants had a mean gestational age (GA) of 40.1 weeks (SD=1.44, range: 37-42 weeks) and a mean birth weight of 3,742,5g (SD 461.1). One-min and 5 min Apgar scores averaged 8.71 and 8.96, respectively (SDs 0.38.6/42.7 weeks 53 and 0.19, respectively). The infants were tested within 34-157 hr (1/6 days) from birth, except for 3 participants, whose GA was below 38 weeks. They were tested at about 40 weeks post conceptional age (and thus within 14-23 days from birth). Thus, the mean conceptional age (CA) at the time of measurement was 40.8 weeks (SD 1.10, range: 38.6-42.7 weeks). All these infants participated in the 1100-Hz-deviant condition of the experiment (Leppänen et al., 1997).

In the study of Leppänen, Eklund and Lyytinen (1997) thirteen of these 28 participants participated in addition to the main experiment, in two control conditions (see Leppänen et al., 1997). The control conditions were performed in order to test whether any significant differences were found in any of the characteristics (see Leppänen et. al., 1997) in an independent two-tailed t-test between the infants who participated in these additional control conditions and the rest of the 28 participants. No such significant differences were found (Leppänen et al., 1997). In our study we focus on the main experiment reported above.

2.2. Stimuli

The stimuli was the same as reported by Leppänen, Eklund and Lyytinen 1997. The stimuli were sinewave pure tones. In the 1100-Hz-deviant condition these were presented in an oddball-sequence, in which an infrequent deviant stimulus of 1100 Hz (probability 12%) was embedded among a repeated frequent standard stimulus of 1000 Hz (probability 88%). The duration of the stimuli was 74 ms (rise and fall times of 24 ms each) and the intensity was 75 dB SPL, calibrated before the experiments using Brüel and Kjaer precision sound level-meter (Type 2235). Four different pseudo random oddball-sequence were created. Each had at least 5-10 standard tones between any two subsequent deviant stimuli. This was done in order to ensure enough repetition of the same stimulus for the formation of neural representation. All four sequences consisted of 65 deviant stimuli. The constant ISI (onset-to-onset) for all stimuli was 425 ms. These sequences were delivered in a random order, but so that two same sequences were never presented in succession.

These blocks of the 1100-Hz-deviant condition alternated with syllable-duration condition blocks in which two consonant-vowel syllables varied (the standard /kaa/ stimulus having a duration of 250 ms and the deviant /ka/ 110 ms; the fixed offset-to-onset ISI was 425 ms). The presentation order of the blocks was counterbalanced between participants. As stated earlier, only the data from the 1100-Hz-deviant condition are reported here.

2.3. Procedure

The experiments were conducted at the neurophysiological laboratory of the Central Hospital of Central Finland in Jyväskylä. The parents were invited to observe the experiments if they wished. The experiments were conducted in a dimly lit EEG-laboratory room. The infants were lying in a slightly reclined position (5.3°) in a crib designed for the purpose, in which the mobility of the infant's head was minimized by a small pillow. The auditory stimuli were delivered through a loudspeaker located at the foot of the crib 39 cm above the bed level and 60 cm from the estimated head position of the infant (the angle between the loudspeaker-head line and bed level was 41°). The recordings were suspended when the infant was either crying or moving excessively (Guttorm, 1999).

The EEG was recorded using disposable Ag/AgCl-electrodes (Blue sensor, Medicotest, Denmark), which were attached to the frontal (F3, F4), temporal (T3, T4), central (C3, C4), and parietal (P3, P4) scalp sites, according to the International 10-20 electrode system. These EEG electrodes were referred to the ipsilateral mastoid, except T3 and T4. All of the electrodes were referred to the corresponding electrode site over the opposite hemisphere (bipolar derivations). However, only the data from monopolar derivations are reported here. The electro-oculogram (EOG) was recorded with two electrodes, one slightly above and lateral to the left eye and the other below the right eye. These EOG electrodes were referred to the left mastoid. A ground electrode was placed on the forehead. ECI Electro-Gel (Electro-Cap International, Inc., Eaton, USA) was used as an electrolyte. The electrical resistance or impedance of the electrodes before measurement was < 10 kiloohms (k), except for five participants where the impedance of the single channel exceeded 10 k. The EEG was recorded and signals were amplified by Nihon Kohden Neurofax EEG-5414K. The EEG-epochs were recorded in the time window from 950 ms before to 950 ms after stimulus presentation, and were stored at the temporal sampling rate of 200 Hz. The time constant was 0.3 and the high frequency filter was 35 Hz. AC-filtering was on (Guttorm, 1999).

2.4 Analysis of ERP data

2.4.1. Sleep state classification

The sleep stages have a considerable influence on cortical auditory responses (Duclaux et al., 1991). For this reason the sleep stages were controlled by classifying the EEG-epochs into four categories according to the infants' sleep states (wakefulness, active sleep, quiet sleep, or indeterminate state). The states were defined according to behavioral criteria defined in the sleep-state scoring manual by Anders, Embde, and Parmalee (1971). In addition, eye movements were monitored at the EOG-channels from the ongoing EEG. Each 1-min. period of the measurement was classified as one of the states. The behavior of the infant was observed and coded on-line during the assessment for the classification of the sleep states, which was done off-line after the measurement. The procedure was the same as in the study reported by Leppänen, Eklund, and Lyytinen (1997), where the interrater agreement of the on-line-coding of the infant's behavior (eyes open or closed, facial or body movements, crying, etc.) between two independent observers was 95 %. This was calculated from the data of five randomly chosen participants and was defined as the percentage of the total number of EEG-epochs that the two observers agreed upon. The comparable interrater agreement of the classification of the EEG-epochs into four sleep states was 92 % (Guttorm, 1999). Only the data classified as quiet sleep are reported in our study.

2.4.2 Principal component analysis

Now we have a presentation of the original data in terms of factor scores. These scores can be subjected to further analysis in order to explain sources of variability. In this

study, the primary interest is actually in the overall variance, when we are analyzing the difference waves, and in possible variance between electrode positions and hemisphere. When analyzing both stimuli, variance between these is of course of a great interest.

In the beginning there was a file mrgpit1.sav with window -950- +950. Timepoints (with 5 ms increments) are used as variables. Standard file consist of variables tn 475- tp 000. Deviant file consist of variables tn 050 - tp 425. Variable names of standard file were renamed to correspond the ones of deviant file. Two files were created for standard and deviant stimuli. These specific files were merged. The stimulus variables were named as stim1 and stim2. PCA was done using covariance matrix procedure. Thus the factor scores form a presentation of the original data, which can be subjected to further analysis of variance. As a criterion for the number of principal components, the eigenvalue-equals-one rule was used (see, e.g. van Boxtel, 1998). As a border point for the region of variability in each factor, value of 0.7 for the factor loadings was used. The number of principal components was limited to 4.

3. RESULTS

Three factors were extracted accounting for 94.7 % of total variance. Latency ranges at which factor loadings were greater than 0.7 determined the time window for each factor. For example, Factor 1, accounting for 59.6 % of total variance, occurred at time window of 250-420 ms. The maximum factor loading (.935) for Factor 1 was reached at 370 ms. The factor loadings are presented in Fig. 1.

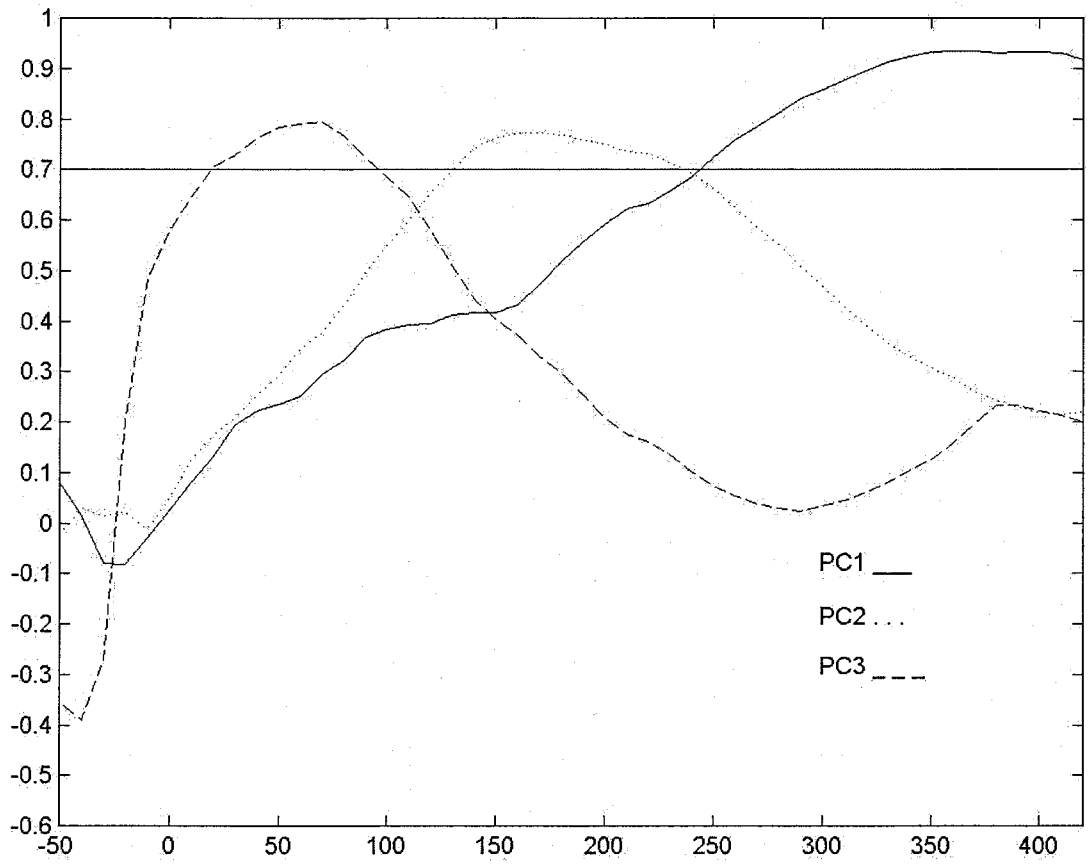

Fig. 1 Principal components.

The multivariate analysis of variance (MANOVA) was done on factor scores. MANOVA assumes the normality of the variables. This was tested by the Kolmogorov-Smirnov-test. This showed that the variables were normally distributed ($p > .05$). The experimental conditions varied across two stimuli (standard, deviant), three electrode positions (central, frontal, parietal) and two hemispheres (left, right). For Factor 1 (250-420 ms), a Stimulus x Electrode Position x Hemisphere interaction was found. A difference contrast indicated that the responses to stimuli differed between frontal and central electrode positions ($F=6,256$, $p < .019$) across hemispheres. Differences occurred at the left hemisphere for the deviant stimulus. For all electrode positions, factor scores were more positive for the deviant stimulus. For Factor 2 (140-230 ms, peak at 170 ms), main effects for stimulus and electrode position were found, ($F(1, 27) = 5.586$, $p < .026$, $\Lambda=.829$ for stimulus, $F(2, 54) = 3.726$, $p < .038$, $\Lambda=.777$ for electrode position). For Factor 3 (20-90 ms), no significant effects were found. Generally, the ERPs showed a slow, positive wave, relating to the difference between responses to deviant and standard stimulus, which increased from the beginning to 300 ms, after which it started slowly decreasing. The difference is seen by comparing deviant and standard stimuli responses averaged for each channel (see Fig. 2.).

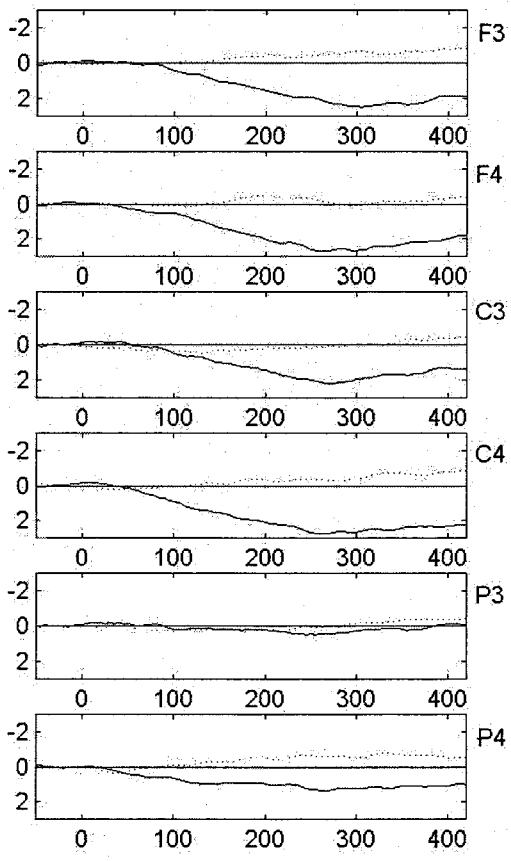

Fig. 2 Mean responses for both stimuli (deviant ___ , standard . .).

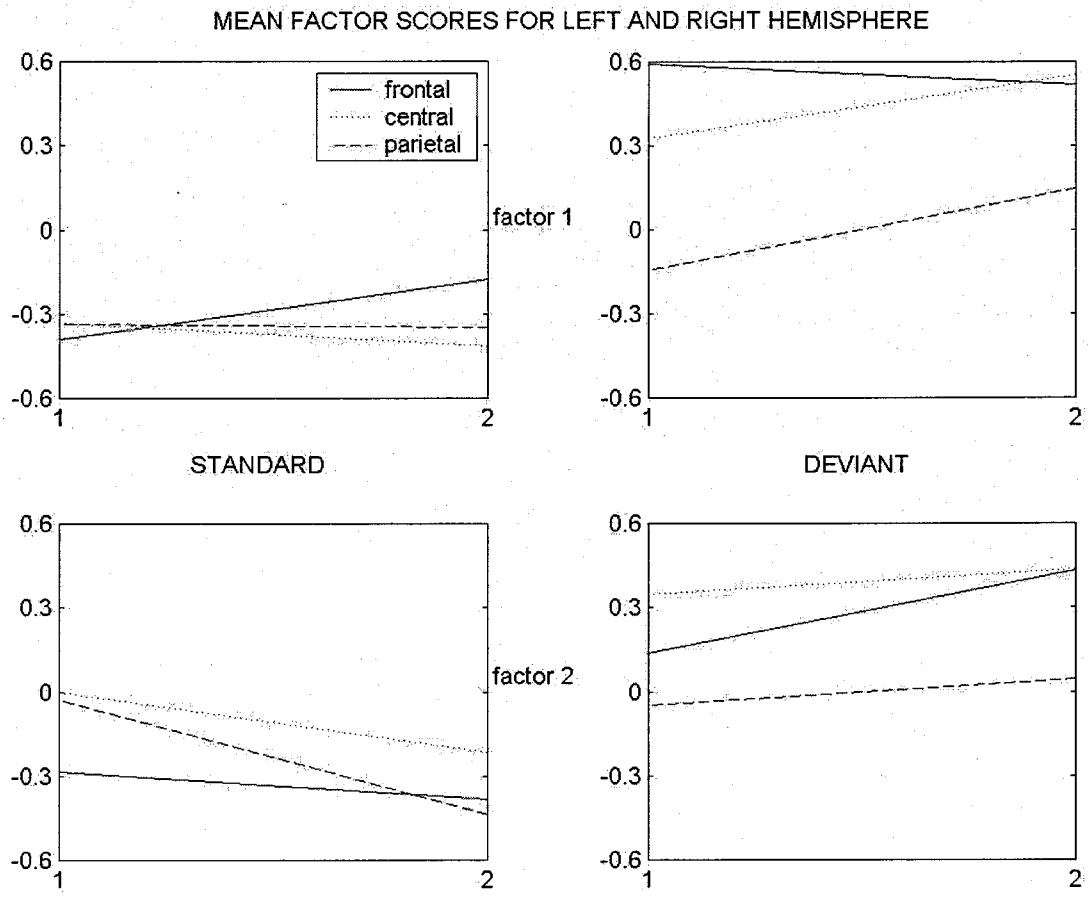

Fig. 3 Mean factor scores for Factors 1 and 2 at different hemispheres (left = 1, right = 2).

Table 1. Analyses of the data.

Factor	**% of total variance**	**Latency (ms)**	**Peak (ms)**	**Manova effects**	**Mean amplitude scores**
1st	59.6	250-425	370	Stim x Elec x Hem	Stim x Elec x Hem
2nd	27.1	140-230	165	Stim	Stim x Hem
3rd	7.9	20-90	70	-	-

Comparison of mean amplitude scores calculated over latency ranges of the three factors revealed similar results than analysis of factor scores (see Table 1.). For latency range of 250-425 ms (Factor 1), a Stimulus x Electrode Position x Hemisphere interaction was found, $F(2,26) = 3.443$, $p < .047$. The contrasts indicate differences between amplitude values at frontal and central channels, $F(1, 27) = 5.425$, $p < .028$. For Factor 2, at latency range of 140-230 ms, a Stimulus x Hemisphere interaction was found, $F(1, 27) = 4.989$, $p < .034$. Analysis of factor scores did not reveal this interaction. However, the p-value was .063 for this interaction when analyzing factor scores.

4. DISCUSSION

The main goal in this study was to explore how PCA would perform in analyzing newborn ERP data. Three different latencies, covering the time point variables almost totally, were extracted and the sources of variation in these latencies were determined. Factor 1 appeared at the latency range of 250-425 ms, Factor 2 at 140-230 ms and Factor 3 at 20-90 ms. In this way, a sensible interpretation of the data was found. The main effects and interactions are in line with previous studies of Leppänen et al. (1997) as can been seen in the following.

In addition to earlier findings by Leppänen et al. (1997), some interesting differences between electrode positions were found in our study. Performing contrasts showed differences between frontal and central channels for Factor 1 ($p < .015$). Mean factor score for frontal channel was greater compared to central channel at left hemisphere for the deviant stimulus, whereas the mean factor score for standard was greater over the right hemisphere. However, paired samples t-tests did not support this finding. Instead, t-tests revealed differences between parietal channels from both frontal and central channels at the left hemisphere. At the right hemisphere difference was significant between parietal and central channels. Results by pairwised tests are in line with earlier study by Leppänen et al. (1997).

Differences in mean factor scores on Factors 1 and 2 for the stimulus effect (see Fig. 2) show that a MMN-like response is present in the data. This response appears in the latencies of 250-420 ms (Factor 1) and 140-230 ms (Factor 2). The peak for loadings of Factor 1, which accounted for 56 % of variance in data, appears at 370 ms. The factor scores for Factor 1 can be used as a measure for the analyses of variance in order to investigate the sources of variation. When using five components instead of three, two factors with negative loadings towards the end of the time window were found. Despite the fact that the loadings were small, and that no sources of variation were found, this finding seems interesting and needs further studies.

4.1 Advantages of using PCA in newborn EEG data

The results of this study confirmed our assumption that PCA is rather suitable method in analysing newborn EEG data. Our aim was to show and locate the variance in the data by PCA from the newborn EEG data. In our study, PCA indicated its strength as a methodological tool by extracting Factor 1, which locates in the timewindow of 260-420 ms, peaking at 360 ms, in which MMN typically exists in an infant EEG data (Alho et al., 1990). In the study by Leppänen et al. (1997) a subtraction method was used as a methodological tool to find a latency for infant the MMN-like response. According to the study of Leppänen et al. (1997) this latency of MMN-like response appeared at about 280 ms. The data in the study of Leppänen et al. (1997) is the same as it is in our study. The reason we decided to use exactly the same data was an aim to compare a traditional subtraction method to PCA. As stated, the results of our study elicited in PCA a maximum loading for Factor 1 at a latency of 360 ms. The use of PCA as a method in our study appears to be theoretically justified by the fact that the MMN needs a methodological tool which has a theoretical basis, as PCA does. Previous studies, for example, Näätänen (1992), Cheour (1998) and Alho et al. (1990) have confirmed the theoretical basis of MMN. According to Näätänen (1992), MMN probably reflects a cerebral mismatch process triggered by the deviant sensory input in an automatic comparison process with a neuronal memory trace left by the repetitive, standard stimulus (Näätänen, 1992). In the very first MMN-study by Alho et al. (1990) neonates were studied in quiet sleep state when frequent 1000 Hz and infrequent 1200 Hz tones were presented to them. As stated earlier, the deviant tones elicited fronto-centrally largest MMN-like response that peaked at the latency of 200-400 ms. Importantly, when the deviant stimuli were presented alone, without intervening standard stimuli, no MMN-like response was elicited. This finding supports the idea that the negativity found in response to deviant tones occurring among standard tones was indeed MMN, which is generated by a comparison of a deviant input with a memory trace formed by a standard stimulus (Näätänen et al., 1989). PCA as a methodological tool has a theoretical basis according to Guttorm (1999, 2001). According to Guttorm, the typical traditional analysis of ERPs examine stimulus

specific or group differences searched from the latencies or time ranges based on the visual identification of the largest deflection on the basis of grand average waves. Grand averaging across individual data may, however, confound important information, especially in infants, in whom there is greater inter-individual variability (Guttorm et al., 2001). In the study by Cheour et al., 1998 the greatest difference between the MMN in adults and children seems to be that there is much larger inter-individual variation in the MMN amplitudes, and especially in the MMN latencies, in infants and even in school-age children than in adults (Cheour et al., 1998). According to Guttorm the PCA is an effective way to identify the initial variance in the data, and it may also provide an objective procedure to locate the response latencies of interest (Guttorm et al., 2001). Therefore, the question whether there is any advantages using PCA in newborn EEG data detecting MMN seems to have given an answer that in some points there is. We found that in the newborn data the MMN was found in the latency of 360 ms by using PCA. The polarity was positive in the latency of 360 ms as the polarity also is in the previous study by Leppänen et al. (1997). According to Leppänen et al. (1997) the negativity is hidden by the slow positive waveform. This finding is likely to gain some support from the PCA in our study with five components extracted, though this phenomenon did not relate to changes in stimuli or electrode positions.

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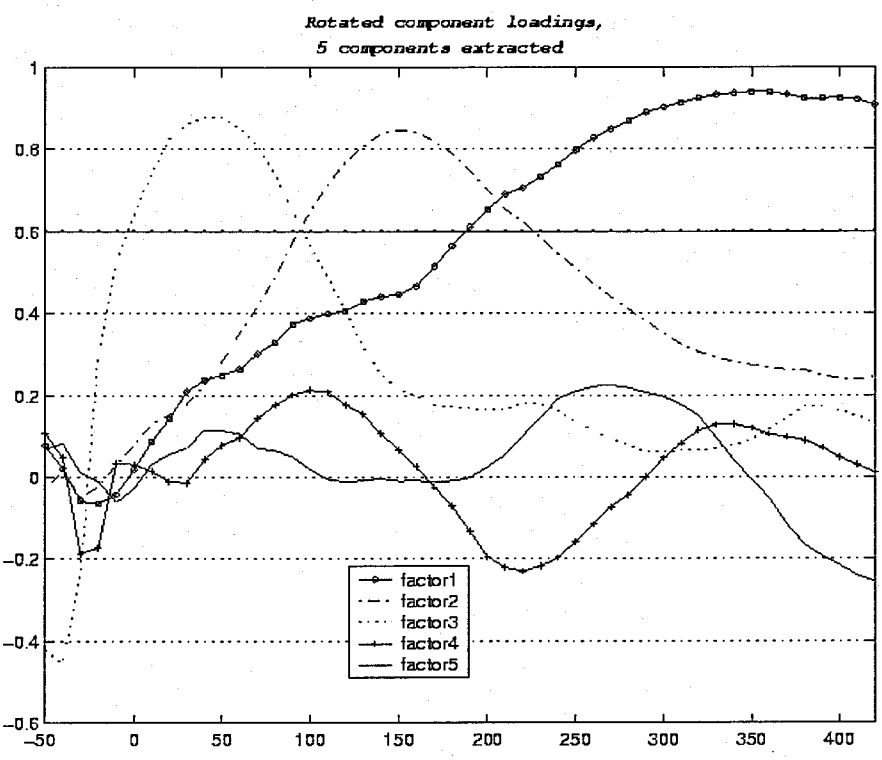
APPENDIX

DATA ACQUISITION

Numerical files containing pitch1-data were formed using Dsamp-command files. The numerical files were transformed to SPSS data file. The two stimuli appear in time window of –950 – 950 ms, so that the standard stimulus is present in the interval –475-(-50) ms and the deviant stimulus in the interval 0-425 ms. Amplitude values were measured in 5 ms intervals. Time points for every 10 ms of each ERP were used as variables. This resulted in 48 variables. The variables were named with concatenated coding; t = time, n /p = negative/positive and number = time point of measurement. Now the variables concerning standard stimulus are tn475, . .,tn050 and for the deviant tp000, . .,tp425. In order to investigate variance between stimuli, the standard variables were renamed to same as the deviant variables (tn475 as tp000, tn470 as tp005, and so on). The number of subjects was 28 the measurement was done using 6 channels for two different conditions. The number of cases treated was thus 336 (28 x 6 x 2).


Rotated component loadings,
5 components extracted
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−50
0
50
100
150
200
250
300
350
400
factor1
factor2
factor3
factor4
factor5