ABSTRACT

There is evidence that the auditory evoked potential (AEP) is altered by malnutrition both in laboratory animals and in humans. The objective of the present study was to determine whether changes in the AEP caused by malnutrition could be reversed by nutritional rehabilitation and sensorymotor and environmental stimulation during hospitalization. Six children aged 5-33 months with severe malnutrition (kwashiorkor, marasmus and marasmic-kwashiorkor) were admitted to the Pediatric Ward of a University Hospital. Normal age and sex-matched children from the hospital day-care center were enrolled as a control group. The AEP was tested in an electrically and acoustically isolated room using a Nicolet CA 2000 microcomputer. Clicks of 90; 80; 70 and 60 dBn HL were presented through earphones. The results suggest that malnutrition leads to an increase in wave I latencies in patients with marasmus, and in waves I, III and V in those with kwashiorkor or marasmic-kwashiorkor type at 90 dB HL. At discharge, all but one patient with kwashiorkor showed reduced latencies of waves I, III and V compared to the values on admission. Despite the small sample, these preliminary results pointed out that the process of sensory stimulation used in our study in a properly directed, systematic and individualized manner showed encouraging results in terms of AEP recovery in these children.

Keywords: malnutrition, infants, nutritional rehabilitation, auditory evoked potential, stimulation.
Many investigators have shown that nutrition is one of the basic factors for the development of the central nervous system (CNS) and that nutritional deficiency impairs CNS functioning (Bedi, 1987; Bedi, Thomas, Davies & Dobbing, 1980; Cragg, 1972; Cravio, Delicardie, & Birch, 1966; Dobbing, 1968; Dobbing & Sands, 1971; Finger & Stein 1982; Lima, 1992; Mourek, Himwich, Myslivec, & Callison, 1967; Quirk, Mejya, Hesse, & Su, 1995; Smart, Dobbing, Adlard, Linch, & Sands, 1973; Sobotka, Cook, & Brodie, 1974; Stein, Finger, & Hart, 1983; Wang & Xu, 2007).

There is evidence since the 1950’s and 1960’s that protein-calorie malnutrition is associated with retarded brain growth (Stock & Smythe, 1963), reduced cerebral cellularity (Winick & Rosso, 1969), reduced myelination (Fishman, Prensky, & Dodge, 1969) and, in the neurophysiological field, changes in electroencephalogram (Engel, 1956; Nelson & Dean, 1959) and evoked potential tracings (Kawai, Nakamura, & Matsuo, 1989; Flinn, Barnet, Lydick, & Lackner, 1993; Hernández et al., in press).

Auditory Evoked Potentials (AEP) are very sensitive measures related to brain functions and have been used by many researchers with diverse objectives in humans, as in the pioneering studies by Hecox and Galambos (1974); and in experimental studies in animals (Buchwald & Huang, 1975; Shipley, Buchwald, Norman, & Guthrie, 1980; Plantz, Williston, & Jewett, 1981).

It has been shown that early malnutrition (marasmus and kwashiorkor) produces marked alterations in the electrophysiological parameters of AEPs (Barnet et al., 1978; Bartel, Robinson, Conradie, & Prinsloo, 1986). The study by Barnet et al. (1978) demonstrated that malnutrition of the marasmic type causes increased latencies of AEP waves that were considered to be irreversible since they were detected not only during the acute phase of malnutrition, but also after a long period of nutritional rehabilitation (12 months). Bartel et al. (1986) detected AEP abnormalities in children with kwashiorkor both during hospitalization and after the period of rehabilitation. The above data suggested that these abnormalities could be due to deficiencies in the myelination process that would result in decreased synaptic efficiency in the auditory system.

Some studies have shown CNS recovery in laboratory animals (Bedi & Bhide, 1988; Renner & Rosenzweig, 1987; Rocinholi, de Oliveira, & Colaêmina, 2001), and it is well known that relatively simple programs of sensory motor stimulation of malnourished children are effective in promoting a better development of these children (Carrazza et al., 1993; Grantham-McGregor, Powell, & Walker, 1991; Grantham-McGregor, Schofield, & Harris, 1983; Grantham-McGregor, Schofield, & Powell, 1987; Grantham-McGregor, Stewart, & Schofield, 1980; Nahar et al., in press; Walker, Chang, Powell, & Grantham-McGregor, 2005).

Studies evaluating electrophysiological parameters have pointed to diverse nervous system compromise of auditory (Durmaz, Karagol, Deda, & Onal, 1999; Vandana & Tandon, 2006), visual (Durmaz et al., 1999; McDonald, Joffe, Barnet, & Flinn, 2007), corticospinal (Karak, Misra, Garg, & Katiyar, 1999), and somatosensory paths (Hesse, Rivera, de Diaz, & Quirk, 1998), as well as inter-hemispheric modulation (Pinto & Guedes, 2008), but no reference to the stimulation effects on evoked potentials in malnourished infants was found in the literature. Thus, the objective of the present study was to determine the effects of malnutrition (kwashiorkor, marasmus and marasmic-kwashiorkor) on AEPs and to establish whether changes could be reversed by nutritional rehabilitation associated with daily and individual sensory-motor and environmental stimulation.

**METHOD**

**Participants**

After approval by the local Committee of Ethics and provision of signed informed consents by the adults responsible for the infants, the study was conducted on six children admitted to the Pediatric Unit of the University Hospital (HC) of Ribeirão Preto, São Paulo University, presenting one of the following types of malnutrition: kwashiorkor (cases N1 and N5), marasmus (N2 and N3) and marasmic kwashiorkor (N4 and N6) according to the classification of McLaren, Pleet, and Read (1967). Data concerning the identification of the children at birth and during hospitalization are presented in Table 1. All patients but one (N4 - 2.300 g - low weight at birth) were born at term with adequate weight for the gestational age, according to Sala (1977). The children were submitted to two AEP evaluations, one at admission and the other just before discharge from the hospital after nutritional rehabilitation. During hospitalization the children were submitted to sensory and environmental stimulation. Six healthy children matched by age and sex from the hospital daycare center were enrolled as controls. Control children were submitted to only one AEP evaluation after investigation of the middle ear by impedance measurement and by the stapes reflex. Subjects with other pathologies interfering with the air or sensorial ear conduction and presenting growth or neurodevelopmental problems were excluded from the study.

**Sensory-motor Stimulation**

Stimulating activities were performed as described by Céledon (1983), taking into account the children’s age and development, evaluated by the Bayley Infant Development Scale (1993), soon after admission to the hospital. Based on the Bayley Scale psychomotor score, each child was assigned to an age group and the stimulation process was started with the activities listed in Table 2.

The children were submitted to daily stimulation sessions of one hour, between 1:30 and 2:30 pm, in a separate room prepared for the stimulation process. Three well-trained professionals were in charge of the stimulation process and received previous training in order to obtain agreement regarding the application of the technique proposed at the beginning of the study. During the first days of hospitalization, before the stimulation program, the three professionals took turns caring for each child, so that he/she would adapt to the new environment (the hospital) and to establish a process of child-stimulator interaction important for the fulfillment of the objectives proposed. Any activity to be performed with the child needed to be accepted by him/her and the sequence of activities was not rigid but depended on the interest and development of each child.
Table 1. Signs and symptoms of malnourished children Mo- months d- days *For the same age and sex of socioeconomic group IV of the table of Marques et al. (1974) **MacLaren et al. (1967) + Present - Absent Kw-kwashiorkor MIII-marasmus degree III Mix - Marasmic-kwashiorkor.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Weight (g)</th>
<th>Expected Weight (g)</th>
<th>Weight (g) deficit</th>
<th>Weight (g) discharge</th>
<th>Edema</th>
<th>Skin lesions</th>
<th>Hair changes</th>
<th>Hepatomegaly</th>
<th>Albamin (g/dl)</th>
<th>Points**</th>
<th>Class</th>
</tr>
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<td>9190</td>
<td>11862</td>
<td>2253</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<td>8278</td>
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<td>6740</td>
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<td>+</td>
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<td>-</td>
<td>6</td>
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<tr>
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<td>8278</td>
<td>5531</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<td>5</td>
<td>M III</td>
</tr>
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<td>7387</td>
<td>4314</td>
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<td>8</td>
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<td>14869</td>
<td>4741</td>
<td>9400</td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>4</td>
<td>10</td>
<td>Kw</td>
</tr>
<tr>
<td>N6 F</td>
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<td>6610</td>
<td>5983</td>
<td>4570</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>6.7</td>
<td>6</td>
<td>Mix</td>
</tr>
</tbody>
</table>

Table 2. Objectives proposed and activities in the process of sensorymotor stimulation of children at different age ranges. DD dorsal decubitus VD – ventral decubitus.

**OBJECTIVES AND ACTIVITIES**

0-3 months

To acquire prehension, visual, auditory integration and cervical control: - hold objects of different shapes, sizes, colors, sounds. DD - follow a light source (large flashlight) and objects in the horizontal, vertical and circular directions. VD - follow light sources and objects in the vertical direction (up and down).

4-5 months

To facilitate object manipulation: - Exploration of different objects; DD: grasp objects at different heights; dorsal decubitus, grasp objects. To acquire schemes of trunk dislocation and balance: - placed now on the right, now the left; DD, with support under the axillae, bring to the sitting position in a movement cadenced on music. To acquisition and reinforcement of sound: DD, rhythmic exercises in limb flexion and extension with the help of the sound, repeating casually emitted sounds.

6-8 months

To reinforce the sitting position and change in decubitus: - sitting up, to manipulate and explore different and new objects. To stimulate crawling: - Sitting up, to turn to a ball thrown now to the left, now to the right. DD, try to reach objects placed now on the left, now on the right. DV, try to reach objects initially near and after more far.

9-12 months

To reinforce hand dexterity: - fit, stack up or group chips locks, rings, beating a drum; hit the keys of a piano with hands and fingers rhythmically imitating the examiner and orders. To place in the standing position and help to walk: - walk with support with two hands, then with one and then holding on to furniture. Acquisition of first words, attitudes: - point and name figures on cards (daddy, mommy, meow, bow wow). To recognize body parts: - Indicate parts of the body on him-herself or on a doll.

13-18 months

To reinforce activities of the previous group, increase coordination, improve fine movements and language: - grasp and arrange balls, stack, fit and group smaller objects, model sand, clay, hug, blow a kiss, say please, say goodbye, dance and mimic.

19-24 months
AEP Evaluation

Following regular otoscopic examination, the infants were evaluated in an electrically and acoustically isolated room. All of them received 16% chlormal hydrate at the dose of 1.2 ml/kg body weight 30 minutes before the AEP test to reduce artifacts of muscular origin to a minimum and thus obtain satisfactory recordings before each evaluation. Brain stem electrical activities were recorded with three disk-shaped Ag/AgCl electrodes using a Nicolet model CA 2000 microcomputer. The positive active electrode (+) was placed on the region of the skull (CZ), the common ground electrode on the frontal region of the head (FZ) and the negative electrode (-) on the earlobe homolateral to the test ear A1 (-) or A2 (-). Before installation of the electrode, the skin was cleaned with detergent and steel wool to remove necrotic and desquamating cells. To facilitate electrical conduction, a gelatinous substance of good electrolytic conduction was applied to the electrodes. The impedance between the electrodes was maintained below 5 kΩ. The sound stimulation was presented through TDH-39 earphones and consisted of monaural rarefaction clicks generated by pulses of 100 μs duration. White noise was presented in the contralateral ear to mask with a differential of 40 dBn HL between the two stimuli. The clicks were presented at stimulation rates of 11.4/sec at intensities of 90, 80, 70 and 60 dBn HL, with a low filter (high - pass) of 150 Hz and high filter (low - pass) of 3000 HZ (12 dB/ octave). The scanning speed was 100ms. Responses to 2000 clicks with a accepted rejection level of up to 20% were averaged and stored in a floppy disk, while wave latencies were simultaneously evaluated with a mobile cursor on the screen with digital reading. The data were plotted using a CITO HM 8510 printer/plotter.

Statistics

A paired t-test was used for the comparison of waves and interpeak latencies at different intensities of dBn HL, and qualitative analysis was performed taking into consideration the small sample.

RESULTS

The mean latencies of the wave I, III and V and the interpeak intervals I-III, III-V and I-V are shown in Table 3 for 70 dBn HL and in Table 4 for 90 dBn HL. Only both levels, 70 and 90 dBn HL, were presented, since the results for 60 and 70 dBn HL were similar, as well as for 80 and 90 dBn HL (t-test p > .05).

Before the stimulation program and the nutritional treatment, patient N3 had a good registry for analysis only at 90 dBn HL, and the latencies of waves I, III and V were lower than those obtained in the control infant. Patients N1 (kwashiorkor) and N4 (marasmic- kwashiorkor, the only one who was small according to the gestational age at birth in the sample) showed increases in latencies of waves I, III and V in both 90 and 70 dBn HL. Patient N2 (marasmus degree III) showed increased latencies of wave I at 90 dBn HL, and of waves I, III, V at 70 dBn HL. Patient N6 (marasmic- kwashiorkor) showed increase in the latency of waves I (90 dBn HL) and V (70 dBn HL); the other latencies were equivalent to those of controls in both levels. Patient N5 (kwashiorkor) showed increased latencies of wave V, and waves I and III similar to control at both 90 and 70 dBn HL. At admission, the interpeak I-III was larger for N4 (both levels) and N1 (90 dBn HL); III-V for N5 (70 dBn HL), N1 and N6 (both levels); and I-V was larger for N6 (70 dBn HL) and N5 (both levels). At discharge, all but one infant (N1 - kwashiorkor) showed decrease in the latencies of waves I, III and V, compared to the values at admission. The latencies of wave I remained above the respective controls for patients N1, N2, N3, N4, and N6 even at discharge (70 dBn HL). At discharge, five patients (N2, N3, N4, N5, N6) at 90 dBn HL and three (N4, N5 and N6) at 70 dBn HL had latencies of waves III or V lower than their respective controls.

DISCUSSION

Evidence that malnutrition causes changes in AEP measurements in children has been provided sparsely in the literature (Barnet et al., 1978; Bartel et al., 1986; Durmaz et al., 1999; Odabas et al., 2005).

Taking into account that the most used AEP reaches the level of the brainstem, studies involving higher paths and cortical areas are necessary, focusing on their vulnerability, damage, and persistence of the effects of malnutrition on higher cortical activity with present and future consequences for development. Flinn et al. (1993) investigated potentials recorded in cortical areas, with the child’s name as a stimulus. They observed that malnourished infants had a lower number of wave peaks and amplitudes than controls, after a nutritional recovery period of about four months.

Although it is not our scope to address specific aspects of the stimulation procedure in this report, the effects of different types of stimuli on behavior (Cabral & Almeida, 2008), and the best results on visual learning when multisensory stimuli are presented in a congruent way (Kim, Seitz, & Shams, 2008) are important issues for stimulation strategies in the clinical field.

As in the present work, in human clinical settings we can not attribute the results exclusively to the environmental and sensory stimulation, since nutritional treatment and medicines were often prescribed. To sort out such variables, methods in animal experiments have been developed. AEP Analysis of handling stimulation without food supplementation in rats (Rocinholi et al., 2001) showed that stimulation alone reduced wave latencies, suggesting the possibility of reversion of some of the damage caused by malnutrition.

Despite different methodologies, it is worth to consider the similarity of our results in the control group to a Chinese sample (Jiang, Zheng, Sun, & Liu, 1991) matched by age with the normative data at 70 dBn HL, as well as to a Brazilian sample (Isaac, 1999) at 90 dBn HL.
Table 3. Latencies of waves I, III, V and interpeak values I-III, III-V and I-V recorded at 90 dB in six malnourished children and controls (C). A – admission; D – discharge; mo – months; d -days.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>I</th>
<th>A</th>
<th>D</th>
<th>III</th>
<th>A</th>
<th>D</th>
<th>V</th>
<th>A</th>
<th>D</th>
<th>I-III</th>
<th>A</th>
<th>D</th>
<th>I-V</th>
<th>A</th>
<th>D</th>
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<tbody>
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<td>N2</td>
<td>6mo27d</td>
<td>2.31</td>
<td>1.72</td>
<td>4.00</td>
<td>3.54</td>
<td>5.93</td>
<td>6.02</td>
<td>1.69</td>
<td>1.72</td>
<td>1.93</td>
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<td>4.34</td>
<td></td>
<td></td>
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<tr>
<td>C2</td>
<td>7mo28d</td>
<td>1.69</td>
<td>4.00</td>
<td>6.36</td>
<td>2.31</td>
<td>2.36</td>
<td>4.67</td>
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</tr>
<tr>
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<td>7mo8d</td>
<td>1.58</td>
<td>1.60</td>
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<td>5.92</td>
<td>5.84</td>
<td>2.44</td>
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<td>1.90</td>
<td>1.92</td>
<td>4.34</td>
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<tr>
<td>C3</td>
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<td>2.31</td>
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<td></td>
</tr>
<tr>
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<td>1.74</td>
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<td>6.72</td>
<td>5.52</td>
<td>2.79</td>
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Table 4. Wave latencies I, III, V and interpeak values I-III, III-V and I-V recorded at 70 dB in six malnourished children (N) and controls (C). A – admission; D – discharge; mo – months; d -days.

<table>
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<th>D</th>
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<td>4.16</td>
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<td>2.06</td>
<td>5.23</td>
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<td>2.37</td>
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<td>2.07</td>
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<td>2.20</td>
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Moreover, considering these two references, we found that among patients aged four to seven months, at admission and discharge, the values of latency of wave I were much higher than those of normal newborns, and that the two oldest patients (20 and 34 months of age) presented latencies of waves III and V around the values observed at 6 months age. Odabas et al. (2005) reported increased wave latencies (mainly for wave I) at all degrees of malnutrition. These findings suggest that malnutrition early in life may have a more marked effect on the caudal myelination of the auditory pathway. Wave I, according to Hecox and Galambos (1974), undergoes a process of maturation at about two or three months of life, and six months according to Isaac (1999). According to Salamy and McKea (1976), the significantly longer latency of wave I in newborns (six weeks of life), as compared to other age groups, suggests that the peripheral acoustic mechanisms are not fully developed in newborns, although it is not clear whether this initial delay in the latency of wave I is the result of immaturity of the middle and inner ear or of neuronal immaturity.

Concerning the interpeak latencies, our findings of kwashiorkorinfants with III-Vand I-Vlatencies longer than those of children with marasmus are in accordance with the findings of Durmaz et al. (1999). Bartel et al. (1986) found I-III, III-V and I-V latencies uniformly distributed among the degrees of malnutrition, studying 22 children, also showing differences between the two ears, which was further supported by Odabas et al. (2005). In the present sample we did not analyze the data for each ear.

The analysis of covariates in malnutrition and AEP studies has been rarely mentioned in the literature. The children’s height was mentioned by Barnett et al. (1978), finding higher AEP waves amplitude in children with shorter stature. Other covariates, such as iron deficiency and albuminaemia, were analyzed by Odabas et al. (2005), showing in malnourished infants with iron deficiency longer than controls the mean latency of wave I on the left side and the mean III-V interpeak on the right side; in malnourished infants without iron deficiency, the mean I-III interpeak on the right ear was longer than controls, and no difference was found between low and normal serum albumin within the malnourished groups.

The SGA (small for gestational age) condition has not been studied among the possible confounding variables in the field of malnutrition. Odabas et al. (2005) mention the exclusion of SGA infants in their criteria for subject selection. In the present study, the five-month-old patient who was born SGA, was the only one presenting increased I-III interpeak interval and above the control values at 70 dBn HL, in both the admission and discharge AEP tracings, suggesting the need to examine the cochlear station since neonatal period for the SGA patients.

According to Bartel et al. (1986), AEP abnormalities were still evident after one year of nutritional follow-up of marasmic children, probably reflecting long-term effects of malnutrition on cerebral function. Durmaz et al. (1999) found different results comparing malnourished infants to controls on admission in a nutritional program and discharge. These authors reported differences at the time of admission, but not at discharge, in line with our results. In spite of the limitations of the present analysis, an interesting finding was the fact that the malnourished group showed lower latencies of waves I, III and V at discharge compared with the latencies at admission, and in three patients (one kwashiorkor) the latencies of waves III or V at discharge were lower than those of their respective controls. Thereby, we can suggest that the process of sensory and environmental stimulation may have been effective in bringing about a recovery from or prevention of the effects of malnutrition.

In conclusion, the present study suggests that the process of sensory stimulation used in a properly directed, systematic and individualized manner showed encouraging results in terms of AEP recovery in these children. Even if they attend day-care centers using some type of stimulation, these children are exposed to a more collective and not individually directed process.

Acknowledgements
The authors owe debts of gratitude to Psychologist Dorothy Bono, Dr Naul Motta de Souza, and the staff of the Pediatrics Ward at the University Hospital of Ribeirão Preto, São Paulo University.

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