Early malnutrition decreases contrast sensitivity to circular concentric gratings

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Abstract
Early malnutrition refers to inadequate nutrition during the critical period of nervous system development followed by nutritional recovery, resulting in a short stature according to age but normal weight according to short stature. We measured the effects of early malnutrition on contrast sensitivity (CS) to concentric circular gratings in 18 children of both sexes, aged 8 to 11 years (mean = 9.2 years, standard deviation = .99 years). Nine of the children were eutrophic (E group), and nine experienced early malnutrition (EM group) based on state healthcare records and Waterlow’s anthropometric parameters. Contrast sensitivity to four spatial frequencies (.25, 1.0, 2.0, and 8.0 cycles per degree [cpd]) was measured using a temporal two-alternative forced-choice psychophysical method with mean luminance of 40.1 cd/m². Statistical analyses showed significant differences between groups and a group × frequency interaction. EM group was significantly less sensitive than the E group to the 8.0 cpd frequency and needed 1.49-times more contrast to detect the gratings. These results suggest that early malnutrition impairs CS to high-spatial-frequency concentric circular gratings in children. Therefore, early malnutrition, which is known to affect primary visual cortical areas, may also affect higher visual cortical areas such as V4 and the inferotemporal cortex. Keywords: early malnutrition; non-Cartesian stimuli; contrast sensitivity; extrastriate processing.

Introduction
Malnutrition is responsible, either directly or indirectly, for the death of more than 6 million children per year (Bryce, 2002; World Health Organization, 2005; Black, Morris, & Bryce, 2003; Caulfield, Onis, Blossner, & Black, 2004; United Nations Children’s Fund, 2007) and has a profound, nonlethal impact on development. It represents the main environmental factor that contributes to mental retardation and permanent changes in fetal and neonatal development (Galler, 2001; Morgane, Mokler, & Galler, 2002). Malnutrition contributes as a latent cause to one in two deaths (53%) in children <5 years of age (World Health Organization, 2005), totaling over 6 million per year (United Nations Children’s Fund, 2007). According to Black et al. (2003), this corresponds to approximately 16,000 children who die each day, one every 5 s.

Proper nutrition is essential for the structural and functional maturation of the central nervous system (Winick, 1975; Morgane et al., 1978; Noback & Eisenman, 1981; Díaz-Cintrón, González-Maciel, Morales, Aguilar, Cintra, & Prado-Alcalá, 2007). Deficits in protein intake result in a shortage of intake of amino acids necessary for the protein synthesis of structural enzymes, neuropeptides, and neurotransmitters (Galler, Shumsky, & Morgane, 1996). Moreover, long-chain polyunsaturated fatty acids (Morales, Hoffman, Castañeda, Wheaton, Burns, & Birch, 2005), omega-3 (Uauy, Birch, Birch, & Hoffman, 1992; Uauy, Hoffman, Birch, Birch, Burns, & Hoffmann, 2003), and docosahexaenoic, arachidonic, and linoleic acids (Berseth et al., 2011; Scalabrin et al., 2011; Uauy, Hoffman, Mena, Llanos, & Birch, 2003), among others, are essential for normal human retinal development. Deficits in these in an infant’s diet are linked to deficits in vision function.

Both behavioral (Santos, Alencar, & Dias, 2009; Santos & Alencar, 2010) and electrophysiological
Evidence from Hoffman et al. (1993; Birch et al., 1992; Uauy et al., 1990; Morale et al., 2005) indicates that inadequate nutrition during development leads to losses in sensitivity to traditional Cartesian sinewave luminance gratings. Does malnutrition affect all of the visual centers in the cortex? Following the lead of recent electrophysiological studies of the primate cortex, we speculated that non-Cartesian grating stimuli may help answer this question.

Prior research indicated that complex stimuli such as non-Cartesian gratings are processed in visual centers beyond V1, such as V2 and V4 (Gallant, Connor, Rakshit, Lewis, & van Essen, 1996; Merigan, 1996; Wilkinson, James, Wilson, Gati, Menon, & Goodale, 2000). David et al. (2006) showed that the majority of neurons in V1 are selective for Cartesian gratings, whereas the majority of V4 neurons are selective for non-Cartesian stimuli. Distinct subpopulations that comprise 16–17% of V4 cells respond at least twice as strongly to concentric, radial, and hyperbolic stimuli than to sinusoidal gratings, whereas very few cells (0–8%) respond best to sinusoidal gratings (Gallant, Braun, & Van Essen, 1993; Gallant et al., 1996). Cell selectivity decreases for Cartesian gratings and increases for non-cartesian gratings from V1 to V2 (see Figure 1 in Wilkinson, James, Wilson, Gati, Menon, & Goodale, 2000).

According to Hegde and Van Essen (2003), however, V2 cells on average respond better to grating stimuli. They are more selective for contour stimuli such as angles, intersections, arcs, and circles. Hegde and Van Essen (2007) found that the percentages of cells that respond better to non-Cartesian gratings than to Cartesian gratings were 65% for V4 and 61% for V2. Cartesian/non-Cartesian selectivity is apparently not an emergent property of V4 cells (Mahon & DeValois, 2001). Hegde and Van Essen (2007) also found that some V1 cells with a relatively large receptive field showed a clear preference for complex contours over simple bars and gratings.

Although several studies found differences in the visual system caused by malnutrition using physiological measurements in animals (Cragg, 1872; Warren, & Bedi, 1982; Gundappa, & Desiraju, 1988; Bedi, Massey, & Smart, 1989; Diaz-Cintra, Cintra, Ortega, Kemper, & Morgane, 1990) and postmortem in humans (Benitez-Bribiesca, Rosa-Alvarez, & Mansilla-Olivares, 1999), fewer behavioral studies have investigated these changes in humans.

Based on prior research indicating that non-Cartesian gratings are processed with increasing selectivity in visual centers beyond V1, such as V2 and V4 (Gallant et al., 1996; Merigan, 1996; Wilkinson et al., 2000), we sought to extend our prior results that examined the effects of early malnutrition in children using Cartesian sinewave gratings (Santos et al., 2009; Santos & Alencar, 2010) by examining contrast sensitivity (CS) to non-Cartesian gratings in children who experienced malnutrition.

**Material and Methods**

**Participants**

The study included 18 children (10 males, 8 females), 8 to 11 years of age (mean = 9.2 years, standard deviation = .99 years) with normal visual acuity (inclusion criterion). They were tested with directional “E” Rasquin optotype cards (Xenônio, São Paulo, SP, Brazil) and were free of eye diseases or neurological disorders other than those that are malnutrition-related (exclusion criterion). The participants were recruited from the Eradication of Child Labor Program in the Santa Clara community, Castelo Branco, João Pessoa, Paraíba, Brazil.

The children were classified into two groups: (i) nine eutrophic children (E group; height/age >95%, weight/height >90%) with normal height according to age and normal weight according to height and (ii) nine early malnourished children (EM group; height/age <95%, weight/height >90%) with short stature according to age but normal weight according to (short) height and who were malnourished during the critical period of brain development (Morgane et al., 1993) for at least 6 months, from birth to 2 years of age, according to Paraíba state healthcare records. Classification was also based on Waterlow anthropometric parameters (Sigulem, Devincenzi, & Lessa, 2000), which utilize ratios of weight, height, and age.

Participation of all volunteers considered ethical aspects relevant to research that involves human subjects according to resolution no. 196/96 of the National Health Council (Health Ministry, Brazil). This plan was submitted to and approved by the Ethics Committee.

**Figure 1.** Examples of non-Cartesian visual grating stimuli of .25, 1.0, 2.0 (above from left to right) and 8.0 (below) cycles per degree. The highest SF grating is shown in a larger size due to print reproduction limitations.
Early malnutrition and non-Cartesian contrast sensitivity

of the Health Sciences Centre, Universidade Federal da Paraíba. Participation of the children was voluntary and occurred only after written informed consent was provided by their parents or legal guardians.

Visual stimuli and equipment

The stimuli (Figure 1) were achromatic concentric circular sinewave gratings (40.1 cd/m² mean luminance) modulated by a cylindrical Bessel profile of zero-order (J₀). The spatial modulation eliminated sharp spatial transients at the edge of the gratings and generated a gradual decrease in contrast between the center and edge of the circular grating. At contrast threshold, one can detect the contrast based on the center of the stimulus; however, the contrast threshold was calculated based on the mean contrast of the entire stimulus.

The diameter of the resulting patterns subtended approximately 7.2 degrees at a distance of 150 cm (i.e., the standardized distance between the monitor and volunteer). The nontest stimulus was gray and contained only average luminance. Test stimuli were concentric, circular, static sinewave gratings (Campbell & Green, 1965) with spatial frequencies of .25, 1.0, 2.0, and 8.0 cycles per degree (cpd). The images (Figure 1) accurately represented the maximum number of cycles visible to the subject. Cycles of 3.6, 7.2, 14.4, and 57.6 were visible to a normal adult at high contrast for .25, 1.0, 2.0, and 8.0 cpd, respectively. Contrast was defined according to Michelson’s formula (Michelson, 1891):

\[ C = \frac{(x)_{\text{max}} - L(x)_{\text{min}}}{L(x)_{\text{max}} + L(x)_{\text{min}}} \]

where \( L(x) \) is the luminance at a given point on the sinewave, with \( x \) measured radially from the center. \( L(x)_{\text{max}} \) and \( L(x)_{\text{min}} \) are the maximum and minimum luminances of the pattern, respectively. \( C \) is the contrast. The spatial average luminance of the grating is given by \( \frac{1}{2}[L(x)_{\text{max}} + L(x)_{\text{min}}] \).

Stimuli were presented on a 19-inch color cathode ray tube video monitor (1024 × 768 resolution, 70 Hz image update). The stimuli were controlled by a microcomputer through VGA and DVI video connectors. The dynamic range of the contrasts that the monitor could present was expanded more than 64 times by a BITS++ digital video processor (Cambridge Research Systems, Rochester, Kent, England, 2002), resulting in 14 bits per channel or 42 bits per pixel, allowing the presentation of more than 16,384 (or 2¹⁴) grayscale levels. This allowed the testing of contrast thresholds while enabling high-contrast resolution. The BITS++ processor was controlled by C++ software developed by our research group. It generated and controlled the stimulus presentation and recorded the observer responses to calculate contrast threshold values.

The monitor was calibrated with LightScan software and an optical photometer (Cambridge Research Systems, Rochester, Kent, England, 2002). The monitor output was gamma-corrected using 48 points from 0 to 255 (gamma = 1.8). The minimum and maximum luminance values of the screen were .20 cd/m² and 80.0 cd/m², respectively (average luminance = 40.1 cd/m²), and the background luminance was the minimum luminance itself. The laboratory room was 2.5 × 2.0 m, with one Philips 20W fluorescent lamp. The walls were gray to better control ambient lighting during the experiment.

Procedure

Observers viewed the stimuli binocularly with natural pupils at a distance of 150 cm. A fixed chair and forehead-chin support were used to control the viewing distance. The visual CS of all of the participants was estimated using a temporal two-alternative forced choice staircase method (Wetherill & Levitt, 1965). The procedure for measuring the threshold for each frequency consisted of presenting successive pairs of simple stimuli, one of which was the test stimulus that should be identified by the participant (i.e., the first or second stimulus of each pair). The order of the presentation of the stimuli and frequencies was randomized by the software. The criterion used to alternate the contrast during the experimental session was three consecutive hits to reduce contrast by 20%, and one error to increase contrast by the same percentage.

Threshold measurements began with the test stimulus contrast set at a suprathreshold level (contrast = 1). Each time the child gave a correct answer, the contrast was decreased successively until he or she made an error. The software recorded the last perceived contrast value. The contrast was then raised until the child could perceive the stimulus again. In this case, the software recorded the first perceived contrast value. The experimental session ended automatically after the software recorded six staircase contrast values. Each threshold measurement was repeated for each spatial frequency. The contrast threshold calculated for each child was the mean of the 12 contrast values (six at the test and six at the retest).

A stimulus sequence was presented during each experimental session starting with a beep and followed immediately by the presentation of the first stimulus for 2 s. A 1-s interval then elapsed between stimuli, followed by the presentation of the second stimulus for 2 s and the volunteer’s response. All children responded within the 3-s interval. A correct response was followed by another beep. The interval between trials was 3 s, regardless of a correct or incorrect response (or choice). The beeps that indicated the beginning of the stimulus pair presentation and correct choice were different.

All children received the following instructions: “Pairs of circles will appear on the screen, one after the other. One of them will be totally gray, whereas the other will contain light and dark circles (rings) inside it. When the circle with light and dark rings appears first, you must press the left mouse button (button 1); when
the circle with light and dark rings appears second, you should press the right mouse button (button 2)."

The experiments began only when the experimenter was confident that the participants understood and responded according to the directions. In this context, instructions were repeated in a training and familiarization session with the experimental conditions.

**Results**

After each experimental session, the software produced a record sheet with the 12 staircase contrast values from each child, six in the test and six in the retest. Values were grouped according to nutritional status (i.e., E and EM groups). All staircase contrast threshold values were converted into CS values (1/threshold). These values were then plotted into a logarithmic scale for statistical analysis. All 360 staircase contrast values (12 from each child) for each spatial frequency were used to conduct a Tukey Two-Sided Outlier Test. The 13, 5, 7, and 20 outlier values for .5, 1.0, 2.0, and 8.0 cpd, respectively, identified by the Tukey test were set to equal the group mean calculated in the absence of the outlier. After the Tukey Outlier Test, the means of the staircase contrast values, converted into CS on a logarithmic scale for each child, were then used to calculate their individual circular CS values.

A one-way analysis of variance (ANOVA) applied to the circular CS revealed significant differences between groups \( F[4, 13] = 7.7, p < .05, \alpha = .97, \eta^2 = .7 \) and a significant interaction \( F[4, 13] = 1912.9, p < .05, \alpha = 1, \eta^2 = 1 \). Thus, eutrophic and malnourished children had different CSs \( (p < .05) \), and approximately 70% of this difference was attributable to their nutritional status \( (\eta^2 = .7) \).

Table 1 shows CSs to .25, 1.0, 2.0, and 8.0 cpd on a logarithmic scale for the E and EM groups. The CSs for .25, 1.0, 2.0, and 8.0 cpd for early malnourished children were 1.07-, 1.03-, 1.03-, and 1.49-fold lower, respectively, than for eutrophic children (Figure 2). The Tukey Honestly Significant Difference post hoc test showed significant differences \( (*p < .05) \) only for 8.0 cpd. For both groups, the CSs for concentric gratings were highest at 1 cpd and lower at both the lower and higher spatial frequencies.

**Discussion**

The present study evaluated the effects of early malnutrition on visual circular CS in 8- to 11-year-old children. This type of malnutrition is characterized by nutritional insult at a crucial time in the development of the central nervous system and visual system when malnutrition presents a higher risk, followed by nutritional rehabilitation. In humans, the critical period extends from the third month of pregnancy to 2 years of age (Morgane et al., 1993). A malnourished child has short stature according to age but normal weight according to (short) stature. The central idea of this

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**Table 1.** Valid \( n \), mean, median, minimum, maximum, standard deviation (SD), and standard error of the mean (SE) of log contrast sensitivity for the E and EM groups for the .25, 1.0, 2.0, and 8.0 cpd gratings

<table>
<thead>
<tr>
<th>Spatial Frequency (cm)</th>
<th>E group Valid ( n )</th>
<th>Mean</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>SE</th>
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<td>.05</td>
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<td>.04</td>
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<td>2.0</td>
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<td>1.40</td>
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<td>1.55</td>
<td>.09</td>
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<td>.67</td>
<td>.69</td>
<td>.51</td>
<td>.83</td>
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<table>
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<tr>
<th>Spatial Frequency (cm)</th>
<th>EM group Valid ( n )</th>
<th>Mean</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
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<td>.36</td>
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</table>
Early malnutrition and non-Cartesian contrast sensitivity

In previous studies we measured CS to linear gratings in children who were malnourished for at least 6 months, from birth to 2 years of age. In early malnutrition and non-Cartesian contrast sensitivity, the inverted U-shape function has been well established and lower [.25 cpd] spatial frequencies). This classic (i.e., peak sensitivity at mid-spatial frequencies [1, range conveys different information about an object. Spatial frequencies, considering that each frequency Bar (2004), stimulus shape is conveyed by low spatial frequencies, and fine details are conveyed by high frequencies, and the shape of the stimulus and fine details. According to the children's ability to process global information about the spatial frequency of 8.0 cpd in the EM group. An interaction between early malnutrition and the visual system in children who were malnourished for at least 6 months, from birth to 2 years of age. The statistical results revealed a decrease in CS for the spatial frequency of 8.0 cpd in the EM group. An interaction between early malnutrition and the visual system of children who were malnourished for at least 6 months, from birth to 2 years of age. According to Wilkinson (2000), parallel patterns provided significantly weaker activation in V4 than either concentric or radial patterns. Faces activated V4 at approximately the same level as concentric and radial patterns. This may suggest that the brain regions involved in face perception may show a bias for concentric patterns because of the importance of quasi-circular structure in defining head shape (Wilson, Wilkinson, & Asaad, 2000) research suggesting that Cartesian visual stimuli are decoded in the primary visual cortex, or V1, and circular visual stimuli are decoded in extrastriate visual cortical areas such as area V4 and the inferotemporal cortex. According to Wilson et al. (2000), concentric gratings produced roughly half as much activation as faces in the fusiform face area, supporting their hypothesis that the analysis of concentric structure may be an important aspect of face perception. However, V4 cells activated by concentric stimuli may contribute to the analysis of a much wider range of objects. Therefore, future research on both the physiology and psychophysics of intermediate-level form vision should benefit from the use of concentric stimuli rather than simple conventional sinusoidal gratings (Wilkinson et al., 2000).
Several studies investigated the normal (Bradley & Freeman, 1982; Adams & Courage, 2002; Benedek, Benedek, Kéri, & Janáky, 2003) and abnormal (amblyopia: Montés-Micó & Ferrer-Blasco, 2001; Down syndrome: Courage, Adams, & Hall, 1997) childhood development of CS to Cartesian stimuli. However, only a few studies, including the present study, examined the development of CS to non-Cartesian gratings (Santos, França, & Cruz, 2007; Santos et al., 2008). The present results suggest that early malnutrition, known to affect primary visual cortical areas (Santos & Alencar, 2010), may affect higher visual cortical areas as well, including V2, V4, and the inferotemporal cortex. To the best of our knowledge, this is the first study to show that early malnutrition decreases CS to non-Cartesian stimuli in later childhood.

In summary, the results of the present study suggest that non-Cartesian stimuli may help evaluate pattern processing in higher visual areas beyond V1 and V2. Additionally, this research also has potential clinical implications. The assessment of non-Cartesian CS may help establish a more nuanced characterization of the effects of malnutrition on the developing visual system and aid in diagnosis, prognosis, and follow-up treatment in children who experienced malnutrition.

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Early malnutrition and non-Cartesian contrast sensitivity


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