Impaired sensitivity to visual contrast in children treated early and continuously for phenylketonuria

Adele Diamond and Chaya Herzberg

1Department of Psychology, University of Pennsylvania and 2Department of Pediatric Optometry, Pennsylvania College of Optometry, Philadelphia, USA

Correspondence to: Adele Diamond, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Building E10-044, Cambridge, MA 02139, USA

Summary

Contrast sensitivity was assessed in 47 children aged 5.4—9.8 years: 12 with phenylketonuria (PKU), six unaffected siblings and 29 children from the general population. Children with PKU, despite early and continuous treatment and despite phenylalanine (Phe) levels within accepted limits, were impaired across the range of spatial frequencies [1.5—18.0 cycles per degree of visual angle (c.p.d.)]. They were most impaired at the next to the highest spatial frequency, where 'group' accounted for 70% of the variance in sensitivity to contrast, controlling for acuity, sex, age and test site. Never, at any spatial frequency, was the contrast sensitivity of any PKU subject better than that of his or her sibling. All subjects were tested under conditions of 20/20 vision, with correction if needed. The mean IQ of PKU subjects was 99; IQ was not significantly related to contrast sensitivity performance. We interpret these findings as support for Diamond's hypothesis that moderately elevated plasma Phe levels (3—5X normal), combined with reduced plasma tyrosine (Tyr), moderately reduce the levels of Tyr reaching the eye and brain, which adversely affects those dopamine neurons that fire and turn over dopamine most rapidly (the dopamine neurons in the retina and those projecting to prefrontal cortex). This would lead to the deficit in contrast sensitivity found here and to the selective deficit in prefrontal cortex cognitive functions previously reported in PKU children under moderately good dietary control.

Keywords: phenylketonuria; dopamine; retina; prefrontal cortex; contrast sensitivity

Abbreviations: c.p.d. = cycles per degree of visual angle; Phe = phenylalanine; PKU = phenylketonuria; Tyr = tyrosine

Introduction

The purpose of the study reported here was to test the prediction that even when PKU is treated, children whose plasma levels of Phe are ≥3X normal (≥6 mg dl⁻¹; ≥360 μmol l⁻¹), are impaired in their sensitivity to contrast.

The importance of this study lies in three areas.

(i) If the prediction is confirmed, the findings would provide independent evidence for the mechanism that has been proposed as the cause of the cognitive deficits in these children (Diamond et al., 1992, 1994). Children with PKU who have been on dietary treatment since shortly after birth and whose plasma Phe levels are maintained at roughly 3—5X normal have a selective impairment in the cognitive functions dependent on prefrontal cortex (e.g. Faust et al., 1986; Welsh et al., 1990; Diamond et al., 1992; Diamond, 1994); other cognitive functions dependent on other neural systems appear to be spared. Diamond has hypothesized that the underlying biological mechanism causing this effect is that mild elevations in the level of Phe relative to Tyr in the bloodstream result in slightly less Tyr crossing from the blood to the brain or eye because of the competition between Phe and Tyr for transport out of the bloodstream. Slight reductions in available Tyr selectively affect those dopamine neurons that fire most rapidly and turn over dopamine most rapidly, such as the prefrontally projecting dopamine neurons and the dopamine neurons in the retina. (Tyr is the precursor of dopamine.) Indeed, in an animal model of early-treated PKU, dopamine metabolism was found to be reduced in prefrontal cortex (Diamond et al., 1994). If retinal dopamine neurons are affected, this should show up as a deficit in contrast sensitivity (Kupersmith et al., 1982; Regan and Neima, 1984; Skrandies and Gottlob, 1986; Bodis-Wollner et al., 1987; Bodisch-Wollner, 1988, 1990; Gottlob and Stangler-Zuschrott, 1990). There is no a priori reason why prefrontal and retinal function would both be selectively affected in...
these children except for the special properties of their dopamine neurons.

(ii) The Vistech test for contrast sensitivity is brief, simple to administer and perform, and easily portable. If PKU children with moderately elevated Phe levels are impaired in their sensitivity to contrast, it might be possible to use a contrast sensitivity test as a measure of whether the children's CNS Tyr levels are low enough that the children's cognitive functions are in danger. The level of dopamine in prefrontal cortex is much lower than in other brain regions such as the caudate or nucleus accumbens; reductions of dopamine or dopamine metabolites restricted to prefrontal cortex might be hardly detectable in cerebrospinal fluid; not to mention the discomfort, cost, and risks involved in a spinal tap. Administering tests of the cognitive functions dependent on prefrontal cortex can be quite difficult and time-consuming, especially when testing young children. Testing contrast sensitivity, on the other hand, is relatively easy for the local paediatric ophthalmologist or optometrist to do after only brief training. Contrast sensitivity testing might be useful (a) as an initial diagnostic tool indicating in whom more intensive diagnostic work might be warranted, (b) in investigating disorders of the Tyr and/or dopamine systems that are much rarer than PKU where it would be uneconomical to fly trained personnel all over the globe to investigate isolated cases, and (c) in evaluating the efficacy of various proposed treatments for PKU to supplement the low-Phe diet, such as tyrosine, L-dopa, or catecholaminergic agonists.

(iii) When children begin to read, impaired contrast sensitivity might make it more difficult for the children to see printed material under conditions of low contrast. Such children might encounter difficulties in school causing them to fall behind or grow discouraged, or causing people to think them less intelligent. Therefore, if a problem in contrast sensitivity can be identified, and compensated for by increasing the visual contrast of the material presented, or corrected by drug treatment, the quality of the child's self-image and of the child's life might be enhanced.

Methods

Experimental procedures

Contrast sensitivity testing

Contrast sensitivity was evaluated by means of the Vistech contrast test system, a well-established and widely used measure of sensitivity to contrast (Ginsburg, 1984; Rogers et al., 1987; Mäntyläri et al., 1989; Tweten et al., 1990; Gilmore and Levy, 1991; Lederer and Bosse, 1992). This test assesses contrast sensitivity at five spatial frequencies (1.5, 3.0, 6.0, 12.0, 18.0 c.p.d.) and eight contrast levels (ranging from 3–260 contrast threshold1).

The 93X68 cm Vistech chart is mounted on a wall. The chart contains sinusoidal grating patterns on circular photographic plates arranged in five rows and nine columns. All plates measure 7.5 cm in diameter and subtend a visual angle of 1.5° at the 3 m viewing distance. Within a row, spatial frequency of the sinusoidal gratings is held constant (i.e. all the plates in a row present black and white lines of a given width; see Fig. 1). Contrast levels decrease logarithmically from left to right across the columns (i.e. the black and white lines are most distinct from one another in the leftmost plates and become progressively less distinct in plates to the right; see Fig. 1). The last photographic plate in each row displays a grey field of equal luminance and contains no gratings. The sinusoidal gratings are oriented vertically, tilted 15° to the left, or tilted 15° to the right; these three orientations are varied randomly over the 40 photographic plates.

On each trial, the subject's attention was drawn to a particular plate and the subject was asked to orient a striped template so that its stripes matched the orientation of the stripes on the test pattern. If the subject oriented the template correctly, the conclusion was drawn that the subject saw the sinusoidal gratings at that level of spatial frequency and contrast. Each subject was tested at each spatial frequency at progressively decreasing levels of contrast until the subject erred. A subject's score for contrast threshold at a given spatial frequency reflected the lowest level of contrast at which the subject correctly oriented the template. Care was taken to make sure that each child understood the instructions (see below). No child was rushed. The tester (C.H.), who has a background in both developmental psychology and paediatric optometry, was encouraging and patient with each child. C.H. was blind to the children's group assignments, their IQs and their plasma Phe and Tyr levels. All contrast sensitivity testing was conducted binocularly.

All children first received a full eye examination. All eye examinations and contrast sensitivity tests were administered by C.H., who obtained informed consent from each child and from at least one parent per child. For those children who could reliably identify letters, visual acuity was measured using a standard Snellen letter chart. For other children, acuity was assessed using the Broken Wheel cards. Retinoscopy and direct, as well as indirect, ophthalmoscopy were also carried out on each child. Because poor vision can impair sensitivity to contrast, refractive errors were corrected using a trial frame before contrast sensitivity testing began (see Subjects, below). Patients with amblyopia (a reduction in visual function secondary to form deprivation in an otherwise healthy eye) also showed impaired sensitivity to contrast. All subjects were, therefore, screened for this during their eye examination (see Subjects, below).

After the eye examination and before contrast sensitivity testing, each subject was administered a pretest with the Vistech apparatus. Here, the subject was asked to turn the stripped template so that its orientation matched that of the three high contrast test patterns with gratings at 6.0 c.p.d. located at the base of the contrast sensitivity chart. If the subject oriented the stripes of the template so that they matched the orientation of the test pattern (up, left or right) on all three trials, the contrast sensitivity test was
Contrast sensitivity in PKU children

administered. All subjects tested for this study passed the pretest. (In pilot work, some subjects 5 years of age and younger had failed the pretest.)

The Vistech test is not without its problems. For example, it is vulnerable to the conditions of testing; performance can vary with different testers and different luminance conditions (e.g. Scialfa et al., 1991; Elliot and Whitaker, 1992). The Vistech test was chosen for this study, however, because of its appropriateness for young children, especially if there is any question of diminished mental capacity or brief attention span. Children, and adults with Alzheimer's disease, have more difficulty with the high-tech, automated methods for testing contrast sensitivity, such as the Nicolet automated procedure or the von Békésy tracking procedure (e.g. Cronin-Golomb et al., 1987; Gilmore and Levy, 1991). Other procedures, such as the Pelli–Robson test (Pelli et al., 1988), require that subjects be able to read letters. All of these other procedures require more time than does the Vistech test. (Although the limited number of trials in the Vistech test provides fewer data points on which to make the contrast sensitivity assessment.) The Vistech test requires only a few minutes, does not require knowledge of the alphabet, and is much less cognitively taxing or intimidating.

In addition, the following modifications were made to the normal Vistech testing procedure to make the test even more appropriate for young children and to help the children perform their best. (i) To avoid confusion about which target on the contrast sensitivity chart the child was being asked to attend, a frame was placed around the target pattern to isolate it.

(ii) Adults are normally asked to say that the lines on the target pattern are oriented to the right, left or straight up and down. To avoid errors because of left–right confusion, no subject in this study had to say which way the lines were oriented. Instead, each child was given a template. The child turned the template so that it matched his or her perception of the target. To do this, a child did not need to know left from right.

(iii) Young children sometimes have difficulty attending to a chart 3 m away as their attention sometimes wanders when asked to do effortful attending to a target at a distance. If a child showed any evidence of this during the pretest, the tester administered the near version of the Vistech test to that child. The near version uses a viewing distance of 0.4 m and a smaller chart (17.5×14 cm) with the stimuli (photographic plates 1.2 cm in diameter) arrayed in the same format as on the distant chart. This was needed for only two subjects, both of whom had PKU.

The eye examinations and contrast sensitivity testing took place at three sites. One site was the Ophthalmology Department at the Children’s Hospital of New Jersey in Newark, NJ, USA. Here, the eye examinations and contrast sensitivity testing were conducted in the same room. Artificial incandescent light was used to illuminate the contrast sensitivity chart, which hung in an alcove in the examination room. The illumination here was the brightest of all three sites (~210–230 cd m⁻²). Another site was the inner city public clinic at Pennsylvania College of Optometry in Philadelphia, Pa, USA. Here, the eye examinations were conducted in one room, and contrast sensitivity was tested in another. The latter room was larger than the one at the other two sites and was illuminated primarily by natural light (~140–160 cd m⁻² on the days of testing). The third site was a private optometry practice in Hamilton Square, NJ, USA.
As at the Philadelphia site, eye examinations were conducted in one room, and contrast sensitivity was tested in another. As at the Newark site, the illumination here was artificial; the lights were fluorescent (~150–170 cd m⁻²). The level of illumination at all three sites was within the established range for the Vistech Test (100–240 cd m⁻²) and was even across the chart. Photometric measurements were taken with a Minolta LS110 photometer before testing on each testday.

**Plasma Phe and Tyr levels**

Mean Phe levels, and the mean ratios of Phe:Tyr, in the bloodstream of each PKU child were calculated for the 6-week period preceding testing and for the 2-year period preceding testing. Mean plasma Phe levels were also calculated for the first month of life and the first year of life. In addition, each child’s Phe levels were plotted for the 2-year period preceding testing, and the percentage of the area under this curve outside the region of 2–6 mg dl⁻¹ was calculated (i.e. the percentage of the child’s Phe levels lower than 2 mg dl⁻¹ and higher than 6 mg dl⁻¹). [This was done because past work has shown that plasma Phe levels <2 mg dl⁻¹ might be dangerous in PKU children and that cognition may be adversely affected by plasma Phe levels >6 mg dl⁻¹ (Smith et al., 1991; Diamond et al., 1992; Costello et al., 1994).] The Phe level measurement taken closest to contrast sensitivity testing was also used; for half of the PKU subjects this measurement was taken on the day of contrast sensitivity testing.

**Statistical methods**

ANOVAs were performed with group, sex, age, acuity and test site as the independent measures, and the log of contrast sensitivity at each spatial frequency as the dependent measure. In addition, MANOVAs were performed using the same independent measures to look at the effect on contrast sensitivity simultaneously at all five spatial frequencies. All analyses were performed comparing the PKU subjects with children from the general population (‘normative sample’), and that cognition may be adversely affected by plasma Phe levels lower than 2 mg dl⁻¹ and higher than 6 mg dl⁻¹. [This was done because past work has shown that plasma Phe levels <2 mg dl⁻¹ might be dangerous in PKU children and that cognition may be adversely affected by plasma Phe levels >6 mg dl⁻¹ (Smith et al., 1991; Diamond et al., 1992; Costello et al., 1994).] The Phe level measurement taken closest to contrast sensitivity testing was also used; for half of the PKU subjects this measurement was taken on the day of contrast sensitivity testing.

In all, 47 children (18 boys and 25 girls) between the ages of 5.4 and 9.8 years were tested. This age range was chosen because pilot work had shown that some children under 5.4 years of age have difficulty understanding the instructions for the Vistech test, or difficulty maintaining their attention throughout testing. Others have found similar problems in attempting to assess contrast sensitivity in children aged 3–5 years (e.g. Rogers et al., 1987; Scharre et al., 1990). Also, our own pilot work and the results of others had indicated that performance on the Vistech test did not vary in children aged between 5.5 and 10 years, although the performance of younger children and of older adults appears to be worse than the performance of children of 5.5–10 years (e.g. Scialfa et al., 1988, 1991; Scharre et al., 1990). The 12 children born with PKU who had been maintained on a diet low in Phe since roughly 14 days of age were recruited from the three PKU clinics in the local area. All of the plasma Phe and Tyr assessments of these children since birth as well as the children’s IQ test scores were obtained from these clinics with the families’ permission. The mean plasma Phe level in these children for the 6-week period prior to contrast sensitivity testing was 7.72 mg dl⁻¹ (SD = 2.57; see Table 1). Two children had mean Phe levels for this period over 10 mg dl⁻¹. All analyses were conducted with and without these two subjects (their mean Phe levels were 12.3 and 15.8 mg dl⁻¹), since the national guideline for acceptable plasma Phe levels has been ≤10 mg dl⁻¹ (Williamson et al., 1981; Koch and Wenz, 1987) and we did not want any group differences unfairly exaggerated by the presence of subjects whose Phe levels had been overly high.

IQ scores at age 5 years were based on performance on the Stanford-Binet or WPPSI-R tests; for older children the WISC-III was used. The mean IQ score for the PKU children was 99.2 (SD = 13.5). Three PKU children had IQs above 110, and two had IQs below 90; this distribution closely approximates that in the general population. All analyses were conducted with and without the two subjects with IQs <90 (IQs of these two subjects were 83 and 79; mean IQ of the two PKU subjects omitting these two subjects was 104.0), since it is conceivable that less intelligent children might have understood the contrast sensitivity testing instructions less well and we did not want any group differences unfairly exaggerated by lower IQ subjects in the PKU group.

Of the 29 children from the general population who were tested, 15 were female and 14 were male. All were healthy, and came from the same communities as the PKU children. The distribution of their ages was comparable to that for the PKU children (see Table 1). Six siblings (two male, four female) of the PKU subjects were also tested. All unaffected siblings were healthy and were tested on the same day at the same site as their PKU sibling.

Reflecting our conservative strategy of correcting even the mildest refractive errors, 15 of the 47 children in the study received corrective lenses when their sensitivity to contrast
Table 1 Background characteristics of the different subject groups

<table>
<thead>
<tr>
<th></th>
<th>Percent female</th>
<th>Age (in years) at testing</th>
<th>Percent of subjects tested in New Jersey</th>
<th>Percent who needed corrective lenses</th>
<th>IQ</th>
<th>Mean phenylalanine level (mg dl⁻¹) during 6-week period 2-year period first month of first year of life</th>
<th>Phe level closest to test date* (mg dl⁻¹)</th>
<th>Mean Phe.Tyr ratio for 6-week period 2-year period prior to testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children treated early and continuously for PKU (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6-week period prior to testing</td>
<td>2-year period prior to testing</td>
<td>first month of life</td>
</tr>
<tr>
<td>Mean</td>
<td>58</td>
<td>7.82</td>
<td>75</td>
<td>17</td>
<td>99.2</td>
<td>8.51</td>
<td>9.64</td>
<td>14.02</td>
</tr>
<tr>
<td>SD</td>
<td>1.12</td>
<td></td>
<td></td>
<td></td>
<td>13.5</td>
<td>3.71</td>
<td>3.22</td>
<td>7.96</td>
</tr>
<tr>
<td>Omitting the 2 PKU children with IQs &lt;90 (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>103.8</td>
<td>7.72</td>
<td>8.78</td>
<td>14.02</td>
</tr>
<tr>
<td>Mean</td>
<td>70</td>
<td>7.73</td>
<td>80</td>
<td>10</td>
<td>10.7</td>
<td>2.57</td>
<td>2.77</td>
<td>7.96</td>
</tr>
<tr>
<td>SD</td>
<td>1.08</td>
<td></td>
<td></td>
<td></td>
<td>10.7</td>
<td>2.57</td>
<td>2.77</td>
<td>7.96</td>
</tr>
<tr>
<td>Omitting the 2 PKU children with mean Phe levels for the 6 weeks prior to testing &gt;10 mg dl⁻¹ (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>101.0</td>
<td>6.68</td>
<td>8.26</td>
<td>14.50</td>
</tr>
<tr>
<td>Mean</td>
<td>60</td>
<td>7.76</td>
<td>70</td>
<td>20</td>
<td>12.9</td>
<td>1.35</td>
<td>2.14</td>
<td>9.05</td>
</tr>
<tr>
<td>SD</td>
<td>1.05</td>
<td></td>
<td></td>
<td></td>
<td>12.9</td>
<td>1.35</td>
<td>2.14</td>
<td>9.05</td>
</tr>
<tr>
<td>Children from the general population (normative sample) (n = 29)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100.0</td>
<td>7.81</td>
<td>8.26</td>
<td>14.50</td>
</tr>
<tr>
<td>Mean</td>
<td>52</td>
<td>7.46</td>
<td>45</td>
<td>10</td>
<td>1.37</td>
<td>1.37</td>
<td>8.26</td>
<td>14.50</td>
</tr>
<tr>
<td>SD</td>
<td>1.23</td>
<td></td>
<td></td>
<td></td>
<td>1.37</td>
<td>1.37</td>
<td>8.26</td>
<td>14.50</td>
</tr>
<tr>
<td>Siblings of the children treated early and continuously for PKU (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100.0</td>
<td>7.81</td>
<td>8.26</td>
<td>14.50</td>
</tr>
<tr>
<td>Mean</td>
<td>67</td>
<td>7.81</td>
<td>100</td>
<td>0</td>
<td>1.37</td>
<td>1.37</td>
<td>8.26</td>
<td>14.50</td>
</tr>
<tr>
<td>SD</td>
<td>1.37</td>
<td></td>
<td></td>
<td></td>
<td>1.37</td>
<td>1.37</td>
<td>8.26</td>
<td>14.50</td>
</tr>
<tr>
<td>Normative sample + siblings of the children treated early and continuously for PKU (n = 35)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>54.0</td>
<td>7.50</td>
<td>54.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Mean</td>
<td>54</td>
<td>7.50</td>
<td>54</td>
<td>11</td>
<td>1.17</td>
<td>1.17</td>
<td>8.26</td>
<td>14.50</td>
</tr>
<tr>
<td>SD</td>
<td>1.17</td>
<td></td>
<td></td>
<td></td>
<td>1.17</td>
<td>1.17</td>
<td>8.26</td>
<td>14.50</td>
</tr>
</tbody>
</table>

*For half of the subjects with PKU, Phe levels were assessed on the day of contrast sensitivity testing.
The children treated early and continuously for PKU were significantly less sensitive to visual contrast than were their peers of the same age across the entire range of spatial frequencies (see Table 3 and Fig. 2). This was true even when the two PKU children with IQs below 90, or the two PKU children with Phe levels above 10 mg dl⁻¹, were omitted from the analyses (see Table 3).

Six comparisons were made: (i) children treated for PKU versus children from the general population; (ii) children treated for PKU omitting the two children with IQs below 90 versus children from the general population; (iii) children treated for PKU omitting the two children with mean plasma Phe levels over 10 mg dl⁻¹ during the 6 weeks preceding...
Table 3  Comparison of the contrast sensitivity performance of the different groups of subjects

<table>
<thead>
<tr>
<th>Spatial frequencies (c.p.d.)</th>
<th>1.5</th>
<th>3.0</th>
<th>6.0</th>
<th>12.0</th>
<th>18.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means (line 1) and standard deviations (line 2) for contrast sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children treated early and continuously for PKU (n = 12)</td>
<td>34.3</td>
<td>93.8</td>
<td>128.8</td>
<td>68.2</td>
<td>20.7</td>
</tr>
<tr>
<td>Omitting the 2 PKU children with IQs &lt;90 (n = 10)</td>
<td>14.2</td>
<td>62.2</td>
<td>74.6</td>
<td>51.5</td>
<td>16.2</td>
</tr>
<tr>
<td>Omitting the 2 PKU children with mean Phe levels for the 6 weeks prior to testing of &gt;10 mg dl⁻¹ (n = 10)</td>
<td>37.0</td>
<td>94.7</td>
<td>141.0</td>
<td>72.2</td>
<td>20.4</td>
</tr>
<tr>
<td>Siblings of the children treated early and continuously for PKU (n = 6)</td>
<td>12.5</td>
<td>57.3</td>
<td>71.4</td>
<td>52.7</td>
<td>15.8</td>
</tr>
<tr>
<td>Normative sample + siblings of the children treated early and continuously for PKU (n = 35)</td>
<td>34.2</td>
<td>91.2</td>
<td>129.6</td>
<td>69.8</td>
<td>19.8</td>
</tr>
<tr>
<td>Omitting the 2 PKU children with IQs &lt;90 versus children from the general population (d.f. = 1,30)</td>
<td>15.7</td>
<td>61.6</td>
<td>82.5</td>
<td>55.2</td>
<td>16.3</td>
</tr>
<tr>
<td>PKU children minus the 2 with IQs &lt;90 versus children from the general population (d.f. = 1,30)</td>
<td>64.7</td>
<td>165.0</td>
<td>186.2</td>
<td>135.2</td>
<td>52.4</td>
</tr>
<tr>
<td>PKU children minus the 2 with higher Phe versus children from the general population (d.f. = 1,30)</td>
<td>35.4</td>
<td>50.8</td>
<td>61.4</td>
<td>36.7</td>
<td>26.2</td>
</tr>
<tr>
<td>PKU children minus the 2 with higher Phe versus children from the general population (d.f. = 1,30)</td>
<td>50.8</td>
<td>135.8</td>
<td>203.3</td>
<td>123.5</td>
<td>48.3</td>
</tr>
<tr>
<td>PKU children minus the 2 with higher Phe versus children from the general population (d.f. = 1,30)</td>
<td>17.7</td>
<td>67.3</td>
<td>30.6</td>
<td>48.3</td>
<td>20.4</td>
</tr>
<tr>
<td>PKU children minus the 2 with higher Phe versus children from the general population (d.f. = 1,30)</td>
<td>62.3</td>
<td>161.4</td>
<td>188.1</td>
<td>130.9</td>
<td>51.7</td>
</tr>
<tr>
<td>PKU children minus the 2 with higher Phe versus children from the general population (d.f. = 1,30)</td>
<td>33.3</td>
<td>53.5</td>
<td>57.1</td>
<td>39.3</td>
<td>25.1</td>
</tr>
</tbody>
</table>

The ANOVAs included age (continuous), acuity (continuous, averaged over the two eyes), sex (2 levels), test site (3 levels) and group (2 levels) in the equation, as well as the two-way interactions for sex×group and group×test site. Analyses were performed both with and without an arcsine transformation of the data; the results were comparable. ANOVAs were performed on the logs of the contrast sensitivity values because that is the convention. The results of ANOVAs using the actual contrast sensitivity values (rather than their log transformation) also yielded significant results across all 30 comparisons (six sets of comparisons across contrast sensitivity at each of five spatial frequencies) as did all six of the MANOVAs; the results of these analyses are available from the first author upon request.

The ANOVAs included age (continuous), acuity (continuous, averaged over the two eyes), sex (2 levels), test site (3 levels) and group (2 levels) in the equation, as well as the two-way interactions for sex×group and group×test site. Analyses were performed both with and without an arcsine transformation of the data; the results were comparable. ANOVAs were performed on the logs of the contrast sensitivity values because that is the convention. The results of ANOVAs using the actual contrast sensitivity values (rather than their log transformation) also yielded significant results across all 30 comparisons (six sets of comparisons across contrast sensitivity at each of five spatial frequencies) as did all six of the MANOVAs; the results of these analyses are available from the first author upon request.
Table 4  Contrast sensitivity performance of sibling pairs: children treated early and continuously for PKU and their unaffected siblings

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Sex</th>
<th>Age (in years) at testing</th>
<th>Test site*</th>
<th>Contrast sensitivity at</th>
<th>Mean difference† across the 5 spatial frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.5 c.p.d.</td>
<td>3.0 c.p.d.</td>
<td>6.0 c.p.d.</td>
</tr>
<tr>
<td>P01†</td>
<td>F</td>
<td>6.75</td>
<td>Newark</td>
<td>35</td>
<td>44</td>
</tr>
<tr>
<td>S01†</td>
<td>F</td>
<td>8.00</td>
<td>Newark</td>
<td>70</td>
<td>85</td>
</tr>
<tr>
<td>P02</td>
<td>M</td>
<td>5.40</td>
<td>Newark</td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td>S02‡</td>
<td>M</td>
<td>7.83</td>
<td>Newark</td>
<td>60</td>
<td>85</td>
</tr>
<tr>
<td>P03</td>
<td>F</td>
<td>9.80</td>
<td>Ham.Sq.</td>
<td>70</td>
<td>85</td>
</tr>
<tr>
<td>S03</td>
<td>M</td>
<td>8.20</td>
<td>Ham.Sq.</td>
<td>70</td>
<td>170</td>
</tr>
<tr>
<td>P04</td>
<td>F</td>
<td>7.88</td>
<td>Newark</td>
<td>35</td>
<td>85</td>
</tr>
<tr>
<td>S04</td>
<td>F</td>
<td>6.83</td>
<td>Newark</td>
<td>35</td>
<td>170</td>
</tr>
<tr>
<td>P05†</td>
<td>F</td>
<td>7.60</td>
<td>Ham.Sq.</td>
<td>35</td>
<td>85</td>
</tr>
<tr>
<td>S05</td>
<td>F</td>
<td>9.67</td>
<td>Ham.Sq.</td>
<td>35</td>
<td>85</td>
</tr>
<tr>
<td>P06</td>
<td>F</td>
<td>9.18</td>
<td>Ham.Sq.</td>
<td>35</td>
<td>220</td>
</tr>
<tr>
<td>S06</td>
<td>F</td>
<td>6.59</td>
<td>Ham.Sq.</td>
<td>35</td>
<td>220</td>
</tr>
<tr>
<td>Mean difference†</td>
<td>0.09</td>
<td></td>
<td></td>
<td>13.8</td>
<td>42.0</td>
</tr>
<tr>
<td>t test‡</td>
<td>1.42</td>
<td></td>
<td></td>
<td>3.16</td>
<td>0.85</td>
</tr>
<tr>
<td>P value</td>
<td>n.s.</td>
<td></td>
<td></td>
<td>&lt;0.03</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Subject numbers beginning with ‘P’ belong to subjects with PKU; subject numbers beginning with ‘S’ belong to unaffected siblings.
*All siblings pairs were tested at Children’s Hospital of New Jersey in Newark or at a private optometry practice in Hamilton Square, NJ.
†Mean differences are always calculated by subtracting the value for the PKU child from the value for his or her unaffected sibling.
‡Needed refractive correction.

Table 3). Consistent with this, we also found that PKU children’s sensitivity to contrast was not significantly related to any of the continuously scaled plasma Phe variables investigated. (The Phe and Phe:Tyr ratio variables investigated were (i) plasma Phe level at the assessment closest to the time of contrast sensitivity testing; (ii) mean plasma Phe level during the 6 weeks preceding testing; (iii) mean level during the 2 years preceding testing; (iv) mean plasma Phe level during the first month of life; (v) mean level during the first year of life; (vi) the area under the Phe versus age curve lying outside the region of 2–6 mg dl−1 during the 2 years preceding testing; (vii) mean plasma Phe:Tyr ratio during the 6 weeks preceding testing; and (viii) mean Phe:Tyr ratio during the 2 years preceding testing.)
The lack of a relationship between plasma Phe variables and sensitivity to contrast may have been due to the small range of Phe levels among the children studied. Most of our PKU children had mean Phe levels between 6–10 mg dl$^{-1}$. Indeed, most mean Phe levels for the 6-week period prior to testing were in the range 7±1 mg dl$^{-1}$. Only two children had mean Phe levels during this period below 6.00 mg dl$^{-1}$ (5.33 and 5.65 mg dl$^{-1}$) and only two children had mean Phe levels >7.60 mg dl$^{-1}$ (12.25 and 15.80 mg dl$^{-1}$).

Or, we might have failed to find significant effects of plasma Phe variables because of the small size of the sample. However, the sample size was sufficient to find other statistically significant differences: among the subjects with PKU, girls had significantly higher IQs than the boys [$F(1,7) = 12.53$, $P < 0.02$] and older children had significantly higher IQs than younger children [$F(1,7) = 7.77$, $P < 0.04$].

When we dichotomized the group of subjects with PKU based on the eight plasma Phe variables, the only variable that differentiated the contrast sensitivity performance of subjects with higher Phe levels from that of subjects with lower Phe levels was the mean level of Phe in the bloodstream during the first month of life. Phenylketonuria children whose Phe levels had been ‘high’ during the first month of their lives (17.5–24.5 mg dl$^{-1}$) were less sensitive to contrast at the two highest spatial frequencies than were PKU children whose Phe levels had been lower during their first month (5.6–10.6 mg dl$^{-1}$; see Fig. 3). At every spatial frequency, PKU children with lower Phe levels during their first month performed more like children from the general population than did PKU children with higher Phe levels during this early period of life; although even the ‘low’ Phe PKU children were significantly impaired at two of the spatial frequencies (see Fig. 3). The PKU children whose Phe levels had been higher during their first month were significantly impaired in their sensitivity to contrast at all spatial frequencies, and especially at the next to the highest frequency.

A median split on other Phe variables, such as the mean plasma Phe level during the 2 years preceding contrast sensitivity testing or the mean plasma Phe:Tyr ratio during the 6 weeks preceding testing, demonstrated that regardless of whether a child’s recent Phe level or recent Phe:Tyr ratio had been ‘high’ or ‘low’, children with PKU performed significantly worse than their peers of the same age. There were no significant differences between the PKU children with ‘high’ versus ‘low’ Phe levels or Phe:Tyr ratios. It would appear that either the range in Phe levels and in the ratios of Phe:Tyr was too small among our subjects with PKU to detect an effect, or the Phe level or Phe:Tyr ratio around the time of testing or in the recent past didn’t matter in the sense that regardless of whether it was ‘high’ or ‘low’, contrast sensitivity was diminished, especially if the child’s plasma Phe levels were high during the first month of life.

A greater percentage of PKU children and of their siblings needed refractive correction (50% of the subjects in each of these two groups) than did subjects drawn from the general population (21%). This difference was statistically significant [$F(1,43) = 4.6$, $P < 0.04$]. There was no significant difference in the size of the refractive errors found among the different groups of children, however. The child with the poorest acuity (20/70 in each eye without correction) came from the general population. There was also no significant group difference in the type of refractive error. However, it is worth noting that five of the subjects with PKU (42% of all PKU subjects; 67% of PKU subjects who required corrective lenses) and two unaffected siblings (33% of all siblings; 67% of those siblings who required correction) were hyperopic. On the other hand, only three of the 29 subjects drawn from the general population (10%; 50% of those who required correction) were hyperopic. The incidence of farsightedness among PKU children and their families thus struck us as high, although it was not sufficiently greater than the incidence in our normative sample to reach statistical significance. Four of the subjects with PKU (33%), two...
unaffected siblings (33%), and one subject from the general population (3%) had astigmatism; again, these differences were not statistically significant, but they might be worth noting nevertheless. Among the group of subjects with PKU, no plasma Phe or Phe:Tyr variable was significantly related to any measure of visual acuity. Acuity decreased significantly over age among children from the general population, but not among the subjects with PKU.

Neither the need for corrective lenses, the size of the refractive error, nor the direction of the refractive error was related to contrast sensitivity performance within any individual subject group or over all subjects, at any individual spatial frequency or over all spatial frequencies. Controlling for these variables, the contrast sensitivity of children with PKU was still significantly worse than that of their peers of the same age at each of the five spatial frequencies investigated.

Children tested at the two New Jersey testing sites (Newark and Hamilton Square) performed significantly better on contrast sensitivity than did the children tested at the Pennsylvania site overall and at three of the five spatial frequencies \( F(2,32) \) at 1.5 c.p.d. = 0.1, n.s.; at 3.0 c.p.d. = 7.3, \( P < 0.003 \); at 6.0 c.p.d. = 0.1, n.s.; at 12.0 c.p.d. = 12.3, \( P < 0.0001 \); at 18.0 c.p.d. = 4.7, \( P < 0.02 \); MANOVA: Wilks' Lambda (0.47) \( F(10,56) = 2.1, \ P < 0.04 \). When the PKU children, or the children from the general population, were considered separately, the effect of test site on sensitivity to contrast was significant at two of the five spatial frequencies (3.0 and 12.0 c.p.d.). (No siblings were tested in Pennsylvania.) There were no significant differences between performance at the two New Jersey sites. The percentage of children tested in New Jersey was higher for the children with PKU than for children from the general population (75% versus 45%). If anything, this should have worked against finding poorer performance among the PKU children (since better performance was generally found among children tested in New Jersey), although the effect of test site was controlled.

The effect of test site might have been due to (i) conditions in the different sites (the New Jersey sites used brighter, artificial illumination; the Pennsylvania site relied on a mix of natural and artificial light) or (ii) differences between the populations of children tested at the different sites. The Philadelphia site was a public clinic that tended to draw people from more economically disadvantaged backgrounds than the New Jersey sites. Subjects tested in New Jersey were older on average than those tested in Pennsylvania \( F(1,42) = 6.84, \ P < 0.003 \). However, there was no significant difference in sensitivity to contrast over age within the age range tested (5.5-10 years) among children from the general population, children with PKU, their unaffected siblings, or all of the subjects combined. The effects noted above for test site were obtained controlling for group, age, sex and acuity. Therefore, the difference in performance by test site cannot be accounted for entirely by differences in those subject characteristics.

**Discussion**

The national guideline has been that plasma Phe levels up to 10 mg dL\(^{-1}\) (i.e. up to 5X normal) are acceptable in children with PKU (e.g. Williamson et al., 1981; Koch and Wenz, 1987). However, in the present study, we found that PKU children whose plasma Phe levels were between 5.33 and 10.02 mg dL\(^{-1}\) (within the acceptable range) showed significantly reduced sensitivity to contrast on the Vistech test at every spatial frequency investigated compared with children of the same age from the general population. The impairment was most marked at the next to the highest spatial frequency (12.0 c.p.d.), where 'group' accounted for 70% of the variance, controlling for acuity, sex, age and test site. At no spatial frequency was the contrast sensitivity of any PKU child better than the performance of his or her own sibling. All children (PKU, normals and siblings) were aged between 5.5 and 10 years, and all had 20/20 binocular acuity with correction.

The poorer contrast sensitivity performance of subjects with PKU was found despite precautions that should have minimized group differences: we repeated all analyses omitting the two subjects with mean plasma Phe levels >10 mg dL\(^{-1}\) and omitting the two subjects with IQ scores <90. A higher percentage of PKU children than children from the general population were tested at the New Jersey sites, where children in all subject groups performed better than at the Pennsylvania site. We made sure all children understood the contrast sensitivity task before testing began by administering a pretest. All children were tested by the same person. All children who needed corrective lenses were given them for contrast sensitivity testing; including four hyperopic children whose acuity was 20/20 even without correction. Two PKU children who had a little difficulty during the contrast sensitivity pretest were tested with the near-chart form of the test because children find that easier. The subject groups were well matched in age, and we found no significant differences in performance by age within the range tested (5.5-10 years).

It is unlikely that the difference in contrast sensitivity performance was due to cognitive differences between the children with PKU and the other children: as detailed in the Methods section, steps were taken to reduce the cognitive demands of the contrast sensitivity test and to minimize its attentional requirements. Indeed, all subjects demonstrated that they understood the task instructions by succeeding on the pretest. We found no significant relationship between IQ and contrast sensitivity performance. Although we do not have IQ scores for our non-PKU subjects, the IQs of our PKU subjects closely resemble national norms for IQ among healthy children. The IQs of the control subjects would have had to be significantly above average for their IQs to be significantly better than those of the PKU subjects (although,
Contrast sensitivity in PKU children

without the actual IQ scores of the control subjects, we cannot rule out that possibility. Many of the control subjects (55%) came from economically disadvantaged areas of the inner city, where children's IQ scores tend to be lower than average, rather than significantly above. In any case, the mean IQ of the PKU subjects was 99 and, omitting the two children with IQs <90, the mean IQ of the PKU subjects was 104. Even omitting the two lower-IQ subjects, the contrast sensitivity performance of children with PKU was significantly depressed at each spatial frequency.

It is unlikely that the difference in contrast sensitivity performance was due to differences in visual acuity or refractive error. The PKU subjects did not have significantly worse acuity than other subjects. All subjects needing refractive correction were tested with correction. All subjects had 20/20 binocular acuity with correction; contrast sensitivity was tested binocularly. Once the refractive error is corrected, any remaining contrast sensitivity deficit should not be related to refractive error. Indeed, since we tested subjects with corrective lenses, we found contrast sensitivity to be unrelated to uncorrected visual acuity, the need for corrective lenses, the size of the refractive error or the direction of the refractive error. This was found overall and within each subject group, over all spatial frequencies and at each individual spatial frequency. Subjects with PKU were found to be significantly less sensitive to contrast than their peers of the same age at every spatial frequency investigated even controlling for acuity and refractive error variables.

It is unlikely that the difference in contrast sensitivity performance was due to differences in the site of testing. We found a significant main effect for test site; children in all groups performed better at the New Jersey sites, probably because those sites had better illumination. A higher percentage of PKU subjects were tested in New Jersey, but they still performed significantly worse than controls, many of whom were tested in the less well-lit Pennsylvania site. Thus, whatever it was about the sites that may have affected performance, this should have worked to reduce the difference between subject groups, rather than enhance it. Not surprisingly, the group differences in contrast sensitivity remain significant at every spatial frequency even controlling for test site.

We found little effect of level of Phe in the bloodstream or of the ratio of Phe to Tyr in the bloodstream on contrast sensitivity. However, there was a narrow range of variability in Phe and in the Phe:Tyr ratio among the PKU children studied here. For example, only two of our PKU subjects had mean plasma Phe levels <6 mg dl⁻¹, and both of them had a mean Phe level >5 mg dl⁻¹. It is possible that a significant relationship might have been obtained if the ranges of plasma Phe and Phe:Tyr levels had not been so truncated. In our study of cognitive development (Diamond et al., 1992; Diamond, 1994), we found that PKU children whose mean plasma Phe levels were 6–10 mg dl⁻¹ were impaired in the cognitive abilities dependent upon prefrontal cortex, whereas PKU children whose mean plasma Phe levels remained <6 mg dl⁻¹ performed normally, and that within these two groups there was little variation in cognitive performance by level of plasma Phe. Our present findings are consistent with that. There were too few PKU subjects in the present study with Phe levels <6 mg dl⁻¹ to be able to determine if Phe levels >2 mg dl⁻¹ but <6 mg dl⁻¹ are compatible with normal contrast sensitivity. Certainly, as found in the cognitive development study, variation in Phe between 6 and 10 mg dl⁻¹ was not found to be associated with significant differences in behaviour in the present study. There may be a threshold, such that once Phe levels reach ~3X normal, contrast sensitivity and the cognitive functions dependent on prefrontal cortex begin to be compromised, but these functions may be no further compromised if Phe levels rise up to 5X normal.

The one Phe level variable that discriminated among the PKU children was the mean level of Phe in the bloodstream during the first month of life. The PKU children whose Phe levels had been 'high' during this period were less sensitive to contrast at the two highest spatial frequencies than were PKU children whose Phe levels had been lower during this period. (Phe levels are often particularly elevated during the first month because 1–3 weeks typically elapse after birth before a PKU infant begins receiving the low-Phe formula.) Mean Phe levels during the first month may have been significantly related to contrast sensitivity performance, whereas other Phe measurements later in life were not, because there was more variation in mean Phe level during the first month than later. Or, it may be that high Phe levels early in life have a significant, irreversible effect on the visual system. Thereafter, contrast sensitivity may be impaired even if plasma Phe levels are kept relatively low. Only further investigation can determine which of these explanations is correct; we will begin to investigate this by testing children with mild hyperphenylalaninemia.

We also found that children with PKU, and their siblings, were more likely to need glasses than were children from the general population, and that the incidence of hyperopia and astigmatism was unusually high among PKU children and their siblings. This needs to be confirmed with larger samples, as do our contrast sensitivity findings. Other visual functions should also be investigated in these children. Is it possible that something happens in utero, or very early in life, to the visual system of children who carry even one gene for PKU?

How the present findings compare to those from other studies

To our knowledge, contrast sensitivity has never been investigated before in children with PKU. In the present study, we found that children with PKU, who had been treated early and continuously, had IQs within the normal range, as have other investigators (e.g. Bickel et al., 1954; Hudson et al., 1970; Dobson et al., 1977; Williamson et al., 1981; Koch et al., 1984; Holtzman et al., 1986; Diamond...
Our subjects perform as well as they did. It is well known that contrast sensitivity performance in general, and performance on the Vistech test in particular, is sensitive to the level of illumination, and several of the other studies that have found poorer performance than here have been conducted with illumination near the minimum acceptable for the Vistech test. Indeed, luminance has a particularly pronounced effect at the higher spatial frequencies (Patel, 1966), and it is at those frequencies that our subjects performed most markedly better than the reports of other subjects.

While the Vistech test clearly seems to be the method of choice for testing contrast sensitivity in young children because it can be administered in such a brief period of time, is simple to administer and perform, does not require knowledge of the alphabet, and is much less cognitively demanding or intimidating than other contrast sensitivity measures, the Vistech test appears to be vulnerable to variation in performance by characteristics of the test site or tester. As noted above, differences in room illumination affect performance. Elliott and Whitaker (1992) report significant differences in the contrast sensitivity scores of the same subjects tested on the Vistech test by different optometrists. It would appear to be critical that individuals in all subject groups be tested under the same conditions by the same person when using the Vistech test; scores obtained in one setting by a particular tester should not be uncritically compared to norms obtained elsewhere by a different tester. One might also want to assess contrast sensitivity on more than one occasion when using the Vistech test, since a single testing consists of a relatively small number of trials.

What impaired contrast sensitivity might suggest about the neural basis of the cognitive deficits observed in children treated early and continuously for PKU

We had predicted that children treated early and continuously for PKU would show diminished sensitivity to contrast because of the following: (i) If plasma levels of Phe are moderately elevated relative to plasma levels of Tyr (as they are in children treated for PKU) then the amount of Tyr reaching the eye and brain should be moderately reduced. The reason for this is that Phe and Tyr compete for the same transporter proteins to cross the blood–brain barrier (Chirigos et al., 1960; Oldendorf, 1973; Pardridge, 1977; Pardridge and Oldendorf, 1977; Miller et al., 1985; Tornquist and Alm, 1986) and blood–retinal barrier (Rapoport, 1976; Hjelle et al., 1978; Fernstrom et al., 1986a; Tornquist and Alm, 1986; Fernstrom and Fernstrom, 1988), and those protein carriers have a higher affinity for Phe than for Tyr. (ii) Moderate reductions in the amount of Tyr reaching the eye or brain disproportionately affect the most active dopamine neurons (those that fire fastest, turn over dopamine fastest and have tyrosine hydroxylase in a particularly activated state).
Contrast sensitivity in PKU children

Site of the defect: retina or brain?

Can we be sure that the site of the contrast sensitivity effect in children treated for PKU is in the retina? No. A behaviour, such as sensitivity to contrast, can be disrupted by problems anywhere within the system that supports it. Research from several laboratories appears to be converging on the conclusion that the site of the contrast sensitivity effect in parkinsonian patients is very early in visual processing, and most likely at the level of the retina (Gawel et al., 1981; Marx et al., 1986; Bodis-Wollner, 1988). However, MRIs of the brains of children with PKU often show white matter abnormalities and sometimes these abnormalities have been described in the occipital lobe (Lou et al., 1992; Meyding-Lamadé et al., 1992), the lobe containing visual cortex. Alzheimer’s disease patients show impaired contrast sensitivity, and here researchers have hypothesized that the deficit is cortical or subcortical in origin rather than retinal (Cronin-Golomb et al., 1987; Gilmore and Levy, 1991). If the site of the contrast sensitivity deficit in PKU children is the brain rather than the retina, then the reason for the deficit would have nothing to do with the special vulnerability of rapidly firing dopamine cells to moderate reductions in available tyrosine. We will investigate this further by studying dopamine levels in the retinas of the mouse model of PKU.

In short, children treated early and continuously for PKU, who have moderately elevated plasma levels of Phe and moderately reduced plasma levels of Tyr, have previously been found to have cognitive impairments specific to the abilities that rely on prefrontal cortex (Faust et al., 1986; Welsh et al., 1990; Diamond, 1994), and have been found in the present study to be impaired in an ability (contrast sensitivity) which is sensitive to the level of dopamine in the retina. The dopamine neurons in the ventral tegmental area that project to prefrontal cortex and the dopamine neurons in the retina are the two populations of dopamine neurons that are unusually active. Thus, dopamine neurons that fire particularly rapidly and turn over dopamine particularly rapidly appear to be vulnerable to sub-normal functioning when levels of Phe in the bloodstream are 3–5× normal (6–10 mg dl⁻¹; 360–600 µmol l⁻¹), especially when plasma Tyr levels are reduced, though such Phe levels had been thought acceptable in treating PKU children. Because contrast sensitivity is so easy to assess, it might prove to be a valuable screening measure to detect a moderate reduction in cerebral Tyr and of a person’s consequent vulnerability to cognitive dysfunction. It might be of help in assessing the efficacy of possible treatments for PKU. Compensating for a child’s poorer contrast sensitivity by better illumination or by the use of higher contrast material might also help some children with PKU in their schoolwork at minimal cost to all involved.

Acknowledgements

We wish to thank Dr Anna Haroutonian and Shaune Bornholdt at the PKU Clinic, Children’s Hospital of New Jersey, United
Hospitals Medical Center, Newark, NJ, USA, and Drs Barbara Evans and Beth Frederick of the PKU Program, Cooper Hospital, University Medical Center, Camden, NJ, USA, for invaluable assistance in identifying and recruiting subjects, and in supplying the information on Phe and Tyr levels and IQ scores in the subjects with PKU. We also wish to thank the Children’s Eye Care Center of New Jersey, directed by Anthony Caputo, MD, Department of Ophthalmology, Children’s Hospital of New Jersey in Newark, for making it possible for us to test subjects there, Weimin Li for running the SAS programs for the statistical analyses and finally, Don Druin for help with the project when help was most needed. This research was supported by NIMH (R01 #MH41842), the March of Dimes Foundation (Grant #12-0718) and NICHD (MRRC P30-HD-26979). A report of the preliminary results of the present study was presented at the annual meeting of the Society for Neuroscience, Washington DC, USA, October 1993.

References


Tweten S, Wall M, Schwartz BD. A comparison of three clinical


Received July 27, 1995. Revised October 19, 1995. Accepted November 6, 1995