Visual event-related potentials in children with phenylketonuria

RM Henderson1, DL McCulloch1, AM Herbert1,2, PH Robinson3 and MJ Taylor4

Department of Vision Sciences, Glasgow Caledonian University1, Glasgow, Scotland; Department of Psychology, University of North Texas2, Denton, TX, USA; Royal Hospital for Sick Children3, Yorkhill, Glasgow, Scotland; CERCO4, Faculté de Médecine de Rangueil, Toulouse, France


Visual event-related potentials (ERPs) were examined in 16 children (aged 5–14 y) with phenylketonuria (PKU) and 16 age- and sex-matched controls. Lifetime median measures of phenylalanine (Phe) were 230–460 μmol/l. The most recent Phe levels were 56–624 μmol/l. ERPs were recorded whilst the children performed a discrimination task. All stimuli were square wave gratings degree, which appeared for 33 ms. A response to an infrequent grating that differed in orientation or spatial frequency was required. The older children with PKU had a delay in the first peak (P1) of the ERP, and age-related changes in the amplitude of P1. There was attenuation of the second peak across age groups in PKU. There was no evidence of reduced response accuracy or longer reaction times in children with PKU. Latencies of the cognitive P3 were not delayed in PKU. The delayed early peaks are consistent with previous studies that have shown delayed visual evoked potentials in PKU. The lack of differences in reaction time and P3 may be due to relatively good Phe control in children with PKU, or to the simplicity of the task. Suggestions are made for future ERP studies of PKU.

Event-related potentials, phenylalanine, phenylketonuria, reaction time, P3, visual evoked potentials

Phenylketonuria (PKU) is an inherited metabolic disorder that prevents the conversion of the amino acid phenylalanine (Phe) to tyrosine. Without treatment from infancy, normal development is severely disrupted (for review, see (1)). Treatment for PKU consists of restriction of dietary Phe and intake of amino acid supplements that contain extra tyrosine. In the past, dietary control was discontinued between 8–14 y of age, because clinical changes are less noticeable after this age with increased Phe (2). In addition, the unpalatable nature of the diet does not encourage compliance.

Currently, there is a growing body of evidence that neurophysiological and neuropsychological changes are associated with high Phe levels in later childhood and even adulthood. High Phe levels affect the intellectual quotient (IQ) up to the age of 10 y (3). There are also changes in reaction time and executive function in childhood and adulthood in early-treated PKU (for review, see (4)). The hypothesis has been advanced that deficits in executive function (defined as anticipation, planning, self-monitoring and flexibility) are due to deficient dopamine levels in the prefrontal cortex (5). Recent advances in brain imaging have also shown abnormalities in patients with PKU. Magnetic resonance imaging (MRI) has shown abnormalities in the white matter of the brain in asymptomatic, early-treated children and adults (6, 7). These MRI abnormalities were related to recent levels of Phe and length of time since dietary treatment ceased, and were independent of Phe levels early in life (6).

Event-related potentials (ERPs) are electrical potentials recorded from the surface of the scalp. Early peaks are sensitive to sensory processing, whilst later peaks are involved with cognitive processing. For instance, the third positive peak (P3) is a late cognitive potential that has a larger amplitude if a stimulus is infrequent and task-relevant (for review, see (8)). ERPs have excellent temporal resolution; changes have been found in the early peaks of scalp-recorded potentials of children and adults with PKU (9–16). PKU may also affect cognitive P3, as recent studies have shown that dopamine monotherapy in Parkinson’s disease causes a reduction in P3 latency (17) for an auditory task. This has led to suggestions that the P3 has a dopaminergic component.

The aim of this study was to measure electrophysiological and behavioural correlates of visual processing in a group of children with early-treated PKU. We wished to determine if the usual age-related changes in latency and amplitude of the ERPs (for review, see (18))
were affected by the relaxation of diet control of PKU during childhood.

Subjects
Twenty-two children with PKU aged 5–14 y participated in the study. Data from five children with PKU were excluded because they were recorded at a coarser sampling rate. Vision screening prior to ERP testing included visual acuity, cover tests and retinoscopy. Subjects were excluded for any one of the following reasons: binocular visual acuity below 6/12 (after correction with lenses), uncorrected hyperopia (> +2D), myopia (> −1.5D), anisometropia (>1D), astigmatism (>1D) and/or manifest squint. Refraction was measured by retinoscopy without cycloplegia. To reduce the possibility of latent hyperopia remaining undetected, all subjects with no vision decrement through +2D lenses were excluded. Subjects with spectacles wore their refractive corrections. One subject with PKU was excluded because of uncorrected moderate hyperopia. These procedures should have prevented children with significant refractive or binocular problems from skewing the data. All remaining children (n = 16) had diet control initiated by 1 mo of age.

Sixteen age- (within 1 y) and sex-matched control subjects were also tested. IQ data was available for 12 of the children with PKU, but not at the time of testing. Full-scale IQ score had a mean of 87 and range of 68–111 (WISC-III scores ±2 y from the data of test). Two subtests (vocabulary and block design) were measured in the controls prior to the test. The average scaled scores for controls were 11.9 for vocabulary and 11.8 for block design. The children with PKU had scaled scores of 9.2 for vocabulary and 5.5 for block design. Phe levels are given in Table 1 for the 14 children with PKU for whom complete lifetime data were available.

Method
Room lights were dimmed while recording took place. Electroencephalogram (EEG) was recorded along the midline of the scalp (Oz, Pz, Cz, Fz) and referenced to an electrode on the chin. An electro-oculogram (EOG) was also measured from the supraorbital ridge referenced to the outer canthus of the right eye. The monitor was positioned 75 cm from the child. The children were seated in a firm chair with arm rests and a headrest. All children were closely observed during the tests to ensure that they maintained the correct viewing distance and fixated on a small target in the middle of the screen.

Square wave gratings of 1.8 or 2.9 cycles per degree (cpd) at 91% contrast appeared on a luminance-matched blank screen (square field of 16.2 degrees) for 33.33 ms. The interstimulus interval was 2 s. A short-pulse stimulus was used to reduce the effects of adaptation (19) and also to reduce the effect of the offset component on later peaks (for review, see (20)).

Two sequences of stimuli were presented, each with two grating stimuli, where one of the stimuli was presented more frequently than the other. These paradigms help to maintain subjects’ attention and allow the measurement of sensory ERPs and cognitive potentials, such as P3 (21):

1. Orientation Discrimination Task. Horizontal gratings (1.8 cpd) appeared with 80% frequency. The children were asked to press a button whenever they saw a vertical grating (1.8 cpd), interspersed randomly within the sequence with a frequency of 20% overall.
2. Size Discrimination Task. Horizontal gratings (1.8 cpd) appeared with a frequency of 80%. The children were asked to press a button whenever they saw horizontal gratings of a higher spatial frequency (2.9 cpd), interspersed randomly within the sequence with a frequency of 20% overall.

The orientation task was always completed first. The size task was not completed in several young children because of limited co-operation.

Raw EEG data were collected and averaged by the Enfant 4010 system (Neuroscientific Co., Farmingdale, NY, USA) running on a Dell Dimension XPS 466V computer. The stimuli were presented on a PixelLink high-resolution monitor (60 Hz refresh rate) triggered by the computer. The signals from each electrode were amplified 10 000-fold, and were recorded with a bandpass between 0.5–100 Hz using isolated amplifiers (AMP 800, Neuroscientific). Raw data were sorted offline to produce separate averages for each stimulus. All 16 PKU and 16 age- and sex-matched control subjects had sufficient EOG artefact-free trials in the first 400 ms of the epoch to allow averages to be made to all 4 grating stimuli (horizontal (1.8 cpd) 80%, vertical (1.8 cpd) 20%, horizontal (1.8 cpd) 20%, horizontal (2.9 cpd) 20%). Nine PKU subjects and 9 age- and sex-matched control subjects had sufficient EOG artefact-free trials between 400–1000 ms to allow averages for scoring the P3 to the vertical grating and narrow grating. All peak amplitudes were scored from baseline to peak using an estimated baseline starting 500 ms before target presentation.

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>n</th>
<th>Highest Phe level in first year</th>
<th>Median lifetime Phe level</th>
<th>Recent Phe level</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–6</td>
<td>5</td>
<td>1935</td>
<td>240</td>
<td>190</td>
</tr>
<tr>
<td>7–9</td>
<td>4</td>
<td>2716</td>
<td>338</td>
<td>375</td>
</tr>
<tr>
<td>10–11</td>
<td>2</td>
<td>1104</td>
<td>360</td>
<td>477</td>
</tr>
<tr>
<td>13–14</td>
<td>3</td>
<td>1653</td>
<td>360</td>
<td>397</td>
</tr>
</tbody>
</table>
Results

Early peaks

All children except for one control subject had a reproducible positive peak at Oz within the first 150 ms for all stimuli (P1). This was followed by a negative peak (N1) and a third peak of positive polarity between 275–400 ms. A positive peak superimposed on the end of the negative peak (150–275 ms) was also seen in some subjects, but was not consistently present (see Fig. 2). The later positive peak between 275–400 ms was not analysed because in the older children, this peak overlapped with cognitive P3.

Peak latencies

Three-way analysis of variance (ANOVA) with stimulus (horizontal 80%, vertical 20%, horizontal 20%, horizontal (2.9 cpd) 20%), age group (5–6 y, 7–9 y, 10–11 y, 13–14 y) and group (PKU, control) was calculated separately for P1 and N1 latencies. The P1 and N1 peak latencies revealed a significant effect of group for the P1 peak latency (F(1,83) = 10.6, p < 0.002). There was also an effect of age on P1 (F(3,83) = 7.75, p < 0.0001). There was an interaction between age and group for P1, due to a relatively delayed P1 latency in the older children with PKU (F(3,83) = 3.29, p < 0.01). N1 was also delayed in the children with PKU (F(1,84) = 10.533, p < 0.01).

Peak amplitude

Three-way ANOVA with stimulus (horizontal 80%, vertical 20%, horizontal 20%, horizontal (2.9 cpd) 20%), age group (5–6 y, 7–9 y, 10–11 y, 13–14 y) and group (PKU, control) was calculated separately for P1 and N1 amplitudes. Both P1 and N1 were significantly affected by age (F(3,83) = 24.9, p < 0.0001; F(3,84) = 4.6, p < 0.005). This was due to older age groups having smaller amplitude components than the youngest age groups.

There was an interaction between age and group for P1 (F(3,83) = 3.493, p < 0.02). The children with PKU had relatively larger amplitudes in the older age groups and smaller amplitudes in the youngest age group compared to controls.

The peak amplitude of N1 was significantly affected by group (F(1,84) = 7.528, p < 0.008), being lower on average in the children with PKU.

Cognitive P3 and behavioural measures

The P3 was measured at Pz as the maximum positive peak 100 ms (±) from the peak latency on the grand average for each age group (see Fig. 3 and Table 3). P3 latency was measured and the amplitude at this latency was measured at the other electrodes. Nine PKU subjects and nine controls had sufficient artefact-free data for the infrequent stimulus in the orientation task and discrimination task. The amplitude at this latency was measured at the other electrodes. Reaction time (RT) was measured from a response histogram, selecting the mode of the distribution as the reaction time, and
accuracy was calculated as a proportion of correct responses.

A three-factor repeated measures ANOVA with stimulus (vertical 20%, horizontal 20%) as a repeated measure and age group (7–9 y, 10–11 y, 13–14 y) and group (PKU, control) as factors was calculated for accuracy of response. There was no difference between the groups for accuracy (F(1,12) = 0.156). There was a significant effect of age group due to the older children responding more accurately than the younger children (F(2,12) = 12.6, p < 0.002).

Reaction time and latency of P3 changed markedly across the age groups tested. There were significant negative correlations with age for both RT and the latency of the P3. Multiple regressions for RT to vertical and horizontal gratings with group (PKU, control) and age as predictors had an $R^2 = 0.75$ (age, $p < 0.0001$) and $R^2 = 0.59$ (age, $p < 0.0003$), respectively. Multiple regressions for latency of P3 to vertical and horizontal gratings with group (PKU, control) and age as predictors had an $R^2 = 0.53$ (age, $p < 0.002$) and $R^2 = 0.57$ (age, $p < 0.0005$), respectively. Because of these strong correlations with age, analysis of covariance (ANCOVA) is more appropriate than ANOVA, given the small numbers of subjects in each age group. Using age as a covariate allows the differences between the children with PKU and the controls to be observed independently from age-related effects. There were no significant differences in RT between the groups (PKU, control) for the vertical grating (F(1,15) = 0.472) or the horizontal grating (F(1,15) = 0.012). There were also no significant differences in latency of P3 between the groups (PKU, control) for the vertical grating (F(1,15) = 2.72) or the horizontal grating (2.9 cpd) (F(1,15) = 0.004).

A three-factor repeated measures ANOVA of P3 amplitude with stimulus (vertical 20%, horizontal 20%) as a repeated measure and age group (7–9 y, 10–11 y, 13–14 y) and group (PKU, control) as factors showed no significant effects in the amplitude of P3.

A three-factor repeated measures ANOVA with amplitude at the midline electrodes (Oz, Pz, Cz, Fz) as the repeated measure and age group (7–9 y, 10–11 y, 13–14 y) and group (PKU, control) as factors was calculated for the orientation and size tasks. There was a significant effect of electrode site for both tasks (F(1,12) = 48.5, $p < 0.0001$) with the amplitude at Pz being larger than the other electrodes. There were no other significant effects.

**Discussion**

Even with early dietary intervention, we noted significant differences in the early ERPs of school-aged

---

**Table 2.** Mean values for early peaks of ERP in control subjects for gratings of identical spatial frequency. Values for PKU subjects in parentheses.

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>n</th>
<th>P1 latency (ms ± SD)</th>
<th>P1 amplitude (µV ± SD)</th>
<th>N1 latency (ms ± SD)</th>
<th>N1 amplitude (µV ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–6</td>
<td>10</td>
<td>115 ± 6</td>
<td>30 ± 9</td>
<td>175 ± 23</td>
<td>−12 ± 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(112 ± 9)</td>
<td>(25 ± 10)</td>
<td>(189 ± 37)</td>
<td>(−4 ± 11)</td>
</tr>
<tr>
<td>7–9</td>
<td>8</td>
<td>108 ± 7</td>
<td>19 ± 7</td>
<td>171 ± 6</td>
<td>−25 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(112 ± 8)</td>
<td>(29 ± 6)</td>
<td>(176 ± 12)</td>
<td>(−15 ± 13)</td>
</tr>
<tr>
<td>10–11</td>
<td>8</td>
<td>113 ± 4</td>
<td>23 ± 8</td>
<td>168 ± 12</td>
<td>−11 ± 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(123 ± 6)</td>
<td>(29 ± 10)</td>
<td>(191 ± 21)</td>
<td>(−8 ± 11)</td>
</tr>
<tr>
<td>13–14</td>
<td>6</td>
<td>99 ± 4</td>
<td>8 ± 4</td>
<td>165 ± 21</td>
<td>−13 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(112 ± 11)</td>
<td>(12 ± 6)</td>
<td>(186 ± 20)</td>
<td>(−9 ± 6)</td>
</tr>
</tbody>
</table>

---

**Fig. 3.** The top three traces are grand averaged data at Pz for one stimulus (vertical, 20% frequency). The bottom trace is the average data for a 14-y-old PKU subject. x-axis: time (ms).
children with PKU. Others studies have also detected delays in the first positive peak of the VEP using pattern reversal and flash VEPs. Direct comparisons of peak times between studies are impossible because early peaks are very sensitive to the type of stimulus used (flash, pattern-reversal or pattern onset) and to the luminance, contrast and spatial frequency of the stimulus (for review, see (20)). However, there appears to be a generalized delay in conduction along the visual pathways, as prolonged VEPs are seen in PKU regardless of the type of stimulus (9–16). Our data are consistent with these reports.

At present, there are two hypotheses that could explain the delayed early ERPs. The first implies a myelin defect, as increased Phe leads to increased turnover of myelin in an animal model (22). Myelin changes in the visual pathways are observed in periventricular regions of the brain in adults and children (23). Lou et al. (7) suggest that this part of the brain may be particularly sensitive to deficient amino acids, as it is served by the longest cerebral arteries, the middle and posterior arteries. However, there is a lack of correlation between these changes and the prolonged VEP latencies measured in adults and children (13). The second hypothesis implies deficient levels of dopamine in the eye and the brain (16). Dopaminergic transmission has been demonstrated in neurones in the retina (24) and the lateral geniculate nuclei (25), and dopamine levels are low in PKU (26). So far Levodopa (L-Dopa) supplementation has produced conflicting results, one study confirming a reduction in latencies with L-Dopa (9) and another failing to find any effect (16). This latter finding may, however, be due to high Phe levels interfering with the production of dopamine or permanent changes to dopaminergic neurones.

The finding that P1 was delayed in the older children with PKU suggests that P1 is sensitive to dietary control after the age of 5 y. Korinthenberg et al. (14) found abnormal pattern reversal VEPs in adolescents off diet that were related to diet control over the first 10 y of life. However, the children in their study had higher levels of Phe than those in this study.

The age-related changes in amplitudes of P1 appear to differ between PKU and control children. There were also attenuated N1 amplitudes across the age groups in PKU. These amplitude changes could be related to sensory or attentional processes. Diamond and Herzig (27) suggested the dopaminergic neurones in the eye as a reason for impaired contrast sensitivity in children with PKU. They suggest that there may be abnormalities in the electrical responses from the retina (the electroretinogram). Unfortunately, this hypothesis has not yet been tested. Selective visual attention may also cause abnormal amplitudes. Mangun and Hillyard (28) have shown that attention to a particular region of the visual field leads to an increase in the amplitude of VEPs between 100–160 ms in adults. They have suggested that altered gating of the sensory input in cortical areas analysing that part of the visual field could account for these findings. However, as we did not manipulate spatial attention, this seems less likely as a possibility. Measures of ERP correlates of attention in children with PKU have not yet been undertaken.

To our knowledge, there have been no previous studies of the effect of PKU on cognitive P3. Our study failed to find an effect of PKU on P3 or RT. One possibility is that Phe levels are low enough to avoid effects on ERPs and task performance. Median levels of control across lifetime are in the range of 230–460 \( \mu \)mol/l, which may be low enough to avoid neurological damage significant enough to affect task performance. Suggested upper limits of Phe are controversial, although 400 \( \mu \)mol/l is an average figure in clinics (2). Possibly effects would become evident if Phe levels were higher. Adults that no longer have controlled diets may exhibit significant differences in the P3 component.

Another possibility is that the P3 elicited by our task represents cortical function that is spared in early-treated PKU. For instance, Welsh et al. (5) found normal picture recognition memory in early-treated PKU and positive correlations between picture recognition memory and infant Phe levels, and they suggested that compensatory development of cognitive skills may occur in the presence of executive function deficits. Recent work suggests that the posterior association cortex contains the neural generators that generate P3 and that prefrontal regions of the brain are not critical in the generation of P3 to simple target stimuli (29–31). P3 may become affected by deficits in prefrontal regions if the task is made more complex or if the stimuli are novel or unexpected (30). It may be that the discrimination tasks used in this study are too simple to elicit abnormal P3 in PKU. This view is supported by previous studies that have found that RT to simple recognition tasks are unaffected by PKU (5, 32, 33). A task dependent on more complex functions, like that used by Lou et al. (7), may elicit ERP differences between PKU and control subjects. Their task involved

---

Table 3. Mean values (ms ± SD) for P3 and RT in control subjects for both tasks. Values for PKU subjects in parentheses.

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>n</th>
<th>P3 latency (orientation)</th>
<th>P3 latency (size)</th>
<th>RT (orientation)</th>
<th>RT (size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7–9</td>
<td>6</td>
<td>554 ± 37 (561 ± 50)</td>
<td>543 ± 46 (582 ± 18)</td>
<td>772 ± 44 (841 ± 50)</td>
<td>693 ± 59 (739 ± 36)</td>
</tr>
<tr>
<td>10–11</td>
<td>6</td>
<td>457 ± 56 (452 ± 72)</td>
<td>522 ± 28 (449 ± 47)</td>
<td>659 ± 69 (604 ± 11)</td>
<td>704 ± 109 (620 ± 69)</td>
</tr>
<tr>
<td>13–14</td>
<td>6</td>
<td>485 ± 36 (398 ± 32)</td>
<td>435 ± 17 (459 ± 28)</td>
<td>564 ± 60 (500 ± 28)</td>
<td>539 ± 63 (544 ± 12)</td>
</tr>
</tbody>
</table>
memorizing an abstract design and subsequently identifying the pattern amongst three similar patterns. Welsh et al. (5) also found deficits on a similar task in preschool children with early-treated PKU.

In summary, we have found subtle differences in the ERPs of early-treated children with PKU. Early ERPs are delayed, whilst cognitive P3 is unaffected by PKU. Future studies looking at early ERPs may also want to measure retinal function (using electroretinograms) and attention, as these are possible sources of early ERP changes. Cognitive P3 was not found to be deficient in PKU, but this might reflect the low lifetime levels of Phe present in the children tested or the simplicity of the discrimination tasks the children were given.

References

Received Feb. 15, 1999; revisions received Aug. 26, 1999; accepted Aug. 30, 1999