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Event-related potential correlates of selective processing in early- and continuously-treated children with phenylketonuria: Effects of concurrent phenylalanine level and dietary control ☆

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ABSTRACT

This study focused on important characteristics of attentional (selective) processing in children with early-treated phenylketonuria (PKU). Seven to 14-year-old children with PKU were allocated to high phenylalanine (Phe) and low Phe groups and compared with control children on amplitudes and latencies of early and late event-related potential (ERP) components elicited during a selective processing task. These components are thought to measure early sensory processes (stimulus encoding/perception) and later selection processes (target detection). The effects of concurrent Phe level and dietary control on brain activity and behavioural performance were studied. Results showed that children with PKU with high Phe levels were less accurate and made more false alarms than controls and children with PKU with low Phe levels. Both children with PKU and controls displayed the expected early fronto-central selection negativity and a late positive peak over posterior sites associated with sensory aspects of the selective attention task. However, in contrast with controls, children with PKU showed an absence of condition differences for selection positivity over anterior sites associated with target detection. Negative and positive selection potentials over fronto-central sites were dependent on concurrent and historical Phe levels, whereas sensory potentials depended more strongly on historical Phe levels. It is concluded that both sensory and selection aspects of attention are affected by Phe levels. The relative predictive strength of historical Phe levels suggests that high Phe levels during sensitive periods for brain maturation may have long-lasting influences on selective attention.

Introduction

Phenylketonuria (PKU[1]; OMIM 261600 and 261630) is an inherited metabolic disease that affects the metabolism of the amino acid phenylalanine (Phe). If untreated, blood Phe levels increase and children with PKU may develop severe mental retardation due to the effects of high cerebral concentrations of Phe and/or low cerebral concentrations of other large neutral amino acids [1], resulting in poor myelination of neuronal connections and decreased concentrations of the neurotransmitters dopamine and serotonin.

For a number of decades, prevention of mental retardation in children with PKU has been accomplished by early screening and using a low Phe diet. Although intellectual development of early-treated children is within the normal range [2,3], a number of studies show a negative relationship between concurrent and lifetime Phe levels and cognitive performance involving, for example, executive functioning [3–11], inter-hemispheric transfer [12,13], sustained attention [14–19], selective attention [19], and speed of processing [20]. To explain these deficiencies, it is assumed that subtle deficits in neural conduction exist, which lead to slower and more error-prone processing.

It is suggested that increased levels of Phe in early-treated children may interfere with the myelination of neuronal connections, particularly in the first months of gestation, and/or lead to deficient levels of dopamine that may specifically affect the

☆ *References to electronic databases:* Phenylketonuria, OMIM 261600 and 261630.

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[1] *Abbreviations used:* PKU, phenylketonuria; Phe, phenylalanine; ERP, event-related potential; Tyr, tyrosine; EEG, electroencephalogram; EOG, electro occulogram; VEP, visual evoked potential; IDC, index of dietary control; cpd, cycles per degree; RT, reaction time; ANOVA, analysis of variance.

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development of prefrontal cortical functions [21,22]. A number of studies using MRI have shown subtle structural brain abnormalities and white matter abnormalities in early-treated PKU patients [23–29]. Recently, it was found that children with PKU with white matter abnormalities showed deficits in cognitive performance [30].

In contrast to the subtle brain abnormalities and the brain dysfunctions inferred from neuropsychological assessments [17–19], more direct evidence for functional disturbances in brain areas involved in cognitive processing is scarce, particularly with regard to electrophysiological correlates of higher cognitive processes such as selective processing and target detection. Leuzzi et al. [21] presented an auditory oddball task to children with PKU and found P300 amplitude, a late positive event-related potential (ERP) associated with stimulus evaluation, to be reduced compared with controls, whereas P300 latency tended to be longer in PKU patients. No correlations with concurrent Phe or tyrosine (Tyr) levels were found. The authors concluded that these P300 effects were attributable to a functional disruption of the dopaminergic system. In contrast, Henderson et al. [31] found that children with PKU differed from controls only in the latency of an early positive visual potential (P1), whereas no group differences in either P300 amplitude or latency or in response accuracy or response time were found. The prolonged latency of P1 has been reported previously and confirms possible deficits in the visual system in children with PKU [31]. Henderson et al. [31] further suggested that the lack of P300 effects may be due to good Phe control in their sample of children with PKU (median lifetime Phe levels varied from 230–460 μmol/L) or low task complexity [31].

In the present study we examined whether differences between children with PKU and controls in both early and late ERP components would emerge during a more complex selective attention task. In addition, the effects of both concurrent and lifetime Phe levels on brain responses elicited during selective processing were evaluated. Some authors have found no correlations between quality of dietary control and electroencephalogram (EEG) features [32] or significant diet-related changes in evoked potentials features [33]. Others, however, have reported correlations between dietary control and visual evoked potential (VEP) [34], and between blood Phe, brain Phe and EEG activity [35]. These studies have primarily focused on associations between EEG parameters and concurrent Phe levels, but several studies provide evidence that historical Phe levels are more strongly associated with cognitive performance [19,30]. Because sustained elevated Phe levels may have a more deleterious effect on cognitive performance and brain activity than concurrent Phe levels, the present study will examine both concurrent and life time Phe levels in relation to the brain activity associated with selective attention.

In summary, the present study investigates selective processing, a fundamental prerequisite for higher cognitive processing. Latency and amplitude of early and late ERP correlates of sensory processes (stimulus encoding/preprocessing) and selective processes (target selection/detection) are expected to be sensitive markers of changes in brain activity due to alterations in neuronal myelination and neurotransmitter concentrations. The goal of the present study was twofold. First, we investigated whether children with PKU differed from age-matched controls in terms of patterns of early and late ERPs elicited during a visual selective processing task. Based on evidence for both sensory deficits and attentional deficits, it was predicted that, in comparison with healthy controls, children with PKU would show prolongation of latencies and reduction in amplitudes of ERP-waves associated with these processes. Second, we examined whether differences in long-term dietary control were related to differences in brain activity. It was predicted that indices of dietary control and concurrent Phe levels would be correlated with latencies and amplitudes of early as well as late potentials.

Methods

The study was approved by the medical ethical committees of all participating treatment centers and the Dutch National PKU Steering Committee. Written informed consent was obtained from patients' parents or caretakers.

Subjects

Sixty-seven children with PKU, sampled from the Dutch population of patients with PKU between 7 and 14 years, participated in a national study on PKU [19]. Of these, 50 children participated in the current study. After data cleaning, the PKU group comprised 42 children (62% female) with complete data, with a mean age of 11.8 (SD = 1.8) years. All children with PKU were treated early (<1 month after birth) and continuously through dietary restrictions to limit Phe intake, and their Phe levels were monitored regularly.

Blood samples were taken from children with PKU on the day of testing to determine concurrent Phe levels (M = 477 μmol/L, SD = 253). Historical Phe levels obtained during the lifetime were provided by TNO Prevention and Health in Leiden, The Netherlands. The Index of Dietary Control (IDC) was computed as the mean of all half-year median blood Phe levels during the lifetime (M = 321 μmol/L, SD = 80). To study the effects of higher versus lower Phe levels, 26 children with PKU were allocated to a high Phe group (PKU-H; Phe > 360 μmol/L; M = 672, SD = 191), and 16 children were allocated to a low Phe group (PKU-L; Phe $\leqslant$ 360 μmol/L; M = 232, SD = 101). These criteria were based on treatment recommendations provided by the NIH Consensus Statement Panel (2000).

Forty-eight healthy control children (44% female) with a mean age of 11.9 years (SD = 1.7) were recruited from PKU patients' families or peer groups or through newspaper advertisements. Subjects for the PKU and control groups were excluded from participation if they had a diagnosis of non-PKU hyperphenylalaninemia or participated in special education programs for learning or behaviourally impaired children.

Task and stimulus material

The stimuli consisted of four square wave gratings that varied on two dimensions: orientation (horizontal vs. vertical gratings) and spatial frequency (low vs. high frequency gratings). High frequency gratings contained 1.63 cycles per degree (cpd), whereas low frequency gratings contained 0.54 cpd. The gratings subtended a total visual angle of approximately 9 degrees horizontally and vertically and were projected on a grey background square of 15 × 15 degrees. Four blocks of 88 trials each were presented. Each block consisted of 22 trials for each of the gratings that were randomly presented at the centre of a computer monitor. Stimulus duration was 50 ms and stimulus offset was followed by a small fixation cross of 700 ms duration. In the first two blocks, one of the gratings was assigned as a target and required a manual response. The other three gratings were either frequency-relevant (same frequency as the target), orientation-relevant (same orientation as the target), or irrelevant (neither orientation nor frequency were shared with the target). One block was performed with the right hand, the other block with the left hand. In the last two blocks another grating was selected as a target that always differed on both dimensions from the target in the previous two blocks. Subjects were instructed to attend to the centre of the computer

screen during trials and refrain from eye blinking as much as possible. Reaction time (RT) to targets and the number and type of errors were recorded.

EEG recording and procedure

During task performance EEG was recorded continuously from 33 electrode locations using AgAgCL electrodes mounted in an electrode cap (ESI Electro Cap Inc.). To control for eye movement artefacts, vertical and horizontal eye movements were also recorded using electrodes above and below the right eye and electrodes placed on the outer canthi of both eyes. A ground electrode was placed on the forehead and linked ear lobes were used as reference. Impedance of all electrodes was kept below 5 kΩ.

Both EEG and electro occulogram (EOG) were pre-amplified and digitized by a portable 20 channel Twente Medical System recorder (REFA-40/DC), controlled by Poly software (Poly 4.9; Inspektor Research Systems, 1993). The time constant was set to 5 s and the low pass filter was set at 35 Hz. EEG and EOG were sampled with a frequency of 200 Hz. Stimulus presentation, timing and data logging were software-controlled (ERTS 3.18) [36]. Subsequently, subjects were administered a visual acuity test (Landholt ogenkaart, TNO Soesterberg, The Netherlands) and a hearing test (detection of 500, 1000 and 2000 Hz probes) to detect gross visual and/or auditory deficits.

Data reduction

The recorded continuous EEG files were analysed using SCAN 4.2 (Neurosoft, 1999). EEG files were first corrected for effects of eye blinks using SCAN 4.2 ocular artefact correction procedures. The EEG files were epoched with an epoch length of 720 ms including a 100 ms pre-stimulus period. In the next step EEG epochs were base-line corrected and trials with EEG artefacts (signals passing ±100 μV) were removed. To study the sensory effects of orientation and spatial frequency of the gratings on early evoked potentials as well as the selective processing effects (relevant vs. irrelevant stimulus dimensions) on both early and late ERP waves, two different methods of averaging were applied. First, to analyze sensory effects, EEG and EOG signals were averaged across epochs separately for vertical-high (VH), vertical-low (VL), horizontal-high (HH) and horizontal-low (HL) frequency trials. Second, to analyze the effects of selective processing of stimulus dimensions, EEG and EOG signals were averaged across epochs separately for targets as well as frequency-relevant (gratings with same spatial frequency as targets), orientation-relevant (gratings with same orientation as targets) and irrelevant (gratings sharing neither frequency nor orientation with targets) stimuli. Finally, the average pre-stimulus baseline was subtracted from all data points in the average ERPs and ERP waveforms were smoothed using a digital filter. To determine peak-values and latencies, grand-average ERPs were obtained by averaging ERPs at the central electrode sites across subjects for controls and children with PKU separately (see Fig. 1).

The VEP over occipital sites consists of a sharp positive peak around 100 ms (P100), followed by a negative peak around 170 ms (N170). The ERP waveforms over frontal sites are characterized by a negative peak around 90 ms (N90) followed by a positive peak around 180 ms (P180). Over fronto-central sites, a vertex negative wave peaking around 280 ms (N280) can be observed. Finally, a pronounced large positive wave peaking around 425 ms (P425) is present at parieto-occipital locations as a response to the presentation of targets. To analyze sensory effects and selective stimulus processing, average amplitudes were calculated for the different peaks within the following intervals: *sensory effects* on VEP: P100 from 75–115 ms and N170 from 150–190 ms at O1, Oz and O2 electrodes and *selective processing* of stimulus features: Frontal selection negativity (N90) from 70–110 ms and frontal selection positivity (P180) from 160–200 ms and Vertex wave (N280) from 260–300 ms at C3, Cz, C4, F3, Fz, F4, Fp1 and Fp2 electrodes. Finally, the interval for posterior target selection (P425) ranged from 375 to 475 ms at P3, Pz, P4, C3, Cz, C4, O1, Oz and O2 electrodes. In addition, latencies for P425 were determined at P3, Pz and P4 sites. Amplitude variations in the selected time intervals are sensitive to different stages of selective processing [37].

Data analysis

Sensory effects of frequency and orientation on P100 and N170 at occipital sites were analyzed using repeated measures analysis of variance (ANOVA) with group (control, PKU-L and PKU-H) as the between subjects factor and grating (HH, HL, VH, VL) as the within subjects factors.

Selective processing effects on frontal selection negativity (N90) and positivity (P180), the vertex negative wave (N280), and the positive wave over parieto-occipital sites (P425) were analyzed employing a similar ANOVA with group as the between subjects factor and frequency relevance (FR: relevant, irrelevant), orientation relevance (OR: relevant, irrelevant), and Topography (outer lateral, inner lateral sites) as within subjects factors; occipital selection positivity (P100) and negativity (N170) were analyzed similarly but without the Topography factor.

Main effects for FR and OR indicate differences between relevant and irrelevant frequencies and orientations, respectively. If the effect is specific for targets, a significant FR × OR interaction would be present. For all analyses involving factors with more than two levels, Greenhouse-Geisser corrected *p*-values are reported to prevent inflation of statistical significance.

Pearson product-moment correlations were computed and multiple regression analyses were performed to assess the relationship between Phe levels (concurrent, lifetime and IDC) and brain activity. Lifetime Phe levels were the median of Phe recordings for each year from 0 to10 years of age. The IDC was represented by the average median Phe levels across the first 10 years (or less if children were younger). In addition, multiple regression analyses were performed to examine whether behavioural variables (RT and accuracy) were associated with ERP activity. Age was entered as a variable in these analyses. To reduce the number of independent variables, difference amplitudes were calculated, subtracting amplitudes elicited by frequency-relevant, orientation-relevant, and irrelevant gratings from target gratings. Regression results are reported only if standardized residuals were below 3 and Cook's distances were below 1 [38]. Effect sizes were estimated using partial eta squared (η_p^2), and interpreted as small ($\eta_p^2 \approx 0.01$), medium ($\eta_p^2 \approx d$ 0.06), and large ($\eta_p^2 > 0.13$) [39].

Results

Sensory effects on VEP

Analyses of sensory effects on occipital P100 amplitude revealed a significant main effect of OR and FR at Oz (both $p = 0.001$), and at O1 and O2 ($p = 0.0001$ and $p = 0.019$, respectively). P100 was larger on vertical than horizontal gratings and larger on low than on high frequency gratings. N170 amplitude was only affected by OR at Oz ($p = 0.039$), and at O1 and O2 ($p = 0.047$), showing larger negativity on horizontal than on vertical gratings. Children with PKU and controls did not significantly differ in occipital P100 and N170 amplitudes.

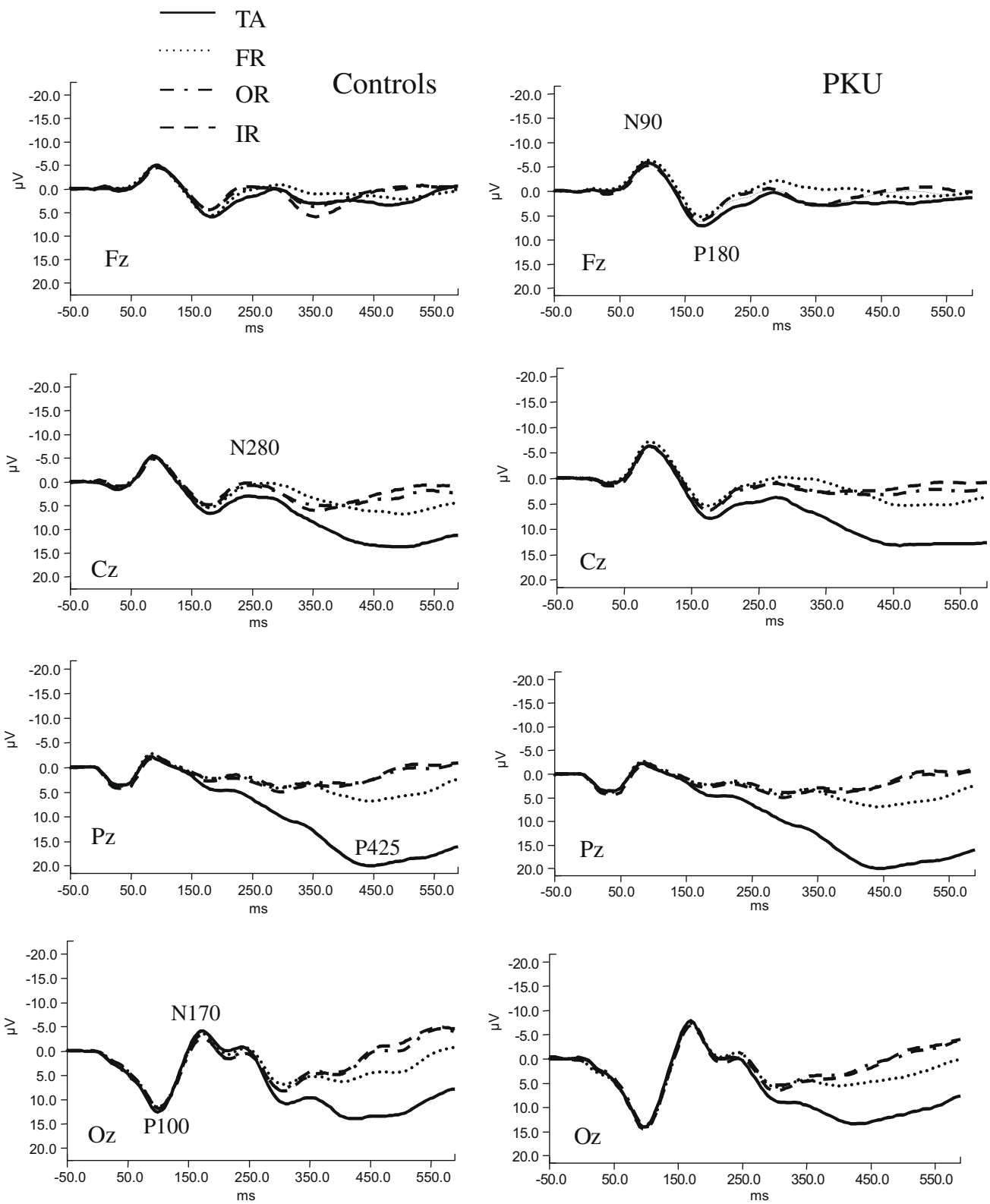

Fig. 1. Grand average ERP waveforms for target (TA), frequency-relevant (FR), orientation-relevant (OR) and irrelevant stimuli (IR) at occipital (Oz), parietal (Pz), central (Cz) and frontal (Fz) sites for controls (left) and children with PKU (right).

Significant correlations were found between IDC and N170 amplitude at O1, Oz and O2 ranging from −0.344 to −0.426 (*p*-values from 0.04 to 0.013), indicating that higher IDC scores (poorer dietary control) were associated with increased negativity of N170. No relationship between concurrent Phe levels and VEP amplitudes was found.

Multiple regression analyses with Phe and IDC scores as independent variables and VEP amplitudes as dependent variables re-

vealed that IDC level explained 15.5% of variance in N170 amplitudes at Oz ($F(1, 31) = 6.891$, $p = 0.013$) (see Table 1). Examination of the relationship between lifetime Phe levels and VEP amplitudes showed that N170 amplitude at O1 and O2 correlated negatively with median Phe levels at ages 2, 6 and 7 years (r varied from −0.371 to −0.497, p-values varied from 0.014 to 0.001 1-tailed), indicating that increased Phe levels at these ages were associated with enhanced N170 amplitudes. Phe level at the age of 2, 6 and 7 years accounted for 21.1%, 22.3% and 17% of variance in N170 amplitudes, respectively.

Selective processing effects on VEP

Analyses of frontal selection negativity (N90) revealed a significant FR effect at lateral ($p = 0.003$) and midline ($p = 0.007$) sites. N90 amplitude was larger for frequency-relevant than irrelevant gratings. Midline frontal selection negativity also showed an effect of group ($F(2, 87) = 3.706$, $p = 0.029$, $\eta_p^2 = 0.079$). Tukey multiple comparisons revealed that PKU-H had larger amplitudes than controls ($p = 0.026$).

Analyses of frontal selection positivity (P180) showed an OR effect and an OR × FR interaction effect at lateral and midline sites. These effects indicate that targets elicited larger positivity than all other gratings at lateral and midline sites (p-values range from 0.006 to 0.0001). At midline sites the target effect appeared most pronounced at central electrodes (OR × FR × Topography; $p = 0.050$). At lateral sites the group × FR interaction was significant ($F(2, 84) = 3.50$, $p = 0.035$, $\eta_p^2 = 0.077$). This interaction indicates that control children have reduced P180 amplitudes on frequency-irrelevant gratings, whereas in both PKU groups P180 amplitude does not differentiate frequency-relevant from irrelevant gratings.

There were no group differences with regard to amplitudes and latencies of the vertex negative wave (N280) or the late positive wave over posterior electrode sites (P425). Multiple regression analyses performed on ERP amplitudes and concurrent Phe levels and IDCs (see Table 1) revealed that IDC explained 9.3% and 37.0% of variance in N90 amplitudes at frontal and central sites, respectively, and accounted for 12.6% and 20.3% of variance in P180 and N280 at frontal locations, respectively. Concurrent Phe level accounted for 11.3% and 10.4% of variance in N280 amplitudes at frontal and central locations, respectively. Concurrent Phe level explained 8.8%, 7.8% and 12.0% of variance in P180 amplitudes at both frontal sites (Fp, F) and at central sites (C), respectively, and 8.5% of variance in N90 amplitudes at frontal locations.

Regression analyses were also performed on median lifetime Phe levels from the first year of life until 10 years of age (see Table 2). Inspection of Table 2 shows that Phe levels explained substantial proportions of variance in N90, P180 and N280 amplitudes over frontal and central sites when the children were 1 year old (9.5–19.8%), 2 years old (8.4–41.2%) and 10 years old (14.8–42.4%). In the period from 3–9 years Phe level does account significantly for variance in amplitudes of some of the same ERP components but percentages are lower.

Behavioural and ERP measures

ANOVAs on target RT and accuracy showed that RT was not significantly different between groups. Significant group differences were found on the number of false positive responses on orientation-relevant ($F(2,83) = 4.410$, $p = 0.015$, $\eta_p^2 = 0.096$) and irrelevant ($F(2, 83) = 4.173$, $p = 0.019$, $\eta_p^2 = 0.091$) trials. Additional comparisons revealed that PKU-H children were less accurate on target trials than controls ($p = 0.021$) and made more false alarms on orientation-relevant and irrelevant trials than either controls ($p < 0.009$ and $p = 0.011$, respectively) or PKU-L children ($p = 0.015$ and $p = 0.033$, respectively).

Multiple regression analyses revealed that RT was strongly related to age and also N280, N90 and N170 amplitudes in children with PKU (see Table 3). In controls, RT was related to age and P425 amplitude, whereas accuracy (number of targets correct) was related to age, P425 and N90 amplitudes in controls, but age only in children with PKU. In children with PKU, the number of false alarms on frequency-relevant stimuli was related to amplitude of sensory potentials over occipital sites, as was RT. In controls no relationship between sensory potentials and behavioural measures was found.

Discussion

Children with PKU differed from controls with respect to accuracy of task performance. Accuracy of performance was related to lifetime/historical/longer-term and concurrent Phe levels with a stronger effect of lifetime/historical/longer-term Phe concentrations.

Children with high concurrent Phe levels (>360 μmol/L) performed more poorly than controls. In some instances, specifically when the numbers of false alarms on orientation-relevant and irrelevant trials were concerned, children with high concurrent Phe levels also performed more poorly than children with low concurrent Phe levels (⩽360 μmol/L).

Children with PKU also differed from controls with respect to brain activity during task performance. These differences were specifically observed for later selective attention-related ERP components. No group differences, however, were observed for early sensory components.

Concurrent Phe levels did not strongly influence amplitudes and latencies of early sensory or later selective attention-related ERP components. In the analyses of early sensory components, there were no group differences at all, whereas in the analyses of the la-

Table 1
Results of regression analyses on IDC and concurrent Phe level and ERP amplitudes at different electrode sites.

	ERP component	Sites	% Variance	df	F	p
IDC	N90	Fp	9.3	1/34	4.59	0.039
		C	37.0	2/32	10.99	0.0001
	P180	Fp	12.6	1/34	6.03	0.019
	N280	Fp	20.3	2/32	5.32	0.010
	SN170	O	15.5	1/31	6.89	0.013
Concurrent Phe	N90	F	8.5	1/37	4.53	0.040
	P180	Fp	8.8	1/38	4.77	0.035
		F	7.8	1/37	4.19	0.048
		C	12.0	1/37	6.19	0.018
	N280	Fp	11.3	1/38	5.96	0.019
		C	10.4	1/37	5.40	0.026

Table 2
Results of regression analyses on lifetime Phe levels (median of each year from 1 to 10 year) and ERP amplitudes at different electrode sites.

Median Phe level	ERP component	Sites	% Variance	df	*F*	*p*
1st year	N90	C	18.6	2/31	4.78	0.016
		Fp	9.5	1/33	4.57	0.040
	P170	Fp	19.8	1/33	9.40	0.004
		C	12.0	1/32	5.50	0.025
	N280	Fp	11.7	1/33	5.52	0.025
2nd year	N90	Fp	34.8	2/33	10.35	0.0001
		C	41.2	2/32	12.93	0.0001
		F	20.2	1/33	9.62	0.004
	P170	Fp	20.0	1/34	9.76	0.004
	N280	Fp	8.4	1/34	4.22	0.048
	SN170	O	21.1	2/30	5.26	0.011
3rd year	P180	Fp	18.5	2/33	4.98	0.013
4th year	P425	O	19.9	2/31	5.11	0.012
	P100	O	10.6	1/32	4.91	0.034
5th year	N90	F	12.4	1/33	5.82	0.022
	N280	F	9.0	1/33	4.37	0.044
	N170	O	21.3	1/35	10.75	0.002
6th year	N90	Fp	9.3	1/35	4.70	0.037
	SN170	O	22.3	1/32	10.50	0.003
7th year	N90	Fp	9.4	1/35	4.75	0.036
		F	29.1	3/32	5.78	0.003
	P180	Fp	8.1	1/35	4.18	0.048
	SN170	O	17.0	1/32	7.74	0.009
9th year	N90	C	25.2	2/31	6.56	0.004
10th year	N90	Fp	14.8	1/27	5.87	0.022
	P180	C	42.4	2/25	10.95	0.0001
		Fp	35.4	1/27	16.34	0.0001
	N280	Fp	22.2	1/27	8.98	0.006
		F	35.9	2/25	8.55	0.001
		C	25.5	2/25	5.62	0.010
	P100	O	37.0	1/27	17.45	0.0001

Table 3
Results of regression analyses on behavioural variables, age, and ERP components for PKU and controls.

Behavioural variable	ERP component	Site	% Variance	df	*F*	*p*	
PKU			Age	ERP			
Reaction time	Age, N280, N90, N170	C, Fp, O	50.5	23.6	4/30	25.3	0.0005
	Age, SN170	O	50.5	7.9	2/33	25.5	0.0005
	Age, P425lat	P	50.5	8.4	2/37	29.0	0.0005
NTARG	Age, P110	O	23.1	18.8	2/34	13.9	0.0005
NFR	SP110	O	34.7		2/34	10.6	0.0005
NOR	P180, N90	F, C		37.4	3/35	8.6	0.0005
NIRR	P180, Age	C	19.6	10.9	2/35	9.1	0.001
	SN170	O	8.8	8.8	1/34	4.4	0.044
Controls							
Reaction time	Age, P425	O	40.3	13.9	2/41	26.4	0.0005
	Age, P425lat	P	40.3	14.7	2/42	27.9	0.0005
NTARG	Age, P425, N90	C, Fp	25.3	17.3	3/39	11.4	0.0005
	Age, P425lat	P	25.3	5.7	2/41	10.7	0.0005
NOR	N90, Age, P180	Fp	6.5	26.5	3/40	8.1	0.0005
NIRR	P180	Fp	12.1		1/41	6.8	0.013

NTARG, number of targets correct; NFR, false alarms on frequency-relevant gratings; NOR, false alarms on orientation-relevant gratings; NIRR, false alarms on irrelevant gratings.

ter components controls differed from children with high and low concurrent Phe levels on one of the frontal components (P180) associated with target detection. More specifically, children with PKU did not differentiate between relevant and irrelevant aspects of the signal, suggesting deficient selective attention, which would explain (at least partly) the finding that they made more false alarms to non-targets. There was some evidence for a concurrent Phe-related effect on another frontal component (N90) associated with target detection, where there was only a significant difference between controls and children with PKU who had high concurrent Phe levels.

Historical Phe levels, as measured by an IDC, appeared to have a much greater influence, both on early sensory components and later selective attention components. Regression with both concurrent and historical Phe levels showed a significant relation between the early sensory component N170 (measured occipitally) and IDC, but not between this component and concurrent Phe level. The IDC was also a better predictor of selective attention components (i.e., N90, P180 and N280, the first measured frontally and centrally; the latter two measured frontally). P180 and N280 were exclusively predicted by IDC, whereas for N90 more variance was explained by IDC than concurrent Phe levels.

It was also evident that task performance was related to different ERP components in controls and PKU patients. For example, both accuracy and RT were related to sensory potentials for children with PKU, whereas such associations were largely absent for controls. For controls, there appeared to be frontal and parietal activity that was associated with task performance. It is not clear why this was the case, but it may be speculated that these differential patterns of brain activity are observed because of Phe-related damage to brain regions associated with task performance. Brain regions associated with sensory processing such as the occipital lobe may have to become more active in individuals with PKU to achieve the same level of sensory processing as controls. On the other hand, possible damage to regions associated with cognitive control (in this case selective attention), such as the prefrontal cortex and the posterior parietal cortex, may result in individuals with PKU using other brain regions (such as the occipital lobe) to compensate for reduced frontal and parietal activity.

Other studies have shown differences in sensory effects between children with PKU and controls [31]. These studies suggest that increased sensory amplitudes may reflect abnormalities in attention or in electrical responses from the retina. Although we did not find group differences, the correlations between N170 and IDC within the group of children with PKU provide some evidence for early Phe-induced harm to sensory systems that has long-lasting effects. Long-lasting effects of Phe levels during the first month of life on visual contrast sensitivity have been reported, suggesting some early structural damage to the visual system possibly due to elevated Phe levels [40]. In the present study, postnatal Phe levels, measured within 2 weeks after birth, were also highly elevated (about 1350 μmol/L) and could have interfered with the normal development of the dopamine-sensitive visual system [41]. It cannot be inferred from the present data, however, that the dopamine-sensitive visual system was responsible for the observed Phe-related differences between children with PKU and controls.

Regarding selective attention, the P425 can be equated with P300 or P3b, which is commonly associated with stimulus evaluation and/or context updating. P425 and P300/P3B have similar latencies and topographical distributions. When both frequency-relevant and orientation-relevant cues are present the stimulus can be classified as a target and P425 amplitude and latency can be seen as indices of this decision process. We expected that children with PKU with elevated Phe levels would show longer P425 latencies than controls and have reduced P425 amplitudes. Group differences in P425 latency were in the expected direction but were not statistically significant. Neither were there significant correlations between P425 latency and concurrent Phe levels or IDC. Similar findings for P300 latency have been reported [31], although Leuzzi et al. [21] did find longer latencies for PKU patients but no correlation with Phe levels.

The lack of significant P300 group differences could mean that the present task was too easy and did not elicit sufficient processing load, although children with elevated Phe levels displayed significantly more false alarms on frequency-relevant gratings and more missed targets than controls and children with low Phe levels. In addition, they tended to have longer RTs compared with controls. Interestingly, performance of controls was associated with P300/P425 amplitudes/latencies, whereas this association was much less evident for children with PKU. Again, this may indicate that, due to Phe-induced damage to brain regions necessary for cognitive control (selective attention), individuals with PKU processed information differently and relied on potentially less effective brain regions while completing the task.

In conclusion, from a behavioural perspective, the results supported our hypothesis that performance in children with PKU with increased Phe levels would be poorer than in patients with lower Phe levels and controls. From an electrophysiological perspective, the evidence that children with PKU with high Phe levels have a deviant pattern of brain activity in terms of amplitude and latency is less clear. Although RT mainly depends on age in both controls and children with PKU, it is remarkable that RT in children with PKU also depends on early brain electrical activity at occipital sites (sensory potentials and N170) and at fronto-central sites (N90 and N280), whereas RT in controls depends also on amplitude and latency of the late positive potential (P425). Similar relationships between accuracy, age and ERP components were found. That is, in children with PKU early potentials were associated with performance, whereas in controls accuracy also depended on amplitude and latency of the late P425. These findings suggest that behavioural performance in children with PKU depends more on the quality of early stages of information processing in which stimulus features have to be selected or rejected than on later processing stages associated with decision making or context updating as reflected by P425. In controls, behavioural performance seems to depend more on the efficiency of later stages of processing.

The most important overall conclusions of this study are that children with PKU differ from age-matched controls with regard to the quality of selective attention, that these differences can be observed in ERP components associated with selective attention (particularly those associated with relatively late attentional processing), and that historical Phe levels are the best predictors of both early and late components of selective attention in children with PKU. A preliminary conclusion is that children with PKU employ different brain regions compared to controls when selective attention is demanded or employ similar brain regions but to different degrees.

There are limitations to our study. The data as presented do not allow further distinction between contributions of different components of the visual system. In addition, although our group of patients with PKU was relatively large, statistical analyses of the many conditions of the task, as well as partitioning into high and low Phe PKU subgroups, would have benefited from a larger sample. The data also do not allow for distinctions between the contributions of the dopamine system and myelination, for which more advanced techniques will be required.

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References

[1] F.J. van Spronsen, M. Hoeksma, D.J. Reijngoud, Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause?, J Inherit. Metab. Dis. 32 (2009) 46–51.
[2] P. Burgard, Development of intelligence in early treated phenylketonuria, Eur. J. Pediatr. 159 (Suppl. 2) (2000) S74–S79.
[3] K. DeRoche, M. Welsh, Twenty-five years of research on neurocognitive outcomes in early-treated phenylketonuria: intelligence and executive function, Dev. Neuropsychol. 33 (2008) 474–504.
[4] K.H. VanZutphen, W. Packman, L. Sporri, M.C. Needham, C. Morgan, K. Weisiger, S. Packman, Executive functioning in children and adolescents with phenylketonuria, Clin. Genet. 72 (2007) 13–18.
[5] S.E. Christ, A. Moinuddin, R.C. McKinstry, M. DeBaun, D.A. White, Inhibitory control in children with frontal infarcts related to sickle cell disease, Child Neuropsychol. 13 (2007) 132–141.
[6] A. Diamond, M.B. Prevor, G. Callender, D.P. Druin, Prefrontal cortex cognitive deficits in children treated early and continuously for PKU, Monogr. Soc. Res. Child Dev. 62 (1997) (4, Serial No. 252).
[7] P. Griffiths, M. Tarrini, P. Robinson, Executive function and psychosocial adjustment in children with early treated phenylketonuria: correlation with historical and concurrent phenylalanine levels, J. Intellect. Disabil. Res. 41 (1997) 317–323.
[8] V. Leuzzi, M. Pansini, E. Sechi, F. Chiarotti, C. Carducci, G. Levi, I. Antonozzi, Executive function impairment in early-treated PKU subjects with normal mental development, J. Inherit. Metab. Dis. 27 (2004) 115–125.

[9] M.C. Welsh, B.F. Pennington, S. Ozonoff, B. Rouse, E.R.B. McCabe, Neuropsychology of early-treated phenylketonuria: specific executive function deficits, Child Dev. 61 (1990) 1697–1713.
[10] D.A. White, M.J. Nortz, T. Mandernach, K. Huntington, R.D. Steiner, Age-related working memory impairments in children with prefrontal dysfunction associated with phenylketonuria, J. Int. Neuropsychol. Soc. 8 (2002) 1–11.
[11] B. Azadi, A. Seddigh, M. Tehrani-Doost, J. Alaghband-Rad, M.R. Ashrafi, Executive dysfunction in treated phenylketonuric patients, Eur. Child Adolesc. Psychiatry 18 (2009) 360–368.
[12] M.L. Gourovitch, S. Craft, S.B. Dowton, P. Ambrose, S. Sparta, Interhemispheric transfer in children with early-treated phenylketonuria, J. Clin. Exp. Neuropsychol. 16 (1994) 393–404.
[13] M.M. Mazzocco, A.M. Nord, W.J. Van Doorninck, C.L. Greene, C.G. Kovar, B.F. Pennington, Cognitive development among children with early-treated phenylketonuria, Dev. Neuropsychol. 10 (1994) 133–151.
[14] E. Schmidt, P. Burgard, A. Rupp, Effects of concurrent phenylalanine levels on sustained attention and calculation speed in patients treated early for phenylketonuria, Eur. J. Pediatr. 155 (Suppl. 1) (1996) S82–S86.
[15] J. Weglage, M. Pietsch, B. Funders, H.G. Koch, K. Ullrich, Deficits in selective and sustained attention processes in early treated children with phenylketonuria: result of impaired frontal lobe functions?, Eur J. Pediatr. 155 (1996) 200–204.
[16] J. Weglage, E. Schmidt, B. Funders, M. Pietsch, K. Ullrich, Sustained attention in untreated non-PKU-hyperphenylalanaemia, J. Clin. Exp. Neuropsychol. 18 (1996) 343–348.
[17] L.M.J. De Sonneville, E. Schmidt, U. Michel, U. Batzler, Preliminary neuropsychological test results, Eur. J. Pediatr. 149 (Suppl. 1) (1990) S39–S44.
[18] S.C.J. Huijbregts, L.M.J. De Sonneville, R. Licht, F.J. Van Spronsen, P.H. Verkerk, J.A. Sergeant, Sustained attention and inhibition of cognitive interference in treated phenylketonuria: associations with concurrent and lifetime phenylalanine concentrations, Neuropsychologia 40 (2002) 7–15.
[19] S.C.J. Huijbregts, L.M.J. De Sonneville, F.J. Van Spronsen, R. Licht, J.A. Sergeant, The neuropsychological profile of early and continuously treated phenylketonuria: orienting, vigilance, and maintenance versus manipulation-functions of working memory, Neurosci. Biobehav. Rev. 26 (2002) 697–712.
[20] J. Albrecht, S.F. Garbade, P. Burgard, Neuropsychological speed tests and blood phenylalanine levels in patients with phenylketonuria: a meta-analysis, Neurosci. Biobehav. Rev. 33 (2008) 414–421.
[21] V. Leuzzi, S. Seri, A. Cerquiglini, CL. Carducci, Ca. Carducci, I. Antonozzi, Derangement of the dopaminergic system in phenylketonuria: study of the event-related potential (P300), J. Inherit. Metab. Dis. 23 (2000) 317–320.
[22] A. Diamond, A model system for studying the role of dopamine in the prefrontal cortex during early development in humans: early and continuously treated phenylketonuria, in: C.A. Nelson, M. Luciana (Eds.), Handbook of Developmental Cognitive Neuroscience, MIT Press, Cambridge, Massachusetts, 2001.
[23] U. Bick, G. Fahrendorf, A.C. Ludolph, P. Vassalo, J. Weglage, K. Ullrich, Disturbed myelination in patients with treated hyperphenylalaninaemia: evaluation with magnetic resonance imaging, Eur. J. Pediatr. 150 (1991) 185–189.
[24] U. Bick, K. Ullrich, U. Stober, H. Moller, G. Schuierer, A.C. Ludolph, C. Oberwittler, J. Weglage, U. Wendel, White matter abnormalities in patient with treated hyperphenylalanaemia: magnetic resonance relaxometry and proton spectroscopy findings, Eur. J. Pediatr. 152 (1993) 1012–1020.
[25] M.A. Cleary, J.H. Walter, J.P. Jenkins, S.M. Alani, K. Tyler, D. Whittle, Magnetic resonance imaging of the brain in phenylketonuria, Lancet 344 (1994) 87–90.
[26] A.J. Thompson, S. Tillotson, I. Smith, B. Kendall, S.G. Moore, D.P. Brenton, Brain MRI changes in phenylketonuria. Associations with dietary status, Brain 116 (1993) 811–821.
[27] J. Weglage, U. Bick, G. Schuierer, M. Pietsch, A. Sprinz, R. Zab, K. Ullrich, Progression of cerebral white matter abnormalities in early-treated patients with phenylketonuria during adolescence, Neuropediatrics 28 (1997) 239–240.
[28] T. Scarabino, T. Popolizio, M. Tosetti, D. Montanaro, G.M. Giannatempo, R. Terlizzi, S. Pollice, A. Maiorana, N. Maggialetti, A. Carriero, V. Leuzzi, U. Salvolini, Phenylketonuria: white-matter changes assessed by 3.0-T magnetic resonance (MR) imaging, MR spectroscopy and MR diffusion, Radiol. Med. 114 (2009) 461–474.
[29] V. Leuzzi, M. Tosetti, D. Montanaro, C. Carducci, C. Artiola, C. Carducci, I. Antonozzi, M. Burroni, F. Carnevale, F. Chiarotti, T. Popolizio, G.M. Giannatempo, V. D'Alesio, T. Scarabino, The pathogenesis of the white matter abnormalities in phenylketonuria. A multimodal 3.0 tesla MRI and magnetic resonance spectroscopy (1H MRS) study, J. Inherit. Metab. Dis. 30 (2007) 209–216.
[30] P.J. Anderson, S.J. Wood, D.E. Francis, L. Coleman, L. Warwick, S. Casanelia, V.A. Anderson, A. Boneh, Neuropsychological functioning in children with early-treated phenylketonuria: impact of white matter abnormalities, Dev. Med. Child Neurol. 46 (2004) 230–238.
[31] R.M. Henderson, D.L. McCulloch, A.M. Herbert, P.H. Robinson, M.J. Taylor, Visual event-related potentials in children with phenylketonuria, Acta. Paediatr. 89 (2000) 52–57.
[32] J. Pietz, E. Schmidt, P. Matthis, B. Kobialka, A. Kutscha, L.M.J. De Sonneville, EEGs in phenylketonuria. I: follow-up to adulthood; II: short-term diet-related changes in EEGs and cognitive function, Dev. Med. Child Neurol. 35 (1993) 54–64.
[33] S.M. Pueschel, L. Fogelson-Doyle, B. Kammerer, Y. Matsumiya, Neurophysiological, psychological, and nutritional investigations during discontinuation of the phenylalanine-restricted diet in children with classic phenylketonuria, J. Ment. Defic. Res. 27 (1983) 61–67.
[34] M. Giovannini, R. Valsasina, R. Villani, A. Ducati, E. Riva, A. Landi, R. Longhi, Pattern reversal visual evoked potentials in phenylketonuria, J. Inherit. Met. Dis. 11 (1988) 416–421.
[35] J. Pietz, R. Kreis, A. Rupp, E. Mayatepek, D. Rating, C. Boesch, H.J. Bremer, Large neutral amino acids block phenylalanine transport into brain tissue in patients with phenylketonuria, J. Clin. Invest. 103 (1999) 1169–1178.
[36] J. Beringer, Experimental Run Time Systems, BeriSoft Cooperation, Frankfurt am Main, Germany, 1996.
[37] J.L. Kenemans, F.T. Smulders, A. Kok, Selective processing of two-dimensional visual stimuli in young and old subjects: electrophysiological analysis, Psychophysiology 32 (1995) 108–120.
[38] J. Stevens, Applied Multivariate Statistics for the Social Sciences, Lawrence Erlbaum Associates, Mahwah, New Jersey, 1996.
[39] J. Cohen, Statistical Power Analysis for the Behavioral Sciences, Lawrence Erlbaum Associates, Hillsdale, NJ, 1988.
[40] A. Diamond, C. Herzberg, Impaired sensitivity to visual contrast in children treated early and continuously for phenylketonuria, Brain 119 (1996) 523–538.
[41] K. Ullrich, J. Weglage, C. Oberwittler, M. Pietsch, B. Funders, H.V. Eckhardstein, Effect of L-Dopa on visual evoked potentials and neuropsychological tests in adult phenylketonuria patients, Eur. J. Pediatr. 155 (Suppl. 1) (1996) S74–S77.