

Age-related working memory impairments in children with prefrontal dysfunction associated with phenylketonuria

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Abstract

The prefrontal cortex of the brain has been shown to play a crucial role in working memory, and age-related changes in prefrontal function may contribute to the improvements in working memory that are observed during childhood. We examined the developmental trajectory of working memory in school-age children with early-treated phenylketonuria (PKU), a metabolic disorder that results in prefrontal dysfunction. Using a recognition procedure, we evaluated working memory for letters, abstract objects, and spatial locations in 20 children with PKU and 20 typically developing control children. Children in both groups ranged from 6 to 17 years of age. Our findings revealed poorer performance across all three types of materials for children with PKU. In addition, there was a significant difference in the developmental trajectory of working memory for children with PKU as compared with controls. Specifically, deficits were not apparent in younger children with PKU. Instead, deficits were observed only in older children, suggesting the presence of a developmental deficit rather than a developmental delay in the working memory of children with PKU. (*JINS*, 2002, *8*, 1–11.)

Keywords: Working memory, Prefrontal cortex, Dopamine, Phenylketonuria, Children

INTRODUCTION

Working memory encompasses a cognitive system through which information may be maintained and manipulated for brief periods of time during the performance of cognitive tasks (Baddeley 1986, 1992). It is well established that the prefrontal cortex plays a prominent role in the mediation of this ability. Functional neuroimaging studies of healthy adults have shown that the prefrontal cortex is activated during the performance of working memory tasks involving verbal (Awh et al., 1996; Fiez et al., 1996; Paulesu et al., 1993; Petrides et al., 1993) and nonverbal (Jonides et al., 1993; Smith et al., 1995) materials. Findings from typically aging adults and patients with brain damage further support the notion that prefrontal integrity affects working memory performance. For example, neuroimaging studies have shown

that the declines in working memory observed in older adults are related to functional changes in prefrontal cortex (Jonides et al., 2000; Reuter-Lorenz et al., 2000; Rypma & D'Esposito, 2000). In terms of patient studies, individuals with prefrontal dysfunction related to dopamine dysregulation, such as adults with Parkinson's disease (Gabrieli et al., 1996) or schizophrenia (Cohen et al., 1999; Park & Holzman, 1992), have been shown to exhibit deficits in working memory.

The majority of research has focused on the relationship between prefrontal function and working memory in adults. It is equally interesting to consider this issue at the earlier end of the developmental spectrum. Working memory improves substantially during childhood (Case et al., 1982; Fry & Hale, 1996; Hitch et al., 1989; Hulme & Tordoff, 1989; White et al., 1994, 1995) and may be facilitated by concomitant improvements in basic cognitive abilities such as processing speed (Case et al., 1982; Fry & Hale, 1996) and prefrontally mediated inhibitory control (Bjorklund & Harnishfeger, 1990). Recent work using functional neuro-

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imaging has shown that, as in adults, the prefrontal cortex of children is activated during the performance of working memory tasks (Casey et al., 1995; Thomas et al., 1999). It has also been shown that cerebral infarcts occurring in the frontal cortex during childhood disrupt the development of working memory (White et al., 2000).

The prefrontal cortex undergoes considerable change during the course of early development and does not reach maturity until late adolescence. Prefrontal alterations in synaptic density, dendritic density, and myelination have all been associated with developmental changes in cognition (for an overview, see Huttenlocher & Dabholkar, 1997). It is also thought that the interconnections between prefrontal cortex and posterior brain regions undergo considerable refinement during childhood, such that higher order executive processes play an ever-increasing role in the enhancement of basic abilities. Thatcher and colleagues (Thatcher, 1991; Thatcher et al., 1987) used electroencephalographic techniques to show that critical periods of cognitive development are accompanied by the elaboration of white matter connections between frontal and posterior brain regions.

Luciani and Nelson (1998) spoke to the possible link between developmental changes in working memory and elaborated interconnectivity in a study of typically developing children from 4 to 8 years of age. These researchers found that younger and older children performed similarly on tasks with minimal working memory demands. Better performance by older children, however, was observed as the working memory demands required in performing cognitive tasks increased. The authors postulated an association between this age-related difference and the increased functional efficiency of the prefrontal cortex and its interconnections with distal brain regions that subservise basic perceptual abilities.

In the current study we examined the hypothesis that prefrontal dysfunction in school-age children with early-treated phenylketonuria (PKU) would be associated with disruptions in the development of working memory. This group is of interest from several perspectives. First, children with PKU experience a depletion of dopamine due to an abnormality in the metabolism of phenylalanine into tyrosine, which is an essential precursor of dopamine (Curtius et al., 1981; Diamond et al., 1994; Krause et al., 1985; Paans et al., 1996). Second, most studies examining the association between dopamine dysregulation and working memory have been conducted with adults (e.g., Parkinson's disease and schizophrenia); here we examined the effects of dopamine dysregulation in school-age children. Third, this population is of interest because, in addition to the abnormalities in dopamine synthesis, white matter abnormalities have been identified (Bick et al., 1991; Dyer et al., 1996; Hasselbalch et al., 1996; Lou et al., 1992; Thompson et al., 1993).

Prior to the implementation of newborn screening for PKU in the 1960s, the brain abnormalities associated with PKU typically resulted in mental retardation (Sandler, 1982). With early identification and dietary restriction of phenyl-

alanine intake, most children with PKU perform within expected limits in terms of general intellectual abilities (Waisbren et al., 1987). Their scores, however, tend to be somewhat lower than their siblings and parents without PKU (Dobson et al., 1977; Koch et al., 1984; Ris et al., 1994). Because it is not possible to completely eliminate phenylalanine from the diet, mild elevations in phenylalanine are typical in children with early-treated PKU (for a comprehensive overview see Scriver et al., 1995) and have been associated with deficits in a range of neuropsychological areas. For example, impairments have been identified in attention (Craft et al., 1992; Lou et al., 1985; Ris et al., 1994), visuospatial ability (Brunner et al., 1987; Fishler et al., 1987; Koff et al., 1977; Pennington et al., 1985; Ris et al., 1994), executive ability (Brunner et al., 1983; Diamond, 1994; Diamond et al., 1997; Faust et al., 1986; Pennington et al., 1985; Weglage et al., 1996; Welsh et al., 1990; White et al., 2001), strategic contributions to long-term memory (White et al., 2001), response speed (Krause et al., 1985; Pietz et al., 1993; White et al., 2001), inter-hemispheric interactions (Banich et al., 2000; Gourovitch et al., 1994), and academic ability (Berry et al., 1979; Brunner et al., 1983).

Given that untreated PKU results in mental retardation (Sandler, 1982), it is clear that elevations in phenylalanine are detrimental to cognitive development. There is, however, inconsistency in the literature regarding the relationship between phenylalanine levels and cognitive performance in children with early-treated PKU. Several studies have demonstrated a negative relationship between phenylalanine level obtained close to the time of cognitive evaluation (i.e., concurrent phenylalanine level) and performance on a variety of cognitive tasks (Brunner et al., 1983; Diamond et al., 1997; Weglage et al., 1996; Welsh et al., 1990). Mazzocco et al. (1994), however, failed to identify a significant relationship between concurrent phenylalanine level and cognitive performance. Results are also inconsistent in terms of life-time phenylalanine level. Welsh et al. (1990) identified a significant negative relationship with cognitive performance, whereas Weglage et al. (1996) did not. Finally, infant phenylalanine level does not appear to be predictive of later cognitive performance (Brunner et al., 1983; Welsh et al., 1990).

In the current investigation we extended our knowledge of the neuropsychological abilities of school-age children with early-treated PKU by examining working memory performance. Because the prefrontal cortex and its interconnections with other brain regions continue to develop during childhood (Huttenlocher & Dabholkar, 1997; Thatcher, 1991; Thatcher et al., 1987), we hypothesized that working memory performance would be poorer for children with PKU compared with uncompromised control children. We also examined the developmental trajectory of working memory and the possibility that children with PKU demonstrate a developmental deficit rather than a developmental delay in working memory. In other words, we evaluated whether or not deficits in working memory would be more apparent in

younger or older children with early-treated PKU. Finally, we examined the relationship between phenylalanine levels and cognitive performance.

METHODS

Research Participants

A total of 20 (11 female, 9 male) children with early-treated PKU were recruited through the Division of Medical Genetics/Department of Pediatrics at St. Louis Children's Hospital in Missouri and through the Metabolic Clinic at the Child Development and Rehabilitation Center at Doernbecher Children's Hospital in Portland, Oregon. For all children with PKU, diagnosis was made and treatment was implemented before 6 weeks of age. At the time of participation, all children were on a dietary control program to limit phenylalanine intake. Phenylalanine levels concurrent with participation in our study ranged from 2 to 16 mg/dl ($M = 8.3$ mg/dl, $SD = 4.0$ mg/dl), which is elevated in comparison with the levels typically observed in individuals without PKU (i.e., 2 mg/dl; Scriver et al., 1995). The highest phenylalanine levels on record at the medical clinics from which children were recruited ranged from 7 to 54 mg/dl ($M = 23.4$ mg/dl, $SD = 12.8$ mg/dl).

The working memory performance of children with PKU was compared with that of 20 (10 female, 10 male) typically developing control children who were recruited from the St. Louis and Portland communities. No children in the control or PKU groups had histories of learning disorder or major medical disorder other than PKU. Children in both groups ranged from 6 to 17 years of age. Mean (SD) years of age for control and PKU groups were 10.5 (3.6) and 11.4 (3.5), respectively. Education ranged from 0.6 to 11.3 years for the control group and from 0.9 to 11.9 years for the PKU group. Mean (SD) years of education for the control and PKU groups were 4.9 (3.4) and 6.0 (3.6), respectively. There were no significant group differences in these variables.

Basic verbal and nonverbal abilities were evaluated using the Picture Vocabulary and Spatial Relations subtests of the Woodcock-Johnson Psycho-Educational Battery-Revised (Woodcock & Johnson, 1989). Verbal standard scores ranged from 85 to 128 for the control group and from 83 to 125 for the PKU group. Mean (SD) verbal standard scores for the control and PKU groups were 108 (13) and 104 (15), respectively. Nonverbal standard scores ranged from 90 to 135 for the control group and from 91 to 131 for the PKU group. Mean (SD) nonverbal standard scores for the control and PKU groups were 111 (14) and 109 (13), respectively. There were no significant group differences in these variables.

Procedure

Working memory tasks were administered as part of a larger neuropsychological test battery. Results from other compo-

nents of this battery are reported elsewhere (White et al., 2001).

Stimuli

Three types of stimuli (letters, objects, and spatial locations) representing 3 experimental conditions were used in constructing the working memory tasks. For each stimulus type, pools containing 16 items were assembled. The letter pool contained the following upper case consonants: B, C, D, F, G, H, J, L, N, P, Q, R, S, V, X, Z. Filled line drawings representing abstract objects comprised the object pool (see Appendix A for examples; adapted from Vanderplas & Garvin, 1959). The spatial pool consisted of the 16 locations represented within a 4×4 grid (see Appendix B for examples).

Perceptual discrimination

To ensure that children could discriminate between the stimuli of the types to be presented in our working memory tasks, we administered recognition trials using a subset of stimuli from each of the pools. The order in which the conditions (letter, object, and spatial) were administered was randomly determined, and each condition was presented as a block of five trials. During each trial, a single stimulus appeared on a computer monitor for 1250 ms. Following an interstimulus interval of 500 ms, a second stimulus replaced the first. Children were asked to indicate whether the second stimulus was identical to the first by saying "same" or "different." The examiner recorded the accuracy of children's responses by depressing the right or left computer mouse button for correct or incorrect responses, respectively.

Working memory

To assess working memory, series of two to nine stimuli were presented on a computer monitor. For each child, the order in which the three stimulus type conditions were administered was identical to that used in the perceptual discrimination trials. Series comprising each condition were administered as a block. Stimuli within a series were presented one at a time, with each stimulus remaining on the monitor for 1250 ms. The interstimulus interval was 500 ms. Following presentation of the last stimulus of a series, an array appeared that included all of the stimuli presented in that series. Children were asked to point to the stimuli in the order in which these were presented in the memory series. The examiner depressed the right or left computer mouse button to record correct or incorrect responses, respectively.

A staircase method (Watkins, 1977) was used in presenting the series of each condition. Administration always began with a series of two stimuli. If children correctly identified the order in which the stimuli were presented, the next series contained one additional stimulus. If children did not correctly identify the order of presentation, the next

series contained one less stimulus. An exception occurred when children responded incorrectly to series containing only two stimuli; in this instance, another series of two stimuli was presented.

As the starting point of each condition, children were administered a pair of practice trials to ensure that they understood the task demands and to ensure that all children could correctly identify series of at least two stimuli. The first practice trial included a series of two stimuli. If children responded correctly, the second practice trial included three stimuli, and no further practice trials were administered. If children responded incorrectly to the first two-stimulus practice trial, the second practice trial again included two stimuli; these children were also administered a second pair of practice trials.

Immediately following the practice trials, the working memory trials were administered using the staircase method. This procedure continued for a total of 15 trials in each condition. Working memory scores were recorded as the mean number of stimuli comprising the last seven series administered in each condition. Scores were calculated in this manner because we wished to reflect performance on trials during which children should have neared their maximal performance; in the first eight trials, children may have been approaching, but not yet have reached, their maximal performance. We also recorded maximum memory scores that reflected the number of stimuli in the longest series correctly recalled for each child.

RESULTS

Perceptual Discrimination

The percentage of correct responses was calculated in each condition. For the control group, mean percent correct in the letter, object, and spatial conditions was 96%, 93%, and 99%, respectively. For the PKU group, mean percent correct in the letter, object, and spatial conditions was 99%, 98%, and 98%, respectively. There were no significant group differences in perceptual accuracy across any of the three conditions. Thus, children were able to discriminate between the stimuli with a high degree of accuracy, and the performance of the two study groups was comparable.

Working Memory

During practice for the working memory tasks, most children correctly completed the series that included two stimuli on the first attempt. There were, however, 6 children in the control group and 3 children in the PKU group who were administered a second pair of practice trials due to an incorrect response on the first practice trial. All of these children responded correctly to the two-stimulus series in the second pair of practice trials. Thus, all of the children who participated in our study were able to perform the tasks and were able to correctly identify at least two stimuli across all conditions.

With regard to the working memory trials, we report results obtained from the analysis of mean scores recorded for the last seven series presented. Means and standard deviations of raw group scores in the letter, object, and spatial conditions are reported in Table 1. An identical pattern of results was obtained using maximum scores; the details of these analyses are not reported for the sake of brevity.

Mixed model analysis of covariance (ANCOVA) was used to examine group differences in memory scores and to examine the effects of stimulus type on memory scores. Group (control, PKU) served as the between-subjects variable and stimulus type (letter, object, spatial) served as the within-subjects variable. Although age was not significantly different between our study groups, children in the PKU group tended to be older than children were in the control group. Because small differences in age can contribute to variations in performance during early development, age was included as a covariate.

Results of ANCOVA revealed a significant main effect of group [$F(1,37) = 7.62, p < .01$], reflecting the fact that memory scores were greater for the control group than for the PKU group. There was no significant effect of stimulus type, indicating that memory scores were approximately equivalent across the letter, object, and spatial conditions. There was also no significant interaction between group and stimulus type. Thus, stimulus type had comparable effects on memory scores for the control and PKU groups. Additional comparisons verified that the scores of the control group were significantly greater than those of the PKU group across all conditions [letter: $F(2,37) = 5.40, p < .05$; object: $F(2,37) = 6.01, p < .05$; spatial: $F(2,37) = 4.73, p < .05$].

As the next step in our analysis of these data, linear regression was performed to determine if the developmental trajectory of working memory was comparable for the control and PKU groups. Because of the absence of a significant effect of condition or a group by condition interaction, we used a summary memory score that represented the mean across letter, object, and spatial conditions (see Table 1). As depicted in Figure 1, summary memory scores were plotted as a function of age for each group. A test for separate regressions revealed that the intercepts of the functions for the control (1.31) and PKU (2.80) groups were not significantly different. The slopes of the functions for the control (.29) and PKU (.08) groups, however, were significantly

Table 1. Means and standard deviations of raw scores for study groups

Variable	Control		PKU	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Verbal score	5.2	1.6	4.6	1.1
Object score	3.9	1.2	3.4	0.7
Spatial score	3.8	1.4	3.3	0.7
Summary score	4.3	1.3	3.8	1.1

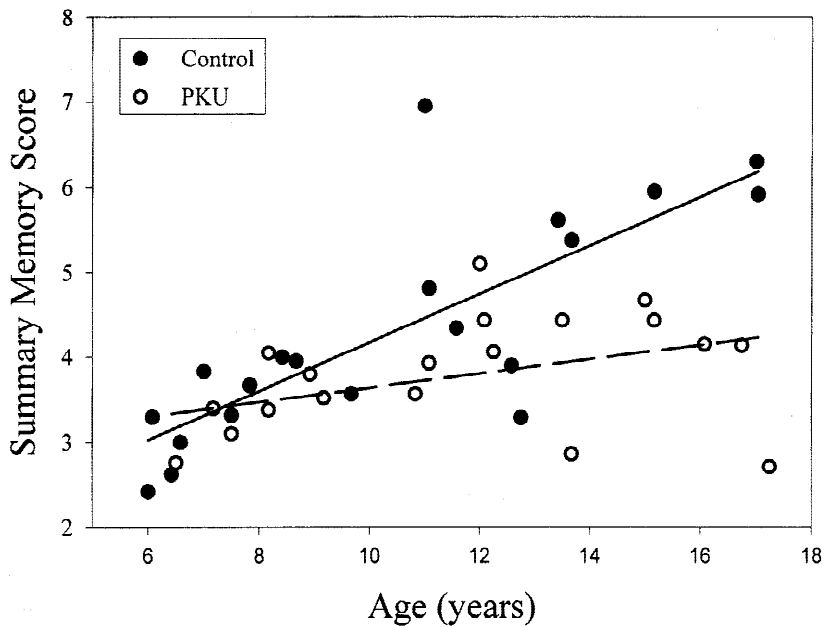


Fig. 1. Summary memory scores plotted as a function of age for each group.

different [$t(15) = -3.05, p < .005$], reflecting poorer memory scores with increasing age for the PKU group.

Because our regression analysis indicated that age had a different effect on working memory for the control and PKU groups, we also examined our data after dividing children into younger (less than 11 years of age) and older (greater than or equal to 11 years of age) subgroups. The resulting younger subgroups included 10 controls (years of age: $M = 7.4, SD = 1.2$) and 9 children with PKU (years of age: $M = 8.1, SD = 1.4$). The resulting older subgroups included 10 controls (years of age: $M = 13.5, SD = 2.2$) and 11 children with PKU (years of age: $M = 14.1, SD = 2.1$). Means and standard deviations of raw summary scores are reported in Table 2. For the sake of completeness, means and standard deviations of raw scores for the letter, object, and spatial conditions are also reported. Using ANCOVA (age was again used as a covariate), no significant differences were observed between the summary scores of the younger control and younger PKU subgroups. The summary score of the older control subgroup, however, was significantly greater than that of the older PKU subgroup [$F(2, 18) = 7.67, p < .05$].

To provide a context for interpreting the clinical significance of our findings, z scores were calculated for children in the younger and older PKU subgroups (see Table 2). These scores were based on the means and standard deviations of the appropriate control subgroups. The effect of age was statistically controlled when performing these calculations. A t test was used to compare the summary z scores of the younger PKU subgroup with those of the older PKU subgroup. This analysis revealed significantly lower scores for children in the older PKU subgroup [$t(18) = 3.01, p < .01$]. Lower z scores for the older PKU subgroup were verified across the letter [$t(18) = 2.55, p < .05$], object [$t(18) = 3.31, p < .005$], and spatial [$t(18) = 2.16, p < .05$] conditions. Taken together, these findings suggest that impairments in working memory are present in older but not younger children with PKU.

In examining Figure 1, there is 1 older control and there are 2 older children with PKU for whom, considering their age, summary working memory scores might be considered outliers. Additional analyses were conducted to ensure that our results were not overly influenced by the performance of these 3 children. Using ANCOVA (age was used as a

Table 2. Means, standard deviations, and z scores for younger and older study subgroups

Variable	Younger					Older				
	Control		PKU			Control		PKU		
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	Z scores	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	Z scores
Verbal score	4.1	1.0	4.0	0.9	-0.4	6.4	1.2	5.1	1.1	-1.0
Object score	3.1	0.6	3.2	0.5	0.1	4.7	1.2	3.5	0.9	-1.1
Spatial score	2.9	0.7	2.9	0.5	-0.4	4.6	1.4	3.6	0.7	-0.7
Summary score	3.4	0.5	3.4	0.5	-0.4	5.2	1.1	4.1	0.7	-1.0

covariate), we compared scores of the control and PKU groups following the exclusion of data for these 3 children. This analysis verified our previous results, revealing a significant between-group difference [$F(1,34) = 5.07, p < .05$], with children with PKU performing more poorly than controls. We also compared the scores of the older control and PKU subgroups and found a trend that fell somewhat short of reaching significance [$F(1,15) = 4.17, p < .06$]. Our finding of a trend was most likely related to the reduction in power resulting from the loss of data for 3 out of 21 children when we conducted this restricted analysis. After excluding the three data points that might be considered outliers, examination of summary working memory scores provides compelling evidence that the older PKU subgroup performed more poorly than the older control group (older controls: $M = 5.05$; older PKU: $M = 4.37$).

Phenylalanine Levels and Cognitive Performance

In our study, it was possible that performance discrepancies between the younger and older subgroups of children with PKU were not attributable to age but instead were attributable to cohort effects. For example, the between-subgroup differences in working memory could have been related to poorer basic cognitive abilities or higher phenylalanine levels in the older PKU subgroup. Additional analyses were conducted to address these issues.

With regard to basic cognitive abilities, t tests revealed no significant differences between the younger and older subgroups in standard scores from the Picture Vocabulary or Spatial Relations subtests of the Woodcock-Johnson Psycho-Educational Battery-Revised (Woodcock & Johnson, 1989). In addition, no significant differences in basic cognitive ability scores were observed when we used ANCOVAs to control for concurrent phenylalanine level or highest phenylalanine level on record. More generally, no significant correlations were observed between phenylalanine levels (concurrent or highest recorded) and Picture Vocabulary or Spatial Relations scores. Similarly, there was no significant correlation between phenylalanine levels and summary working memory z scores.

With regard to possible discrepancies in phenylalanine levels, we found no significant differences between the younger and older PKU subgroups in terms of concurrent phenylalanine level (younger: $M = 7.0, SD = 4.2$; older $M = 9.3, SD = 3.8$) or the highest phenylalanine level on record at our participating clinics (younger: $M = 22.6, SD = 12.5$; older $M = 24.1, SD = 13.5$). To verify that discrepancies in working memory z scores for the younger and older PKU subgroups were not attributable to differences in phenylalanine levels, we reanalyzed our data using ANCOVA, with phenylalanine levels as covariates. After controlling for concurrent phenylalanine level, the between-subgroup differences remained statistically significant for the summary [$F(1,17) = 8.39, p < .01$], letter [$F(1,17) = 6.48, p < .05$], object [$F(1,17) = 8.06, p < .05$], and spatial [$F(1,17) =$

$5.70, p < .05$] z scores. Significant differences were also observed for the summary [$F(1,17) = 9.47, p < .01$], letter [$F(1,17) = 7.42, p < .05$], object [$F(1,17) = 10.39, p < .005$], and spatial [$F(1,17) = 4.61, p < .05$] z scores after controlling for the highest phenylalanine level on record. Thus, phenylalanine levels did not account for the between-subgroup differences in working memory performance.

DISCUSSION

In the current investigation we examined working memory in children with early-treated PKU. Because PKU results in an early depletion of dopamine that in turn affects the function of the prefrontal cortex (Curtius et al., 1981; Diamond et al., 1994; Krause et al., 1985; Paans et al., 1996), it was hypothesized that these children would demonstrate impairments in working memory compared with typically developing control children. This hypothesis was strongly supported. Across three types of materials (letters, objects, and spatial locations), children with PKU obtained significantly poorer working memory scores than did controls.

A particularly intriguing finding from our study was that PKU-related deficits in working memory were prominent during later childhood but not earlier childhood. There were three converging lines of evidence in support of this view. First, in examining memory scores as a function of age, we found a discrepancy in the developmental trajectories of working memory for children with PKU and control children. Second, after dividing children into younger and older subgroups, we found that the memory scores of younger children with PKU were comparable to those of younger control children. Older children with PKU, however, demonstrated a clear deficit in working memory. Finally, by transforming memory scores into z scores, we demonstrated that the performance of younger children with PKU was within one-half standard deviation of that of younger control children, whereas the performance of older children with PKU was 1 standard deviation below that of older control children.

Taken together, our findings suggest the presence of a *developmental deficit* rather than a *developmental delay* in working memory for children with PKU. If our findings were attributable to a developmental delay, one might have expected to see a pattern of results opposite to that observed. That is, as depicted in Figure 2 (see PKU delay), the slope describing memory scores as a function of age would have been steeper rather than shallower for children with PKU compared with control children. In other words, our results would have suggested that, with age, the working memory abilities of children with PKU were catching up with those of control children. Instead, working memory abilities appeared to decline with advancing age (see PKU deficit in Figure 2). The cross-sectional design of our study, however, precludes us from definitively stating that working memory impairments *emerge* with age in children with PKU. We plan to longitudinally follow the children who participated in our study to further explore this issue.

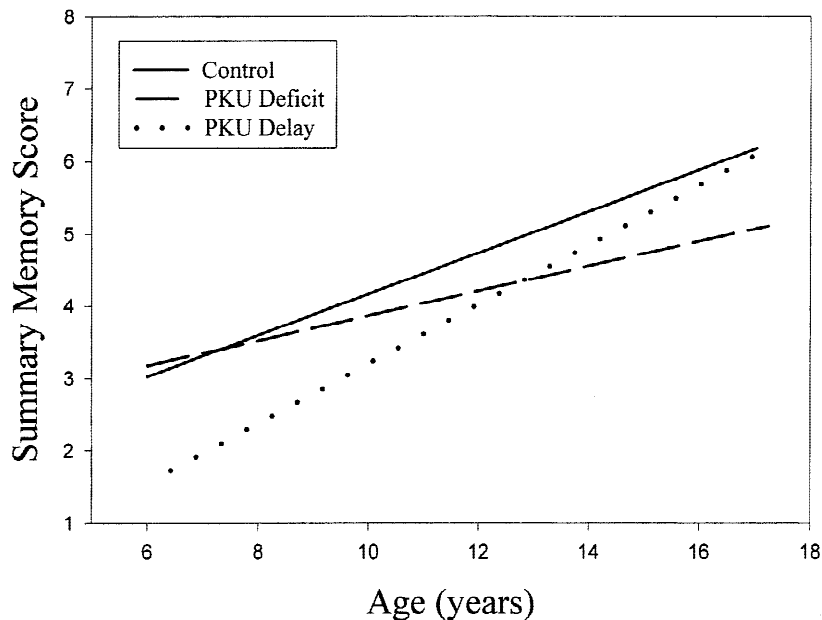


Fig. 2. Depiction of regression lines representing developmental delay *versus* developmental deficit.

Within the context of our cross-sectional design, it is possible that the developmental deficit in working memory for children with PKU was a cohort effect and was attributable to variables other than or in addition to age. For example, it could be hypothesized that differences in basic cognitive abilities or phenylalanine levels contributed to the discrepancy in working memory performance between younger and older children with PKU. This, however, does not appear to be the case. The verbal and nonverbal abilities of younger and older children with PKU were comparable, as were their phenylalanine levels. In addition, although contradictory to findings from some previous studies (Brunner et al., 1983; Diamond et al., 1997; Weglage et al., 1996; Welsh et al., 1990), phenylalanine levels were not significantly correlated with cognitive performance in our study. The reasons for discrepant findings across studies regarding phenylalanine levels remain unclear, but could be associated with differences in the cognitive domains assessed, the specific tasks administered, the ages of participating children, the range of phenylalanine levels of participating children, and the quality of dietary control.

It is also important to keep in mind that, as demonstrated by the performance of our control group, there is an age-related progression in working memory that occurs during the course of uncompromised development (Case et al., 1982; Fry & Hale, 1996; Hitch et al., 1989; Hulme & Tordoff, 1989; Luciani & Nelson, 1998; White et al., 1994, 1995). This likely reflects ongoing development in the prefrontal cortex (Luciani & Nelson, 1998). As discussed previously, maturation of the interconnections between prefrontal cortex and posterior brain regions also may contribute to the age-related progression. Thus, it is possible that working memory deficits were not apparent in the performance of younger school-age children with PKU because the contributions of executive processes subserved by prefrontal cor-

tex and its interconnections are less pronounced earlier in development. That is, younger children with PKU did not exhibit deficits in working memory because they had not yet reached the age at which strategic, prefrontal contributions to working memory are crucial to age-typical performance. We previously reported a similar pattern in the development of organizational strategies that facilitate long-term episodic memory for verbal information (White et al., 2001). Deficits in the use of organizational strategies were apparent only in older children with PKU, after the age at which the use of such strategies is expected to be evident in typically developing children (e.g., Bjorklund & Douglas, 1997).

It should be noted that previous work with infants, toddlers, and very young children (6 months to 7 years of age) with PKU has pointed to possible deficits in working memory (Diamond et al., 1997). These findings may appear contradictory to our findings that suggested deficits in working memory are not apparent until later childhood. The demands of the tasks used to assess working memory by Diamond et al. and by our research group, however, were quite different. For example, Diamond et al. described their *A not B* task (administered to infants and toddlers) and their Stroop-like *Day-night* task (administered to children 3.5 to 7 years of age) as “tests of working memory and inhibitory control, dependent on dorsolateral prefrontal cortex” (pp. 28–29). It is possible that inhibitory control makes a greater contribution to performance on these tasks than working memory during very early development and that the poorer performance of infants, toddlers, and very young children with PKU was attributable to deficits in inhibitory control rather than working memory.

Future investigation will be necessary to more thoroughly address the development of working memory in children with PKU. A variety of methods are used to assess

working memory (e.g., n-back, sentence span, or traditional memory span tasks) in children and adults, some permitting assessment of the ability to manipulate rather than simply maintain information (as was the case in our study). Converging evidence using such tasks would ensure that our findings are indicative of a general deficit in working memory rather than task-specific. In addition, a prominent component of the tasks used in our study was the requirement that temporal order be maintained. Along with working memory, this is an ability that has been strongly linked to function of the prefrontal cortex (Cabeza et al., 2000; Shimamura et al., 1990; Vriezen & Moscovitch, 1990). In future studies, it will be of interest to elucidate the distinct contributions of working memory and maintenance of temporal order to performance deficits in children with PKU.

To better ascertain the developmental point at which deficits in working memory become apparent in children with PKU, it will be useful to administer tasks specifically designed to assess working memory across a broad age range (e.g., infancy through childhood). Studies of children and adults with PKU will also permit us to determine if the developmental deficit we identified does in fact persist into adulthood. In turn, longitudinal study will permit us to determine if deficits in working memory emerge at the level of individual children as they age.

Neuroimaging studies of children and adults with PKU would also be of interest, as it is possible that the brain regions activated during working memory tasks in these individuals are more consistent with those activated in typically developing, younger children rather than typically developing adults. Neuroimaging technology might also provide information regarding the relative contributions of the prefrontal cortex versus other brain regions during the course of uncompromised and compromised working memory development. Within this neuroanatomical context, it would be of interest to consider the white matter abnormalities (Bick et al., 1991; Dyer et al., 1996; Hasselbalch et al., 1996; Lou et al., 1992; Thompson et al., 1993) that are associated with PKU. It is possible that the disruption of interconnections between prefrontal cortex and distal brain regions contributes not only to impairments in working memory, but to a range of cognitive deficits associated with PKU.

Finally, in future research it will be of interest to examine the contributions of other areas of cognition to working memory in children with PKU. For example, it has been suggested that developmental changes in processing speed (Case et al., 1982; Fry & Hale, 1996) or inhibitory control (Bjorklund & Harnishfeger, 1990) may underlie the improvements in working memory that occur in typically developing children. Perhaps very early deficits in inhibitory control (as identified by Diamond et al., 1997) or processing speed foreshadow the later emergence of deficits in working memory in children. By extension, it is possible that delays or deficits in these abilities underlie the developmental deficit in working memory that we have identified in school-age children with early-treated PKU.

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APPENDIX A

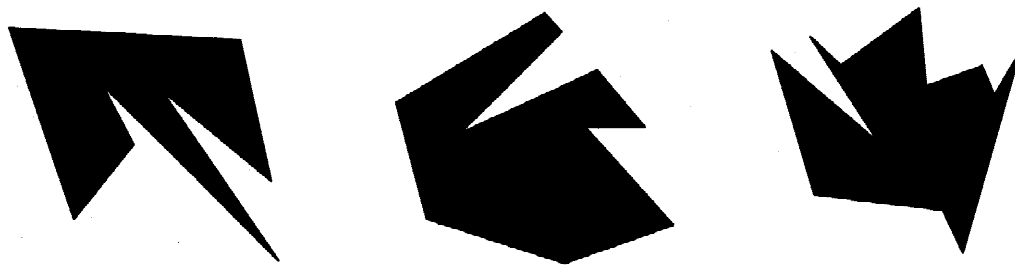


Fig. A1. Examples from the object stimulus pool (adapted from Vanderplas & Garvin, 1959).

APPENDIX B

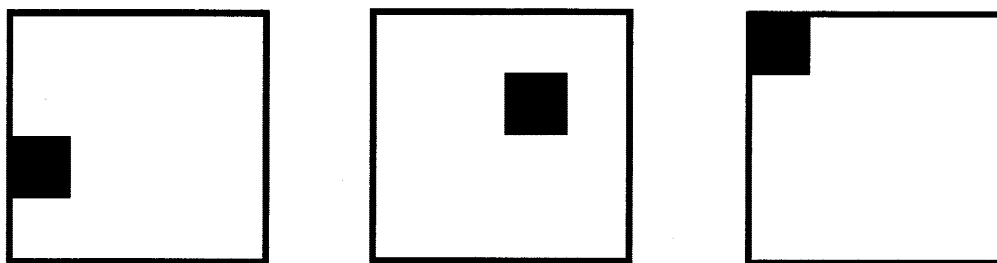


Fig. B1. Examples from the spatial locations stimulus pool.