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An Animal Model of Early-treated PKU

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Phenylketonuria (PKU) is a genetic disorder in which the hydroxylation of phenylalanine (Phe) to tyrosine is severely disrupted. If PKU is left untreated, severe mental retardation results. The accepted treatment is to restrict dietary intake of Phe. It has generally been thought that cognitive impairments are prevented if levels of Phe in plasma are maintained at or below five times the normal level. However, we recently documented that children treated early and continuously for PKU or children mildly hyperphenylalaninemic, who have levels of Phe in plasma approximately three to five times normal, still have cognitive impairments. These impairments are specific to the functions of frontal cortex (A. Diamond, W. Hurwitz, E. Lee, W. Grover, and C. Minarcik, unpublished observations). To investigate the mechanism underlying these cognitive deficits, an animal model of this condition was developed and characterized.

Thirty-six rat pups were divided into three groups. The first group was treated pre- and postnatally with Phe and α-methylphenylalanine (a phenylalanine hydroxylase inhibitor). The second group was injected postnatally with Phe and α-methylphenylalanine. The third group received postnatal control injections. The mild plasma Phe elevations in the two experimental groups produced significant behavioral and neurochemical effects. Both experimental groups were impaired on a task dependent on frontal cortex, delayed alternation. Levels of dopamine, homovanillic acid (HVA), norepinephrine, and 5-hydroxyindole acetic acid (5-HIAA) were measured in medial prefrontal cortex, anterior cingulate cortex, striatum, and nucleus accumbens. The largest neurochemical reductions observed were in HVA and were in the two frontal cortical areas (medial prefrontal cortex and anterior cingulate cortex). There were modest reductions in HVA in the nucleus accumbens but no significant changes in HVA, or in any other metabolite or neurotransmitter, in the striatum. The levels of 5-HIAA were also reduced in all brain regions examined. There was no effect on norepinephrine in any of the four regions examined. Reduced levels of HVA in medial prefrontal cortex were the only neurochemical effect that significantly correlated with every measure of performance on the delayed alternation task.

This study provides evidence of deleterious effects from mild elevations in the levels of Phe in plasma previously considered small enough to be safe. These effects include impaired performance on a cognitive task dependent on frontal cortex and reduced HVA levels in frontal cortex. It is not possible to rule out a role for other neurotransmitter systems from this study alone, but the results of other investigations suggest that the behavioral impairment is due to the alterations in the dopamine system in frontal cortex.

[Key words: phenylketonuria, phenylalanine, tyrosine, prefrontal cortex, anterior cingulate, dopamine, homovanillic acid, 5-HT, inherited metabolic disorder, delayed alternation, memory]

Phenylketonuria (PKU) is most commonly caused by mutations of the gene in chromosome 12 that codes for phenylalanine hydroxylase (Woo et al., 1983; Lidsky et al., 1985; DiLella et al., 1986). Phenylalanine hydroxylase is essential for the conversion of phenylalanine (Phe) to tyrosine. Other mutations in this gene are the most common cause of milder forms of hyperphenylalaninemia (Ledley et al., 1986; Levy et al., 1971). These deficits in Phe metabolism result in increases in the level of Phe in the bloodstream (e.g., Krause et al., 1985) and often cause decreases in the level of tyrosine in blood. If untreated, PKU results in widespread brain damage and severe mental retardation (e.g., Hsia, 1970; Cowie, 1971). The treatment for PKU is a diet low in Phe. When begun early and consistently maintained, this diet averts the most severe consequences of PKU. Neither gross structural brain damage nor global cognitive impairments are found (e.g., Bickel et al., 1954; Hudson et al., 1970; Williamson et al., 1981). Unfortunately, recent data indicate that there are deficits in the cognitive abilities dependent on frontal cortex even in children who have been on a restricted diet since birth, if those children have three- to fivefold elevations in plasma Phe (e.g., Welsh et al., 1990; A. Diamond, W. Hurwitz, E. Lee, W. Grover, and C. Minarchik, unpublished observations). These elevations were previously thought to be benign. The cognitive deficits appear to be specific; the children's performance on tasks dependent on parietal cortex or the medial temporal lobe is normal (Diamond, Hurwitz, Lee, Grover, and Minarchik, unpublished observations).

While the Phe-restricted diet succeeds in greatly lowering Phe concentrations in plasma from their untreated levels (>20 mg/

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Table 1. Summary of experimental groups

Experimental conditions	Prenatal treatment	Postnatal treatment
Mildly elevated Phe levels pre- and postnatally ($N = 12$; 6 M, 6 F)	Mothers fed liquid diet supplemented with 0.5% α-methylphenylalanine[a] + 2% Phe beginning on gestational day 3.	Daily injections of α-methylphenylalanine (24 μmol/10 gm body wt) + Phe (12 μmol/10 gm body wt) beginning on P4.
Mildly elevated Phe levels postnatally ($N = 12$; 6 M, 6 F)	Mothers fed normal liquid diet.	Daily injections of α-methylphenylalanine (24 μmol/10 gm body wt) + Phe (12 μmol/10 gm body wt) beginning on P4.
Controls ($N = 12$; 6 M, 6 F)[b]	Mothers fed normal liquid diet.	Daily control injections of saline beginning on P4.

[a] α-Methylphenylalanine is an inhibitor of the enzyme phenylalanine hydroxylase.

[b] Every subject assigned to the control group was matched to a littermate of the same sex and size in the second experimental group. One male rat in the control group died of unknown causes in early infancy.

dl; >1200 μmol/liter), it rarely succeeds in bringing them down to normal (2 mg/dl). For this reason, even children treated early for PKU and maintained on diet have moderately elevated plasma levels of Phe (4–10 mg/dl). Children with mild hyperphenylalaninemia have comparable elevations in plasma Phe while on a normal diet. Because these levels had been thought acceptable, mildly hyperphenylalaninemic children are not usually placed on a restricted diet.

Since Phe and tyrosine compete for the same transporter proteins to cross the blood–brain barrier, increases in the ratio of Phe to tyrosine in plasma result in less tyrosine crossing into the brain (Chirigos et al., 1960; Pardridge and Olendorf, 1977; Miller et al., 1985), especially since the transporters have a higher affinity for Phe than for tyrosine (Pardridge and Olendorf, 1977; Miller et al., 1985). Most areas of the brain receiving dopaminergic input are insensitive to small changes in the level of tyrosine; prefrontal cortex is an exception. The dopaminergic neurons innervating prefrontal cortex appear to have higher levels of activity and higher dopamine turnover than most other dopaminergic neurons, and may also lack the synthesis-modulating autoreceptors present on most other dopaminergic neurons (Bannon et al., 1981a,b, 1982; Chiodo et al., 1984; Roth, 1984). This makes prefrontal cortex acutely sensitive to even a small change in tyrosine levels (Thierry et al., 1977; Chiodo et al., 1984; Tam et al., 1991). Indeed, reductions in tyrosine that have little or no effect on dopamine systems in much of the brain, profoundly reduce the amount of dopamine metabolized in prefrontal cortex (e.g., Bradberry et al., 1989). In addition, dopamine synthesis can also be affected by the competitive inhibition exerted by high levels of Phe on tyrosine hydroxylase activity (Levitt et al., 1965; Ikeda et al., 1967; McKean, 1972).

Furthermore, the cognitive functions subserved by frontal cortex are acutely sensitive to reductions in dopamine in frontal cortex. Selectively depleting prefrontal cortex of dopamine produces cognitive impairments as severe as those found after removal of frontal cortex (Brozoski et al., 1979). Indeed, local injection of dopamine antagonists into frontal cortex impairs performance on tasks dependent on prefrontal cortex in a precise, dose-dependent manner (Sawaguchi and Goldman-Rakic, 1991).

Given that (1) children with early-treated PKU or mild hyperphenylalaninemia have moderately elevated plasma levels of Phe, (2) moderate elevations in plasma Phe are capable of reducing brain levels of tyrosine only mildly, (3) the levels of dopamine in frontal cortex are affected by mild reductions in tyrosine too small to affect dopamine levels in most other brain regions, and (4) reductions in frontal cortex dopamine levels produce cognitive deficits similar to those observed in children with early-treated PKU or mild hyperphenylalaninemia, we hypothesized that the cognitive deficits in these children might be caused by reductions in dopamine metabolism in frontal cortex. To test this hypothesis, we developed what we believe to be the first animal model of *early-treated* PKU. The cognitive performance of these treated animals was compared to littermate controls using a task dependent on frontal cortex and the levels of biogenic amine neurotransmitters and their metabolites in the treated animals were measured.

Materials and Methods

Pharmacological treatment. Thirty-six Long Evans Hooded rats were bred from multiparous dams obtained from Charles River Breeders (Wilmington, MA). These rats were divided into three groups (see Table 1).

For some children with PKU, imbalances in the levels of Phe and tyrosine begin *in utero* (Lenke and Levy, 1980; Bessman et al., 1988). This can happen, for example, if the mother's plasma Phe levels are moderately elevated. To examine effects that begin prenatally and continue postnatally, animals in experimental group 1 were exposed to moderately elevated Phe levels beginning *in utero*. Beginning on day 3 after mating, their mothers were fed a diet supplemented with 0.5% α-methylphenylalanine (a partial inhibitor of phenylalanine hydroxylase; Greengard et al., 1976) plus 2% Phe (chemicals supplied by Sigma Chemical, St. Louis, MO). To keep Phe levels elevated for as much of the day as possible, the pregnant dams were fed in small amounts six times daily. Prior work has already demonstrated that elevated maternal levels of Phe are transmitted to the fetus via the placenta (e.g., Kerr et al., 1968; Wapnir and Dierks-Vertling, 1971; Levy and Waisbren, 1983) and that dietary supplementation such as used here raises Phe levels in the plasma and brain of both mother and fetus (Brass and Greengard, 1982; Brass et al., 1982). These investigators employed the same percentage of α-methylphenylalanine as used in the present study, but more Phe (3–5%; Brass and Greengard, 1982; Brass et al., 1982). The reason higher doses of Phe were used in these prior studies is that the investigators were trying to model *untreated* PKU.

Another nine dams received the same diet without the addition of α-methylphenylalanine or Phe. Because the liquid diet lacked fiber and the added Phe reduced its palatability, both the normal and Phe-enriched diets were supplemented with 5% fiber and chocolate (diets prepared by Bioserve Co., Frenchtown, NJ). To ensure that pregnant dams in the control and experimental groups consumed equal amounts of food, each dam on the normal diet was yoked to a dam on the Phe-enriched diet. Those on the Phe-enriched diet were provided with food

ad lib. Each dam on normal diet was fed the amount her match had eaten on the preceding day. Upon parturition, all dams were placed on a normal solid diet of Purina rat chow.

Pups of dams on the Phe-enriched diet were injected daily with α-methylphenylalanine and Phe, beginning on postnatal day 4 (P4) and continuing through the day of death (see Table 1). This is experimental group 1; they were exposed to elevations of Phe *in utero* and after birth. Pups of dams on normal diet were divided into two groups: one group received the same daily injections (experimental group 2: elevations in Phe only postnatally); the final group received daily injections of saline (the controls).

Both experimental groups received the same postnatal treatment; they differed only in the diet their mothers had been fed during pregnancy. Experimental group 2 and the controls were littermates, matched for sex, weight, and size; they differed only in their postnatal treatment. The daily subcutaneous injections were prepared according to the protocol of DelValle and Greengard (DelValle et al., 1978; Brass and Greengard, 1982) and began on postnatal day 4 (P4). P4 is when dopamine innervation of rat prefrontal cortex appears to begin (Kalsbeek et al., 1988) and is roughly the same age when previous investigators began their treatments to cause elevations in Phe (Greengard et al., 1976; DelValle et al., 1978; Brass and Greengard, 1982). Each experimental animal received two consecutive injections, one of α-methylphenylalaninc and one of Phe. Previous work had demonstrated that treatment results in Phe levels that remain elevated for at least 16–18 hr postinjection (DelValle et al., 1978), and that daily injections continue to be effective even after several weeks (Greengard et al., 1976; DelValle et al., 1978). Each control animal received two consecutive injections of saline isovolumetric with the α-methylphenylalanine and Phe injections for that animal's body weight. As was indicated above for the dietary treatment of pregnant dams, previous investigators injected the same percentage of α-methylphenylalanine as employed in the present study, but four times as much Phe (Greengard et al., 1976; DelValle et al., 1978; Brass and Greengard, 1982). In these prior studies, the dosage of Phe was higher because these studies were designed to model the higher Phe concentrations found in *untreated* PKU.

To determine the effectiveness of the Phe manipulation, plasma Phe levels were measured in blood samples drawn from the tail vein 2 hr postinjection at three different ages [the day before infant behavioral testing (P19), the day following the conclusion of infant testing (P27), and the day following juvenile testing (P47)]. Plasma Phe analyses were performed by Roche Biomedical Labs (Raritan, NJ) using the Guthrie modified bacterial inhibition assay procedure (Guthrie and Susi, 1963).

Behavioral testing. All animals were tested on delayed alternation, a task sensitive to frontal cortex damage in both rats (e.g., Wikmark et al., 1973; Larsen and Divac, 1978; Bubser and Schmidt, 1990) and primates (e.g., Jacobsen and Nissen, 1937; Bättig et al., 1960; Kubota and Niki, 1971). On trial 1 of each delayed alternation session, the reward was delivered at whichever goal arm of the T-maze the animal entered. Thereafter, the animal was rewarded only for entering the goal arm *not* chosen on the preceding trial. The name of the task derives from the fact that animals must learn to *alternate* goal arms, and must remember which goal arm was last entered over the *delay* imposed between trials. No visual or olfactory cues within the maze signaled the correct choice since (1) the goal arms were identical, (2) the reward was delivered only after an animal had selected a goal arm, and (3) the maze was thoroughly cleaned between animals to remove scent markings. Testing began immediately after weaning and continued for 1 week (infant testing: P20–P26; delays of 0, 10, 30, and 90 sec), with retesting 1 month later (juvenile testing: P45–P46; delays of 0 and 90 sec).

Rat pups were removed from their mothers on the evening of P18, although they continued to be group housed with same-sex littermates. As infants, male rats were run in the same infant T-maze used by Green and Stanton (1989), and females were run in a replica of that maze. Both mazes were made of opaque Plexiglas and consisted of a start box (23 × 8 cm) that opened to a straight central runway (8 × 8 cm) leading to left and right goal arms (each 23 × 8 cm). Opaque doors separated the start box from the choice area and the choice area from each of the goal arms. The mazes were placed on a long table with one of the two 7.5 W bulbs illuminating the room above each maze.

Familiarization to these mazes occurred on postnatal day 19 and consisted of two goal box training sessions and one forced choice training session [same procedure used by Freeman and Stanton (1991)]. In the first goal box training session, each subject received six 3 min exposures to the same goal arm, receiving 0.05 ml of cream each time, with 9 min between each exposure. The second session was the same except that each animal was placed in the other goal arm of the same maze. For forced choice training, an animal was placed in the start box, and the door of only one goal arm was raised. The pup was rewarded for entering the goal arm. This was repeated for a total of 12 trials, six to the right and six to the left, in random order.

Each testing session consisted of a block of 12 trials at a given delay. The criterion for passing the task at a given delay was 10 out of 12 trials correct. Delay was only incremented if the subject passed criterion at the current delay. At the end of each trial, the subject was confined to the goal arm for 20 sec (for consumption of the 0.075 ml cream reward if correct, or as punishment if incorrect). Animals were placed in individual holding cages for at least 1 hr prior to testing (to allow the stress of removal from group housing to subside), and for 2 hr following testing, during which time they were weighed and fed. A given animal was tested by one experimenter and injected by another; the three testers were blind to the group assignments of their animals (two males and two females per tester in each of the three conditions).

Previous work has demonstrated that the levels of Phe in plasma are maximally elevated during the few hours after injection (DelValle et al., 1978; Brass and Greengard, 1982). To determine whether there was an acute effect of higher levels of Phe superimposed on the effect of chronic Phe elevation.Performance on the delayed alternation task 3.5 hr after injection (postinjection) was compared to performance 20 hr after injection [equivalent to 4 hr prior to injection (preinjection)]. For a given delay, the first testing session was always administered preinjection.

Juvenile rats were tested in the adult-size T-maze, consisting of a start box (20 × 15 cm), central runway (22.5 × 15 cm), and left and right goal arms (each 51.25 × 15 cm), described by Zhang et al. (1984). Familiarization training occurred on P44 and consisted of one 2 min goal box training trial per goal arm and six forced-choice trials in the order L-R-L-R-L-R. Because of the shortness of the retest period, animals were tested only postinjection. The criterion for passing a given delay was still 10 out of 12 trials correct within a block. Only animals that succeeded at the shorter delay were tested at the longer delay. The reward was liquid rat diet supplemented with fiber and chocolate, a treat that the rats had not had since the days of infant testing.

Biochemical analyses of the brains. Two cortical and two subcortical areas were dissected bilaterally. The two cortical areas were medial prefrontal cortex and anterior cingulate cortex, the two cortical areas highest in dopamine in the rat (Thierry et al., 1973; Berger et al., 1974, 1976; Lindvall and Bjorklund et al., 1978; Berger et al., 1985). Medial prefrontal cortex in the rat is generally considered the homolog of the dorsolateral prefrontal cortex in the primate (Leonard, 1969; Domesick, 1972; Kolb, 1984; Groenewegen, 1988). The two subcortical areas dissected, the caudate-putamen (striatum) and the nucleus accumbens, are the two subcortical areas that have the highest levels of dopamine (Fuxe, 1965).

The four brain regions were anatomically defined as follows. *Medial prefrontal cortex* corresponds to Brodmann's area 32 (e.g., Brodmann, 1909). It is located immediately in front of the genu and extends down midway through the brain in coronal section. *Anterior cingulate cortex* (area 24 or 24B of Vogt and Peters, 1981) is also part of frontal cortex and is located immediately behind medial prefrontal cortex and above the genu (Berger et al., 1976; Lindvall and Bjorklund, 1978; Lindvall et al., 1978). The *caudate-putamen, or striatum,* is the large area immediately below the corpus callosum and is especially easy to discern in the rat. For dissection of the *nucleus accumbens,* we used the anterior commissure (above) and the olfactory tubercle (below) as landmarks. An excellent diagram illustrating the location of all of these areas is provided in Deutch et al. (1985).

Given the sensitivity of dopamine in frontal cortex to stress (e.g., Thierry et al., 1976; Reinhard et al., 1982; Roth, 1984), precautions were taken to keep the animals as calm and undisturbed as possible prior to dissection (similar to precautions used by A. Y. Deutch, personal communication). All animals were killed by decapitation 2 hr postinjection (range, 1.97–2.25 hr) on P62 and the four brain regions dissected in coronal section over ice. The tissue was frozen in dry ice immediately after dissection and stored at −70°C. The order of death was one female from each of the three experimental groups, and then one male from each of the three groups, with order within each group of three randomized across experimental conditions.

The levels of dopamine, homovanillic acid (HVA; a dopamine metabolite), norepinephrine, and 5-hydroxyindole acetic acid (5-HIAA; a metabolite of 5-HT) were measured using reverse-phase HPLC analysis with an electrochemical detector as described in Robinson et al. (1992). At the time of analysis, the samples were reweighed and diluted 10-fold

in 0.1 M cold acetate buffer, containing 100 nM dihydroxybenzylamine as an internal standard. The tissue was then sonicated for 10 sec and centrifuged at 29,000 × g for 10 min. The bilateral dissections for each brain region from a given animal were analyzed independently and then averaged. First, all the tissues from two animals in each group were analyzed. The remaining analyses were performed by assaying one region at a time from all animals. The person performing these analyses was kept blind as to the group assignments of the animals.

A model 510 pump in conjugation with a WISP model 712 (Waters Co., MA), and a pulse damper (ESA, MA) served as the solvent delivery system. The chromatographic column was a Brownlee 10 cm, 4.6 i.d. C18 reverse-phase column, preceded by Brownlee Guard OD-GU 3 cm, 4.6 mm i.d. (Rainin, CA). An electrochemical detector model 5100A Coulochem with a model 5011 analytical cell was used for the analysis of the biogenic amines and catecholamines. The detector setting was the following: detector 1, +0.03 V; detector 2, +0.55 V. The mobile phase consisted of sodium acetate at 50 mM (Baker Inc., NJ), disodium EDTA at 50 μM (Sigma), sodium 1-octanesulfonate at 6 mM (Kodak, NY), 5 vol% acetonitrile (Baker), and 950 ml of double distilled deionized water (in-house unit). The pH was adjusted to 3.3 with 85% phosphoric acid. The mobile phase was filtered using a 0.22 μm filter (Millipore, MA) and degassed under vacuum. All the chemicals were of analytic grade. Under these conditions, serotonin elutes as a broad peak late in the chromatogram and, therefore, was not analyzed. 3,4-Dihydroxyphenylacetic acid (DOPAC), another metabolite of dopamine, was not analyzed because it too elutes close to the front under the chromatographic conditions used here.

Results

Phenylalanine manipulation. The mean Phe level for control rats was 2.2 mg/dl. Our manipulation raised the mean Phe level 6.4 times control levels in the first experimental group and 6.0 times control levels in the postnatally treated group (see Table 2; mean for the two groups = 13.7 mg/dl).

Phe levels at P19 and P27 were within, or close to, our goal of raising Phe <5 times normal, although at P47 Phe levels were higher. At P19, measured 2.25 hr after injection, the mean Phe level of rats pre- and postnatally treated (group 1) was 13.3 ± 5.8 mg/dl, the mean level in rats postnatally treated (group 2) was 15.6 ± 4.7 mg/dl, and the mean for controls was 2.7 ± 2.4 mg/dl. At P27, 1.8 hr postinjection, the mean Phe levels for the experimental groups were 8.8 ± 4.8 mg/dl and 9.2 ± 4.7 mg/dl, respectively; control levels were 2.0 ± 0 mg/dl. At P47, measured 2 hr postinjection, the mean Phe level was 17.6 mg/dl (SD_{G1} = 5.7, SD_{G2} = 4.7) for both experimental groups and 2.0 (SD = 0) for the controls. While plasma Phe levels were raised slightly more than intended, these Phe levels are much lower than those found in untreated PKU (e.g., 36–43 mg/dl, Berry et al., 1979; 21–48 mg/dl, Cabalska et al., 1977) and much less than the increase in Phe levels produced in animal models of untreated PKU (e.g., 12–18 times normal, DelValle et al., 1978; 10–33 times normal, Greengard et al., 1976). No significant difference was found between the Phe levels of the two experimental groups at any age, nor was there an overall sex difference, although among the rats treated only postnatally females showed a larger elevation in plasma Phe than did males at both P27 and P47 (females vs males: P27, $t = 3.7$, $p < 0.005$; P47, $t = 2.17$, $p = 0.05$). Brass and Greengard (1982) also report a greater effect in female rats.

The prenatal manipulation also had an effect on mortality and body weight, although the postnatal manipulation did not. Two of the seven litters from dams on the α-methylphenylalanine + Phe diet died of unknown causes within 24 hr after birth. Shortly after birth (P4), the pups chosen from the remaining α-methylphenylalanine + Phe litters (group 1) weighed roughly 20% less than pups chosen from the other litters [mean weights = 8.3 ± 0.8 gm (group 1), 10.0 ± 1.3 gm (group 2), 10.2 ± 1.6 gm (controls); $F(1,32) = 17.05$, $p < 0.0003$, regression analysis of P4 weights in group 1 vs the other two groups]. This difference in infant mortality and weight is similar to that reported by others (e.g., Kerr et al., 1968; Brass et al., 1982; McDonald et al., 1990), and occurred despite the yoking of food intake. By P12 the difference in weight among the groups of rat pups had disappeared. From then on, the weight gains of all groups of rats were comparable (e.g., mean weight at P19: 37 gm, 37 gm, and 38 gm, respectively; at P44: 156 gm, 155 gm, and 160 gm, respectively; and at P62: 236 gm, 241 gm, and 242 gm, respectively). During the period between infant and juvenile behavioral testing, the weights of the male rats in all conditions began to exceed that of the female rats and continued to do so throughout the duration of the experiment.

Delayed alternation performance. When tested as infants (P20–P26), there was no significant difference among the groups at the 0 sec or 10 sec delays in percent correct or in mean number of trials needed to pass criterion (see Table 2). All animals passed the 0 sec delay and all but one passed the 10 sec delay. At the 30 sec delay, however, both experimental groups performed significantly worse than controls (see Table 2). At the 90 sec delay, animals treated both pre- and postnatally (group 1) were severely impaired, but the impairment in the animals treated only postnatally (group 2) was not sufficient to achieve statistical significance.

On retesting as juveniles, the performance of group 1 did not differ from that of controls at the 0 sec delay, but group 2 performed significantly worse (Table 2). The performance of group 2 at the 0 sec delay may indicate that several of those animals failed to transfer what they had learned in the infant maze to the new adult maze. At the 90 sec delay, the two experimental groups combined performed significantly worse than controls.

There was no effect of tester or of sex of the animal, and no significant interaction of either of these with any other variable. There were also no noticeable differences in activity or emotionality among animals in the different groups. Indeed, response latency was measured on every trial during both infant and juvenile testing, and there were no group differences in this whatsoever.

All animals in the experimental groups had chronically elevated plasma Phe levels. The elevations are highest soon after injection and lowest before the next day's injection (DelValle et al., 1978; Brass and Greengard, 1982). Animals were tested shortly after injection (3.5 hr after) and shortly before the next day's injection (4 hr before) to determine if performance is worse when Phe levels are higher. We calculated the difference in performance between all adjacent sessions within a given delay (percentage correct in later session minus percentage correct in earlier session). We compared the difference obtained when the earlier session was preinjection to the difference when the earlier session was postinjection. If animals are learning, one would expect performance to be better on later sessions. Indeed, all adjacent session comparisons (whether pre- to postinjection or post- to preinjection) showed improved performance in later sessions. If there is an effect of acute elevation in Phe, one would expect less improvement in later sessions that occur postinjection, when Phe levels are higher, than in later sessions that occur preinjection, when Phe levels are lower. Indeed, performance improved more when the later session occurred at a time when Phe levels are lower than when the later session occurred shortly after injection (across all delays for the two experimental groups:

Table 2. Results of animal model of effects of early-treated PKU

Experimental conditions	Mildly elevated levels of Phe pre- & postnatally ($N = 12$)[a]	Mildly elevated levels of Phe postnatally ($N = 12$)[a]	Controls (normal Phe levels) ($N = 11$)[a]	Results of significance testing: Both elevated Phe groups vs controls[b]	Pre- & postnatally elevated vs controls	Postnatally elevated vs controls[c]
Mean plasma Phe levels (mg/dl)	14.1 (4.6)	13.3 (5.43)	2.2 (0.74)			
Infant delayed alternation testing (P20–P26)[d]						
0 sec delay						
Mean percentage correct	81	89	91	$F = 1.80$, NS	$F = 3.45$, $p < 0.07$	$F = 0.38$, NS
Percentage passing criterion[e]	100	100	100	$F = 0$, NS	$F = 0$, NS	$F = 0$, NS
Mean trials to criterion	63	66	60	$F = 0.01$, NS	$F = 0.07$, NS	$F = 0.01$, NS
10 sec delay						
Mean percentage correct	89	85	81	$F = 0.49$, NS	$F = 0.23$, NS	$F = 0.98$, NS
Percentage passing criterion[e]	92	100	100	$F = 0.68$, NS	$F = 0.49$, NS	$F = 0$, NS
Mean trials to criterion	34	32	20	$F = 2.73$, NS	$F = 2.02$, NS	$F = 2.11$, NS
30 sec delay						
Mean percentage correct	68**	73*	81	$F = 12.71$, $p < 0.001$	$F = 16.12$, $p < 0.0004$	$F = 5.58$, $p < 0.03$
Percentage passing criterion[e]	92 ($N = 11$)[f]	83	100	$F = 1.80$, NS	$F = 0.49$, NS	$F = 1.97$, NS
Mean trials to criterion	59*	57*	27	$F = 9.48$, $p < 0.005$	$F = 7.59$, $p < 0.01$	$F = 7.68$, $p < 0.01$
90 sec delay						
Mean percentage correct	63* ($N = 9$)[f]	74 ($N = 10$)[f]	81	$F = 7.95$, $p < 0.01$	$F = 9.38$, $p < 0.005$	$F = 1.65$, NS
Percentage passing criterion[e]	33**	75	100	$F = 12.96$, $p < 0.001$	$F = 16.60$, $p < 0.0003$	$F = 2.34$, NS
Mean trials to criterion	55*	36	24	$F = 7.82$, $p < 0.01$	$F = 10.27$, $p < 0.004$	$F = 2.86$, $p < 0.10$
Juvenile delayed alternation retesting (P45–P46)[d]						
0 sec delay						
Mean percentage correct	83	70*	84	$F = 1.17$, NS	$F = 0.60$, NS	$F = 7.65$, $p < 0.01$
Percentage passing criterion[e]	100	58	100	$F = 3.54$, $p < 0.07$	$F = 0.00$, NS	$F = 6.04$, $p < 0.02$
Mean trials to criterion	19	28	18	$F = 3.19$, $p < 0.09$	$F = 0.26$, NS	$F = 5.63$, $p < 0.03$
90 sec delay[g]						
Mean percentage correct	70	69 ($N = 7$)[f]	85	$F = 4.57$, $p < 0.05$	$F = 3.44$, $p < 0.08$	$F = 2.78$, $p < 0.10$
Percentage passing criterion[e]	27	8*	80	$F = 8.20$, $p < 0.008$	$F = 6.25$, $p < 0.02$	$F = 7.86$, $p < 0.01$
Brain monoamine levels[d,h]						
Homovanillic acid levels (HVA is a metabolite of dopamine)						
Medial prefrontal cortex	0.12 (0.04)***	0.11 (0.04)***	0.25 (0.06)	$F = 30.73$, $p < 0.0001$	$F = 39.64$, $p < 0.0001$	$F = 30.75$, $p < 0.0001$
Anterior cingulate cortex	0.14 (0.13)	0.09 (0.03)**	0.33 (0.21)	$F = 11.25$, $p < 0.003$	$F = 3.87$, NS	$F = 13.68$, $p < 0.001$
Caudate nucleus	2.29 (1.05)	2.01 (0.69)	2.50 (0.68)	$F = 0.46$, NS	$F = 0.71$, NS	$F = 3.63$, NS
Nucleus accumbens	0.90 (0.22)	0.88 (0.34)	1.52 (0.69)	$F = 7.41$, $p < 0.01$	$F = 4.20$, $p < 0.05$	$F = 6.40$, $p < 0.02$
Dopamine levels[i]						
Medial prefrontal cortex	0.24 (0.10)	0.34 (0.26)	0.54 (0.15)	Reduced to 54%	Reduced to 54%	Reduced to 63%
Anterior cingulate cortex	0.64 (0.65)	0.85 (0.15)	1.45 (0.45)	Reduced to 51%	Reduced to 44%	Reduced to 59%
Caudate nucleus	21.50 (0.10)	27.40 (1.33)	28.92 (10.69)	Reduced to 78%	Reduced to 74%	Reduced to 95%
Nucleus accumbens	11.28 (1.67)	10.25 (1.68)	11.83 (2.91)	Reduced to 91%	Reduced to 95%	Reduced to 87%
Norepinephrine levels						
Medial prefrontal cortex	1.04 (1.06)	1.32 (1.60)	1.60 (2.04)	$F = 0.49$, NS	$F = 0.86$, NS	$F = 0.68$, NS
Anterior cingulate cortex	0.90 (0.32)	0.86 (0.20)	0.94 (0.02)	$F = 0.30$, NS	$F = 0.17$, NS	$F = 0.57$, NS

Table 2. Continued

Experimental conditions	Mildly elevated levels of Phe pre- & postnatally ($N = 12$)[a]	Mildly elevated levels of Phe postnatally ($N = 12$)[a]	Controls (normal Phe levels) ($N = 11$)[a]	Results of significance testing: Both elevated Phe groups vs controls[b]	Pre- & postnatally elevated vs controls	Postnatally elevated vs controls[c]
Caudate nucleus	1.90 (0.50)	1.72 (0.84)	2.32 (1.04)	$F = 2.83$, NS	$F = 1.16$, NS	$F = 1.77$, NS
Nucleus accumbens	1.52 (0.26)	1.42 (0.94)	1.42 (0.46)	$F = 0.32$, NS	$F = 0.32$, NS	$F = 0$, NS
5-Hydroxyindole acetic acid (5-HIAA is a metabolite of 5-HT)						
Medial prefrontal cortex	0.72 (0.30)	0.62 (0.20)	0.94 (0.31)	$F = 3.41, p < 0.08$	$F = 1.45$, NS	$F = 4.18, p = 0.05$
Anterior cingulate cortex	0.15 (0.14)	0.15 (0.09)	0.29 (0.12)	$F = 7.45, p < 0.01$	$F = 5.77, p < 0.03$	$F = 5.40, p < 0.03$
Caudate nucleus	0.26 (0.11)*	0.27 (0.09)*	0.40 (0.07)	$F = 10.23, p < 0.004$	$F = 8.46, p < 0.01$	$F = 7.39, p < 0.01$
Nucleus accumbens	0.49 (0.21)*	0.55 (0.22)*	1.05 (0.52)	$F = 9.91, p < 0.005$	$F = 8.00, p < 0.01$	$F = 9.27, p < 0.01$

[a] SDs appear in parentheses.

[b] Regression analyses were performed, which included litter, sex, and two orthogonal linear contrasts in the equation (one for the experimental groups vs the controls and one for the two experimental groups vs one another).

[c] Regression analysis of the postnatally treated group versus the controls included litter in the equation, as well as sex and the orthogonal contrast since more than one animal per group came from the same litter (although any animal assigned to the postnatal group was always paired with a littermate assigned to the control group). (All animals in the group treated both pre- and postnatally came from litters different from those of the animals in either of the other two conditions.)

[d] ***, significantly worse than the control group at $p \leq 0.0001$; **, worse at $p \leq 0.001$; *, worse at $p \leq 0.01$.

[e] Criterion = 10 correct responses out of the 12 trials in a session.

[f] Animals that did not pass criterion at a brief delay were not tested at the longer delay. Had they been, the percentage correct for their group at the longer delay would presumably have been even lower.

[g] Mean trials to criterion is not given here because juveniles were only tested at the 90 sec delay for one session; those that passed did so in 12 trials.

[h] Dissections were performed on P62; units = pmol/mg of tissue.

[i] Dopamine levels are based on only two animals per group. We gratefully acknowledge the assistance of Larry DiStefano in the laboratory of Jay Schneider in this work.

within-subject $t = 4.59$, $p < 0.0001$). This difference was greater for group 2 than for group 1 (across all delays, group 1: within-subject $t = 1.59$, $p = 0.07$; group 2: within-subject $t = 7.66$, $p < 0.0001$). As expected, the time interval between saline injections and testing yielded no significant effects for the control group (within-subject $t = 1.1$, NS). Within individual delays the difference in performance for both experimental groups was significant only at the 0 sec delay (both groups together: $t = 4.69$, $p = 0.0003$; group 1: $t = 2.27$, $p = 0.041$; group 2: $t = 4.95$, $p = 0.002$). Given the marginal effect across delays for the pups in group 1 and given that at only one individual delay was the effect large enough to achieve significance, we conclude that effects of acute elevation, while present, were not prominent.

Neurotransmitter and metabolite levels. First, and most important, small elevations in plasma levels of Phe, previously thought to be benign, produced significant neurochemical changes. The strongest effect was on HVA in the frontal cortex. In both frontal cortical areas (medial prefrontal cortex and anterior cingulate), the levels of HVA in each experimental group were reduced to less than half (27–48%) of those observed in control animals (see Table 2). In medial prefrontal cortex, there was almost no overlap between HVA levels of controls and of either experimental group: all control animals but one had higher HVA levels than *any* animal in either experimental group. The levels of HVA in the nucleus accumbens were reduced to approximately 60% of control levels. HVA was unaffected in the striatum. The levels of 5-HIAA were reduced to approximately 70% of control levels in medial prefrontal cortex and the striatum. 5-HIAA was reduced to approximately 50% of control levels in anterior cingulate cortex and nucleus accumbens. The levels of norepinephrine were not significantly affected in any of the brain regions examined.

HVA levels in medial prefrontal cortex were the only brain monoamine levels significantly related to all measures of performance on the delayed alternation task (see Table 3). The greater the reduction in HVA levels in prefrontal cortex, the worse the animal's performance on delayed alternation. HVA levels in the other three brain regions investigated were not significantly related to performance (except for one performance measure and HVA in the cingulate). No neurotransmitter or metabolite level studied in the anterior cingulate, striatum, or nucleus accumbens was significantly related to performance (with the single exception just noted). 5-HIAA levels in prefrontal cortex were significantly related to measures of performance during juvenile testing but not infant testing.

Discussion

In the present study, rats were treated with an inhibitor of phenylalanine hydroxylase plus Phe. This treatment caused an elevation in plasma Phe levels roughly comparable to those observed in children with mild hyperphenylalaninemia or with early and continuously treated PKU. While accepted opinion has maintained that these mild elevations in Phe are benign, our results document significant neurochemical and behavioral effects. These results were found in both experimental treatments, the group treated pre- and postnatally and the group treated only postnatally. Although group 1 came from different litters than the controls (and so differed genetically from the controls), the study's conclusions are the same if one compares only experimental group 2 to their same-sex littermates in the control group. Because the Phe manipulation was effective for

Table 3. Relation between delayed alternation performance and brain metabolite levels

	Infant testing (90 sec delay)		Juvenile testing (90 sec delay)	
	Percentage correct	Trials to criterion	Percentage correct	Trials to criterion
HVA				
Medial prefrontal cortex	0.45**	−0.44**	0.42*	−0.37
Anterior cingulate cortex	0.39*	−0.35	0.20	−0.33
Caudate nucleus	0.21	−0.04	0.21	−0.13
Nucleus accumbens	0.20	−0.21	0.06	−0.33
5-HIAA				
Medial prefrontal cortex	0.01	−0.16	0.41*	−0.41*
Anterior cingulate cortex	0.09	−0.13	0.09	−0.11
Caudate nucleus	0.26	−0.31	0.01	−0.05
Nucleus accumbens	0.26	−0.33	0.30	−0.26

There were no group differences in norepinephrine levels, and no significant correlation between level of norepinephrine and any performance measure.

* $p < 0.05$.

** $p < 0.01$.

group 2, we think it likely that the Phe manipulation (rather than the genetic differences) was also responsible for the observed effects in group 1. The results were comparable for males and females.

In the present study we found a significant deficit on the delayed alternation task, a well-established measure of the cognitive functions dependent on prefrontal cortex (e.g., Jacobsen and Nissen, 1937; Bättig et al., 1960; Gross, 1963; Kubota and Niki, 1971; Wikmark et al., 1973; Niki, 1974; Larsen and Divac, 1978; Bubser and Schmidt, 1990). Success when no delay is imposed, but failure with a delay (as we found in infant rats in groups 1 and 2, and in juvenile rats in group 1), is characteristic of the performance of primates with lesions of dorsolateral prefrontal cortex (e.g., Bättig et al., 1960), although these lesioned primates often fail at even short delays (such as 10 sec or less), which we did not find here. [dorsolateral prefrontal cortex in primates is generally considered to be the homolog of medial prefrontal cortex in rats (Leonard, 1969; Domesick, 1972; Kolb, 1984; Groenewegen, 1988)]. Lesions of medial prefrontal cortex in rats often produce deficits on delayed alternation even at the 0 sec delay (e.g., Freeman and Stanton, 1992), presumably because it takes 1–2 sec to move the rat from the goal arm to the start box even when no additional delay is imposed. This is the pattern of performance we found in juvenile rats in group 2. Thus, our findings in animals with moderately elevated Phe levels are comparable to those previously reported in animals with lesions of prefrontal cortex. This deficit on a prefrontal cortex task produced by mild elevations in Phe is also consistent with the report by Diamond, Hurwitz, Lee, Grover, and Minarchik (unpublished observations) of deficits on nine of nine measures of the cognitive functions dependent on prefrontal cortex in children with mild, chronic elevations in plasma Phe due to mild hyperphenylalaninemia or treated PKU.

Neonatal lesions of medial prefrontal cortex in the rat severely impair delayed alternation performance in P19 and P23 infant rats, mildly impair performance in P27 infant rats, and no longer significantly affect delayed alternation performance by P33 (Freeman and Stanton, 1992; see also Kolb and Nonneman, 1978; Nonneman and Corwin, 1981; Vicedomini et al., 1982a,b, 1984). We found the severest deficits in group 1 when they were tested as infants. For example, their impairment at the 90 sec delay at P26 was more severe than their impairment at the same delay at P46, consistent with the effects found after lesions of prefrontal cortex by Freeman and Stanton (1992). Group 2 showed some difficulty transferring what they had learned in the infant maze to the adult maze. Hence, their impaired performance in the adult maze on initial sessions at the 0 sec delay. Although the animals in group 2 showed some impairment at the 90 sec delay, the impairment might have been more pronounced had they been tested closer to P20 on this delay, when lesions of prefrontal cortex have their most severe effects on performance of this task.

We found that dopamine metabolism was depressed in frontal cortex. In fact, the strongest neurochemical effect was on HVA levels in the two frontal cortical areas. It was not the only effect, however. For example, decreased HVA levels were also found in the nucleus accumbens, although no significant effect was found on HVA levels in the striatum. The different results for the accumbens and striatum are consistent with other findings (Acworth et al., 1988) and are to be expected given the higher rate of dopamine turnover in the accumbens (Bannon and Roth, 1983). The absence of any effect on norepinephrine is also consistent with previous work demonstrating that norepinephrine levels are relatively insensitive to alterations in precursor (Irie and Wurtman, 1987).

The levels of 5-HIAA were reduced in all brain regions examined. Some effect on 5-HIAA was to be expected given that 5-HT levels in the brain are sensitive to levels of the serotoninergic precursor (tryptophan; Fernstrom and Wurtman, 1971), and given that tryptophan, being a large neutral amino acid, must (like tyrosine) compete with Phe to cross the blood–brain barrier. The protein-carrier's affinity for tryptophan is roughly midway between that for Phe and tyrosine; the effect that we found on prefrontal 5-HIAA was roughly half that of the effect we found on prefrontal HVA. The plasma ratio of Phe:tryptophan should not be as imbalanced in those treated for PKU as the ratio of Phe:tyrosine. That is because the absolute level of tryptophan in blood should be unaffected, whereas the absolute level of tyrosine is often lowered due to the impairment in converting Phe to tyrosine. Because the absolute levels of tryp-

tophan are not reduced and because tryptophan competes more successfully with Phe than does tyrosine in crossing the blood–brain barrier, we think the main effect of mild Phe elevations in those with impairments in the Phe hydroxylating system should be on cerebral levels of the dopamine precursor, tyrosine. The additional effect on 5-HT metabolism in the present study may have occurred because plasma Phe levels were raised slightly more than intended, especially since high concentrations of Phe in the brain competitively inhibit tryptophan hydroxylase activity (in much the same way that these concentrations inhibit the activity of tyrosine hydroxylase; Levitt et al., 1965; Lovenberg et al., 1968; McKean, 1972).

The results of the present study contrast with previous studies that have failed to document effects of mild elevations of Phe. In such studies, generalized measures of performance, such as IQ tests, and global measures of metabolites (from cerebrospinal fluid) were used. On measures as global as IQ tests even patients in whom frontal cortex has been surgically removed score within the normal range (e.g., Stuss and Benson, 1986, 1987). Since there is much less HVA in frontal cortex than in the striatum and other brain regions, a change in HVA in frontal cortex alone would be unlikely to significantly affect the levels of HVA in cerebrospinal fluid. Similarly, when previous work has failed to find an effect of acute, mild elevations of Phe in animals this has been because the investigators were looking in regions that are relatively unaffected (e.g., the striatum) or in whole brain tissue that could miss region-specific effects. For example, Arvin et al. (1992) found that increased Phe in plasma had no effect on extracellular dopamine levels in the striatum. The striatum was a logical brain region in which to investigate possible effects of Phe on dopamine because the striatum is the brain region with the highest levels of dopamine. However, striatal dopaminergic neurons appear to be protected against small perturbations in the level of precursor by their autoreceptors, their lower activity, and their slower rates of dopamine turnover.

Although frontal cortex has less dopamine than areas such as the striatum, this does not mean that dopamine is unimportant for frontal cortex function. On the contrary, depletion of dopamine or inhibition of dopamine receptors in prefrontal cortex produces behavioral impairments comparable to those produced by destruction or removal of prefrontal cortex (Brozoski et al., 1979; Simon et al., 1980; Stam et al., 1989; Sawaguchi and Goldman-Rakic, 1991). In one study with nonhuman primates, depletion of nearly 90% of the dopamine in prefrontal cortex was required to achieve this effect (Brozoski et al., 1979), but when this has been studied in the rat, the deficit in delayed alternation performance has been found with dopamine reductions more comparable to those reported here (32% of controls, Simon et al., 1980; 29% of controls, Stam et al., 1989).

It is unlikely that effects on the dopaminergic system outside of frontal cortex or effects on other neurotransmitter systems were responsible for the observed impairment in delayed alternation performance for the following reasons. (1) Only dopamine metabolism in prefrontal cortex was significantly related to every measure of performance on the delayed alternation task. Previous work has demonstrated similar correlations (Sahakian et al., 1985). Indeed, Sahakian and colleagues found *no* significant correlations between 5-HT levels in cortex or in subcortical areas and delayed alternation performance, whereas they found that the correlation between cortical dopamine levels and delayed alternation performance was significant. (2) Our conclusion that the observed impairment in delayed alternation performance is due to effects on the dopaminergic system in frontal cortex is consistent with previous work demonstrating that while success on delayed alternation is highly dependent on the level of dopamine specifically in prefrontal cortex, it is not dependent on 5-HT or norepinephrine levels in the same region (Brozoski et al., 1979). Brozoski and colleagues found that depletions of norepinephrine or 5-HT in prefrontal cortex, comparable in magnitude to the depletions of prefrontal dopamine, had no effect on delayed alternation performance. (3) Our conclusion is also consistent with previous work demonstrating that success on delayed alternation is not dependent on dopamine levels elsewhere in the brain, such as in the striatum, hippocampus, or hypothalamus, or on norepinephrine or 5-HT concentrations in those regions (Simon et al., 1980; Sahakian et al., 1985). (4) The lack of correlation we found between observed neurochemical changes in the nucleus accumbens and delayed alternation performance is consistent with evidence that ablation of the nucleus accumbens does not affect performance on a similar task (Annett et al., 1989).

There is some evidence linking the caudate nucleus, which receives a strong projection from prefrontal cortex, to delayed alternation performance (e.g., Bättig et al., 1960; Goldman and Rosvold, 1972; Vicedomini et al., 1982b). However, in the present study, HVA levels in the caudate were not significantly lowered and dopamine levels were only minimally affected (reduced less than half as much as those in prefrontal cortex). Therefore, it is unlikely that the observed impairment in delayed alternation performance was due to effects on the striatal dopaminergic system.

The levels of HVA and 5-HIAA in the anterior cingulate cortex were significantly reduced in the present study. The effect on HVA in the anterior cingulate is consistent with reports that the segregation between the dopamine neurons projecting to medial prefrontal cortex and anterior cingulate cortex is less complete than once thought (e.g., Loughlin and Fallon, 1984). The role of the anterior cingulate in performance of delayed alternation or similar tasks, such as delayed response, has received little attention. This region, like medial prefrontal cortex, is an association area in frontal cortex. Given recent evidence demonstrating the importance of the anterior cingulate for cognitive performance in humans (Pardo et al., 1990, 1991; Corbetta et al., 1991; Grossman et al., 1992; Paus et al., 1993), it is not as unlikely as it once seemed that this region might be important for delayed alternation performance as well. This may deserve further study. There is evidence, however, that partial damage to anterior cingulate cortex has no effect on performance of delayed alternation (Larsen and Divac, 1978; Thomas and Brito, 1980).

The role of 5-HT and the interrelations between 5-HT and dopamine in the functions of prefrontal cortex have not received a great deal of attention; it too might merit closer examination. (In the present study, 5-HIAA levels in prefrontal cortex also showed some relation to delayed alternation performance.) Future work is also needed to confirm the present findings using tasks that do not require prefrontal cortex in addition to tasks that do, to look at other areas of the brain, to measure tyrosine as well as Phe, and to measure DOPAC and 5-HT levels as well as the neurochemical variables assessed here. Future work should be done to confirm that the prefrontal dopaminergic system is selectively affected by Phe levels in the range of 6–10 mg/dl, and that the effect on dopamine metabolism is due to elevations

in Phe and not to the α-methylphenylalanine that was administered here along with Phe.

In summary, the results of the present study accord well with the mechanism we hypothesized to account for the children's cognitive deficits. We had predicted that moderate elevations in Phe would depress dopamine metabolism in prefrontal cortex, impairing the cognitive functions prefrontal cortex subserves. We had predicted this would occur because moderate increases in Phe relative to tyrosine in plasma *moderately* reduce the amount of tyrosine crossing the blood–brain barrier. Most dopaminergic systems in the brain are insensitive to modest changes in available tyrosine, but not the prefrontal dopaminergic system, whose neurons have higher levels of activity and of dopamine turnover. In our model of mild, chronic elevations in plasma Phe early in life, performance on a task dependent on frontal cortex was impaired and dopamine metabolism in frontal cortex (medial prefrontal cortex and anterior cingulate cortex) was reduced. For children whose Phe levels remain moderately elevated despite strict adherence to a low-Phe diet, these results provide reason for hope—the problem appears to be primarily in the frontal dopaminergic system and should be amenable to a drug treatment targeted to that system. Additional evidence of the specificity of the effects comes from Diamond, Hurwitz, Lee, Grover, and Minarchik (unpublished observations): children with mild plasma Phe elevations were impaired on all tests of frontal cortex function but were unimpaired on control tasks that required the functions of parietal cortex or the medial temporal lobe. In any future treatment that includes tyrosine supplementation, it will be important to monitor whether this exacerbates the changes observed here in the serotoninergic system, since tryptophan would then have to compete with elevated levels of both tyrosine and Phe. For those concerned with the possible deleterious effects of food additives high in Phe (such as Nutrasweet or Equal), the present results indicate where to look to see if there is indeed cause for concern.

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