Evidence for the importance of dopamine for prefrontal cortex functions early in life

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SUMMARY

There is considerable evidence that dorsolateral prefrontal cortex subserves critical cognitive abilities even during early infancy and that improvement in these abilities is evident over roughly the next 10 years. We also know that (a) in adult monkeys these cognitive abilities depend critically on the dopaminergic projection to prefrontal cortex and (b) the distribution of dopamine axons within dorsolateral prefrontal cortex changes, and the level of dopamine increases, during the period that infant monkeys are improving on tasks that require the cognitive abilities dependent on prefrontal cortex. To begin to look at whether these cognitive abilities depend critically on the prefrontal dopamine projection in humans even during infancy and early childhood we have been studying children who we hypothesized might have a selective reduction in the dopaminergic innervation of prefrontal cortex and a selective impairment in the cognitive functions subserved by dorsolateral prefrontal cortex. These are children treated early and continuously for the genetic disorder, phenylketonuria (PKU). In PKU the ability to convert the amino acid, phenylalanine (Phe), into another amino acid, tyrosine (Tyr), is impaired. This causes Phe to accumulate in the bloodstream to dangerously high levels and the plasma level of Tyr to fall. Widespread brain damage and severe mental retardation result. When PKU is moderately well controlled by a diet low in Phe (thus keeping the imbalance between Phe and Tyr in plasma within moderate limits) severe mental retardation is averted, but deficits remain in higher cognitive functions. In a four-year longitudinal study we have found these deficits to be in the working memory and inhibitory control functions dependent upon dorsolateral prefrontal cortex in PKU children with plasma Phe levels 3–5 times normal. The fact that even infants showed these impairments suggests that dopaminergic innervation to prefrontal cortex is critical for the proper expression of these abilities even during the first year of life. To test the hypothesis about the underlying biological mechanism we have created the first animal model of early and continuously treated PKU. As predicted, the experimental animals had reduced levels of dopamine and the dopamine metabolite, homovanillic acid (HVA), in prefrontal cortex and showed impaired performance on delayed alternation, a task dependent on prefrontal cortex function. Noradrenaline levels were unaffected; however some reduction in serotonin levels and in dopamine levels outside the prefrontal cortex was found.

If prefrontal cortex functions are vulnerable in children with a moderate plasma Phe:Tyr imbalance because of the special properties of the dopamine neurons that project to prefrontal cortex, then other dopamine neurons that share those same properties should also be vulnerable in these children. The dopamine neurons in the retina share these properties (i.e. unusually high firing and dopamine turnover rates), and we have found that PKU children with plasma Phe levels 3–5 times normal are impaired in their contrast sensitivity, a behavioural measure sensitive to retinal dopamine levels.

1. INTRODUCTION

One of the first demonstrated, and still one of the strongest, links between cognitive development and brain function is the improvement in infants' performance on the A-not-B, delayed response, and object retrieval tasks between $7\frac{1}{2}$ and 12 months of age and the dependence of successful performance on these tasks on the proper functioning of dorsolateral prefrontal cortex (see table 1). Human infants of $7\frac{1}{2}$–9 months, infant macaques of $1\frac{1}{2}$–$2\frac{1}{2}$ months, and adult macaques with bilateral removals of dorsolateral prefrontal cortex fail all three tasks under the same conditions and in the same ways. For reviews of this work see Diamond (1990*a*, *b*, 1991*a*, *b*). If maturation of the prefrontal neural system helps make possible cognitive advances reflected in improved performance on these tasks, in what aspects of the prefrontal system might one expect these maturational changes to be occurring? One candidate is in the levels of dopamine in prefrontal cortex. We know that (a) in adult monkeys these cognitive abilities depend critically on the dopaminergic projection to prefrontal cortex (e.g., Brozoski *et*

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Table 1.

	AB	delayed response	Object retrieval
Human infants show a clear developmental progression from 7½–12 months.	Diamond 1985	Diamond & Doar 1988	Diamond 1988
Infant monkeys show a clear developmental progression from 1½–4 months.	Diamond & Goldman-Rakic 1986	Diamond & Goldman-Rakic 1986	Diamond & Goldman-Rakic 1986
Adult monkeys with lesions of prefrontal cortex fail.	Diamond & Goldman-Rakic 1989	Diamond & Goldman-Rakic 1989	Diamond & Goldman-Rakic 1985
5-month-old infant monkeys, who received lesions of prefrontal cortex at 4 months, fail.	Diamond & Goldman-Rakic 1986	Diamond & Goldman-Rakic 1986	
Adult monkeys with lesions of parietal cortex succeed.	Diamond & Goldman-Rakic 1989	Diamond & Goldman-Rakic 1989	Diamond & Goldman-Rakic 1985
Adult monkeys with lesions of the hippocampal formation succeed.	Diamond *et al.* 1989	Squire & Zola-Morgan 1983	Diamond *et al.* 1989

al., 1979; Taylor *et al.*, 1990; Sawaguchi & Goldman-Rakic, 1991) and (b) the distribution of dopamine axons within dorsolateral prefrontal cortex changes, and the level of dopamine increases, during the period that infant monkeys are improving on tasks that require the cognitive abilities dependent on prefrontal cortex (Brown & Goldman, 1977; Lewis & Harris, 1991). After briefly considering the abilities required for success on the A-not-B, delayed response, and object retrieval tasks, this paper will focus on recent work with children treated early and continuously for phenylketonuria (PKU), and with a corresponding animal model. That work sheds light on the importance of dopamine for prefrontal cortex function during early human life.

2. THE COGNITIVE ABILITIES REQUIRED BY THE A-not-B, DELAYED RESPONSE, AND OBJECT RETRIEVAL TASKS

A-not-B (Piaget 1954, original french edition 1937) and delayed response (Jacobsen 1935) are two-choice hiding tasks. The ability of the subject to keep his or her mind on where the reward was hidden (whether one calls this 'working memory' or 'sustained attention') during the delay until retrieval is permitted appears to be essential. Evidence for this is, for example, that human infants, infant macaques, and infant or adult macaques with lesions of dorsolateral prefrontal cortex generally perform well on the A-not-B and delayed response tasks if there is no delay, if the delay is shortened by 2–3 s, if they can look at the correct well during the delay, or if allowed to reach or move toward the correct well during the delay (in monkeys with dorsolateral prefrontal cortex ablations see Harlow *et al.* 1952; Bättig *et al.* 1960; Miles & Blomquist 1960; Pinsker & French 1967; Goldman & Rosvold 1970; Fuster & Alexander 1971; Diamond & Goldman-Rakic 1989; in human infants 7½–9 months of age see Gratch & Landers 1971; Evans 1973; Harris 1973; Gratch *et al.* 1974; Cornell 1979; Fox *et al.* 1979; Diamond 1985).

The role of working memory or sustained attention is less prominent in the object retrieval task (Diamond 1988, 1990*b*), where the subject must detour around a small transparent box open on one side to retrieve the reward inside. Yet even here the ability to hold information in mind appears to be important in attaining mastery of the task. Infants of 6–8 months reach only at the side of the clear box through which they are looking. Thus, to retrieve the reward they must look through the opening and continue to do so as they reach inside. However, by 8½–9 months when the front of the box is open, and by 9½–10 months when the left or right side of the box is open, infants can look through the opening, then sit up and reach in while looking through a closed side; that is, the memory of having looked through the opening is enough. Looking along the route the hand will reach appears to be critical; at these ages infants still fail if they have not looked through the opening on a given trial. However, it is a major achievement to be freed from having to continue to do so. Even so, being able to divide or switch one's attention between the box and the reward appears to be more critical on the object retrieval task than does sustained attention or working memory.

The most obvious requirement of the object retrieval task is the ability to resist the strong pull to reach straight to the visible reward. For example, human infants, and macaques with dorsolateral prefrontal cortex ablations, perform much better when the box is opaque than when it is transparent (Diamond 1990*b*). Inhibitory control also seems to be required for success on the A-not-B and delayed response tasks. Infants, and macaques with dorsolateral prefrontal cortex

ablations, perform fine at the first hiding place, although the delay is just as long there as it is on subsequent trials. It would appear that errors occur when the reward is hidden in the other hiding place because, having been positively reinforced for finding the reward at 'A' the tendency of subjects to reach to 'A' has been strengthened and that must now be inhibited or overridden if the subject is to reach to 'B'.

Some errors can be elicited simply by taxing working memory or sustained attention, for example by using a long delay at the first hiding location (e.g. Sophian & Wellman 1983). Similarly, some errors can be elicited simply by taxing inhibitory control, for example some infants err on the reversal trials to 'B' even when the covers are transparent (e.g. Butterworth 1977; Willatts 1985). However, the vast majority of errors occur when subjects must both hold information in mind and also exercise inhibitory control over their behaviour; that is on reversal trials to 'B' when the covers are transparent and a delay is imposed.

An analogue of this type of error in normal adults might be a scenario such as: 'On going to the telephone to call an old friend, I reminded myself of my friend's new phone number and was resolved not to make the mistake of dialling the old number. However, as the old and new phone numbers began with the same initial digits, as I began dialling the number I got into the routine of dialling the number I had dialled so many times in the past and dialled the old number though I knew full well my friend's new phone number'. In infants this type of error is often manifest in the A-not-B task by the infant reaching to 'A' when the infant had seen a toy being hidden in 'B' just before the delay, but not looking in the A well to see if the toy were there, and instead uncovering 'B' and looking in there for the toy. It is as if at some level the infant knew the toy was not in 'A' since the infant did not look in that well after uncovering it, but for some reason that knowledge did not direct where the infant first reached. A less common but more dramatic error is for the infant to look straight at the correct well ('B'), indicating with the eyes that he or she 'knows' where the toy is, while at the same time reaching to the previously correct well ('A') and uncovering it. The error can also happen earlier in the behaviour sequence as when you forget that your friend's phone number has changed and so go to the telephone with the intention of dialling the old phone number, or when an infant looks genuinely surprised not to find the toy in well 'A'.

With effort we can all dial our friends' changed numbers correctly, but it certainly requires less effort to remember and correctly dial other phone numbers (even if they are less well-practised) that do not require the inhibition of another number. Similarly, it requires less effort to dial a friend's new phone number if we are looking at the number written down as we dial it. It is when we must *both* hold the number in mind and resist or override a strong tendency to dial a different number that errors are most likely to occur, and for that, I would contend, dorsolateral prefrontal cortex is most clearly required. To repeat, it is when we must act *in a different way than our first inclination* **and** *when at least some of the information needed for action must be held in mind that dorsolateral prefrontal cortex is most clearly required.*

While the studies summarized in table 1 provide some evidence that important maturational changes may be occurring within the dorsolateral prefrontal cortex neural system during the first year of life, dorsolateral prefrontal cortex is probably not fully mature until at least 10 years of age. Not surprisingly, then, if one uses slightly more difficult tasks, one can elicit A-not-B-type errors beyond the infancy period. For example, 3-year-old children can sort cards correctly by the first criterion, whether that criterion is colour or shape (just as infants correctly retrieve the reward from wherever it is first hidden). However, when instructed to switch and sort by the other criterion, 3-year-olds persist in sorting by the first criterion (Zelazo *et al.* 1995, 1996; just as infants persist in reaching to the first hiding place when the reward is later hidden at the other hiding location), even though the experimenter reminds the child of the new sorting criterion at the outset of every trial. Children 4–5 years of age fail other tasks that require holding information in mind plus inhibitory control, such as the day-night Stroop-like task (Gerstadt *et al.* 1994). On that task, the subject must hold two rules in mind ('Say "Night" when you see a white card with a picture of the sun, and say "Day" when you see a black card with a picture of the moon and stars') and must resist or override the tendency to say what the images on the cards really represent. In Diamond & Taylor (1996), we present additional evidence that the working memory and inhibitory control abilities dependent on dorsolateral prefrontal cortex may reach a critical level of maturity by 6 years of age.

If it is true that maturational changes in prefrontal cortex underlie some of the cognitive advances during early childhood, as I have suggested, what exactly is changing in the prefrontal system during the early years of postnatal life? One candidate is increasing levels of the neurotransmitter, dopamine. For example, dopamine levels increase in the rhesus macaque brain during the period when infant rhesus macaques are improving on the A-not-B, delayed response, and object retrieval tasks (Brown & Goldman 1977; Lewis & Harris 1991). As an initial way of looking at the role of dopamine in prefrontal cortex function in humans early in development, we have been investigating children who, there was reason to believe, might have a selective reduction in dopamine in prefrontal cortex without other abnormalities in the brain – children treated early and continuously for phenylketonuria (PKU). We have also been investigating animal models of this condition.

3. WHY THE DOPAMINE SYSTEM IN PREFRONTAL CORTEX MIGHT BE SELECTIVELY AFFECTED IN EARLY AND CONTINUOUSLY TREATED PKU (ECT-PKU)

The core problem in PKU is an inability to convert the amino acid Phe, into another amino acid, Tyr. This problem is caused by a mutation of the gene on

chromosome 12 (12q22–12q24.1) which codes for the enzyme, phenylalanine hydroxylase (Woo *et al.* 1983; Lidsky *et al.* 1985). Levels of Phe in the bloodstream rise to well over 10 times normal ($\geqslant 20$ mgdl^{-1} (1200 μmol L^{-1})), levels of Tyr in the bloodstream drop, and severe mental retardation results (Krause *et al.* 1985). Treatment consists of a diet low in Phe. This results in lower plasma Phe levels than if the child ate normally, and it succeeds in averting severe mental retardation. However, the reduced-Phe diet does not result in completely normal plasma Phe levels (roughly 2 mgdl^{-1}). That is because the low-Phe diet reflects a compromise between the need to minimize Phe intake and the need for protein. The advice of the U.S. National Collaborative Study of Treated PKU has been that plasma Phe levels should not exceed five times normal (10 mgdl^{-1}; Williamson *et al.* 1981; Koch & Wenz 1987). Thus, dietary treatment for PKU reduces the plasma Phe elevation, but does not erase it, and does nothing to ameliorate the plasma Tyr reduction. Because plasma levels of Phe and Tyr are not fully normal in children treated for PKU, the door is open for neurological and cognitive problems to still be present, at least in some children.

There have been reports of cognitive impairments in children treated early and continuously for PKU (i.e. children on the low-Phe diet since shortly after birth). For example, the IQs of these children are often significantly lower than the IQs of their siblings. Children with PKU, even when they have been on the special diet since shortly after birth, typically have IQs in the 80s or 90s; that is, IQs in the normal range, but just barely (e.g. Dobson *et al.* 1976; Berry *et al.* 1979; Williamson *et al.* 1981). More recently, studies have reported problems in attentional control, problem-solving, or 'executive functions'. For example, children with early and continuously treated PKU tend to be more distractible, to be more limited in the amount of information they can hold in mind at one time and manipulate, and to have more difficulty maintaining set until a problem is solved or a goal attained (e.g. Krause *et al.* 1985; Pennington *et al.* 1985; Faust *et al.* 1986; Brunner *et al.* 1987; Smith & Beasley 1989). These problems are reminiscent of the deficits seen after damage to prefrontal cortex. Indeed, damage to prefrontal cortex typically results in IQs lowered to the 80s or 90s (Stuss & Benson 1986, 1987), that is, the same range as one sees in children treated for PKU.

Why might the cognitive deficits be specific to the functions of prefrontal cortex? The mild imbalance in the plasma Phe:Tyr ratio results in mildly reduced levels of Tyr reaching the brain. Phe and Tyr compete for the same transporter proteins to cross the blood-brain barrier (Chirigos *et al.* 1960; Oldendorf, 1973; Pardridge 1977). Indeed, those proteins have a higher affinity for Phe than for Tyr (Pardridge & Oldendorf 1977; Miller *et al.* 1985). Hence, a modest imbalance between Phe and Tyr in the bloodstream results in mildly reduced CNS Tyr levels.

The dopamine system in prefrontal cortex is more sensitive to a mild reduction in Tyr than are the dopamine systems in most other brain regions. Tyr is the precursor of dopamine. Most brain regions receiving dopaminergic input are unaffected by modest changes in CNS Tyr levels. However, prefrontal cortex is acutely sensitive to even a small reduction in Tyr. That is because the dopamine neurons that project to prefrontal cortex fire so rapidly and turn over dopamine so quickly (Thierry *et al.* 1977; Bannon *et al.* 1981, 1983; Wurtman *et al.* 1981; Tam *et al.* 1990). Indeed, moderate reductions in CNS levels of Tyr, that have little effect on dopamine synthesis in other neural regions (such as the striatum), profoundly reduce dopamine synthesis in prefrontal cortex (Bradberry *et al.* 1989). Hence, we hypothesized that here was a mechanism by which the moderate Phe:Tyr imbalance in the bloodstream of children treated for PKU might selectively affect prefrontal cortex.

It has also been known for some time that reducing dopamine in prefrontal cortex produces deficits in the cognitive abilities dependent on prefrontal cortex. Selectively depleting dorsolateral prefrontal cortex of dopamine can produce deficits as severe as those found when dorsolateral prefrontal cortex is removed altogether (Brozoski *et al.* 1979). Indeed, local injection of dopamine antagonists into dorsolateral prefrontal cortex impairs performance in a precise, dose-dependent manner on tasks dependent on prefrontal cortex (Sawaguchi & Goldman-Rakic 1991). Similarly, destruction of the dopamine neurons in the ventral tegmental area (VTA) that project to prefrontal cortex impairs performance on these tasks (Simon *et al.*, 1980). Injections of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) result in reduced levels of dopamine in the prefrontal-striatal neural system. Cumulative doses of 15–75 mg of MPTP do not produce Parkinsonian-type motor deficits in rhesus macaques, although larger doses do. At the lower doses of MPTP, the monkeys are impaired on delayed response and object retrieval (Schneider & Kovelowski 1990; Taylor *et al.* 1990), although they perform normally on other tasks such as visual discrimination. Hence, we hypothesized, the moderate Phe:Tyr imbalance in the bloodstream of children treated for PKU might result in deficits in the cognitive abilities dependent upon prefrontal cortex because of the effect of the Phe:Tyr imbalance on prefrontal dopamine levels.

4. EVIDENCE OF PREFRONTAL CORTEX COGNITIVE DEFICITS IN ECT-PKU CHILDREN

In a large, four-year longitudinal study (Diamond 1994; Diamond *et al.* 1996) we found that PKU children, who had been on a low-Phe diet since the first month of life, but who had moderately elevated plasma Phe levels (levels roughly 3–5 times normal (6–10 mgdl^{-1}; 360–600 μmol L^{-1})), were impaired on all six tests that required both holding information in mind and resisting or overriding a dominant response, that is, tasks dependent on dorsolateral prefrontal cortex. The tests were the A-not-B and the object retrieval tasks for infants; A-not-B with invisible displacement for toddlers; the day-night Stroop-like test, the tapping

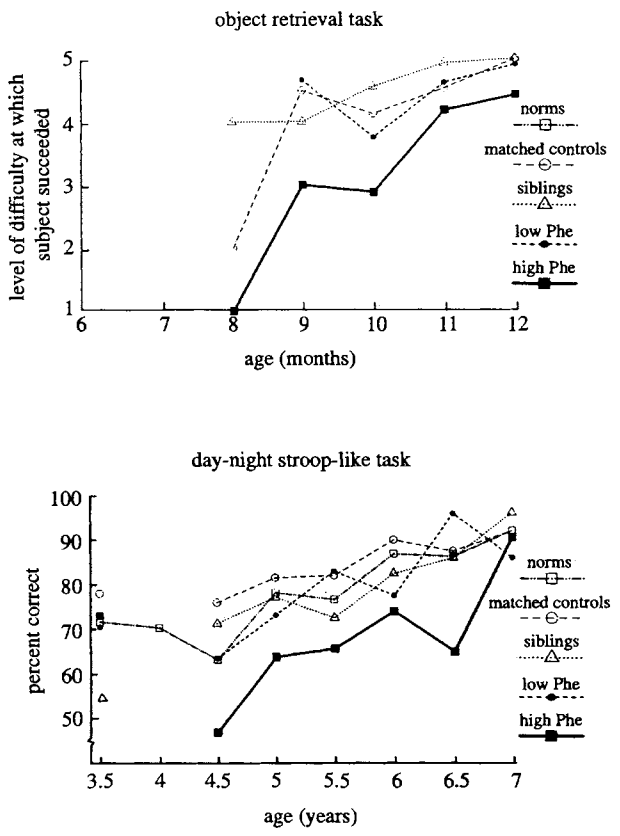

Figure 1. ECT-PKU Children with 'higher' plasma Phe levels (Phe levels 3–5 times normal) performed significantly worse on tasks that required both working memory and inhibitory control than did other PKU and hyperphe children with Phe levels closer to normal, their siblings, matched controls, and children from the general population. Examples of this can be seen in infants on (a) the object retrieval task (Diamond 1990, 1991) and (b) in young children on the day-night Stroop-like task (Gerstadt *et al.* 1994). The day-night Stroop-like task was not administered at 4 years of age to any of the groups followed longitudinally, because the control version of the task was administered instead. All groups performed normally on the control version, where they had to remember two rules, but did not have to resist the tendency to say what the scenes really represented (since the stimuli were abstract designs).

test, and the three pegs test for young children; (see figure 1).

This is consistent with the results of other studies, the most relevant ones being those by Welsh *et al.* (1990) and Smith *et al.* (1996), as they too used cognitive tasks tailored to the functions of dorsolateral prefrontal cortex. The deficits we observed were evident in all age groups (infants, toddlers, and young children), and remained significant even after controlling for IQ, sex, health variables, and background characteristics. The deficits were clear whether the children were compared to (a) other ECT-PKU children with lower Phe levels, (b) their own siblings, (c) matched controls, or (d) children from the general population. Of the 24 comparisons between ECT-PKU children with Phe levels 3–5 times normal and the other groups of children on these six tasks (6 tasks × 4 comparisons per task), ECT-PKU children with higher Phe levels performed significantly worse on 21 of the 24 comparisons. The group differences obtained are robust because the children were tested repeatedly over time, which yielded several data points per subject and provided a more accurate and reliable indication of each child's ability and developmental trajectory than would have been possible had we tested each child only once. (Infants were tested every month from 6 to 12 months of age, toddlers every 3 months from 15 to 30 months, and young children every 6 months from $3\frac{1}{2}$ to 7 years of age.) In contrast to the marked differences between PKU children and other subjects on these tasks, of the 36 comparisons among the control groups (groups a–d above) on these six tasks (6 tasks × 6 comparisons per task), only six comparisons revealed a significant difference. That is, of the 60 pairwise group comparisons, 51 yielded results in the predicted direction. The cognitive deficits documented in several of the other studies of PKU children could be explained away by saying that (a) the plasma Phe levels of many of the children were outside the 'safe' range (i.e. > 5 times normal), (b) even if concurrent Phe levels were not excessively elevated earlier Phe levels had been (during the years the children had been off diet), and/or (c) the low-Phe diet had been started too late to avert early brain damage. None of those disclaimers are applicable here.

The higher a child's Phe level, the worse that child's performance tended to be on tasks that required acting counter to his or her initial tendency on the basis of information held in mind. Performance on these tasks was most strongly and consistently related to concurrent plasma Phe levels, rather than to mean Phe levels over a wide age range, during the first year of life, or during the first month. As current Phe levels varied so too, inversely, did behavioural performance.

Three tasks were administered that require memory of the choices one has already made or temporal order memory. The tasks were the three boxes test (boxes scrambled after each reach; Petrides 1988, 1989, 1991), the six boxes test (boxes scrambled after each reach), and the Corsi-Milner test of temporal order memory (Milner *et al.* 1991)). These tests tax working memory and require the functions of prefrontal cortex, but they do not tax inhibitory control and they do not require precisely the same region of prefrontal cortex as do tasks that tax both working memory + inhibitory control. Of the 12 comparisons between ECT-PKU children with Phe levels 3–5 times normal and the other groups of children on these three tasks (3 tasks × 4 comparisons per task), the children with higher Phe levels performed significantly worse than the comparison groups on only 0–2 of these 12 comparisons. Similarly, when plasma Phe level was treated as a continuous variable, no significant relationship between plasma Phe levels and any measure of performance on these three tasks was found. Perhaps the boxes and Corsi-Milner tasks failed to show an effect because of ceiling and floor effects, perhaps taxing both working memory and inhibitory control is critical, perhaps taxing inhibitory control is critical in and of itself, perhaps it has to do with regional differences within prefrontal cortex in the dopamine innervation,

or perhaps the boxes and Corsi-Milner tasks did not lend themselves to the use of a strategy and so did not really require prefrontal cortex.

Dorsolateral prefrontal cortex consists primarily of Areas 9 and 46. In the rhesus macaque, Area 9 is much more densely innervated by dopamine than is Area 46 (Lewis *et al.* 1986; Lewis & Morrison 1989). The tasks on which we found no effect are more strongly linked to Area 9 than Area 46 (Petrides 1995). Perhaps the subregion of dorsolateral prefrontal cortex that enables us to hold information in mind and inhibit interfering actions or thoughts is more acutely sensitive to any small fluctuations in dopamine because it has so relatively little dopamine to start with, and hence might need all of its allottment all of the time.

Robbins and colleagues (Robbins this volume) and Mangels (1996) have found that prefrontal cortex is not required for memory of the choices one has already made or for temporal order memory when that information is processed incidentally, rather than intentionally, or when the task does not lend itself to use of a strategy. It is possible that the versions of self-ordered and temporal memory tasks we have used here might not require prefrontal cortex involvement. Certainly, on the Corsi-Milner test of memory for temporal order there was no meaningful order to the stimuli; the stimuli told no story, reflected no underlying organizing principles, and in general did not relate one to another. Perhaps if there had been some reason to remember the order in which the stimuli were being presented, other than because one would be tested on it, a group difference might have emerged.

Milner and her colleagues (Milner *et al.* 1991) found that patients with prefrontal cortex excisions were impaired on the temporal order test on which we found no impairment, but the excisions in their patients extended across subregions within prefrontal cortex and were not restricted to Areas 46 and 9. Robbins *et al.* found that prefrontal cortex was critically involved in remembering which stimuli one had already chosen when normal subjects were able to apply a strategy to the task. However, in a condition very much like our multiple boxes tasks, where the boxes were scrambled after each reach, Robbins *et al.* found that subjects were not able to develop a strategy to solve the task, and prefrontal cortex did not appear to be required. However, Petrides (1995) found that monkeys were impaired on the three boxes tasks (boxes scrambled after each reach) after lesions of dorsolateral prefrontal cortex. The literature is contradictory on this point. Perhaps if we had included a test where temporal order memory, or memory of the reponses one has already made, was more amenable to the use of a strategy, we might have found an impairment among subjects whose Phe levels were moderately elevated.

The same children who were impaired on all six working memory + inhibitory control tasks, performed normally on most of the ten control tasks, most of which required the functions of parietal cortex or the medial temporal lobe. The cognitive deficits, thus, appeared to be selective. The functions of parietal cortex and of the medial temporal lobe appeared to be spared. ECT-PKU children with higher Phe levels performed worse on only six out of the 40 comparisons on these tasks. The consistency of the deficits of the ECT-PKU children with Phe levels 3–5 times normal on the working memory + inhibitory control tasks and the paucity of deficits on all other tasks is unlikely to be a chance occurrence.

Visual paired comparison, delayed nonmatching to sample, and the Corsi-Milner test of recognition memory were the measures used of the recognition memory ability dependent on the medial temporal lobe. Of the 12 comparisons between children with Phe levels 3–5 times normal and the other groups of children on these three tasks (3 tasks × 4 comparisons per task), children with higher Phe levels performed significantly worse on only three of these 12 comparisons. However, at the longest delays on the visual paired comparison task (10 min), ECT-PKU infants, regardless of their plasma Phe level, performed significantly worse than infants from the general population and tended to perform worse than matched controls. Plasma Phe levels covaried inversely with performance on the task as well. Hence, on this condition of this one control task an impairment was found.

ECT-PKU children whose plasma Phe levels were closer to normal ($< 3 \times$ normal) performed comparably to all control groups on our cognitive tests. This is consistent with another recent report that, when plasma Phe levels were kept below three times normal from birth to the present, ECT-PKU children showed no cognitive impairments (Stemerdink *et al.* 1995). This indicates that the cognitive deficits may well be preventable.

Are the cognitive deficits observed here indicative of a developmental delay or of absolute, lasting deficits? On the one hand, all children, even ECT-PKU children with higher Phe levels, improved over time on our tasks. On the other hand, the impression that ECT-PKU children may 'catch up' to other children is probably misleading. In almost all cases this 'catch up' was due to ceiling effects because the same tasks were administered over a wide age range, and these tasks tended to be too easy for children at the upper end of an age range. Many tests, such as IQ measures, increase the difficulty of the test as children get older, although the test may be called by the same name throughout. We found that when all groups of children appeared to be performing comparably, because all were near the ceiling, when we went to the next battery of tasks for the next age range, the differences between the groups reappeared. The impairment of the ECT-PKU children with higher Phe levels in working memory and inhibitory control was as evident in our oldest age range ($3\frac{1}{2}$–7 year olds) as it was in our youngest age range (6–12 month olds). There was little evidence of any narrowing of the gap between ECT-PKU children with Phe levels 3–5 times normal and their same-aged peers, at least from 6 months to 6 years of age. We stopped studying children at 7 years of age. One cannot tell from this study whether sometime after 7 years of age ECT-PKU children whose Phe levels remain only moderately elevated might no longer show the kinds of cognitive deficits we have documented.

However, early cognitive deficits or developmental delays, especially when they extend over a long period (6 months–6 years) are likely to have profound and enduring effects, even if the cognitive deficits themselves are subsequently resolved. For example, children who have repeatedly seen themselves struggle with cognitive tasks which their peers find easy, come to believe themselves to be less able, and may continue to see themselves in this way long after the ability gap has been narrowed. Similarly, others around the child (parents, teachers, and peers) typically expect a child to perform as he or she always has, and people often perform as others expect them to perform. Because ECT-PKU children whose Phe concentrations are moderately elevated appear to have difficulty in getting their actions to reflect their intentions, because they sometimes get stuck in behavioural ruts from which they cannot easily extricate themselves, these children may be wrongly labelled as 'bad', 'intentionally difficult', or 'wilful' – and such labels can affect a child for life.

5. IS DORSOLATERAL PREFRONTAL CORTEX ONLY REQUIRED WHEN THE INFORMATION TO BE HELD IN MIND CONCERNS SPATIAL LOCATION?

The tasks on which the infants and toddlers with plasma Phe levels 3–5 times normal failed (A-not-B, object retrieval, and A-not-B with invisible displacements), required holding information in mind about spatial location. One of the most influential and intriguing hypotheses about the functions of dorsolateral prefrontal cortex is that this region is required for holding spatial information in mind (Goldman-Rakic 1987). According to that hypothesis, information about object features is processed in ventrolateral prefrontal cortex, whereas dorsolateral prefrontal cortex is specialized to help us hold in mind information about spatial location. Also according to this hypothesis, a subject acts according to a prepotent response when the correct answer has been forgotten; the prepotent response is sort of a default when one does not know what to do. That is, subjects with dorsolateral prefrontal cortex damage do not err, even in part, because of a failure to exercise inhibitory control; they err because of a failure of working memory, specifically spatial working memory.

There were no spatial tasks on which ECT-PKU infants and toddlers with plasma Phe levels 3–5 times normal performed as well as controls, but there were also no spatial tasks that did not require both holding information in mind and inhibition of a strong response tendency. The prefrontal tasks on which we found no deficits (the multiple boxes and temporal order memory tasks) do not require memory of spatial information. That would be consistent with the spatial memory hypothesis. These tasks also required little or no inhibition; however, the main findings that are inconsistent with the spatial memory hypothesis are that at least two of the tasks on which young children with moderate plasma Phe elevations failed (the day-night Stroop-like task and the tapping task) do not seem to require holding any spatial information in mind. (On the tapping task, as on the day-night task, the subject must hold two rules in mind and must resist making the response that would ordinarily be made (Diamond & Taylor 1996).)

In general, evidence is mixed concerning the possible specialized role of dorsolateral prefrontal cortex in handling spatial information in particular. Evidence in support of this role includes findings such as the following: rhesus monkeys with lesions of dorsolateral prefrontal cortex fail spatial alternation tasks but not object alternation tasks (Mishkin & Manning 1978; but see Bauer & Fuster 1976), and in rhesus monkeys neurons in dorsolateral prefrontal cortex have been found which are active during the delay period of the spatial delayed response task whereas neurons in the inferior convexity are active during the delay period of a conditional pattern discrimination task (Wilson *et al.* 1993). However, evidence contrary to the dorsolateral spatial memory hypothesis includes the following: In positron emission tomography (PET) studies with human subjects, Petrides and his colleagues report activation in ventrolateral prefrontal cortex, but not in dorsolateral prefrontal cortex, on a spatial memory span task (Petrides this volume), and activation in dorsolateral prefrontal cortex, but not in ventrolateral prefrontal cortex, on a non-spatial self-ordered memory task (Petrides *et al.* 1993 and this volume).

The tasks that most reliably and robustly elicit activation of dorsolateral prefrontal cortex in neuroimaging studies, such as the two-back task (e.g. Cohen *et al.* 1993 and this volume), require both holding information in mind (whether spatial or non-spatial) and inhibiting a response tendency. For example, on the two-back test, the subject must remember the rule, 'Respond when, and only when, you see an "x" that follows two items after an "a",' and subjects must resist or override the propensity to respond when they see an "x" under all other circumstances. Some of the evidence on the centrality of inhibitory control problems in patients with damage to dorsolateral prefrontal cortex seems hard to reconcile with a model that proposes that this neural region is important only for working memory. For example, even when the current rule is displayed on all trials so one does not need to hold it in mind, patients with damage to dorsolateral prefrontal cortex have more difficulty switching from one rule to another (e.g. to switch between sorting by size to sorting by shape; Rubinstein *et al.* 1996) than do patients with more posterior damage. It has long been known that patients with damage to dorsolateral prefrontal cortex can sometimes tell you the correct criterion even as they continue to sort cards on the Wisconsin card sorting test incorrectly by the old criterion (e.g. Milner 1963; Luria & Homskaya 1964). In these instances, there is a failure of knowledge to result in the prepotent response being overridden. It is as if subjects get stuck in a behavioural rut from which they cannot readily extricate themselves despite their best intentions and despite knowing what correct performance entails.

6. AN ANIMAL MODEL OF MILD, CHRONIC PLASMA PHE ELEVATIONS

With children it was only possible to measure cognitive performance and plasma amino acid levels. To more directly investigate our hypothesis that the cognitive deficits associated with moderately elevated plasma Phe levels are produced by a selective reduction in dopamine metabolism in prefrontal cortex, we turned to an animal model. Building on work modelling the untreated PKU condition (Greengard *et al.* 1976; Brass & Greengard 1982), we developed and characterized the first animal model of ECT-PKU (Diamond *et al.* 1994). By administering a phenylalanine hydroxylase inhibitor (α-methylphenylalanine) plus a small supplement of Phe we were able to mildly and chronically elevate the plasma Phe levels of rat pups.

The affected rats were impaired on a behavioural task dependent on prefrontal cortex (delayed alternation). On this task, the animal must remember which goal arm was last entered over the *delay* between trials and the animal is rewarded only if it *alternates* goal arms (i.e. if it selects the goal arm *not* selected on the previous trial). The hallmark of what one finds after ablation of prefrontal cortex is that subjects fail when a delay is imposed between trials, although they are unimpaired at learning the task or when no delay is used (in rats see for example Wikmark *et al.* 1973; Larsen & Divac 1978; Bubser & Schmidt 1990; in monkeys see for example Jacobsen & Nissen 1937; Bättig *et al.* 1960; Kubota & Niki 1971). Thus, they are impaired when they must hold in mind which arm of the maze they have just entered and when they have to inhibit repeating that response in order to alternate. We found that the rats with moderately elevated plasma Phe levels learned the delayed alternation task normally and performed well when there was no delay between trials, but failed when a delay was imposed between trials. That is, they showed the pattern of error associated with prefrontal cortex dysfunction.

As predicted, lower levels of dopamine and the dopamine metabolite, HVA, were found in prefrontal cortex in the PKU-model animals than in controls. (Medial prefrontal cortex in the rodent is considered the homologue of dorsolateral prefrontal cortex in the primate (Leonard 1969; Domesick, 1972 Kolb, 1984; Groenewegen 1988), although Preuss (1995) has recently called this homology into question. Medial prefrontal cortex is located immediately in front of the genu.) There was almost no overlap between HVA and dopamine levels in prefrontal cortex of controls and of either experimental group. In contrast, there was no effect of elevated plasma Phe levels on noradrenaline in any of the neural regions investigated (prefrontal cortex, anterior cingulate, caudate-putamen, and nucleus accumbens).

Our predictions were not perfectly confirmed, however. We found some effect on the serotoninergic system, and some effects on dopamine metabolism outside of prefrontal cortex. This could be because plasma Phe levels were raised a bit more than intended (6.5 times normal, rather than < 5 times normal) or because the neurochemical effects of moderately elevated plasma Phe levels are not as localized as we have hypothesized. Our lab is presently investigating this further with the genetic mouse model of PKU created by McDonald & Shedlovsky (McDonald *et al.* 1990; Shedlovsky *et al.* 1993). These mice have a mutation of the gene that causes PKU in humans; these mice have a mutation of the gene homologous to the one that causes PKU in humans; these mice, too, are severely impaired in converting Phe to Tyr.

7. CONFIRMING EVIDENCE FROM RESEARCH ON VISION

If we are correct that the special properties of the dopamine neurons that project to prefrontal cortex make the functions of that area particularly vulnerable to moderate increases in the ratio of Phe: Tyr in the bloodstream, then any other dopamine neurons that also have those properties should also be affected. It turns out that the dopamine neurons in the retina share those properties. They too have rapid firing and dopamine turnover rates (Iuvone *et al.* 1978, 1989; Fernstrom *et al.* 1986). Moreover, the competition at the blood-retinal barrier is comparable to that at the blood-brain barrier (Rapoport 1976; Hjelle *et al.* 1978; Fernstrom *et al.* 1986; Tornquist & Alm 1986). Therefore, we predicted that retinal functions dependent on dopamine should also be affected in PKU children with plasma Phe levels 3–5 times normal; that prediction has recently been confirmed.

If the retina is depleted of dopamine, one finds impaired sensitivity to contrast (Kupersmith *et al.* 1982; Regan & Neima 1984; Skrandies & Gottlob 1986; Bodis-Wollner *et al.* 1987; Bodis-Wollner 1990). (Contrast sensitivity refers to the threshold of how much contrast between black lines (sinusoid gratings) and a white background is required for a person to be able to detect the black lines.) Diamond and Herzberg (1996) found that children treated early and continuously for PKU were impaired in their sensitivity to contrast across all five spatial frequencies tested (1.5–18.0 cycles per degree). Indeed, at the next to highest spatial frequency (12 cycles per degree), the 'group' variable accounted for 70% of the variance, controlling for acuity, sex, age, and test site. At no spatial frequency was the contrast sensitivity performance of any child with PKU superior to the performance of his or her sibling. The poorer contrast sensitivity performance of subjects with PKU was found even when the analyses were repeated omitting the two subjects with mean plasma Phe levels above five times normal and omitting the two subjects with IQ scores less than 90. In short, we have found two superficially unrelated behavioural effects, a selective deficit in cognitive functions dependent on dorsolateral prefrontal cortex and a selective visual defect in contrast sensitivity, both of which had been predicted based on the same underlying hypothesis (that a moderate imbalance in the plasma Phe:Tyr ratio selectively affects those dopamine neurons that have the most rapid firing and dopamine turnover rates).

We knew, from work with rats, that the dopamine neurons that project to prefrontal cortex have unusual properties that set them apart from most other dopamine-containing neurons in the CNS. However, there was no evidence concerning this in humans. The results we have obtained with children treated early and continuously for PKU provide what is probably the first evidence suggesting that the prefrontally-projecting dopamine neurons may also have these special properties in humans. We knew, from work with rhesus macaques, that during the period that infant monkeys are improving on the A-not-B, delayed response, and object retrieval tasks, striking changes in the distribution of tyrosine hydroxylase-immunoreactive fibres in prefrontal cortex (Area 9) are occuring (Lewis & Harris 1991), and the level of dopamine is increasing in the monkey brain (Brown & Goldman 1977). It is possible that one of the reasons that human infants are able to succeed on these tasks by 12 months of age, although they fail these tasks even under the simplest conditions below 8–9 months of age, has to do with developmental changes in the dopaminergic innervation of prefrontal cortex.

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REFERENCES

Bannon, M. J., Bunney, E. B. & Roth, R. H. 1981 Mesocortical dopamine neurons: rapid transmitter turnover compared to other brain catecholamine systems. *Brain Res.* **218**, 376–382.

Bannon, M. J., Wolf, M. E. & Roth, R. H. 1983 Pharmacology of dopamine neurons innervating the prefrontal, cingulate, and piriform cortices. *Eur. J. Pharmacol.* **92**, 119–125.

Bättig, K., Rosvold, H. E. & Mishkin, M. 1960 Comparison of the effects of frontal and caudate lesions on delayed response and alternation in monkeys. *J. Comp. Physiol.* **53**, 400–404.

Bauer, R. H. & Fuster, J. M. 1976 Delayed-matching and delayed-response deficit from cooling dorsolateral prefrontal cortex in monkeys. *J. Comp. Physiol. Psychol.* **90**, 293–302.

Berry, H. K., O'Grady, D. J., Perlmutter, L. J. & Bofinger, M. K. 1979 Intellectual development and achievement of children treated early for phenylketonuria. *Devl. Med. Child Neurol.* **21**, 311–320.

Bodis-Wollner, I. 1990 Visual deficits related to dopamine deficiency in experimental animals and Parkinson's disease patients. *TINS* **13**, 296–302.

Bodis-Wollner, I., Marx, M. S., Mitra, S., Bobak, P., Mylin, L. & Yahr, M. 1987 Visual dysfunction in Parkinson's disease. Loss in spatiotemporal contrast sensitivity. *Brain* **110**, 1675–1698.

Bradberry, C. W., Karasic, D. H., Deutch, A. Y. & Roth, R. H. 1989 Regionally-specific alterations in mesotelencephalic dopamine synthesis in diabetic rats: association with precursor tyrosine. *J. Neural Transmission* **78**, 221–229.

Brass, C. A. & Greengard, O. 1982 Modulation of cerebral catecholamine concentrations during hyperphenylalaninaemia. *Biochem. J.* **208**, 765–771.

Brown, R. M. & Goldman, P. S. 1977 Catecholamines in neocortex of rhesus monkeys: regional distribution and ontogenetic development. *Brain Res.* **124**, 576–580.

Brozoski, T. J., Brown, R. M., Rosvold, H. E. & Goldman, P. S. 1979 Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science* **205**, 929–932.

Brunner, R. L., Berch, D. B. & Berry, H. 1987 Phenylketonuria and complex spatial visualization; an analysis of information processing. *Devl. Med. Child Neurol.* **29**, 460–468.

Bubser, M. & Schmidt, W. J. 1990 6–hydroxydopamine lesion of the rat prefrontal cortex increases locomotor activity, impairs acquisition of delayed alternation tasks, but does not affect uninterrupted tasks in the radial maze. *Behav. Brain Res.* **37**, 157–168.

Butterworth, G. 1977 Object disappearance and error in Piaget's stage IV task. *J. Exptl. Child Psychol.* **23**, 391–401.

Chirigos, M., Greengard, P. & Udenfriend, S. 1960 Uptake of tyrosine by rat brain *in vivo*. *J. Biol. Chem.* **235**, 2075–2079.

Cohen, J. D., Forman, S., Casey, B. J., Servan-Schreiber, D., Noll, D. C. & Lewis, D. A. 1993 Activation of dorsolateral prefrontal cortex in humans durng a working memory task using functional MRI. *Soc. Neurosci. Abstr.* **19**, 1285.

Cornell, E. H. 1979 The effects of cue reliability on infants' manual search. *J. Exptl. Child Psychol.* **28**, 81–91.

Diamond, A. 1985 Development of the ability to use recall to guide action, as indicated by infants' performance on A$\bar{B}$. *Child Development* **56**, 868–883.

Diamond, A. 1988 Differences between adult and infant cognition: is the crucial variable presence or absence of language? In *Thought without language* (ed. L. Weiskrantz), pp. 337–370. Oxford University Press.

Diamond, A. 1990*a* The development and neural bases of memory functions, as indexed by the A$\bar{B}$ and delayed response tasks, in human infants and infant monkeys. *Ann. N. Y. Acad. Sci.* **608**, 267–317.

Diamond, A. 1990*b* Rate of maturation of the hippocampus and the developmental progression of children's performance on the delayed non-matching to sample and visual paired comparison tasks. *Ann. N. Y. Acad. Sci.* **608**, 394–426.

Diamond, A. 1991*a* Frontal lobe involvement in cognitive changes during the first year of life. In *Brain maturation and cognitive development: comparative and cross-cultural perspectives* (ed. K. R. Gibson & A. C. Petersen), pp. 127–180. New York: Aldine de Gruyter.

Diamond, A. 1991*b* Neuropsychological insights into the meaning of object concept development. In *The epigenesis of mind: essays on biology and knowledge* (ed. S. Carey & R. Gelman), pp. 67–110. Hillsdale, New Jersey: Lawrence Erlbaum Associates.

Diamond, A. 1994 Phenylalanine levels of 6–10 mg/dl may not be as benign as once thought. *Acta Pædiatrica* **83** (Supplement 407), 89–91.

Diamond, A. 1995 Evidence of robust recognition memory early in live even when assessed by reaching behavior. *J. Exptl. Child Psychol.* **59**, 419–456.

Diamond, A., Ciaramitaro, V., Donner, E., Djali, S. & Robinson, M. 1994 An animal model of early-treated PKU. *J. Neurosci.* **14**, 3072–3082.

Diamond, A. & Goldman-Rakic, P. S. 1989 Comparison of human infants and rhesus monkeys on Piaget's A$\bar{B}$ task: evidence for dependence on dorsolateral prefrontal cortex. *Exptl. Brain Res.* **74**, 24–40.

Diamond, A. & Herzberg, C. 1996 Impaired sensitivity to visual contrast in children treated early and continuously for PKU. *Brain* **119**, 523–538.

Diamond, A., Prevor, M., Callender, G. & Druin, D. P.

1996 Prefrontal cortex cognitive deficits in children treated early and continuously for PKU. *Monogr. Soc. Res. Child Devel.* (In the press).

Diamond, A. & Taylor, C. 1996 Development of an aspect of executive control: development of the abilities to remember what I said and to "Do as I say, not as I do". *Devtl. Psychobiol.* **29**, 315–334.

Dobson, J. C., Kushida, E., Williamson, M. L. & Friedman, E. G. 1976 Intellectual performance of 36 phenylketonuric patients and their non-affected siblings. *Pediatrics* **58**, 53–58.

Domesick, V. B. 1972 Thalamic relationships of the medial cortex in the rat. *Brain Behav. Evol.* **6**, 457–483.

Evans, W. F. 1973 The stage IV error in Piaget's theory of concept development: an investigation of the rise of activity. Doctoral dissertation, University of Houston.

Faust, D., Libon, D. & Pueschel, S. 1986 Neuropsychological functioning in treated phenylketonuria. *International J. Psychiatry Med.* **16**, 169–177.

Fernstrom, M. H., Volk, E. A., Fernstrom, J. D. & Iuvone, P. M. 1986 Effect of tyrosine administration on dopa accumulation in light- and dark- adapted retinas from normal and diabetic rats. *Life Sciences* **39**, 2049–2057.

Fox, N., Kagan, J. & Weiskopf, S. 1979 The growth of memory during infancy. *Genetic Psychology Monographs* **99**, 91–130.

Fuster, J. M. & Alexander, G. E. 1971 Neuron activity related to short-term memory. *Science* **173**, 652–654.

Gerstadt, C., Hong, Y. & Diamond, A. 1994 The relationship between cognition and action: Performance of $3\frac{1}{2}$–7 year old children on a Stroop-like day-night test. *Cognition* **53**, 129–153.

Goldman, P. S. & Rosvold, H. E. 1970 Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. *Exptl. Neurology* **27**, 291–304.

Goldman-Rakic, P. S. 1987 Circuitry of primate prefrontal cortex and regulation of behavior by representational knowledge. In *Handbook of physiology* (ed. F. Plum & V. Mountcastle), vol. 5, pp. 373–417.

Gratch, G., Appel, K. J., Evans, W. F., LeCompte, G. K. & Wright, N. A. 1974 Piaget's stage IV object concept error: evidence of forgetting or object conception? *Child Devl.* **45**, 71–77.

Gratch, G. & Landers, W. F. 1971 Stage IV of Piaget's theory of infant's object concepts: a longitudinal study. *Child Devl.* **42**, 359–372.

Greengard, O., Yoss, M. S. & DelValle, J. A. 1976 α-methylphenylalanine, a new inducer of chronic hyperphenylalaninemia in suckling rats. *Science* **192**, 1007–1008.

Groenewegen, H. J. 1988 Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. *Brain Res.* **24**, 379–431.

Harlow, H. F., Davis, R. T., Settlage, P. H. & Meyer, D. R. 1952 Analysis of frontal and posterior association syndromes in brain-damaged monkeys. *J. Comp. Physiol. Psychol.* **54**, 419–429.

Harris, P. L. 1973 Perseverative errors in search by young infants. *Child Devl.* **44**, 28–33.

Hjelle, J. T., Baird-Lambert, J., Cardinale, G. Specor, S. & Udenfriend, S. 1978 Isolated microvessels: the blood-brain barrier *in vitro*. *Proc. Nat. Acad. Sci.* **75**, 4544–4548.

Iuvone, P. M., Galli, C. L., Garrison-Gund, C. K. & Neff, N. H. 1978 Light stimulates tyrosine hydroxylase activity and dopamine synthesis in retinal amacrine neurons. *Science* **202**, 901–902.

Iuvone, P. M., Tigges, M., Fernandes, A. & Tigges, J. 1989 Dopamine synthesis and metabolism in rhesus monkey retina: development, aging, and the effects of monocular visual deprivation. *Visual Neurosci.* **2**, 465–471.

Jacobsen, C. F. 1935 Functions of frontal association areas in primates. *Arch. Neurol. Psychiat.* **33**, 558–560.

Jacobsen, C. F. & Nissen, H. W. 1937 Studies of cerebral function in primates. The effects of frontal lobe lesions on the delayed alternation habit in monkeys. *J. Comp. Physiol. Psychol.* **23**, 101–112.

Koch, R. & Wenz, E. 1987 Phenylketonuria. *A. Rev. Nutr.* **7**, 117–135.

Kolb, B. 1984 Functions of the frontal cortex of the rat: a comparative review. *Brain Res. Rev.* **8**, 65–98.

Krause, W. L., Helminski, M., McDonald, L., Dembure, P., Salvo, R., Freides, D. & Elsas, L. J. 1985 Biochemical and neurophysiological effects of elevated plasma phenylalanine in patients with treated phenylketonuria, a model for the study of phenylalanine in brain function in man. *J. Clin. Invest.* **75**, 40–48.

Kubota, K. & Niki, H. 1971 Prefrontal cortical unit activity and delayed alternation performance in monkeys. *J. Neurophysiol.* **34**, 337–347.

Kupersmith, M. J., Shakin, E., Siegel, I. M. & Lieberman, A. 1982 Visual system abnormalities in patients with Parkinson's disease. *Arch. Neurol.* **39**, 284–286.

Larsen, J. K. & Divac, I. 1978 Selective ablations within the prefrontal cortex of the rat and performance on delayed alternation. *Physiological Psychol.*, **6**, 15–17.

Leonard, C. M. 1969 The prefrontal cortex of the rat. I. Cortical projections of the mediodorsal nucleus. II. Efferent connections. *Brain Res.* **12**, 321–343.

Lewis, D. A., Campbell, M. J., Foote, S. L. & Morrison, J. H. 1986 The monoaminergic innervation of primate neocortex. *Hum. Neurobiol.* **5**, 181–188.

Lewis, D. & Harris, H. W. 1991 Differential laminar distribution of tyrosine hydroxylase-immunoreactive axons in infant and adult monkey prefrontal cortex. *Neurosci. Lett.* **125**, 151–154.

Lewis, D. A. & Morrison, J. H. 1989 The noradrenergic innervation of monkey prefrontal cortex: A dopamine-β-hydroxylase immunohistochemical study. *J. Comput. Neurosci.* **282**, 317–330.

Lidsky, A. S., Law, M. L., Morse, H. G., Kao, F. T. & Woo, S. L. C. 1985 Regional mapping of the human phenylalanine hydroxylase gene and the PKU locus on chromosome 12. *Proc. Nat. Acad. Sci.* **82**, 6221–6225.

Luria, A. R. & Homskaya, E. D. 1964 Disturbance in the regulative role of speech with frontal lobe lesions. In *The frontal granular cortex and behavior* (ed. J. M. Warren & K. Akert), pp. 353–371. New York: McGraw-Hill.

Mangels, J. A. 1996 The relationship between strategic processing, interference and memory for temporal order in patients with frontal lobe lesions. *Neuropsychol.* (Submitted).

McDonald, J. D., Bode, V. C., Dove, W. F. & Shedlovsky, A. 1990 Pah^{hph-5}: a mouse mutant deficient in phenylalanine hydroxylase. *Proc. Nat. Acad. Sci.* **87**, 1965–1967.

Miles, R. C. & Blomquist, A. 1960 Frontal lesions and behavioral deficits in monkey. *J. Neurophsiol.* **23**, 471–484.

Miller, L., Braun, L. D., Pardridge, W. M. & Oldendorf, W. H. 1985 Kinetic constants for blood-brain barrier amino acid transport in conscious rats. *J. Neurochem.* **45**, 1427–1432.

Milner, B. 1963 Effects of brain lesions on card sorting. *Arch. Neurol.* **9**, 90–100.

Milner, B., Corsi, P. & Leonard, G. 1991 Frontal-lobe contribution to recency judgements. *Neuropsychologia* **29**, 601–618.

Mishkin, M. & Manning, F. J. 1978 Nonspatial memory

after selective prefrontal lesions in monkeys. *Brain Res.* **143**, 313–323.

Oldendorf, W. H. 1973 Stereospecificity of blood brain barrier permeability to amino acids. *Am. J. Physiol.* **224**, 967–969.

Pardridge, W. 1977 Regulation of amino acid availability to the brain. In *Nutrition and the brain* (ed. R. J. Wurtman & J. J. Wurtman), pp 141–204. New York: Raven Press.

Pardridge, W. M. & Oldendorf, W. H. 1977 Transport of metabolic substrates through the blood-brain barrier. *J. Neurochem.* **28**, 5–12.

Pennington, B. F., VanDoornick, W. J., McCabe, L. L. & McCabe, E. R.B. 1985 Neuropsychological deficits in early treated phenylketonuric children. *Am. J. Mental Deficiency* **89**, 467–474.

Petrides, M. 1988 Performance on a nonspatial self-ordered task after selective lesions of the primate frontal cortex. *Soc. Neurosci. Abstr.* **14**, 4–8.

Petrides, M. 1989 Frontal lobes and memory. In *Handbook of neuropsychology* (ed. F. Boller & J. Grafman), pp. 75–90. Amsterdam: Elsevier.

Petrides, M. 1991 Functional specialization within the dorsolateral frontal corex for serial order memory. *Proc. R. Soc. Lond.* B **246**, 299–306.

Petrides, M. 1995 Impairments on nonspatial self-ordered and externally ordered working memory tasks after lesions of the mid-dorsal part of the lateral frontal cortex in the monkey. *J. Neurosci* **15**, 359–375.

Petrides, M., Alivisatos, B., Evans, A. C. & Meyer, E. 1993 Dissociation of human mid-dorsalateral from posterior dorsolateral frontal cortex in memory processing. *Proc. Nat. Acad. Sci.* **90**, 873–877.

Piaget, J. 1954 *The construction of reality in the child.* New York: Basic Books. Original French edition, 1937.

Pinsker, H. M. & French, G. M. 1967 Indirect delayed reactions under various testing conditions in normal and midlateral frontal monkeys. *Neuropsychologia* **5**, 13–24.

Preuss, T. M. 1995 Do rats have prefrontal cortex? The Rose-Woolsey-Akert Program reconsidered. *J. Cog. Neurosci.* **7**, 1–24.

Rapoport, S. I. 1976 Sites and functions of the blood-aqueous and blood-vitreous barriers of the eye. In *Blood-brain barrier in physiology and medicine* (ed. S. I. Rapoport), pp. 207–232. New York: Raven Press.

Regan, D. & Neima, D. 1984 Low-contrast letter charts in early diabetic retinopathy, ocular hypertension, glaucoma, and Parkinson's disease. *Br. J. Opthalmol.* **68**, 885–889.

Rubinstein, J., Evans, J. E. & Meyer, D. E. 1996 Executive control of cognitive processes in task switching. (In preparation).

Sawaguchi, T. & Goldman-Rakic, P. S. 1991 D1 dopamine receptors in prefrontal cortex: Involvement in working memory. *Science* **251**, 947–950.

Schneider, J. S. & Kovelowski, C. J., II. 1990 Chronic exposure to low doses of MPTP. I. Cognitive deficits in motor asymptomatic monkeys. *Brain Res.* **519**, 122–128.

Shedlovsky, A., McDonald, J. D., Symula, D. & Dove, W. F. 1993 Mouse models of human phenylketonuria. *Genetics* **134**, 1205–1210.

Simon, H., Scatton, B. & LeMoal, M. 1980 Dopaminergic A10 neurones are involved in cognitive functions. *Nature, Lond.* **286**, 150–151.

Skrandies, W. & Gottlob, I. 1986 Alterations of visual contrast sensitivity in Parkinson's disease. *Hum. Neurobiol.* **5**, 255–259.

Smith, M. L., Klim, P., Mallozzi, E. & Hanley, W. B. 1996 A test of the frontal-specificity hypothesis in the cognitive performance of adults with phenylketonuria. *Devtl. Neuropsychol.* (In the press).

Smith, I. & Beasley, M. 1989 Intelligence and behaviour in children with early treated phenylketonuria. *Eur. J. Clin. Nutrition* **43**, 1–5.

Sophian, C. & Wellman, H. M. 1983 Selective information use and perseveration in the search behavior of infants and young children. *J. Exptl. Child Psychol.* **35**, 369–390.

Stemerdink, B. A., van der Meere, J. J., van der Molen, M. W., Kalverboer, A. F.*et al.* 1995 Information processing in patients with early and continuously-treated phenylketonuria. *Eur. J. Pediatrics* **172**

Stuss, D. T. & Benson, D. F. 1986 *The frontal lobes.* New York: Raven Press.

Stuss, D. T. & Benson, D. F. 1987 The frontal lobes and control of cognition and memory. In *The frontal lobes revisited* (ed. E. Perecman), pp. 141–158. New York: IRBN Press.

Tam, S. Y., Elsworth, J. D., Bradberry, C. W. & Roth, R. H. 1990 Mesocortical dopamine neurons: high basal firing frequency predicts tyrosine dependence of dopamine synthesis. *J. Neural Transmission General Section* **81**, 97–110.

Taylor, J. R., Roth, R. H., Sladek, J. R., Jr. & Redmond, D. E., Jr. 1990 Cognitive and motor deficits in the performance of the object retrieval detour task in monkeys (*Cercopithecus aethiops sabaeus*) treated with MPTP: Long-term performance and effect of transparency of the barrier. *Behav. Neurosci.* **104**, 564–576.

Thierry, A. M., Tassin, J. P., Blanc, A., Stinus, L., Scatton, B. & Glowinski, J. 1977 Discovery of the mesocortical dopaminergic system: Some pharmacological and functional characteristics. *Adv. Biomed. Psychopharmacology* **16**, 5–12.

Tornquist, P. & Alm, A. 1986 Carrier-mediated transport of amino acids through the blood-retinal and blood-brain barriers. *Graefe's Archive for Clinical and Experimental Ophthalmology* **224**, 21–25.

Welsh, M. C., Pennington, B. F., Ozonoff, S., Rouse, B. & McCabe, E. R.B. 1990 Neuropsychology of early-treated phenylketonuria: specific executive function deficits. *Child Devl.* **61**, 1697–1713.

Wikmark, R. G.E., Divac, I. & Weiss, R. 1973 Delayed alternation in rats with lesions in the frontal lobes: Implications for a comparative neuropsychology of the frontal system. *Brain Behav. Evol.* **8**, 329–339.

Willatts, P. 1985 Adjustment of means-ends coordination and the representation of spatial relations in the productions of search errors by infants. *Br. J. Devtl. Psychol.* **3**, 259–272.

Williamson, M. L., Koch, R., Azen, C. & Chang, C. 1981 Correlates of intelligence test results in treated phenylketonuric children. *Pediatrics* **68**, 161–167.

Wilson, F. A., Scalaidhe, S. P. & Goldman-Rakic, P. S. 1993 Dissociation of object and spatial processing domains in primate prefrontal cortex. *Science* **260**, 1955–1958.

Woo, S. L. C., Lidsky, A. S., Güttler, F., Chandra, T. & Robson, K. J. H. 1983 Cloned human phenylalanine hydroxylase gene allows prenatal diagnosis and carrier detection of classical phenylketonuria. *Nature, Lond.* **306**, 151–155.

Wurtman, R. J., Hefti, F. & Melamed, E. 1981 Precursor control of neurotransmitter synthesis. *Pharmacological Rev.* **32**, 315–335.

Zelazo, P. D., Frye, D. & Rapus, T. 1996 An age-related dissociation between knowing rules and using them. *Cog. Devt.* **11**, 37–63.

Zelazo, P. D., Reznick, J. S. & Piñon, D. E. 1995 Response control and the execution of verbal rules. *Devetl. Psychol.* **31**, 508–517.

Discussion

A. Baddeley (*Department of Psychology, University of Bristol, 8 Woodland Road, Bristol BS8 1TN, U.K.*). Inter-correlations between tests of frontal lobe or executive function have typically not been very high. What is your experience with the battery of cognitive tests you have used to study PKU in children?

A. Diamond. The intercorrelation between performance on the frontal tasks for infants, AB and object retrieval, was 0.35 ($P < 0.001$); both of these tasks require holding information in mind+inhibitory control. There were no significant intercorrelations among any of the tasks administered to toddlers, but only one of those tasks required holding information in mind + inhibitory control. The intercorrelation between performance on the tapping and three pegs tasks was 0.53 ($P < 0.0001$), on tapping and the day-night Stroop-like tasks it was 0.35 ($P < 0.0001$), and on the day-night Stroop-like task and the tapping task it was 0.20 ($P = 0.01$); all three of these tasks require holding information in mind+inhibitory control. Performance on the other frontal tests (the six boxes task and the Corsi-Milner test of temporal order memory), which did not require holding information in mind+inhibitory control, did not correlate significantly with performance on any other test.