Chapter 29. A Model System for Studying the Role of Dopamine in the Prefrontal Cortex during Early Development in Humans: Early and Continuously Treated Phenylketonuria

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Abstract

After a brief overview of the anatomy of dorsolateral prefrontal cortex (DL-PFC) and of its anatomical connections with other brain regions, findings are summarized that DL-PFC subserves working memory and inhibitory control abilities even during infancy. Evidence suggests that one change in the prefrontal neural circuit helping to make possible some of the cognitive advances seen in infants between 6 and 12 months of age might be changes in the dopaminergic innervation of prefrontal cortex. The period of 3-6 years is then examined as a period when there is particularly marked improvement in the working memory and inhibitory control abilities thought to depend upon DL-PFC. Perhaps that improvement is made possible, in part, by maturational changes in DL-PFC, perhaps even in its dopamine projection, although that remains to be demonstrated. (To propose that changes in the dopamine innervation of prefrontal cortex play a role in making possible some cognitive advances is not to negate the role of experience nor the role of other maturational changes in the prefrontal neural system.)

As an initial way to begin looking at the role of dopamine in prefrontal cortex function in human beings during infancy and early childhood, we studied a group of children who, there was reason to believe, have reduced levels of dopamine in prefrontal cortex but otherwise normal brains. These are children treated early and continuously for phenylketonuria (PKU), whose phenylalanine levels are 3-5 times normal and whose tyrosine (Tyr) levels are below normal. The rationale for studying these children and the results obtained in those studies are summarized, as are the results from our work with an animal model of early, and continuously, treated PKU. Evidence on dissociations among tasks that require DL-PFC but are differentially sensitive to the dopamine content of DL-PFC is discussed. The evidence shows that not all cognitive tasks dependent on DL-PFC are dependent on dopamine in DL-PFC. It is my hope that this review will offer some insight into cognitive development, into the role of prefrontal cortex in cognitive development, and into the role of dopamine in prefrontal cortex function.

Dorsolateral prefrontal cortex (DL-PFC) undergoes an extremely protracted period of maturation and is not fully mature until adulthood (Huttenlocher, 1979, 1984, 1990; Huttenlocher et al., 1982; Orzhekhovskaya, 1981; Rosenberg and Lewis, 1994; Sowell et al., 1999; Thatcher, Walker, and Giudice, 1987; Yakovlev and Lecours, 1967). Growing evidence indicates, however, that some of the cognitive advances seen as early as the first year of life (6-12 months) are made possible, in part, by early changes in DL-PFC (e.g., Bell and Fox, 1992, 1997; Diamond, 1991a,b; Fox and Bell, 1990). One maturational change in DL-PFC that might help make

possible these early cognitive advances is increasing levels of the neurotransmitter dopamine in DL-PFC.

Prefrontal cortex is richer in dopamine than any other region of the cerebral cortex (Bjorklund, Divac, and Lindvall, 1978; Brown, Crane, and Goldman, 1979; Gaspar et al., 1989; Levitt, Rakic, and Goldman-Rakic, 1984; Lewis et al., 1988, 1998; Williams and Goldman-Rakic, 1993, 1995). Not surprisingly, given its high concentration in prefrontal cortex, dopamine plays an important role in DL-PFC function in adult human and nonhuman primates (Akil et al., 1999; Brozoski et al., 1979; Luciana et al., 1992; Sawaguchi, Matsumura, and Kubota, 1988; Sawaguchi and Goldman-Rakic, 1991; Watanabe, Kodama, and Hikosaka, 1997).

We know that during the period that infant rhesus macaques are improving on tasks dependent on DL-PFC (the A-not-B, delayed response, and object retrieval tasks) the level of dopamine is increasing in their brains (Brown, Crane, and Goldman, 1976; Brown and Goldman, 1977), the density of dopamine receptors in their prefrontal cortex is increasing (Lidow and Rakic, 1992), and the distribution within their DL-PFC (Brodmann area 9) of axons containing the rate-limiting enzyme for the production of dopamine (tyrosine hydroxylase) is markedly changing (Lewis and Harris, 1991; Rosenberg and Lewis, 1995). Moreover, in adult rhesus macaques, the cognitive abilities that depend on DL-PFC (as indexed by tasks such as delayed response) rely critically on the dopaminergic projection to prefrontal cortex (Brozoski et al., 1979; Sawaguchi and Goldman-Rakic, 1991; Sawaguchi, Matsumura, and Kubota, 1990; Taylor et al., 1990a,b).

Evidence such as that summarized here makes it plausible that one change in the prefrontal neural circuit helping to make possible some of the cognitive advances that occur in infants between 6 and 12 months of age might be changes in the dopaminergic innervation of prefrontal cortex. Maturational changes in the prefrontal dopamine system are protracted, and therefore it is conceivable that later maturational changes in that system might help make possible subsequent improvements in the cognitive abilities dependent on prefrontal cortex as well. (To propose that changes in the dopamine innervation of prefrontal cortex play a role in making possible some of the cognitive advances during development is not to negate the role of experience nor the role of other maturational changes in the prefrontal neural system, such as in the communication between prefrontal cortex and other neural regions.)

To begin to look at the role of the dopamine projection to DL-PFC in helping to subserve cognitive functions early in life in humans, we have been studying children who, the evidence suggests, have reduced levels of dopamine in prefrontal cortex but otherwise remarkably normal brains. These are children treated early and continuously for the genetic disorder phenylketonuria (PKU) in whose bloodstreams the levels of the amino acid phenylalanine (Phe) are 3-5 times normal (6-10 mg/dL).

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Section 1. Where is DL-PFC?

The cerebral cortex is distinguished from subcortex by generally having six different layers of cells (subcortical regions have fewer layers) and by being the outer mantle of the brain (closer to the surface), whereas subcortical structures are buried deep inside the brain below the cortex. In general, cortical regions are phylogenetically newer regions of the brain than subcortical regions, mature later during development, and receive more highly processed information that has already passed through subcortical structures. During primate evolution, the cerebral cortex changed from being smooth to having marked "hills" (gyri) and "valleys" (sulci). This infolding permitted an extraordinary expansion in cerebral cortex size despite a much less marked expansion in cranium size -- an adaptive solution that increased surface area in a limited space.

The central sulcus divides the front of the brain from the back. All of the cerebral cortex in front of the central sulcus is frontal cortex (see figure 29.1). The most posterior region of frontal cortex, directly in front of the central sulcus, is primary motor cortex (Brodmann's area 4). The anterior boundary of motor cortex is the precentral sulcus. In front of that is premotor cortex and the supplementary motor area (SMA), two distinct subregions of Brodmann's area 6. All of the cortex in front of that is prefrontal cortex (areas 8, 9, 10, 12, 44, 45, 46, 47, and 9/46). Prefrontal cortex is not only the most anterior region of frontal cortex, but the only region of frontal cortex with a granule cell layer.

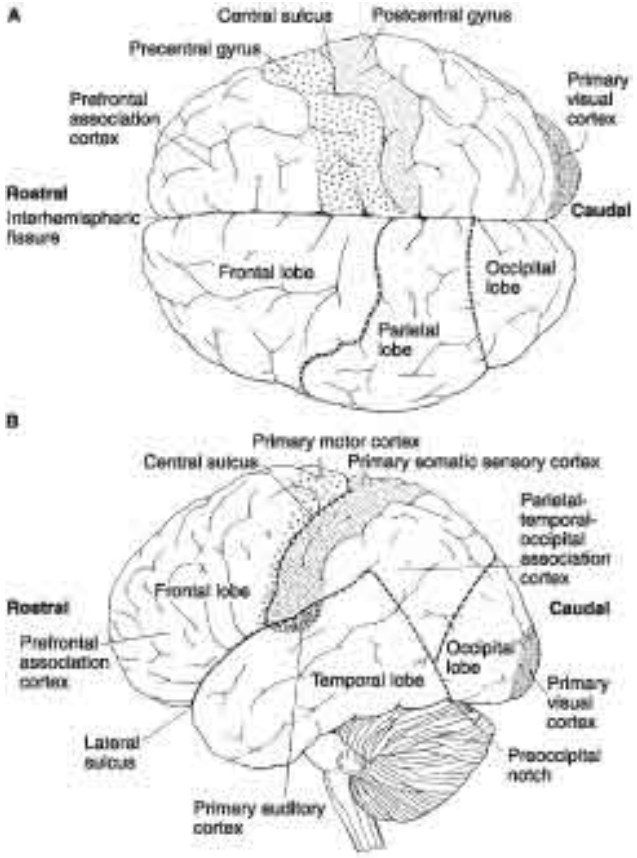

Figure 29.1. Diagram of the human brain, indicating the location of dorsolateral prefrontal cortex. (Reprinted with permission from Kandel, E. R., J. H. Schwartz, and T. M. Jessell, eds., 1991. *Principles of Neural Science*, 3rd ed. New York: McGraw Hill.)

While the brain as a whole has increased in size during evolution, the proportion of the brain devoted to prefrontal cortex has increased much more dramatically, especially in humans (Brodmann, 1912). For example, prefrontal cortex makes up 25% of the cortex in the human brain, but only 15% in chimpanzees, 7% in dogs, and 4% in cats. Prefrontal cortex is an association area; its functions are primarily integrative, neither exclusively sensory nor motor. In accord with its late maturational timetable and massive expansion during primate evolution, prefrontal cortex is credited with underlying the most sophisticated cognitive abilities, often called "executive processes," such as reasoning, planning, problem-solving, and coordinating the performance of multiple tasks (Goldman-Rakic, 1987; Pennington and Ozonoff, 1996; Postle, Berger, and D'Esposito, 1999; Shallice, 1988; Warren and Akert, 1964).

Within prefrontal cortex, the mid-dorsolateral subregion (areas 9, 46, and 9/46) has increased disproportionately in size during evolution even compared to the other regions of prefrontal cortex. Mid-DL-PFC consists of the middle section of the superior and middle frontal gyri, extending from behind the frontal pole (area 10) to area 8 (see figure 29.1; Petrides and Pandya, 1999). DL-PFC has historically been defined by its reciprocal connections with the parvocellular subdivision of the mediodorsal nucleus of the thalamus (Akert, 1964; Goldman-Rakic and Porrino, 1985; Jacobson, Butters, and Tovsky, 1978; Kievit and Kuypers, 1977; McLardy, 1950; Rose and Woolsey, 1948; Siwek and Pandya, 1991; Tobias, 1975; Walker, 1940). The size of the parvocellular portion of the mediodorsal nucleus has increased phylogenetically in proportion to the increase in size of DL-PFC and disproportionately compared even to other regions of the mediodorsal nucleus (Clark, 1930; Khokhryakova, 1979; Pines, 1927).

No area of the brain acts in isolation. A neural region functions as part of system of functionally and anatomically interrelated structures. Through its reciprocal connections with the *superior temporal cortex* (Petrides and Pandya, 1988; Seltzer and Pandya, 1989), *posterior parietal cortex* (area 7a; Cavada and

Goldman-Rakic, 1989; Goldman-Rakic and Schwartz, 1982; Johnson et al., 1989; Petrides and Pandya, 1984; Schwartz and Goldman-Rakic, 1984; Selemon and Goldman-Rakic, 1988), *anterior and posterior cingulate* (Vogt and Pandya, 1987; Vogt, Rosene, and Pandya, 1979), *premotor cortex* (Barbas and Mesulam, 1985, 1987; Künzle, 1978), SMA (McGuire, Bates, and Goldman-Rakic, 1991; Wiesendanger, 1981), *retrosplenial cortex* (Morris, Pandya, and Petrides, 1999; Morris, Petrides, and Pandya, 1999; see also Petrides and Pandya, 1999, concerning all of these interconnections), and the *neocerebellum* (Diamond, 2000; Leiner, Leiner, and Dow, 1989; Middleton and Strick, 1994, 1997; Sasaki et al., 1979; Schmahmann and Pandya, 1995; Yamamoto et al., 1992), mid-DL-PFC can modulate the activity of those regions, as well as receive information from, and be modulated by, these regions. In addition, mid-DL-PFC sends a strong projection to the caudate nucleus (Arikuni and Kubota, 1986; Goldman and Nauta, 1977; Kemp and Powell, 1970; Selemon and Goldman-Rakic, 1985). The projections from DL-PFC, posterior parietal cortex, and the superior temporal cortex are intricately interdigitated throughout the brain, including in the caudate nucleus, providing multiple opportunities for these neural regions to communicate with, and influence, one another (Goldman-Rakic and Schwartz, 1982; Johnson et al., 1989; Schwartz and Goldman-Rakic, 1984; Selemon and Goldman-Rakic, 1985, 1988).

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Section 2. Evidence that DL-PFC subserves cognitive abilities even during infancy

The A-not-B task has been used in scores of laboratories throughout the world to study cognitive development in infants since it was first introduced by Piaget in 1936 (see Piaget, 1954). Under the name "delayed response," an almost-identical task has been the classic paradigm for studying the functions of DL-PFC in macaques since it was first introduced for that purpose by Jacobsen (1935, 1936). In the A-not-B/delayed response task, the participant watches as a desired object is hidden in one of two hiding places that differ only in their left-right location, and then a few seconds later is allowed to reach to find that object. The participant must hold in mind over those few seconds where the object was hidden. Over trials, the participant must update his or her mental record to reflect where the reward was hidden last. When the participant reaches correctly, he or she is rewarded by being allowed to retrieve the desired object. In this manner, the behavior of reaching to that hiding location is reinforced, and hence the tendency to emit that response is strengthened. When the reward is then hidden at the other location, the participant must inhibit the natural tendency to repeat the rewarded response and instead respond according to the representation held in mind of where the reward was just hidden. Thus, the A-not-B task requires holding information in mind (where the reward was last hidden) and inhibition of a prepotent response tendency. By roughly 7½-8 months of age, infants reach correctly to the first hiding location with delays as long as 3 s. When the reward is then hidden at the other hiding place, however, infants err by going back to the first location (called the "A-not-B error"). As infants get older, they are able to succeed at longer and longer delays. Thus, for example, one sees the A-not-B error (correct at the first location, but incorrectly repeating that response on the reversal trials) at delays of 5 s in infants of 9 months and at delays of 7-8 s in infants of 10 months (Diamond, 1985; Diamond and Doar, 1989).

In the object retrieval task (Diamond, 1981, 1988, 1990a), nothing is hidden and there is no delay. A toy is placed within easy reach in a small, clear box, open on one side. Difficulties arise when the infant sees the toy through one of the closed sides of the box. Here, the infant must integrate seeing the toy through one side of the box with reaching through a different side. There is a strong pull to try to reach straight for the toy; that prepotent response must be inhibited when a detour reach is required. The following variables are manipulated: (1) which side of the box is open (top, front, left, or right), (2) distance of the toy from the box opening, (3) position of the box on the testing surface (e.g., near the front edge of table or far), (4) box size, and (5) box transparency. The experimental variables jointly determine through which side of the box the toy is seen. Initially, infants reach only at the side through which they are looking. They must look through the opening, and continue to do so to reach in and retrieve the toy. As they get older, the memory of having looked

through the opening is enough; infants can look through the opening, sit up, and reach in while looking through a closed side. Still older infants do not need to look along the line of reach at all. Infants progress through a well-demarcated series of 5 stages of performance on this task between 6 and 12 months of age (Diamond, 1981, 1988, 1990a).

Although the A-not-B/delayed response task and the object retrieval task appear to share few surface similarities, human infants improve on these tasks during the same age period (6-12 months; Diamond, 1988, 1991a,b) and so do infant rhesus macaque (1½-4 months; Diamond, 1988, 1991a,b; Diamond and Goldman-Rakic, 1986). Indeed, although there is considerable individual variation in the rate at which different infants improve on any of these tasks, the age at which a given infant reaches "phase 1B" on the object retrieval task is remarkably close to the age at which that same infant can first uncover a hidden object in the A-not-B/delayed response paradigm (Diamond, 1991a,b). Developmental improvements on both in human infants are related to the same changes in the EEG pattern over frontal leads and in frontal-parietal EEG coherence (*re:* A-not-B: Bell and Fox, 1992, 1997; Fox and Bell, 1990; *re:* object retrieval: Fox, personal communication). Both the A-not-B/delayed response task and the object retrieval task depend on DL-PFC and are sensitive to the level of dopamine there.

There is no behavioral task more firmly linked to DL-PFC than the A-not-B/delayed response task. Lesions that destroy DL-PFC disrupt performance of A-not-B and delayed response in adult macaques (Butters et al., 1969; Diamond and Goldman-Rakic, 1989; Goldman and Rosvold, 1970) and infant macaques (Diamond, 1990b; Diamond and Goldman-Rakic, 1986; Goldman, Rosvold, and Mishkin, 1970), while performance of other tasks such as delayed nonmatching to sample (Bachevalier and Mishkin, 1986) and visual discrimination (Goldman, Rosvold, and Mishkin, 1970) is unimpaired. Lesions of other brain regions do not affect A-not-B or delayed response performance at the same brief delays (e.g., medial temporal lobe [Diamond, Zola-Morgan, and Squire, 1989]; posterior parietal cortex [Bauer and Fuster, 1976; Diamond and Goldman-Rakic, 1989; Harlow et al., 1952]). Successful delayed response performance has been linked to dorsolateral PFC by techniques as varied as *reversible cooling* (where function is only temporarily disrupted, and an animal can serve as his own control; e.g., Bauer and Fuster, 1976; Fuster and Alexander, 1970), *single unit recording* (where the functions of individual neurons are studied in the intact brain; e.g., Fuster, 1973; Fuster and Alexander, 1971; Niki, 1974), and *2-deoxyglucose metabolic labeling* (where the functions of diverse neural regions are studied in the intact brain; Bugbee and Goldman-Rakic, 1981). Blocking dopamine receptors in DL-PFC produces deficits on the delayed response task as severe as when DL-PFC is removed altogether (Brozoski et al., 1979). Indeed, there is a precise dose-dependent relation between how large a dose of the dopamine antagonist is injected and performance on the delayed response task (Sawaguchi and Goldman-Rakic, 1991). Disruption of the prefrontal dopamine system by injections of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) also impairs performance on the task (Schneider and Kovelowski, 1990). Destruction of the dopamine neurons in the ventral tegmental area (VTA) that project to prefrontal cortex impairs performance on the task as well (Simon, Scatton, and LeMoal, 1980). Pharmacological activation of D2 dopamine receptors in normal human adults has been found to facilitate performance on the task (Luciana et al., 1992).

DL-PFC lesions in the macaque also disrupt performance on the object retrieval task (Diamond, 1990b; Diamond and Goldman-Rakic, 1985), while lesions of the medial temporal lobe (Diamond, Zola-Morgan, and Squire, 1989) or of posterior parietal cortex (Diamond and Goldman-Rakic, 1989) do not. MPTP injections, which reduce the level of dopamine in prefrontal cortex, also produce deficits on the task (e.g., Saint-Cyr et al., 1988; Schneider and Roeltgen, 1993; Taylor et al., 1990a,b). (MPTP also affects the level of dopamine in the striatum, but lesions of the striatum do not impair performance on the object retrieval task [Crofts et al., 1999].) 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 (15-75 mg), monkeys are impaired on the object retrieval and A-not-B/delayed response tasks (Schneider and Kovelowski, 1990; Taylor et al., 1990a,b), although they perform normally on other tasks such as visual discrimination.

Importantly, human infants, infant rhesus macaques, and infant and adult rhesus macaques with lesions of DL-PFC fail the A-not-B/delayed response task under the same conditions and in the same ways. The same is true for the object retrieval task. Thus, for example, on A-not-B, macaques with lesions of DL-PFC and human infants of 7½-9 months succeed when there is no delay (*macaques:* Bättig, Rosvold, and Mishkin, 1960; Goldman, Rosvold, and Mishkin, 1970; Harlow et al., 1952; *infants:* Gratch et al., 1974; Harris, 1973), succeed when allowed to circumvent the memory requirements by continuing to stare at or strain toward the correct well during the delay (*macaques:* Bättig, Rosvold, and Mishkin, 1960; Miles and Blomquist, 1960; Pinsker and French, 1967; *infants:* Cornell, 1979; Fox, Kagan, and Weiskopf, 1979), succeed if a landmark reliably indicates where the reward is located (*macaques:* Pohl, 1973; *infants:* Butterworth, Jarrett, and Hicks, 1982), fail even at brief delays of only 2-5 s (*macaques:* Diamond and Goldman-Rakic, 1989; Goldman and Rosvold, 1970; *infants:* Diamond, 1985; Diamond and Doar, 1989; Fox, Kagan, and Weiskopf, 1979; Gratch and Landers, 1971), and fail if the hiding places differ either in left-right or up-down location (*macaques:* Fuster, 1980; Goldman, Rosvold, and Mishkin, 1970; *infants:* Butterworth, 1976; Gratch and Landers, 1971). See figure 29.2 for details.

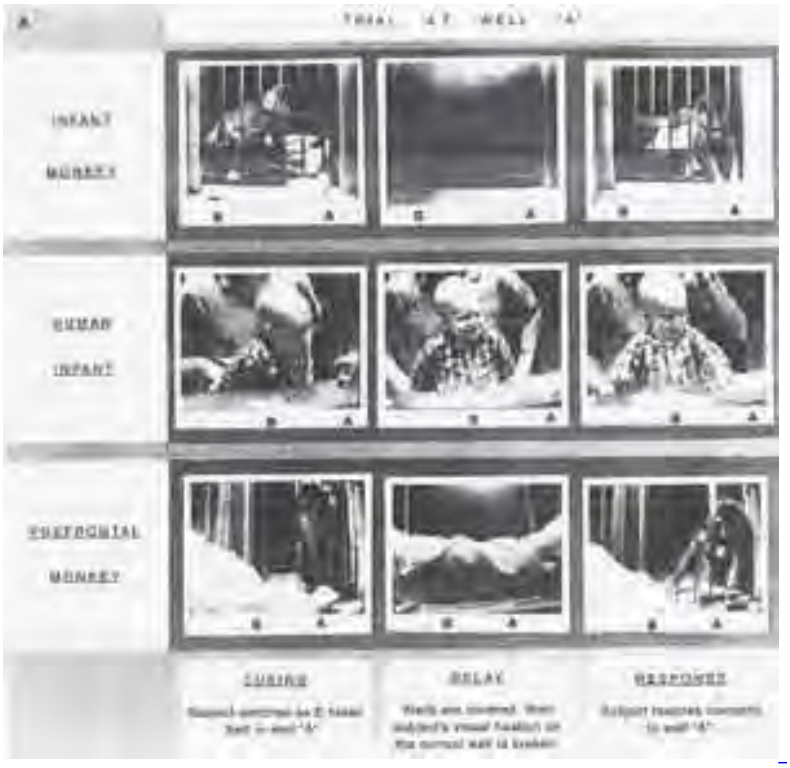

Figure 29.2. Illustration of a 1½-month-old infant rhesus macaque, 8-month-old human infant, and an adult rhesus macaque in whom dorsolateral prefrontal cortex had been removed bilaterally performing the A-not-B/delayed response task. All are correct at the first hiding place (**A**).*(continued)*

Figure 29.2 ***(Continued).***. After 2 trials, there is a switch and the reward is hidden at the second hiding place (**B**). Although they all watch the hiding at B, and although the delay at B is no longer than at A, they all err by reaching back to A. Thiserror is called the A-not-B error because they are correct on the A trials, but not on the B trials; they reach to A, not B. (Reprinted with permission from Diamond, 1990a,b.)

Note that most of these manipulations indicate that the presence of the delay is critical, since even very young infants and prefrontally-lesioned macaques perform well when there is no delay or when the requirements of the delay can be circumvented. This suggests that the ability to hold in mind the information on where the reward was last hidden (this might be termed "sustained attention" or the information-maintenance component of "working memory" [Baddeley, 1992]) is critical to success on this task. The information that must be held in mind is relational (Was the reward hidden on the right or the left most recently? "Left" is only left in relation to right and, similarly, "recent" implies a before-and-after relation.) There is also a characteristic pattern to the errors made by infants and by prefrontally-lesioned macaques on the A-not-B task: Their errors tend to be confined to the reversal trials and to the trials immediately following a reversal error when the reward continues to be hidden at the new location (Diamond, 1985, 1990a, 1991a,b). If the only source of error on the task were failure to keep the critical information in mind, one would expect errors to be random; but they are not (Diamond, Cruttenden, and Neiderman, 1994). The nonrandom pattern of errors, and the fact that participants occasionally look at the correct location (as if they remember that the reward is there) while at the same time reaching back to the previously correct location (Diamond, 1990a, 1991a; see also Hofstadter and Reznick, 1996), suggests that success on the task also requires resisting, or inhibiting, the tendency to repeat the previous response. (I have suggested that there is a predisposition to repeat the previous response because it had been rewarded. Smith and colleagues &1999; suggest that there is a predisposition to repeat the previous response simply because the response was made before [not because of reinforcement], just as it is easier for neurons in visual cortex to process a visual stimulus if they have previously processed that visual stimulus. Either account of the source of the predisposition works equally well for my theoretical position. The important

point is that there is a tendency to repeat the previous response; the source of that predisposition is unimportant for my argument.)

A few errors can be elicited simply by taxing how long information must be held in mind even when no inhibition is required, such as by using a long delay at the first hiding location (Sophian and Wellman, 1983). Similarly, a few errors can be elicited simply by taxing inhibitory control even when the participant does not have to remember where the reward was hidden; for example, a few infants err on the reversal trial even when the covers are transparent (Butterworth, 1977; Willatts, 1985). However, the overwhelming majority of errors occur when participants must both hold information in mind and also exercise inhibitory control (i.e., on reversal trials when the covers are opaque and a delay is imposed).1

Similar close parallels in the parameters determining success or failure, and in the characteristics of performance, hold for the object retrieval task (*infants:* Diamond, 1981; 1990b, 1991a; *macaques with lesions of DL-PFC:* Diamond, 1990b; 1991a; Diamond and Goldman-Rakic, 1985; *MPTP-treated macaques:* Saint-Cyr et al., 1988; Schneider and Roeltgen, 1993; Taylor et al., 1990a,b). Human infants of 7½-9 months, rhesus macaques with lesions of DL-PFC, and macaques treated with MPTP all succeed on the object retrieval task when they are looking through the open side of the box. They fail when they are looking through a closed side, and they fail by trying to reach straight through the transparent barrier instead of detouring around it. Human infants of 7½-9 months, rhesus macaques with lesions of DL-PFC, and macaques treated with MPTP perform better when the box is opaque than when the box is transparent. They lean over to look in the box opening when the left or right side of the box is open and recruit the contralateral hand to reach in the opening (see figure 29.3) and show that "awkward reach" on both the left and right sides of the box.

Figure 29.3. Illustration of a 2-month-old infant rhesus macaque, 9-month-old human infant, and an adult rhesus macaque in whom dorsolateral prefrontal cortex had been removed bilaterally performing the object retrieval task. They lean and look in the side opening of the transparent box, and then while continuing to look through the opening, recruit the contralateral hand to reach in and retrieve the reward. This is seen on both sides of the box and does not reflect a hand preference. Because of its appearance, the recruitment of the contralateral arm is dubbed the "awkward reach. (Reprinted with permission from Diamond, 1990a,b.)"

This pattern of performance highlights the importance, for success on the object retrieval task, of being able to inhibit the strong tendency to reach straight in the side of the box through which one is looking. Behaviors such as the "awkward reach" also highlight the importance of holding the location of the box opening in mind when looking at the reward and holding the location of the reward in mind when looking at the box opening, and of integrating the two pieces of information. Focusing exclusively on the reward or the box will not work for this task; both must be taken into account. Reaching through the opening when looking through a closed side requires integrating in one's mind looking at the reward along one route with reaching for the reward along a completely different route. Infants of 8½-9 months and prefrontally-lesioned macaques are only able to

succeed when the left or right side of the transparent box is open by simplifying the task. They lean over to look in the opening, hence lining up the opening and the reward so that they can see both at once and so that their line of sight is the same as the line along which they will reach.

In sum, human infants of 7½-9 months, infant macaques of 1½-2½ months, adult macaques with bilateral removals of DL-PFC, infant macaques of 5 months in whom DL-PFC was removed at 4 months, and adult macaques who have received MPTP injections to disrupt the prefrontal dopamine system fail the A-not-B/delayed response and object retrieval tasks under the same conditions and in the same ways (table 29.1). This does not prove that maturational changes in DL-PFC during infancy contribute to the emergence of success on these tasks during infancy, but this body of work makes that hypothesis plausible.

Table 29.1 : Performance of human infants, infant rhesus monkeys, and adult rhesus monkeys with selective ablations on the same three tasks

	A-not-B	**Delayed Response**	**Object Retrieval**
Human infants show a clear developmental progression from 7½ to 12 months	Diamond, 1985	Diamond and Doar, 1989	Diamond, 1988
Adult monkeys with lesions of prefrontal cortex fail	Diamond and Goldman-Rakic, 1989	Diamond and Goldman-Rakic, 1989	Diamond and Goldman-Rakic, 1985
Adult monkeys with lesions of parietal cortex succeed	Diamond and Goldman-Rakic, 1989	Diamond and Goldman-Rakic, 1989	Diamond and Goldman-Rakic, 1985
Adult monkeys with lesions of the hippocampal formation succeed	Diamond, Zola-Morgan, and Squire, 1989	Squire and Zola-Morgan, 1983	Diamond, Zola-Morgan, and Squire, 1989
Infant monkeys show a clear developmental progression from 1½ to 4 months	Diamond and Goldman-Rakic, 1986	Diamond and Goldman-Rakic, 1986	Diamond and Goldman-Rakic, 1986
5-month-old infant monkeys who received lesions of prefrontal cortex at 4 months fail	Diamond and Goldman-Rakic, 1986	Diamond and Goldman-Rakic, 1986	
Disruption of the prefrontal dopamine system impairs performance in monkeys		Taylor et al., 1990a,b; Schneider and Roeltgen, 1993	Schneider and Kovelowski, 1990; Sawaguchi and Goldman-Rakic, 1991

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Section 3. Evidence of improvement in the cognitive abilities that depend on DL-PFC during early childhood

DL-PFC continues to mature until early adulthood. Marked improvements on tasks that require working memory plus inhibition (tasks thought to require the functions of DL-PFC) are seen in children between 3 and 6 years of age. At 3 years of age, one can see errors reminiscent of the A-not-B error seen in infants and in prefrontally-lesioned macaques, but with a slightly more difficult task. On this task, children who are 3 years old can sort cards correctly by the first criterion they are given (either color or shape; Kirkham, Cruess, and Diamond, submitted; Zelazo, Frye, and Rapus, 1996; Zelazo and Reznick, 1991; Zelazo, Reznick, and Piñon, 1995), just as infants of 7½-9 months and prefrontally-lesioned macaques are correct at the first hiding place, and just as adults with prefrontal cortex damage are correct at sorting cards according to the first criterion (Wisconsin Card Sort test: Drewe, 1974; Milner, 1963, 1964). Three-year-old children err when correct performance demands switching to a new criterion, i.e., when cards previously sorted by color (or shape) must now be sorted according to the other criterion (shape or color), just as infants of 7½-9 months and prefrontally lesioned macaques err when required to switch and search for the reward at the other location, and just as adults with prefrontal cortex damage err when required to switch to a new sorting criterion.

Although 3-year-old children fail to sort by the new sorting criterion (sticking steadfastly to the previously correct criterion), they can correctly state the new sorting criterion (Kirkham et al., submitted; Zelazo, Frye, and Rapus, 1996). Similarly, infants of 7½-9 months can sometimes tell you with their eyes that they know the reward is in the new hiding place even as they persist in reaching back to the previously correct location (Diamond, 1990a, 1991a,b; Hofstadter and Reznick, 1996), and patients with prefrontal cortex damage can sometimes tell you correctly the new sorting criterion even as they persist in sorting by the previously correct criterion (Luria and Homskaya, 1964; Milner, 1963, 1964). When there are only two sorting criteria (color and shape) and only two values for each criterion (e.g., red/blue, truck/star) children are able to succeed at the card sorting task by 4-5 years of age. If the task is made more complicated, by, for example, adding a third sorting dimension, then children cannot succeed until they are 5-6 years old. The problem for the children appears to be in relating two or more dimensions to a single stimulus (thinking of a stimulus as either red or blue and also thinking about the same stimulus as either a truck or a star) and in inhibiting the tendency to repeat their previously correct way of categorizing the stimulus.

Similarly, children 3 years old have great difficulty with "appearance-reality" tasks (Flavell, 1986, 1993) where,

for example, they are presented with a sponge that looks like a rock. Three-year-olds typically report, for example, that it looks like a rock and really is a rock, whereas a child of 4-5 years correctly answers that it looks like a rock but really is a sponge. The problem for the younger children is in relating two conflicting identities to the same object (Rice et al., 1997) and in inhibiting the response that matches their perception (thus manipulations that reduce the perceptual salience, by removing the object during questioning, find significantly better performance by children of 3-4 years [e.g., Heberle, Clune, and Kelly, 1999]). "Theory of mind" and "false belief" tasks are other tasks that require holding two things in mind about the same situation (the true state of affairs and the false belief of another person) and inhibiting the impulse to give the veridical answer. For example, the child must keep in mind where the hidden object is now and where another person saw it placed before, and the child must inhibit the inclination to say where the object really is and instead say where the other person would think it is, even though the child knows that answer to be "wrong" because the object is not there now. Here, as well, manipulations that reduce the perceptual salience of the true state of affairs aid children of 3-4 years (Fritz, 1991; Zaitchik, 1991). Carlson, Moses, and Hix (1998) reasoned that pointing veridically to true locations and identities is likely to be a well-practiced and reinforced response in young children, and that children of 3-4 years have trouble inhibiting that tendency when they should point to the false location, as is required on false belief tasks. Carlson, Moses, and Hix (1998) found that when they gave children a novel response by which to indicate the false location, children of 3-4 years performed much better on the false belief task.

Many of the advances of Piaget's "preoperational" child of 5-7 years over a child of 3-4 years, who is in the stage of "concrete operations," similarly reflect the development of the ability to hold more than one thing in mind and to inhibit the strongest response tendency of the moment. Evidence that children 3 or 4 years old have difficulty keeping two things in mind at the same time, or that they tend to focus on only one aspect of a problem, can be seen in (1) their failure on tests of liquid conservation (they fail to attend to both height and width, attending only to height), (2) their difficulty on tests of perspective-taking where they must mentally manipulate a scene to say what it would look like from another perspective and must inhibit the strong tendency to give the most salient response (i.e., their current perspective), (3) their difficulty in comparing an old idea with a new one and hence seeing the contradiction, and (4) their difficulty in working through a two-step problem without losing track of what they are doing. By 5 or 6 years of age, children are capable of doing all of these things. Certainly, part of the difficulty posed by Piaget's liquid conservation task (Piaget and Inhelder, 1941) is the salience of the visual perception that the tall, thin container appears to have more liquid in it. Thus, if an opaque screen is placed between the child and the containers before the child answers, younger children are much more likely to answer correctly (Bruner, 1964).

Many investigators have similarly found evidence of improved ability to exercise inhibitory control over one's behavior between 3 and 6 years of age, especially when children must hold two things in mind and relate them to one another. For example, in the delay of gratification paradigm, when faced with the choice of a smaller, immediate reward or a later, larger reward, children of 3-4 years are unable to inhibit going for the immediate reward although they would prefer the larger one. By 5-6 years of age, children are much better at waiting for the bigger reward (Mischel and Mischel, 1983). Similarly, on the windows task, where children are rewarded for pointing to a box that is visibly empty, and are not rewarded for pointing to a box in which they can see candy, 3-year-olds fail to inhibit the tendency to point to the baited box (Russell et al., 1991). Children 3-4 years of age also tend to fail go/no-go tasks because they cannot inhibit responding. They appear to understand and remember the task instructions (e.g., they can verbalize the instructions), but cannot get themselves to act accordingly. By 5-6 years, they succeed on these tasks (Bell and Livesey, 1985; Livesey and Morgan, 1991).

Difficulty in holding two things in mind can also be seen in persons with frontal cortex damage. For example, they can have difficulty when asked to do two things (such as clean the windshield and change the oil). They are inclined to focus on only one aspect of a story, instead of on the story as a whole. Indeed, Goldstein (1936, 1944) considered the fundamental disorder caused by damage to the frontal lobe to be an "inability to grasp the entirety of a complex situation." Patients with frontal cortex damage also have a well-documented difficulty

inhibiting a strong response tendency. For example, they are impaired on the Stroop task, which requires inhibiting the normal tendency to say the word when one is reading; one is instructed instead to say the color of the ink in which the word is printed (Perret, 1974; Richer et al., 1993). They fail a perspective-taking task much like Piaget's, and make the same error as do the younger children (they give as their answer their current perspective, when the current answer is the scene as viewed from a different perspective; Price et al., 1990).

We have followed the developmental improvement in these abilities between 3½ and 7 years of age using three tasks, the "day-night Stroop-like" task (Gerstadt, Hong, and Diamond, 1994), the tapping task (Diamond and Taylor, 1996), and the three pegs task (Diamond et al., 1997). These three tasks were also used in our research on the role of dopamine in prefrontal cortex function early in life in treated PKU children and so will be described briefly here.

For the day-night task, children 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 inhibit the tendency to say what the stimuli really represent; instead they must say the opposite. Children of 3½-4½ years find the task terribly difficult; by 6-7 years of age, the task is trivially easy. Children younger than 6 years of age err often, whereas children of 6-7 years are correct on roughly 90% of the trials (see figure 29.4). Children of 3½ and 4 years show long response latencies on the task (roughly 2 s); older children take roughly half as long (1 s). The age-related increase in the percentage of correct responses is relatively continuous from 3½ to 7 years of age, but the decrease in speed of responding occurs primarily between 3½ and 4½ years. Passler, Isaac, and Hynd (1985) tested children on a similar, though slightly easier, variant of this task, which required children to recognize the correct answer, whereas our task requires that they recall the correct answer. They found that children of 6 years were performing at ceiling on their task, which is consistent with the excellent performance that we found at 6-7 years of age.

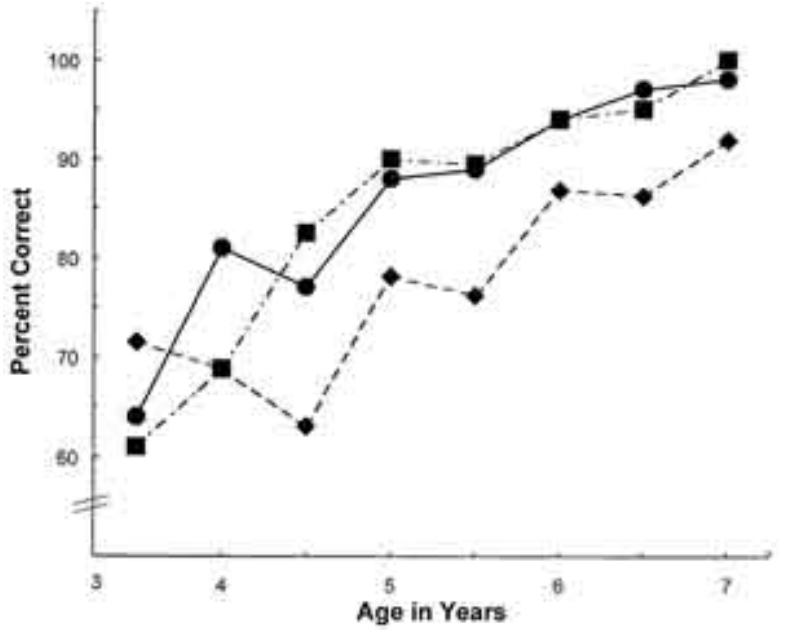


Figure 29.4. Performance of children, 3½ through 7 years of age, on the day-night, tapping, and three pegs task. Note the close parallels in performance on all three tasks throughout this age range.

To test whether the requirement to remember two rules alone is sufficient to cause the younger children difficulty, we tested a version of our day-night test where each card contained one of two abstract designs (Gerstadt, Hong, and Diamond, 1994). Children were instructed to say "day" to one design and "night" to the other. Here the children were still required to hold two rules in mind, but they did not also have to inhibit the tendency to say what the stimuli really represented because the stimuli were abstract designs. Even the

youngest children performed superbly here. Thus, the requirement to learn and remember two rules is not in itself sufficient to account for the poor performance of the younger children on the day-night task.

Moreover, children's difficulty with the task depends critically on the correct responses being semantically related to the responses that must be inhibited. When we used the same white/sun and black/moon cards, but instructed the children to say "dog" to one and "pig" to the other, even the youngest children again performed well (Diamond, Kirkham, and Amso, submitted). The task of holding two rules in mind and inhibiting one's natural inclination is sufficiently hard for the younger children that they need a long time to formulate their answers in order to respond correctly. Although we gave children unlimited time, they tended to speed up their responses over the 16 test trials, and the accuracy of the youngest children correspondingly fell. When we made the children wait to respond, by singing a brief ditty to them on each trial after the stimulus was presented, the younger children were able to perform well, even though the period before their response was filled with potentially interfering verbal stimulation (Diamond et al., submitted). It is not simply that slowing down the testing helped because when the children were made to wait before the start of each trial, they performed poorly. The day-night task is sufficiently difficult for young children that it takes them several seconds to compute the answer. Often they do not take the needed time; when forced to take extra time they can perform well.

Luria's tapping test (Luria, 1966) also requires (1) remembering two rules and (2) inhibiting the response you were inclined to make, making the opposite response instead. Here, one needs to remember the rules, "Tap once when the experimenter taps twice, and tap twice when the experimenter taps once," and one needs to inhibit the tendency to mimic what the experimenter does. Children improve on this task over the same age period as they do on the day-night task (see figure 29.4). Over the period of 3½-7 years, children improve in both speed and accuracy on the tapping task, with most of the improvement occurring by the age of 6 (Becker, Isaac, and Hynd, 1987; Diamond and Taylor, 1996; Passler, Isaac, and Hynd, 1985).

Adults with large frontal lobe lesions fail this same tapping task (Luria, 1966). They have similar problems when instructed to raise a finger in response to the experimenter's making a fist and to make a fist in response to the experimenter's raising a finger (Luria, 1966). The most common error by young children is always tapping once, or always tapping twice, regardless of what the experimenter does. It may be that the young children are able to keep in mind only one of the two rules. Or, it may be that they lack the ability to switch flexibly between the two rules, although they remember both. (It cannot be because they do not understand what they should do, because no child is tested who does not demonstrate understanding during training of what he or she should do when the experimenter taps once or twice.) This error is reminiscent of a characteristic error Luria (1966) observed in his patients. For example, when asked to draw, alternately, a circle and a cross, patients with extensive frontal lobe damage start out performing correctly (as do even the youngest children), but the patients soon deteriorate into following only one of the rules (i.e., drawing only circles or only crosses).

Other errors by the children seem more clearly to reflect inadequate inhibitory control. One common error among the younger children is to be unable to resist tapping many times, instead of just once or twice. Again, this error is reminiscent of behavior Luria noted in patients with excessive damage to the frontal lobe: "[When asked] to tap three times or to squeeze the doctor's hand three times … although the patient retains the verbal instruction and repeats it correctly, he taps many times or squeezes the doctor's hand five, six, or more times instead of three" (Luria, 1966: p. 252). Another error made by the younger children is to match what the experimenter does, instead of doing the reverse. Luria (1966; Luria and Homskaya, 1964) has extensively described such "echopractic" errors in frontal lobe patients. Indeed, on the tapping task itself, Luria found that although the patients could correctly comply with the instructions for a short while (like the younger children), they very soon began to imitate the experimenter's movements. Luria also found that the frontal patients could verbalize the rules even as they failed to act in accord with them.

Since Luria introduced the tapping test more than 30 years ago, it has been widely used in neurological

assessments of frontal lobe damage in patients. However, much of the work with this test comes from old studies with patients with massive damage. It is not clear from such studies which regions within frontal cortex are critical for the task, or even whether the cortex, rather than the basal ganglia, is the critical site.

For the three pegs task (Balamore and Wozniak, 1984) a child is shown a pegboard containing three pegs arranged in the order red, yellow, green. The child is asked to tap the pegs in the order red, green, yellow. This task requires remembering a three-item sequence and inhibiting the tendency to tap the pegs in their spatial order. The tapping and day-night tasks are more similar to each other than is the three pegs task, but it, too, requires acting counter to one's initial tendency on the basis of information held in mind. Children show developmental improvements on the three pegs task during the same age period that they are improving on the tapping and day-night tasks (see figure 29.4; Diamond et al., 1997), and performance on the three tasks is correlated (tapping and three pegs: *r*&144; = .53, *p* = .0001; tapping and day-night: *r*&144; = .35, *p* = .0001; day-night and three pegs: *r*&151; = .20, *p* = .01; Diamond et al., 1997).

Clearly, improvement in the performance of tasks requiring memory plus inhibition occurs between 3 and 6 years of age. Perhaps that improvement is made possible, in part, by maturational changes in DL-PFC, although that remains to be demonstrated. Perhaps one of those maturational changes in DL-PFC is in its dopamine system, although little is known about what is happening in the dopamine system in prefrontal cortex during this period. To begin to look at the role of the dopamine innervation of DL-PFC in helping to subserve cognitive functions during infancy and early childhood, we have been studying children who, we had good reason to believe, have reduced levels of dopamine in prefrontal cortex but otherwise remarkably normal brains -- children treated early and continuously for phenylketonuria (PKU), whose phenylalanine (Phe) levels are 3-5 times normal (6-10 mg/dL [360-600 mmol/L]).

At the time we began this work, there were almost no data on the role of dopamine in prefrontal function in humans, and no data on the role of dopamine in aiding prefrontal function early in development in any species. As an initial way of beginning to look at the role of dopamine in prefrontal cortex function in humans early in development, we conducted a large, longitudinal study of children treated early and continuously for PKU (Diamond et al., 1997). We complemented that with work with an animal model of early-and continuously-treated PKU, where we could investigate the underlying biological mechanism (Diamond et al., 1994). Additionally, we sought to obtain converging evidence from a study of visual contrast sensitivity in children treated early and continuously for PKU (Diamond and Herzberg, 1996), where we postulated that the same underlying mechanism was at work.

Chapter 29. A Model System for Studying the Role of Dopamine in the Prefrontal Cortex during Early Development in Humans: Early and Continuously Treated Phenylketonuria

Section 4. The reasoning and evidence leading to the hypothesis of a selective deficit in dopamine in prefrontal cortex in children treated early and continuously for PKU

Phenylketonuria (PKU) Defined The core problem in PKU is a mutation of the gene on chromosome 12 (12q22-12q24.1) that codes for the enzyme phenylalanine hydroxylase. Phenylalanine hydroxylase is essential for hydroxylating (i.e., converting) the amino acid phenylalanine (Phe) into the amino acid tyrosine (Tyr) (DiLella et al., 1986; Lidsky et al., 1985; Woo et al., 1983; see figure 29.5). In the roughly 1 in every 10,000 people born with PKU, phenylalanine hydroxylase activity is either absent or markedly reduced. Hence, PKU is a member of the class of disorders called "inborn [i.e., genetic] errors of metabolism." In the case of PKU, the error is in the metabolism of Phe.

Figure 29.5. Diagram illustrating the reasoning leading to the hypothesis that children treated early and continuously for PKU, whose blood Phe levels are 6-10 mg/dL, would have a selective decrease of dopamine in prefrontal cortex and a selective deficit in the cognitive abilities dependent on prefrontal cortex.

Since little, if any, Phe is metabolized, Phe levels in the bloodstream reach dangerously high levels. Indeed, when PKU is untreated, levels of Phe in the bloodstream rise to well over 10 times normal (>20 mg/dL [>1200 mmol/L]). Since little or no Tyr is produced from Phe, the level of Tyr in the bloodstream is low (Nord, McCabe, and McCabe, 1988). (Tyr levels would be still lower were it not for the availability of Tyr directly through the foods we eat.) This imbalance in blood levels of Phe and Tyr, if not corrected early, causes widespread brain damage and severe mental retardation (Cowie, 1971; Hsia, 1967; Koch et al., 1982; Krause et al., 1985; Tourian and Sidbury, 1978). Indeed, PKU is the most common biochemical cause of mental retardation. The primary cause of the widespread brain damage is thought to be the toxic effects of grossly elevated levels of Phe in the brain.

The Treatment for PKU: A Diet Low in Phenylalanine The treatment for PKU consists of a diet low in Phe. Since Phe is a constituent of protein, the low-Phe diet severely restricts the intake of milk and milk products (such as ice cream, butter, and cheese), and all meat and fish. When PKU is treated early and continuously by a diet low in Phe, gross brain damage and severe mental retardation are averted (Bickel, Hudson, and Woolf, 1971; Holtzman et al., 1986). Note that here is an example of how a behavioral change (changing what you eat) can profoundly affect your biochemistry and your brain.

Section 4. Limitations of the diet: Why problems might still exist when PKU is treated

The low-Phe diet rarely results in fully normal levels of Phe or Tyr. This is because the need to minimize Phe intake must be balanced with the need for protein. Eliminating all Phe from the diet would require eliminating all protein. Outside of protein, Phe is not present in any naturally occurring food. The human body needs to ingest protein; moreover, the body needs a small quantity of Phe to produce its own protein. Hence, because persons with PKU need protein, blood Phe levels remain somewhat elevated in a person with PKU, even with conscientious adherence to the recommended diet, as an inevitable consequence of consuming even a small amount of protein. The advice of the U.S. National Collaborative Study of Treated PKU has been that as long as Phe levels in the bloodstream do not exceed 5 times normal (10 mg/dL [600 mmol/L]), persons with PKU are considered to be under adequate control (Koch and Wenz, 1987; Williamson et al., 1981). The diet has historically done little to correct the reduction in Tyr, although recently the companies that manufacture the "formula" that persons with PKU drink instead of milk have added additional Tyr to their formulas. Still, Tyr levels are below normal in most children treated for PKU.

Section 4. Even with a low-Phe diet, there are moderate elevations in the Phe:Tyr ratio in the bloodstream and deficits in certain cognitive abilities

For PKU children, following a dietary regimen of reduced Phe intake and mild Tyr supplementation results in moderately elevated levels of Phe and moderately reduced levels of Tyr in their bloodstreams. (Were they not following this dietary regimen, the elevation in their Phe:Tyr ratio be huge, rather than moderate, and they would likely incur brain damage and become severely cognitively impaired.)

Given that the low-Phe diet does not return Phe and Tyr levels to normal, one can see how the possibility for problems could still exist. Indeed, a number of studies have found significant cognitive deficits in PKU children on the low-Phe diet (Dobson et al., 1976; Faust, Libon, and Pueschel, 1986; Pennington et al., 1985; Smith and Beasley, 1989; Williamson et al., 1981). 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 -- lower than the mean score of 100 of their same-age peers, though still within the normal range (Berry et al., 1979; Dobson et al., 1976; Williamson et al, 1981).

In the 1980s, studies reported problems in holding information in mind, problem-solving, and "executive functions" in children with PKU on the low-Phe diet (Brunner, Berch, and Berry, 1987; Faust, Libon, and Pueschel, 1986; Krause et al., 1985; Pennington et al., 1985; Smith and Beasley, 1989). These problems are reminiscent of the deficits seen after damage to prefrontal cortex, and that similarity did not escape the notice of others (see, especially, Welsh et al., 1990). Indeed, damage to prefrontal cortex typically results in IQs lowered to the 80s or 90s (Stuss and Benson, 1986, 1987) -- the same range one sees in children treated for PKU. The impact of these findings was muted, however, because people were not sure how to make sense of them. No one had suggested a mechanism whereby the cognitive functions dependent on prefrontal cortex might be impaired in treated PKU children, while other cognitive functions appeared normal. Actually, the facts needed for understanding the underlying mechanism were already available. However, the neuroscientists

working on the prefrontal dopamine system in the rat and the cognitive neuropsychologists and pediatricians working with PKU children did not know of one another's work, so no one had put the facts together.

Proposed Mechanism: How a Modest Imbalance in the Levels of Phe and Tyr in the Bloodstream Might Produce Deficits Specific to the Cognitive Abilities Dependent on Prefrontal Cortex Children treated early and continuously for PKU have a moderate increase in the ratio of one amino acid (Phe) to another (Tyr) in their bloodstreams (Tyr is the precursor of dopamine.) We predicted that when the imbalance is moderate it would selectively affect the dopamine projection to prefrontal cortex.

Why should a modest imbalance in the levels of Phe and tyrosine in the bloodstream produce deficits in the cognitive abilities dependent on DL-PFC? And why should deficits be confined to that neural system and not extend to other functions of the brain?

Section 4. Modest reduction in the level of Tyr reaching the brain

The modest elevation in Phe relative to Tyr in the bloodstream results in a modest reduction in the level of Tyr reaching the brain. This is because Phe and Tyr compete for the same limited supply of proteins to transport them across the blood-brain barrier (Chirigos, Greengard, and Udenfriend, 1960; Oldendorf, 1973; Pardridge, 1977). Indeed, those transport proteins have a higher binding affinity for Phe than for Tyr (Miller et al., 1985; Pardridge and Oldendorf 1977). Thus, elevations in blood levels of Phe relative to Tyr place Tyr at a competitive disadvantage in finding transport into the brain. Because the ratio of Phe to Tyr in the bloodstream is only modestly increased in those on dietary treatment for PKU, the decrease in the amount of Tyr reaching the brain is correspondingly modest. In this way, the moderate plasma imbalance in Phe:Tyr results in modestly reduced Tyr levels in the brain.

Section 4. The dopamine neurons that project to prefrontal cortex are unusually sensitive to modest reductions

The special properties of the dopamine projection to prefrontal cortex make prefrontal cortex more sensitive to small changes in the level of Tyr than other brain regions. The brain needs Tyr to make dopamine (see figure 29.6). Indeed, the hydroxylation of Tyr is the rate-limiting step in the synthesis of dopamine. Most dopamine systems in the brain are unaffected by small decreases in the amount of available Tyr. Not so prefrontal cortex. The dopamine neurons that project to prefrontal cortex are unusual in that they have a higher firing rate and higher rate of dopamine turnover than other dopamine neurons (Bannon, Bunney, and Roth, 1981; Roth, 1984; Thierry et al., 1977). These unusual properties of the prefrontally projecting dopamine neurons in the ventral tegmental area (VTA) make prefrontal cortex acutely sensitive to even a modest change in the supply of Tyr (Tam et al., 1990; Wurtman et al., 1974). Reductions in the availability of Tyr too small to have much effect on other dopamine systems in other neural regions (such as the striatum) have been shown to profoundly reduce dopamine levels in prefrontal cortex (Bradberry et al., 1989).

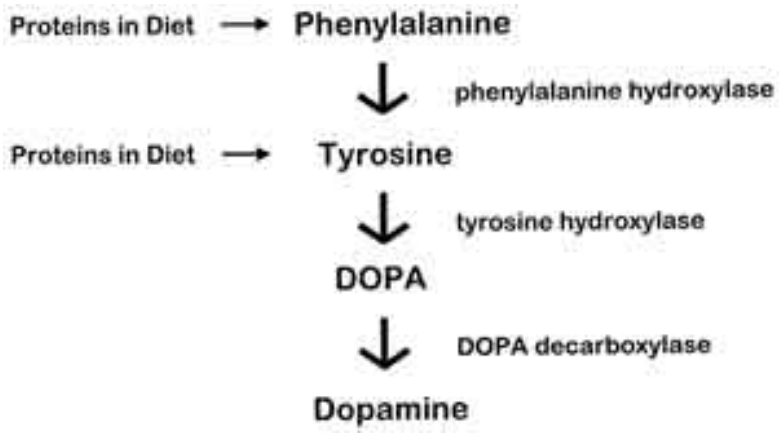


Figure 29.6. Diagram illustrating the mechanism by which the neurotransmitter dopamine is produced in the body. Persons with PKU either lack the enzyme phenylalanine hydroxylase or have it in an inactive form. Note that the body acquires tyrosine via two routes, the hydroxylation of Phe and directly through diet. The hydroxylation of tyrosine is the rate-limiting step in the production of dopamine.

Section 4. Reducing the level of dopamine in prefrontal cortex produces deficits in the cognitive abilities dependent on prefrontal cortex

As mentioned earlier, selectively depleting DL-PFC of dopamine can produce cognitive deficits as severe as those found when DL-PFC is removed altogether (Brozoski et al., 1979). Local injection of dopamine antagonists into DL-PFC impairs performance in a precise, dose-dependent manner (Sawaguchi and Goldman-Rakic, 1991). Destruction of the dopamine neurons in the VTA that project to prefrontal cortex also impairs performance on tasks dependent on DL-PFC (Simon, Scatton, and LeMoal, 1980). Similarly, injections of MPTP that disrupt the dopamine projection to prefrontal cortex, but are of sufficiently low dose that motor deficits are avoided, impair performance on the A-not-B/delayed response and object retrieval tasks (Schneider and Kovelowski, 1990; Taylor et al., 1990a,b).

Summary of the Reasoning Leading to the Prefrontal Dopamine Hypothesis in Treated PKU For these reasons it seemed plausible that the moderate imbalance in the Phe:Tyr ratio in the bloodstreams of children treated early and continuously for PKU might well result in deficits in the cognitive abilities dependent on prefrontal cortex (because of the unusual vulnerability of the dopamine projection to prefrontal cortex to a moderate reduction in the amount of available tyrosine) without significantly affecting other brain regions or other cognitive abilities. Hence, we hypothesized that here was a mechanism by which the modest elevation in the Phe:Tyr ratio in the bloodstream of some children treated for PKU, which results in moderate reductions in the level of Tyr reaching the brain, might selectively affect prefrontal cortex (by modestly decreasing the level of Tyr reaching the brain).

Chapter 29. A Model System for Studying the Role of Dopamine in the Prefrontal Cortex during Early Development in Humans: Early and Continuously Treated Phenylketonuria

Section 5. A 4-year longitudinal study of children treated early and continuously for PKU

To investigate our prediction that children treated early and continuously for PKU have selective deficits in the cognitive functions dependent on prefrontal cortex, we tested 148 children longitudinally and 364 children cross-sectionally (Diamond et al., 1997). Included were children treated early and continuously for PKU, siblings of the PKU children, matched controls, and children from the general population. Children from the general population were tested cross-sectionally; all other groups were tested longitudinally.

If a PKU child starts dietary treatment too late or discontinues it, the very high plasma Phe levels during those off-treatment periods can cause permanent, widespread brain damage. Therefore, we were careful to include in this study only those PKU children who started dietary treatment soon after birth (80% began the low-Phe diet within 14 days of age; all had been placed on a low-Phe diet within 1 month of birth) and who had been continuously maintained on the diet thereafter (i.e., children with early- and continuously-treated PKU).

Because no control group is perfect, we included three different control groups. Siblings provide a partial control for family background and genetic make-up. However, they are an imperfect control group because, except for twins, they are not matched on age or birth order, and often not on gender or health status. Therefore, we also studied children who, though unrelated to our PKU participants, matched them on a host of background and health variables -- gender, gestational age at birth, birthweight, ethnic background, religion, age at beginning of testing, community of residence, childcare arrangements, number of siblings, and birth order, as well as the age, level of education, and occupational status of each parent. Selecting control subjects by matching on a list of variables is imperfect as well, however, because the children thus selected may not match on other critical variables that one had not considered. Therefore, we complemented the inclusion of siblings and matched controls with a normative sample of children from the general population. With this last group we attempted to get an estimate of the "normal" developmental progression on each of our tasks.

All children studied had normal birthweights, IQs within the normal range, and no known learning disabilities or serious medical problems. Almost all were full-term (100% of the children tested cross-sectionally; 96% tested longitudinally). PKU is found primarily among Caucasians, so almost all of our participants were Caucasian (95% of the children tested cross-sectionally; 93% tested longitudinally). Because of the large age range studied (6 months to 7 years), three different batteries of cognitive neuropsychological measures were used --

one for infants (6-12 months), one for toddlers (15-30 months), and one for young children (3½-7 years). A total of 19 cognitive neuropsychological measures were administered (see table 29.2). Infants were tested every month, toddlers every 3 months, and young children every 6 months. At each age, each child was tested on multiple tasks linked to prefrontal cortex and on multiple control tasks not linked to prefrontal cortex.

Table 29.2 : List of tasks

Tasks Used with Infants (ages 6-12 months)	
Tests of working memory + inhibitory control, dependent on DL-PFC	
A-not-B	A hiding task requiring working memory and inhibition of a previously rewarded response. Subject sees a reward hidden to left or right (2 identical hiding wells); after a delay, subject is allowed to search one well. Linked to DL-PFC by work with rhesus monkeys (Diamond and Goldman-Rakic, 1989).
Object retrieval	A transparent barrier detour task. Subject can see the reward through all sides of a transparent box, but can reach through only the one open side (Diamond, 1981, 1990a,b). Linked to DL-PFC by work with rhesus monkeys (Diamond and Goldman-Rakic, 1985).
Tests that do not require working memory + inhibitory control	
Spatial discrimination	An associative rule-learning and memory task. Hiding done unseen; subject must learn and remember that reward is always hidden to left or right (2 identical hiding places); after delay between trials, subject is allowed to reach. *Not* impaired by lesions to prefrontal cortex (Goldman and Rosvold, 1970).
Visual paired comparison	A recognition memory task in which a sample is presented, a delay imposed, and then subject is given a choice of that stimulus or something new. Linked to medial temporal lobe (Bachevalier, Brickson, and Hagger, 1993; McKee and Squire, 1992).
Tasks Used with Toddlers (ages 15-30 months)	
Tests of working memory + inhibitory control, dependent on DL-PFC	
A-not-B with invisible displacement	A hiding task requiring memory of where the container-with-reward was last moved and inhibition of a previously rewarded response. Similar to A-not-B for infants, but not independently, directly linked to prefrontal cortex.
Tests that do not require working memory + inhibitory control	

Three boxes (boxes scrambled after each reach)	A memory task in which subjects are to try to open all boxes without repeating a choice; a delay is imposed between reaches. S must remember color/shape of the boxes; spatial location is irrelevant. Linked to DL-PFC by work with rhesus monkeys (Petrides, 1995b).
Three boxes (stationary)	Here, uncovering the boxes in spatial order will suffice. Similar to a condition *not* impaired by damage to DL-PFC (Petrides and Milner, 1982).
Delayed nonmatching to sample	A recognition memory task in which one is rewarded for reaching to the stimulus not matching the sample that was presented shortly before. Linked to the medial temporal lobe by work with rhesus monkeys and amnesic patients (Meunier, Bachevalier, Mishkin, and Murray, 1993; Squire, Zola-Morgan, and Chen, 1988; Zola-Morgan, Squire, and Amaral, 1989).
Global-local (preferential looking procedure)	A visual-spatial attention task. Assesses attention to the global and local features of composite stimuli (e.g., and H made up of S's). Similar to task linked to parietal cortex by work with brain-damaged patients (Lamb, Robertson, and Knight, 1989; Robertson, Lamb, and Knight, 1988) and to a task linked to parietal cortex through functional magnetic imaging (fMRI) of neural activity in normal adults.

Tasks Used with Young Children (ages 3½-7 years)

Tests of working memory + inhibitory control, dependent on DL-PFC

Day-night Stroop-like test	Requires holding 2 rules in mind and exercising inhibitory control. S must say "night" when shown a white-sun card, and say "day" when shown a black-moon card. Hypothesized to require the functions of DL-PFC, but has yet to be studied in relation to brain function.
Tapping	A conflict test requiring memory of 2 rules and inhibitory control. When E taps once, S must tap 2 times; when E taps twice, S must tap twice. Linked to prefrontal cortex by work with brain-damaged patients (Luria, 1966).
Three pegs	S is shown a board containing 3 colored pegs arranged in the order red, yellow, green. S is instructed to tap the pegs in the order red, *green,* yellow. This requires remembering the instructed sequence and inhibiting the tendency to tap the pegs in their spatial order. It has yet to be studied in relation to brain function.

Tests that do not require working memory + inhibitory control

Corsi-Milner test of temporal order memory	Subject is shown a series of stimuli one at a time, and is periodically shown 2 previously presented stimuli and asked, "Which of these two pictures did you see last?" Linked to prefrontal cortex by work with brain-damaged human adults (Milner, Corsi, and Leonard, 1991).

Six boxes (boxes scrambled after each reach)	A memory task in which subject must try to open all boxes without repeating a choice; a delay is imposed between reaches. Similar to tasks linked to prefrontal cortex in rhesus monkeys (Petrides, 1995a,b) and in brain-damaged human adults (Petrides and Milner, 1982).
Stroop control condition	Requires learning and remembering 2 rules (as does the Stroop task above), but requires no inhibition (unlike the Stroop task above) -- 2 arbitrary patterns used; to one, subject must say "day"; to the other, subject must say "night."
Corsi-Milner test of recognition memory	S is shown a series of pictures and periodically asked, "Among the pictures I've shown you, which of these two have you already seen?" Linked to medial temporal lobe by work with brain-damaged patients (Milner, 1982; Milner, Corsi, and Leonard, 1991).
Six boxes (stationary)	Here, uncovering the boxes in spatial order will suffice. Similar to a condition *not* impaired by damage to DL-PFC (Petrides and Milner, 1982).
Global-local (forced choice procedure)	A visual-spatial attention task. Assesses attention to the global and local features of composite stimuli (e.g., an H made up of S's). Linked to parietal cortex by work with brain-damaged patients (Lamb et al., 1989; Robertson, Lamb, and Knight, 1988) and by fMRI with normal adults.
Line bisection	A spatial perception task. Subject is asked to indicate the middle of each line. Linked to parietal cortex by work with brain-damaged patients (Benton, 1969).

Section 5. Deficits in the working memory and inhibitory control abilities dependent on DL-PFC in children treated early and continuously for PKU

We found that PKU children, who had been on a low-Phe diet since the first month of life but who had moderately elevated blood Phe levels (levels roughly 3-5 times normal [6-10 mg/dL; 360-600 •mol/L]) were impaired on all six tests that require both holding information in mind and overriding or resisting a dominant response, i.e., tasks dependent on DL-PFC. These six tasks 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 test, and the three pegs test for young children (see figure 29.7). The fact that even *infants* showed these impairments suggests that the dopaminergic innervation to prefrontal cortex is critical for the proper expression of these abilities even during the first year of life.

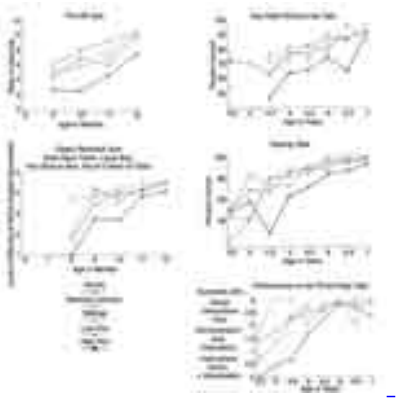


Figure 29.7. Performance of PKU children whose blood Phe levels are 6-10 mg/dL (3-5 times normal) on tasks requiring both working memory and inhibitory control. Note that they are significantly impaired compared to each comparison group: other PKU children with Phe levels closer to normal, siblings of the PKU children, control children matched to the PKU children on a large number of variables, and children from the general population. Note also that they are significantly impaired in the youngest age range investigated (as infants they are impaired on the A-not-B/delayed response and object retrieval tasks) and in the oldest age range investigated (as young children on the day-night, tapping, and three-pegs tasks).(Reprinted with permission from Diamond et al., 1997.)

These deficits in the working memory and inhibitory control abilities dependent on DL-PFC were evident in all age groups (infants, toddlers, and young children), and remained significant even controlling for IQ, gender, health variables, and background characteristics. The deficits were clear whether the PKU children with blood Phe levels 3-5 times normal were compared to (1) other PKU children with lower Phe levels, (2) their own siblings, (3) matched controls, or (4) children from the general population.

One way to summarize the many comparisons across the three age groups and 19 tasks is to look at the results on one dependent measure for every task. For each task (the control tasks as well as those requiring working memory plus inhibitory control) we selected the dependent measure that yielded the strongest between-group differences on that particular task. This gave each task the best possible opportunity to yield a difference between groups, whether we had predicted a group difference or not. Of the 24 comparisons between PKU children with blood Phe levels 3-5 times above normal and the four other groups of children (PKU children with Phe levels closer to normal [<3× normal], siblings of PKU children, matched controls, and children from the general population) on the six tasks requiring working memory plus inhibitory control (6 tasks × 4 comparisons per task), PKU children with higher Phe levels performed significantly worse than the comparison groups on 79% of these comparisons using the stringent criterion of $p \leq .005$ for each test to correct for multiple comparisons (see table 29.3). This pattern of 19 out of 24 comparisons in the predicted direction would be very unlikely to occur by chance ($p < .004$ [binomial distribution]). In short, the impairment of the PKU children, whose blood Phe levels were 3-5 times above normal, on the tasks that require the working memory and inhibitory control functions dependent on DL-PFC was clear and consistent.

Table 29.3 : Pairwise comparisons between subject groups significant at p ≤ .005*

	The 6 tasks that required the working memory and inhibitory control abilities dependent on DL-PFC	**The 10 control tasks that are not dependent of PFC**
PKU children whose plasma Phe levels were 3-5× normal performed significantly worse than the other groups of children on …	19 out of 24 comparisons (79%)	4 out of 40 comparisons (10%)
The other groups of children performed significantly different from one another on …	2 out of 36 comparisons (5%)	3 out of 60 comparisons (5%)

*The .005 significance level was chosen to correct for multiple comparisons. This is similar to what a Bonferroni correction would do.

This finding of deficits in the working memory and inhibitory control abilities dependent on DL-PFC in PKU children whose blood Phe levels are mildly elevated (3-5× normal) is consistent with the results of a number of other studies. The most relevant are those by Welsh and colleagues (1990) and Smith and colleagues (1996), as these investigators used cognitive tasks tailored to the functions of DL-PFC.

The cognitive deficits documented in many studies of children treated for PKU could be explained away by saying that (1) the blood Phe levels of many of the children were outside the "safe" range (i.e., >5× normal); (2) even if current Phe levels were not excessively elevated, earlier Phe levels had been (during the years the children had been off diet); and/or (3) the low-Phe diet had been started too late to avert early brain damage. Those disclaimers are not applicable to the study done by Diamond and colleagues (1997).

Section 5. A linear relationship between Phe level and performance

The higher a PKU child's current Phe level (the higher a child's Phe:Tyr ratio), the worse that child's performance on the tasks that required the working memory and inhibitory control functions dependent on DL-PFC. PKU children whose blood Phe levels had been maintained at 2-6 mg/dL performed comparably to all control groups on our tasks. Thus, at least in this subgroup of PKU children, deficits in the ability to exercise working memory and inhibitory control simultaneously did not appear to be a necessary, unavoidable consequence of being born with PKU. The effect of elevated Phe levels appeared to be acute rather than chronic: Performance on these tasks was most strongly and consistently related to *current* blood Phe levels, not mean Phe levels over a wide age range during the first year of life or during the first month of life. As current Phe levels varied, so too, inversely, did behavioral performance on five of the six tasks that required acting counter to one's initial tendency on the basis of information held in mind (the exception being A-not-B with invisible displacement). Indeed, over time, changes in blood Phe levels *within the same child* were accompanied by concomitant, inverse changes in performance on these cognitive tasks.

The findings that performance is most closely tied to current blood Phe levels (rather than to Phe levels earlier in life) and that performance covaries with a child's current blood Phe levels is consistent with the aforementioned biological mechanism concerning the cause of the cognitive deficits. That is, these findings are consistent with an effect of reduced dopamine on prefrontal cortex function, which would vary directly with changes in the Phe:Tyr ratio in the bloodstream, as opposed to structural, neuroanatomical changes, which might be more fixed.

Like us, Welsh and colleagues (1990) and Smith and colleagues (1996) found that performance on measures of DL-PFC function was significantly and negatively correlated with concurrent Phe levels and less so with lifetime Phe levels. Brunner, Jordan, and Berry (1983) found that cognitive neuropsychological performance was significantly correlated with concurrent Phe levels but not with Phe levels during infancy. Using IQ and school achievement as the outcome measures, Dobson and colleagues (1976) also found a significant, negative correlation with concurrent blood Phe levels, and a much weaker association with Phe levels earlier in life. Like us, Stemerdink and colleagues (1995) found that when blood Phe levels were kept below 3× normal from birth to the present, PKU children showed no cognitive deficits. The only contrary finding is the report of Sonneville and colleagues (1990), who found that Phe levels during the 2 years preceding cognitive testing were a better predictor of speed of responding on a continuous performance test than were concurrent Phe levels.

The relationship found between blood Phe level and performance in three studies (Diamond et al., 1997; Smith and Beasley, 1989; Welsh et al., 1990) is particularly impressive considering the truncated range of Phe levels; all PKU children in those studies were on a dietary regimen and their Phe levels were generally within the "acceptable" range. Because participants in the study by Diamond and colleagues (1997) were followed longitudinally, we are able to present evidence for the first time that performance on tasks requiring the working memory and inhibitory control functions of DL-PFC covaried inversely with Phe levels in the same child over time. Because of the evidence of cognitive deficits in PKU children whose blood Phe levels are 6-10 mg/dL, the national guidelines for the treatment of PKU have been changed in the United Kingdom, the Netherlands, and Denmark: Phe levels higher than 6 mg/dL are no longer considered acceptable. In addition, several clinics in the United States have similarly revised their guidelines.

Section 5. A developmental delay or absolute, lasting deficits?

Are the cognitive deficits in treated PKU children indicative of a developmental delay or of lasting deficits? On the one hand, all children, even PKU children with Phe levels 3-5 times above normal, improved over time on our tasks. On the other hand, the impression that PKU children may "catch up" to other children is probably misleading. In almost all cases this "catch up" was due to ceiling effects: The same tasks were administered over a wide age range, and these tasks were often too easy for children at the upper end of an age range. We have repeatedly found that the between-group differences reappeared on the next battery of tasks for the next age group. The impairment of the PKU children with higher Phe levels in simultaneously holding information in mind and inhibiting a prepotent response was as evident in our oldest age range (3½-7 years) as it was in our youngest age range (6-12 months). The deficit showed no evidence of subsiding within the age range we studied (6 months to 7 years).

The oldest children tested by Diamond and colleagues (1997) were 7 years old. One cannot tell from our study whether some time after 7 years PKU children whose Phe levels remain only moderately elevated might no longer show the kinds of cognitive deficits we have documented. Many studies of elementary school-age PKU children on the low-Phe diet have found cognitive deficits (Smith and Beasley, 1989; Weglage et al., 1995; Welsh et al., 1990). Recent studies by Ris and colleagues (1994) and Smith and colleagues (1996) report deficits in the cognitive abilities dependent on prefrontal cortex in young adults with PKU. However, dietary compliance tends to become progressively more lax after children enter school, so that these studies have included participants whose blood Phe levels were higher than 10 mg/dL. What would happen if blood Phe levels were maintained at 3-5 times normal: Would the cognitive deficits eventually disappear? The data to answer that question do not currently exist. Amino acid uptake across the blood-brain barrier changes during development, offering more protection against blood Phe elevations as children get older (Greengard and Brass, 1984; Lajtha, Sershen, and Dunlop, 1987). Thus, it is quite possible that the blood Phe levels we found to be detrimental during infancy and early childhood might be more benign in later childhood or adolescence.

Early cognitive deficits or developmental delays -- especially when they extend over a long period (such as the 6-year period we have documented) -- are likely to have profound and enduring effects, even if the cognitive deficits themselves are subsequently resolved. They affect children's perceptions of, and expectations for, themselves and the perceptions and expectations of others for the children. Such perceptions and expectations can be inordinately difficult to change and can have major effects in shaping development and behavior.

Section 5. Selective, rather than global, cognitive deficits

The same children who were impaired on all six working memory plus inhibitory control tasks performed well on the ten control tasks, which required other cognitive abilities dependent on other neural systems such as parietal cortex or the medial temporal lobe. Performance on the control tasks, moreover, was not related to current blood Phe levels. For each of the ten control tasks, we compared the performance of PKU children with higher blood Phe levels (6-10 mg/dL; 3-5× normal) to that of the four comparison groups: other PKU children with lower blood Phe levels, siblings of the PKU children, matched controls, and children from the general population. This yielded a total of 40 pairwise comparisons (10 tasks × 4 comparisons per task). PKU children with higher Phe levels performed worse on only 10% of these comparisons (see table 29.3). This pattern of 36 out of 40 comparisons in the predicted direction would be extremely unlikely to occur by chance ($p < .001$ [z distribution]). The consistency of the deficits of the PKU children with Phe levels 3-5 times normal on the working memory plus inhibitory control tasks and the paucity of deficits on the control tasks is quite striking: 55 out of 64 comparisons in the predicted direction (86%), $p < .0001$, z distribution.

Thus, for children treated early and continuously for PKU whose blood Phe levels are 3-5 times normal, the cognitive deficits appear to be selective. The functions of parietal cortex and of the medial temporal lobe appear to be spared, even if the children's Phe levels go up to 6-10 mg/dL. This is consistent with reports by Welsh and colleagues (1990) and Smith and colleagues (1996) who found (1) greater impairments on tasks dependent on prefrontal cortex than on tasks dependent on parietal cortex or the medial temporal lobe in those treated early and continuously for PKU, and (2) an inverse relationship between Phe levels and performance on tasks dependent on prefrontal cortex function but no such relationship for tasks dependent on parietal cortex or the medial temporal lobe. This is an example of a very specific, selective effect resulting from a global insult (a moderate elevation in Phe and a moderate reduction in Tyr in the bloodstream that feeds the entire body, and moderately too little Tyr in the entire brain). The reason for the specificity is the differential, unique sensitivity of prefrontally projecting dopamine neurons to a mild reduction in the dopamine precursor, tyrosine.

This finding of a deficit in the working memory and inhibitory control functions of DL-PFC, but not in the cognitive functions dependent on other neural systems, is consistent with the mechanism I have hypothesized as the cause of the cognitive deficits: A moderate imbalance in the Phe:Tyr ratio in blood (as when Phe levels are 3-5 times normal in PKU children) adversely affects the dopamine concentration in prefrontal cortex but not other dopamine systems in the brain because of the special properties of the prefrontally projecting dopamine neurons, which makes them unusually vulnerable to modest reductions in the level of Tyr reaching the brain. The specificity of the deficits suggests that the cause of those deficits is probably too little Tyr reaching the brain, rather than too much Phe reaching the brain, because all neural regions would be equally vulnerable to the negative effects of too much Phe; the functions of DL-PFC would not be disproportionately affected. That is, if the cause of the cognitive deficits were too much Phe in the brain, the cognitive deficits should be global, rather than limited to the prefrontal neural system.

Section 5. Findings we had not predicted: Preserved performance on self-ordered pointing and temporal order memory tasks

The mechanism I have proposed to explain the cause of the cognitive deficits in children treated early and continuously for PKU, whose Phe levels are 3-5 times normal, rests on the special properties of the dopamine

neurons that project to prefrontal cortex. I had not hypothesized that only certain cognitive functions dependent on DL-PFC would be affected. I was surprised, therefore, when we found that PKU children with Phe levels 3-5 times normal performed normally on three tasks dependent on DL-PFC: the three- and six-boxes tasks (boxes scrambled after each reach), which are adaptations of the Petrides and Milner self-ordered pointing task; and the Corsi-Milner test of temporal order memory. These tasks require working memory (remembering what choices one has already made or remembering the order in which stimuli have been presented) but not inhibitory control. Thus, the treated PKU children with moderately elevated Phe levels were only impaired on the subclass of prefrontal cortex tasks that required *both* working memory and resisting a prepotent action tendency.

These findings were puzzling since nothing in my hypothesis would lead one to predict that certain cognitive functions dependent on dorsolateral PFC should be affected but not others. Although I have emphasized the conjunction of working memory plus inhibitory control as the hallmark of tasks dependent on DL-PFC, I had no explanation at the time for why performance on only certain cognitive tasks that require the functions of prefrontal cortex should be affected in PKU children. The evidence linking self-ordered pointing and temporal order memory to DL-PFC is strong, with converging evidence from lesion studies in rhesus macaques, human adult patients with damage to DL-PFC, and neuroimaging studies in normal human adults (Milner, Corsi, and Leonard, 1991; Petrides, 1995a; Petrides and Milner, 1982; Petrides, Alivatos, Meyer, and Evans, 1993). It was extremely unlikely that the failure to find a deficit on these tasks was due to their not requiring DL-PFC.

An excellent recent study by Collins and colleagues (1998) begins to make sense of what we found. They compared the effect of lesioning prefrontal cortex to the effect of depleting prefrontal cortex of dopamine. Their anatomical lesions were excitotoxic, which is a technique that destroys the cell bodies in the target region, but not the fibers of passage, so that one can have more confidence than with traditional lesioning methods that the observed effect is due to damage to the target region specifically. They depleted prefrontal cortex of dopamine by injecting it with 6-hydroxydopamine (6-OHDA). The concentrations of norepinephrine and serotonin in prefrontal cortex were not similarly reduced because the investigators pre-injected prefrontal cortex with a norepinephrine antagonist (talsupram) and a serotonin antagonist (citalopram). Although their work is in the marmoset, they replicated the findings of others in the rhesus macaque; plus, they added one important new finding.

Replicating the work of others (Butters et al., 1969; Diamond and Goldman-Rakic, 1989; Goldman and Rosvold, 1970), Collins and colleagues (1998) found that their lesions of prefrontal cortex impaired performance on the delayed response task. Similarly, like others (Petrides, 1995a; Petrides and Milner, 1982), they found that their lesions of prefrontal cortex impaired performance on the self-ordered pointing task, and to the same degree as the same lesions impaired performance on delayed response. Finally, as others had reported (Sawaguchi and Goldman-Rakic, 1991), they found that depleting prefrontal cortex of dopamine impaired performance on delayed response. No one before had looked at the effect of dopamine depletion on self-ordered pointing, however. Collins and colleagues (1998) found that, when they depleted the same region of prefrontal cortex of dopamine, performance on the self-ordered pointing task was *not* impaired. (See table 29.4 for a summary of this set of results.)

Table 29.4 : Summary of the results of the 1998 study by Collins, Roberts, Dias, Everitt, and Robbins

Type of lesion to frontal cortex	Behavioral Task: Delayed response (requires working memory + inhibition)	Behavioral Task: Self-ordered pointing (requires working memory
Excitotoxic (cell bodies destroyed)	Performance impaired	Performance impaired
6-OHDA (dopamine depleted)	Performance impaired	Performance *spared*

Thus, even though prefrontal cortex is necessary for successful performance on self-ordered pointing (as can be seen from the lesion results), the dopamine innervation of prefrontal cortex is not necessary for successful performance of the task. Luciana and Collins (1997) found a dissociation that is perhaps similar in that performance on one of their working memory tasks appeared to rely critically on dopamine while performance of the other working memory task did not. They found that a dopamine agonist (bromocriptine) improved performance on delayed response and a dopamine antagonist (haloperidol) impaired performance on delayed response, but neither affected performance on a nonspatial working memory task. Unfortunately, though, there is no evidence that Luciana and Collins' nonspatial working memory task requires DL-PFC.

The effects we documented in children treated with PKU whose blood Phe levels were 6-10 mg/dL are (we contend) due to reduced dopamine in prefrontal cortex. Consistent with the results that Collins and colleagues (1998) obtained after our study was completed, we found that these treated PKU children were impaired on our delayed response task (A-not-B) but not on our self-ordered pointing tasks (three- and six-boxes [boxes scrambled after each reach]). The results that had seemed puzzling at the time end up providing additional support for our hypothesis. It appears that the dopamine content of prefrontal cortex is critical for certain cognitive functions dependent on prefrontal cortex (working memory plus inhibition) but not for others (when working memory is taxed alone). We still do not understand, however, *why* that is the case. Luciana and Collins (1997) suggested that dopamine might be critical when the information that must be held in mind is spatial. Such an explanation cannot account for our results, however: Although the prefrontal tasks on which we found sparing were nonspatial, we also found impairments on the day-night and tapping tasks, which are also nonspatial.

Chapter 29. A Model System for Studying the Role of Dopamine in the Prefrontal Cortex during Early Development in Humans: Early and Continuously Treated Phenylketonuria

Section 6. An animal model of mild, chronic plasma Phe elevations

With children it was possible only to measure blood levels of Phe and Tyr and cognitive performance. To investigate the biological mechanism underlying the cognitive deficits of children treated for PKU more directly, we developed and characterized the first animal model of treated PKU (Diamond et al., 1994) and subsequently worked with the genetic mouse model of PKU (Zagreda et al., 1999). The animal model enabled us to study the effect of moderate, chronic plasma Phe elevations on neurotransmitter and metabolite levels in specific brain regions. Thus, we could directly investigate our hypothesis that the cognitive deficits associated with moderately elevated plasma Phe levels are produced by a selective reduction in dopamine synthesis in prefrontal cortex.

Building on work modeling the untreated PKU condition (Brass and Greengard, 1982; Greengard, Yoss, and Del Valle, 1976), Diamond and colleagues (1994) administered a phenylalanine hydroxylase inhibitor (α-methylphenylalanine) plus a small supplement of Phe to mildly and chronically elevate the blood Phe levels in rat pups. (The Phe supplement was needed because α-methylphenylalanine does not inhibit phenylalanine hydroxylase completely.) There were two experimental groups: (1) pups whose blood Phe levels were elevated postnatally, and (2) pups whose blood Phe levels were elevated pre- and postnatally. Control animals came from the same litters as the first group and received daily control injections of saline.

All were tested on delayed alternation, a task sensitive to prefrontal cortex dysfunction (Bättig, Rosvold, and Mishkin, 1960; Bubser and Schmidt, 1990; Kubota and Niki, 1971; Larsen and Divac, 1978; Wikmark, Divac, and Weiss, 1973). Testers were blind to the group assignment of their animals. Each of the testers was assigned four animals in each group and the order of testing was randomized across experimental condition. Blood samples were collected at multiple time points to determine the animals' Phe levels. High-performance liquid chromatographic (HPLC) analyses of the brain tissue assessed the distributions and concentrations of dopamine, serotonin, norepinephrine, and their metabolites in various brain regions (prefrontal cortex, caudate-putamen, and nucleus accumbens).2

The most dramatic neurochemical effects of the moderate elevation in blood Phe levels were the reduction in dopamine and in the dopamine metabolite HVA in prefrontal cortex in each of the PKU-model animals. There was almost no overlap between HVA levels in the prefrontal cortex of controls and either PKU-model group: All control animals but one had higher HVA levels in prefrontal cortex than *any* animal in either experimental

group. In contrast, as predicted, the levels of dopamine and dopamine metabolites were not reduced elsewhere in the brain, and norepinephrine levels were not reduced elsewhere in the brain or in prefrontal cortex. We had predicted that norepinephrine levels would be unaffected (even though norepinephrine is made from dopamine) because previous work had shown that norepinephrine levels are relatively insensitive to alterations in precursor availability (Irie and Wurtman, 1987).

The PKU-model animals were impaired on delayed alternation in the same ways and under the same conditions as are animals with prefrontal cortex lesions. On the delayed alternation task, the animal is rewarded only for alternating goal arms (i.e., for selecting the goal arm *not* selected on the previous trial). Thus, the animal must remember which goal arm was last entered over the delay between trials and must inhibit repeating that response. The hallmark of performance after prefrontal cortex is removed is that subjects fail when a delay is imposed between trials, although they are unimpaired at learning the alternation rule or in performing the task when no delay is imposed (*in rats:* Bubser and Schmidt, 1990; Larsen and Divac, 1978; Wikmark, Divac, and Weiss,1973; *in monkeys:* Bättig, Rosvold, and Mishkin, 1960; Jacobsen and Nissen, 1937; Kubota and Niki, 1971). We found that the animals with moderately elevated plasma Phe levels learned the delayed alternation task normally and performed well when there was no delay, but failed when a delay was imposed between trials (see figure 29.8), just as do prefrontally lesioned animals.

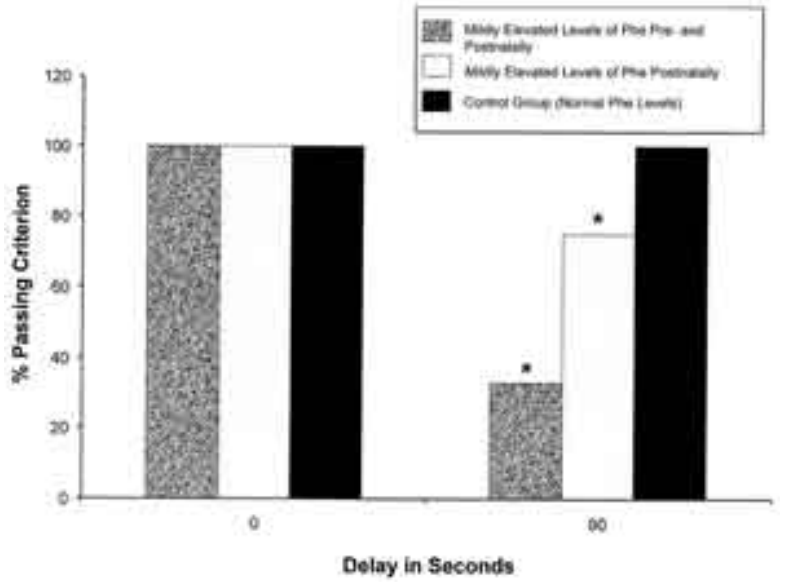


Figure 29.8. Rats with chronic, mild elevations in their blood Phe levels, to create an animal model of early and continuously treated PKU, show the same pattern of performance on the delayed alternation task, as do monkeys whose dorsolateral prefrontal cortex has been lesioned and rats whose homolog to dorsolateral prefrontal cortex has been lesioned. That is, they can learn the delayed alternation rule and perform well when there is no delay, but are impaired when a delay is introduced.

Moreover, we found that the lower an animal's prefrontal dopamine levels, the worse that animal performed on the delayed alternation task. The neurochemical variable most strongly and consistently related to performance on delayed alternation was the level of HVA in prefrontal cortex. This is consistent with previous work, which has demonstrated that delayed alternation performance is highly dependent on the level of dopamine in prefrontal cortex, and is uncorrelated with serotonin or norepinephrine levels (Brozoski et al., 1979; Sahakian et al., 1985; Simon, Scatton, and LeMoal, 1980) or with dopamine elsewhere in the brain (Sahakian et al., 1985; Simon, Scatton, and LeMoal, 1980).

Thus, Diamond and colleagues (1994) found the neurochemical changes (reduced levels dopamine and the dopamine metabolite HVA in prefrontal cortex) and the cognitive deficits (impaired performance on a behavioral task dependent on prefrontal cortex [delayed alternation]) predicted by our model in both groups of

PKU-model animals with moderately elevated blood Phe levels. The only result that deviated from those predicted was an effect on the serotoninergic system in PKU-model animals. The lack of complete specificity may have been because blood Phe levels were a bit more elevated than intended (6.5× normal, rather than ≤ 5× normal) or because the neurochemical effects of moderately elevated blood Phe levels are not quite as localized as I have hypothesized. We are investigating this further with the genetic mouse model of PKU created by McDonald and colleagues (McDonald et al., 1990; Shedlovsky et al., 1993).

Chapter 29. A Model System for Studying the Role of Dopamine in the Prefrontal Cortex during Early Development in Humans: Early and Continuously Treated Phenylketonuria

Section 7. What we thought was independent, confirming evidence from visual psychophysics for the proposed causal mechanism

If it is the special properties of the dopamine neurons projecting to prefrontal cortex that make the functions of prefrontal cortex particularly vulnerable to moderate increases in the Phe:Tyr ratio in the bloodstream, then any other dopamine neurons that share those special properties should also be affected by moderately elevated blood Phe:Tyr ratios. It so happens that the dopamine neurons in the retina share all those same unusual properties. They, too, have unusually rapid firing and dopamine turnover rates (Fernstrom, Volk, and Fernstrom, 1986; Iuvone et al., 1978, 1989). Moreover, the competition between Phe and Tyr at the blood-retinal barrier is fully comparable to their competitive uptake at the blood-brain barrier (Fernstrom, Volk, and Fernstrom, 1986; Hjelle et al., 1978; Rapoport, 1976; Tornquist and Alm, 1986). Indeed, it has been shown that a small reduction in the level of Tyr reaching the retina dramatically reduces retinal dopamine synthesis (Fernstrom and Fernstrom, 1988; Fernstrom, Volk, and Fernstrom, 1986), mirroring the effect on dopamine synthesis in prefrontal cortex. Therefore, to be consistent, we had to predict that retinal function should also be affected in PKU children who have been on a low-Phe diet since the first month of life, but who have moderately elevated blood Phe levels (levels roughly 3-5 times normal [6-10 mg/dL; 360-600 •mol/L]), even though no visual deficit had been reported in these children before.

The aspect of retinal function most firmly linked to the level of dopamine in the retina is contrast sensitivity. Contrast sensitivity refers to the ability to detect differences in luminance (brightness) of adjacent regions in a pattern. Your contrast sensitivity threshold is the limit of how faint items printed in gray can become before you fail to perceive them at all. People with good contrast sensitivity can perceive fainter lines than those who require a greater luminance difference between foreground and background. Patients with Parkinson's disease, who have greatly reduced levels of dopamine, have impaired contrast sensitivity (Bodis-Wollner, 1990; Bodis-Wollner et al., 1987; Kupersmith et al., 1982; Regan and Neima, 1984; Skrandies and Gottlob, 1986). It is thought that this occurs because dopamine is important for the center-surround organization of retinal receptive fields (Bodis-Wollner, 1990; Bodis-Wollner and Piccolino, 1988).

To investigate contrast sensitivity, we (Diamond and Herzberg, 1996) tested children between the ages of 5.4 and 9.8 years on the Vistech test (Gilmore and Levy, 1991; Ginsberg, 1984; Lederer and Bosse, 1992; Mäntyjärvi et al., 1989; Rogers, Bremer, and Leguire, 1987; Tweten, Wall, and Schwartz, 1990). We found that children treated early and continuously for PKU, whose blood Phe levels were 6-10 mg/dL (3-5× normal), were

impaired in their sensitivity to contrast at each of the five spatial frequencies tested (1.5-18.0 cycles per degree; see figure 29.9). Even though all children had been tested under conditions of 20/20 acuity, the PKU children were significantly less sensitive to visual contrast than their same-aged peers across the entire range of spatial frequencies. These group differences remained robust even when the two PKU children whose IQs were below 90 were omitted from the analyses. Indeed, at the next to the highest spatial frequency (12 cycles per degree), the "group" variable accounted for 70% of the variance, controlling for acuity, gender, age, and test site. At no spatial frequency was the contrast sensitivity of any PKU child better than that of his or her own sibling. Standard eye exams had never detected a problem in this population because acuity is normally tested under conditions of high contrast; an impairment in contrast sensitivity was not revealed before because no one had tested for it.

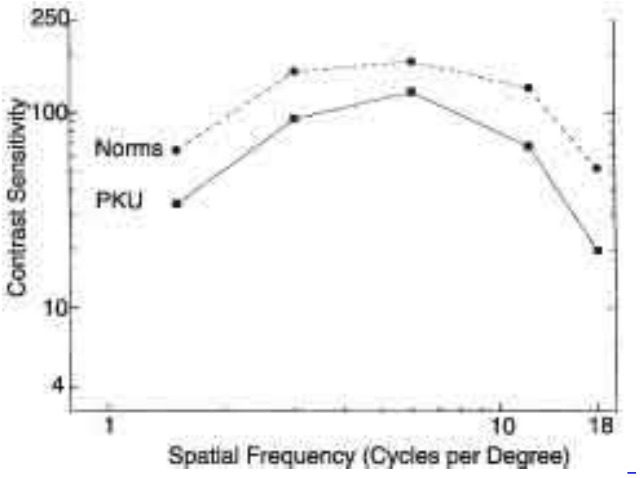


Figure 29.9. PKU children whose blood Phe levels are 6-10 mg/dL were found to be significantly impaired in contrast sensitivity compared to children of the same age at every spatial frequency investigated.

At the time, we interpreted these results as providing converging evidence in support of the biological mechanism I had proposed. I had predicted the contrast sensitivity deficit for the same reason I had predicted DL-PFC cognitive deficit. Both predictions had been based on the special sensitivity of dopamine neurons that fire rapidly and turn over dopamine rapidly to moderate reductions in the level of available tyrosine. We had found two superficially unrelated behavioral effects, a selective deficit in cognitive functions dependent on DL-PFC and a selective visual defect in contrast sensitivity, both of which had been predicted based on the same underlying hypothesis.

However, I was troubled by one lack of convergence. In the Diamond and Herzberg study (1996), we found that contrast sensitivity performance did not correlate with children's current blood Phe levels, but rather with their Phe levels during the first month of life. In another study (Diamond et al., 1997), however, we had found that, on the cognitive tasks that required the working memory and inhibitory control functionsdependent on DL-PFC, performance had correlated with children's current blood Phe levels, not their Phe levels during the first month of life.

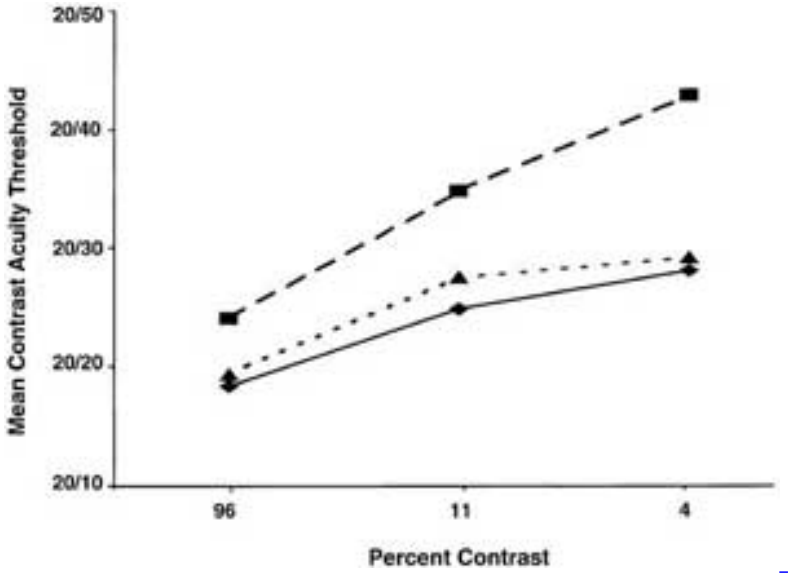
If contrast sensitivity was poor because the retina was low on "fuel" (i.e., low in dopamine), then contrast sensitivity performance should have covaried with current blood Phe levels. The failure to find such a relationship might have been due simply to the truncated range of concurrent Phe levels in the contrast sensitivity study. Only PKU children whose current Phe levels were 6-10 mg/dL had been included in that study, whereas the cognitive study had included PKU children with lower Phe levels as well as those with Phe levels of 6-10 mg/dL. On the other hand, the range in Phe levels during the first month of life was great and so included sufficient variability to find a relationship with contrast sensitivity performance. The possibility also existed, however, that long-lasting structural damage might occur to the visual system during the first weeks of

life, when the visual system is maturing rapidly, and when the Phe levels of PKU infants are dramatically elevated. PKU infants in the United States are generally not placed on the low-Phe diet until they are about 2 weeks old; thus, for the first 2 weeks of life, their Phe levels can easily reach 20-30 mg/dL. Might those extremely high Phe levels, at a time of very rapid maturation of the visual system, cause irreparable damage to the visual system? (In utero, the fetus's levels of Phe and Tyr depend upon the mother's levels, so it is believed that the detrimental effects of PKU begin postnatally.)

One way to test for the latter possibility is to study pairs of siblings, both of whom have PKU. Since more than 150 different mutations of the phenylalanine hydroxylase gene can cause PKU, amniocentesis testing for PKU is extremely expensive. Therefore, fetuses are not usually tested for PKU unless there is already one child with PKU in the family. In the United States, the older sibling with PKU (in whom it was detected postnatally) usually starts the low-Phe diet at about 1½-2 weeks of age, while the younger sibling (in whom PKU was detected prenatally) usually starts on the diet by 2 or 3 days of age. Thus, for this study we have been flying in pairs of PKU siblings from all over the USA and UK (Diamond et al., 1999a). Within each of these families, the earlier-born child (mean age at testing 13 years, range 9-16 years) was exposed to extremely high levels of Phe for a mean of 11 days (range 8-14 days before initiation of diet), while the later born sibling (mean age at testing 10 years, range 6-14 years) was exposed to extremely high levels of Phe for a mean of only 3 days (range 1-5 days). All of the children began the low-Phe diet within the first month of life and have remained on it continuously ever since.

Our preliminary results indicate that earlier-born PKU siblings show worse contrast sensitivity (as measured by the Regan low contrast letter acuity charts [Regan and Neima, 1983]) than their later born siblings under conditions of low contrast (4% contrast; see figure 29.10). This is striking because contrast sensitivity usually improves with age. For example, among siblings pairs without PKU, older siblings performed significantly *better* than their younger siblings, the reverse of the pattern seen in the PKU sibling pairs. The earlier born PKU siblings (who started the diet at 1½-2 weeks of age) also showed worse contrast sensitivity than their same-age peers.

Figure 29.10. *Top,* Partial schematic representation of Regan Low Contrast Acuity Charts. The panel on the left represents high contrast (96%) and the panel on the right represents low contrast (11%). *Bottom,* Discrimination performance for the PKU sibling pairs and a normal comparison group are shown for the three Regan Low Contrast Acuity Charts, each chart was presented at a different level of contrast (96%, 11%, and 4%). Thresholds were calculated using the least squares error method to apply a linear curve fit to the obtained data and selecting the 75% correct discrimination value as the threshold. Earlier-born PKU children, who started on dietary treatment at roughly 11 days of age, showed impaired acuities compared to the other children, as can be seen by their elevated line on the graph. Since the charts differed only in contrast, the sharper drop-off in performance of the earlier-born PKU children when tested at lower contrast, indicates that these children have a deficit in contrast sensitivity. Earlier-born PKU siblings (13 years) (began diet at 11 days) -- squares --; later-born PKU siblings (10 years) (began diet at 3 days) --triangles--; children from the general population (mean age 11 years) --diamonds--.



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These results suggest that extremely high Phe levels during the first weeks of life, even if subsequently lowered and maintained at lower levels, may cause long-lasting damage to the visual system. Evidently, a short-term exposure to high concentrations of Phe of only a couple of weeks during the sensitive neonatal period can have long-lasting effects on contrast sensitivity, evident 9-16 years later. This is significant because it suggests that the current practice of allowing up to 2 weeks to pass before beginning treatment for an infant born with PKU may be ill-advised. Since the blood sample to test for PKU is taken at birth, it would be feasible to start the diet earlier. These results also suggest that although we obtained the results I had predicted for contrast sensitivity in the Diamond and Herzberg (1996) study, we may have obtained those results for reasons *other* than the ones predicted. The deficits in the working memory and inhibitory control abilities dependent on DL-PFC do indeed appear to occur for the reason hypothesized (because of reduced levels of dopamine in DL-PFC due to elevated blood Phe:Tyr ratios). Those deficits covary with concurrent levels of Phe in the bloodstream. However, the retinal deficit in contrast sensitivity appears to be caused, at least in part, by the inordinately high levels of Phe in the first weeks of life, does not covary with current levels of Phe, and appears to be structural.

PREVIOUS NEXT

Chapter 29. A Model System for Studying the Role of Dopamine in the Prefrontal Cortex during Early Development in Humans: Early and Continuously Treated Phenylketonuria

Section 8. Conclusions

DL-PFC begins subserving cognitive functions even during the first year of life. Even as infants, we are thinking problem-solvers. Prefrontal cortex continues to mature over the next 15-20 years of a child's life, just as the child's cognitive development, while remarkable by 1 year, continues to unfold over the next 15-20 years. Tasks that require simply holding one piece of information in mind (such as delayed nonmatching to sample) are too simple to require DL-PFC (Bachevalier and Mishkin, 1986). I have emphasized that DL-PFC is recruited when one must both hold information in mind and inhibit a prepotent response. Other investigators have characterized the functions of DL-PFC more broadly, proposing that when one must both hold information in mind and manipulate or process that information, then DL-PFC becomes critical (D'Esposito, 1995; D'Esposito et al., 1999; Owen et al., 1996, 1999; Petrides, 1994, 1995b; Postle, Berger, and D'Esposito, 1999; Smith and Jonides, 1999; Smith et al., 1998). Under such conceptualizations, holding information in mind plus inhibiting a dominant response becomes part of a subset of "holding information in mind plus another cognitive operation." I am in complete accord with such formulations. In general, tasks that require DL-PFC are more difficult than tasks that do not. However, if one increases how much information must be held in mind so that the task is as difficult as one that requires both holding information in mind plus either inhibition (Diamond, O'Craven, and Savoy, 1998) or alphabetizing the information held in mind (Postle, Berger, and D'Esposito, 1999; Rypma and D'Esposito, 1999), then that task, too, will activate DL-PFC.

There may be differential developmental trajectories for the working memory and inhibitory control abilities dependent on DL-PFC. Between 7½-12 months of age there is evidence of clear age-related improvements in inhibitory control (from results with the object retrieval task) and in how long infants can hold information in mind (from results with the A-not-B task) (Diamond, 1988, 1991). For example, infants make the A-not-B error at delays under 2 s at 7½-8 months and at delays of 10 s or more at 12 months, an average improvement of about 2 s per month in their ability to hold information in mind (see figure 29.11). On the other hand, between 4 and 22 years of age there are marked improvements in inhibitory control but little improvement in the ability to hold information in mind, which appears to be quite robust even by the age of 4 (see figures 29.12 and 29.13). The periods of 7½-12 months and 3-6 years appear to be times of particularly dramatic improvement in the abilities dependent on DL-PFC.

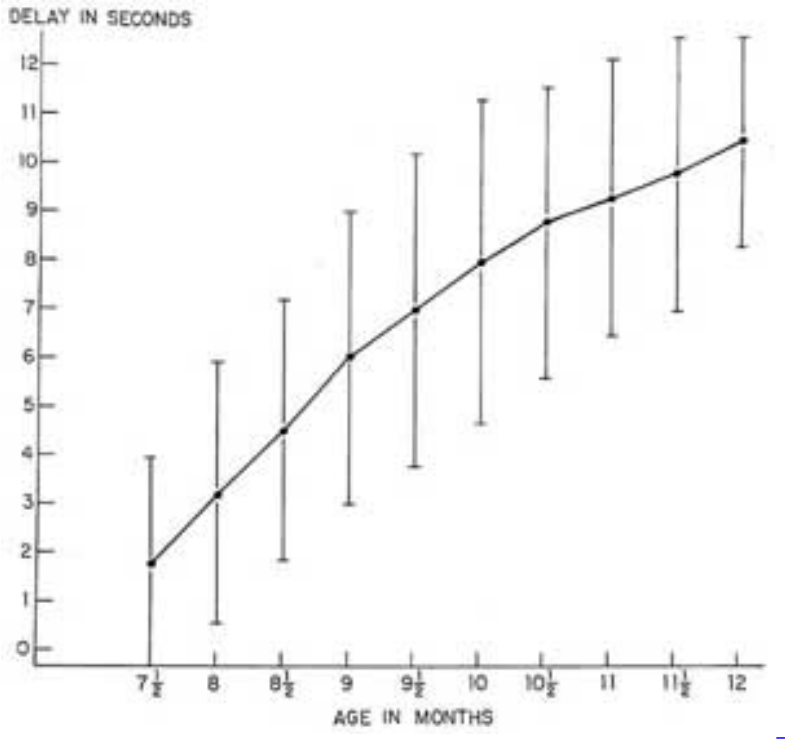


Figure 29.11. The delay at which 25 infants studied longitudinally made the A-not-B error during the first year of life. (Reprinted with permission from Diamond, 1985.)

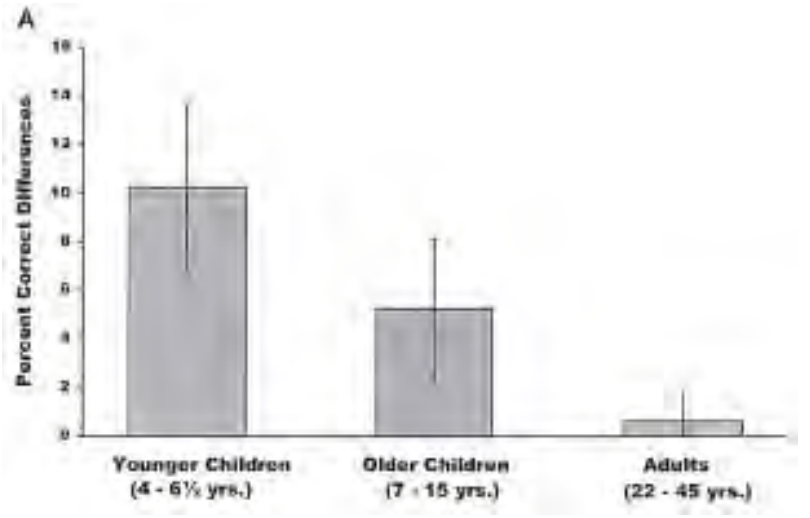
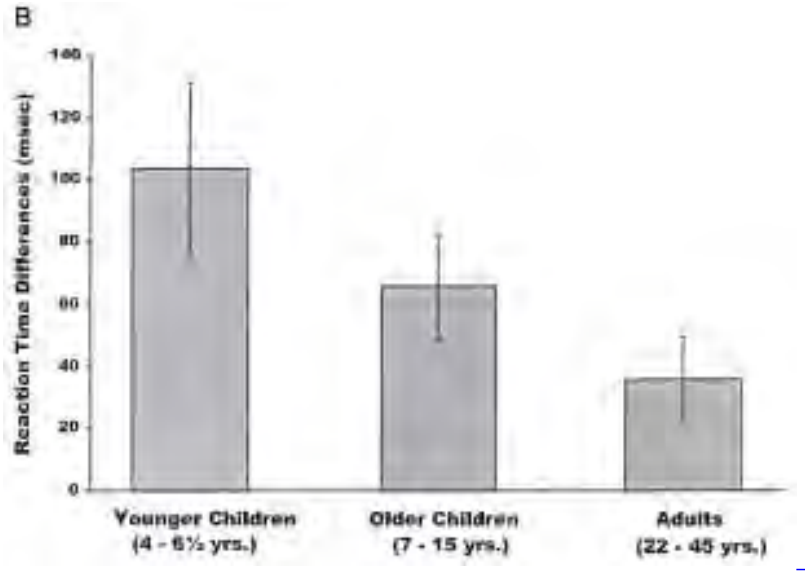


Figure 29.12. Performance on our directional Stroop task when inhibitory control was *not* required minus when it was required. Trials that required inhibitory control were trials where participants had to resist the tendency to respond with the hand on the same side as the stimulus ("spatial incompatibility"). The addition of an inhibitory requirement lowered the accuracy (panel A) and increased the reaction times (panel B) of younger children more than older children and older children more than adults.



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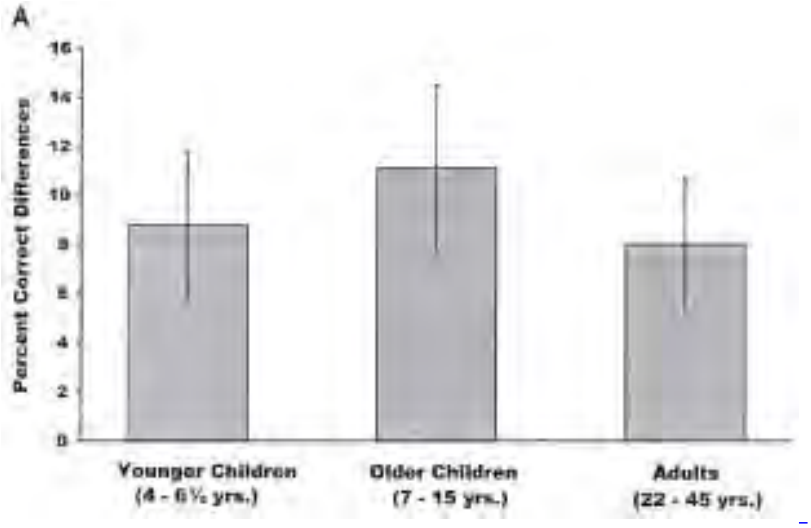
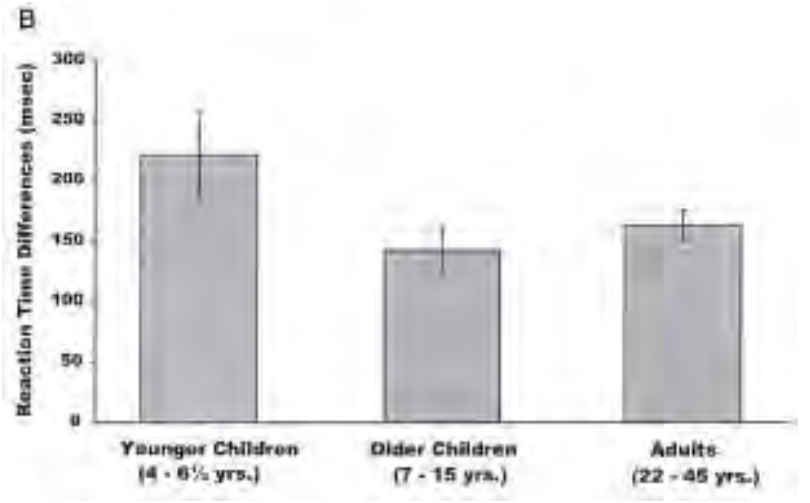


Figure 29.13. Performance on our directional Stroop task when memory load was greater minus when it was less. Memory load was greater when the rules for 6 abstract shapes had to be remembered than when the rules for only 2 abstract shapes had to be remembered. The condition where memory load was greater was harder for all participants, but it was not differentially harder for any age group. Hence, the effect on the accuracy (panel A) and on reaction times (panel B) of younger children, older children, and adults were comparable.



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Dopamine is a particularly important neurotransmitter in prefrontal cortex. The level of dopamine increases in the brains of rhesus macaques during the period when infant rhesus macaques are improving on the A-not-B/delayed response and object retrieval tasks, tasks linked to DL-PFC. To begin to look at the role of dopamine in prefrontal cortex function early in life in humans, we studied children treated early and continuously for PKU because we predicted that they would have lower levels of dopamine in prefrontal cortex but otherwise normal brains, a prediction we were able to confirm in an animal model. We predicted this because the children have moderately elevated levels of Phe (3-5 times normal [6-10 mg/dL]) and moderately reduced levels of Tyr in their bloodstreams. Since Phe and Tyr compete to cross from blood to brain, and since the transporter proteins have a higher affinity for Phe than for Tyr, the upshot of a moderate increase in the Phe:Tyr ratio in the bloodstream is a moderate reduction in the amount of Tyr reaching the brain. Most dopamine systems in the brain are insensitive to modest decreases in the amount of precursor (i.e., Tyr). However, the dopamine neurons that project to prefrontal cortex are different. They fire faster and turn over dopamine faster, and are acutely sensitive to even a modest change in the level of Tyr. Because of the special properties of this dopamine projection, we predicted and found a specific, localized effect (prefrontal cortex affected but not other regions of the brain) even though the insult is global (a mildly increased Phe:Tyr ratio throughout the bloodstream and mildly reduced Tyr levels throughout the brain).

The dopamine neurons in the retina share the same special properties as the dopamine neurons that project to prefrontal cortex. We predicted, therefore, that retinal function would also be affected in early and continuously treated PKU children whose Phe levels are 3-5 times normal. Indeed, we found the predicted impairment in contrast sensitivity. However, this effect appears to be due to the exceedingly high Phe levels during the first two weeks of life, when most children with PKU have not yet begun treatment.

Cognitive deficits in children treated early and continuously for PKU whose blood Phe levels are 3-5 times normal went officially unrecognized for years, despite the protestations of parents and teachers that something was wrong, in part because the children performed within the normal range on IQ tests. Their IQ scores were in the 80s and the 90s, just as are the IQ scores of patients in whom prefrontal cortex has been damaged or removed. IQ tests, or any general tests of intellectual functioning, can easily miss specific deficits. The global cognitive measures that had been in use in clinics were too imprecise to detect the children's deficits. Global measures, such as overall IQ score, are poor indices of *specific* cognitive functions and poor indicators of what particular neural system might be affected if there is a problem. Developmental cognitive neuroscientists now have precise measures of specific cognitive functions, sensitive to the functions of particular neural subsystems. These measures can help in the study and treatment of diverse developmental disorders.

The other reason for the lack of official recognition of the cognitive deficits in children treated early and continuously for PKU was the lack of any hypothesized causal mechanism whereby a global insult might produce a selective effect on the functions of only one neural system (prefrontal cortex). The information on such a causal mechanism already existed in neuropharmacology through the work of Anne-Marie Thierry, Robert Roth, Michael Bannon, and colleagues, but the clinicians working on PKU and the neuroscientists working on the properties of the dopamine projection to prefrontal cortex did not know of one another's work.

To study DL-PFC function in children treated early and continuously for PKU, we used tasks linked to DL-PFC by work in macaques (by methods that disrupt the functioning of, or destroy, a specific neural region, then look at what deficits the animals show; and by methods that look at functioning in the intact brain, such as single cell recording) and by work with brain-damaged adults. It is not a foregone conclusion, however, that a task that requires a particular neural system in one species (say, macaques) will be solved in the same way, and require the same neural system, in a different species (say, humans). Nor is it a foregone conclusion that a task that requires a particular neural system in adults will be solved in the same way, and require the same neural system, in children. In addition, there are a number of reasons why inferring what functions a neural region might subserve from what functions are disrupted when that region is damaged, inactivated, or functioning improperly might lead to erroneous conclusions about structure-function relations.

Thus, it is critical to obtain evidence in *children,* looking at functioning in the *intact brain*. To begin to do this, we have developed a directional Stroop task that is appropriate for children as young as 4 years as well as adults (and even adults are not at ceiling in their performance). The task permits demands on working memory and inhibitory control to be varied independently (see table 29.5), and can be used in the magnetic resonance (MR) scanner. Thus, the neural systems activated under the various conditions of the task can be studied noninvasively in the intact brain using fMRI [functional magnetic resonance imaging] (Diamond, O'Craven, and Savoy, 1998; Diamond et al., 1999b; Savoy et al., 1999). Participants are given a response box with two buttons -- one for the left hand and one for the right. In the "dots-side" variant, when a large gray dot appears to the left or right, the child (or adult) is to press the button on the same side as the dot. When a large black-and-white striped dot appears to the left or right, the child (or adult) is to press the button on the side opposite the stimulus (see figure 29.14a). In the "mixed" condition of dots-side, the two kinds of dots are randomly intermixed over trials, requiring the participant to remember two rules and to inhibit the tendency to respond on the same side as the stimulus when the dot is striped. (The tendency to respond on the same side as a stimulus has been well-documented. People are slower and less accurate when required to respond on the side opposite a stimulus than when required to respond on the same side. This is called "spatial incompatibility" or the "Simon effect" (Craft and Simon, 1970; Fitts and Seeger, 1953; Simon and Berbaum, 1990). In the "arrows-side" variant, when an arrow pointing straight down appears to the left or right, the participant is to press the button on the same side as the arrow. When the arrow points diagonally toward the opposite side, the participant is to press the button on the side opposite the stimulus (see figure 29.14b). This still requires inhibiting the tendency to respond on the same side as the stimulus when a diagonal arrow appears, but it requires little or no working memory, as the arrow points directly to the correct response button on all trials. In a third variant of the directional Stroop task, "abstract-center-six," the participant sees one of six abstract figures at the midline, and needs to remember the rule associated with each figure ("press right" or "press left"; see figure 29.14c). The abstract-center-six variant taxes working memory heavily (participants must hold six rules in mind), but it requires little or no inhibition (as the stimuli appear at the center of the screen and do not preferentially activate the right or left hand). It is work with this task that produced the results on the age-related improvements between 4 and 22 years in inhibitory control, but not in the amount of information that could be held in mind, as illustrated in figures 29.12 and 29.13.

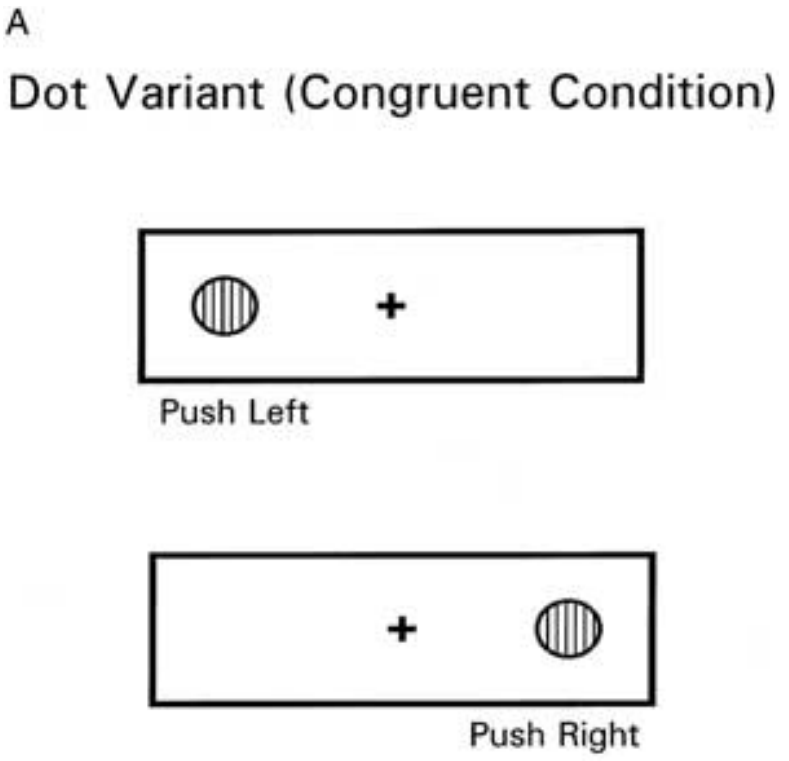


Figure 29.14 (A). Figure illustrating the stimuli in the "dots-side" variant of the directional Stroop task. Here, half the participants were instructed to press the response button on the same side as the dot if the dot is striped and on the opposite side if the dot is gray. (Half the participants were given the opposite instructions. Participants were given a two-button response box: one button for their right thumb and one for their left.) *(continued on following page)*

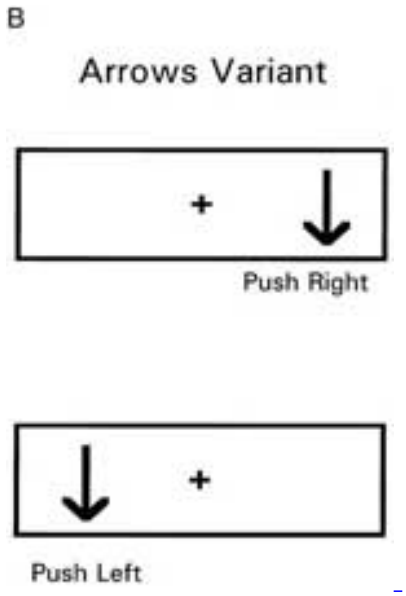
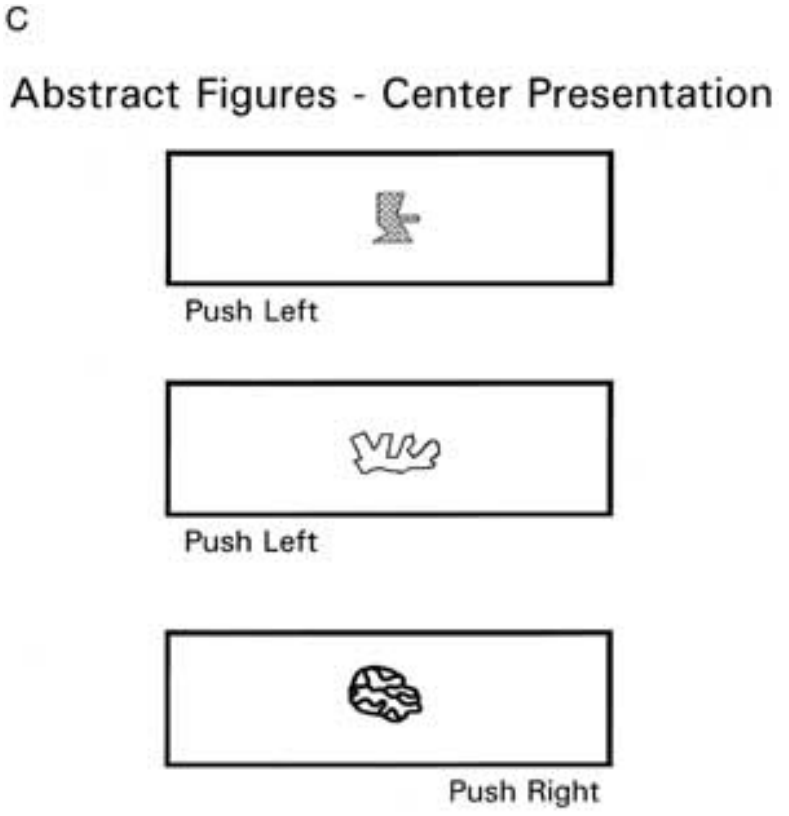


Figure 29.14 *(Continued).* (B). Figure illustrating the stimuli in the "arrows-side" variant of the directional Stroop task. Here, participants were instructed to press the response button to which the arrow was pointing. If the arrow pointed straight down, they were to press the button on the same side as the arrow; and if the arrow pointed diagonally to the opposite side, they were to press the button on the side opposite the arrow.



(C). Figure illustrating some of the stimuli in the "abstract-center-six" variant of the directional Stroop task. Participants were taught a rule for each of six abstract shapes ("press right" or "press left"). The stimuli were presented at the midline, not peripherally as in the dots-side and arrows-side variants.

Table 29.5 : Inhibitory demand and memory load are independently varied in different conditions of the directional Stroop task

	Inhibition	
Memory Load	***Low***	***High***
Low	Arrows-center	Arrows-side
Medium	Abstract-two-center	Abstract-two-side
High	Abstract-six-center	Dots-side-mixed

Because inhibitory control undergoes such a protracted development, young children often fail to inhibit the prepotent response, despite their best intentions and despite knowing what they should do. It would be a shame to mistakenly label such a young child as "bad," "stupid," or "willful." It is not enough to know something or remember it; one must get that knowledge into one's behavior. Infants and young children, in whom prefrontal cortex is not yet mature, sometimes do the wrong thing even though they know what they should do and are trying to do it. Their attention is sometimes so captured by the desired goal object that they either cannot inhibit responding (as in delay of gratification or Go-NoGo paradigms) or cannot override the strong tendency to go straight to that goal when an indirect route is required (as in the object retrieval and windows tasks). To sustain the focused concentration required for hearing someone in a noisy room or for a difficult task, one needs to be able to resist distraction; to relate multiple ideas to one another, one needs to resist focusing exclusively on only one idea; when visual perception is misleading, one needs to be able to resist acting in accord with what one sees; and to act in new ways, one needs to resist falling back into one's usual way of acting or thinking. That is, one needs inhibitory control, dependent upon prefrontal cortex. The ability to exercise inhibitory control, which prefrontal cortex makes possible, frees us to act according to what we choose to do rather than being simply creatures of habit or immediate perception. The ability to hold information in mind, which also depends upon prefrontal cortex, enables us to remember what we are supposed to do, to consider alternatives, to remember the past and consider the future, and to use what we know -- not just what we see -- to help guide our actions and choices. These abilities make it possible for us to solve new, undreamed-of challenges, permitting us to exercise free will and self-determination. Not that it's easy, of course; but prefrontal cortex helps make it possible.

29. A Model System for Studying the Role of Dopamine in the Prefrontal Cortex during Early Development in Humans: Early and Continuously Treated Phenylketonuria

Acknowledgements

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