Delayed Recovery of Function following Orbital Prefrontal Lesions in Infant Monkeys

Abstract. *Monkeys given orbital prefrontal lesions at 1, 4, or 8 weeks of age exhibited a severe learning disability when they were tested at 1 year of age, but showed substantial recovery by the time they were 2 years old. These results suggest that the protracted maturation of intact cortical regions is important in recovery of function after early brain injury.*

Recovery of function following cortical injury is generally greater if the injury is sustained in infancy rather than in adulthood (*1, 2*). An exception to this rule has been described in the case of monkeys given orbital prefrontal lesions at 2 months of age (*3, 4*). These monkeys were initially tested on a variety of behavioral tasks when they were 1 year old and later retested on one of the most sensitive tasks when they were approximately 2 years old. Initially, the monkeys operated on in infancy were impaired on the same tests and to the same degree as monkeys given identical lesions as juveniles, but later, at the 2-year stage of development, the performance of the early operated monkeys showed substantial recovery.

One explanation of these findings is in terms of the widely held view that recovery of function will occur only if injury is sustained before the region of the brain involved matures (*2*). The finding that monkeys given orbital lesions at 2 months of age were impaired at the 1-year stage of development suggests that the orbital cortex may develop relatively early in ontogeny and that by 2 months of age it may already be committed to its adult functions. Injury at 2 months, then, may have an effect characteristic of older rather than younger animals. It is reasonable, therefore, to ask whether lesions made before 2 months of age would lead to sparing rather than impairment of function. If they do, such sparing would be consistent with other findings in the literature, and the differences in the particular age after which recovery did not ensue would reflect differences among cortical areas in their developmental status at the time of injury. However, this explanation, which emphasizes the relative maturity of the cortical area at the time it is damaged, would still leave unexplained the phenomenon of recovery observed at the 2-year stage of development.

An alternative view, which may explain both the initial impairments and the later recovery, emphasizes the importance of the maturational status of the parts of the brain that survive injury. The relative maturity of intact cortical areas also varies with age at surgery, and whether recovery of function takes place may depend on the readiness of this remaining cortex to assume the functions of the injured area. One way to estimate the contribution to recovery mechanisms of this developmental factor is to compare the effects of early injury at different periods of life subsequent to surgery.

In order to evaluate these alternative views, we compared the behavior of monkeys given orbital prefrontal lesions within the first, fourth, or eighth week of life with that of age-matched unoperated controls at two subsequent stages of development: initially between 1 and 1½ years of age and again later on one of the tests at 2 years of age.

Our results indicate that recovery of orbital function is not governed by the developmental maturity of the area removed but is a function instead of the maturation of uninjured brain tissue. Taken together with other recent findings, the evidence suggests that age at surgery is importantly related to recovery, not, as has been tacitly assumed, because an area is removed before its own development, but because the surgery is performed before the development of the regions that may eventually substitute for the damaged region.

The subjects for this investigation were 26 rhesus monkeys (*Macaca mulatta*). Nine were unoperated controls, five sustained bilateral orbital lesions within the first week of life, five were operated on at 4 weeks of age and seven at 8 weeks of age. The histological findings in four of the monkeys operated on at 8 weeks have been reported previously and the lesions were as intended (*4*). The remaining monkeys are subjects in continuing investigations and reconstructions of their lesions will not be available until these studies are completed. We feel justified in reporting some of our findings at the present time both because the surgical procedure has been performed reliably in dozens of animals in this laboratory and also because the finding of principal interest in this study emerges from comparisons of the same subjects over time rather than from comparisons between subjects. A diagram illustrating the boundaries of the lesion is shown in Fig. 1. The orbital resection was produced by aspirating the cortex from a line 3 to 5 mm below and roughly parallel to the principal sulcus, including the inferior cortex of the lateral surface, and all the tissue on the ventral surface save for the lateral olfactory stria. The bilateral lesions were performed in a single stage under aseptic conditions. The monkeys were anesthetized with methoxyflurane administered by nasal inhalation.

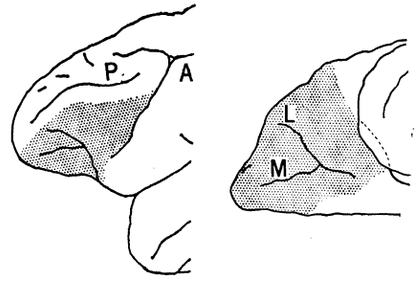

Fig. 1. Diagrammatic representation of the orbital prefrontal lesions; *P*, principal sulcus; *A*, arcuate sulcus; *L*, lateral orbital sulcus; *M*, medial orbital sulcus.

The infants were separated from their mothers at birth and reared in pairs or triplets until they reached 1 year of age. They were then housed singly to facilitate behavioral testing, which began at this time. All testing was conducted in a Wisconsin General Test Apparatus, containing a stationary test tray with two food wells, placed 33 cm apart. Initially, the monkeys were taught to retrieve halved peanuts from the food wells by displacing the mat board plaques which covered them. They were then given the following sequence of tests: spatial delayed response, visual pattern discrimination, spatial delayed alternation, and object discrimination reversal. Training was conducted daily in sessions of 30 trials and, unless otherwise noted, the criterion for learning was 90 correct responses in 100 consecutive trials.

The delayed-response test required the monkey to remember for as long as 5 seconds which of the two food wells he had just observed being baited. As may be seen in Fig. 2A, the degree of impairment on delayed response was moderate and a few operated monkeys performed as well as the unoperated

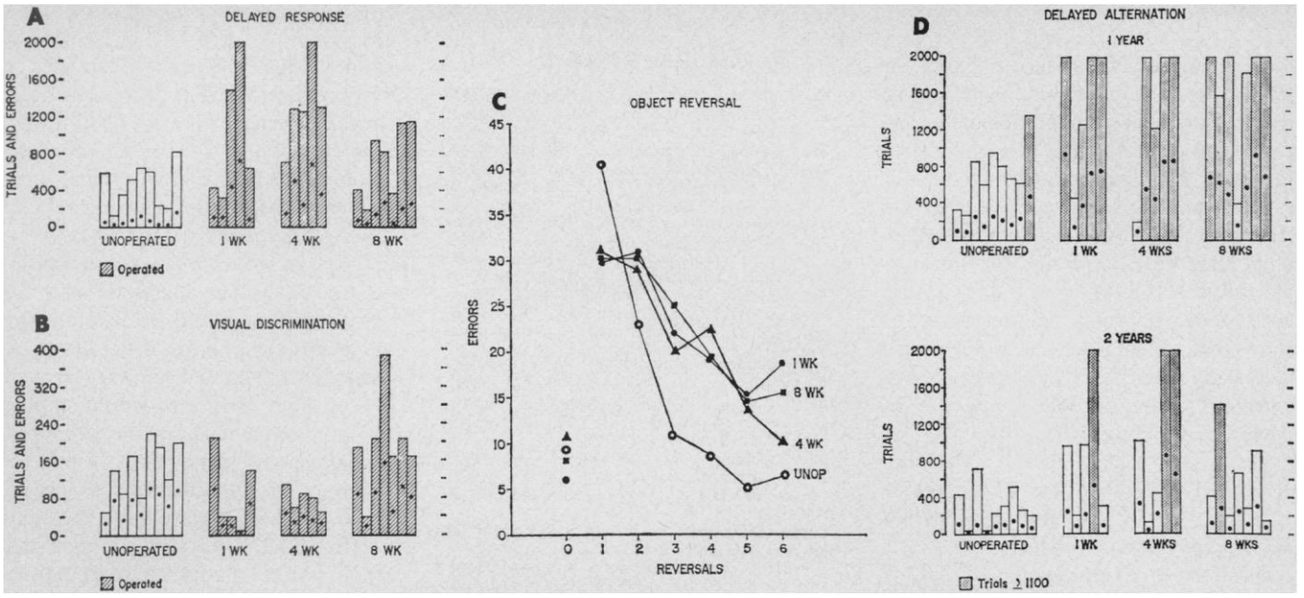

Fig. 2. (A) Trials and errors on delayed response. (B) Trials and errors on visual pattern discrimination. (C) Errors on the initial discrimination and on each of the six reversals in the object reversal problem. (D) Trials and errors on delayed alternation at 1 year and 2 years of age. Bars indicate trials; dots, errors. Trial and error scores do not include the criterion trials.

controls. Such monkeys were distributed among all of the operated groups. Even including them in the statistical analyses, the operated groups were significantly more impaired than the controls in both the number of trials and the number of errors made during learning ($P < .01$, analysis of variance).

The second task was a control task—simultaneous visual pattern discrimination. Figure 2B shows that 16 of the 17 operated animals performed well on this task, providing evidence that the monkeys were well motivated and that learning abilities not dependent on the frontal cortex were intact.

For object discrimination reversal, a task selectively sensitive to orbital damage, the monkeys were first taught to discriminate between two objects. Then the reward contingencies were reversed and the previously negative object was made positive. Six such reversals were given, with the criterion for learning each reversal consisting of 27 correct responses in 30 trials on two consecutive days. Figure 2C presents the results obtained on this test in terms of the numbers of errors made per reversal. There were no differences among the operated groups and all were significantly impaired compared with the unoperated controls ($P < .01$, Newman-Keuls test).

The task most sensitive to prefrontal injury is spatial delayed alternation. This test requires the monkey to alternate his responses to the right and left food wells on succeeding trials. A 5-second delay interposed between trials forces the monkey to remember which food well he responded to last. As shown in the upper part of Fig. 2D, the orbital lesions produced striking deficits on this task. There were no significant differences among the three operated groups and each was significantly impaired relative to the unoperated controls ($P < .05$, Newman-Keuls test). Although testing on delayed alternation was continued for 2000 trials, most of the operated monkeys failed to learn the task, and those that did manage to reach criterion were evenly distributed among the groups.

Thus, when the monkeys were tested between 1 and 1½ years of age, the operated groups were moderately impaired on delayed response, seriously impaired on object reversal, and in general failed to learn delayed alternation irrespective of age at operation. These results are in marked contrast to those obtained when the monkeys were retested on delayed alternation at 2 years of age. At this age, all but three of the operated monkeys were able to solve the problem and their performance was not significantly different from that of unoperated controls (Fig. 2D, lower). Such recovery is peculiar to young monkeys since in monkeys operated on as juveniles, ability to solve delayed alternation is not restored even after equivalent training and test-retest intervals (*4*). The recovery phenomenon thus seems to require a developmental explanation.

We believe that the long-range recovery exhibited by monkeys given orbital prefrontal lesions in infancy may be due to the maturation of another cortical region during the second year of life, perhaps the dorsolateral prefrontal cortex. Both the dorsolateral and orbital regions are granular cortical areas, both receive projections from the dorsomedial nucleus of the thalamus, and both are necessary for normal performance on delayed-response tasks (*3–5*). However, whereas monkeys given orbital lesions in infancy are impaired on these tasks at 1 to 1½ years, monkeys given dorsolateral lesions in infancy do not exhibit deficits of this type until they are 2 years old (*4*). The late emergence of abnormality in these monkeys at 2 years of age suggests that the dorsolateral cortex would normally become functionally mature at about this age. It has been proposed that the dorsolateral and orbital prefrontal cortical areas have nonreciprocal capacities for compensation of each other's injuries because they develop ontogenetically at different rates (*4, 5*). That is, in the case of orbital prefrontal injury in infancy, the dorsolateral cortex or some other late-developing region might derive a capacity for compensation by virtue of being relatively immature at the time of orbital injury. However, if the orbital cortex develops early in ontogeny, as the behavioral evidence suggests, its capacity for compensation would be limited in the event of dorsolateral injury.

Most of the evidence for plasticity of

the central nervous system is based on relative sparing of functions following early brain damage. Paradoxically, the cases with orbital lesions presented here, in which initially orbital functions fail to be spared, may turn out to be stronger models for developmental plasticity than cases in which the immediate result is lack of impairment. In the latter case, it would be necessary to show that deficits do not appear with increasing age before absence of impairment could be interpreted as recovery of function.

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Carbon Dioxide and pH: Effect on Species Succession of Algae

The report of Shapiro (*1*) raises a number of perplexing questions regarding the role of inorganic carbon in the eutrophication process. Although there is considerable circumstantial evidence (*2*) to support Shapiro's hypothesis that blue-green algae predominate at high *p*H levels ($p\mathrm{H} > 9$), it is difficut to accept his or King's (*3*) conclusion that the predominance of blue-green algae results from a lowering of the CO_2 (aqueous) concentration as the *p*H rises in natural waters. Thus, the implication from their work is that blue-green algae have a higher affininty for CO_2 (aqueous) than green algae.

I contest this point on several grounds. First, I know of no evidence in the literature that the growth rate of algae is controlled solely by the CO_2 (aqueous) concentration. Rather, as I have demonstrated (*4*), algal growth rates in an inorganic carbon–limited growth situation are controlled by the total inorganic carbon concentration ($C_T = CO_2 + H_2CO_3 + HCO_3^- + CO_3^{2-}$) regardless of the *p*H. As I pointed out (*4*), on the basis of the established values of the rate constant for the dehydration of H_2CO_3, only in the most dense algal cultures (for example, stabilization ponds or controlled laboratory cultures) would such a high demand be placed on the C_T reservoir that the rate reaction for the dehydration of H_2CO_3 to CO_2 (aqueous) would become a limiting step for algal growth. In reality for most natural waters, regardless of the *p*H, it is not the CO_2 (aqueous) concentration that controls algal growth rates, but rather the C_T concentration.

In addition, if the dehydration of H_2CO_3, or, more accurately, the direct conversion of HCO_3^- to CO_2 (aqueous) at high *p*H ($p\mathrm{H} > 10$), is not a rate-limiting step, then from an ecological point of view it should make little difference what form of inorganic carbon is directly taken up by algae. As an example, it has been suggested by a number of researchers that certain species of both green and blue-green algae can utilize HCO_3^- directly (*5*). Because in the *p*H range from 10 to 11 HCO_3^- and CO_3^{2-} are the major forms of inorganic carbon, it could be argued that at high *p*H algal species capable of using HCO_3^- directly would be able to win out in competition with species that use only CO_2 (aqueous). It can easily be shown (*4*), on the basis of a few simple calculations, that for practically all natural waters the rate at which CO_2 (aqueous) is supplied from HCO_3^- via chemical reactions is still considerably greater than the rate at which it is assimilated by algae. Thus, at high *p*H, regardless of what form of carbon an algal species uses, only the C_T concentration is of importance.

The factor that is important, however, is the relative affinities for C_T of green and blue-green algae, as represented by their respective half saturation coefficients (K_s) for C_T-limited growth. As I have shown (*4*), in the *p*H range from 7.1 to 7.6 the K_s values for C_T-limited growth of two typical green algae were 0.2 to 0.6 mg/liter (*Scenedesmus quadricanda*) and 0.4 to 1.3 mg/liter (*Selenastrum capricornutum*), and the K_s values increased with increasing *p*H. It might be possible that blue-green algae have even lower K_s values for C_T-limited growth, particularly at high *p*H; this would explain their predominance in some situations. This explanation, however, would be valid only for conditions of C_T-limited growth. Many researchers, including Shapiro and I, are of the opinion that carbon is not limiting in most natural waters (*6*). The ability of blue-green algae to thrive at high *p*H must then be explained by other factors. Shapiro's evidence that low CO_2 concentrations (that is, high *p*H) resulted in the predominance of blue-green algae, and that through the addition of 100 percent CO_2 and a concomitant reduction in the *p*H green algae succeeded blue-green algae must be considered highly circumstantial.

My second line of reasoning to counter Shapiro's claims concerns the effect of *p*H on the availability of several algal nutrients. At high *p*H levels the solubility of such essential nutrients as phosphorus, iron, and many trace elements is very low (*7*). The possibility that blue-green algae have a higher affinity for one or more of these nutrients could explain their predominance at high *p*H values. Shapiro (*1*) actually alluded to this possibility by suggesting that blue-green algae have a greater affinity (that is, lower K_s value) than green algae for phosphorus. Therefore, is it not possible that, as the *p*H rises as a result of algal growth, the phosphorus concentration in a sample of natural water decreases through chemical precipitation, and phosphorus, if it is not already the limiting nutrient, becomes limiting? Then could not blue-green algae, because of their ability to utilize lower concentrations of phosphorus, acquire an ecological advantage over green algae? In view of the fact that the chemistry of phosphates and other sparingly soluble compounds is extremely complex and so influenced by *p*H, it is crucial that this point be clearly defined and investigated in any experiments dealing with the effect of *p*H on species succession.