Recovery of function after brain damage: The effects of nimodipine on the chronic behavioral deficit

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It is commonly found, although not often documented or studied, that while the performance of brain-injured individuals may achieve some measure of recovery, this recovery is nonetheless below the performance of normal individuals. In the present research the drug nimodipine, which has been shown to be effective in treating several different brain pathologies, was evaluated as a possible candidate for reducing the long-term deficit that prevents the brain-injured individual from reaching normal levels of performance.

Investigators interested in the recovery of learned behaviors disrupted by neocortical injury typically employ an experimental paradigm in which animals are (1) required to learn some behavior, (2) subjected to brain injury, and (3) required to recover—that is, perform—the previously learned behavior. In the usual experiment, the criteria for original preoperative learning and postoperative recovery are the same. As intuitively correct as this procedure may seem, and notwithstanding its incredibly significant contribution to our understanding of brain function, it has a rather severe drawback—namely, that simply assessing whether a brain-injured animal is able to attain some preoperative learning criterion does not necessarily mean that complete recovery has occurred, that the effects of the brain injury have been reversed, and that the animal is now able to behave normally.

For example, if lesioned animals are tested for long periods of time subsequent to reaching criterion performance after brain injury, it is quite apparent that the recovery is often incomplete. In fact, their performance is usually some 5 to 15 percentage points lower than that of normal subjects tested on the same problem (Chappell & LeVere, 1988; Cooper, Freeman, & Pinel, 1967; Lashley, 1930; LeVere & Mills, 1977). And the data indicate that this deficit is chronic and long-term and does not change or subside, even when testing continues for many months after brain injury.

Notwithstanding the tenacious character of this long-term deficit, the deficit can nonetheless be manipulated by controlling certain conditions of the test situation. Reducing the time between individual test trials, for example, will significantly lower the number of errors committed by a visual decorticate rat tested on a learned

brightness-discrimination task, whereas increasing the time between test trials will significantly increase the number of errors (Chappell & LeVere, 1988). Normal animals are, however, quite unaffected by either of these manipulations. While the effect of these sorts of maneuvers may have some theoretical importance, they are, unfortunately, of little practical consequence and provide little insight into the cause or nature of the long-term deficit.

A more useful strategy might be to approach the chronic long-term deficit with drugs. One compound that has shown considerable promise in alleviating some of the acute consequences of brain pathology is the dihydropyridine calcium channel blocker nimodipine, which reduces cell death following stroke (Scriabine, Schuurman, & Traber, 1989; see also Young, Teasdale, Tettenborn, & Scriabine, 1991) and also facilitates behavioral recovery of function after brain injury (Finger, in press; LeVere, Brugler, Sandin, & Gray-Silva, 1989), as well as improves short-term memory in aged rabbits (Deyo, Straube, & Disterhoft, 1989), rats (LeVere & Walker, 1991), and subhuman primates (Jasmin, Szeliga, & LeVere, in press; Sandin, Jasmin, & LeVere, 1990). These data strongly imply that brain calcium regulation is an important factor contributing to the behavioral consequences of a number of brain pathologies. The present research was an attempt to determine whether such might also be the case with the long-term consequences of cerebral injury.

METHOD

Subjects

The subjects were 20 6-month-old male hooded rats born and raised in our laboratory colony, which is temperature controlled and maintained on a 10:14-h reversed light:dark cycle. These animals had previously participated in an experiment designed to evaluate whether nimodipine-supplemented diets would facilitate the initial recovery of a learned brightness discrimination following visual neocortical injury (LeVere, Ford, & Sandin, 1992). In that study, 10 of them had been maintained on a normal diet and 10 had been maintained on a diet supplemented with nimodipine (1,000 ppm), and all 20 had postoperatively reestablished a 9/10 criterion performance.

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Apparatus

The apparatus was our computer-controlled rat automated training system, which has been previously described in detail (see LeVere & Fontain, 1978). Briefly, the apparatus is an equilateral Y-maze with walls and ceiling fabricated from plastic and a floor of brass rods that form a shock grid. Translucent panels at the end of each arm of the Y-maze can be illuminated by a 7-W bulb (110 V ac), or not illuminated, to produce the brightness cues that the animals must learn to discriminate. The maze itself is housed in a large acoustical chamber to mask external sounds.

Operationally, the apparatus is used in a discrete-trial training mode. A trial begins when the brightness of the rear panel of the alleys changes and a mild pulsating footshock (1 sec on, 1 sec off; 0.1–0.5 mA) is initiated. All animals are trained to escape footshock by entering the alley that has the lighted rear panel. Thus, a trial begins when the light in the alley occupied by the rat is extinguished and the rear panel of one of the two unoccupied alleys is illuminated. The rat is then required to enter the alley with the lighted rear panel and interrupt a photocell circuit some 5 in. from the end of the alley. If the animal enters an incorrect—that is, a dark—alley, the trial is scored by the computer as incorrect but continues until the animal enters the correct, lighted, alley. Once the animal is in the correct alley, a 15-sec intertrial interval (ITI) begins, during which the cue conditions remain constant. During the ITI, the animal is required to remain in the alley. Any entry into another alley suspends the ITI and reinstates the pulsating footshock until the animal returns to the previously chosen, lighted, alley, at which time the ITI is resumed.

The diets that the animals were fed were manufactured by Dyets Inc. (Bethlehem, PA) from the same lot of Purina (No. 5001) powdered Rat Chow. The nimodipine pellets contained a supplement of 1,000 ppm nimodipine; the control pellets were plain Purina Rat Chow. Thus, with the exception of nimodipine, the pellets were identical.

Procedure

The 10 animals previously fed a nimodipine-supplemented diet and the 10 animals previously fed a plain diet during LeVere et al.'s (1992) procedure were each randomly divided into two groups of 5, so that the present experiment consisted of four groups. Two of the four groups continued on their original diet: Group N-N had the nimodipine-supplemented diet; Group P-P had the plain diet. The diet of the two remaining groups was switched—from a nimodipine-supplemented diet to a plain diet for Group N-P; and from a plain to a nimodipine-supplemented diet for Group P-N.

Each group was then maintained on its respective diet and tested in the same apparatus, under the same conditions, that was used when the animals originally learned, and *recovered*, the brightness discrimination after visual neocortical injury (see LeVere et al., 1992). Testing was carried out over a 5-week period, for 5 days each week. Each testing session consisted of 25 trials. The major dependent measure was the percentage of first-choice-correct responses during each day of testing.

At the end of the 5-week test period, each animal was deeply anesthetized and perfused through the heart with saline followed by buffered formalin. The brain of each animal was then removed and the extent of the neocortical injury drawn on a standard surface diagram with the aid of a dissecting microscope. The brains with the largest and smallest lesions in each group were sunk in formalin and sucrose, frozen, sectioned at 50 μ , and stained with cresyl violet for microscopic analysis.

RESULTS

One of the subjects in the N-P group died during the extended training period, so the following data represent 19 rats.

Histology

The neocortical lesions in LeVere et al.'s (1992) study were produced by aspiration with a small glass pipet after a bone flap of the necessary size had been detached. It was intended that all neocortex posterior to a bilateral line beginning at bregma and becoming tangential to the rhinal sulcus be removed. The surface diagrams of these lesions demonstrated that they were as intended, with little variation among the four groups. The mean neocortical injury, as determined by planeometry, for the N-N, P-P, N-P, and P-N groups was 47.0%, 45.8%, 47.5%, and 46.0%, respectively. An analysis of variance (ANOVA) of these data indicated no significant differences between the groups ($F < 1$). In all cases, all six layers of the neocortex had been destroyed without damage to any subcortical structures.

Microscopic analysis of the cresyl violet material indicated complete degeneration of principal cells within the dorsal lateral geniculate nucleus in all cases. The ventral lateral geniculate nucleus appeared normal. As would be expected from lesions of this size, there was considerable loss of the corpus callosum and some cell loss in the posterior thalamic nucleus and pretectal area. However, there were no visually detectable differences among the groups.

Behavior

We were unable to detect any feeding differences between the groups maintained on plain pellets and pellets supplemented with nimodipine. Whatever the animals were fed, they consumed similar amounts of food and gained similar amounts of weight. In addition, each animal showed similar response latencies during testing. This replicated the results of LeVere et al.'s (1992) study and reaffirmed that diets supplemented with nimodipine do not have any general excitatory or inhibitory influences on behavior or any gross motor consequences.

The 5-week behavioral test data are shown in Figure 1, where the animals are grouped according to their diet dur-

Figure 1. Mean percent incorrect for each of the four experimental groups during the 5-week test period. The animals are grouped according to their diet prior to and during the present experiment. See text for additional details.

ing LeVere et al.'s (1992) procedure and during the present experiment. For example, the N-N group was fed a nimodipine-supplemented diet in LeVere et al.'s study prior to the present experiment and continued on a nimodipine-supplemented diet during the experiment. The P-N group was fed a plain diet prior to the present experiment and then switched to a nimodipine-supplemented diet during the experiment. The other groups are similarly labeled.

Inspection of Figure 1 suggests that during the 1st test week the groups performed about equally, which is as it should have been, since all animals had "recovered" the visual habit in LeVere et al.'s study. It is important, however, that over the 5-week test period there was considerable improvement in the performance of the groups maintained on a nimodipine-supplemented diet. To quantify this, we first calculated a repeated measures ANOVA for each group, using mean daily percent incorrect responses during each of the 5 weeks as the dependent measure. For the N-N group, overall $F(4,16) = 4.28$, $p < .05$. Linquist's (1953) critical difference score ($p < .05$, two-tailed) for these data was 1.75, which indicated that the significant overall F value was due to the fact that the N-N animals made significantly fewer errors during Weeks 3 and 5 than during Week 1. Weeks 2 and 4 did not differ significantly from Week 1 or from Weeks 3 and 5. The performance during Weeks 3 and 5 did not differ significantly.

For Group P-N, overall $F(4,16) = 12.90$, $p < .01$. The critical difference score for these data was 1.06, indicating that the animals committed significantly fewer errors during Weeks 4 and 5 than during Week 1. Weeks 2 and 3 did not differ significantly from Week 1 or from Weeks 4 and 5. The performance during Weeks 4 and 5 did not differ significantly.

Overall, for the N-P and P-P groups, $F(4,16) = 1.56$ and $F(4,16) = 2.31$, respectively. Neither value is significant according to conventional standards.

The preceding analysis indicates that there was significant improvement during the 5-week test period by the animals maintained on a nimodipine diet as opposed to plain food pellets. To confirm this improvement, the terminal, Week 5, performance of the groups that were fed the nimodipine diet in the present research (N-N and P-N) and the terminal Week 5 performance of groups that were fed the plain diet in the present experiment (P-P and N-P) were compared in two separate t tests. Both tests failed to reach significance ($t < 1$ for both tests). Accordingly, the 5th-week performance of the nimodipine diet groups was pooled and the 5th-week performance of the plain diet groups was pooled. Another t test was then performed, to determine whether the terminal performance of the animals fed the nimodipine-supplemented diet was different from that of the animals that received a plain diet. This t value was significant [$t(17) = 2.38$, $p < .05$, two-tailed], confirming that a nimodipine-supplemented diet reduced the chronic long-term deficit of visual decorticate rats given extended testing on a brightness discrimination. These data are graphically presented in Figure 2.

Figure 2. Pooled mean percent incorrect performance of all animals fed a nimodipine-supplemented and all animals fed a plain diet in the present experiment.

DISCUSSION

Few would argue that some amount of behavioral recovery is to be expected after most types of brain injury. The amount of this recovery, however, is an entirely different matter, and it is often hotly debated. The issue underlying these debates is not really how much behavioral recovery can, and/or will, occur, but rather how to define behavioral recovery (see LeVere, 1988).

For example, if one endorses the notion that behavioral compensation is an instance of behavioral recovery, then behavioral recovery can be quite extensive if not complete. Unfortunately, to accept compensation as an instance of recovery of function says little about the nature of the behavioral deficit. On the other hand, if one is somewhat more of a purist and adheres to the definition suggested by Almli and Finger (1988) that "recovery is a theoretical construct that implies a complete regaining of identical functions that were lost or impaired after brain damage," then behavioral recovery is an elusive, if at all attainable, goal (see Finger, LeVere, Almli, & Stein, 1988). Although it is difficult to adamantly take one position or the other without reducing the number of colleagues who are interested in what one has to say, it is possible to walk a middle path. That is, without denying that compensatory behaviors may serve to benefit the brain-injured individual, it is also possible to try to understand the nature of the behavioral dysfunction and to determine the parameters that control behavioral recovery as it is more strictly defined by Almli and Finger. The present research was carried out under this aegis. Specifically, it is accepted here that reattaining some preoperative criterion of performance after brain injury is certainly beneficial. It is also accepted that this does not represent behavioral normality, because of the chronic long-term deficit. The purpose of the present research was thus an attempt to understand what underwrites the long-term deficit and perhaps to improve the ledger.

Severely limiting the conditions under which a behavior may be expressed is one strategy to reduce the chronic

long-term consequences of brain injury (Chappell & LeVere, 1988). Unfortunately, although these controls may produce a statistically significant improvement in behavior and be of some conceptual importance, they reveal little about the cause of the long-term deficit and are so restrictive that they are clinically irrelevant. The present research represents a more physiological approach to the problem, an attempt to reduce the long-term deficit with drugs. The reason for this was, of course, that pharmacological intervention may be more clinically useful and that the known pharmacology of a drug might provide some indication of why the long-term deficit occurs in the first place.

In this regard, the chronic administration of the drug nimodipine shows some promise. Dietary supplements of this drug not only significantly speed the initial recovery of a learned behavior (LeVere et al., 1992), but also, in the present research, improved long-term performance over that of brain-injured animals whose diet was not supplemented with nimodipine. It must be quickly added, however, that diets high in nimodipine are not a prophylactic against the devastation of neocortical brain injury, as is indicated by the lack of difference between the N-N and P-N groups and the poor performance of the N-P group. Thus, nimodipine does not, within the limits of the present paradigm, provide protection against the consequences of brain injury. Rather, the drug seems to be effective only after the brain pathology has occurred, which means that the drug is able to restore some functions that were disrupted by the brain injury.

The major question for some then becomes why this should be so, and its answer must rest on the physiological nature of the behavioral deficit and the actions of the drug itself. To a behaviorist, this issue is perhaps an annoyance and not at all relevant. The drug will change behavior in a predictable and lawful manner, and, for the behaviorist, nothing is left to do but determine the behavioral limits of this change. To a physiologist, or a molecular biologist, on the other hand, the important issue has just been defined, and it is now necessary to find an explanatory mechanism. Unfortunately, the present investigation does not, and cannot, speak to this directly. Yet certain possibilities may be worth considering.

First, it is well known that nimodipine will reduce cell death by blocking potential activated L-type calcium channels and prevent calcium overloading following cerebral ischemia (Scriabine et al., 1989). But this does not seem to have been the critical mechanism here, because the drug did not provide any protection against the visual neocortical insult (Group N-P)—the animals were fed a nimodipine diet for many weeks prior to sustaining visual decortication. Rather, the mechanism is more likely related to a possible restoration of normal calcium regulation following an event that disrupts this regulation.

Pursuing this line of argument, but not necessarily championing it, one is reminded of the recent suggestions that the dementias associated with aging may be due in part to a loss of calcium regulation (see Davies & Finch, 1987; Khachaturian, Cotman, & Pettegrew, 1989). For

example, there is a massive calcium influx when neurons are depolarized, which the aged brain is unable to correct efficiently (Michaelis, 1989; Smith, 1987). Now if localized brain injury leads to long-term changes in calcium regulation similar to the brain pathologies associated with aging, the restorative effects of nimodipine may be understood or at least guessed at. That is, nimodipine's ability to block potential gated L-type calcium channels may serve to reduce internal calcium accumulation by moderating calcium influx during neural depolarization.

The fundamental point of this suggestion is a simple, but often overlooked, truism. Behavioral deficits following any brain pathology represent the functioning of surviving neural cells, not neural cells that have been lost to the pathology. Neural cells lost because of injury or age do not directly produce aberrant behavior; logically they cannot, simply because they do not exist. Yet it is possible that the neural cells surviving the direct impact of localized injury might biologically respond to the insult in ways that are not always beneficial. Once such response might be to adjust calcium regulation. This is, of course, exactly the circumstance that produces calcium overload and the additional cell death that immediately follows an ischemic event. The affected cells simply cannot expel calcium fast enough to prevent overload and death. Nimodipine corrects this condition by blocking calcium influx. The proposition here is simply an extension of this and suggests that the inability to expel calcium from neural cells may continue, albeit moderated, long after brain injury; and while this does not lead to cell death, it most certainly must impair normal neural function. Put otherwise, the young brain with a circumscribed injury may have more than little in common with the aged brain. A spice to add to the conceptual sauce of this line of thought is that nimodipine appears to benefit both the aged brain (Deyo et al., 1989; Jasmin et al., in press; Sandin et al., 1990; Schuurman & Traber, 1989) and the young brain that has been injured (Finger, in press; LeVere et al., 1989).

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