

AD-A172 466 NEUROTROPHIC SUBSTANCES AND BEHAVIORAL RECOVERY FROM
BRAIN DAMAGE(U) CLARK UNIV WORCESTER MA BRAIN RESEARCH
LAB D G STEIN JUL 83 DAND17-82-C-2205

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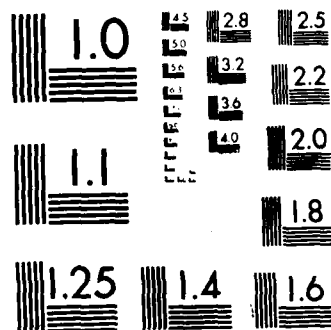
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NEUROTROPHIC SUBSTANCES AND BEHAVIORAL RECOVERY
FROM BRAIN DAMAGE

ANNUAL REPORT

DONALD G. STEIN

July, 1983

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-82-C-2205

Clark University
950 Main St.
Worcester, Massachusetts 01610

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) We have examined the question of whether Nerve Growth Factor (NGF), polyamines, gangliosides and transplants of embryonic neural tissue, can be used to promote recovery from severe, traumatic injury to the head in young and adult laboratory rats. Our data have shown that neurotrophic substances facilitate behavioral recovery from brain wounds if conditions are correctly manipulated. For example, in young laboratory animals, single intracerebral injections of NGF to obtain good results. (Continued on reverse)		

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lead to significant recovery from brain injuries long after the treatment has been terminated. In adults, recovery is less effective following single injections and the subject may require multiple, post-traumatic treatments of NGF to obtain good results. In contrast, intracerebral injections of polyamines promote functional recovery from damage inflicted early in life, but are ineffective in the treatment of brain-damaged adults.

Both repeated, systemic, ganglioside injections as well as implants of fetal brain cells into damaged adult brains, lead to very significant improvements in behavioral performance in brain-damaged subjects. In the present experiments, the methodological parameters have just begun to be examined in detail. Future projects will study the physiological substrates of behavioral compensation following CNS lesions and extend our findings to other parts of the brain.

TABLE I

	INITIAL LEARNING Mean Scores		1st REVERSAL Mean Scores	
	Mean Trials to Learn	Mean Avoidances & Escapes /Trials	Mean Trials to Learn	Mean Avoidances & Escapes /Trials
Sham	50	.77	52	.88
Lesion Only	74	.66	86	.64
Lesion + NGF	40	.84	50	.81
Lesion + Cyto- chrome C	53	.72	87	.62
	P = >.05	>.05	>.05	>.05

TABLE II

	ACQUISITION		RETEST	
	Mean Trials to Learn	Mean Persevera- tive Errors /Trials	Mean Trials to Learn	Mean Persevera- tive Errors /Trials
Sham	58	.04	60	.02
EC Lesions + Saline	42	.03	197	.21
EC Lesions + Polyamine	67	.06	262	.27

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SUMMARY

This contract began on 15 July, 1982, and since that time we have made considerable progress on our research concerning the role of neurotrophic factors in promoting functional recovery from injury to the central nervous system. We have used both behavioral and anatomical measures to evaluate the ability of rats with severe brain injuries to respond to specific treatments that facilitate recovery of behavioral functions. The substances we employ either stimulate damaged neuronal membranes or stimulate neuronal growth and regeneration in both the peripheral and central nervous systems.

Thus, during the past year we have begun to examine the question of whether Nerve Growth Factor (NGF), gangliosides (GM-1), polyamines (putrescine) and embryonic neuronal tissue transplanted to damaged host brains, can ameliorate the symptoms caused by severe brain wounds.

Briefly stated, we were able to show that NGF can facilitate recovery from subcortical lesions in those areas of the brain involved in spatial and motor performance. Our data suggest that multiple injections of NGF in adults result in better recovery than single injections of this substance given at the time of injury. In young subjects, however, a single injection of NGF can produce long-lasting, beneficial consequences.

We have also been able to demonstrate that systemic injections of GM-1 gangliosides can also promote partial recovery from CNS injuries. Likewise, transplants of fetal brain tissue into the damaged areas of adult host brains has led to significant improvement in behavioral performance on a complex, spatial task.

Although our studies have been successful, not every experiment yielded significant results; however, this is not surprising because we are just beginning to understand some of the neural mechanisms involved in recovery from brain damage and many of the specific parameters that can affect or influence this recovery, remain to be evaluated.

It is important to point out that although we requested three years of support, only one year was approved. This was then extended to March of 1984, giving us an additional eight months. As a result, we were required to change our priorities and delay the initiation of some of the projects because of lack of funding and the time to conduct them. If the renewal application is approved for a longer period of support, these studies will be completed in accordance with the contract.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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For the purposes of this report, brief summaries of experiments completed to date are presented. Figures and tables follow the report.

Effects of purified nerve growth factor (NGF) on recovery from caudate nucleus lesions:

In our first experiment, adult male rats were subjected to bilateral lesions of the caudate nucleus (n = 40). One group of rats (n = 10) received sham operations in which no brain damage was inflicted; these animals served as the normal controls. Another group of 10 rats received bilateral caudate lesions followed immediately by bilateral injections of highly purified nerve growth factor directly into the region of injury. A third group with lesions was given an equivalent volume of the protein, cytochrome C, as a control for the NGF treatments, and a 4th group of brain-damaged animals were given only lesions.

All of these rats were tested on a delayed spatial reversal task. Upon completion of all behavioral testing, the rats were killed and their brains removed for histological verification of the lesions and for counting and measuring of neurons and reactive astrocytes in the caudate nucleus. At the present time we are preparing a statistical analysis of the extent of the damage in the groups with lesions and will soon begin our counting techniques to determine if the NGF treatments altered the neuron/glia ratio or the presence of reactive astrocytes in the treated area. This aspect of the research will be completed in October, 1983.

Table I shows the results of the behavioral assays in terms of trials to learn the initial task as well as the percentage of escapes and avoidances made by the 4 groups of rats. Scores for the first reversal are also presented. These measures indicate the extent to which a single injection of NGF, or of cytochrome C, was effective in facilitating the recovery from the caudate lesions.

In brief, clear trends can be seen in the treated animals. In initial learning, the group given NGF took fewer trials to learn than the lesion-only group; the animals were comparable to the sham-operated controls. I was surprised to note that, in initial acquisition, the group given cytochrome C also performed as well as the NGF group; however, it was recently found (James Turner Bowman Gray School of Medicine) that a specific brain growth factor just isolated from pig brain had the same molecular weight as cytochrome C. At present I cannot account for this finding, but it is interesting that on the reversal task, only the NGF-treated animals had scores identical to the sham controls in both trials to learn the reversal and the number of escapes and avoidances of the footshock. Thus, it may be concluded that the cytochrome C effect was temporary while that of the NGF was more long lasting.

NGF Treatments facilitate recovery from lesions inflicted in early life - the effects are long lasting:

With respect to the question of whether NGF effects in brain-damaged subjects are of long duration, we decided to study the effects of single NGF injections in rats given lesions as neonates and tested until they were 80 days of age. This experiment was done in collaboration with Dr. Françoise Eclancher, who visited my laboratory as a guest scientist from the National Center for Scientific Research in Strasbourg, France.

Seven days after birth, groups of rats were given lesions of either the ventromedial nucleus of the hypothalamus or the septal nucleus. Immediately after surgery, half of the animals in each surgical group (n=10/group) received a single, intraventricular injection of 50µg NGF. These animals were then compared to counterparts given identical lesions followed by buffer solution, or to intact controls given either NGF or buffer at the same age. All of the rats began testing on an active avoidance to shock (shuttle-box task) at 20 days of age (20 trials/day) and continued testing every 10 days until they reached 80 days of age.

We hypothesized that if NGF were effective in promoting functional recovery, the rats with septal lesions followed by the treatment should show a diminished capacity for active avoidance learning (rats with septal lesions are paradoxically better on shock avoidance tasks and tend to be much more "reactive" to environmental stimuli than normals). In contrast, the rats with early VMH lesions would be very impaired on the A.A. task so NGF treatment would be expected to improve their performance to the level of the normals.

Figure 1 shows the results of this study which is now being prepared for submission to Developmental Brain Research. Most of the details can be seen in the preliminary draft of the paper appended to this report (Appendix A). Briefly stated, it can be seen in Fig. 1, that the mean number of crossings (a measure of the animal's ability to learn to run at the sound of a tone to escape shock) is significantly decreased for animals with septal lesions given a single NGF treatment early in life. However, it is also clear that the septal "syndrome" in these animals still prevails. Consistent with our hypothesis, the animals with VMH lesions alone were impaired on learning the A.A. task from 30-80 days of age, but there was a clear improvement beginning at 40 days of age in VMH animals given the NGF. It is also interesting to note that the normal animals given a single injection of NGF into the ventricle at 7 days of age tended to have higher numbers of crossings from 40 to 80 days of age than saline-injected counterparts; however, the differences between the two groups on this measure were not significant. In contrast, when latencies of running were examined (a measure of reactivity to stimuli), normal rats given NGF in infancy had much shorter response times than those given saline. This was also true for the NGF-treated rats with VMH lesions (i.e., they improved consistently by about 40

days of age--some 33 days after the NGF injection, and remained better throughout the testing).

Thus, the NGF treatments once again, showed that this trophic substance is capable of facilitating recovery from severe brain injuries. Although the recovery is not complete (the animals do not often perform as well as completely intact animals), it is often significantly better in treated brain-injured subjects than in untreated controls with identical lesions. The effects may be long-lasting or relatively short-lived, requiring perhaps multiple doses for better results. We hope to address this question in much more detail if the contract is renewed.

Effects of polyamine injection (putrescine) on recovery from entorhinal cortex lesions in adults:

We have now completed our experiments examining the effects of putrescine on recovery from brain damage. In this experiment, rats were given lesions of the entorhinal cortex followed by injections of .2 molar solution of putrescine, isotonic saline, or no injection at all. After the treatments the rats began testing on a spatial alternation task for food reinforcement. The behavioral data indicate that the polyamine treatments are not effective in adults so the only histology done in these animals was to verify the lesions.

The experiments on the behavioral effects of polyamine administration (putrescine) to animals with brain lesions made in infants or adult rats have been completed. At present, we are finishing the histological analyses of the brain tissue. In one study, we examined the questions of whether intraventricular administration of putrescine could facilitate recovery from lesions of the ventromedial nucleus of the hypothalamus (VMH). The damage was created at 7 days of age and the rats began testing on an active avoidance task (A.A.) at 20 days of age. Our behavioral results indicated that the rats with VMH lesions, treated with putrescine, and tested at different ages, were able to solve the A.A. task better than their untreated counterparts (see Figure 2). However, they were not as good as the intact controls following the single injection.

As mentioned above, we also examined the effects of putrescine on recovery from hippocampal damage in adult, male rats. We chose the hippocampus because of its involvement in spatial and short-term memory and because previous pilot data with putrescine seemed to show that this substance might facilitate recovery from this brain injury.

In this study our results were disappointing. Putrescine failed to provide any evidence for functional recovery in adult rats with bilateral hippocampal lesions. The reasons for the differences between the developmental study and the present experiment are being examined. The polyamines are important in growth and development but may be less critical in nerve repair.

Given the fact that our work is primarily with adult and perhaps aging subjects and that our other projects are more successful in promoting recovery, I propose to try and publish the present findings and then discontinue our studies of polyamine and concentrate more extensively on the role of the GM-1 gangliosides in aiding functional recovery from CNS injuries. The specific details and rationale are given in the objectives section of the renewal application (See Table 2).

NGF facilitates recovery from hippocampal lesions:

In order to test for the generality of the effects of neurotrophic factors in promoting functional recovery following brain injury, we decided to apply NGF to rats with bilateral lesions of the hippocampus. The animals were given a single, intrahippocampal injection of 25ug purified NGF and one week later began testing on an 8-arm radial maze designed to measure working and spatial memory in rats.

As expected, the hippocampectomized rats with no treatment made the most errors. As trials proceeded, the sham-operated controls visited an average of 7.5 arms before making an error while the rats with hippocampal lesions and no treatments entered only 4 arms before committing their first error. These data suggest that NGF exerts a facilitatory effect on recovery from hippocampal damage. Figure 3 shows that the rats with NGF improve in their ability to learn the radial maze to a greater extent than buffer-treated controls. Thus, only NGF-treated rats showed a significant difference from a zero-slope (regression analysis). This finding can be taken to indicate that neither the buffer-treated nor the control group changed their level of performance over the test sessions. Our data, taken in conjunction with the results of others (Hefti, et al., 1983)¹, suggests that the initial, and perhaps temporary effects, of NGF may be due to the fact that intracerebral administration of this substance can increase choline acetyltransferase, an enzyme necessary for the production of neurotransmitter.

This project has been completed, including histological evaluation of the lesions. In collaboration with Dr. Bruno Will, Laboratoire de Psychophysiologie, Universite Louis Pasteur, Strasbourg, France, we have drafted a manuscript to be submitted to Brain Research, this fall (please see Appendix B).

Multiple injections of NGF via intracerebral cannulas:

Because the results we obtained in several projects that examined the role of NGF in treating recovery from limbic system

¹Hefti, F., Dravid, A. and Hartikka, J. Chronic intraventricular injections of nerve growth factor elevate hippocampal choline acetyltransferase activity in adult rats with partial septo-hippocampal lesions. Basel, Switzerland, Sandoz, Ltd., (In press).

lesions in adult animals were temporary, we decided to explore the possibility that multiple injections of this substance could produce better results. Accordingly, and in collaboration with investigators in Strasbourg and in Basel, Switzerland, we have initiated a new project to test this hypothesis. Six groups of rats received either sham operation or lesions of the fimbria/fornix, the fiber system that carries many of the nerve fibers to and from the hippocampus. The animals were also implanted with indwelling ventricular cannulas for the repeated injections of the NGF. Control groups received either injections of buffer control solutions or thyroxine, another putative, growth-promoting neurotrophic substance. All surgery was completed on these animals, and they will begin testing following a series of alternate-day injections of the neurotrophic factors (Ss are given a total of 8 injections via cannula). Animals will be examined for their ability to solve the 8-arm radial maze (details are described in Stein and Will manuscript, Appendix B).

Pilot data that my colleagues and I have collected so far (Figure 4) suggests that repeated NGF injections can promote a much more significant degree of functional recovery following limbic system lesions than single injections. Upon completion of all behavioral testing, all histological and anatomical testing will be performed in my laboratory. To determine if the treatments have altered the metabolic activity of the damaged structures, we will employ cytochrome oxidase staining techniques along with a ^{14}C -2DG labeled method. The ^{14}C -2DG analyses will be performed by Dr. Ami Isseroff of the Weizmann Institute, Rehovot, Israel. Dr. Isseroff will use computer assisted, false coloring techniques to determine and quantify the density of grains exposed by the isotope. This technique will permit us to evaluate the metabolic activity of the damaged areas in comparison to intact regions of the same animal. The effects of the NGF injections on neuronal and glial metabolism can then be quantified. My collaborators will be responsible for keeping some of the animals to examine levels of ChAT in the different groups. This may tell us whether the repeated doses of NGF have helped to restore adequate levels of ACH, which in turn, would suggest an increase in neuronal sprouting in response to the NGF treatments.

Multiple injections of NGF and caudate nucleus lesions:

In a recent study to be published by my colleagues in Basel and Strasbourg, it was found that repeated injections of NGF through cannulas implanted into the ventricles, produce better recovery of a spatial learning task following lesions of the fimbria/fornix regions in adult rats. Because of these results, we decided to investigate the possibility of facilitating long-term recovery after caudate nucleus damage to a greater extent than we have seen previously with single, intracaudate, NGF treatment. We have now developed the implantation techniques and plan to begin a full-scale study in the 2nd week of September.

For this experiment, three groups of animals will be used. The sham-operated group will consist of adult rats receiving no

treatment and no cannulas. Another group will be given bilateral caudate lesions and implantation of an indwelling intraventricular cannula. Thrice weekly injections of buffer, control solution will be administered to this group for a period of three weeks. Finally, a third group will be given caudate lesions, cannulas and thrice weekly injections of 25ug NGF. Three days after the last injection, the animals will begin testing on the learned avoidance task described for previous experiments. Upon completion of the testing, the animals will be killed and their brains prepared for the histological analyses described earlier in this report and in the text of the reapplication (i.e., lesion verification, neuron/glia ratios, GFAP, etc.). The histological procedures will not be completed until early 1984 since we do not have completely automated equipment to assist us in our measurements.

Development of GFAP immunocytochemistry:

In previously published research, I noted that NGF treatment may alter the response of reactive astrocytes to brain injury. Using the Cajal gold sublimate method, we found that NGF injection produces a time-dependent increase in the size and number of astrocytes in the area of the wound. Although we are convinced that the Cajal method is sound, colleagues have suggested that we corroborate our findings through the use of an immunocytochemical technique that uses specific, glial fibrillary acidic protein (GFAP) antibody to mark astrocytes. Thus, to verify our findings, we have worked with Dr. Amico Bignami of the Boston VA Hospital to develop this procedure. We are currently applying it to brain sections taken from treated and untreated animals with lesions of the caudate nucleus; we have already demonstrated clear evidence of reactive astrocytes with this method and will begin counting these cells as a replication of our previous work. Since glial cells are thought to be a possible source of trophic factors in damaged brains, the demonstration that NGF injections could induce a more intensive, if temporary, glial reaction at the site of injury could be an important finding.

Gangliosides can facilitate recovery from brain injury:

As mentioned earlier in this report, we decided to examine the role of GM-1 ganglioside in facilitating recovery from bilateral brain injuries. Recent experimental reports suggested that repeated, systemic injections of GM-1 were effective in promoting partial regeneration from spinal cord crush. Other investigators showed that GM-1 could stimulate neurite outgrowth in explants of neurons taken from the superior cervical ganglion. Finally, there were also a few reports that GM-1 treatments were effective in facilitating behavioral recovery from limbic system lesions. The in vivo experiments were very interesting to us because the functional recovery was promoted by giving repeated, systemic injections to the brain-damaged animals. Thus the technique avoids the need for intracerebral or intraventricular treatments which are technically more demanding, more time consuming and more dangerous for the subject.

In this study, three experimental groups were employed. One group received no brain damage, whereas the two other groups received bilateral lesions of the caudate nucleus. Of the latter two groups, one was treated with GM-1 ganglioside, whereas the other group was injected only with the vehicle solution. After caudate surgery and 14 days of IP injections of vehicle solution or 30mg/kg GM-1, the rats began to be tested on the active avoidance task. Figure 5A shows that the GM-1 treated group performed the active avoidance task significantly better than untreated animals. However, the sham-operated animals acquired the new behavioral response better than both groups with brain damage.

We have now completed the analysis of long-term effects of ganglioside treatment in our animals. Briefly, the superior performance of ganglioside-treated animals remained stable some three months after the initial task had been completed. This period is roughly equivalent to a 12-year, post-traumatic period in the human subject. In all three groups of rats there was some improvement in the task. Even some non-treated animals recovered from the surgery, thus reducing the differences between the ganglioside-treated and non-treated group (Fig. 5B).

The animals were all sacrificed after retesting was completed, and we are preparing the brains for further histological analyses.

Using three pilot animals, radioactively labeled gangliosides were injected IP 7 days after bilateral caudate nucleus damage (1, 3 and 6 microcuries). The animals were sacrificed 24 hours later and the brains were removed for autoradiography. Mounted brain sections were coated with photographic emulsion, and they are presently being stored for 4 weeks of photographic development. The first results are expected shortly.

Cerebral Isotonic saline injections may alter neuronal degeneration following brain lesions:

As I noted in an earlier progress report and previous publications, we have observed that intracerebrally administered isotonic saline can facilitate recovery from lesions of the caudate nucleus. Admittedly, this is a curious and puzzling phenomenon, but one that we have now seen in several replications conducted prior to work on this contract. Although we cannot yet speculate on specific mechanisms, we decided to determine whether the saline treatment might alter the extent of anterograde degeneration produced by the lesion and then to correlate these anatomical changes with the degree of behavioral recovery observed in a shock avoidance learning situation.

In this study, the animals received a single injection of physiological saline (groups S7 and S31) or no injection (groups L7 and L31) after the brain had been damaged. The groups survived either 7 days (groups S7 and L7) or 31 days (groups S31 and L31). For the short survival group, behavioral testing was done 2-6 days after surgery, whereas for the long-time survival groups, a

9-day postoperative recovery period was permitted and behavioral testing was done on day 10-25. In order to determine the number of intact, striato-nigral projections, the animals received a second lesion caudal to the first lesion 25 days after the first surgery. After the animals were sacrificed, the brain was cut and stained with cresyl-echt violet (to determine the lesion size) and with Fink-Heimer staining procedure for secondary degeneration (to determine the extent of degeneration).

Whereas no significant differences were found in the short survival group, saline-treated animals which survived for 31 days (S31) initially performed significantly better than untreated brain-damaged rats (L31). Animals of group C did not receive any damage. These results are summarized in Fig. 6.

With respect to the extent of damage, groups S7 and L7 had lesions of comparable size and location (caudate nucleus). The same is true when groups S31 and L31 are compared. Due to the secondary lesion, animals which survived for 31 days (S31 and L31) had significantly larger lesions than animals which survived only 7 days (S7 and L7). The second lesion extended into caudal parts of the caudate nucleus and also damaged the globus pallidus partially.

The analysis of the Fink-Heimer material revealed significantly less anterograde, secondary degeneration in the substantia nigra, pars reticulata in saline-treated animals (S7) than in non-treated animals (L7) ($F=7.3$, $p<.03$) (Fig. 7). In group S31, a trend towards more degeneration was observed compared to group L31, indicating that there may have been more intact connections to the substantia nigra which were destroyed by the second lesion (no saline treatment was given then). The degeneration of the short- and long-survival groups adds up to approximately the same amount in saline-treated and non-treated animals (Fig. 8). It is interesting to note that several significant correlations between lesion parameters and behavioral measures could be found in saline-treated animals. In untreated animals there were no such correlations.

Our findings can be taken to indicate that saline injections may help to overcome some of the behavioral deficits that often accompany brain damage. These data are in agreement with our previous results. On the anatomical level, saline prevents anterograde, secondary degeneration following brain injury in structures which are connected to the zone of trauma.

Transplants of fetal brain tissue facilitate recovery in brain-damaged adults:

As interest in the problem of recovery from brain damage continues to grow, a number of different approaches to the problem have been tried. One of the more novel and interesting tactics involves the transplantation of embryonic brain tissue directly into the damaged brain of mature adults. Although others have examined the problem of using embryonic transplants to promote

functional recovery following small cuts of subcortical fiber systems, little had been done to investigate the possibility that brain grafts could mediate behavioral recovery after large, bilateral cortical lesions.

To examine this question in detail, frontal cortex and cerebellar tissue from 21-embryonic-day-old rats were implanted into the damaged frontal cortex of adults. As a control for the specificity of the graft, another group of brain-damaged adults received transplants of cerebellar tissue taken from the embryonic brain. All of the grafts were made 8 days after the host frontal cortices were removed. Following the transplants, the rats were given a 4-day recovery period and then began testing on a delayed spatial alternation task sensitive to lesions of the medial frontal cortex. Normal controls served to provide baseline data against which the performance of the brain-damaged rats, with or without transplants could be compared. The details of this experiment have been published in SCIENCE and are appended (Appendix C).

Briefly stated, we found that the cognitive deficits in spatial alternation learning that accompany frontal cortex lesions were reduced by transplants of fetal frontal cortex but not by implants of age-matched cerebellar material. Subsequent histological evaluation using horseradish peroxidase techniques showed that the transplants that were successful ((i.e., those of frontal cortex) formed continuous bridges connecting the injured hemispheres or formed separate grafts, each adhering to the host cortex. The HRP technique revealed that there were functional connections that developed between the two pieces of transplanted cortex as well as in the medial and dorsal thalamic nuclei; areas of the brain which normally project to the frontal cortex in intact rats. These findings show that the brain is capable of reestablishing contacts with the newly implanted cortical materials. In addition, the survivability of the transplants show that when they do take, they must receive microcapillary and vascular support from the host brain.

Since the recovery was not complete, we decided to examine the possibility that the addition of NGF or cytochrome C might improve the postoperative performance of animals with transplants.

In this experiment rats were given the same lesions, but just prior to the implant, NGF was placed directly into the wound cavity or injected directly into the transplant immediately after it was placed into the host brain.

Our behavioral analysis is almost complete. We found that NGF or cytochrome C supplements do not increase functional recovery over the transplant alone. Of the 31 animals receiving injections in addition to transplants, 22 have been used for histochemical (horseradish peroxidase and cytochrome oxidase staining) and autoradiographic evaluation. The remaining, injected animals as well as the animals without transplants are being used for histological analysis using staining for Nissl substance. When all of the animals are evaluated, we may be able to draw some conclusions

about the relationship between the injections and anatomical parameters such as survivability and size of the transplants, the connectivity of the transplant and the host brain. In addition, we are preparing 2-DG autoradiography on 10 of the transplant animals and the 2-DG uptake will be quantified. Seven of the 31 animals with transplants and no injections have been used for single-unit electrophysiology. We currently have recordings of single neurons within the transplant and hope to expand this to demonstrate synaptic connections between the transplant and thalamic and cortical structures in the host brain that provide afferent fibers to the frontal cortex.

During the past three months, we have also expanded our focus with regard to transplants and their role in functional recovery. We first added a group of animals with medial-frontal cortex lesions and transplants of fetal, frontal cortex from 15-day fetuses. It has been demonstrated that younger tissue, although smaller initially, will grow much larger than older, fetal tissue. These animals have not been evaluated anatomically, but behaviorally they are not significantly different than animals with transplants taken from 18-, 19-, or 20-day fetuses. We also have added a group of animals given GM-1 ganglioside treatment in addition to transplants and a group receiving lactated ringer's injections to control for the injection. These animals are currently being tested.

During the next three months, we will complete the testing and anatomical evaluation for all of the animals mentioned in this report.

In summary, I believe that this laboratory has made considerable progress in meeting the objectives that were outlined in the initial application. As mentioned earlier, this contract has been in operation for just over one year. Given the initial delays in start-up of the projects as well as reductions in time and funding for the experiments, I am pleased that my colleagues, students and I were able to accomplish our goals. Of course, much more basic research needs to be done before we can begin to extrapolate our findings to the amelioration of the human condition. The projects we have completed, as well as those still in progress, are giving us a much better understanding of some of the factors involved in the organization and the repair of the damaged central nervous system. Our work with trophic substances as well as with transplants of embryonic tissue to damaged host brains, may, in the long run begin to provide us with the therapeutic tools necessary to relieve much of the suffering that the brain-damaged patient must face. I very much hope that I will be permitted the opportunity to continue this line of research, and very much appreciate the support that I have received thus far.

LEGENDS FOR FIGURES AND TABLES

Figure 1

This figure shows the number of crossings made by rats in a two-way, shuttle avoidance box. The open triangles and circles show the performance of rats with lesions of the septal nucleus. Throughout most of the testing periods (days of age), the animals given a single injection of NGF at time of surgery performed more like normal controls (black circles), although they were still impaired. The rats with VMH lesions given NGF performed the avoidance task better than saline-treated counterparts (half-filled circles and triangles) from 30 days of age until the end of testing at 80 days of age. Thus the NGF treatments were demonstrated to have long-lasting and beneficial consequences for brain-damaged rats.

Figure 2

This figure shows that rats with VMH lesions created at 7 days of age are impaired on a two-way shuttle avoidance task (open circles), but that a single injection of the polyamine, putrescine, at the time of surgery can significantly improve performance throughout the entire period of testing (black circles) in rats with VMH lesions.

Figure 3

Figure 3 shows that a single intracerebral injection of NGF given at the time of hippocampal injury, gradually improves the rate of learning in comparison to similarly injured animals given buffer control solution. In this experiment the fully mature rats were tested in a complex, 8-arm radial maze.

Figure 4

Figure 4 shows that repeated injections of NGF (administered intraventricularly) significantly enhance performance in a complex, 8-arm radial maze. By the second session of testing, NGF-treated rats with fimbria/fornix lesions (triangles) are performing significantly better than the controls (X---); however, they still are not performing at the level of the completely intact (circles) age-matched controls.

Table I

Table I shows initial learning and reversal learning scores of adult rats given single, intracaudate injections of NGF at the time they received bilateral lesions of the caudate nucleus. The scores presented are for those animals that reached criterion on the initial learning task. The animals given NGF treatment were consistently better than untreated rats with similar lesions or those with brain damage given cytochrome C as a control.

LEGENDS FOR FIGURES AND TABLES (Continued)

Table II

Table II shows acquisition, perseveration and retest scores for adult rats with lesions of the entorhinal cortex given treatments of putrescine to facilitate recovery from the brain lesions. The polyamine administered did not aid the animals with the brain injuries.

Figure 5

This figure shows that repeated, systemic injections of the ganglioside, GM-1, can markedly improve performance of a spatial reversal task in adult rats given bilateral lesions of the caudate nucleus. The effects of this treatment, beginning at the time of the injury, have been shown to have long-lasting beneficial consequences; the treated rats continue to show improved behavioral performance some 3 months after the treatments had terminated.

Figure 6

This figure shows that rats with caudate nucleus lesions given isotonic saline injections directly into the zone of injury perform an active avoidance task better than untreated controls with the same lesions. The lesion-only group (solid black line) had the highest number of escape "failures," while the saline-treated groups (diagonal stripes) performed as well as unoperated controls on this task.

Figure 7

This reconstruction of the region of the substantia nigra shows that the anterograde degeneration caused by caudate nucleus lesions is greater in untreated rats than in those given saline injections into the lesion zone at the time the damage was inflicted.

Figure 8

This figure shows that the area of anterograde degeneration in the substantia nigra of rats with caudate lesions, is less in saline-treated than in untreated controls. The second lesion (open bars) is made to trace the remaining, healthy nerve fibers after the initial damage had been inflicted.

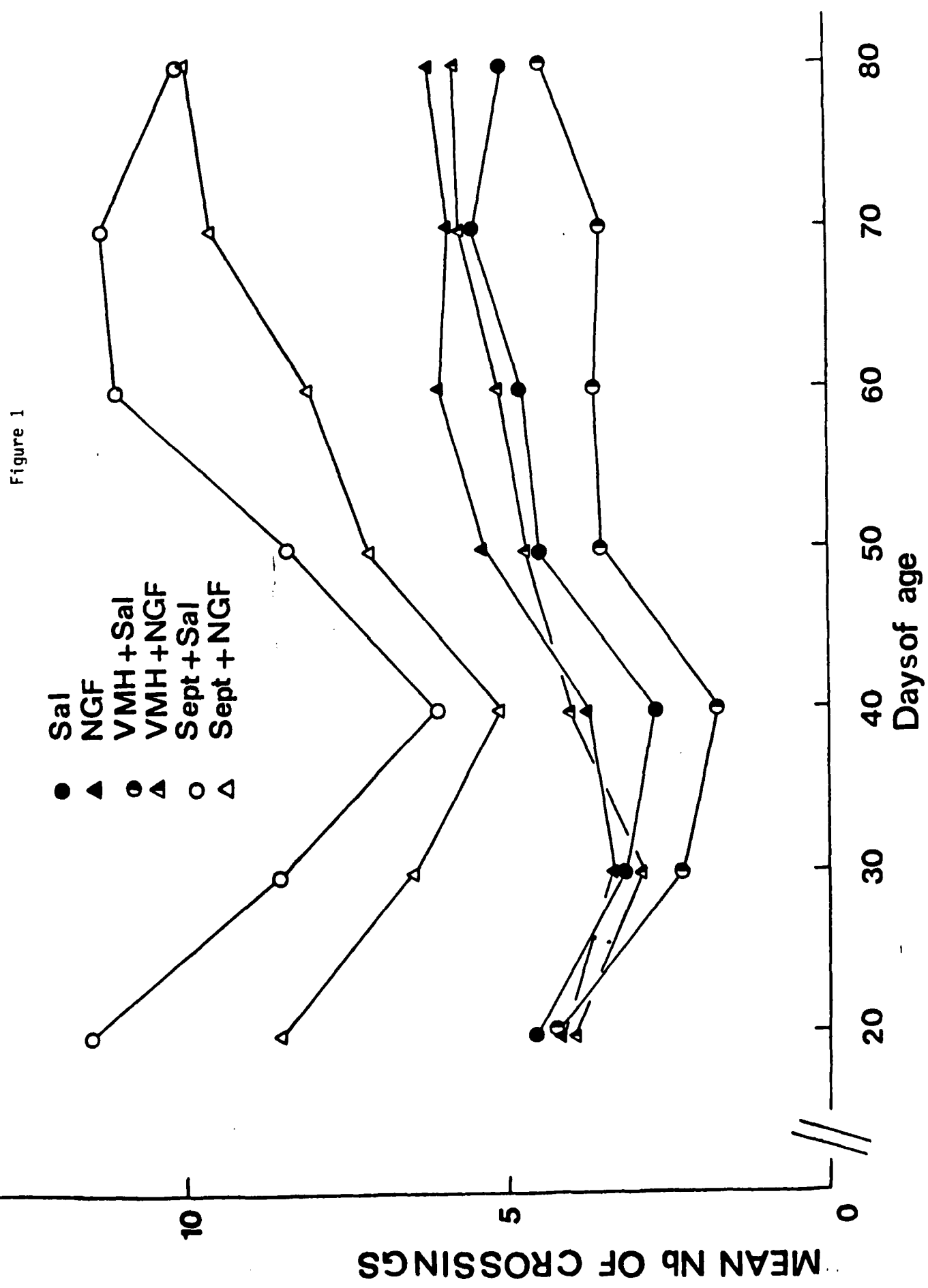


Figure 1

Figure 2

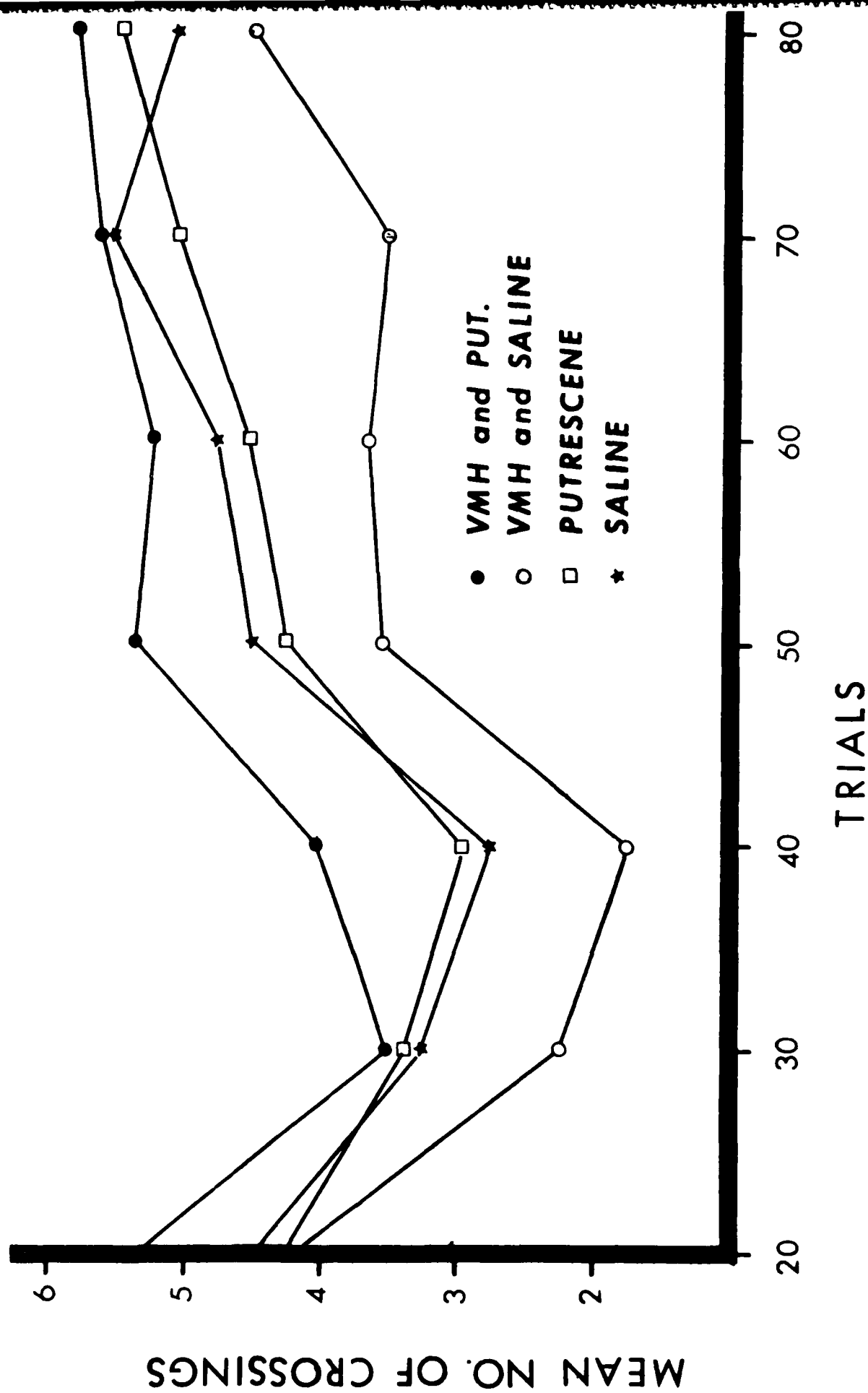
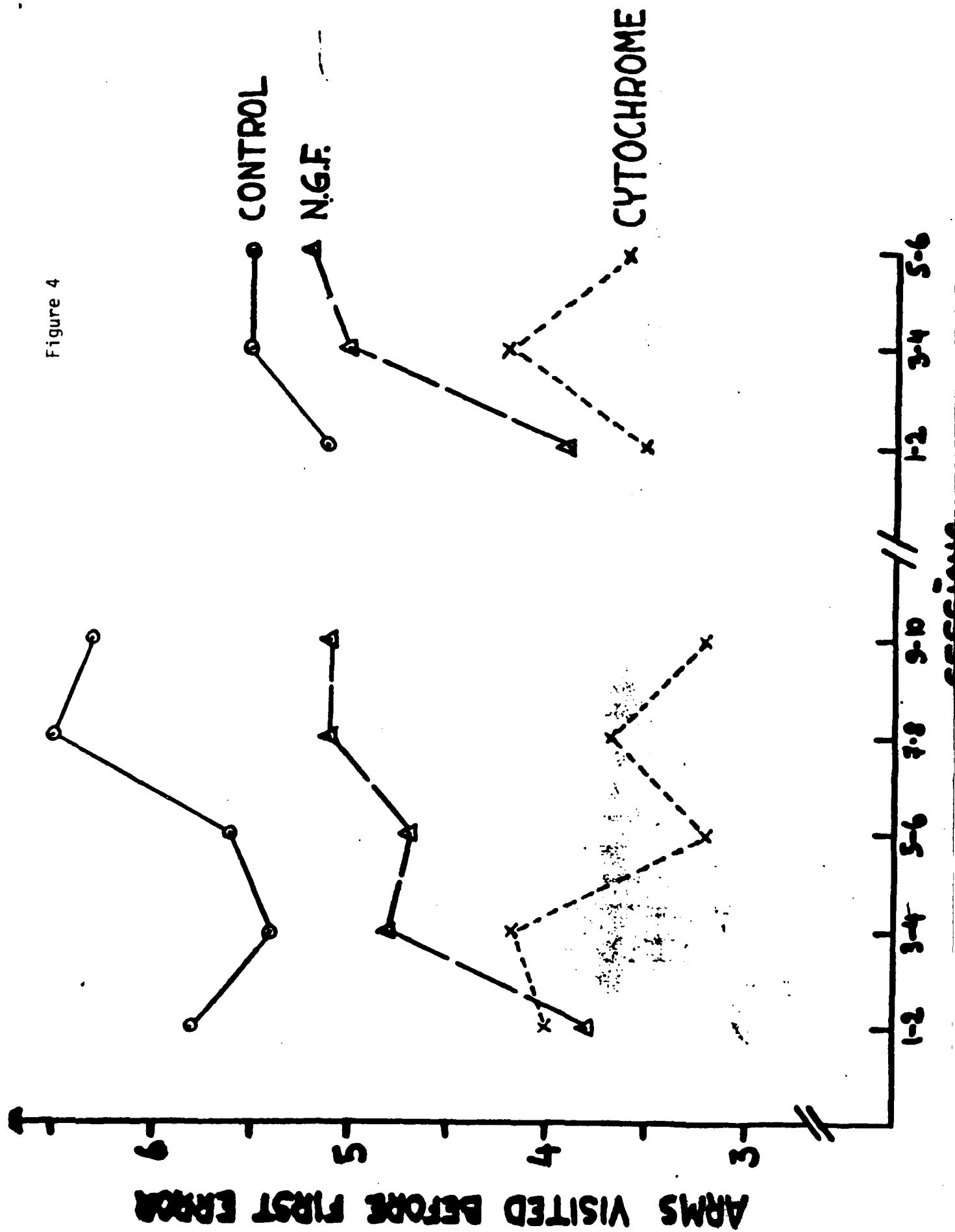


Figure 3



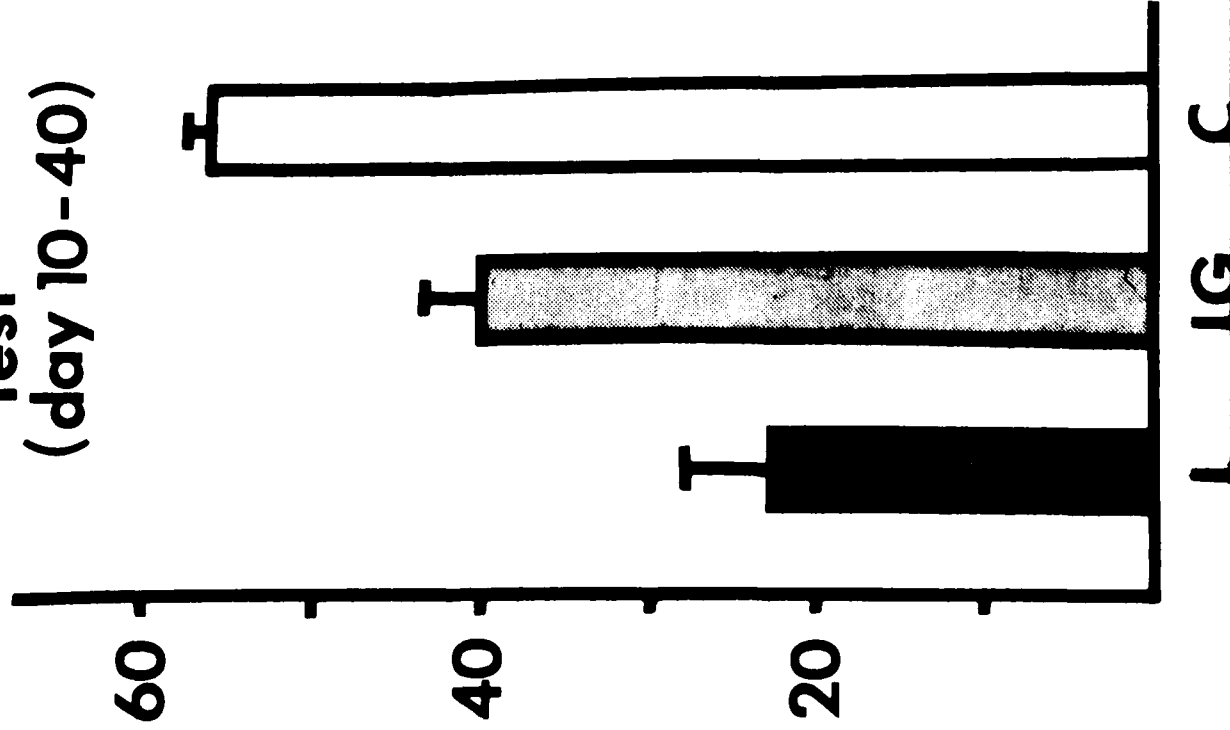
Figure 4



A

**Test
(day 10-40)**

**Percent of days reaching criterion
(9/10 correct responses)**



B

**Retest
(day 90-104)**

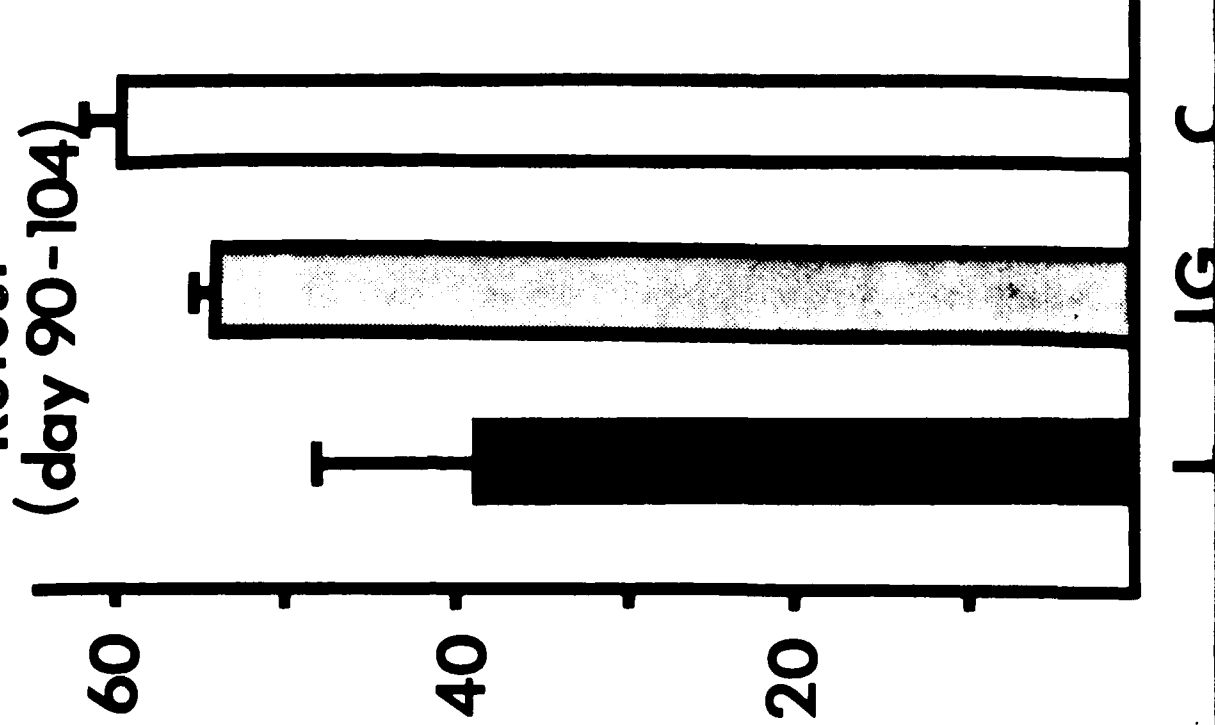


Figure 5

Figure 6

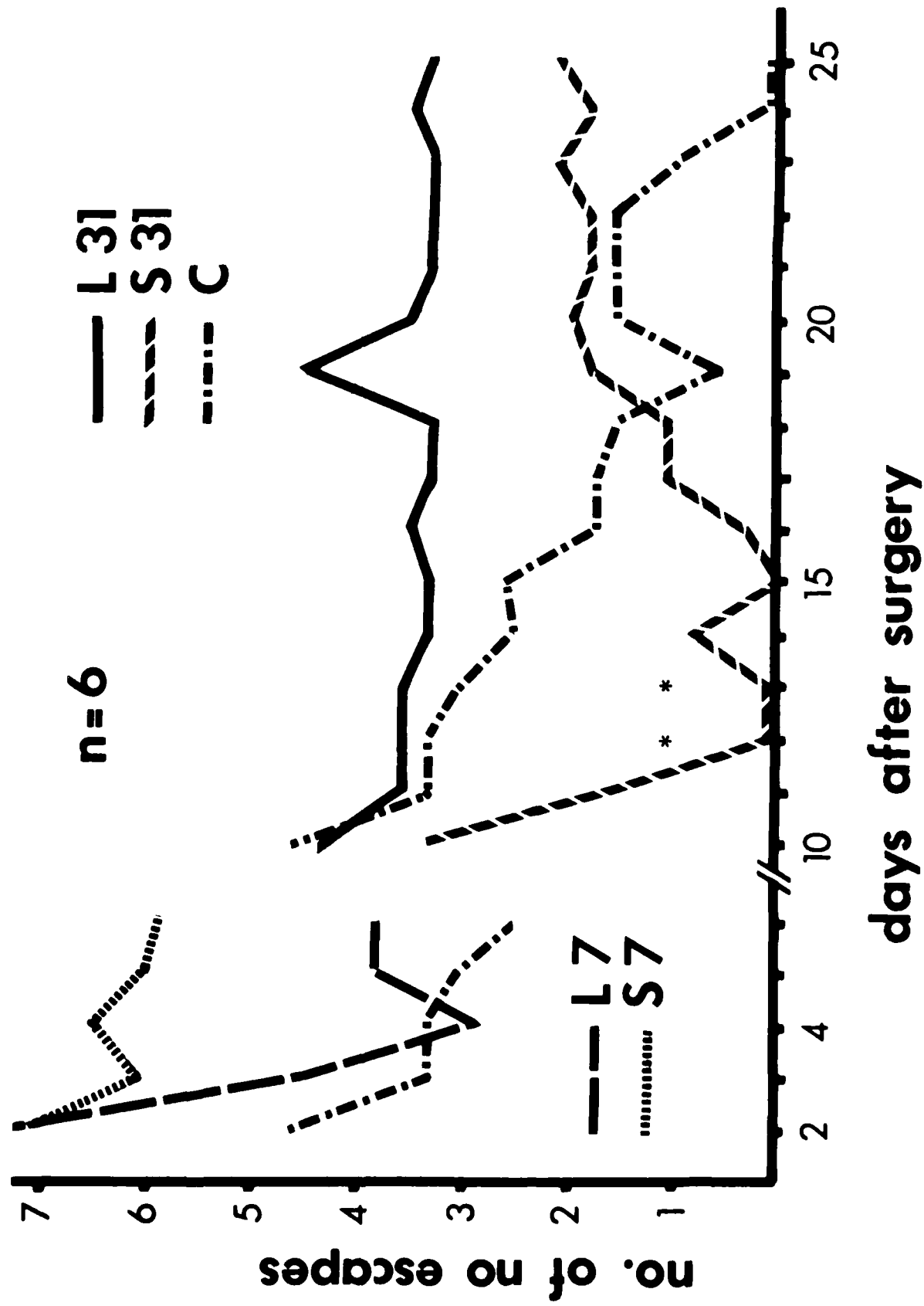


Figure 7

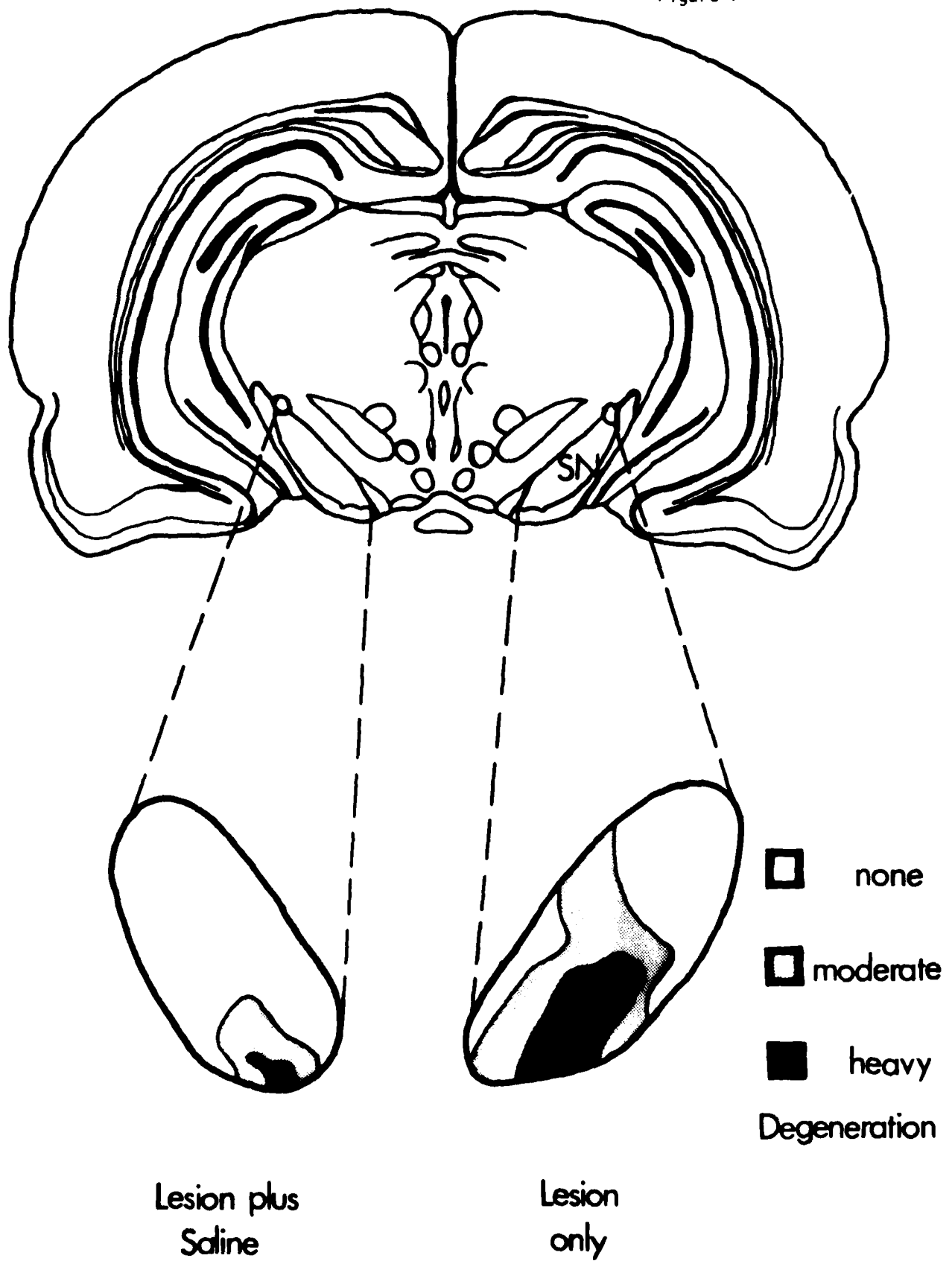
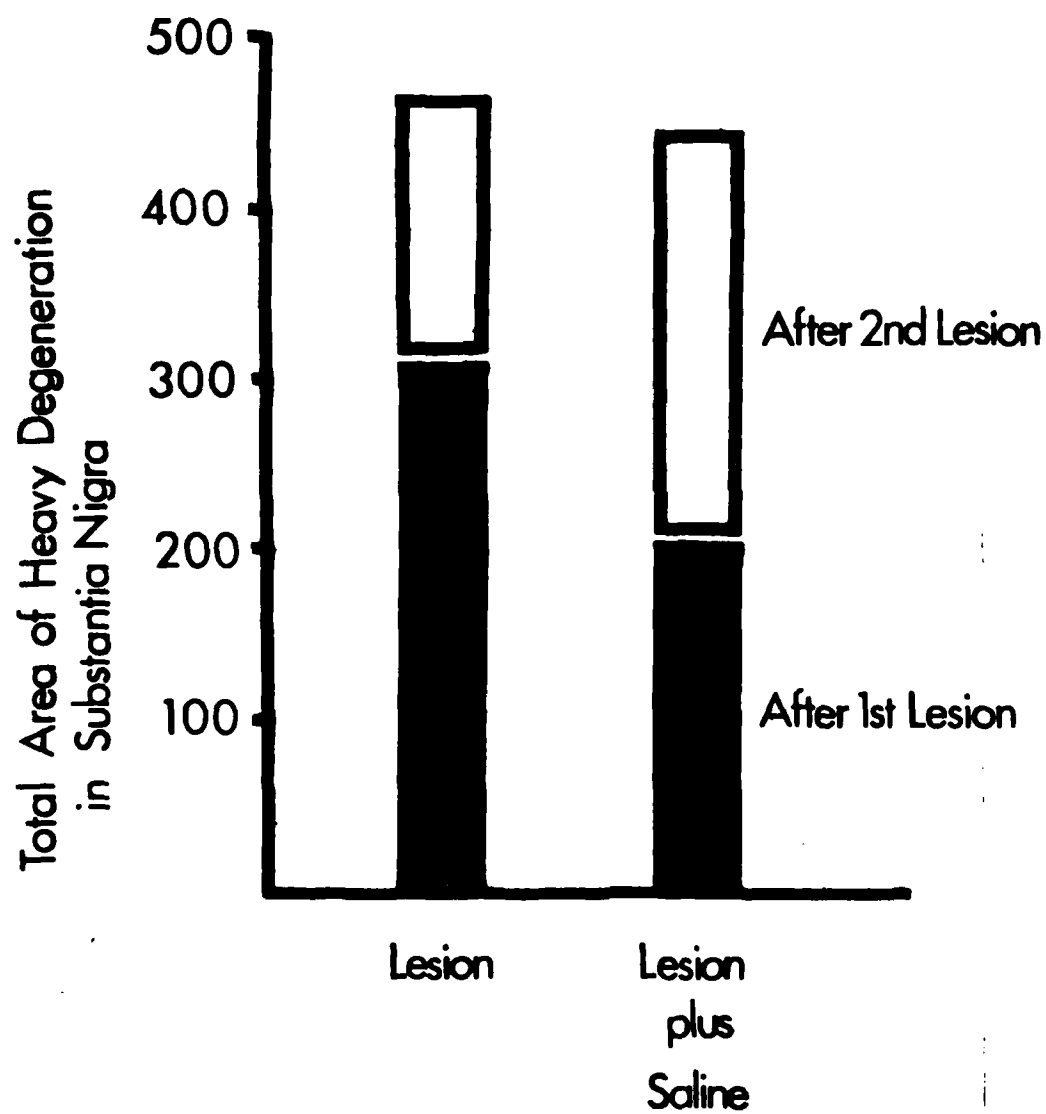


Figure 8



Stein, D.G.
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APPENDIX A

**NEONATAL BRAIN DAMAGE AND RECOVERY: INTRAVENTRICULAR INJECTION
OF NGF AT TIME OF INJURY ALTERS PERFORMANCE OF ACTIVE AVOIDANCE**

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ABSTRACT

Rats were given lesions of either the VMH or septal nucleus at 7d of age and then were tested repeatedly in an active avoidance task (A.A.) from 20-80d. VMH rats were consistently impaired on the A.A. task beginning at 40d of age. The animals with septal lesions performed the A.A. task consistently better than VMH or control animals throughout the entire test period; the septal syndrome becoming more pronounced as the rats reached maturity. In intact rats a single, intraventricular injection of NGF given at 7d of age resulted in a greater reactivity, especially as the rats approached maturity. NGF, given at time of surgery, also improved performance of the A.A. task in rats with VMH lesions tested at 40-80d. In rats given septal lesions, NGF treatment at time of injury attenuated the septal syndrome of improved A.A. performance. The data indicate that NGF treatment, given to neonatal rats, can produce long-lasting effects on CNS functions and can contribute to functional recovery from brain lesions.

INTRODUCTION

Although NGF has been characterized primarily by its effects on the development and maintenance of sympathetic and peripheral sensory neurons, there is now a growing body of evidence demonstrating that this protein may also play a role in the C.N.S. as well. More than ten years ago, Bjorklund and Stenevi (1972) showed that intracerebrally injected NGF could promote the regeneration of central norepinephrine containing neurons. Shortly after their study appeared, Berger, Wise and Stein (1973) hypothesized that the enhanced regeneration might have behavioral correlates. In particular, they were interested in studying whether intraventricular administration of NGF could facilitate behavioral recovery from the debilitating effects of lateral hypothalamic lesions. Damage to this brainstem area can result in severe aphagia and adipsia; NGF administration, however, prompted a more rapid and complete remission of symptoms in rats with this syndrome. Recently, other investigators have shown that in fully mature rats, single, intracerebral injections of NGF can promote at least partial recovery from damage to the caudate nucleus (Hart, et al., 1978), nucleus accumbens (Lewis, et al., 1979) or entorhinal cortex (Stein and Will, 1982).

While studies on the behavioral effects of NGF and other neurotrophic substances in brain-damaged adults are progressing, much less is known about the consequences of such treatments during the early stages of development. Accordingly, in the present study we decided to examine the question of whether NGF treatment can promote functional recovery from brain injury sustained in early life.

We chose to damage the ventromedial hypothalamus (VMH) in 7-day old rats for two reasons. First, the VMH contains catecholaminergic neurons and fibers, and it has already been shown that intracerebral NGF injections given during development will stimulate the growth of sympathetic terminals in the brain (Levi-Montalcini and Cossari, 1979). Second, in the absence of any special pre- or post-operative treatments, rats sustaining VMH lesions as neonates will present a deficit in the acquisition of a two-way active avoidance task (A.A.) (Eclancher and Karli, 1981).

Thus, any functional recovery produced by NGF treatment, should permit animals with bilateral VMH lesions to perform two-way A.A. in a manner similar to that of controls. Since there have been some reports that NGF injections serve to increase both general activity and reactivity to stimulation (Lewis, et al., 1979; Stein, et al., 1980), we needed to control for the possibility that rats given NGF would simply cross more frequently from one compartment to the other because they become more active and behave more like intact animals.

An increase in reactivity to normal levels in rats with VMH damage could then be attributed to NGF treatment given early in life. That is why we also observed the behavioral consequences of intracerebral NGF injections in rats who received early lesions of the septal nucleus, known to increase the reactivity of animals in the shuttle box. Bilateral septal lesions have been shown to improve the rat's ability to acquire 2-way active avoidance, regardless of the age at which the animals receive the injury (Eclancher & Karli, 1981). Thus rats with septal lesions made as early as 7 days of age, will show higher reactivity in the shuttle box, lower running latencies and higher footshock avoidance. Accordingly, we hypothesized that, if

recovery was mediated by NGF injections, the treated animals should actually show a decrease in ability to master the A.A. task--to reach the level of intact controls. If, however, the NGF merely increased reactivity and activity by acting as a general stimulant, then the rats with septal damage would show an even greater enhancement in A.A. acquisition than they would show normally in comparison to intact controls.

It has only been within the last few years that NGF has even been considered to play a role in the C.N.S. itself. Recently, for example, Hefti and colleagues have shown that NGF might act as a specific trophic factor for cholinergic neurons (Hefti, et al., 1983). These workers found that NGF injected into the rat hippocampus was specifically taken up by nerve terminals and transported to the cholinergic neurons in the medial septal nucleus as well as the nucleus of the diagonal band of Broca. Furthermore, ChAT activity was shown to have increased by 78% in the septal area of neonatal rats treated with NGF. The present experiments, then, have given us the opportunity to explore the effects of NGF on functional recovery following neonatal damage to a primarily cholinergic system of the brain. Finally, in order to have a developmental perspective on the effects of NGF in animals with septal or VMH lesions, we decided to test the rats repeatedly until they were 80 days of age.

SUBJECTS

Pregnant female rats (CD strain) were received from Charles River Breeding Laboratories on their 14th to 16th day of gestation. The dams were housed individually in large, fiberglass breeding cages and were provided approximately 3 cm of wood shavings as floor covering and nesting material. Food and water were provided ad libitum. After the rat pups were delivered, all of the females were eliminated on the second post-operative day, creating litters of from 5 - 8 males. By the sixth day of age, the pups were randomly assigned to one of the six treatment groups as listed below:

Sham operation + 1 μ l of saline injection;	Saline rats : n=10
Sham operation + 1 μ l of NGF injection;	NGF rats : n=10
VMH lesion + 1 μ l of saline injection;	VMH + saline: n=10
VMH lesion + 1 μ l of NGF injection;	VMH + NGF : n=10
Septal lesion + 1 μ l of saline injection;	Sept + saline: n=10
Septal lesion + 1 μ l of NGF injection;	Sept + NGF : n=10

At 20 days of age, the rat pups were weaned from the dams and housed individually in standard, suspended metal cages. The animals were maintained on a 12:12 h light/dark cycle with food and water provided ad libitum.

SURGERY

On their 7th postnatal day, the rat pups were anesthetized by an IP injection of Nembutal and loosely fixed in a David Kopf stereotaxic apparatus. The septal or VMH lesion was performed by lowering an epoxylite-coated stainless steel electrode (.15 mm diameter) through holes drilled in the cartilaginous skull. Two lesion sites were defined by the following stereotaxic coordinates:

For the septum:	Antero Posterior (AP)	5.0 mm and 4.7 mm
	Medio Lateral (ML)	0.3 mm
	Dorso Ventral (DV)	5.0 mm and 4.5 mm
For the VMH:	(AP)	3.6 mm and 3.3 mm
	(ML)	0.3 mm
	(DV)	7.5 mm

For both lesions, a 2 mA D.C. current was passed through the electrode for 10 seconds.

The surgical procedures (anesthesia, lowering the electrode, etc.,) for the sham operation were the same as for the lesions except that the current was not passed through the electrodes.

Immediately after the lesions or the sham operations, 1 μ l of saline or of 30 μ g of highly purified, renin-free NGF (3 ng/ μ l) was injected into the third ventricle by hydraulic infusion at controlled low speed (.2 μ l/min.). The 2.5S NGF we injected was prepared according to the procedures of Bocchini and Angeletti (1969) from adult mouse salivary glands suspended in sodium acetate (.05 M at PH 5.0 at 1:1 concentration) and kept frozen until just before use.

Upon completion of the surgery, the pups were returned to the dam and allowed to remain with her until they were weaned at 20 days of age.

BEHAVIORAL TESTING IN THE SHUTTLE BOX:

All rats were tested in a wooden shuttle box every 20 days from 20 days of age until 80 days of age.

The shuttle box used for the 20-, 30- and 40-day-old rats was 42.5 cm long x 12 cm wide x 33 cm high and divided into two compartments separated by a partition with a 6 cm high x 8 cm wide opening. The grid floor of each compartment consisted of 3 mm diameter stainless steel rods mounted 4 cm apart. The 50-, 60-, 70- and 80-day-old rats were tested in a larger shuttle box of 60 cm long x 18 cm wide x 35 cm high; the opening was 10.5 cm high x 14 cm wide and 5 mm diameter stainless steel rods were mounted 7 cm apart.

An 80 dB buzzer, 6 sec. in duration, served as the conditioned

stimulus (CS) and was placed on one wall of the shuttle box. The unconditioned stimulus (US) was a .3 mA scrambled footshock.

Each rat was placed in one of the two lighted compartments of the shuttle box and was allowed 2 min. free exploration. After this adaptation period, the CS (tone) was presented. If the animal did not respond within the 2 sec. of sound presentation, the US was administered along with the buzzer for 4 sec. During each trial, termination of both CS and footshock was contingent upon crossing over to the opposite compartment or upon the end of the 6 sec. buzzer presentation. Responding with a latency of less than 2 sec. from CS onset enabled the rat to avoid the shock. Crossings occurring during the 14 sec. intertrial interval (ITI) were recorded but not punished. Training sessions consisted of 20 trials (i.e., 4 series of 5 trials each) on the 20th, 30th, 40th, 50th, 60th, 70th and 80th day of age.

HISTOLOGY

Upon completion of behavioral testing, the animals were killed with an overdose of anesthetic (Nembutal) and perfused intracardially with saline-formalin solution. Their brains were prepared with Cresyl-echt violet stain for routine microscopic evaluation of the lesions. Tissue was cut in the coronal plane at 30 μ on freezing microtome and every 5th section was saved for reconstruction of the injury.

DATA ANALYSES

The data from these experiments were subjected to 2-way analysis of variance for independent groups followed by Scheffé tests for individual comparisons between the groups. As dependent variables,

we measured the number of crossings in an open field marked off in squares, the number of shocks received in the A.A. task, response latency and the number of runway crossings in response to the CS or to footshock.

RESULTS

Considering the various parameters measured in the 2-way A.A. acquisition (i.e., response latencies, number of crossing and number of shocks received), the analysis of variance revealed a lesion and treatment effect as well as an age effect. Individual comparisons made with the Scheffe test showed that VMH-damaged rats had higher response latencies than control rats from 40 to 80 days of age (Fig. 1). They also crossed the partition between both compartments less frequently than controls, from 40 to 80 days of age. The difference is significant for 40, 50, 60 and 80 days (Fig. 2).

Concerning the number of shocks received, the VMH-damaged rats were similar to controls since the latter were already at the highest level, receiving the maximum of shocks possible within the testing protocol (i.e., 5 in 5 trials) (Fig. 3). Looking at the effects of the septal lesion, we can see that they are clearly observable as early as 20 days of age, and that they endure over the entire 80-day test period. The differences were highly significant between the septal rats and the control rats for each day of test and for each of the three parameters considered. The septal syndrome of enhanced acquisition of A.A. (number of shocks received) became even more pronounced as the rats began to approach maturity at about 50 days of age (Fig. 3).

In control rats, a single, intraventricular injection of NGF just after the sham operation at 7 days of age, resulted in a higher reactivity to tones and to shocks than controls receiving an injection of saline. The reaction time of the NGF rats to escape or to avoid the shock was greater than that in saline control rats from 40 to 80 days of age. The differences were significant at each of the ages tested. The NGF-treated control rats also crossed more frequently than the control rats given saline, at 40, 50, 60 and 80 days of age. However, they did not acquire the A.A. task more easily than the saline control rats since the number of shocks received was similar in both groups.

When NGF was injected just after the VMH lesion, it resulted in an enhanced reactivity in the shuttle box. From 40 to 80 days of age, the rats with VMH lesions, given intraventricular NGF injection at the time of injury, crossed more frequently and reacted more rapidly to the stimuli than their saline-injected counterparts. Indeed the NGF treatment helped the VMH-damaged rats to perform like intact control rats; the number of shocks received was similar in both groups at each day of testing (Fig. 3).

The rats with early septal lesions immediately followed by intraventricular NGF injection made less crossings in the shuttle box than counterparts receiving an injection of saline at 7 days of age and then tested at 20, 30, 40 and 60 days of age. The NGF treatment (at least at 30 and 40 days of age) appeared to compensate for the "septal" rats' enhanced ability to avoid shocks typically induced by the lesions. From 50 days of age, the number of shocks received did not differ in the two groups of rats (Fig. 3).

If we look at the performances of the animals in more detail, by series of 5 trials at each day of testing, it appears that at least by 60 days of age, the septal NGF-treated rats received significantly fewer shocks over the 4 series of 5 trials, while their saline-treated counterparts received the same number of shocks over these 4 series of 5 trials: The NGF rats appeared to learn more rapidly for a given day of testing but would also forget more rapidly from one day to the other (Fig. 4).

Even though NGF-treated septal rats differed somewhat from saline-treated counterparts, they behaved more like septal rats than like the intact rats tested on the A.A. task.

An analysis of the extent of lesions in both the VMH and the septal groups revealed that, within each condition, the lesions were generally well localized to the appropriate brain areas. Figure 5 shows the maximum and minimum extent of damage for the rats with septal and VMH lesions: No differences in the locus or extent of damage were observed between animals given NGF or saline. In general, the septum was almost completely destroyed in both groups.

With respect to the VMH and septal lesions, no statistical differences were seen in size or locus of the lesions in the NGF- or buffer-treated animals. Representative lesions in the 2 groups are presented in Fig. 5.

DISCUSSION

The results of our experiments show that rat pups given VMH lesions at 7 days of age will present a lower reactivity to stimuli received in the shuttle box and also cross less frequently from one

compartment to the other than sham controls of the same age. These changes were already observable at 20 days of age and persisted into adult life (at 80 days of age). Our results confirm the deficit in the 2-way active avoidance acquisition that Eclancher and Karlí (1981) noticed in adult rats that were given VMH lesions at 7 days of age.

The 20 trials given to the rats, every 10 days from 20 days of age until 80 days of age, were not sufficient for controls to acquire the 2-way A.A., i.e., to avoid the shocks when they reached adult age. That is why the VMH-damaged rats did not show any impairment in their capacity to avoid the shocks compared to control rats; the latter simply did not perform well.

In contrast, the infant rats that sustained septal lesions at 7 days of age, showed at 20 days of age, improved performances in the shuttle box; that is, lower response latencies, higher number of crossings, as well as increased ability to avoid the shocks. This early improvement persisted throughout life. In an earlier experiment, Eclancher and Karlí (1980) had observed that, in rats, early septal lesions made at 7 days of age were as efficient as the same lesions created in adults in causing an improvement in the 2-way A.A. acquisition.

The results of our study also show that a single injection of NGF given after VMH damage sustained at 7 days of age facilitated recovery from that brain damage. In the rats given early VMH lesions followed by NGF injection, there was a significant increase in the number of crossings as well as a decrease in the latency to respond to the stimuli received in the shuttle box. These changes appeared at 40 days of age and were still observable at maturity. Similarly, intact

rats also showed increased reactivity to stimuli in the shuttle box which began to be significant from 40 days of age and persisted into adult life. These findings are consistent with others who reported that NGF administration can increase reactivity to stimuli when given intracerebrally (Lewis, et al., 1979; Stein, Blake and Wald, 1980). Based on these results, one might be tempted to speculate that the NGF simply serves as a neural stimulant that increases general activity in intact or in brain-damaged subjects, much like amphetamine might do.

When the data from the animals with septal lesions are considered, a different picture begins to emerge. The rats with septal lesions given a single injection at 7 days of age actually show a decrease in the number of crossings that they make in the A.A. situation, as well as a tendency toward longer latency of response before reaching maturity. Thus, the "recovery" we observed is in accord with our original prediction that NGF treatment, if successful, would increase response/performance in rats with VMH lesions and decrease those same responses in rats with neonatal septal damage. In addition, it should be pointed out again that our rats began testing 14 days after the initial treatments and differences among the groups could be observed for as long as 70-75 days after the single injections had been given.

Our study is the first to show that a single, intracerebral injection of NGF can facilitate recovery from both VMH and septal damage inflicted in early life. Furthermore, the effects of NGF on recovery need not be limited to primarily catecholaminergic systems in the brain, as has sometimes been suggested. Our data show that animals with septal lesions also respond to NGF treatment, even though

such damage deprives structures such as the hippocampus of its cholinergic innervation. While the specific mechanisms of NGF action in the CNS are not yet known, there is mounting evidence that NGF does have direct effects on CNS metabolism. For example, Hefti and his colleagues (1983) have shown that NGF treatments will alter ChAT activity after lesions of the fimbria/fornix in adult rats. Others have shown that NGF may alter the glial response to injury and further that glial cells, when stimulated by NGF, can be induced to secrete neurotrophic substances that facilitate neuronal regeneration (Stein, 1980; Cotman and Nieto-Sampedro, 1983).

In summary, and as we noted earlier, neonatal animals with VMH or septal lesions will often show persistent alterations in active avoidance performance, even with repeated testing on this task. In rats with VMH lesions, a single, intraventricular injection of NGF given at the time of injury increased their reactivity in the shuttle box to the level of intact rats. In contrast, as a result of the NGF treatments, the rat with septal damage showed only a partial compensation that persisted until adult age, if we consider the number of crossings, but transitory, if we consider the number of shocks received. These animals continued, nevertheless, to show the "septal syndrome" of greater reactivity and enhanced performance of two-way active avoidance.

Recently, Will, et al., (1983) have shown that repeated intraventricular doses of NGF given to rats with fimbria/fornix damage, can produce a steady and significant improvement in 8-arm, radial maze performance, a task which is complex and difficult for a rat with this type of cerebral injury. Had we injected our animals with repeated

doses of NGF throughout their development, our findings, too, might have been more dramatic. However despite the limitations of our study, we have been able to show that the nerve growth factor does indeed exert long-lasting and beneficial effects in subjects with brain injuries inflicted early in life. We have also demonstrated that NGF injections can have some effects in the intact animal as well. Although we can only speculate at this time, the changes in reactivity seen after NGF treatments in normal animals may be due to its capacity to alter ChAT activity (Hefti, et al., 1983) or tyrosine hydroxylase activity in the short term. Likewise, these alterations in enzymes that play an important role in neurotransmitter functions may be partially responsible for the compensatory effects of the NGF in our brain-damaged animals and we are currently examining this question in more detail.

FIGURE LEGENDS

Figure 1

This figure shows mean response latencies in a shuttle avoidance learning task. Rats with VMH lesions followed by saline injection had the highest latencies of running across the alley. Rats with VMH lesions given NGF were significantly better than their untreated counterparts but slower than saline-treated, intact controls. As expected, rats with septal damage, regardless of subsequent treatments, had the lowest response latencies of all the groups tested.

Figure 2

This figure shows the number of crossings made by rats in a two-way, shuttle avoidance box. The open triangles and circles show the performance of rats with lesions of the septal nucleus. Throughout most of the testing periods (days of age), the animals given a single injection of NGF at time of surgery performed more like normal controls (black circles), although they were still impaired. The rats with VMH lesions given NGF performed the avoidance task better than saline-treated counterparts (half-filled circles and triangles) from 30 days of age until the end of testing at 80 days of age. Thus the NGF treatments were demonstrated to have long-lasting and beneficial consequences for brain-damaged rats.

Figure 3

This figure shows the number of shocks received in the shuttle task. Only animals with septal lesions took less shocks than controls.

Figure 4

This figure shows the number of shocks received at each age of testing. Rats with septal lesions treated with NGF, beginning at 50 days of age show the most rapid rate of decline in the number of shocks received.

Figure 5

(Upper figures) - Representative section of septal lesion. Notice the virtually complete absence of the septal nucleus.

(Lower figures) - Representative lesion of the VMH. Under microscopic examination, some sparing of the lateral aspect of the VMH was observed, but there were no statistical differences in the extent or locus of damage between untreated rats and those given NGF treatment.

FIGURE 1

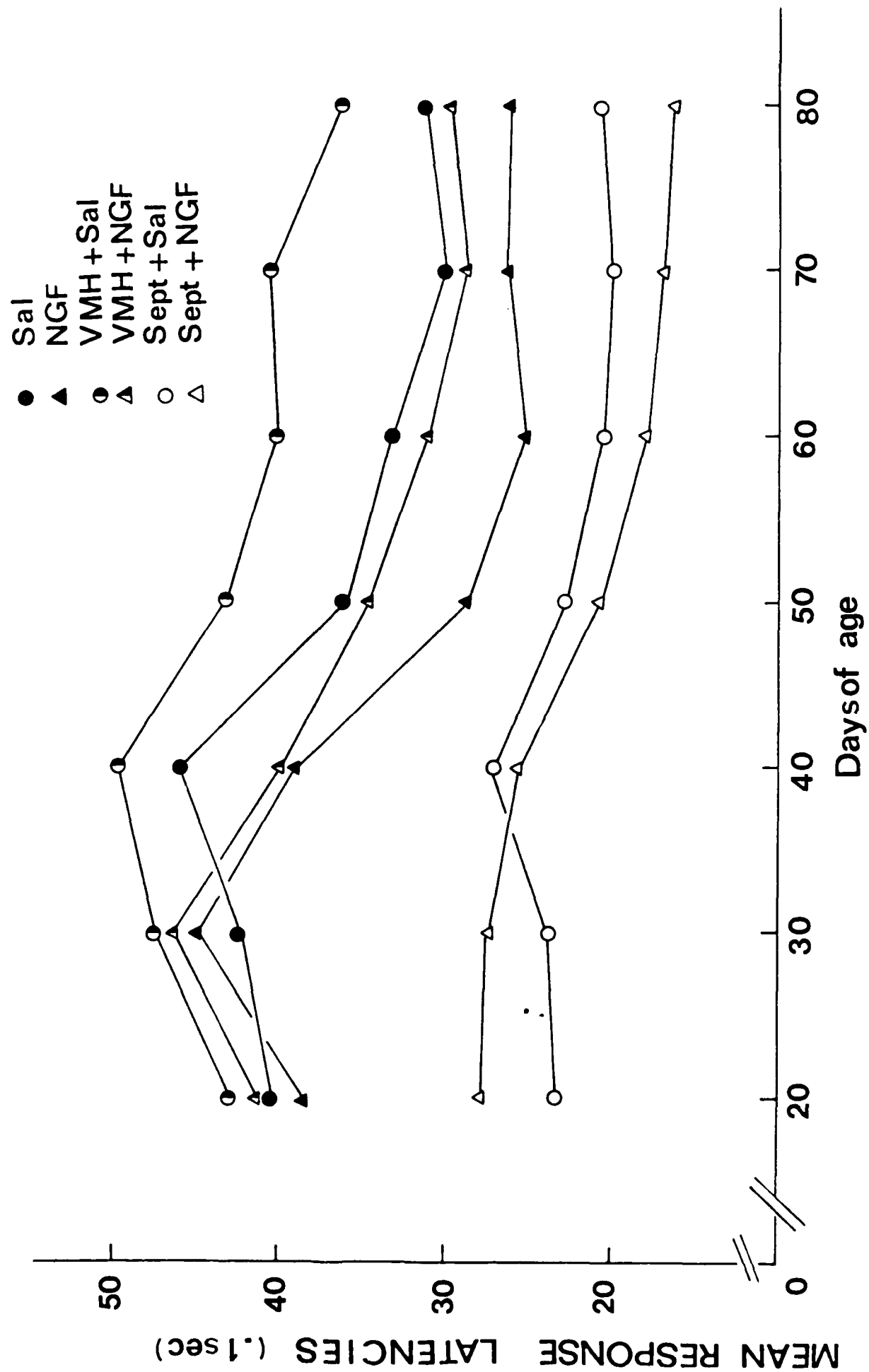


Figure 2

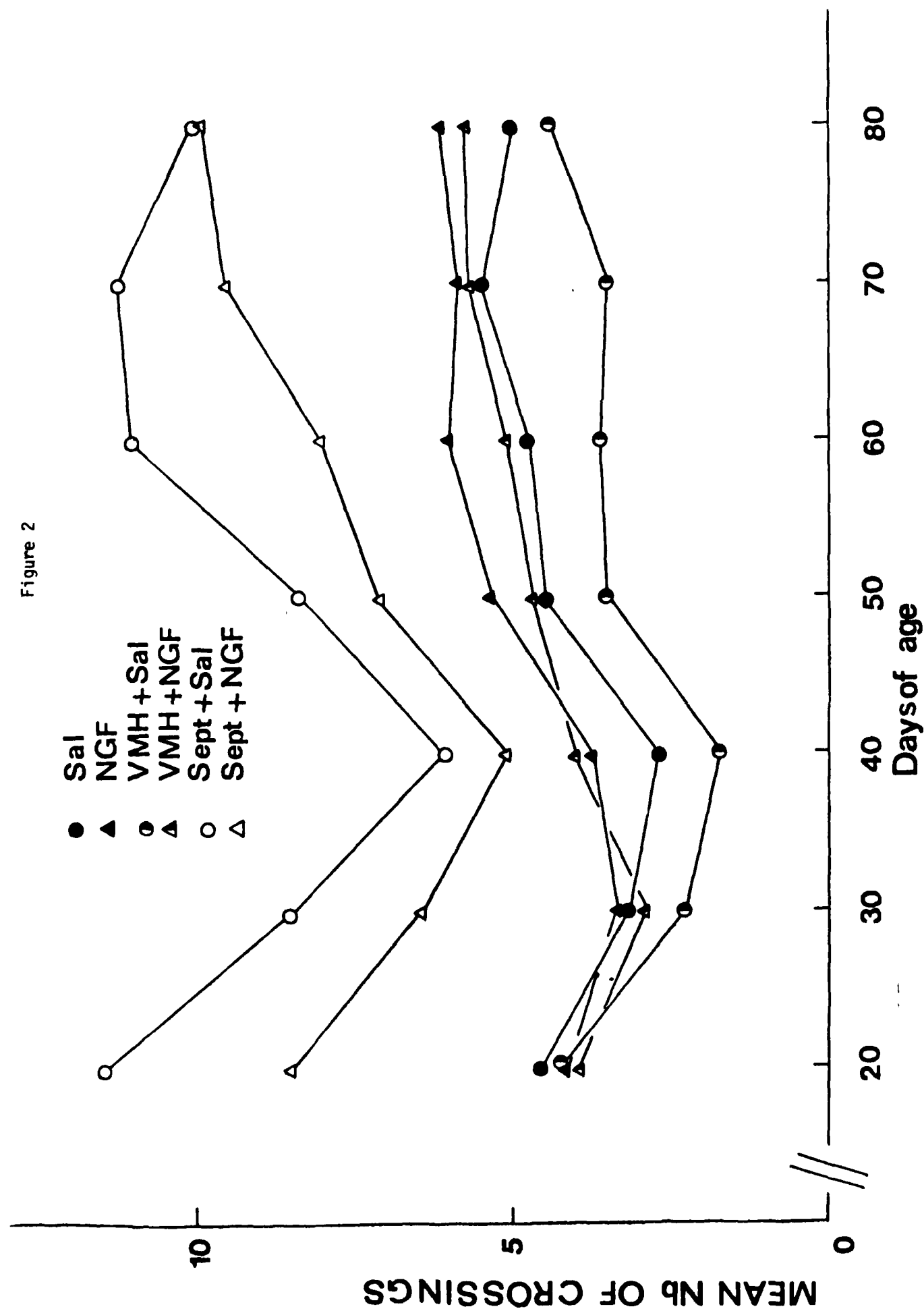


FIGURE 3

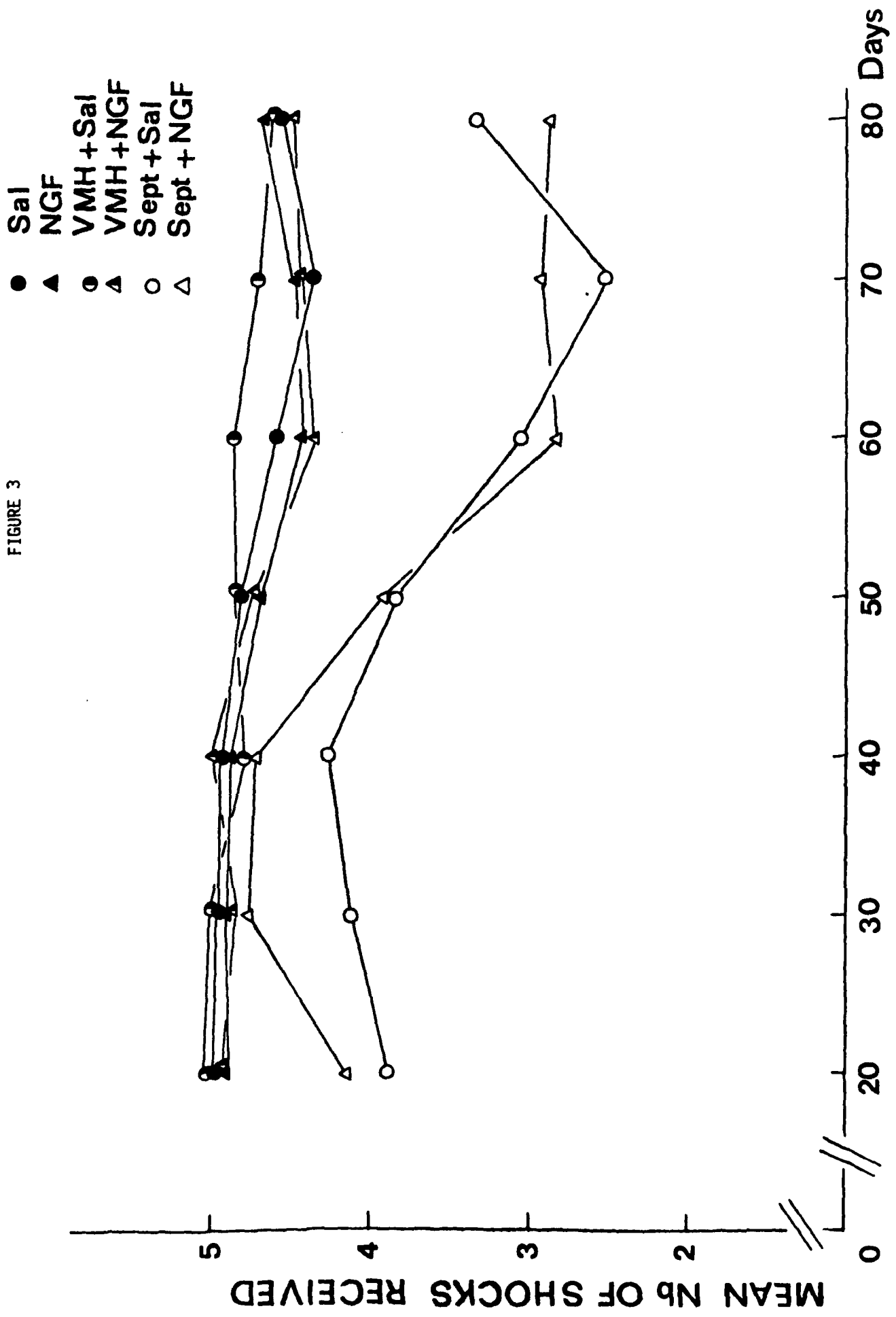


FIGURE 4

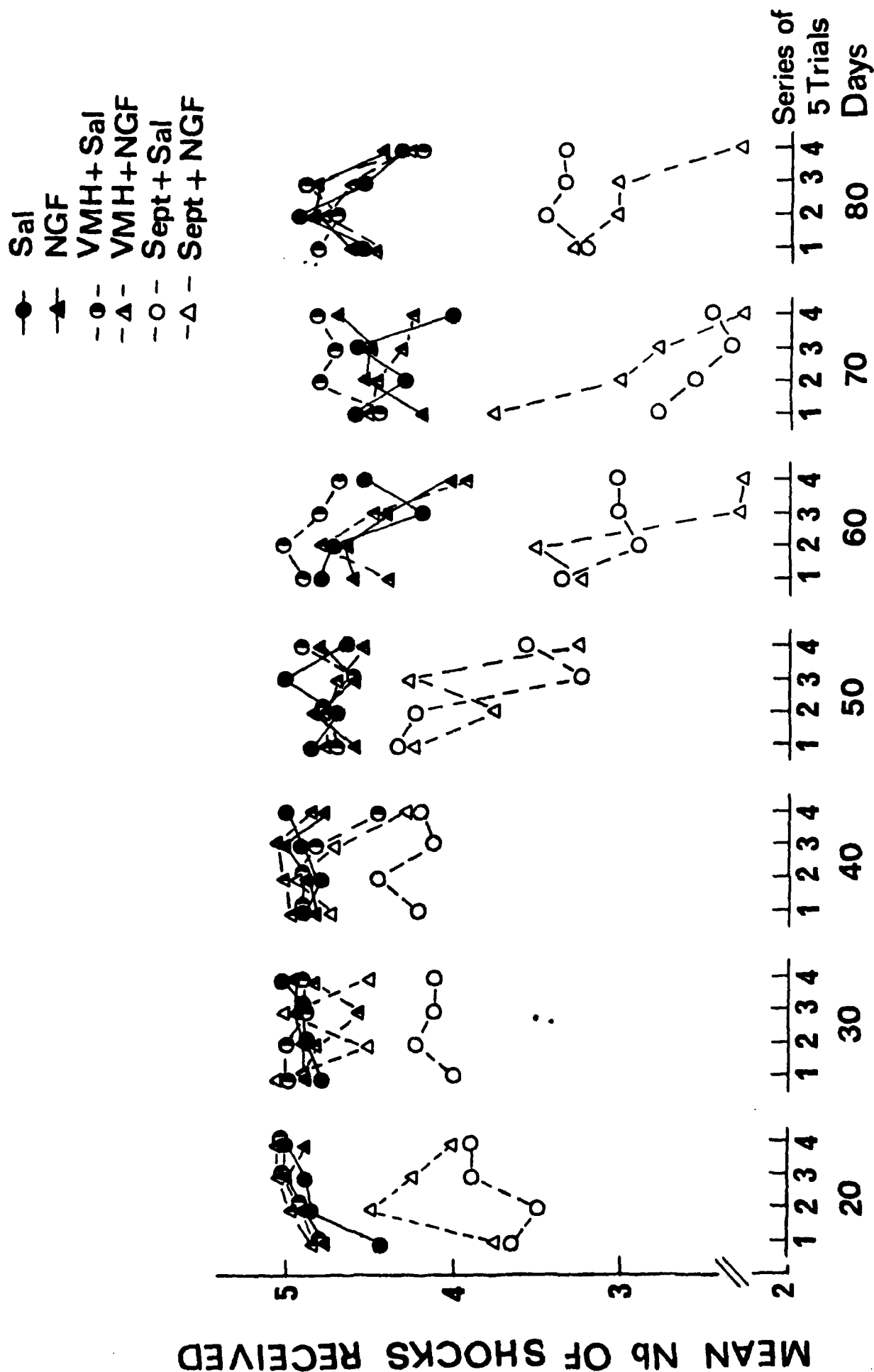


FIGURE 5



APPENDIX B

NERVE GROWTH FACTOR IMPROVES RADIAL MAZE PERFORMANCE
IN ADULT RATS WITH HIPPOCAMPAL LESIONS

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ABSTRACT

Rats with dorsal hippocampal lesions were impaired in their ability to learn a radial arm maze. Nerve Growth Factor (NGF) injected into the damaged zone at the time of surgery enabled the rats to learn the maze problem more rapidly than untreated animals with the same injury. This finding shows that damaged, cholinergic regions of the brain also respond to NGF therapy.

INTRODUCTION

Nerve Growth Factor (NGF) has long been known to have an effect on the development and maturation of sympathetic nervous system and on at least those primary sensory neurons containing substance P (Thoenen and Barde, 1980). NGF protein can also increase the rate of regeneration of central noradrenergic neurons (Bjerre, et al., 1973). Along with its capacity to stimulate regeneration or anomalous growth into the CNS (Levi-Montalcini and Calissano, 1979), others have recently demonstrated that intracerebrally administered injections of NGF can improve behavioral functions in brain-damaged subjects. For example, over 10 years ago, Berger, Wise and Stein (1973) showed that intraventricular injection of NGF could attenuate the aphagia and adipsia caused by bilateral lesions of the lateral hypothalamic area. Berger, et al., 1973, thought that the behavioral recovery was due to NGF - induced supersensitivity to noradrenaline or to sprouting of noradrenergic neurons in the brain.

Others, such as Hart, Chaimas, Moore and Stein (1978), found that a single intrastriatal injection of NGF, given at the time of injury, can promote recovery from bilateral damage to the caudate nucleus. A short time later, Lewis, et al. (1979) found that NGF could increase locomotor activity in normal rats as well as restore normal activity levels in rats given chemotoxic lesions of the nucleus accumbens. Lewis and colleagues suggested that the NGF-induced changes in the turnover of monoamines might have played a role in mediating the behavioral recovery they observed.

More recently, Eclancher and Stein (1983), noted that neonatal rats given either lesions of the septal nucleus or the ventromedial

hypothalamus at 7 days of age, showed altered performance in the learning and retention of an active avoidance task. Intraventricular injections of NGF at the time of the injury, diminished the "septal syndrome" of more rapid active avoidance learning, and it also improved avoidance learning in the animals with VMH lesions. These effects were observed up to 80 days after lesions and NGF treatments.

Up to this point, most work on the central effects of NGF has focused on its neurotrophic role in catecholaminergic systems. However, recent evidence is beginning to suggest that NGF may also affect cholinergic neurons in the brain. For example, Schwab and Thoenen (1983) have shown that NGF injected directly into the hippocampus is transported retrogradely into the cholinergic cell bodies of the medial septum and diagonal band. Weil, et al. (1980), using immunofluorescence techniques, have shown that antibody to beta-NGF was associated with neurons in the hippocampus, subiculum and dentate gyrus, areas of the brain which receive cholinergic projections from the nuclei of the medial septum and diagonal band of Broca. In the most recent study, Hefti and his colleagues (1983) have demonstrated that NGF increases ChAT activity in the hippocampus and the septum following partial damage to the septal/hippocampal fiber system in adult rats. This effect was primarily a temporary one lasting only about two weeks.

Accordingly, we designed a series of studies in which we damaged either the entorhinal cortex, the hippocampus or the dorsal fimbria/fornix bundle to determine if NGF treatment could facilitate recovery from these lesions. Thus, in the first experiment (Stein and Will, 1982), we injected the NGF directly into the hippocampus because it is the primary projection site of the entorhinal cortex and of the cholinergic

terminals of the medial septal nucleus and the nucleus of the diagonal band of Broca. In this first study, we found that a single injection of NGF given at the time of surgery, had a temporary, ameliorative effect on Hebb-Williams maze performance.

In the present experiment, we decided to explore the question of whether a single injection of NGF could attenuate the learning deficits produced by bilateral damage to the dorsal hippocampus. We chose to examine the relationship between NGF treatment and hippocampal function because this structure receives a number of cholinergic projections. In addition, Heftl and his coworkers (1983) have shown that exogenous NGF administration selectively alters the neurochemistry of this structure in both neonatal and adult rats and that the altered levels of chAT activity last for about two weeks. With respect to behavioral assays, small lesions of the dorsal hippocampus lead to severe deficits on radial maze performance (Will, 1983). The radial maze is known to be an effective measure of the animal's capacity to remember where it was last and it does not require one to change the problems repeatedly as is the case with the Hebb-Williams maze. We thought that, in our first study (Stein and Will, 1982), this variable could have accounted for the limited recovery of the rats with entorhinal cortex lesions since the constant novelty of the changing maze patterns may have made them more reactive, distracted and less likely to respond appropriately in the test situation.

In this study we will show that rats with dorsal hippocampus lesions who are given NGF have more rapid improvement in radial maze performance in comparison to rats with the same lesions treated with a control solution.

METHODS

Twenty-nine male hooded rats of the Long-Evans strain were used. They were weaned at 25 days of age and kept thereafter 2 per cage (40 x 26 x 15 cm). They were maintained at a 12:12h light/dark cycle with food and water ad libitum until they began behavioral pretraining. The rats were 65 (± 5) days at the time surgery and NGF injections.

The 2.5S purified NGF (without renin) was prepared according to the procedure of Bocchini and Angeletti (1969) from adult male mouse salivary glands suspended in sodium acetate and sodium bicarbonate (0.1M and 0.2M respectively at pH 7.35 at 1:1 concentration).

Twenty rats sustained bilateral electrolytic lesions of the dorsal hippocampus by passing a rectified 1 mA DC current through an epoxylite-coated stainless steel electrode (.15 mm diameter) whose tip was exposed after coating. Details of this method and the coordinates for the lesions have recently been published (Will, Delezarche and Kelche, 1983).

Immediately after the lesions were created, and while the rats remained under deep anesthesia (Nembutal, 37mg/kg, IP, and atropine sulfate, 2 mg/kg, IP), 2 μ l of NGF (25ug/ μ l) were injected directly into each dorsal hippocampus by hydraulic infusion at controlled low speed (0.4 μ l/min).

Behavioral testing began 3 days after surgery had been completed. An 8-arm radial open elevated maze, painted in grey was used. The central platform was 30 cm in diameter and the goal arms radiating from the platform measured 60 cm in length and 10 cm in width. The goal arms were separated from each other at the central platform by

plywood walls, 15 cm high and 20 cm long. At the end of each arm, a hole, 2 cm in diameter and 1 cm in depth served as a food cup. Two adjoining red lights, located approximately 70 cm above the center platform, provided the only source of illumination during testing.

For six days, the rats were familiarized with the new apparatus by being placed in the maze for a given time per day (days 1 and 2: 15 min.; day 3: 12 min.; days 4 and 5: 15 min.), in pairs for the two first days, alone for the next three days. During these five days of pretraining, rats were free to eat three calibrated food pellets (45 mg) placed in the eight food cups.

Rats were tested once daily for four consecutive days. Timing of each daily trial session began when the rat was placed on the center platform. An entry into a goal arm was defined as the placement of all four paws in the arm. If the rat did not eat the pellet (1 pellet per arm during testing) upon entering a given arm, such an entry was considered to be exploratory (and accounted for only a small percentage of total entries). If the rat ate the pellet, the arm was noted as visited. Explored arms could be re-entered, but these re-entries were not counted as errors. However, once a rat had eaten the pellet, subsequent entries into the same arm were counted as errors.

Six weeks after completion of testing, the rats were retested for 10 daily sessions, according to the test procedure already described.

At the end of testing, rats were killed with an overdose of Nembutal. Brains were placed in 10 percent formaldehyde solution for 24h. Frozen coronal sections were cut at 52 μ m, mounted on slides and examined for extent of damage.

The data were evaluated by an analysis of variance followed by a least significant difference test for two-by-two comparisons.

By an analysis of covariance, we determined whether the behavioral changes expressed as a function of time (Session #) were the same for the different groups. We tested the statistical significance of the parallelism of the regression lines after having tested the linearity of each of them. The slope (regular coefficient) of each was also compared to a zero slope.

A behavioral test was used to compare the performance of the rats in each treatment group to chance level of performance. The probability of visiting n out of 8 arms before entering an already chosen arm was calculated.

RESULTS

In this experiment, rats with dorsal hippocampal lesions were impaired in learning an 8-arm radial maze in comparison to intact controls. The brain-damaged animals visited significantly fewer arms before they made their first error (Fig. 1).

It was also interesting to note that by the first day of individual pretraining in the radial maze (when the rats were given 3 pellets per maze arm instead of one), the rats with hippocampal lesions given NGF, consistently ate more of the pellets than their control counterparts ($p < .02$, two-tailed, Mann-Whitney U test). In this respect, the NGF-treated rats performed in a manner almost identical to unoperated controls.

With respect to NGF treatment given at the time of surgery, individual comparisons between the groups revealed that initially, NGF-treated rats with hippocampal lesions showed a greater deficit than buffer-treated counterparts ($p < .03$). Neither the buffer-treated

nor the intact rats showed any improvements in performance over the four days of initial testing, while the NGF-treated rats showed a significant increase in the number of arms they visited before making their first error.

A regression analysis revealed that all 3 regression lines could be considered as linear. However, only the NGF groups showed a significant difference from the zero slope ($F_{1/38} = 24.5$, $p < .001$), indicating that the other 2 groups did not change their level of performance over test sessions. Further, an analysis of covariance showed that the 3 regression lines could not be considered as parallel because the regression coefficient for the NGF group was significantly different.

We also wanted to determine whether NGF enabled the rats with brain damage to solve the Olton maze significantly above the chance level of performance. We found that rats with dorsal hippocampal lesions given buffer solution never performed above chance on any of the four test days (i.e., entering more than 3-4 alleys successively). In contrast, the NGF-treated rats showed significantly above chance performance on day 3 ($p < .05$) and day 4 ($p < .001$) of testing. The normal controls performed above chance level on all testing days ($p < .002$) but day 3 ($p < .08$).

Finally, individual comparisons revealed that, once the rats with lesions had attained criterion during the initial testing, they were able to retain what they learned even after a six-week delay in testing. Any differences between the lesion groups had disappeared. These findings could be interpreted to suggest that a single injection of NGF at the time of injury can exert only a short-term effect on functional recovery.

Histological evaluation of the mean lesion size ($\bar{x}_{NGF}=4.46$; $\bar{x}_B=2.95$; $F=2.28$; $p=NS$) revealed no significant differences among the treated and untreated rats with brain lesions. If anything, the NGF group had somewhat larger lesions than animals given buffered control solution. The dorsal hippocampus was successfully damaged. Figure 2* shows the maximum and minimum extent of damage in the two lesion groups.

DISCUSSION

Our results show that a single injection of NGF given at the time of injury, can accelerate the rate of recovery from dorsal hippocampal lesions. It should be noted, however, that the improved performance of the rats in the Olton maze was not as good as that of the normal controls.

Our findings are consistent with an earlier report (Stein and Will, 1982) in which we demonstrated that NGF injection can attenuate the symptoms induced by entorhinal cortex lesions, although the animals did not recover completely. In the present study, the rats given the NGF injection made fewer errors on the last day of testing than buffer-treated counterparts. The former also performed at better than chance levels on days 3 and 4 of testing, while buffer controls never performed the radial maze task at better than chance throughout testing.

We are unable to explain why the animals given the NGF at the time of surgery show an initial, significant deficit in radial maze performance in comparison to the buffer-treated controls. Despite the initial handicap, the NGF -treated rats did show a significant improvement by the end of testing; the slope of their learning curve was clearly

*Fig. 2 in preparation as of this writing.

different than that of both other treatment groups. From a physiological perspective, there does not appear to be any one mechanism that might account for the initially severe deficit followed by gradual improvement in NGF-treated rats with dorsal hippocampal lesions. From a behavioral viewpoint, it is nonetheless interesting to note that the NGF seems to increase reactivity in both normal and brain-damaged rats. For example, Lewis et al. (1979), were able to show that normal rats given NGF injections into the substantia nigra, were significantly more active when given subsequent amphetamine injections than saline-treated controls. In another study, Stein, Blake and Wiener (1980) found that intraventricular NGF in normal rats tended to make the rats hyper-emotional in a stressful, "jumping stand," visual discrimination task.

As we noted earlier, if we examine the first few days of pretraining in the Olton, radial arm maze, we can count the number of arms in which the rats enter and consistently eat the food pellets. When this simple analysis is performed, it is quite clear that the NGF-treated rats are substantially more active than buffer-treated counterparts. It is thus very likely that the NGF treatment, given at the time of surgery, can have multiple effects on the damaged and intact brain. The hyperactivity we have seen in this and other experiments may account for the disrupted behavior seen in the initial stages of learning. Once the reactivity dissipates, the animals do show an accelerated rate of recovery on this task.

Recently, Hefti et al., (1983) extended this present study by giving multiple, intraventricular injections of NGF to animals that had suffered partial fimbria/fornix transections (partial lesions).

Their rats also showed an initial deficit which exactly paralleled the one we have seen here. Likewise, the animals given NGF showed an accelerated improvement in radial maze performance that was simply not evident in the buffer-treated controls.

In the brain-damaged adult rat, the effects of NGF treatments appear to be somewhat transitory. In the present study, the rats repeat the same problem daily until they complete 10 days of testing. Six weeks later the animals were retested on the same radial maze. Under these conditions, we noted that once the rats had learned the task, regardless of lesion condition, they were able to retain the solution to the maze as well as nonoperated controls. Thus, the principal effects of the single, intrahippocampal injection of NGF were to modify the rate at which the rats were able to recover from the brain injury.

The initial changes in performance observed in brain-damaged rats treated with NGF may be due to the fact that the protein causes a significant increase in choline acetyltransferase activity (up to 60 percent higher than in buffer-treated controls with the same lesions; Hefti, et al., 1983). Apparently, with adults there must first be injury to the brain for this increase to occur since Hefti and his colleagues found no change in ChAT on the undamaged side of the brain.

Since ChAT is one of the rate-limiting enzymes in the formation of AChE, it would be tempting to speculate that NGF treatments might serve to induce anomalous sprouting to replace fibers lost as a result of the lesion. However, since Hefti, et al. found no elevations in AChE activity in NGF-treated rats with fimbria lesions, it is difficult to assume that sprouting is the mechanism by which NGF mediates partial functional recovery from brain lesions.

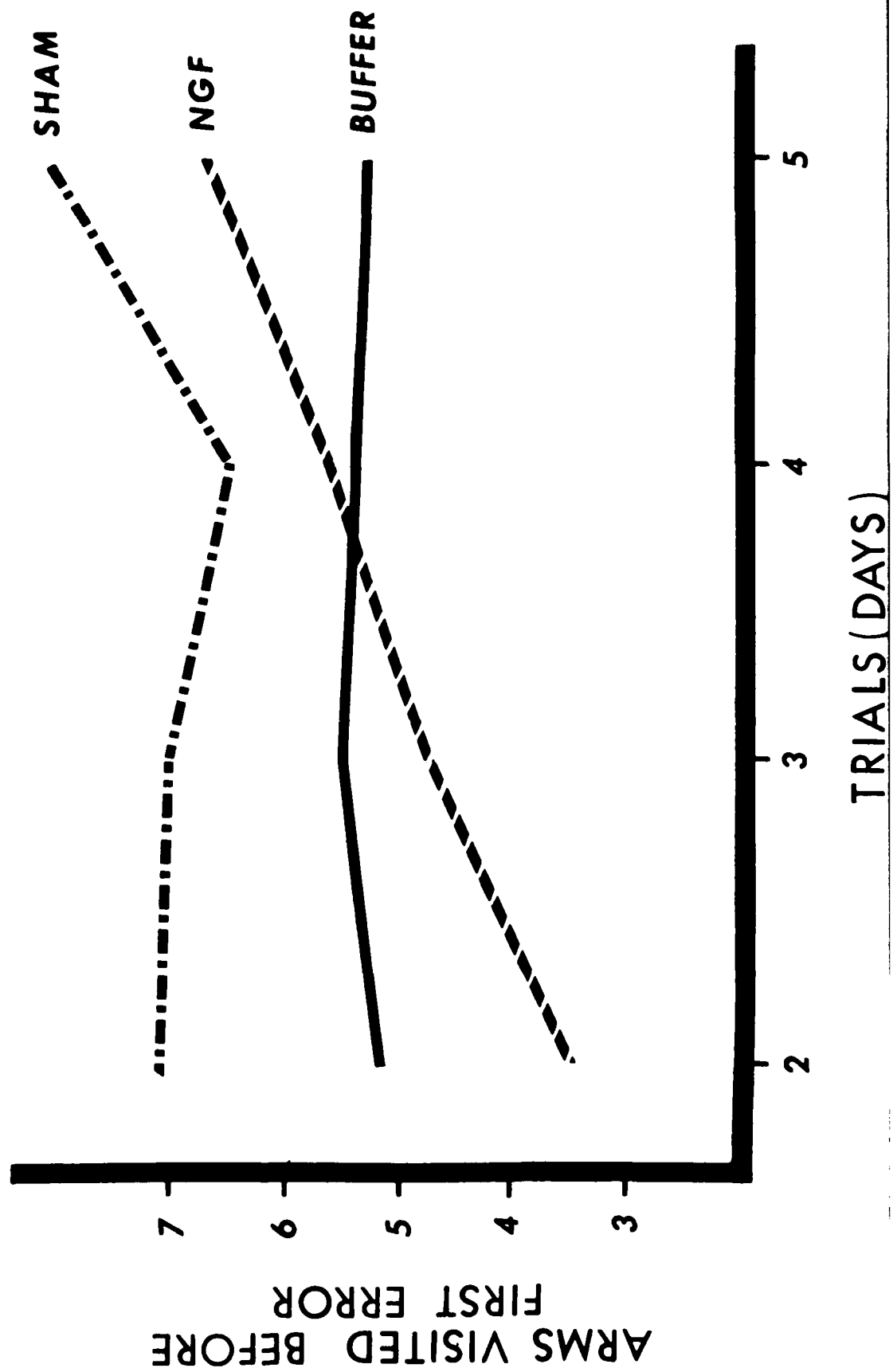
Although the specific mode of action by which NGF facilitates behavioral recovery is not yet known, the protein may act to increase the level of specific neurotrophic substances that are released by glia (Nieto, Sampedro and Cotman, 1982) following brain injury and that accumulate in the wound area. NGF has also been shown to increase the size and the number of reactive astrocytes in the area of damage (Stein, 1980) and these all, in turn, may secrete the brain-specific neurotrophic substances which enhance the successful "take" of transplants of embryonic brain tissue into damaged adult brains, at least in respect to the hippocampal system (Varon and Cotman, 1983). Thus, NGF may exert its primary effects on repair of remaining (but damaged?) neuronal membranes in the area of the injury rather than by altering sprouting or neurotransmitter levels per se.

FIGURE LEGEND

Figure 1

Figure 1 shows that a single intracerebral injection of NGF given at the time of hippocampal injury, gradually improves the rate of learning in comparison to similarly injured animals given buffer control solution. In this experiment the fully mature rats were tested in a complex, 8-arm radial maze.

Figure 1



Fetal Brain Transplants: Reduction of Cognitive Deficits in Rats with Frontal Cortex Lesions

Randy Labbe, Arthur Firl, Jr., Elliott J. Mufson, and Donald G. Stein

Fetal Brain Transplants: Reduction of Cognitive Deficits in Rats with Frontal Cortex Lesions

Abstract. *Frontal cortex and cerebellar tissue from fetal rats was implanted into the damaged frontal cortex of adults. Cognitive deficits in spatial alternation learning that follow bilateral destruction of medial frontal cortex were reduced in rats with frontal cortex implants but not in those with implants of cerebellum. Histological evaluation showed that connections were made between the frontal cortex implants and host brain tissue.*

Interest in the problem of recovery from brain injury is growing, and a number of new approaches are being tried (1). One of the more novel and interesting of these involves the transplantation of embryonic brain tissue directly into the damaged brain of a mature recipient (2). In recent experiments the behavioral deficits associated with damage to the nigrostriatal or fimbria-fornix systems have been diminished by implanting fetal dopaminergic neurons or solid embryonic septal grafts, respectively, into the lesion sites (3, 4). Anatomical studies with anterograde and retrograde tracers have also shown that the transplants can establish connections with the host brain (5), while electrophysiological experiments show that the neural implants are capable of forming functional synapses (6).

Despite these achievements, the ability of brain grafts to mediate behavioral recovery after bilateral cortical ablations

has still not been systematically investigated. We report here that the impairments in cognitive functioning caused by damage to the medial frontal cortex are significantly reduced by the implantation of fetal frontal cortex into the lesion site. Furthermore, injections of the enzyme horseradish peroxidase (HRP) show that the transplants and the host brains establish afferent neuronal connections.

Twenty-nine male Sprague-Dawley rats (Charles River; CD) approximately 105 days old at the time of surgery were used. Eight animals served as unoperated controls with sham incisions. The medial frontal cortex of the remaining 21 animals was damaged bilaterally by aspiration (7). Seven days after surgery 14 animals were implanted with fetal frontal cortex ($N = 8$) or fetal cerebellar tissue ($N = 6$) (8). (The unoperated controls and the seven lesion animals not receiving implants were anesthetized at this point and their wounds were reopened.)

The transplanted neural tissue was obtained from CD rat fetuses on day 21 or 22 of gestation and placed into the cavity created by the removal of the medial frontal cortex (9). The implants had a volume of approximately 6 mm³ and were placed bilaterally directly into the area of damage.

On the fourth day after transplantation all 29 animals began training on a spatial alternation task in a T-maze (10). Spatial alternation requires the water-deprived rat to enter the goal arm opposite the one entered on the previous trial in order to receive a 0.15-ml water reward. This test has been used to determine the effects of frontal cortex damage (11). Ten trials per day constituted a testing session, and animals were tested 5 days per week. When an animal made 19 of 20 choices correctly during two consecutive test sessions, or when 30 test sessions had been completed, testing was terminated for that rat. After behavioral testing, and between 78 and 155 days after transplantation, the rats that had received frontal cortex or cerebellar tissue were given injections of the retrograde transport marker HRP in the transplant or the host brain to determine whether afferent connections had been established between these neural regions (12).

We found that transplants of frontal cortex, but not cerebellar tissue, facilitated recovery from the lesions (Fig. 1). An analysis of variance revealed significant differences among the four groups in the number of days needed to meet our most stringent criterion, the making of 19 of 20 choices correctly in two consecutive days [$F(3, 25) = 10.91$, $P < 0.01$]. Randomization tests for two independent groups revealed that rats receiving frontal cortex performed significantly better than the lesion group that did not receive brain transplants in terms of number of days needed to make nine of ten choices correctly in 1 day ($P < 0.01$), number of days needed to make 18 of 20 choices correctly in two consecutive days ($P < 0.05$), total number of errors divided by number of trials needed to meet the most stringent criterion ($P < 0.05$), and number of perseverative errors divided by number of trials needed to meet the most stringent criterion ($P < 0.05$).

Animals that received frontal cortex scored significantly better than the group given cerebellar tissue on days needed to make nine of ten choices correctly in 1 day ($P < 0.05$) and number of perseverative errors divided by number of trials needed to make 18 of 20 and 19 of 20 choices correctly over 2-day periods

($P < 0.05$). Four of the six animals that received cerebellar transplants and three of the seven animals with cortical injuries and no transplants never met our most stringent criterion. In contrast, only one of the eight lesion animals receiving frontal cortex tissue failed to reach this criterion.

The unoperated control animals scored significantly better than the three lesion groups on all of the measures we employed. No significant differences were observed between the cerebellar transplant group and the group with lesions only.

After the behavioral testing, five animals with frontal cortex transplants and three with cerebellar transplants were used for an anatomical evaluation of afferent connections. In the exposed brains the grafts were clearly visible only in those animals that had been implanted with frontal cortex tissue. These grafts were located in the rostral portion of the lesion cavity and appeared to the naked eye as oval, whitish lumps. HRP was then injected ipsilaterally into the graft ($N = 3$) or the host cortex ($N = 2$). In cerebellar transplant rats we made unilateral injections of HRP into the cortex ($N = 3$) since no transplanted tissue was visible. Rejection of the cerebellar transplants may have been caused by a difference in specific growth factors between the frontal cortex of the host and the cerebellar tissue (13). The HRP-injected brains were processed by the highly sensitive tetramethylbenzidine procedure and the remaining brains were prepared for histological analysis by staining for Nissl substance (12).

Histological examination revealed that transplants either formed continuous bridges connecting the injured hemispheres or formed separate grafts, each adhering to the host cortex. At the points of attachment between graft and host there were areas of continuity, some of which exhibited glial scarring (Fig. 2, A to C). Light microscopic evaluation of the cresyl violet-stained sections revealed little internal order in the transplants and no laminar arrangement of neurons characteristic of the frontal cortex. The perikarya in the grafts varied in size and occasional large neurons were seen (Fig. 2C). In all brains with lesions, bilateral damage included the medial frontal portion of the cortex from the tip of the frontal pole to at least the genu of the corpus callosum, and in several cases there was some minor involvement to the head of the caudate nucleus.

In all three brains with HRP injections into the frontal grafts the HRP was re-

stricted to one side of the transplant. In one animal the HRP reaction was confined to the transplant, while in the other two there was some minor involvement of the adjacent cortex. In each case, however, labeled neurons were observed in the adjacent host cortex as well as in the medial dorsal and anterior thalamic nuclei (Fig. 2D). Areas of host brain found to project to frontal transplants

were areas known to have efferent connections with portions of normal frontal cortex (7). In addition, retrogradely labeled cells were found in the contralateral portion of the transplant, suggesting that intratransplant connections had been established.

In each brain with a frontal cortex transplant and an HRP injection into the host cortex, labeled perikarya were ob-

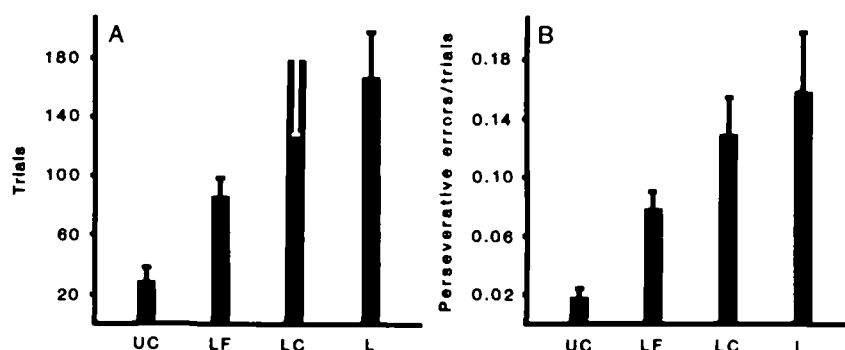


Fig. 1. (A) Mean number of trials needed to make nine of ten choices correctly during one test session. (B) Mean number of perseverative errors divided by number of trials needed to meet the most stringent criterion. Abbreviations: UC, unoperated controls; LF, frontal cortex lesions with grafts of fetal frontal cortex; LC, frontal cortex lesions with grafts of fetal cerebellar tissue; and L, frontal cortex lesions without implants of fetal tissue.

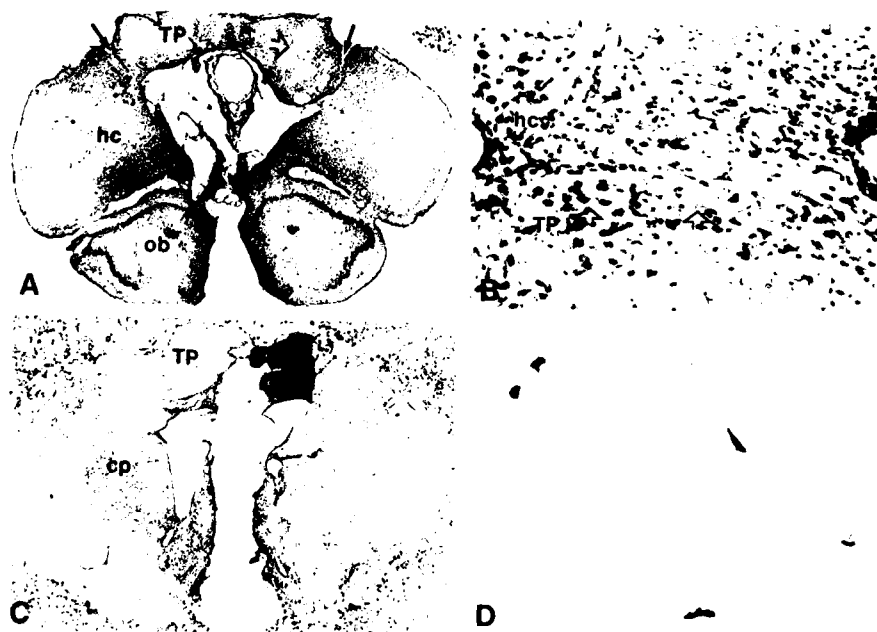


Fig. 2. (A) Transverse section of rat brain stained for Nissl substance, showing the position of a fetal cortex transplant (TP) in the anterior region of the frontal pole 78 days after transplantation. The transplant has bridged the cavity produced by aspiration. Neuron-free patches appear in the transplant (open arrow). Filled arrows indicate the points of attachment between the host tissue and the transplant. Abbreviation: ob, olfactory bulb ($\times 10$). (B) Transplant tissue stained for Nissl substance, showing the area of attachment between host tissue and implant in another animal. The host cortex (hc) is situated in the upper portion of the micrograph and the transplant in the lower portion. Note the large neurons located at the host-transplant border (open arrows) ($\times 50$). (C) Coronal brain section counterstained with cresyl violet, showing a cortical transplant unilaterally injected with HRP. The injection site appears black and there is virtually no spread of injectate into the adjacent cortex. At this level the transplant appears as two separate islands of tissue; however, at more anterior levels these islands are joined. Abbreviation: cp, caudate-putamen ($\times 10$). (D) Uncounterstained section showing retrogradely labeled anterior thalamic neurons in the host after the injection of HRP into the cortex transplant ($\times 100$).

served in the transplant. However, there was a slight diffusion of the HRP from the host cortex into the transplant.

Functional recovery from brain damage in animals with transplants of neural tissue may be due to factors other than connectivity between the transplant and host brain. It is possible that fetal brain grafts release neuronotrophic substances, such as polyamines and specific nerve growth factors. These may promote functional recovery by altering glial activity or neurotransmitter levels or by changing membrane receptor properties in the tissue surrounding the graft. Although the specific mechanisms remain to be discovered, these findings indicate that transplants of cortical tissue in adult rats are capable of enhancing behavioral recovery after bilateral brain injury.

Dunnett *et al.* (4) found that implants of fetal septal tissue promoted recovery of a learned discrimination in rats with damage to the fimbria-fornix system. These animals were able to solve a rewarded, spatial alternation task significantly faster than rats with similar damage but without transplants. However, animals with grafts did not perform as well as intact control animals on the spatial alternation task. These findings are similar to our own, despite the fact that Dunnett *et al.* waited 7 months before beginning behavioral training whereas we began testing just 4 days after transplantation. It is reasonable to conclude that the transplanted tissue begins to mediate behavioral recovery soon after transplantation and remains functional for almost a year, and perhaps for the rest of the animal's life.

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7. C. M. Leonard, *Brain Res.* **12**, 321 (1969); *Brain Behav. Evol.* **6**, 524 (1972).
8. We waited 7 days after inflicting the lesions to implant the fetal tissues because early responses to brain injury may hinder survival of such implants [E. R. Lewis and C. W. Cotman, *J. Neurosci.* **2**, 66 (1982)].
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11. J. V. Corwin *et al.*, *Neurobiol. Aging* **3**, 69 (1982).
12. Pressure injections of HRP conjugated to wheat

germ agglutinin (Sigma) were made into the host cortex of transplant tissue. After 48 hours the rats were perfused transcardially and their brains were prepared by the tetramethylbenzidine procedure [M. M. Mesulam, Ed., *Tracing Neural Connections with Horseradish Peroxidase* (Wiley, Chichester, England, 1982)]. Animals that did not receive HRP injections were perfused with 0.9 percent saline and Formalin and their brains were cut into 40- μ m sections and stained with cresyl violet acetate.

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14. We thank P. Curley, M. L. Valentino, R. Plourde, and D. Gash for their assistance and advice. Supported by United States Army Medical Research and Development Command Contract DAMD-82-C-2205.

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