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The Effects of Differential Therapies on the Functional Integration of Fetal Cerebellar Grafts

by

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Abstract

An aspirative lesion was made in the ventral and caudal cerebellum of rats. Fetal cerebellum was implanted into a lesion cavity in some of the rats. These rats were then trained in either a control condition, an exercise condition, or an acrobatic condition. The rats were then tested for behavioral recovery following a 25-day training period. The rats with acrobatic training committed more errors than the other groups regardless of the presence or absence of a transplant. These results show an effect of behavioral therapy (but not a transplantation therapy) at the time period studied, with respect to the functional recovery after mechanical damage to the cerebellum.

Introduction

Traditionally, physical therapy has been used to partially restore function to brain-damaged individuals. Recently, however, the transplantation of viable neurons from one brain to another has been shown to restore function to damaged brains (see below for some examples). These transplants (or grafts) show an ability to restore function in certain types of injury (cf. Zager & Black, 1988 for a review). The present study hopes to compares the effects of these different theraputic approaches to discover the best therapy in the case of damage to the cerebellum.

The mechanism by which grafts exert their ameliorative effects has not been determined, although investigators have been interested in the interplay of factors in the survival of grafted tissue for many years. To this date, scientists have elucidated six major factors affecting the functional viability of transplanted cells. These are: the age of the donor, the age of the host, the placement of the graft, the status of the graft's target, the vascularization of the transplant, and genetic and immunological problems.

The effects of the donor's age have been investigated in a large number of paradigms that relate to the transplantation of neural tissue. It is generally agreed that only tissue obtained from fetal or

very young animals is likely to establish functional connections with the host brain (Stenevi et al., 1976). In this classic study, various types of monoamine neurons were transplanted into a multitude of brain sites in adult rats. The transplants were then analyzed by fluorescence histochemistry to assess the survival of monoamine-containing neurons. Enhanced survival of embryonic tissue (relative to adult tissue) was observed for one, three, and six months post operation. The authors even suggested that transplanted embryonic neurons were capable of migrating into host tissue. In contrast, the tissue from the adult donors did not survive well, if at all, in many sites. By far the vast majority of subsequent grafting studies employ fetal tissue for the best results.

Gopal Das and colleagues (1980) examined the effects of the maturity of the embryonic tissue on the growth of the graft. These scientists transplanted neocortical tissue of constant volume from rats in the later stages of development (each embryonic day from fifteen to twenty-one, inclusive) into the cerebellum of adult hosts. It was shown that the transplants from embryonic day fifteen were the largest (a twenty-one-fold increase over original volume), with a definite graded effect being observed (less than a two-fold increase in donors at embryonic day twenty-one). In addition, the total number of neurons in the graft also decreased steadily with

increasing age of the donor. The cell density, however, was constant in all animals. The authors discussed their results in the context of the neurohistogenesis of the neocortex. The grafts from the fifteen day-old embryos were composed mostly of neuroepithelial cells which are capable of mitosis, then differentiation and growth, resulting in large transplants that became functionally integrated with the host. This could easily explain the increased volume of younger transplants. The older transplants consisted mostly of neuroblasts which simply differentiated and grew within the host brain. Thus, the older grafts did not divide.

The age of the animal into which the cells are implanted has also been shown to play a critical role in the survival of grafted neural tissue. Lund and associates (1987) looked at the comparative connections made when embryonic retina was implanted in the cortex of hosts of differing ages. The retinas were taken from rats at gestational age of days twelve and thirteen. When implanted into neonatal hosts, the retinas differentiated into all of the layers found in normal retinas and showed connections with the host tectum (the normal target of the retinal projections). It is interesting to note that all of the transplants followed the course of differentiation of the donor rather than the host. In adult hosts, however, the transplants did not differentiate or establish connections as well as

the transplants in the neonates. In both populations of hosts, it appeared that the retinal/tectal connections were highly specific, with many projections traced to the pretectum, dorsal lateral geniculate nucleus, and optic nuclei in neonates.

Similarly, Hallas (1980) found that neocortical transplants from embryonic rats at gestational day fifteen were removed and implanted into rat hosts of different ages. These investigators found that the transplants in five day-old hosts were considerably larger than those implanted into older hosts. The authors postulated that the relatively undifferentiated state of the brain parenchyma in animals of that age allowed the transplants the space to grow and integrate with the host. This study also found that transplants in the cerebellum occupied more space than those in the forebrain. This may reflect the later histogenesis of the cerebellum.

Access to a suitable target is vital to graft survival. Transplants that are implanted where their access to a target is limited often show increased necrosis and, if they survive, they remain confined to the cavity formed by the transplanting instrument (Foster et al., 1988). Gage and Björkland (1986) did a landmark study on the effects of the denervation of the area which supplies the graft area. They lesioned the fimbria-fornix area of rats destined to receive hippocampal transplants. They found that the volume in grafts in

animals with lesions was more than twice the size of those in their non-lesioned counterparts. They also found that the number of acetylcholinesterase-positive cells found in the graft areas of animals with the lesion was also more than twice that of the animals without a lesion. As may be expected from the above data, the cell density was nearly constant for all animals. The differences can be even more basic. Grafts were found in all of the animals with fimbria-fornix lesions, yet in two of the non-lesioned animals, no graft was ever found. Because the volume of the transplant is twice as large, but the cell density is the same in lesioned hosts, the authors postulated that the trophic factors released by the damaged fimbria-fornix acted upon all the cells in a graft (including glia), and not just the neurons (although the neuronal soma sizes in lesioned rats were significantly higher than those in non-lesioned animals). Gage and Björkland also ran a control experiment where the retrosplenial cortex was aspiratively lesioned. They found that this lesion had no significant effect on the growth of the hippocampal grafts. This led the investigators to imply that the effects of a fimbria-fornix lesion were specific to the hippocampal formation and not simply the effects of a more general wound-derived trophic factor, discussed subsequently (Nieto-Sampedro et al., 1984). This and other studies have demonstrated the powerful capabilities of

selective denervation as a tool for the enhancement of graft survival.

As noted above, the speed with which a graft is vascularized is critical to the graft's further development. This is intuitively obvious when one notes that no cell can survive for any extended period of time without some source of nutrition and waste removal. The first unchallenged report of neural transplantation by Dunn in 1917 found the greatest reports of graft survival when the grafts were placed adjacent to the choroid plexus, which is the highly vascularized site of the manufacture of cerebro-spinal fluid (Dunnett, 1990). Stenevi and associates (1976), as a corollary to their definitive study of the necessary conditions for the survival of neural transplants, found that transplants to the pial covering in the choroidal fissure demonstrated consistent survival and were all highly vascularized. In contrast to this finding, transplants to the caudate nucleus were almost totally necrotic or resorbed, save one. This sole survivor was the site of very few surviving neurons, however. The authors also used a vessel-rich iris as a transplantation bed in the hippocampus or the caudate nucleus to confirm these results. Survival of these grafts was greatly enhanced, with survival rates easily approaching those in the pia mater. This is probably due to the functioning of the iris as a "vascular bridge".

As stated above, immunological factors do not play as important a role in the survival of brain transplants as they do in transplants of other types. There is a general consensus among leading investigators that the brain is an immunoprivileged site, and thus not susceptible to invasion by the immune system (Dunnett & Björkland, 1987). Recently, however, certain investigators have begun to challenge this doctrine. As the second part of a paper, Lund and colleagues demonstrated the immunological consequences of cross-species transplantation (Lund et al., 1987). They dissected embryonic mouse cerebral cortex, dissociated it, and placed it into the occipital cortex of the host rats (aged from zero days to twelve weeks). They also examined the stability of mouse retinal transplants in rat hosts. As other studies have shown, survival was increased in those animals that were between zero and eight days post-natal. These transplants appeared to be integrated with the host brain to some degree. Transplants in animals that were between twelve and twenty days post-natal were all infiltrated by macrophages and lymphocytes. These transplants also failed to show any real connections with the host cortex. Transplants into adult hosts (aged eight to twelve weeks) were rejected almost immediately. If cyclosporin A was administered to these rats, the transplants were capable of surviving for months. The authors

looked at the rupturing of the blood/brain barrier as a possible explanation of these results. It is also possible to interpret these data by assuming that the transplants to neonates fail to be rejected due to the immaturity of the animal's immune system and the ability of the blood/brain barrier to quickly be reestablished quickly in younger animals, possibly because of the trophic interactions during development. These studies were done with xenografts and provide a model for understanding the complex immunological interactions during the grafting process. This in turn may allow us to examine the cause of allograft failure (failure of a same-species graft).

The ability to repair a damaged circuit in the brain is very important, given the central nervous system's inability to regenerate completely following a very early period. Neurotransplantation holds much promise for neurologists interested in curing many neurodegenerative diseases. These diseases often cause devastating (and usually incurable) behavioral deficits. But some of these deficits can be at least partially ameliorated through the use of brain transplants. In particular, neural transplantation has been proposed as a possible treatment for Parkinson's disease and Alzheimer's disease. The direction in which the search for a cure for these two ailments is progressing will be demonstrated. In addition, there has

recently been some progress in the understanding of the lesioned brain. This literature will be reviewed as well.

Alzheimer's disease has been studied in rats with both lesion-induced and age-related cognitive deficits (Gash, 1987). One study found that fetal locus coeruleus grafts were effective in the amelioration of age-related deficits in rats (Collier et al., 1985). Grafts from cholinergic cell lines have also been used to reduce cognitive deficits in rats (Gash et al., 1986). These avenues are promising, although there is no undebatable animal model for Alzheimer's disease. At the very least, such research has encouraged other researchers to begin looking for the treatment of the disease in the direction of neurotransplantation.

The most widely studied clinical application of grafting techniques is the treatment of Parkinson's disease. Although recent studies have shown serious flaws in the lesion model of this disease, it continues to be used as a paradigm, guiding research today (Dunnett et al., 1987). It was certainly the first of the clinical applications, as it was in use (in animal models) by 1979 (Perlow et al., 1979). The test animals are typically lesioned by the stereotaxic injection of 6-hydroxydopamine into the striatum and cells are transplanted into the lesioned area (Dunnett and Björkland, 1987). Some of the

crucial studies are reviewed below (cf. Freund, 1980 and Dunnett et al, 1987).

The understanding of the brain's response to mechanical damage is critical, keeping in mind the large number of people who suffer from in head injuries every year. Nieto-Sampedro and associates at the University of California have made significant advances in this area (1983; 1984). The authors found that the damaged brain released a number of neuronotrophic substances, and that these substances are released on a reproducible timetable. They caused a wound in the brain of the animal and then filled the cavity with Gelfoam (a inert, absorbant substance). The Gelfoam was later removed at various time points and assayed for neurotrophic activity. The activity began to increase at about postlesion day six and reached a maximum at sixteen days after the lesion was made. They also found that this increase of neuronotrophic activity was accompanied by the proliferation of glia. This suggests that glia may be responsible for the production of these trophic factors. An in vivo test of the effects of this factor was run. Cells transplanted into the entorhinal cortex of adult rats failed to survive if the surgery was performed immediately after the lesion was made. If the transplantation surgery was performed sixteen days postlesion, survival was greatly enhanced (Nieto-Sampedro, 1983). Supplying

the transplants with an extract from a wound site also promoted survival. The in vitro survival of corpus striatum neurons was also enhanced by this method (Nieto-Sampedro, 1984). These data obviously have clinical significance, and show that, although plain transplantation often fails in adults, perhaps a combination of treatments will provide better success.

The idea that grafts are able to enhance function through the establishment of one- or two-way connections with the host brain provides us with the most intuitive hypothetical mechanism of graft function. Since normal synaptic connections have been damaged in a lesioned animal, the reestablishment of these connections would appear to be necessary for normal behavior to resume. There is evidence that this can, in fact, occur. Freund and collaborators (1985) did an ultrastructural survey of the synapses in rats that showed recovery in behavioral tasks. Freund unilaterally lesioned the nigrostriatial dopamine pathway with 6-hydroxydopamine. He then grafted dopaminergic neurons to the neostriatum of the lesioned rats. After the rats showed functional recovery of normal turning behaviors (rats with a unilateral striatal lesion show a highly characteristic turning towards the side that is ipsilateral to the lesion), he sacrificed the animals and examined the grafts and their connections. He found that the cells in the graft made abundant

synapses with the neurons in the host. The vast majority (85-90%) of these synapses were axon/dendrite synapses. By retrograde tracing with horseradish peroxidase from the graft, Freund found that no labelled cells could be found in the normal dopaminergic centers in the host. This implies that the grafts function without normal input. He also found that the transplanted neurons also formed axon/cell body synapses in the striatum that have never been observed in a normal (ungrafted) animal.

In another study, the ability of dopaminergic transplants to fully restore host circuitry is questioned (Dunnett et al., 1987). These authors found that grafts to the striatum in dopamine-depleted animals are capable of restoring only simple behaviors, such as motor asymmetry. More complex behaviors, such as independent limb use, remain resistant to graft restoration. The authors question the ability of the graft to fully reconstruct lost synaptic connections when the cells are transplanted ectopically in the striatum. Obviously, a much more detailed study needs to be done in this area. New connections between cortical (occipital) grafts and the host thalamus and neocortex in the rat were demonstrated by retrograde labelling as well (Chang et al. 1984). It is important to note that these connections need not be synapses, because only a

light microscope was used. Synapses can only be verified through the use of electron microscopy.

Experience has also been shown to affect the restorative properties of central nervous system transplants (Kelche et al., 1988). In this study, rats that received a fimbria-fornix lesion were give a combination of behavioral and biological therapies. Some rats received transplants, some received experience in a complex environment, and others received a combination of the two treatments. Those rats that received both a transplant and enriched experience performed better on maze-learning tasks than animals that received only one of the therapies.

In the visual cortex, learning is accompanied by the formation of new synapses as well as new blood vessels (Turner and Greenough, 1985; Sirevaag et al., 1988). Because these two effects are inseparable in this system, the cerebral cortex is not an appropriate place to examine the interaction between the trophic effects of synaptogenesis and angiogenesis. In the cerebellar cortex, however, such a separation of effects may occur. Black and associates (1990) have developed a methodology that allows one to carefully manipulate the experimental variables that allow the formation of new synapses or new blood vessels. By training rats to perform acrobatic tasks, an investigator can induce synaptogenesis.

Likewise, by allowing rats free access to a running wheel, the formation of new blood vessels is enhanced. There is an almost total dissociation of the two desired effects.

The physiology of cerebellar transplants has been extensively characterized, most notably by Sotelo and co-workers (1990, 1987). They found that grafted Purkinje cells integrate synaptically with host climbing fibers and cerebellar interneurons. These synapses responded to electrical stimulation in the same way that adult Purkinje cells respond as early as 15 to 17 days after the graft was placed. All of these responses recapitulate those found during the normal ontogeny of the cerebellum.

Using these findings, an experimental design can be devised to take advantage of the peculiarities of the cerebellum, while answering some very important theoretical and clinical questions. The current study seeks to elucidate the psychobiological mechanisms underlying the integration of neural grafts, and to identify the most effective therapy (or combination of therapies) for recovery of cerebellar function will be discussed. The most effective method for the promotion of graft survival and growth will also be analyzed at a behavioral and histological level.

Methods

Subjects

Young Long-Evans hooded rats (aged 60-100 days) were used in this study. The animals were divided into seven groups, each containing six animals (see Fig. 1).

Surgery

To evaluate *in vivo* the effects of different types of therapy on graft integration, rats were lesioned in the paramedian lobule of the cerebellum. The animals had all surgery done under sterile conditions while anesthetised with sodium pentobarbital (60 to 75 mg/kg depending on the weight of the animal). The animals were given injections of atropine (.2 ml) and sodium pentobarbital while under light ether anesthesia to reduce discomfort and stress. The animals were placed in a head holder of a stereotaxic apparatus. The corneas were covered in ophthalmic ointment and surgical prep and surgical scrub was applied to the surgical area. An incision was made at the midline of the head and the scalp and periosteum is reflected. A hole was drilled over the cerebellum to provide the necessary access for the lesion. An aspirative lesion was performed, confining the lesion to the most caudal and dorsal areas of the

cerebellum, including, but not limited to, vermis and paramedian lobule. During the entire procedure, bleeding was controlled primarily by pressure, but epinephrine was used in some cases. A piece of bonewax, or the original bone flap (if intact), was then placed over the hole and the entire area was sealed with wound clips. The animal was then placed on its ventral side in its cage under a heat lamp. Its tail was covered with bedding to prevent hypothermia. The animal was monitored at ten minute intervals for postsurgical complications for at least one hour, then at least once every hour until consciousness was regained. During this time, atropine, electrolytes, and heat are administered to prevent fluid buildup in the lungs, dehydration, and hypothermia, respectively.

Transplant tissue source

Pregnant breeders (at 18-19 days post-fertilization) were anesthetized with ether. The fetuses were then removed from the uterus and placed into sterile media (F-10 from Gibco). The breeder was then allowed to overdose on ether. The fetal brains were removed and the rhombic lip (the cerebellum primordium) was dissected out. This fragment of tissue was then placed in a Hamilton Syringe and injected into the lesion cavity of a freshly lesioned animal (see Surgery above).

Training

After the surgery, the animals were split into three groups. All animals had food and water *ad libitum* and a 12/12 light/dark cycle for the entire training period. The first group (individual condition or IC) was housed individually according to standard animal care protocols. They were handled by experimenters for two minutes per day for 25 days and then behaviorally tested. The second group (voluntary exercise or VX) had free access to a running wheel and was also handled for two minutes per day for 25 days and then behaviorally tested. The final group (acrobatic condition or AC) was trained to negotiate a progressively more difficult "obstacle course". Animals were encouraged to traverse the obstacles by a gentle pinch of the hindquarters when performance declines. When the animals were incapable of traversing an obstacle, they were in some cases supported in part by the experimenter, though care was taken to avoid this. Animals that fell were caught by the experimenter and those that landed on the ground were cushioned by one inch of foam rubber. This training was continued for 25 days and the animals were then behaviorally tested.

Behavioral Testing

On the day before the behavioral testing began, the experimental animals were placed on a feed restricted diet. This restriction continued to the end of testing (which is variable). The weight of each rat was monitored each day so that none of the rats fell below 80% of their *ad libitum* weight. The animals underwent a three-day training period to familiarize them with the rotating rod apparatus. The animals began on a six inch start platform, and ended upon a six inch goal platform where a food reward (consisting of regular chow mixed with sugar that has been baked) had been placed. During the first day, the rats were shaped by progressively increasing the distance (in one foot increments) that they must travel along a stationary rod that has been covered with sand to ensure a good grip. During the successive training days, the animals were encouraged to traverse the entire (six foot) rod without hesitation by using gentle tail and hindquarter pinches. When all animals were successfully exploring the length of the rod, testing began. Animals were given six trials at each speed beginning with 6 rpm and increasing according to a second degree polynomial. Animals were allowed to continue with testing if they were able to traverse the rod four out of six times at any given speed in less than ten seconds without error. Errors are 1) a fall from the rod, 2) returning to the start platform, or 3) refusal to run within three minutes. If the

animal failed to pass even the slowest speed, the animal was removed from the testing apparatus, and given another day of training. If the animal still was unable to perform, the animal was scored on their second attempt and treated as the others. Otherwise, the animal was scored on only the second and subsequent attempts. On the subsequent testing days, animals were given one trial at each previously achieved speed and then tested as above until three errors occur at a single speed. If the animal was able to improve its performance by the attainment of a higher speed, the new scores were used in the analysis. When an animal was no longer improving the maximum speed attained, the animal was given food *ad libitum* and euthanized the next day.

Euthanasia and Histology

Animals were prepared for microscopic analysis by a standard perfusion. Animals were anesthetised with an overdose of sodium pentobarbital (100 mg/kg) and intracardially perfused with saline followed by a 4% paraformaldehyde solution. The brains were post-fixed in the perfusion solution, and then allowed to saturate with a sucrose solution to facilitate cutting.

Results

Three measures were used on the rotating rod: the speed that the rod was rotating (rpm), the time that it took the rats to cross the rod (seconds), and the errors that the rat made. Errors included falling off the rod, refusing to leave the start platform within three minutes, stepping onto the rod and then returning to the start platform, and taking longer than ten seconds to cross the rod.

Speed

This measure is simply a comparison of the average of the highest speed completed across groups. These data are shown in a scatter plot in Fig. 2. This measure failed to reach significance in both one-way and two-way ANOVAs.

Running Time

These data are the raw means of each animal's running time on successful trials. The data is presented as a scatter graph in Fig. 3. This measure also failed to reach significance within the limits of this experiment.

Errors

This measure was obtained by taking the total number of errors that each rat made and analyzing across groups. There was a significant physical therapy effect ($p<.05$), but there was no significant transplant effect. The lesioned (without transplant) acrobatic condition made more errors than either of the individual cage groups. A summary of errors is in Fig 4. Major errors include falls and refusals to run within three minutes. To better understand this effect, three other analyses were performed.

The first and second analyses involved comparisons between learning and non-learning groups (Fig. 5 and 6). The learning group was comprised of all the acrobatic condition groups, whereas the non-learning group was a combination of the voluntary exercise and the individual cage groups, regardless of whether they had a transplant or not. The learning group made significantly more errors than the non-learning group ($p<.01$). The learning group also had more trials greater than ten seconds ($p<.025$).

Because the animals were required to perform a criterion-based task, they each had a different number of trials. Because this may bias the results, another analysis was run. Here, the number of trials greater than ten seconds was divided by the total number of trials. These data are presented in Fig. 7. There was a nearly

significant difference between physical therapies ($p<.055$), but there was no difference between the groups with or without a transplant in the same physical therapy group.

Discussion

The most obvious finding in analyzing the data presented is that in none of the measures was a transplant effect significant, nor was interaction between the two types of therapy (transplantation and physical therapy) evident in any of the analyses performed. The effects of physical therapy, however, reached statistical significance on two measures (both measuring the ability to recover after an error), and showed near significance on one of the more robust tests ($p<.055$). For instance, for an animal to remain on the rotating rod for longer than ten seconds, the rat must be either running perpendicular to the axis of rotation, or rolling around the rod trying to gain a foothold. Both of these behaviors require a significant amount of motor coordination. Also, acrobatic animals tended not to be as upset by a fall from the rod. Non-learning animals would become highly agitated after a fall, refusing to leave the start platform on subsequent trials, thus disqualifying them from the next speed. This observation is easily explainable when one considers that the acrobatic rats had slipped before, whereas the others had never had any trouble with unstable surfaces. This could lead one to believe that this result could be extended further if more animals were used.

One must examine the entire experiment to determine why more conclusive results were not obtained. The length of the training (25 days) is a serious problem. In that time, a large amount of restoration may have already occurred. But this is a problem with the training paradigm itself, rather than this particular experiment. At that length of training the anatomical correlates of the experience are detectable. At shorter intervals, these correlates have not been experimentally verified (Black et al., 1989).

Another source of variation in this particular study is the size of the lesion. Because the aspirative lesions were done with a limited view of the cerebellum, the lesion was not necessarily confined to the target areas. A stereotaxic injection of excitotoxin (e.g. kainic acid) would yield a much more precise lesion, while also limiting the amount of incidental damage. Perhaps a histological examination of the brains of the animals will yield a better understanding of the processes involved in the recovery that takes place after mechanical damage to the cerebellum. This would allow the present animals to be used, and statistical methods could be applied to the present data. These comparisons would take into account both the raw volume of the lesion and the particular areas that are lesioned.

The final methodological problem that we will consider is the problem of behavioral assessment. The choice of the rotating rod as the test of motor function is justified on the basis of the literature supporting it as a measure of cerebellar damage. In the pilot study, it was found to be sensitive (as defined above) to the particular type of damage induced by our lesion. The failure of these results to replicate may be a function of the instability of the effects of the lesion and the few animals that we used. Still, a battery of tests designed to measure cerebellar dysfunction is almost definitely preferable to a single test. Also, the pretraining period was designed to ensure that all animals began the testing at a minimal level of competence, thus limiting the animal only by its ability, rather than its understanding of the task. However, recent unpublished data in our laboratory by Goldberg suggest that this may not be the best approach. She found a performance difference on the first day between various groups in the acrobatic training paradigm. This same difference did not apply to subsequent days, however. Note that this does not imply that such differences do not exist (few animals were used), but it does imply that group differences are most pronounced at early test points. The test on the rod when it is rotating is a different task than the training on a stationary rod, yet

it would be premature to assume that no transference of knowledge occurs.

With the correct application of the techniques described above, the variability of the behavioral data could be reduced, and one would hope that any variation remaining would be the result of the experimental manipulations of the independent variables. This paradigm is still in the process of development, and much work on the control of extraneous factors must be done. The potential benefit of this experimental design is great, and it should not be dismissed out of hand because the first results ever obtained from it did not yield significant effects. The results discussed in this paper, in reality, only chronicle the development of an experimental approach rather than the objective reporting of data based upon a well-established investigative technique. With further development, this approach could be modified to examine other questions in basic and applied psychobiology.

Endnotes

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Figure Legends

In all figures, S=sham-operated controls, LIC=individual cage without transplant, TIC=individual cage with transplant, LVX=voluntary exercise without transplant, TVX=voluntary exercise with transplant, LAC=acrobatic condition without transplant, and TAC=acrobatic condition with transplant.

Figure 1: The experimental design.

Figure 2: Highest speed achieved before failing to meet criterion.

Figure 3: Average running time.

Figure 4: Summary of errors made over all trials.

Figure 5: Total errors made over all trials between learning and non- learning groups.

Figure 6: Trials that took longer than ten seconds between learning and non-learning groups.

Figure 7: Trials that took longer than ten seconds divided by total number of trials.

Figure 1
Experimental Design

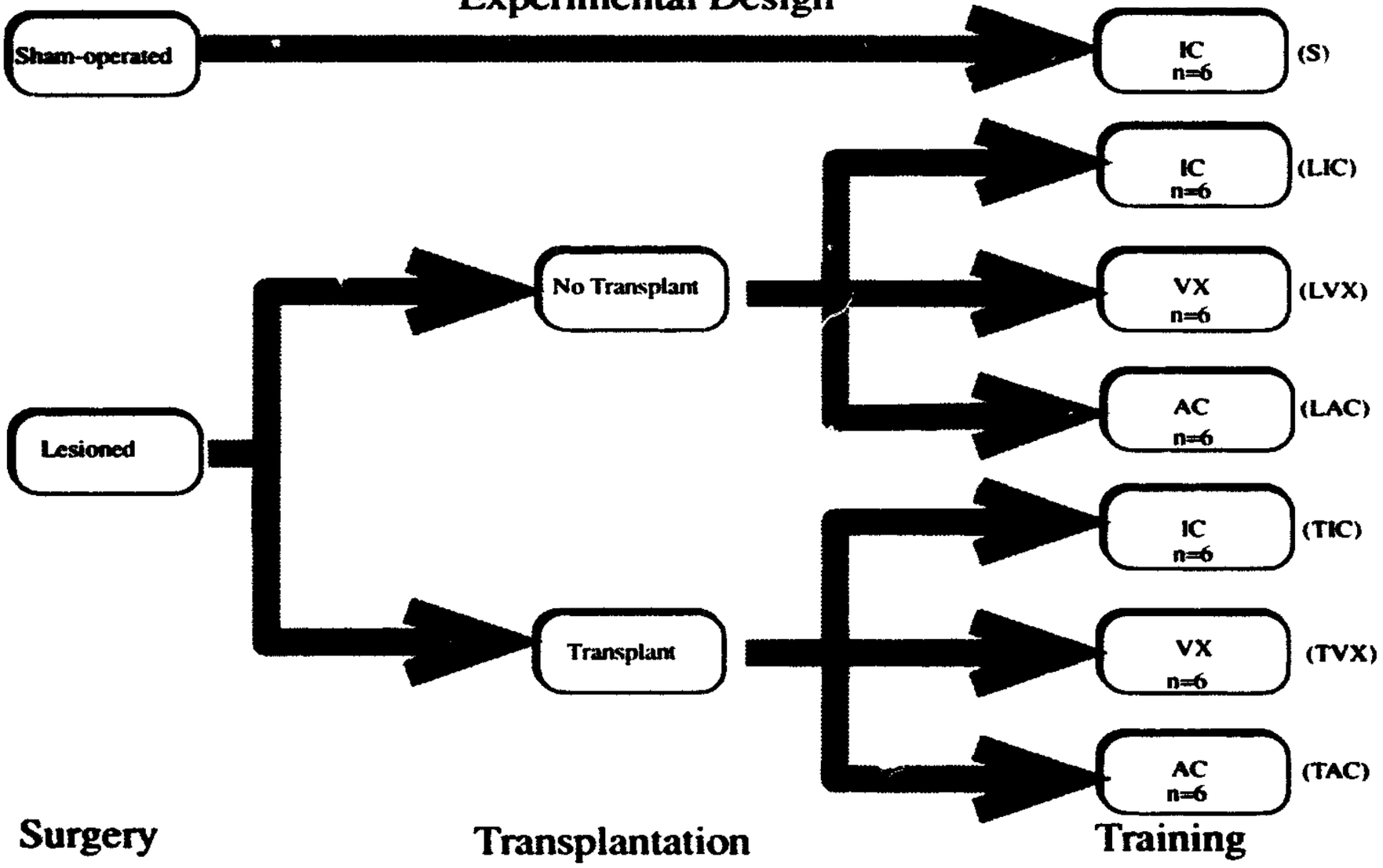

Figure 2

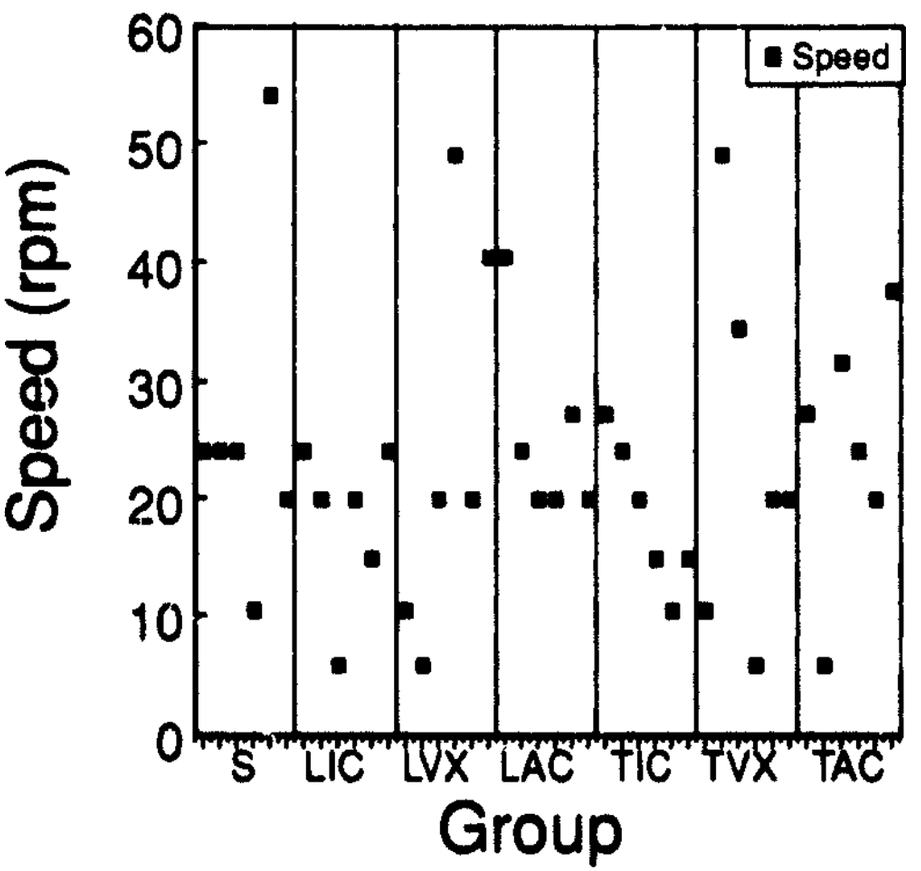
Top Speed Achieved
Speed (rpm)
60
50
40
30
20
10
0
Speed
S
LIC
LVX
LAC
TIC
TVX
TAC
Group

Figure 3

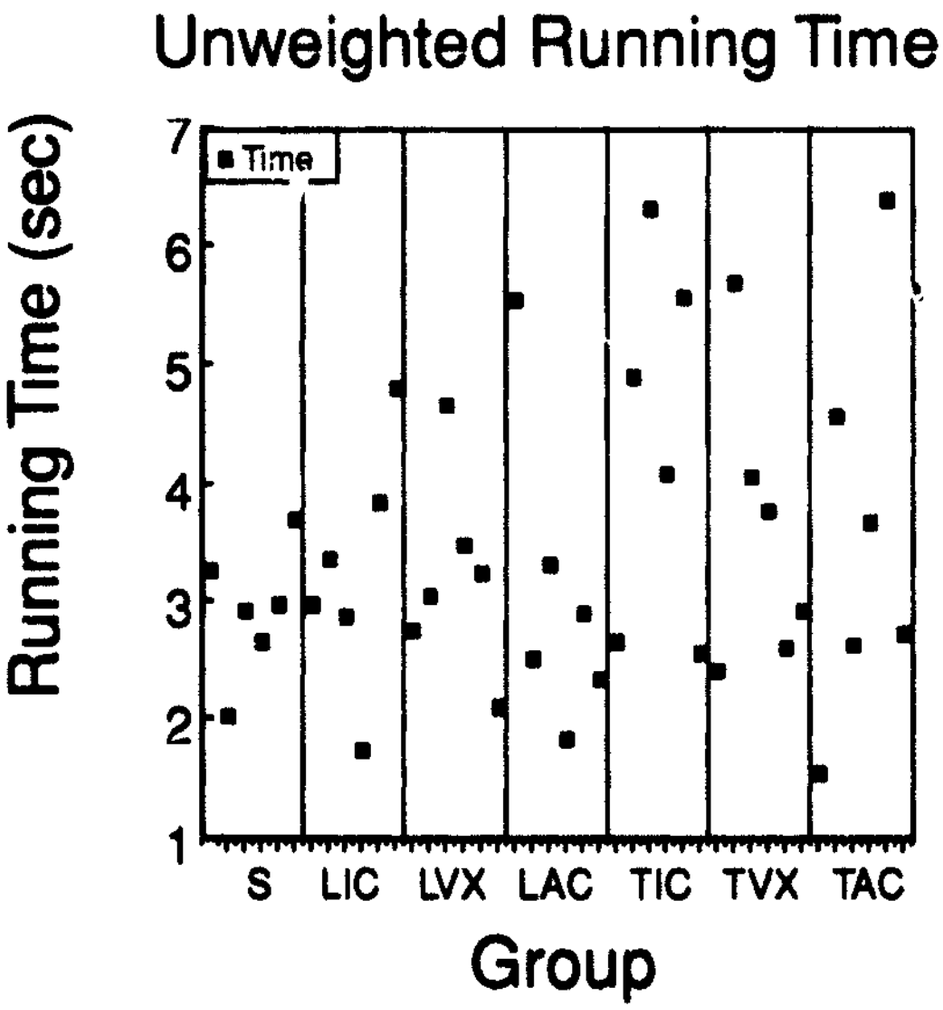
Unweighted Running Time
Running Time (sec)
Time
7
6
5
4
3
2
1
S
LIC
LVX
LAC
TIC
TVX
TAC
Group

Figure 4

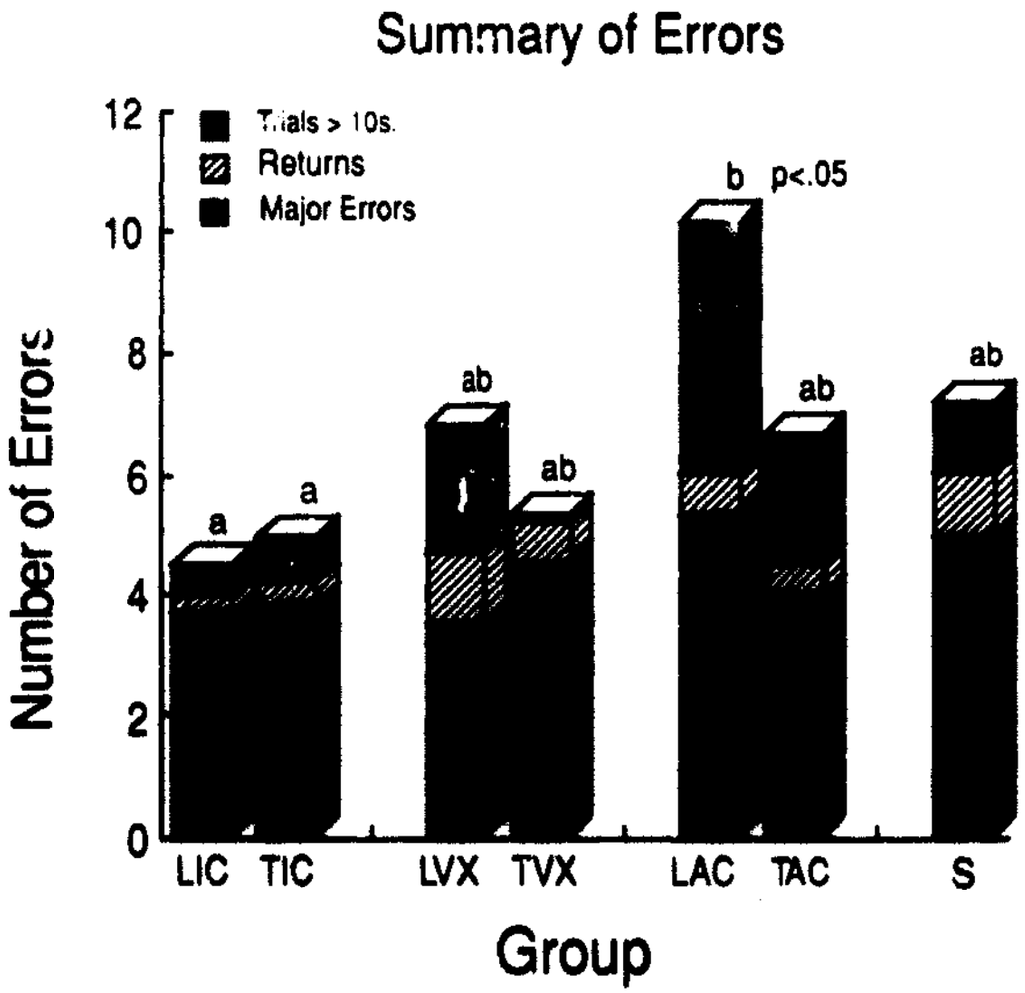
Summary of Errors
Trials > 10s.
Returns
Major Errors
Number of Errors
12
10
8
6
4
2
0
a
a
ab
ab
b
p<.05
ab
ab
LIC
TIC
LVX
TVX
LAC
TAC
S
Group

Figure 5

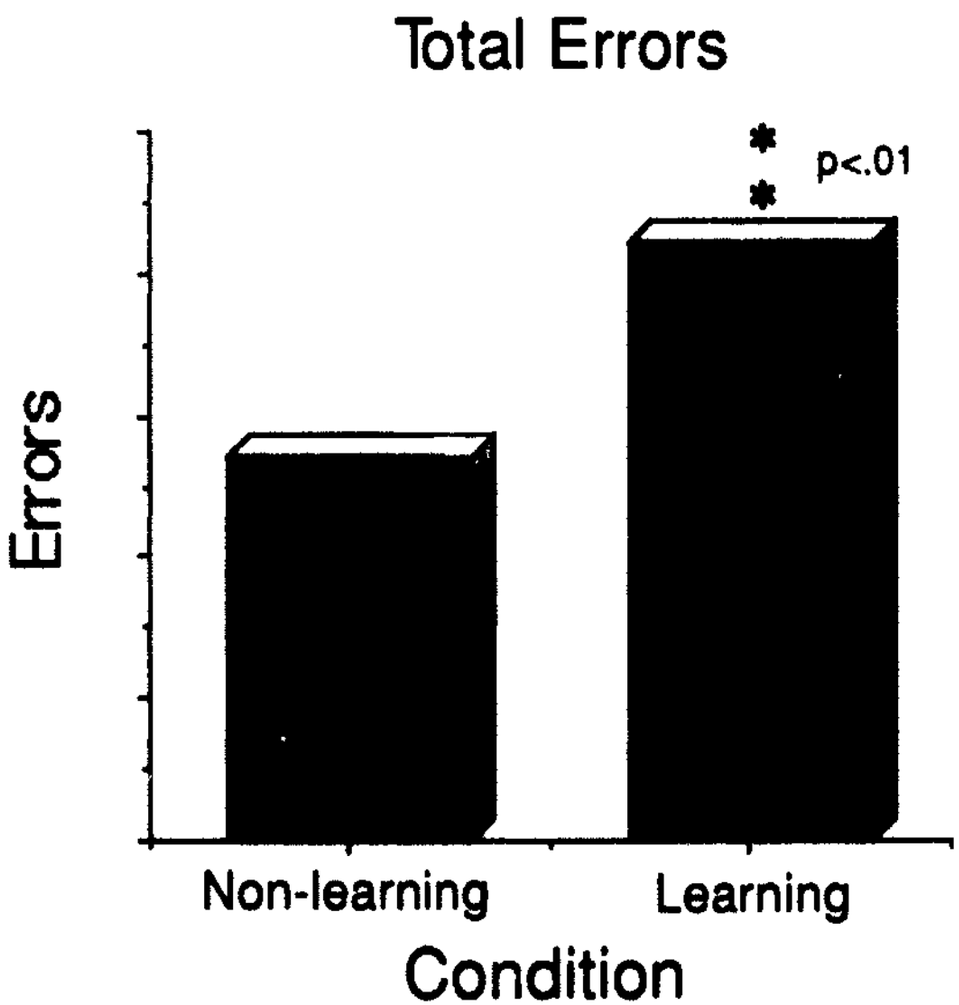

Figure 6

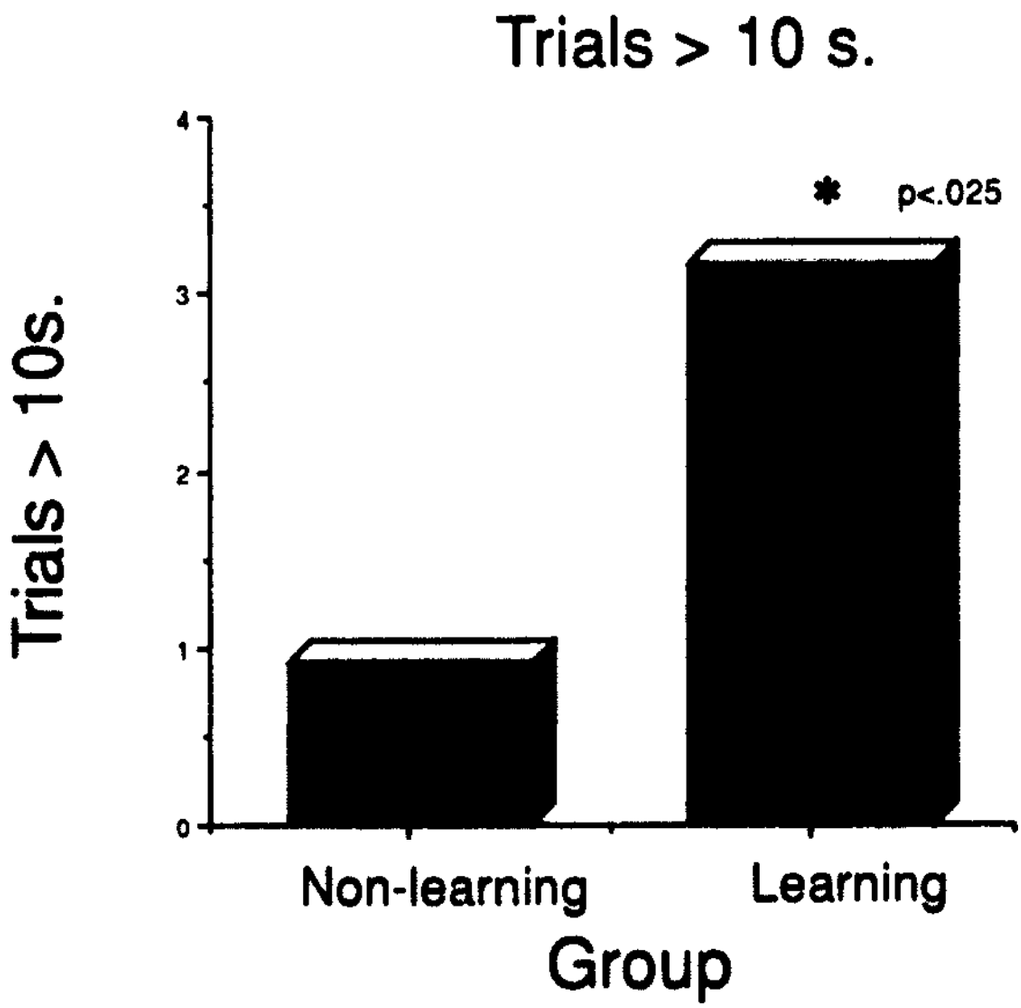
Trials > 10 s.
Trials > 10s.
4
3
2
1
0
* p<.025
Non-learning
Learning
Group

Figure 7

Trials>10 s. Weighted by Total Trials