TITLE PAGE

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ABSTRACT

Effects of weightlessness on vestibular development of quail.

The lack of gravity is known to alter vestibular responses in developing and adult vertebrates. One cause of these altered responses may be changes in the connections between the vestibular receptor and the brain. Therefore we propose to investigate the effects of gravity on the formations of connections between the gravity receptors of the ear and the brain in developing quail incubated in space beginning at an age before these connections are established (incubation day three) until near the time of hatching, when they are to some extent functional. This investigation will make use of a novel technique, the diffusion of a lipophilic dye, DiI, in fixed tissue. This technique can thus be used to analyze the connections in specimens fixed in orbit, thus eliminating changes due to the earth's gravity. The evaluation of the data will enable us to detect gross deviations from normal patterns as well as detailed quantitative deviations.

PROJECT DESCRIPTION

Weightlessness alters vestibular responses in developing and adult vertebrates. These behavioral changes may, in part, be due to the development of aberrant connections between the vestibular receptor and the brain. Therefore we propose to investigate the effects of gravity on the brain in quail embryos and hatchlings raised in space from a stage before these connections are present until approximately hatching, when they are, to some extent, functional. A novel technique, the diffusion of a lipophilic dye (DiI) in fixed tissue, will be used to analyze the connections immediately after landing of the space shuttle. The evaluation of the data will enable us to detect gross deviations from normal patterns as well as detailed quantitative deviations. The specific aims are:

Aim 1: To examine the central projection from gravistatic receptors (utricle and saccule) into the brainstem in quail raised in zero gravity from egg laying to approximately hatching (17 days) and to compare these results with those from synchronously incubated eggs and control groups.

Aim 2: To examine the peripheral termination of vestibular fibers at the gravistatic receptors within the sensory epithelia and to compare these results with those from control quail.

Background: Gravity appears to be a critical factor in the normal development of the vestibular system (Vinnikov, 1983; Jones et al., 1993). In particular, this evidence suggests that the parts of the vestibular system that monitor the position with respect to gravity (saccule and utricle) may require exposure to gravity for normal development. Thus, weightlessness during critical developmental periods may result in aberrant development. Alternatively, it is possible that the vestibular development relies on cues other than gravity to achieve its adult-like organization. According to the latter scenario one would not expect any developmental deviation of this vestibular organ in animals reared under reduced gravity. Thus, we can not presume a priori that rearing in weightlessness will necessarily result in structural defects of the vestibular system. Indeed, the existing body of experimental embryological data suggests that the vestibular system develops in a very rigid manner (Yntema, 1955). However, given that critical phases are well known in the developing visual, auditory and somatosensory system, we suspect that the developing vestibular system will require an adequate environmental stimulus (i.e., gravity) at a critical stage of development for functional verification of connections.

In this context, evidence among various vertebrates indicates that weightlessness during various developmental stages induces a temporary (tadpoles and embryonic zebrafish) or prolonged (chick embryos) spatial disorientation (Vinnikov, 1983; Jones et al., 1993). Even adult monkeys and humans exhibit a temporary spatial disorientation after even brief space flights (COSMOS 2044 Mission, 1992; Paloski et al., 1993). . This suggests that some degree of abnormal physiological changes occur in the vestibular system. This behavioral disorientation has been observed in animals exposed to weightlessness during a variety of different developmental stages (Vinnikov, 1983) in particualr in chicken which were unable to graps and feed (Jones et al., 1991, 1993) as well as in adult monkeys and humans after even brief space flights (COSMOS 2044 Mission, 1992; Paloski et al., 1993). This indicates that some degree of abnormal physiological and structural change occurs in the vestibular system. However, little is known of anatomical changes that underlie this abnormal behavior. In fact the peripheral gravistatic receptors in the ear appear to develop normally under weightless conditions (Vinnikov, 1983; Jones et al., 1993) suggesting that the abnormal behavior may be due to connectional defects between the ear and the vestibular nuclei. In support of this possibility weightlessness has been suggested to produce synaptic plasticity in connections of the developing vestibular system of fish (Rahmann et al., 1992). Moreover, weightlessness appears to induce quantifiable differences in synapse formation in the saccule and utricle of rats (preliminary results, SLS-1 mission). We propose to further test this possibility in the developing vestibular system of quails, in particular in developing animals that are reared under zero gravity from a time before the developing vestibular periphery establishes contact with the brain (approximately incubational day 2.5; Fritzsch et al., 1993) and before the functional connections are needed during hatching movements.

1. **Specific tissue requested:** Heads of aldehyde immersed quail embryos from flight group, synchronous control, and vivarium control (10 embryos from each group).

2. **Scientific rationale for requesting this tissue:**

We propose to study the development of vestibular connections with the lipophilic dye, DiI, in fixed tissue. The technique requires the heads of aldehyde perfused or immersed embryos. We would need the entire head so that we can expose the appropriate tissue (i.e. the vestibular sensory epithelia or the brain stem), allow the DiI to diffuse along the nerve fibers, and later examine the DiI-labeled fibers to determine where they terminate in the brain stem or the vestibular sensory epithelia. The diffusion of the DiI can only

occur in fibers that have been left undisturbed. Tissue from embryos from the synchronous control group and vivarium control group, in addition to the flight group, are essential controls for interpreting the effect of gravity on development.

3a. How analysis of the tissue will complement other ongoing research:

The current proposal is related to an ongoing analysis of ear development in chicken, quail, mouse and rat currently undertaken in our laboratories with respect to the auditory system. This NIH funded project has already provided new insights into early pattern formation (Bruce et al., 1993; Bruce and Christensen, 1993; Fritzsch and Nichols, 1993; Fritzsch et al., 1993). Both the current proposal and the NIH-funded project use the same technique of tracing connections with the lipophilic dye DiI in fixed tissues. In addition, we have requested support for a comparable analysis of the vestibular system development of rats in wieghtlessness (Fritzsch and Bruce, pending support). Clearly, expanding this analysis to the vestibular part of the ear and adding the dimension of potential ontogenetic changes in the connections caused by the exposure to zero gravity would tremendously compliment the ongoing research. Moreover, it is expected that the comparison between rats (support pending) and quail vestibular development in weightlessness will allow to draw a broader conclusion about possible effects of weightlessness on the development of vestibular connections.

3b. Scientific questions being addressed:

As outlined above, there is evidence for disturbed function of gravistatic responses in animals reared under reduced gravity. Whether these behavioral deviations are at all related to structural alterations of the otic receptors is currently doubtful (Vinnikov et al., 1983; Johns et al., 1993). The next logical system that could be affected would be the connection between the otic receptors and the brain. In this context the recent finding of more numerous synaptic profiles at vestibular hair cells appears to indicate some plastic response with respect to gravity (preliminary results, SLS-1 mission). Based on these findings as well as the suggestions of gravity-induced plasticity in the vestibular nuclei of fish raised under zero gravity (Rahmann et al., 1992), we believe that examining in detail the connections between the vestibular sensory receptors and the vestibular nuclei will clarify whether or not the behavioral defects are manifested in structural changes at this level. Our rational then would be to test the likely hypothesis that behavioral disorientation following exposure to weightlessness is due to the development of structural changes in these connections.

3c. Scientific questions to be answered:

Finding any structural correlates for the known behavioral disorientation in space-reared animals would certainly be a major step towards understanding the effect of gravity in the development of vertebrate vestibular systems. In a more general way, our investigation would contribute towards an understanding of the role of environmental versus genetic influences in the acquisition of appropriate neuronal connectivity.

4. Required tissue processing and handling.

Quail embryos which were incubated under reduced gravity from begin of incubation wiould be fixed in orbit short before hatching (around 17 days). The embryos would be fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) and storred until landing. Control embryos (synchronous control and vivarium control) would be fixed in a similar way. The aldehyde fixed animals would need to have the haeds surgically removed after landing and these heads would then be brought to our laboratories for further analysis. We would need at least ten heads of embryos of each group (space flight, synchronous control and vivarium control) to obtain statistically significant information.

5. General plan and methods used:

Our labs are fully equipped for the necessary histological and fluorescent microscopic techniques needed to evaluate the tracer data. Both PI's have extensive experience in studying neuronal development in the ear and other systems with this technique. We are both currently involved in a somewhat related, NIH funded project that investigates comparable aspects in the development of the auditory system of rats and mice with the technique proposed here.

Once the fixed (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2) embyo heads have arrived in our labs, we will surgically expose the desired area (brainstem or ear). The neuronal tracer DiI (Molecular Probes, Oregon) will be dissolved in dimethylformamide and filter strips or resin beads will be soaked in this solution. After evaporation of the solvent, the filter strips will be cut into appropriate-sized pieces and either these strips or resin beads of the appropriate size will be inserted into the desired sensory epithelium or specific areas of vestibular nuclei. For this procedure we would need an additional dissecting scope (see budget) because the available dissecting scopes (one in each laboratory of the PI's) are constantly used in other projects. The heads will subsequently be incubated in an 36 °C oven for accelerated diffusion of the dye (2-4 weeks) to obtain either a retrograde or anterograde filling of the cells and profiles. The brain and/or the ear will

then be dissected from the bony capsule, embedded in 10% gelatin, and hardened in 4% paraformaldehyde overnight. The blocks will be sectioned on a vibratome at 60-100 μm, the sections mounted on a slide (requiring a dissecting microscope), coverslipped, and examined with an epifluorescence microscope. All of the tissue handling would be performed by the research assistant requested. Data would be documented by the PI's with color and black/white photography at various magnifications and videotaped for future computer enhanced analyses of fiber distribution in the vestibular nuclei and the sensory epithelia.

The distribution of the labeled profiles from the experimental and control tissues would be plotted and compared for qualitative differences, i.e. fibers growing beyond normal target areas). At this level of our analysis we would not necessarily attempt to evaluate the data quantitatively because we hope to find some qualitative deviation from normal projection patterns (i.e. fibers going to aberrant targets). If that is not the case in the first series of animals processed (4 heads from each group), we would proceed to look at more minute differences. To do so we will counterstain the vestibular nuclei in the second batch of animals with a blue fluorescent dye to be able to simultaneously investigate the nuclear boundaries and cytoarchitectonic details of the vestibular nuclei and the distribution (area and density) of labeled profiles. For statistical evaluation the images would be grabbed and processed using appropriate software for area and density analysis. This software, computers, and image grabbers are available in our laboratory. If all of these analyses show similar results in the experimental and control groups, we would need to conclude that higher-order connections (i.e. vestibulo-spinal or vestibulo-ocular connections) may be affected, but not the receptor to vestibular nuclei connections. Given the number of animals that we would be able to analyze and the general reliability of the technique employed we are confident that our objective could either be verified or falsified with the numbers of pups requested (10 embryos in each of the three groups). If our data would show structural aberrations in the vestibular projection patterns due to the reduction of gravity, further investigations of whether and how fast these aberrations could be reversed under terrestrial gravity conditions would be needed. In addition identification of the critical stages in development that gravity is essential for the formation of normal axonal growth and terminations and for the recovery from the effects of weightlessness would need further investigation. Should there be no detectable aberration in the projection patterns, one could conclude that higher-order connections must be quantitatively or qualitatively modified to achieve the known behavioral defects of space flight.

LITERATURE CITED

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Bruce, L.L., B. Fritzsch, and M.A. Christensen (1993) Advantages and pitfalls of using DiI for electron and light microscopy. Europ. J. Neurosci. Suppl. 6: 75.

COSMOS 2044 Mission (August 1992) Journal of Applied Physiol., 73(2), Suppl.

Fritzsch, B., and D.H. Nichols (1993) DiI reveals a prenatal arrival of efferents at developing ears of mice. Hearing Res., 65: 51-60.

Fritzsch, B., M.A. Christensen, and D.H. Nichols (1993) Fiber pathways and positional changes in efferent perikarya of 2.5 to 7 day chick embryos as revealed with DiI and dextran amines. Journal of Neurobiol., 24: 1481-1499.

Harrison, R.G. (1945) Relations of symmetry in the developing embryo. Trans. Conn. Acad. Arts Sci., 36: 277-330.

Jones, T.A., J. Vellinger, P.Y. Hester, and C. Fermin (1991) Weightlessness and the ontogeny of vestibular function: evidence for persistetn vestibular threshold shifts in chicks incubated in space. The Physiologist, 34: 143-144.

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Paloski, W.H., F.O. Black, M.F. Reschke, D.S. Calkins, and C. Shupert (1993) Vestibular ataxia following shuttle flights: Effects of microgravity on otolith-mediated sensorimotor control of posture. Am. J. Otology, 14: 9-17.

Rahmann, H., K. Slenzka, K.H. Kortje, and R. Hilbig (1992) Synaptic plasticity and gravity: Ultrastructural, biochemical and physicochemical fundamentals. Adv. Space Res., 12: (1)63-(1)72.

SLS-1 mission (1993) Preliminary results. NASA Announcement of Opportunity AO 93-OLMSA-01, pg. 6.

Vinnikov, Ya. A., O.G. Gazeno, D.V. Lychakov, and L.R.> Palmbach (1983) Formation of the vestibular apparatus in weightlessness. In: Development of Auditory and Vestibular Systems (ed. R. Romand), pp. 537-560. Academic Press, New York.

Yntema, C.L. (1955) Ear and nose. In: Willier BH, Weiss PA, Hamburger V (eds) Analysis of Development. Philadelphia: Saunders, pp. 415-428.

Personnel:

Bernd Fritzsch, Ph.D. Professor 5/1/48

University of Darmstadt, Darmstadt, Germany	M.A.	1974	Biology
University of Darmstadt, Darmstadt, Germany	Ph.D	1978	Zoology
University of Darmstadt, Darmstadt, Germany	Postd.	1981	Neuroanat.

Res. Assistant	University of Darmstadt, Germany	1978-1981
Assistant Prof.	University of Bielefeld, Germany	1981-1986
Heisenberg Stip.	University of Bielefeld, Germany	1986-1990
Assoc. Prof.	Creighton University, NE, USA	1990-1993
Professor	Creighton University, NE. USA	1993-present

Honors and Awards:
Funded continuously by the German Science Foundation from 1978 to 1991. Graduation award of the Studienstiftung (1975). Heisenberg-Award (equivalent to the RCDA). Distinguished Research Career Award, Creighton, 1993. NIH program project grant: Neurobiology of the Auditory System (1992-1997).

Selected Publications of 1991-1993 (out of a total of 89).

1. Manns, M. and **Fritzsch, B.** (1992) Retinoic acid affects the organization of reticulospinal neurons in developing Xenopus. Neuroscience Letters, 139:253-256.
2. Wilm, C. and **Fritzsch, B.** (1993) Ipsilateral retinopetal projections of the nucleus olfacto-retinalis (NOR) during development and regeneration: A DiI study in fixed tissue. Journal of Neurobiology, 24: 70-79.
3. **Fritzsch, B.** and Nichols, D.H. (1993) DiI reveals a prenatal arrival of efferents at developing ears of mice. Hearing Res. 65: 51-60.
4. **Fritzsch, B.**, and Northcutt, R.G. (1993) Origin and migration of trochlear, oculomotor and abducent motoneurons in Petromyzon marinus L. Developmental Brain Research. 74:122-126.
5. **Fritzsch, B.**, (1993) Fast diffusion of 3000 molecular weight dextran amines. Journal of Neuroscience Methods. 50: 95-103.
6. **Fritzsch, B.**, M. A. Christensen and D.H. Nichols (1993) Fiber pathways and positional changes in efferent perikarya of 2.5 to 7 day chick embryos as revealed with DiI and dextran amines. Journal of Neurobiology, 24: 1481-1499.
7. **Fritzsch, B.** and Northcutt, R.G. (1993) Cranial and spinal nerve organization in amphioxus and lampreys. Acta Anatomica, 148: 96-109.

Laura L. Bruce, Ph.D. Associate Professor

Cornell College, IA	B.A.	Biology	1975
Georgetown University, DC	Ph.D.	Anatomy	1983
Medical College of Virginia	Postdoc. Fellow	Physiology	1982-1987
Creighton University, NE	Assist. Professor	Anatomy	1987-

Honors and Awards:
Distinction for Ph.D. thesis work (1983); NIH postdoc. fellow: Ontogeny of Visual Corticotectal Influences (1983-1986); NIH program project grant: Neurobiology of the Auditory System (1992-1997)

Publications:

1. Loop, M.S., and **L.L. Bruce** (1978) Cat color vision: The effect of stimulus size. Science, 199: 1221-1222.
2. Loop, M.S., **L.L. Bruce**, and S. Petuchowski (1979) Cat color vision: The effect of stimulus size, shape and viewing distance. Vision Res., 19: 507-512.
3. Butler, A.B., and **L.L. Bruce** (1981) Nucleus laminaris of the torus semicircularis: Projection to spinal cord in reptiles. Neurosci. Lett. 25: 221-231.
4. Kicliter, E., and **L.L. Bruce** (1983) Ground squirrel ventral lateral geniculate receives laminated retinal projections. Brain Res., 267: 340-344.
5. Newman, D.B., W.L.R. Cruce, and **L.L. Bruce** (1983) The sources of supraspinal efferents to the spinal cord in a variety of limbed reptiles. I. Reticulospinal systems. J. Comp. Neurol., 215: 17-32.
6. **Bruce, L.L.**, and A.B. Butler (1984) Telencephalic connections in lizards. I. Projections to the cortex. J. Comp. Neurol., 229: 585-601.
7. **Bruce, L.L.**, and A.B. Butler (1984) Telencephalic connections in lizards. II. Projections to anterior dorsal ventricular ridge. J. Comp. Neurol., 229: 602-615.
8. **Bruce, L.L.**, J.G. McHaffie, and B.E. Stein (1987) The organization of trigeminotectal and trigeminothalamic neurons in rodents: A double-labeling study with fluorescent dyes. J. Comp. Neurol., 262: 315-330.
9. **Bruce, L.L.**, and B.E. Stein (1988) The postnatal maturation of transient projections from the lateral geniculate to the posteromedial lateral suprasylvian visual cortex in kittens. J. Comp. Neurol., 278: 287-302.
10. **Bruce, L.L.** (1993) Postnatal development and specification of the cat's visual corticotectal projection: efferents from the posteromedial lateral suprasylvian area. Dev. Brain Res., 73: 47-61.

Facilities and Equipment Available:

All of the work described in this proposal will be done by faculty and staff of the Anatomy Division in the Department of Biomedical Sciences at Creighton University School of Medicine using laboratory facilities there. Those facilities are listed below.

Dr. Bernd Fritzsch has a 450-sq. ft. embryology laboratory with two laminar flow hoods that are equipped with appropriate optical instruments for embryonic surgery. Dr. Fritzsch also has an anatomy laboratory with an Olympus photomicroscope (BH2) equipped for fluorescence and DIC microscopy, an Olympus SZH Macroscope with photographic equipment, and a refrigerator (17.2 cu. ft., with freezer compartment). Documentation of the DiI labeled material will be done with a videocamera (World video camera HR-1 (resolution 480x350), a VHS recorder (Mitsubishi super VHS, BV 1000 with freeze frame), and a monitor (World video NTSC 14" CDM-14). Software for the detailed analysis is available (Waytek). In addition he has shared access to the histology laboratory described below and to an SGI IRIS 210/VGX workstation with 1.8 gigabytes of storage which can be used for image processing and 3D-reconstruction. This workstation is connected with the regional computer network, MidNet.

Dr. Laura L. Bruce has a 510-sq. ft. laboratory equipped with a Nikon Optiphot microscope with epi-fluorescent, photographic, drawing tube, and polarizing light attachments, as well as a tri-simplex overhead projector, a vibratome, oven, and IBM compatible Gateway 4DX-33 and Dell 286 computers with a LaserJet II printer. A multi-user laboratory adjacent to Dr. Bruce's laboratory is available for histological processing. It is fully equipped with sinks, hoods, benches, and adequate storage space and tissue processing. Additional equipment includes a glass water distillery, sledge and rotary microtomes, pH meters, ovens, LKB Ultramicrotome III and glass breaker, and a Reichert ultramicrotome for TEM-sections. There is also a fully equipped dark room for photography.

CURRENT SUPPORT

(1) Currently Active Support:

Source: NIH-NIDCD /P50 DC00215-09 - W.B.Warr, P.I.
Title: *Neurobiology of the auditory system. Project I: Postnatal development of the efferent system*
% of appointment: 20% - L.L. Bruce, co-PI
Project period: 04/1/92 - 03/31/97
Annual direct cost: $87,900
Description: This program project grant (PPG) investigates the postnatal development of the connections of the neurons providing the efferent and afferent innervation of the cochlea in rats during the period the auditory system becomes functionally active. There is no overlap with the current project.

Source: NIH-NIDCD /P50 DC00215-09 - B. Fritzsch, P.I.
Title: *Neurobiology of the auditory system. Project II: Embryonic development of the efferent system*
% of appointment: 40% - Fritzsch
Project period: 04/1/92 - 03/31/97
Annual direct cost: $ 82,555
Description: This program project grant (PPG) investigates the embryonic development of the olivocochlear efferent system in mice, chicken and frogs. Both descriptive development of the connections as well as experimental manipulations (in chicken and frogs only) will be used to investigate the time table of developmental events and the mechanisms. There is no overlap with the current application.

Source: NIH-NIDCD /P50 DC00215-09 - B. Fritzsch, P.I.
Title: *Effect of weightlessness on vestibular development of rats.*
% of appointment: 20% - Fritzsch, 20% Bruce Co-PI
Project period: 01/94 - 12/94
Annual direct cost: $ 56,744
Description: This application proposes to examine the effect of weightlessness on the differentiation of the vestibular connections in rats under reduced gravity. There is no overlap with the current application but both investigations are complementary in scope.

PROPOSED COSTS:

Labor costs

	Hours	Rate	Amount
Chief investigator	416	$44.90	$18678
Co-investigator	416	$43.74	$18192
Research assistant	1040	$17.53	$18231
Total	**1872**		**$55101**

Justification.

Personnel: Both PI's will be evaluating the tissue processed by the research assistant. Both PI's are involved in other projects and teaching so that the presence of a research assistant for the tissue processing is paramount for the speedy processing of the tissue to finish the project within the time requested.

Indirect costs: See enclosed copies of historical and current rates. The current rate is 41% and does not include equipment costs.

Travel: As discussed, the PI's need to be annually at two workshops (7 days; $525 room, $266 board, $120 car, $850 airfare; total $1,1761 x 2 = $3,522). In addition, each PI will attend one meeting per year (5 days; $381 room, $190 board, $100 car, $850 airfare; total $1,521 x 2 = total $3,042). The total of travel will come to $6,564 per year.

Supplies

We are requesting support for the neuroanatomical tracer (DiI, $260), two high intensity microscope bulbs ($616), for slides, coverslips and other laboratory material ($575), for photographic supplies (films, developer, tapes; $1200), for publication costs ($500). Supplies will run at a total of $3151 per year.

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FORM C

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Fritzsch, B. Ph. D.

DETAILED BUDGET FOR 12-MONTH BUDGET PERIOD DIRECT COSTS ONLY		FROM 7/1/96	THROUGH 6/30/97		
Duplicate this form for each year of grant support requested		DOLLAR AMOUNT REQUESTS *(Omit cents)*			
PERSONNEL *(Applicant Organization Only)*		**EFFORT ON PROJECT**	**SALARY**	**FRINGE BENEFITS**	**TOTALS**
NAME	**ROLE IN PROJECT**				
B. Fritzsch	Principal Investigator	20%	15,042	3,836	18,878
L.L. Bruce	Co-Investigator	20%	14,496	3,696	18,192
M.Christensen	Res. Assistant	50%	14,527	3,704	18,231
	SUBTOTALS →		44,065	11,236	55,101
CONSULTANT COSTS					
EQUIPMENT *(Itemize, use additional sheet if needed)*					
SUPPLIES *(Itemize by category, use additional sheet if needed)*					3,151
TRAVEL	DOMESTIC				6,564
	FOREIGN				
OTHER EXPENSES *(Itemize by category, use additional sheet if needed)*					

TOTAL DIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD ***(Item 8a, Form A)***	**$**	64,819
INDIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD 41% of 64,819	**$**	26,576
TOTAL COSTS FOR FIRST 12-MONTH BUDGET PERIOD ***(Item 8b, Form A)***	**$**	91,395

G0018RM

FORM D

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: B. Fritzsch, Ph. D.

BUDGET FOR ENTIRE PROJECT PERIOD DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		1st BUDGET PERIOD	ADDITIONAL YEARS OF SUPPORT REQUESTED	
			2nd	3rd
PERSONNEL *(Salary and Fringe Benefits)* *(Applicant organization only)*			55,101	
CONSULTANT COSTS				
EQUIPMENT				
SUPPLIES			3,151	
TRAVEL	DOMESTIC		6,564	
	FOREIGN			
OTHER EXPENSES				
TOTAL DIRECT COSTS FOR this BUDGET PERIOD				$ 64,819
TOTAL INDIRECT COSTS FOR this BUDGET PERIOD				$ 26,576
TOTAL DIRECT + INDIRECT COSTS FOR this PERIOD				$ 91,395
TOTAL DIRECT + INDIRECT COSTS FOR ENTIRE PROJECT				$ 183.030

JUSTIFICATION FOR UNUSUAL EXPENSES (Detail Justification in Cost Section of Proposal)

One year progress report on:
"Effects of weightlessness on vestibular development in Quail"
B. Fritzsch and L.L. Bruce

In our original application we proposed to investigate the effects of gravity on the formations of connections between the gravity receptors of the inner ear and the brain in quail raised in space beginning at an age before these connections are made until near the time of hatching, when they are to some extent functional. We proposed to use the neuronal tracer, DiI, which can be applied to tissue fixed in orbit, thus blocking changes in connections due to the earth's gravity. We hoped to determine whether the vestibular system develops in two phases as do other sensory systems (such as the visual system). In these other systems the first phase of development is controlled genetically and the second phase is controlled by environmental stimulation. Unfortunately we have not yet received tissue that was exposed to microgravity. In the absence of such tissue we have started to conduct an analysis of the projection patterns of the inner ear sensory epithelia at the developmental stages during which we had planned to study the development of vestibular projections in microgravity.

We have obtained quail from Dr. Hester and have incubated them for the appropriate times. These animals were then fixed by transcardiac perfusion with 4% paraformaldehyde in PBS. The inner ears were surgically exposed and the lipophilic dye, DiI, was introduced into selective sensory epithelia. After appropriate diffusion times the ears with the ganglia attached were prepared and analyzed. Thus far we have concentrated on issues of distribution of ganglion cells that project to different sensory epithelia and the distribution of efferent fibers between the different sensory epithelia. The latter aspect has previously been reported based on double labeling techniques, but its full extent has never been described. DiI is a tracer that allows us to study this. This issue is important as our preliminary data in rats that developed in microgravity suggest some developmental aberrations in these connections.

The first two launches of quail eggs were unsuccessful because of equipment failure. This was found out after we had dissected the eggs and

found no or already partially degenerated embryos. Our third group of quail eggs is currently on the MIR station, and will be transported to earth in the next shuttle. Owing to the delay in the launch of Atlantis we could not work on this third, hopefully successfully incubated batch of quail eggs. These microgravity exposed eggs are already fixed and we will be dissecting them as soon as they arrive. We expect that we will have in the second year the material to verify our suspicion of changes in the efferent system in microgravity and to extend other findings obtained in microgravity exposed rats into another species. We are also hopeful that additional eggs will be launched and incubated to replace those that were lost due to equipment failure.

Bernd Fritzsch, Ph. D.

L. Bruce

Laura L. Bruce, Ph. D.