Summary of Research

Investigation Title: Effects of weightlessness on vestibular development of quail.

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Additional Investigators: Laura L. Bruce, Ph. D.

Investigation Objectives:

A. Hypothesis: In our original application we proposed to investigate the effects of gravity on the formation 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 preventing changes in connections due to reentry into 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. In the case of the vestibular system this environmental stimulus could be gravity induced linear acceleration.

B.

C. Objectives of Experiment: The long range importance of this research is to find out whether or not there is a critical phase during development of the vestibular system in which appropriate stimuli are needed to fine tune synaptogenesis. These data will be crucial for future long range space explorations that require multi-generation flights.

Phase 1 Missions: Flown on MIR, total of three flights, only one flight (Atlantis) resulted in some successfully incubated quail eggs of sufficient age to be used by us.

Results:

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 received tissue that was exposed to microgravity and was fixed suitable for this analysis.

Completeness/quality of data:

a) # of quail embryos at 14 days of incubation obtained: 2 heads and brains.
b) # of quail embryos at 16 days of incubation obtained: 1.5 heads and brains.

These numbers were not sufficient and far off from the requested 10 animals per stage. Technically, we wanted to analyze the central projection of the vestibular end organs such as saccule, lagena and utricle and compare this with non-gravity sensing end organs such as the angular accelerometers of the semicircular canals. The method used is rapid diffusion of the lipophilic dye DiI in the vestibular nerve fibers after selective implantation of DiI crystals into the appropriate organs. This technique has been used extensively by us in the past on a variety of tissues (Bruce et al., 1997a,b; Fritzsch et al., 1997) and was previously successfully applied to an analysis of the vestibular connections in microgravity exposed rat embryos (Fritzsch and Bruce, 1997).

In the absence of a fixation suitable for DiI tracing, we tried to analyze the ears using immunohistochemical techniques. Initial stains indicated in control quail that we would be able to label the nerve fibers using and antibody against β-acteylated tubulin. However, using this antibody we did not get any staining in the microgravity exposed ears. Again, the insufficient fixation is likely to blame. As a last resort we embedded the ears in plastic for a thick section analysis of hair cell numbers and degree of maturation. Unfortunately even this rather simple issue could not be analyzed in the

microgravity-exposed chicken due to inadequate fixation. Both the incubator and the fixation technique are currently being revised by NASA.

Conclusions:
The data confirm previous findings that quail embryos can, under proper circumstances, develop until hatching in microgravity. There were no gross abnormalities in the few ears of the late embryos (we received 3 ears at E14.5 and 4 ears at E16.5). Due to inadequate numbers of samples returned and their fully insufficient fixation, no conclusions could be reached that warrant any publications.

Publications from related projects in 1997-99 (*denotes papers supported by this and other NASA grants):

1. Fritzsch, B., I. Silos-Santiago, L. Bianchi, and I. Fariñas (1997) The role of neurotrophic factors in regulating inner ear innervation. TINS, 20: 159-165.
2. Fritzsch, B (1997) On the role played by ontogenetic remodeling and functional transformation in the evolution of terrestrial hearing. Brain, Behav. Evol., 50:38-49.
3. Fritzsch, B., I. Silos-Santiago, L. Bianchi, and I. Farinas (1997) Neurotrophins, neurotrophin receptors and the maintenance of the afferent inner ear innervation. Seminars in Cell and Developmental Biology, 8:277-284.
4. Bruce, L.L., Christensen, M.A., and Fritzsch, B. (1997)a Electron microscopic differentiation of directly and transneuronally transported DiI and applications for studies of synaptogenesis. J. Neurosci. Meth.,73: 107-112.
5. Bruce, L.L., J. Kingsley, D.H. Nichols and B. Fritzsch (1997b) The development of vestibulocochlear efferents and cochlear afferents in mice. Int. J. Dev. Neurosci., 15; 671-692.
6. Fritzsch, B., Farinas, I. and Reichardt, L.F. (1997) Lack of NT-3 causes losses of both classes of spiral ganglion neurons in the cochlea in a region specific fashion. J. Neurosci. 17: 6213-6225.
7. Hallböök, F. and Fritzsch, B. (1997) Distribution of BDNF and trkB mRNA in the otic region of 3.5 and 4.5 chick embryos as revealed with a combination of in situ hybridization and tract tracing. Int. J. Dev. Biol. 41:725-732.
8. Silos-Santiago, I., Fagan, A.M., Garber; M., Fritzsch, B., and Barbacid, M. (1997) Severe sensory deficits but normal CNS

development in newborn mice lacking TrkB and TrkC tyrosine protein kinase receptors. Eur J Neurosci. 9: 2045-2056.

9. *Bruce, L.L. and Fritzsch, B. (1997) The Development of Vestibular Connections in Rat Embryos in Microgravity. J. Gravit. Physiol. Vol. 4: 59-62.
10. *Fritzsch, B., Barald, K. and Lomax, M. (1998) Early embryology of the vertebrate ear. In: Rubel, E.W., Popper, A.N., Fay, R.R. (eds.) Springer Handbook of Auditory Research. Vol XII. Development of the Auditory System. Springer, New York, 80-145.
11. *Fritzsch, B. (1998) Evolution of the Vestibulo-Ocular system. Otolaryngology - Head and Neck Surgery, 119:182-196.
12. Fritzsch, B. (1998) Of mice and genes: Evolution of vertebrate brain development. Brain, Behav. Evol., 52: 207-217.
13. Fritzsch, B., Barbacid, M. and Silos-Santiago, I. (1998) Nerve dependency of developing and mature sensory receptor cells. Ann. N.Y. Acad. Sci., 855: 14-27.
14. *Fritzsch, B. and Beisel, K. (1998) Development and maintenance of ear innervation and function: Lessons from mutations in mouse and man. Am J. Hum. Gen., 63: 1263-1270.
15. Fritzsch, B., M. Barbacid and I. Silos-Santiago (1998) The combined effects of trkB and trkC mutation on the innervation of the inner ear. Int. J. Dev. Neurosci., 16: 493-505.
16. Fritzsch, B. and Neary T.J. (1998) The octavolateral system of mechanosensory and electrosensory organs. In: H. Heatwole, E. M. Dawley (eds.) Amphibian Biology, Vol 3, Surrey Beatty & Sons, Australia. pp. 878-922.
17. Fritzsch, B. (1999) Hearing in two worlds: Theoretical and realistic adaptive changes of the aquatic and terrestrial ear for sound reception. In: A.N. Popper, R.R. Fay (eds). The ear of fishes and amphibians. Springer, Verlag, New York, pp 15-42.
18. Fritzsch, B. Pirvola U. and Ylikoski J. (1999) Making and breaking the innervation of the ear: Neurotrophic support during ear development and its clinical implications. Cell & Tissue Res, 295 369-382.
19. *Fritzsch, B. (1999) Ontogenetic and evolutionary evidence for the motoneuron nature of vestibular and cochlear efferents. In: "The Efferent Auditory System: Basic science and clinical applications". C. I. Berlin (ed.). Singular Publishing, p 31-59.

20. Barritt, L.C., Fritzsch, B. Beisel, K. (1999) Characterization of G-protein bg expression in inner ear. Molecular Brain Res. 68: 42-54.
21. *Maklad, A., Fritzsch, B.(1999) Incomplete segregation of endorgan-specific vestibular ganglion cells in mice and rats. J. Vestib. Res., in press.