Summary of Research on NIH.R1 (Ames #: NCC2-861)
Effects of weightlessness on vestibular development
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In our original application we proposed to investigate the effects of gravity on the formation of connections between the gravity receptors of the ear and the brain in rat pups raised in space beginning at an age before these connections are made until near the time of birth, when they are to some extent functional. We used the neuronal tracer, DiI, which could be applied to tissue obtained immediately after landing of the space shuttle, thus minimizing changes 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. Our data collected strongly supports the idea that the vestibular system has these same two phases of development.

The tissue obtained from the NIH.R1 experiment was of exceptionally high quality for our analysis. Therefore, we expanded our investigation into the ultrastructural effects of microgravity on vestibular development. For the sake of clarity we will subdivide our summary into two categories: (1) analysis of the branching pattern of axons between the vestibular nerve and the gravistatic receptors of the ear in flight and control animals, and (2) analysis of the branching pattern of axons between the vestibular nerve and the brain in flight and control animals.

1. Analysis of branching pattern between the vestibular nerve and receptors

To verify that the DiI was applied to the intended gravistatic receptors in the ear, we routinely checked the application site. If the injection was in the saccule we expected to find little labeling in the utricle and vice versa. Much to our surprise we found that in both normal and flight animals there were extensive collaterals to other receptors which appeared to be derived from (efferent) collaterals of the vestibular nerve. Thus neurons in the brain send efferent fibers to the periphery that branch, sending one branch to the receptors of the saccule and another branch to the basal turn of the cochlea. Previously each neuron was believed to innervate a single sensory receptor target. Some of these data on the efferent branching pattern was presented at the Society for Neuroscience meeting in San Diego [Nichols, D.H., J.D. Kingsley, **L.L. Bruce**, and **B. Fritzsch** (1995) Incomplete target segregation of facial, vestibular and cochlear efferents in rodents. Soc. Neurosci. Abstr., 21: 1040]. These findings have now appeared as a publication [**Bruce, L.L.,** J. Kingsley, D.H. Nichols

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and **B. Fritzsch** (1997) The development of vestibulocochlear efferents and cochlear afferents in mice. Int. J. Dev. Neurosci., 15; 671-692].

Although this finding was unexpected, it is consistent with our other data showing that efferent axons within the VIIth (facial) nucleus also have multiple branches in both control and flight animals. However microgravity appears to alter the peripheral sensory (afferent) innervation of the facial nerve. We found that the utricle was innervated by many facial ganglion cells in almost all of our flight animals, but only in one of our control animals. These data suggest that (1) microgravity allows facial ganglion cells to innervate the utricle, or (2) facial ganglion cells may grow to the utricular receptors in both microgravity and control fetal rats, but exposure to gravity causes them to be eliminated. Further work on animals exposed to microgravity for shorter and longer periods of time are needed to distinguish between these two possibilities.

2. Analysis of the branching pattern between the vestibular nerve and brain

Our analyses of axonal projections from gravistatic (linear acceleration) receptors into the brain showed that axons in the control animals had greater numbers of branches and more elaborate branches than the flight animals. Moreover, in control animals these axons had terminal swellings suggestive of synapses, but in the flight animals the axons ended in small growth cones. The control and flight animals were matched for size, weight, and sex to account for possible variables. These data suggest that these projections develop much more slowly in microgravity than in normal gravity.

To further assess this tentative conclusion, we have compared the development of projections from the gravistatic (linear acceleration) receptors to that of the angular-acceleration receptors. In the fetuses the angular-acceleration receptors, *semicircular canals*, should be stimulated by the movements of the mother in both microgravity and normal gravity. However the gravistatic (linear-acceleration) receptors, the *utricle and saccule*, should receive very little stimulation in microgravity, as compared to normal gravity. Other studies of neuronal development suggest that stimulation increases the formation of axonal branches at the expense of less stimulated fibers. Thus we hypothesize that our flight animals with the underdeveloped gravistatic projections may have overdeveloped angular-acceleration projections, as compared to the controls. We are currently testing this hypothesis by comparing the projections of flight and control animals. This idea could be proposed

only after we analyzed the first data proposed in our original grant application. Thus this new avenue of research represents a novel addition to our original hypothesis.

Complete list of Inventions: N/A.

In summary, our data have opened an exciting new avenue of research into the anatomical basis of microgravity-related orientation deficits. Our study examined fetuses exposed to microgravity during a period when the vestibular system is just beginning to function. Behavioral studies (see Alberts) show that their littermates regained partial or complete responsiveness to gravity over time. However, we predict that the magnitude of these anatomical changes will be age related, and the deficits may be more pronounced and possibly permanent in animals exposed to microgravity soon after birth. Further anatomical and behavioral experiments are necessary to identify a possible critical period where gravity may be essential for the development of normal vestibular connections.

As a result of these findings we are now seeking answers for two new questions:
A) Is the projection of the non-gravistatic vestibular receptors of flight animals not only as mature as but even ahead of the control animals. In other words, is there a reciprocal effect of microgravity on the gravistatic versus non-gravistatic projections of the vestibular system.
B) What is the degree of maturation of synaptic contacts between vestibular fibers and second order neurons.

These new questions will allow us to obtain answers to the more general question: Is there a critical period during which the gravistatic and non-gravistatic components of the vestibular system compete for the targets in the vestibular nuclei, as demonstrated in other maturing sensory systems.

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