Ribbon Synaptic Plasticity in Gravity Sensors of Rats Flown on Neurolab

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ABSTRACT

Previous spaceflight experiments (Space Life Sciences-1 and -2 (SLS-1 and SLS-2)) first demonstrated the extraordinary ability of gravity sensor hair cells to change the number, kind, and distribution of connections (synapses) they make to other cells while in weightlessness. The number of synapses in hair cells in one part of the inner ear (the utricle) was markedly elevated on flight day 13 (FD13) of SLS-2. Unanswered questions, however, were whether these increases in synapses occur rapidly and whether they remain stable in weightlessness. The answers have implications for long-duration human space travel. If gravity sensors can adapt quickly, crews may be able to move easily between different gravity levels, since the sensors will adapt rapidly to weightlessness on the spacecraft and then back to Earth's gravity when the mission ends. This ability to adapt is also important for recovery from balance disorders. To further our understanding of this adaptive potential (a property called neuronal synaptic plasticity), the present Neurolab research was undertaken. Our experiment examined whether: (a) increases in synapses would remain stable throughout the flight, (b) changes in the number of synapses were uniform across different portions of the gravity sensors (the utricle and saccule), and (c) synaptic changes were similar for the different types of hair cells (Type I and Type II).

Utricular and saccular maculae (the gravity-sensing portions of the inner ear) were collected in flight from rats on FD2 and FD14. Samples were also collected from control rats on the ground. Tissues were prepared for ultrastructural study. Hair cells and their ribbon synapses were examined in a transmission electron microscope. Synapses were counted in all hair cells in 50 consecutive sections that crossed the striolar zone. Results indicate that utricular hair cell synapses initially increased significantly in number in both types of hair cells by FD2. Counts declined by FD14, but the mean number of synapses in utricular Type II cells remained significantly higher than in the ground control rats. For saccular samples, synaptic number in Type I and Type II cells declined on FD2, but returned to near-baseline values by FD14. These findings indicate that: (a) synaptic plasticity occurs rapidly in weightlessness, and (b) synaptic changes are not identical for the two types of hair cells or for the two maculae.

INTRODUCTION

There are two gravity sensors on each side of the head. These are the utricular and saccular maculae located in the vestibular (balance organ) part of the inner ear. The two maculae are situated at roughly right angles to one another, the utricular macula being approximately horizontal and the saccular macula nearly vertical. The paired maculae have sensory hair cells that detect linear accelerations (gravitational and translational) acting on the head. However, the functional orientation of the hair cells relative to a stripe (called a "striola") that bisects each macula differs between the two maculae. In the utricular maculae, hair cell orientation (and greatest sensitivity) is in the direction of the striola; in the saccular maculae, hair cell orientation (and greatest sensitivity) is away from the striola. These anatomical distinctions also have physiological consequences (Baird, 1986; Goldberg, 1990a,b).

The hair cells communicate via ribbon synapses with primary vestibular neurons (afferents). These afferent neurons carry information to the brain about the direction and force of linear accelerations. This information is used centrally to coordinate eye movements and antigravity muscle activity, to maintain balance whether one is at rest or in motion, and to sustain eye focus on a target (tracking) during head movement. Disturbances in the reflex pathways lead to motion sickness and to the balance disorders frequently encountered in the aged. Space adaptation syndrome (space motion sickness) is a further manifestation of a disturbance in the reflex pathways that occurs when the peripheral gravity sensors are challenged by weightlessness.

Previous spaceflight experiments, Space Life Sciences-1 and -2 (SLS-1 and SLS-2), were the first to demonstrate the extraordinary ability of the ribbon synapses of gravity sensor hair cells to change in number, kind, and distribution when the gravitational environment was perturbed by weightlessness (Ross, 1993, 1994, 2000). The mean number of synapses doubled in Type II hair cells of utricular maculae collected on flight day 13 (FD13) when all cells in a 100 two-dimensional section series were considered, and tripled in completely three-dimensionally reconstructed Type II cells taken from the same series (Ross, 2000). Synaptic increments were lower in Type I hair cells in the same series, and were insignificantly different from controls in complete cells.

A rise in the number of synaptic ribbons, whether by clustering at a synaptic site or by establishing new sites, would augment the possible number of vesicles available for releasing neurotransmitters. This could then increase primary vestibular afferent nerve activity to the brain both under resting conditions and when the cell is stimulated. Synaptic increments were, therefore, interpreted to indicate an adaptive response to improve hair cell output under a condition of a reduced stimulus of gravity (10^{-3} to 10^{-5} G).

The present Neurolab research was undertaken to further our understanding of the potential of the hair cells to alter their ribbon synapses (a property called neuronal synaptic plasticity). A question to be answered by the Neurolab experiment was whether the numerical increments in ribbon synapses noted late in the SLS-2 flight in utricular maculae occurred rapidly upon insertion into weightlessness as an early adaptive response. The answer to this question has implications for human space travel to the Moon and distant planets. That is, if gravity sensors adapt rapidly to a new gravitational environment such as exists on the Moon (1/6-G) or Mars (1/3-G), work could begin quickly upon landing. When such a mission ends, the sensors would re-adapt rapidly again to weightlessness on the spacecraft and, eventually, to Earth's one-G. Thus, crews could move easily among all these different gravity levels.

The present research, therefore, focused on FD2 and FD14 of the Neurolab mission. We wanted to determine whether increments on the order of those seen previously in Type II hair cells on FD13 (Ross, 2000) would be duplicated early in the mission and would be sustained. To expand the previous work, a portion of the macula that included the striolar zone, where sensitivity to phasic linear accelerations increases, was to be included in the study; and saccular maculae were analyzed for the first time. The purpose was to learn whether all portions of a macula, and the two maculae, would respond similarly to weightlessness.

MATERIAL AND METHODS

Rats used in this study were specific pathogen free (SPF) Fischer 344 rats obtained from Taconic Farms, Germantown, New York. Ten rats served as ground controls. Inner ear tissues were obtained on FD2 from four rats and on FD14 inflight from nine rats. Other tissues that are still under study were obtained from six rats on postflight day two (PF2) and from six rats on PF14. The manner of dissection of the labyrinths, of microdissection and tissue preparation for transmission electron microscopy, and of statistical analysis SuperANOVA™ software have been described in detail previously (Ross, 2000). The only differences for the present study were that, with the exception of the basal controls that were fixed by technicians of the Ross laboratory for their own specific use, tissue samples were collected by technical staff on the ground and by crewmembers inflight for shared use by several scientists. All maculae we received were from the right side of the head.

Only one of the utricular maculae fixed in space on FD2 and another on FD14 were suitable for transmission electron microscopy. This necessitated a truncated study of the utricular maculae, which is reported here. The investigation then focused on the saccular maculae.

Inflight data were obtained from one utricular macula and two saccular maculae fixed on FD2 and FD14. Basal samples were obtained on the ground on FD2. The utricular maculae were sectioned from the lateral border; saccular maculae were sectioned from the superior border. One hundred sixty-five sections 1-µm thick cut inward from the utricular lateral border were discarded and then 150 thin sections (~160-nm thick) were collected. In the case of the saccular macula, distance from the superior border to the striolar zone was shorter. Seventy-seven sections were discarded before collecting 250 thin sections. More sections were collected from the saccular maculae to

permit later three-dimensional reconstructions. For this study, 50 consecutive (serial) sections were used routinely to obtain counts. However, 100 sections of one FD2 utricular and one FD2 saccular sample were used in two sets of 50 sections each to compare findings between striolar (set 1) and juxtastriolar zones (set 2), with the second set on the internal side of the striola (pars interna). The striolar zone was determined by the presence of M-type terminals in which the myelin reached the base of the calyx and calyceal processes were not present.

Ribbons were classified as rodlike or as spherules. Teardrop-shaped ribbons were considered to be rodlike for counting purposes (Ross, 2000).

RESULTS

Synapses – Ultrastructurally, ribbon synapses are characterized by a central electron-opaque body (ribbon) that is generally rodlike (Figure 1) or spherical in shape (Figure 2) and is surrounded by a halo of vesicles. The vesicles are tethered to the central body by slender filaments. The ribbon is continuous with one or more foot-like processes that proceed into an arc-shaped density that attaches the synapse to the cell membrane. The synaptic vesicles are docked at the synaptic site alongside the arc-shaped density. Synapses are considered to be multiple when the vesicles are shared by more than one ribbon (Figure 3). Figure 4 illustrates two synapses, one sphere-like and one rodlike (arrows), ending separately on a collateral process terminating on a Type II hair cell.

Utricular hair cell synapses; Type I – The total number of ribbon synapses in the 151 Type I cells was 448. The ranges in synaptic number per cell for the samples were: basal, 1-5; FD2, set one, 1-11; FD2, set two, 1-9; FD14, 1-11.

By FD2, the mean number of ribbon synapses in Type I cells had risen from the basal value (Table 1) and was still slightly higher than the basal value on FD14. The FD2 mean values differed significantly from the control (Table 1). The only other significant differences in kind or distribution of the ribbon synapses were the increases in spherical synapses in both FD2 samples ($p<0.0082$ for set 1; and $p<0.0309$ for set 2) and on FD14 ($p<0.0093$) (not illustrated).

Utricular hair cell synapses; Type II – There were 1035 ribbon synapses in the 149 Type II hair cells counted. The ranges in synaptic number per cell for the data sets were: basal, 1-14; FD2, set one, 1-23; FD2, set two, 1-17; FD14, 1-25.

The mean numbers of synapses in the FD2 and FD14 samples were significantly higher than the basal values (see Table 2). Although not illustrated, spherical synapses had increased significantly in all of the inflight samples compared to the basal values: FD2, set one, $p<0.0034$; FD2, set two, $p<0.0008$; FD14, $p<0.0093$ (not illustrated).

Saccular hair cells; Type I – There were 602 synapses in the 167 Type I hair cells counted. The ranges in number of synapses per data set were: basal, 1-13; FD2, set one, 1-5; FD2, set two, 1-8; FD2 total, areas with striola, 1-11; FD14, 1-6.

The mean number of synapses in Type I cells had declined significantly on FD2 ($FD2_t$, total data, areas with striola), but had returned to a near-normal mean value on FD14

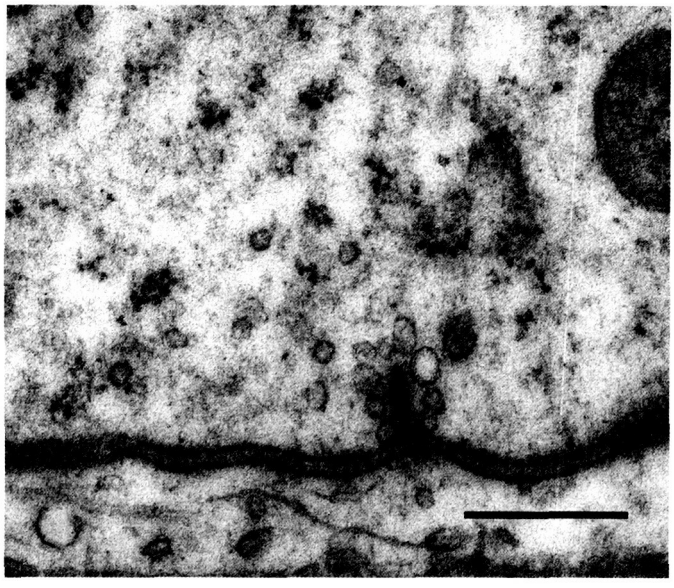

Figure 1. This figure shows a transmission electron micrograph of a synapse. The double black lines at the bottom of the figure are where the Type I cell and its calyx intersect. The black vertical structure along the lines illustrates a rodlike ribbon synapse between the Type I cell and its calyx. This picture was taken from an FD2 utricular sample, striolar zone. The bar equals 0.5 µm.

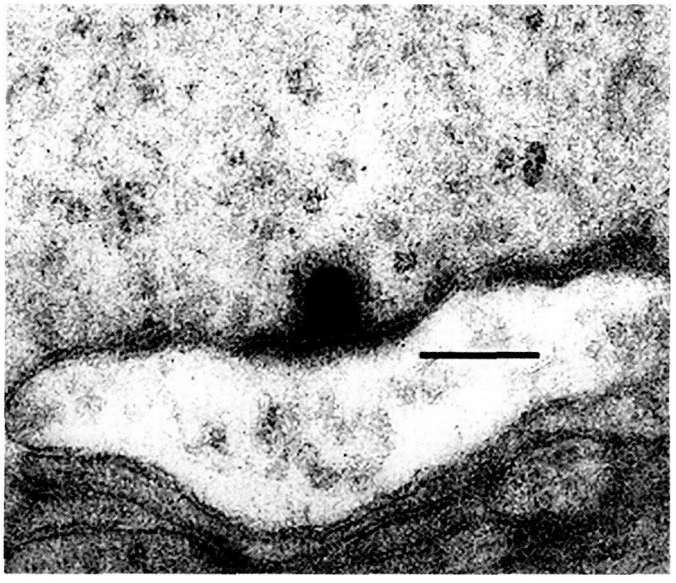

Figure 2. This figure illustrates a sphere-like ribbon synapse in a Type II hair cell of the utricle. Data from FD14. The bar equals 0.5 µm.

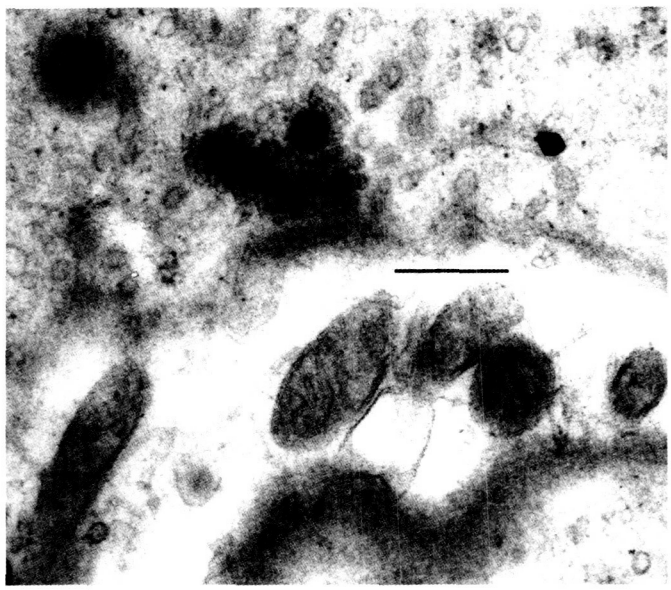

Figure 3. A multiple synapse is illustrated here. The rodlike ribbon synapse on the left is flanked by two sphere-like ribbons on the right. The vesicles containing neurotransmitter are shared. Picture from a ground control rat saccular macula. The bar equals 0.5 μm.

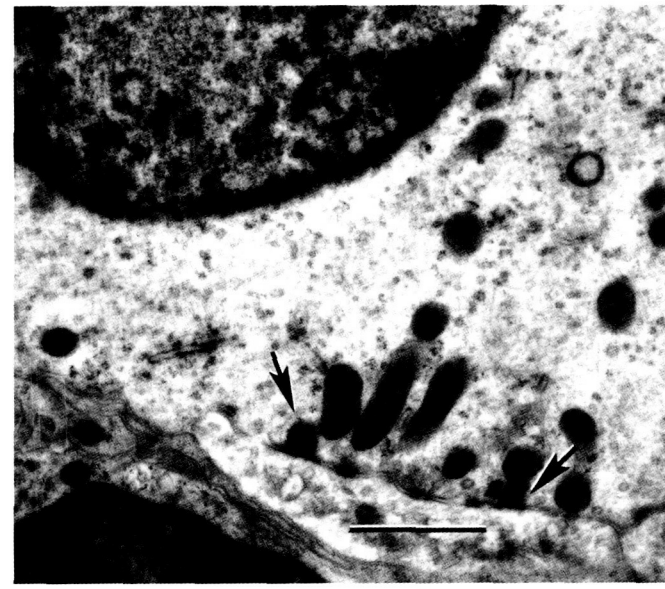

Figure 4. Some processes ending on Type II hair cells receive more than one synaptic input. The arrow on the left indicates a sphere-like ribbon synapse, and the arrow on the right indicates a rod-like synapse with a vesiculated process. Picture from a ground control rat saccular macula. The bar equals 1.0 μm.

(Table 1). There was a statistically significant decline in mean values of sphere-like ribbons ($p>0.0170$) in the FD2 total data ($FD2_t$, not illustrated). No other significant differences were noted.

Saccular hair cells; Type II – There were 208 Type II hair cells with 1434 synapses. The ranges in number of synapses per cell were: basal, 1-14; FD2 set one, 1-18; FD2 set two, 1-18; FD2 total, areas with striola, 1-23; and FD14, 1-16.

The synaptic mean value in the Type II inflight FD2 areas with striola declined as compared to ground controls ($FD2_t$, Table 2). There was also a significant decline in the mean value of rods ($p<0.0376$) in the $FD2_t$ sample (not illustrated).

Table 1. Type I Hair Cells

	Utricle				Saccule			
Day	**N**	**MVS**	**SD**	**S**	**N**	**MVS**	**SD**	**S**
Basal	29	2.034±1.239		*	45	4.133±3.334		*
$FD2_1$	41	3.512±2.491		*0.0044	28	3.036±2.349		ns
$FD2_2$	46	3.543±2.208		*0.0013	22	3.545±2.483		ns
$FD2_t$	41	3.512±2.491		*0.0044	48	2.646±2.037		*0.0105
FD14	35	2.400±2.103		ns	52	3.788±2.953		ns

This table compares data for Type I hair cells from utricle and saccule. Experimental day is given in the left-hand column. Basal = data from ground control rat, $FD2_1$ = data from one sample, striolar zone, on FD2, $FD2_2$ = data from same sample, juxtastriolar zone (internal side of the striola) on FD2, $FD2_t$ = FD2 total data, striolar zones only, N = hair cell number, MVS = mean value, synapses, SD = standard deviation, S = significance compared to the basal, (*) = difference between basal and listed value is significant, ns = not significant.

Table 2. Type II Hair Cells

	Utricle				Saccule			
Day	**N**	**MVS**	**SD**	**S**	**N**	**MVS**	**SD**	**S**
Basal	43	4.744±3.793		*	61	6.939±3.968		*
$FD2_1$	35	9.000±5.122		*0.0001	37	5.865±3.441		ns
$FD2_2$	28	7.929±4.626		*0.0023	29	7.207±5.081		ns
$FD2_t$	35	9.000±5.122		*0.0001	63	6.317±3.750		*0.0435
FD14	43	6.884±5.128		*0.0306	55	6.382±5.237		ns

This table compares data for Type II hair cells from utricle and saccule. Experimental day is given in the left-hand column. Basal = data from ground control rats, $FD2_1$ = data from one striolar zone on FD2, $FD2_2$ = data from same sample, juxtastriolar zones (internal side of the striola) on FD2, $FD2_t$ = FD2 total data, N = hair cell number, MVS = mean value, synapses, SD = standard deviation, S = significance compared to the basal, (*) = difference between basal and listed value is significant, ns = not significant.

DISCUSSION

The most important findings of the Neurolab research were the early elevations in the mean number of synaptic ribbons in utricular hair cells, particularly in Type II cells; and the relative stability of mean values in hair cells of the saccular macula. The results in the utricular macula support previous findings that, in flight, synapses are elevated numerically in hair cells of this macula, particularly in Type II hair cells (Ross, 1993, 1994, 2000). However, the extent of synaptic change is affected by macular location. Increments in Type II hair cell synapses in the posterior part of the utricular macula were greatest there on FD13 of the SLS-2 14-day flight (11.4±7.2, $p<0.0001$) for all Type II cells analyzed (Ross, 2000). On FD14 of the present Neurolab experiment, the mean value of Type II hair cell synapses in utricular striolar zone was 9.000±5.122 (Table 2). Mean values also differed in the FD2 samples, depending on striolar or juxtastriolar (pars interna) zones (Table 2). Hair cell type (I or II), macular location, and macula all provide internal controls for the findings since important differences in synaptic plasticity exist in each case.

The generation and loss of synapses (synaptogenesis and synaptic deletion) in weightlessness may be reactive responses to altered influences from the nerves connecting to the hair cells (Ross, 2000). This effect can be seen during normal development. Initially during development, more synapses than are needed are generated. These are reduced in number (pruned) once innervation to the brain is established (Sobkowicz, 1982; Sobkowicz, 1992). Results from a similar system (the developing organ of Corti) indicate that an interaction between the afferent innervation and the hair cells determines final synaptic number. This is likely in the case of macular synapses in an adult animal as well.

In an adult, the level of normal synaptic activity and turnover has been established for a particular environment through development and maturation. When a decline in feedback occurs (due to a lack of stimulation from gravity, for example), this should stimulate synaptogenesis. An opposite effect would occur when discharge rates are high, such as in a high-gravity environment (hypergravity). That is, synaptogenesis would be shut down until an efficacious number of synapses is reached and balance is restored. Pilot research on hypergravity effects on utricular hair cells supports this thesis, since exposure to two-G for 14 days lowered the synaptic number in Type II hair cells of the rat utricular macula but left Type I cells unaffected (Ross, 1994).

A question raised by macular differences in responses to weightlessness is whether the synaptic changes reported here have relevancy to possible physiologic differences between the utricular and saccular macular afferents. In a series of papers, Fernandez et al. (Fernandez, 1972) and Fernandez and Goldberg (Fernandez, 1976a,b) dealt with differences in response characteristics between superior vestibular nerve (utricular) and inferior nerve (saccular) afferents. Fernandez et al. (Fernandez, 1972) originally described saccular afferents as having unexplained much lower resting discharge rates and lower sensitivity to static tilt. In a later paper (Fernandez and Goldberg, 1976b), use of a larger sample indicated that resting discharge rates did not differ much between the two maculae. Both end organs responded to linear accelerations. Fernandez and Goldberg reported that utricular afferents were most responsive to static tilt in the X-direction (side-to-side) while saccular afferents were primarily sensitive to Z-direction (vertical) tilt. Both kinds of afferents were less sensitive to tilt in the Y-direction (nose forward, backward). The results on tilt in the sacculus are supported by recent findings of Uchino et al. (Uchino, 1997), who reported that saccular but not utricular influences on neck muscles stabilize relative head and body positions against the vertical linear acceleration of gravity.

Overall, our findings indicate that utricular macular synaptic number, kind, and distribution were altered early in weightlessness. Additionally, there were differences in degree and kind of synaptic change based on macula, intramacular location, and day during flight. We conclude that synaptic plasticity occurs rapidly in weightlessness; and that the synaptic changes are not identical for the two types of hair cells or for the two maculae. The changes probably result from a profound change in vestibular input to the macula that occurs in weightlessness.

Impact on space travel – The results continue to be good news for space travelers. Trips to the Moon (1/6-G) or to Mars (1/3-G) should not have a major or a long-lasting effect on the vestibular system, as prior Moon missions have already indicated. The periphery should readily adapt to a novel partial G-force, and space motion sickness should not be a major problem.

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