FINAL REPORT

Vestibular Influences on Autonomic Cardiovascular Control

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i) Abstract of original proposal

The general goal of this proposal is to test the hypothesis that the vestibular system via the autonomic system is involved in the control of cardiovascular function. There is clinical, physiological and anatomical evidence indicating that the vestibular, autonomic and cardiovascular systems interact. For example, motion sickness induced by head movements induces characteristic changes in autonomic outflow. It is postulated that the vestibular system is also important in cardiovascular orthostatic control. In space, central nervous system processing of vestibular input is altered by adaptation to 0G environment and for several days after returning to earth there is reduced sensitivity of the vestibular otolith organs to gravity stimuli (Dai et al 1994). We postulate that the reduced sensitivity of the vestibular system to gravity is an important mechanism responsible for post flight cardiovascular orthostatic intolerance. The goal of this proposal is to determine if activation of vestibular afferents triggers reflexes that engage autonomic pathways which regulate orthostatic cardiovascular adaptation. In **Specific Aim I,** we will test the hypothesis that gravitational stimulation of the vestibular system changes autonomic outflow. To that end, we will measure sympathetic outflow using microneurography during vestibular stimulation induced by pitch movements (nose up-down head rotations) in normal subjects, patients with baroreflex failure and patients with vestibular dysfunction. In **Specific Aim II,** we will test the hypothesis that only vestibular stimulation induced by changes in body orientation with regard to gravity is capable of inducing an autonomic response that is important in orthostatic cardiovascular control (i.e., linear acceleration along the long axis of the body, or along the naso-occipital axis cause an increase in sympathetic outflow to stabilize blood pressure). To test this hypothesis, we will compare the cardiovascular effects of "radial" centrifugation and rotation about axes tilted from the vertical (off-vertical axis rotation, OVAR) maneuvers that change the body orientation with regard to gravity with those produced by on-center rotation about a vertical axis and "tangential" centrifugation, maneuvers that stimulate the vestibular system but do not change the body orientation with regard to gravity. We will measure blood pressure and heart rate continuously and perform spectral analysis of these signals to determine changes in sympathetic and parasympathetic activity. In **Specific Aim III** we will test the hypothesis that normal otolith input is required for autonomic regulation of orthostatic cardiovascular stress. To mimic the reduced sensitivity of the vestibular system induced by exposure to 0G, we will use radial centrifugation (+Gz) a maneuver that temporarily decreases vestibular sensitivity because of adaptation of the otolith organs to +Gz. The response will be compared with that evoked by a similar decrease in central volume induced by lower body negative pressure. We predict that orthostatic tolerance will be less impaired following lower body negative pressure than following +Gz because of normal otolith output in the former. In **Specific Aim IV** we will explore the effect of promethazine, an agent successfully used in the treatment of space motion sickness, on vestibular and autonomic reflexes and reflex interactions. **In summary**, this proposal will use state of the art techniques that will allow us to distinguish between stimulation of semicircular canals and otolith organs to determine whether vestibular reflexes have an important cardiovascular autonomic component and contribute to orthostatic tolerance.

Understanding the interaction between vestibular and autonomic pathways is of importance to spaceflight because it may help explain both space motion sickness and post flight orthostatic intolerance.

ii) Specific aims and objectives of original proposal, including a discussion of changes arising out of the research

It has been postulated that stimulation of the vestibular system by changes in posture or gravity produce changes in heart rate and vascular tone that are important in the maintenance of blood flow to the brain during orthostatic stress (Essandoh et al, 1988; Doba and Reis 1974). This suggests that there is a vestibulo-autonomic reflex involved in cardiovascular control (Yates and Miller, 1994). We hypothesize that abnormalities in this vestibulo autonomic reflex resulting from vestibular adaptation to 0G could account, at least in part, for the cardiovascular orthostatic intolerance experienced by astronauts upon return to earth. Because very little is known about the pathways of this vestibular-autonomic cardiovascular reflex, and indeed, whether the reflex exists in humans at all is unclear, the goal of this proposal is to establish the existence of this reflex in humans and determine its importance in the regulation of cardiovascular orthostatic adaptation. In **Specific Aim I,** we will test the hypothesis that gravitational stimulation of vestibular structures changes autonomic outflow. To that end, we will measure sympathetic outflow directly using microneurography during vestibular stimulation elicited by head up and head down neck flexion in normal subjects. Because in normal subjects autonomic changes are quickly buffered by baroreflexes, we will also study patients with baroreflex failure. We will also study patients with vestibular dysfunction to determine if these reflexes are absent or deficient. In **Specific Aim II,** we will test the hypothesis that only vestibular stimulation induced by changes in body orientation with regard to gravity increases sympathetic outflow whereas vestibular stimulation produced by angular acceleration alone, which does not change the body orientation with regard to gravity, do not produce changes in sympathetic outflow. To test this hypothesis, we will use complimentary paradigms of vestibular stimulation and compare their cardiovascular effects. First, we will use lying-on-back, "radial" centrifugation to produce acceleration along the long axis of the body (Z axis), and rotation about axes tilted from the vertical (off-vertical axis rotation, OVAR) to induce gravitational stress and selective stimulation of the otolith organs. Second, we will use on-center rotation about a vertical axis and "tangential" centrifugation, two maneuvers that do not change the body orientation with regard to gravity and stimulate the semicircular canals. Microneurography cannot be performed when the subject is moving, therefore, we will measure blood pressure and heart rate continuously and perform spectral analysis of these signals in order to determine changes in sympathetic activity. In **Specific Aim III** we will test the hypothesis that normal otolith input is required for autonomic regulation of orthostatic cardiovascular stress. Therefore, we will study baroreflex function and orthostatic tolerance during the period of decreased vestibular sensitivity that follows radial centrifugation (+Gz), a maneuver that mimics the vestibular changes induced by exposure to 0G. Cardiovascular response to orthostatic stress and the responsiveness of the carotid cardiac baroreflex will be compared with that evoked by a similar decrease in central volume induced by lower body negative pressure (LBNP). We

postulate that orthostatic tolerance will be less impaired following LBNP than following +Gz because of normal otolith output in the former. In **Specific Aim IV** we will use promethazine, a known antimotion sickness agent, as a pharmacological probe to explore our general hypothesis that cardiovascular orthostatic intolerance is a consequence of vestibular alterations induced by exposure to 0G.

iii) Progress achieved against the specific aims and objectives

TESTING SETUP

We refined and customized our testing system for vestibular autonomic studies. Adding a miniaturized microneurography device, the system is now capable of monitoring real-time sympathetic activity during movement (Figure 1).

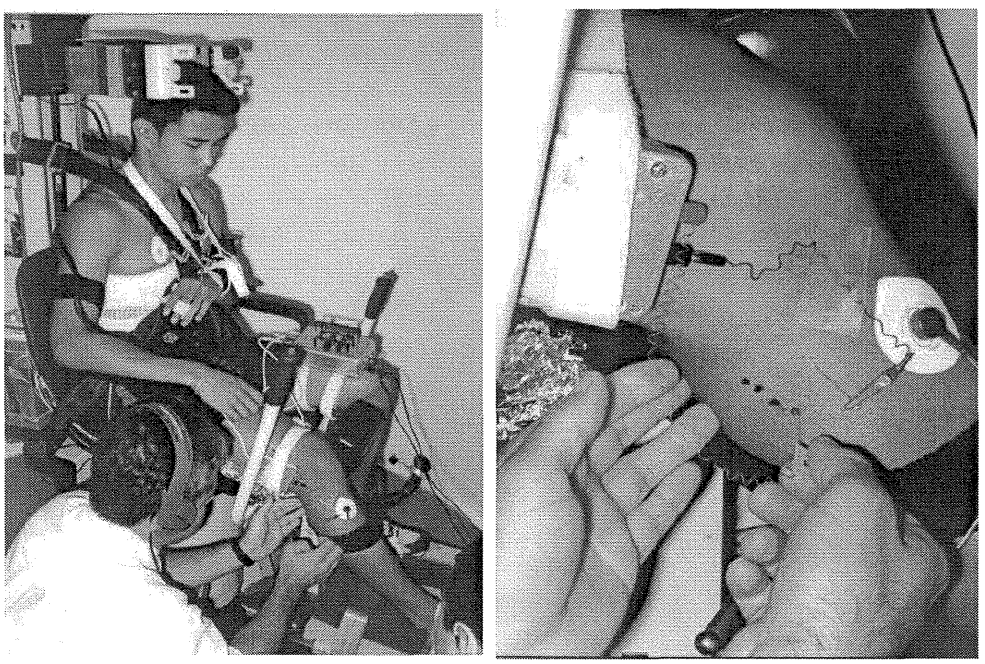

Figure 1. Microneurography procedure customized for the rotating chair.

Our system allowed collection of cardiovascular parameters (blood pressure and heart rate), respiratory frequency, sympathetic nerve activity, pupillary diameter and eye movement data during selective stimulation of semicircular canals and otolith organs. The system consisted of a darkened enclosure containing a computer–controlled vestibular chair. An infrared close-circuit video system was used to monitor the subjects' condition. A two-way intercom system provided audio communication between the subject and experimenter throughout testing. The chair was equipped with an emergency button to allow the subject to interrupt the testing procedure at any time.

The test setup included:

1. Infrared goggles to record eye movements by means of infrared video-oculography (Two Pupil Corneal Reflection Tracking System RK-426).
2. Continuous blood pressure monitoring system based on the volume clamp method (Portapres, TNO Amsterdam, Netherlands).
3. Heart rate monitoring system consisting of surface electrodes attached to a portable high-gain amplifier (Teledyne Electronic Technologies, Los Angeles, California) .
4. Respiration monitoring system (Respitrace, Ambulatory Monitoring, Ardsley, New York) consisting of a pair of elastic bands around the subject's thoracic and abdominal regions, connected to a selective gain differential amplifier and a summing box.
5. Portable microneurography system.

Data acquisition/storage. Data collection was conducted using ASYST data acquisition software. Analog data are digitized online using DI-220 data acquisition system with WinDaq Pro+ software (DATAQ Instruments, Inc., Akron, Ohio) at a sampling rate of 500 Hz and stored on a Pentium-650 computer (Gateway 2000, North Sioux City, South Dakota) for subsequent analysis. Periods of nystagmus were identified in real time by observing the pupillary video images and verified through off-line examination of the stored eye movement records.

In sum, our new testing setup provided unique capabilities previously unavailable to neurovestibular research. Specifically, it made it possible to correlate vestibular activity (monitored by eye movements) with direct autonomic measurements and target organ effects, i.e. muscle sympathetic nerve activity, heart rate, blood pressure and pupil diameter.

EXPERIMENTAL PROTOCOL

We studied subjects during on- and off-vertical axis rotation (VAR and OVAR). In previous ancillary experiments we found 15° tilt to be a good neurovestibular stimulus. During off-vertical axis rotation (OVAR), this projects 0.26 g onto all axes in the horizontal plane of the head. The original testing protocol consisted of four consecutive experimental conditions, each lasting six minutes:

1. Vertical axis, stationary.
2. Vertical axis rotation (VAR) clockwise at 60°/s.
3. Off-vertical axis (tilted 15°, right ear down), stationary.
4. Off-vertical axis rotation (OVAR) (tilted 15°), clockwise at 60°/s.

During OVAR, we discovered a striking entrainment between the frequency of chair rotation and the variability of blood pressure, heart rate and sympathetic nerve traffic. This entrainment was present only during OVAR but not during VAR. To investigate this phenomenon further, we added two additional rotation frequencies to our experimental protocol (clockwise at 24°/s and clockwise at 110°/s, experimental conditions "5" and "6", respectively, each lasting three minutes).

In addition, in order to distinguish between head orientation and body fluid shifts as the primary cause for the observed sympathetic activation, which was at a peak during naso-occipital linear acceleration, we tested additional subjects during both on- and off-vertical axis rotation at 60°/s with their heads turned to the side 40° to 60° in the direction opposite to the direction of rotation (experimental conditions "7" and "8", respectively, each condition lasting six minutes).

Subjects were informed of the beginning and end of each experimental condition. Acceleration and deceleration of the chair were $240°/s^2$ and $-240°/s^2$, respectively.

DATA ANALYSIS

Statistical and spectral analyses of the data were performed using custom-written software. ECG data were processed according to the Berger algorithm and treated with lowpass filter at 40 Hz. QRS complexes were initially identified by the program and then verified by an independent observer. Program-generated ECG time series were treated with a linear interpolation filter set at 300 ms of R-R interval deviation within one second of the time series.

R-R intervals, systolic and diastolic blood pressure and respiratory frequency were analyzed for their mean values and power spectral density (Blackman Tukey spectral type with linear detrending at 4 Hz resolution). Frequency bands were set as follows: (1) 0 – 0.07 Hz (very low frequency), (2) 0.07 – 0.148 Hz (low frequency), and (3) 0.148 – 0.301 Hz (mid-band frequency).

Once an adequate recording site for MSNA was found the signal was filtered (bandwidth = 700-2000 Hz), and integrated with a time constant of 0.1 seconds, from which a mean voltage was recorded and used for measurements. Since voltage amplitude varies between subjects, in order to minimize potential changes in recorded activity over time due to small changes in electrode placement, sympathetic activity was expressed as percent changes from a "baseline", a mean activity over a given rotation cycle. We developed an automated method, based on PV-Wave software, to analyze sympathetic nerve activity independent of observer bias.

To allow for independent analysis of the activity of semicircular canals and otolith organs, all data were divided into two categories: (1) *SCC-induced nystagmus period*, which was defined as the period of occurrence of per- and post-rotatory nystagmus, as determined by the response to vertical axis rotation at a constant velocity, and (2) *the otolith-activation period*, which was defined as the steady-state response to constant velocity rotation about a tilted axis (OVAR). For selective analysis of data during steady state velocity OVAR conditions, we used compiled information from 10 consecutive rotation cycles per subject, starting at the 50th second after rotation began for each experimental condition. For analysis of spectral data we used a 256 second wide data window starting at 50th second for experimental conditions "1" through "4", "7" and "8", and a 128 second wide data window starting at 50th second for experimental conditions "5" and "6". Using data series of 256 and 128 seconds allowed analysis of spectral

frequencies as low as 0.015 Hz and 0.031 Hz, respectively (i.e., minimally characterized frequencies).

To test our main hypothesis that linear acceleration increases sympathetic activity, we compared the effects of vertical axis versus off-vertical axis rotation (VAR vs. OVAR). We used VAR as our negative control, and steady state OVAR as a paradigm to selectively stimulate otolith organs. During steady state OVAR, the hair cells of the otoliths are displaced according to a vector that results from the projection of gravity onto the horizontal plane of the head. The magnitude of the resulting vector, which we call gravito-inertial acceleration (GIA), i.e., the sum of the linear accelerations acting on the head, was 0.26 g in this series of experiments. During OVAR, the GIA rotates relative to the head in the direction opposite to that of the rotation, and it is opposite in the nose-up and nose-down conditions. We further used head on body rotations to determine whether the gravity-related MSNA was more closely related to activation of the otoliths or of body tilt receptors.

RESULTS

Altogether we have tested 60 healthy volunteers (age range 18-42 years, mean ± SD 28 ± 9 years) with no history of cardiovascular or vestibular disorders. All subjects were provided with a detailed explanation of the experiment and they signed informed consent. Quality data, adequate for subsequent analysis, were obtained from seven subjects for experimental conditions "1" through "6", and from five subjects for experimental conditions "7" and "8".

Cardiovascular Spectral Variability. Analysis of power spectra of the R-R intervals revealed the presence of a prominent new spectral peak during OVAR. The location of this peak coincides with the frequency of chair rotation. In contrast, during VAR no such peak was present. We found that not only heart rate, but systolic and diastolic blood pressure and respiration were affected as well, and a new peak occurred in all these variables only during OVAR. Furthermore, when the angular velocity of OVAR was changed (experimental conditions "5" and "6"), the corresponding spectral peak shifted so as to match the new frequency of chair rotation. Since the otoliths are sinusoidally stimulated during OVAR but idle during vertical axis rotation, the observed peaks of power spectra during OVAR are likely to be the result of otolith stimulation.

Sympathetic Nerve Activity. During VAR at constant velocity we found no obvious pattern in MSNA activity. In contrast, during OVAR at constant velocity, a clear pattern was immediately apparent: bursts of MSNA occurred with the frequency of rotation, with one big burst during each complete 360 degree cycle. These bursts of sympathetic activity occurred immediately following the "nose-up" position of the subject and remained synchronous with this position at all three different OVAR velocities.

These data strongly suggest that otolith or body tilt receptors sensitive to tilt along specific axes trigger sympathetic efferent nerve activity: OVAR-specificity of the new spectral peak is an indication of otolith or body tilt receptor involvement, because during VAR the otoliths or the body tilt receptors are not stimulated. Coupling of MSNA bursts

with the "nose-up" position of the subject suggests that gravireceptors are involved in the response and that vestibular-induced changes in MSNA are related to changes in the gravito-inertial vector relative to the head, as it is likely that only changes in body orientation with regard to gravity are capable of eliciting an autonomic response important in cardiovascular orthostatic control.

We used Lissajous patterns to determine the optimal linear correlations between changes in MSNA during OVAR and blood pressure (BP), and between changes MSNA and the projection of linear acceleration along the naso-occipital axis. The best correlation between MSNA and BP was achieved by advancing MSNA by 1.6 s to account for baroreflex latency (a well-documented signal conduction delay). In the nose-up position, however, correlation between MSNA and BP was very poor. Instead, MSNA correlated most closely with the naso-ocipital projection of the gravity vector, the best delay being 0.6 s, which was much too short to be baroreflex-driven.

We conducted a further analysis of the MSNA signal spectrum using wavelets. Energies of the respiration and BP signals, derived from the Haar-wavelet transform, were predominantly confined to lower frequency bands, with little high frequency content. The energy of the MSNA was distributed between higher and middle-level frequency bands, and was closely correlated to the energy distribution of pulses occurring close to the time when the naso-occipital acceleration was maximal. These results support our previous conclusion that MSNA during OVAR was not as well correlated with respiration or blood pressure as with head and body position re gravity and further indicate that MSNA during OVAR is closely linked to a vestibulo-sympathetic reflex, most probably originating in the otolith organs.

Evidence for the primary involvement of the otolith organs in generating this sympathetic response to the GIA came from analysis of the data obtained in experimental conditions "7" and "8" in which the head was turned on the body during OVAR. Our results indicate that during OVAR but not VAR turning the head in the direction opposite the direction of rotation leads to a delay in the peak of muscle sympathetic activity and blood pressure. There was also a stronger correlation of sympathetic activity with the axis of the head rather than with the axis of the body during OVAR, arguing against the impact of the body fluid shifts. Thus, our findings support a primary role for otolith receptors rather than body fluid shifts or body tilt receptors in generating the increase in sympathetic activity during acceleration along the naso-occipital axis.

In summary, our results strongly indicate that vestibular afferents arising in the otolith organs modulate sympathetic outflow according to the direction of tilt of the gravito-inertial vector relative to the head. These findings were presented in several publications in the peer-reviewed journals (see attached).

Publications

Kaufmann H, Biaggioni I, Voustianiouk A, Diedrich A, Costa F, Clarke R, Gizzi M, Raphan T, Cohen B. Vestibular control of sympathetic activity: an otolith-sympathetic reflex in humans. Exp Brain Res 2002; 143:463-469.

Silverberg J, Kaufmann H, Biaggioni I, Voustianiouk A, Diedrich A, Costa F, Clarke R, Gizzi M, Cohen B, Raphan T. Otolith-induced muscle sympathetic nerve activity (MSNA): a multiresolution approach using Haar wavelets. Soc Neurosci Abstr 2001;27:170.7.

Dai M, Kaufmann H, Raphan T, Cohen B. Promethazine affects optokinetic but not vestibular responses in monkeys. Aviat Space Environ Med 2000;71:1003-1012.