

Cardiovascular and sympathetic neural responses to handgrip and cold pressor stimuli in humans before, during and after spaceflight

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Astronauts returning to Earth have reduced orthostatic tolerance and exercise capacity. Alterations in autonomic nervous system and neuromuscular function after spaceflight might contribute to this problem. In this study, we tested the hypothesis that exposure to microgravity impairs autonomic neural control of sympathetic outflow in response to peripheral afferent stimulation produced by handgrip and a cold pressor test in humans. We studied five astronauts ~72 and 23 days before, and on landing day after the 16 day Neurolab (STS-90) space shuttle mission, and four of the astronauts during flight (day 12 or 13). Heart rate, arterial pressure and peroneal muscle sympathetic nerve activity (MSNA) were recorded before and during static handgrip sustained to fatigue at 40 % of maximum voluntary contraction, followed by 2 min of circulatory arrest pre-, in- and post-flight. The cold pressor test was applied only before (five astronauts) and during flight (day 12 or 13, four astronauts). Mean (\pm S.E.M.) baseline heart rates and arterial pressures were similar among pre-, in- and post-flight measurements. At the same relative fatiguing force, the peak systolic pressure and mean arterial pressure during static handgrip were not different before, during and after spaceflight. The peak diastolic pressure tended to be higher post- than pre-flight (112 ± 6 vs. 99 ± 5 mmHg, $P = 0.088$). Contraction-induced rises in heart rate were similar pre-, in- and post-flight. MSNA was higher post-flight in all subjects before static handgrip (26 ± 4 post- vs. 15 ± 4 bursts min^{-1} pre-flight, $P = 0.017$). Contraction-evoked peak MSNA responses were not different before, during, and after spaceflight (41 ± 4 , 38 ± 5 and 46 ± 6 bursts min^{-1} , all $P > 0.05$). MSNA during post-handgrip circulatory arrest was higher post- than pre- or in-flight (41 ± 1 vs. 33 ± 3 and 30 ± 5 bursts min^{-1} , $P = 0.038$ and 0.036). Similarly, responses of MSNA and blood pressure to the cold pressor test were well maintained in-flight. We conclude that modulation of muscle sympathetic neural outflow by muscle metaboreceptors and skin nociceptors is preserved during short duration spaceflight.

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Astronauts adapt to the microgravity environment of space appropriately and effectively (Rummel *et al.* 1976; Michael *et al.* 1977; Nicogossian & Garshnek, 1989). However, on return to Earth, they have reduced orthostatic tolerance (Hoffler *et al.* 1974; Blomqvist & Stone, 1983; Bungo *et al.* 1985; Fritsch-Yelle *et al.* 1994; Buckey *et al.* 1996; Levine, 1996a) and exercise capacity (Convertino, 1996; Levine *et al.* 1996b). The underlying mechanisms remain unclear

and are probably multifactorial. Alterations in autonomic nervous system and/or neuromuscular function after spaceflight might contribute to this problem.

Static handgrip causes marked increases in heart rate, arterial pressure, and muscle sympathetic nerve activity (MSNA) (Mark *et al.* 1985; Wallin *et al.* 1989; Seals & Victor, 1991). The increases in MSNA are thought to result

primarily from activation of the metaboreflex in the exercising muscle (Victor *et al.* 1988; Sinoway *et al.* 1989; Rowell, 1990). This reflex originates in sensory receptors which appear to be sensitive to ischaemic metabolites generated during muscular contraction, via small myelinated or unmyelinated (group III or IV) afferent fibres, and elicits cardiovascular and vasomotor reflexes (Coote *et al.* 1971; McCloskey & Mitchell, 1972; Mitchell *et al.* 1977; Tibes, 1977; Mitchell *et al.* 1983). Central command (Mitchell & Schmidt, 1983; Victor *et al.* 1989; Mitchell, 1990) and the intramuscular mechanoreflex (McClain *et al.* 1993, 1994) also contribute to the exercise pressor response, particularly to the increase in heart rate (Gladwell & Coote, 2002).

The cold pressor test elicits remarkable increases in arterial pressure and MSNA with no significant changes in heart rate (Victor *et al.* 1987; Yamamoto *et al.* 1992). The reflex pathway to activate MSNA may originate from cold nociceptors in the skin which conduct afferent signals by unmyelinated C-fibres, and the pathway may involve central vasomotor centres that serve to regulate MSNA (Yamamoto *et al.* 1992). Thus, cold pressor tests are often performed in the clinical evaluation of autonomic disorders to ensure the integrity of central vasomotor processes and their efferent pathways.

Impairment of pressor responses to isometric muscle contraction has been observed both during and after bed rest, and after spaceflight in some (Pagani *et al.* 2001; Spaak *et al.* 2001) but not all studies (ten Harkel *et al.* 1992; Essfeld *et al.* 1993; Kamiya *et al.* 2000). However, direct measurements of sympathetic activation by muscle contraction have been made only rarely after bed rest (Kamiya *et al.* 2000) and never after spaceflight. Differences in both duration and magnitude of relative and absolute force may explain at least some of the published discrepancies (Seals, 1993), since muscle mass and strength may change during spaceflight and simulated microgravity (Zhang *et al.* 1997; Antonutto *et al.* 1999). In addition, no direct measurements of MSNA during the cold pressor test have been reported during or after microgravity exposure, making the specific neural pathways that might be affected by microgravity uncertain.

This study follows three reports from the Neurolab space shuttle mission which documented the integrity of baroreflex activation of sympathetic nerve activity during and after spaceflight (Cox *et al.* 2002; Ertl *et al.* 2002; Levine *et al.* 2002). We tested the hypothesis that exposure to microgravity impairs autonomic neural control of sympathetic outflow responses to two different types of peripheral afferent stimulation in humans. To accomplish this objective, static handgrip sustained to fatigue was carried out before, during and after the 16 day Neurolab (STS-90) space mission; the cold pressor test was also performed before and during spaceflight.

METHODS

Subjects

We studied five male astronauts of the 16 day Neurolab space mission, described in detail by Cox *et al.* (2002). Their mean age (\pm S.E.M.) was 41 ± 1 years, height 185 ± 2 cm, and weight 84 ± 6 kg. All astronauts were in excellent health, as determined by comprehensive National Aeronautics and Space Administration (NASA) Class III physical examinations. None smoked or used any medication regularly. The subjects were informed of the purpose and procedures used in the study and gave their written informed consent. The present study was conducted under the guidelines proposed by the NASA Johnson Space Center Human Research Policies and Procedures Committee and the human subjects' institutional review boards at the home institutions of the principal investigators (The University of Texas Southwestern Medical Center at Dallas, Vanderbilt University, and the Medical College of Virginia), and in accordance with the Declaration of Helsinki. All five astronauts participated in both pre- and post-flight sessions, and four joined in the in-flight session.

Instrumentation

Experiments were performed 73–70 and 24–21 days pre-flight, 12–13 days in-flight and on landing day. Subjects were studied at least 2 h after a meal, and more than 12 h after the last caffeinated or alcoholic beverage. All experiments on earth were carried out in a quiet, environmentally controlled laboratory with an ambient temperature of 25 °C. Heart rate was measured from the electrocardiogram (HP 78801 B, Hewlett-Packard, Andover, MA, USA), and beat-by-beat arterial pressure was derived by finger photoplethysmography (on Earth, Finapres, Ohmeda, Englewood, CO, USA; in space, Finapres, as modified by TNO Biomedical Instrumentation, Amsterdam, The Netherlands, for the European Space Agency). Arm blood pressure was measured intermittently by electrophygmomanometry (Suntech 4240, Suntech Medical Instruments, Raleigh, NC, USA), with a microphone placed over the brachial artery to detect Korotkoff sounds.

Multiunit recordings of postganglionic MSNA were obtained ~72 days pre-flight, 12–13 days in-flight and on landing day with tungsten microelectrodes inserted into muscle fascicles of a peroneal nerve (Vallbo *et al.* 1979). Briefly, a recording electrode was placed in the peroneal nerve at the fibular head or the popliteal fossa, and a reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The nerve signals were amplified (total gain: 70 000–160 000), band-pass filtered (700–2000 Hz), full-wave rectified and integrated with a resistance–capacitance circuit (time constant: 0.1 s). Criteria for adequate MSNA recording included: (1) pulse synchrony; (2) facilitation during Valsalva straining, and suppression during the hypertensive overshoot after release; (3) increases in response to breath holding; and (4) insensitivity to emotional stimuli, i.e. loud noise (Vallbo *et al.* 1979; Wallin & Eckberg, 1982).

Protocol

Static handgrip to fatigue. Each subject was studied in the supine position (on Earth and in space), with his lower body enclosed in a chamber fitted with a removable window to allow placement of microneurography electrodes. The chamber was used for another study in the Neurolab autonomic investigations (Ertl *et al.* 2002), and was open to air during static handgrip. Each subject performed three brief (~3 s) maximal contractions to determine his maximal voluntary contraction by using a handgrip dynamometer prior to microneurography. The highest value of these three contractions was used as the maximal voluntary contraction.

Static handgrip was performed after controlled-frequency breathing and Valsalva manoeuvres (Cox *et al.* 2002).

After a sufficient recovery period to allow all signals to return to baseline values (approximately 5 min) following the preceding intervention, baseline heart rate, arterial pressure and MSNA were recorded for 1 min. Static handgrip was then performed with the dominant hand at 40 % of maximal voluntary contraction until fatigue, followed by 2 min of post-handgrip forearm circulatory arrest with an upper arm cuff inflated to 250 mmHg. During all sessions, the subject was presented with a bar graph display that was proportionate to the achieved force, with a colour-coded target of 40 % of maximal voluntary contraction. When the achieved force declined to < 80 % of this target for ≥ 2 s the cuff was inflated automatically during flight and manually during pre- and post-flight experiments. During exercise, the subjects were instructed to avoid the Valsalva manoeuvre (breath-holding strain), as well as leg or abdominal muscle tension. Post-handgrip forearm circulatory arrest was performed 10 s before the release of static handgrip and maintained for 2 min.

Cold pressor test. The cold pressor test was carried out after a sufficient recovery of static handgrip until all variables returned to baseline values (a minimum of 7–10 min), according to a protocol modified for use in space. Because the standard technique of immersing the hand in ice water was not practical for use in space flight, the astronaut placed his hand between two gel-packs confined inside an insulated mitt that was pre-cooled to -12°C . Since the thermal conductivity of the gel-mitt was significantly less than water, this greater degree of cooling was used to mimic a standard cold pressor test. Extensive pilot testing confirmed the equivalency of these two stimuli. The same gel-in-mitt system was used for pre- and in-flight experiments. Baseline measurements were recorded during 1 min, and then the right hand was placed in the mitt up to the wrist for 2 min. Subjects were instructed to avoid isometric contraction and performance of the Valsalva manoeuvre or held expiration during the test. The recovery period lasted for 2 min. Data were recorded continuously throughout the procedures.

Data analysis. Physiological signals were digitized on-line during pre- and post-flight sessions with Windaq hardware and software (DA-220, DATAQ Instruments, Akron, OH, USA), and during the in-flight session with data acquisition programs developed by NASA. In-flight signals were down-linked to Earth to allow investigators to monitor cardiovascular parameters and assist astronauts to evaluate the quality of sympathetic nerve recordings.

Sympathetic bursts were identified by a computer program, and then were confirmed by an experienced microneurographer who was blinded to the experimental context. Bursts were selected if the signal-to-noise ratio was greater than 2:1, and bursts occurred about 1.3 s after the previous (one removed) electrocardiographic R wave. The number of bursts per minute (burst frequency) and the number of bursts per 100 heart beats (burst incidence) were used as quantitative indexes. To obtain an index of the change in total sympathetic nerve activity during static handgrip and the cold pressor test, the areas under all bursts were integrated during each recording period. Nerve activity recorded during the baseline recording was assigned a value of 100 %, and subsequent changes of integrated MSNA were expressed as percentages of this baseline value.

Heart rate, arterial pressure and MSNA were averaged for 1 min of baseline, 1 min of the initial static handgrip, the last 30 s of handgrip

prior to fatigue (presented as 'at fatigue'), the intermediate period after the initial 1 min and before the last 30 s of handgrip, and for each 1 min of post-handgrip circulatory arrest and recovery. Data were averaged for 1 min before the cold pressor test (baseline), each 30 s (for MSNA) or each 1 min (for heart rate and arterial pressure) during the cold pressor test, and every 1 min during the recovery.

Statistical analysis

Data are expressed as means \pm s.e.m. Data across conditions (pre-, in- and post-flight) were compared by using a repeated measures analysis of variance (ANOVA) with Bonferroni (with ~ 72 days pre-flight as baseline condition) and Tukey (for comparison among all conditions) *post hoc* tests for multiple comparisons. All statistical analyses were performed with a personal computer-based analysis program (SigmaStat, SPSS, Chicago, IL, USA). A *P* value of < 0.05 was considered significant.

RESULTS

Heart rate and arterial pressure responses to static handgrip and the cold pressor test were similar during the two pre-flight sessions (~ 72 and 23 days before spaceflight), and not significantly different for any variable. Therefore, we report here only the results from the former session, during which MSNA was recorded.

Cardiovascular response to handgrip

The maximal voluntary contraction was not altered significantly by spaceflight (from 50 ± 2 pre- to 45 ± 3 kg post-flight, $P = 0.318$). However, the time to fatigue during static handgrip was shortened both in- and post-flight (183 ± 21 s pre- vs. 120 ± 9 in- and 125 ± 17 s post-flight, $P = 0.04$ and 0.03). Table 1 shows individual cardiovascular responses to static handgrip before, during and after 16 days of spaceflight. Baseline heart rates and mean arterial pressures were similar among pre-, in- and post-flight measurements (Fig. 1). At the same relative (and absolute) forces, heart rate gradually increased during static handgrip, reached its peak at fatigue, and immediately returned to the baseline level during post-handgrip circulatory arrest before, during and after spaceflight. The contraction-induced rises in heart rate were not different among pre-, in- and post-flight (Fig. 1, upper panel), documenting similar degrees of central command. Mean arterial pressure increased progressively during static handgrip, reached its peak at fatigue, and decreased but remained higher than the baseline level during post-handgrip circulatory arrest (Fig. 1, bottom panel). The peak systolic pressure and mean arterial pressure during static handgrip were not different among pre-, in- and post-flight (Table 1 and Fig. 1), though the peak diastolic pressure tended to be higher post- than pre-flight (Table 1, $P = 0.088$).

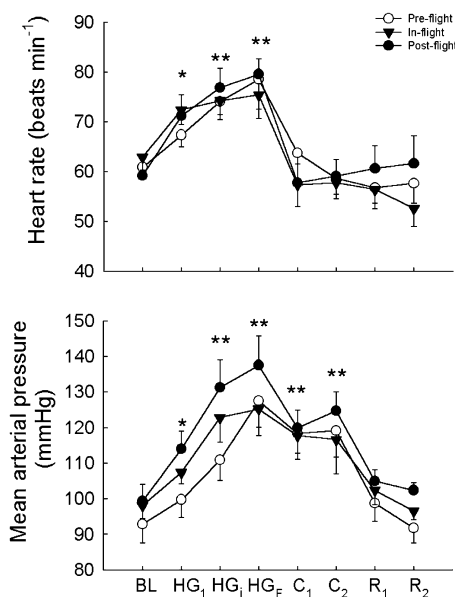
Sympathetic neural response to handgrip

Figure 2 shows MSNA responses to static handgrip and post-handgrip circulatory arrest of a representative astronaut. Baseline MSNA was significantly higher post- compared with pre-flight ($P = 0.017$, Fig. 3). MSNA burst frequency

Table 1. Individual cardiovascular response to static handgrip

Variable	Astronaut	Pre-flight				In-flight				Post-flight			
		BL	HG _F	C	R	BL	HG _F	C	R	BL	HG _F	C	R
SBP (mmHg)	1	138	189	167	132	122	191	175	147	156	200	182	157
	2	146	211	204	159	128	195	175	137	139	209	194	145
	3	123	151	152	127	148	185	189	155	122	155	147	129
	4	162	204	197	157	149	211	198	151	122	166	171	154
	5	136	177	162	143	—	—	—	—	162	214	182	156
	Mean ± S.E.M.		141 ± 6	186** ± 11	176** ± 10	144 ± 6	137 ± 7	196** ± 6	184** ± 6	148 ± 4	140 ± 8	189** ± 12	175** ± 8
DPB (mmHg)	1	66	95	87	62	72	117	102	81	89	121	105	85
	2	77	114	106	81	71	104	94	69	88	125	115	88
	3	63	83	80	66	85	118	105	88	74	95	88	78
	4	90	108	104	78	80	125	105	80	72	101	95	81
	5	69	94	79	67	—	—	—	—	85	118	95	79
	Mean ± S.E.M.		73 ± 5	99** ± 5	91** ± 6	71 ± 4	77 ± 3	116** ± 4	102** ± 3	80 ± 4	82 ± 4	112** ± 6	100** ± 5
MAP (mmHg)	1	85	125	112	82	106	136	124	100	109	147	132	107
	2	97	147	141	103	103	140	138	101	103	152	139	106
	3	81	103	102	85	91	116	93	91	90	114	109	95
	4	111	139	133	100	93	109	112	94	87	122	119	101
	5	91	124	108	89	—	—	—	—	108	153	125	103
	Mean ± S.E.M.		93 ± 5	128** ± 8	119** ± 8	92 ± 4	98 ± 4	125** ± 8	117** ± 10	97 ± 2	99 ± 5	138** ± 8	125** ± 5
HR (beats min ⁻¹)	1	61	73	43	42	68	83	51	45	49	73	47	48
	2	61	81	68	61	57	83	60	48	53	74	57	49
	3	60	71	63	62	61	72	59	58	69	78	66	72
	4	65	101	61	65	66	64	61	59	63	90	64	75
	5	58	68	58	58	—	—	—	—	62	83	62	64
	Mean ± S.E.M.		61 ± 1	79** ± 6	59 ± 4	58 ± 4	63 ± 2	76** ± 5	58 ± 2	53 ± 4	59 ± 4	80** ± 3	59 ± 3

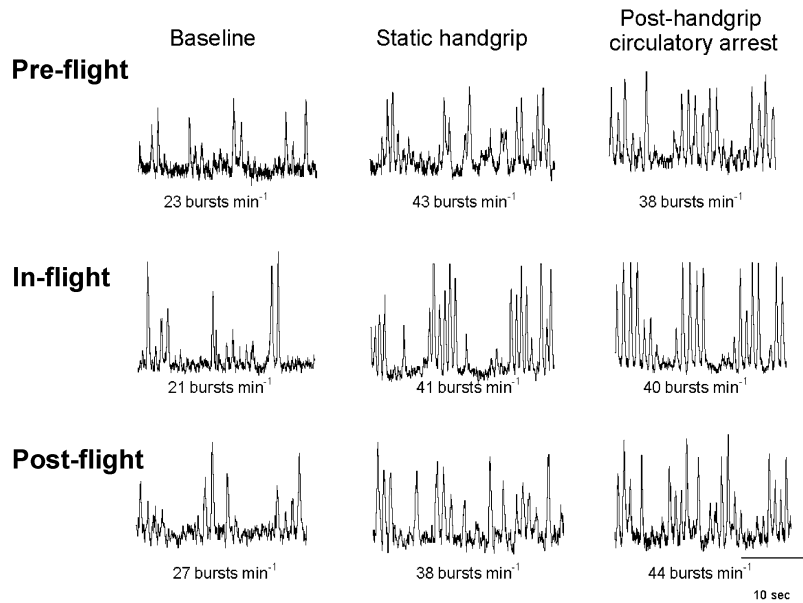
Means ± S.E.M. Data from five astronauts pre- and post-flight, and from four astronauts in-flight. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; BL, baseline; HG_F, static handgrip at fatigue; C, post-handgrip circulatory arrest; R, recovery. ** $P < 0.01$, compared with baseline.

**Figure 1. Heart rate and mean arterial pressure responses to static handgrip**

Mean (± S.E.M.) responses to static handgrip sustained to fatigue at 40 % of maximal voluntary contraction, followed by 2 min post-handgrip circulatory arrest pre-flight ($n = 5$), in-flight ($n = 4$) and post-flight ($n = 5$). Data were averaged for 1 min of baseline, 1 min of the initial static handgrip, the last 30 s of handgrip prior to fatigue (presented as 'at fatigue'), the intermediate period after the initial 1 min and before the last 30 s of handgrip and for each 1 min of post-handgrip circulatory arrest and recovery. There were no significant differences in responses on Earth and in space. BL, baseline; HG₁, first minute of static handgrip; HG₂, intermediate handgrip period; HG_F, static handgrip at fatigue; C₁ and C₂, first and second minutes of post-handgrip circulatory arrest; R₁ and R₂, first and second minutes of recovery. * $P < 0.05$ and ** $P < 0.01$, compared with baseline pre-, in- and post-flight.

Figure 2. Muscle sympathetic nerve activity response to static handgrip of one astronaut

Muscle sympathetic nerve activity recordings from one astronaut at baseline, static handgrip sustained to fatigue and during post-handgrip circulatory arrest pre-flight, in-flight and post-flight.



gradually increased during static handgrip and reached its peak at fatigue under all conditions. The contraction-evoked peak MSNA responses were not different among pre-, in- and post-flight. MSNA remained elevated during post-handgrip circulatory arrest, and was markedly higher post- than pre- and in-flight ($P = 0.038$ and 0.036 , Fig. 3).

MSNA was expressed as bursts $(100 \text{ heart beats})^{-1}$ (burst incidence) before, during and after spaceflight to control for the tachycardia during exercise. Baseline burst incidence was significantly higher post- than pre- and in-flight (44 ± 8 vs. 25 ± 6 and 32 ± 4 bursts $(100 \text{ heart beats})^{-1}$, both $P < 0.01$). MSNA burst incidence gradually increased

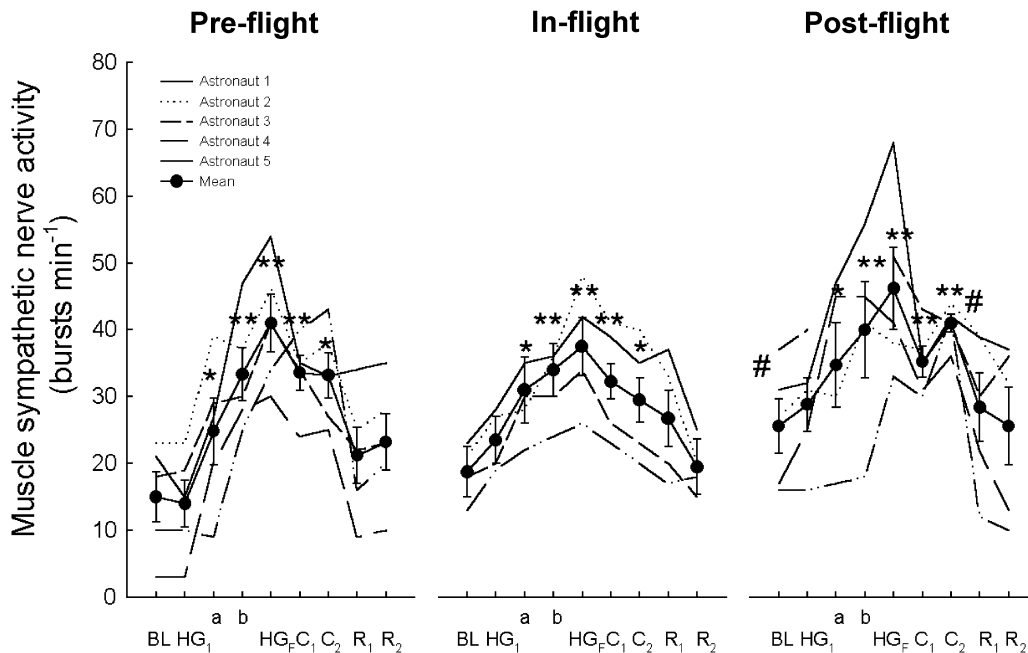


Figure 3. Muscle sympathetic nerve activity burst frequencies for all astronauts in response to static handgrip

Individual and mean (\pm S.E.M.) responses to static handgrip sustained to fatigue at 40% of maximal voluntary contraction, followed by 2 min post-handgrip circulatory arrest pre-flight ($n = 5$), in-flight ($n = 4$) and post-flight ($n = 5$). Data were averaged for 1 min of baseline, 1 min of the initial static handgrip, the last 30 s of handgrip prior to fatigue (presented as 'at fatigue') and for each 1 min of post-handgrip circulatory arrest and recovery. The period between the end of the first minute and 30 s before fatigue was broken up into two equal periods and are depicted as 'a' and 'b' in the figure. BL, baseline; HG₁, first minute of static handgrip; HG_F, static handgrip at fatigue; C₁ and C₂, first and second minutes of post-handgrip circulatory arrest; R₁ and R₂, first and second minutes of recovery. * $P < 0.05$ and ** $P < 0.01$, compared with baseline; # $P < 0.05$, compared with pre-flight.

Table 2. Individual and mean cardiovascular response to the cold pressor test

Variable	Astronaut	Pre-flight					In-flight				
		BL	CPT ₁	CPT ₂	R ₁	R ₂	BL	CPT ₁	CPT ₂	R ₁	R ₂
SBP (mmHg)	1	135	136	148	145	132	149	159	176	165	151
	2	151	161	172	165	160	153	168	188	188	174
	3	127	132	136	128	117	145	154	165	157	140
	4	133	145	145	133	129	140	143	160	150	140
	5	137	145	145	138	132	—	—	—	—	—
	Mean	136	143	150*	143	134	147†	156*	172**‡	165*†	151
	± S.E.M.	± 5	± 6	± 8	± 8	± 9	± 3	± 5	± 6	± 8	± 8
DBP (mmHg)	1	64	69	78	74	65	77	83	94	86	75
	2	76	79	87	82	80	73	81	90	85	74
	3	69	71	74	68	65	76	81	89	84	78
	4	68	71	75	70	73	72	78	88	83	75
	5	68	67	71	67	62	—	—	—	—	—
	Mean	69	73*	78*	74	71	75	81*†	90**†	85*†	76
	± S.E.M.	± 2	± 2	± 3	± 3	± 4	± 1	± 1	± 1	± 1	± 1
MAP (mmHg)	1	88	91	101	98	87	101	108	122	113	100
	2	101	107	115	110	107	100	110	123	119	108
	3	88	91	95	88	83	100	105	115	108	98
	4	90	96	98	91	91	94	99	112	105	97
	5	91	93	96	91	85	—	—	—	—	—
	Mean	92	96*	102**	97*	92	99	106*†	118**†	111*†	101
	± S.E.M.	± 3	± 4	± 4	± 5	± 5	± 2	± 2	± 3	± 3	± 2
HR (beats min ⁻¹)	1	46	48	39	44	41	50	50	48	47	50
	2	56	59	53	56	61	50	53	53	52	51
	3	62	63	62	65	62	57	60	60	57	56
	4	57	57	59	58	63	59	62	59	60	62
	5	52	53	53	52	53	—	—	—	—	—
	Mean	55	57	53	56	57	54	56	55	54	55
	± S.E.M.	± 3	± 3	± 5	± 4	± 5	± 2	± 3	± 3	± 3	± 3

Means ± S.E.M. Data from five astronauts pre-flight and from four astronauts in-flight. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; BL, baseline; CPT₁, first minute of cold pressor test; CPT₂, second minute of cold pressor test; R₁, first minute of recovery; R₂, second minute of recovery. * $P < 0.05$ and ** $P < 0.01$, compared with baseline; † $P < 0.05$ and ‡ $P < 0.01$, compared with pre-flight.

during static handgrip, further increased during post-handgrip circulatory arrest, and reached its peak during the last minute of post-handgrip circulatory arrest. The peak MSNA burst incidences were not different during pre-, in- and post-flight testing ($P > 0.05$). Total sympathetic nerve activity gradually increased during static handgrip, reached its peak at fatigue, and remained higher during post-handgrip circulatory arrest. These responses were not different among pre-, in- and post-flight tests (Fig. 4).

Cardiovascular and sympathetic neural responses to the cold pressor test

Table 2 shows individual and mean cardiovascular responses to the cold pressor test. As expected, the cold pressor test increased systolic, mean and diastolic blood pressure in all subjects, before and during spaceflight. Heart rate did not change during the cold pressor test during both pre- and in-flight testing.

Figure 5 depicts representative astronaut's MSNA in response to the cold pressor test. MSNA was enhanced during the cold pressor test, and the enhancement was similar during pre- and in-flight tests (Fig. 6).

DISCUSSION

We report here, for the first time, the effect of the microgravity of space on human muscle sympathetic nerve activity and cardiovascular responses to two types of reflex stimulation in astronauts. We found that: (1) the contraction-evoked peak muscle sympathetic nerve activities during static handgrip were not different among pre-, in- and post-flight conditions; (2) muscle sympathetic nerve activity during post-handgrip circulatory arrest (stimulation of metaboreceptors without central command) was higher post- than pre- and in-flight; (3) heart rate and blood pressure responses were virtually identical among

all conditions; and (4) muscle sympathetic nerve activity and arterial pressure responses to the cold pressor test were well maintained during spaceflight. Thus, our results reject the hypothesis that exposure to microgravity impairs autonomic neural control of sympathetic outflow in response to peripheral afferent stimulation in humans. Muscle sympathetic nerve activity and cardiovascular responses to static handgrip and to the cold pressor test were preserved in space and after spaceflight.

Earlier studies

Heart rate and arterial pressure responses to isometric exercise have been reported as enhanced (Essfeld *et al.* 1993), unchanged (ten Harkel *et al.* 1992; Kamiya *et al.* 2000) or impaired (Pagani *et al.* 2001; Spaak *et al.* 2001) during and/or after microgravity exposure. Spaak *et al.* (2001) investigated the pressor response to static handgrip before, during and after 120 days of bed rest as well as 179–389 days of spaceflight, and found that at the same relative force (30 % of maximal voluntary contraction), the changes of heart rate and mean arterial pressure during static handgrip were significantly reduced after bed rest and spaceflight. Similarly, Pagani *et al.* (2001) reported that the pressor response to static handgrip at 25 % of maximal voluntary contraction was blunted after 42 days of bed rest. However, the evidence for their conclusions was not

definitive. First, in these two studies, static handgrip was performed for fixed durations (2 and 5 min) at fixed relative work rates (30 and 25 % of maximal voluntary contraction), but not sustained to fatigue. However, responses to isometric exercise, particularly submaximal isometric exercise, are affected both by the percentage of maximal strength maintained, as well as the absolute force and muscle mass engaged (Seals *et al.* 1988; Seals, 1989). Second, post-handgrip forearm circulatory arrest was not performed immediately upon the release of static handgrip in either study, so that it was impossible for the authors to isolate the muscle metaboreflex from other factors, such as central command or stimulation of the muscle mechano-reflex, and to determine which component was affected by simulated microgravity.

In contrast, ten Harkel *et al.* (1992) observed that heart rate and arterial pressure in response to static handgrip were not affected by 10 days of bed rest. Kamiya *et al.* (2000) found that increases in muscle sympathetic nerve activity were similar during static handgrip sustained to fatigue, but significantly lower during post-handgrip circulatory arrest after 14 days bed rest. Heart rate and mean arterial pressure during static handgrip and post-handgrip circulatory arrest were not influenced by bed rest. These somewhat contradictory results (decreased

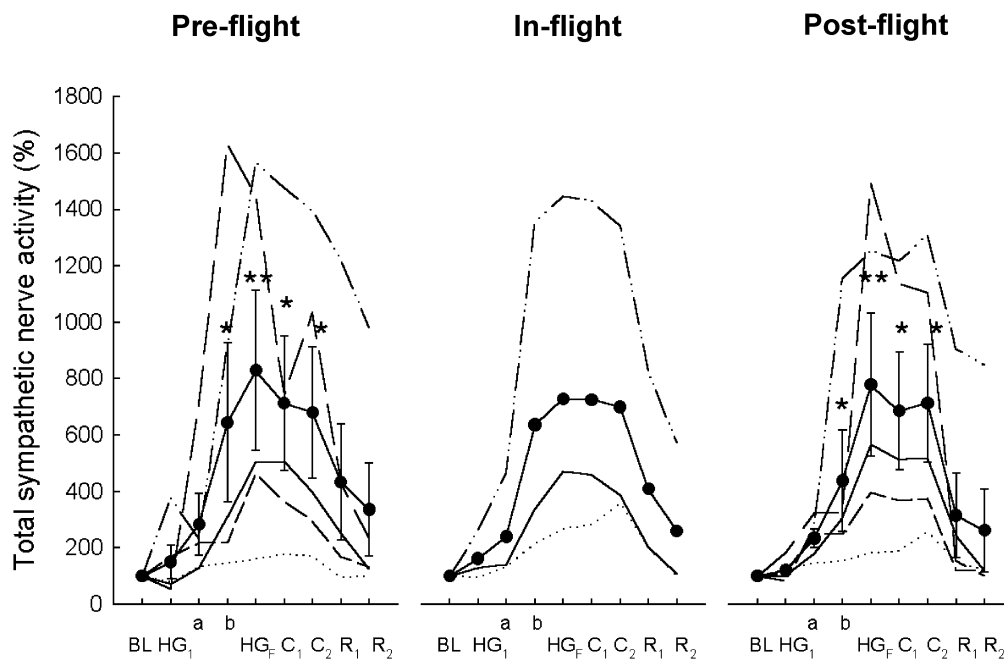


Figure 4. Total sympathetic nerve activity responses to static handgrip

Data for individual astronauts (1–5) are depicted as the same line type in each panel as in Fig. 3. In-flight mean values (●) represent the mean of data from three subjects. The data from astronaut 3 were excluded in this figure because his baseline nerve activity tracing was shifted upward during static handgrip in space; although we could count the burst frequency as well as the burst incidence, we could not obtain the accurate total sympathetic nerve activity from his tracings. BL, baseline; HG₁, first minute of static handgrip; HG_F, static handgrip at fatigue; a and b, the first and second halves of the intermediate period after the first minute of static handgrip and before fatigue; C₁ and C₂, first and second minutes of post-handgrip circulatory arrest; R₁ and R₂, first and second minutes of recovery. * $P < 0.05$ and ** $P < 0.01$, compared with baseline.

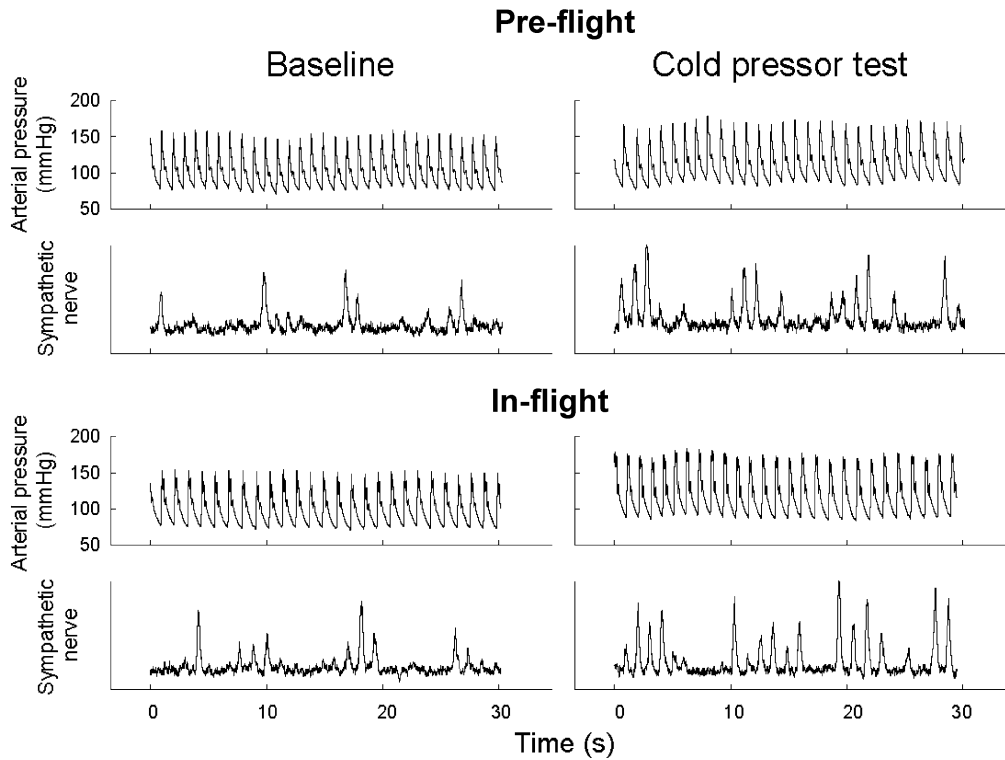


Figure 5. Muscle sympathetic nerve activity and arterial pressure responses to the cold pressor test of one astronaut

Muscle sympathetic nerve activity and arterial pressure recordings from one astronaut at baseline and during the cold pressor test under pre-flight and in-flight conditions.

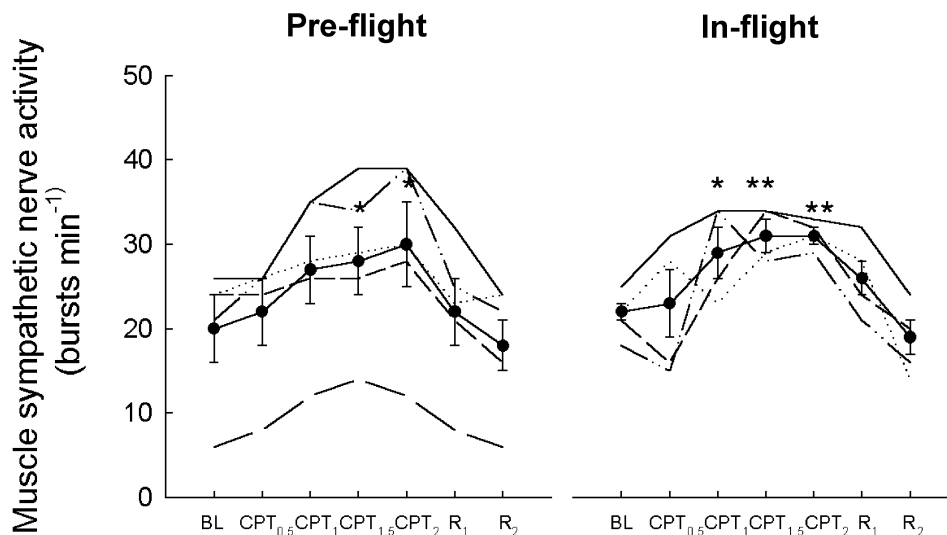


Figure 6. Muscle sympathetic nerve activity burst frequencies in response to the cold pressor test for all astronauts

Data for individual astronauts (1–5) are depicted as the same line type in each panel as in Fig. 3. Individual and mean (\bullet ; \pm S.E.M.) responses to the cold pressor test pre-flight ($n = 5$) and in-flight ($n = 4$). BL, baseline; CPT, the cold pressor test (mean data are shown every 30 sec); R, recovery (mean data are shown every 1 min). * $P < 0.05$ and ** $P < 0.01$, compared with baseline.

sympathetic nerve activity but similar blood pressure response to the post-handgrip circulatory arrest) suggest that the muscle metaboreflex may actually be enhanced by short-term bed rest.

Neurolab results

Our data showing preserved vasomotor sympathetic neural and cardiovascular responses to sustained handgrip in astronauts in space and after spaceflight are consistent with some of these reports (ten Harkel *et al.* 1992; Kamiya *et al.* 2000), but different from the others (Spaak *et al.* 2001; Pagani *et al.* 2001). This is the first report of direct recordings of muscle sympathetic nerve activity in astronauts during isometric handgrip in space. The well-preserved muscle sympathetic nerve activity responses to static handgrip in space and after spaceflight indicate that the autonomic nervous system and neuromuscular system function normally during forearm isometric exercise during and after microgravity exposure.

In the present study, static handgrip sustained to fatigue was performed pre-, in- and post-flight. This protocol was chosen for the following reasons: (1) to standardize the end-point; (2) to elicit peak MSNA responses; and (3) to be insensitive to minor differences in timing, and absolute or relative force among the pre-, in- and post-flight conditions. Since active muscle mass and strength may change during spaceflight (Zhang *et al.* 1997; Antonutto *et al.* 1999), it is extremely important to examine the cardiovascular and sympathetic neural responses to isometric exercise at similar perceptual and performance time points before, during and after microgravity exposure. When normalized to endurance time (i.e. fatigue), the regulation of arterial pressure and MSNA is independent of the force above 20 % of maximal voluntary contraction in humans (Seals, 1993). In addition, unlike submaximal dynamic exercise, in which steady-state conditions can be attained, strenuous isometric exercise elicits progressively greater sympathetic–circulatory adjustments, with peak responses attained only at fatigue (Funderburk *et al.* 1974; Saito *et al.* 1989; Seals & Enoka, 1989). Activation of sympathetic supply to non-active skeletal muscle during static handgrip has been found to be directly related to the development of fatigue (Seals & Enoka, 1989). More importantly, any dependency of MSNA on muscle mass can diminish or disappear if the contractions are prolonged to fatigue (Seals & Enoka, 1989; Saito *et al.* 1993).

Muscle metaboreflex

During exercise, heart rate, arterial pressure, and muscle sympathetic nerve activity drives can be elicited by metaboreceptors situated in the interstitial space of the muscles (Freysschuss, 1970; Martin *et al.* 1974; Victor *et al.* 1989). These receptors are sensitive to the local state of hydration and muscle atrophy. For example, it has been observed that the cardiovascular response to leg exercise

can be augmented by local dehydration and muscle atrophy during spaceflight (Baum *et al.* 1991; Essfeld *et al.* 1991; Essfeld *et al.* 1993). Skeletal muscle is highly sensitive to loading conditions. It has been found that intracellular processes governing protein synthesis in antigravity muscles are transformed (Edgerton *et al.* 1995), such that myofibre size and maximum torque potential are diminished, and susceptibility to fatigue is increased after spaceflight (Day *et al.* 1995; Zhou *et al.* 1995). However, one recent study showed that calf muscle strength and morphology did not change after a 17 day spaceflight (Life and Microgravity Sciences Spacelab Shuttle Transport System – 78 mission) and a control 17 days of bed rest (Trappe *et al.* 2001).

Augmented cardiovascular and vasomotor sympathetic responses to static handgrip were not found in space or after spaceflight in the present study. Although burst frequency was significantly higher post-flight, this difference was not evident when the data were corrected for the heart rate (burst incidence) or total sympathetic nerve activity. One possible explanation for differences between studies examining arms and legs is that exposure to microgravity leads to a persistent fluid loss mainly in the lower body. It is likely that the upper part of the body, such as the forearm, does not suffer as much from dehydration during and/or after microgravity. The second possibility is that in contrast to the leg, arm muscles, because they are not primarily antigravity muscles, may not atrophy during spaceflight, as suggested by the absence of a significant change in maximal voluntary contraction after spaceflight in our study.

Muscle mechanoreflex

Experimental animal studies and a recent study on humans have suggested that the mechanoreceptor effect is mainly an inhibition of cardiac vagal tone during muscle contraction (Coote *et al.* 1971; McCloskey & Mitchell, 1972; Gladwell & Coote, 2002). It is possible that activation of mechanosensitive muscle afferents also contributes to the increase in muscle sympathetic nerve activity during isometric exercise in humans (McClain *et al.* 1993, 1994). In contrast to the metaboreflex, the mechanoreflex ceased during post-handgrip circulatory arrest, yet muscle sympathetic nerve activity remained elevated. Moreover, since muscle sympathetic nerve activity did not increase significantly during the mechanical stimulation of the first minute of static handgrip in this study (Fig. 3), as also noted by others (Wallin *et al.* 1989; Seals & Victor, 1991), we speculate that the muscle mechanoreflex plays a minor role, if any, in the enhancement of muscle sympathetic nerve activity under these conditions.

Central command

A third mechanism involved in the exercise pressor reflex is ‘central command’, which refers to activation of the cardiovascular centre by descending central neural pathways

involved in initiation of somatomotor activity (Krogh & Lindhard, 1913; Goodwin *et al.* 1972; Hobbs, 1982; Mitchell, 1985). Both central command and reflex neural mechanisms have been postulated to explain the cardiovascular responses that occur during exercise, and the two mechanisms appear to affect the same neural circuits and to be capable of working either in conjunction with one another or independently (Mitchell, 1985). For example, at the same muscle tension, decreasing (Goodwin *et al.* 1972) or increasing (Leonard *et al.* 1985) central command elicits corresponding decreased or increased cardiovascular responses to isometric exercise. However, central command has a large influence on parasympathetic outflow to the heart but relatively little effect on vasomotor sympathetic activity (Victor *et al.* 1989), and it produces different effects on heart rate and muscle sympathetic nerve activity during sustained isometric exercise in humans (Seals & Victor, 1991; Rowell, 1993). Based on the observation that the gradual increase in heart rate during static handgrip and the rapid return of heart rate to the baseline level during post-handgrip circulatory arrest were not different among pre-, in- and post-flight, we conclude that central command was well preserved in space and after spaceflight in the present study.

Cold pressor test

Lastly, we found that the muscle sympathetic nerve activity and cardiovascular responses to the cold pressor test were well maintained in astronauts in space. The cold pressor test increases sympathetic activity through a central neural mechanism (Victor *et al.* 1987; Schobel *et al.* 1995). Furthermore, it is independent of the baroreflex and can be used to test the efferent limb of the sympathetic arc (Johnson & Spalding, 1974). Therefore, the well preserved sympathetic outflow responsiveness and arterial hypertension in response to the cold pressor test in space confirm the integrity of central reflex activation of muscle sympathetic nerve activity and appropriate vasomotor responses.

Limitations

There are at least three limitations in this study. First, the number of subjects was small. We studied five astronauts with microneurography before and after the Neurolab mission, and only four astronauts during the late mission. Hence, we present the individual data as much as possible. Second, as indicated by Cox *et al.* (2002), our study was performed after controlled-frequency breathing and Valsalva manoeuvres, which might have influenced the muscle sympathetic nerve activity and cardiovascular responses to static handgrip. Although sufficient recovery time was allowed to return all variables to baseline values, we cannot completely exclude the possibility that the previous protocols influenced our results. We should note, however, that the same experimental order was followed during all experiments, thereby minimizing the impact of

any order effect on pre-, in- or post-flight comparisons. Third, we also cannot exclude the possibility that NASA fluid-loading protocols affected our study on landing day. However, previous studies have suggested fluid-loading minimally influences cardiovascular responses (Buckey *et al.* 1996). Moreover, the preserved static handgrip and cold pressor responses in-flight without fluid loading further suggest a negligible effect of this practice on activation of peripheral afferent nerves.

In conclusion, we found that at the same absolute and relative force (i.e. at fatigue), the contraction-evoked peak muscle sympathetic nerve activity and cardiovascular responses were not different before, during and after spaceflight, suggesting preservation of the exercise pressor reflex via central command and muscle afferent stimulation. Muscle sympathetic nerve activity during post-handgrip circulatory arrest was higher post- than pre- and in-flight, confirming the preservation or even enhancement of the muscle metaboreflex. Similar increases in muscle sympathetic nerve activity and blood pressure during the cold pressor test were observed pre- and in-flight, documenting the integrity of the central reflex activation of muscle sympathetic nerve activity and appropriate vasomotor responses. We conclude that modulation of muscle sympathetic neural outflow by muscle metaboreceptors and skin nociceptors is preserved during short duration spaceflight.

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