Mars 520-d mission simulation reveals protracted crew hypokinesis and alterations of sleep duration and timing

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The success of interplanetary human spaceflight will depend on many factors, including the behavioral activity levels, sleep, and circadian timing of crews exposed to prolonged microgravity and confinement. To address the effects of the latter, we used a high-fidelity ground simulation of a Mars mission to objectively track sleep–wake dynamics in a multinational crew of six during 520 d of confined isolation. Measurements included continuous recordings of wrist actigraphy and light exposure (4.396 million min) and weekly computer-based neurobehavioral assessments ($n = 888$) to identify changes in the crew's activity levels, sleep quantity and quality, sleep–wake periodicity, vigilance performance, and workload throughout the record-long 17 mo of mission confinement. Actigraphy revealed that crew sedentariness increased across the mission as evident in decreased waking movement (i.e., hypokinesis) and increased sleep and rest times. Light exposure decreased during the mission. The majority of crewmembers also experienced one or more disturbances of sleep quality, vigilance deficits, or altered sleep–wake periodicity and timing, suggesting inadequate circadian entrainment. The results point to the need to identify markers of differential vulnerability to hypokinesis and sleep–wake changes during the prolonged isolation of exploration spaceflight and the need to ensure maintenance of circadian entrainment, sleep quantity and quality, and optimal activity levels during exploration missions. Therefore, successful adaptation to such missions will require crew to transit in spacecraft and live in surface habitats that instantiate aspects of Earth's geophysical signals (appropriately timed light exposure, food intake, exercise) required for temporal organization and maintenance of human behavior.

sleep–wake regulation | astronaut

The success of human interplanetary spaceflight, which is anticipated to be in this century, will depend on the ability of spacefarers to remain confined and isolated from Earth much longer than previous missions or simulations, while maintaining the intensity and timing of behavioral activity necessary to accomplish the mission and mitigate the effects of microgravity. A total of four people have spent >1 y in space, with the record of 437 consecutive days on the Mir space station set by Valery Polyakov. The longest Earth-based spaceflight simulation involved four Russians confined in connected hyperbaric chambers for 240 consecutive days. Antarctic winter-over missions have extended up to 363 d. Prediction of how prolonged confinement affects activity levels and sleep–wake dynamics of space explorers is needed to inform spacecraft habitability requirements, crew selection, and behavioral countermeasures during interplanetary missions (1–3). To address this need, we obtained objective neurobehavioral data on the activity patterns of a multinational, culturally diverse crew of six males with backgrounds in engineering, medicine, physiology, and space training, who participated in a high-fidelity ground simulation of a 520-d mission to Mars. Ecological validity of the simulation included a spaceship-like habitat; continuous isolation from Earth's environment; realistic mission activities; a midmission landing on a simulated Mars surface; accurate mission duration and timeline; operations between crew and mission controllers; communication delays inherent in interplanetary travel; limited consumable resources; exercise equipment for physical fitness; diurnal weekly work schedule; crew control of habitat lighting; and video monitoring of crew in habitat common areas. The simulation was developed and operated by the Institute for Bio-Medical Problems (IBMP) of the Russian Academy of Sciences. Photos of the simulation facility and detailed descriptions of the crew, mission timeline, and work–rest schedule are provided in *SI Appendix*, Fig. S1 and Tables S1–S3).

Results

Movement acceleration of crewmembers was continuously recorded at a 1-min resolution throughout the 520-d mission to track the intensity and duration of active wakefulness, rest, and sleep by using validated wrist actigraphy devices that also recorded light intensity (*SI Appendix*, *SI Text* and Fig. S2). The result was 4.396×10^6 min of data constituting 98.0% of time in mission. Behavioral alertness of the crew was probed twice weekly by using psychomotor vigilance test (PVT-B) performance (4, 5) with simultaneous video of the face. Weekly crew ratings were obtained for workload, tiredness, and sleep quality (*SI Appendix*, *SI Text*).

Changes in Crew Rest–Activity Dynamics During the Mission. Profiles of the crew's time and movement intensity in discrete behavioral states indicated increasing sedentariness across the mission. Time spent in active wakefulness per 24 h dropped sharply during the first 3 mo, then more gradually across the next 13 mo

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Data deposition: The data reported in this paper are deposited in a Microsoft Excel file. To access the data: *i*) Go to the www.med.upenn.edu/uep/user_documents/PNAS_Basner_et_al.xlsx. *ii*) When the Save File window appears, choose a destination location to save the file.

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of the mission (Fig. 1*A*). In the final 20 mission days, wake time and intensity rose sharply, whereas sleep and rest times decreased sharply relative to two preceding sequential 60-d periods ($P < 0.002$). Scheduled workload and crew ratings of workload did not increase in these final 20 d. Mission managers indicated that the increased activity of the crew was due to their psychological anticipation of mission end.

Actigraphically estimated mean sleep per 24-h day throughout the mission was 7.39 h (SE = 0.20 h; *SI Appendix*, Table S5*A*). When corrected by −26.4 min (0.44 h) based on our validation study of the Actiwatch algorithm (*SI Appendix*, *SI Text* and Fig. S2), mean mission sleep duration is estimated to have been 6.95 h. Because the correction estimate has a 16.8-min 95% confidence interval, all results reported are for uncorrected actigraphy values. Sleep and rest times showed an inverse pattern to wake time throughout the mission, increasing across the mission until the final 2 mo (Fig. 1*A*). Analyses by mission quarter (MQ; i.e., 130-d periods) revealed a 7.0% decrease in active wakefulness across the mission, equivalent to 1.12-h less active waking per day per crewmember in the last compared with the first MQ (Fig. 1*B*; $P < 0.0001$).

Sleep time increased by 8.4% across the mission, equivalent to 0.59-h more sleep per day per crewmember in the last compared with the first MQ (Fig. 1*C*; $P < 0.0001$). Rest time increased by 50.4% across the mission, equivalent to 0.54-h more rest per day per crewmember in the last relative to the first MQ (Fig. 1*D*; $P < 0.0001$). The findings were confirmed by analyses confined to only the nocturnal (*SI Appendix*, Fig. S3*A*) or only the diurnal portion of each day (*SI Appendix*, Fig. S3*B*), which indicates that the time of day that active wake, rest, and sleep were obtained, did not alter the progressive sedentariness of the crew across the mission. For 90% of mission time awake, crewmembers were exposed at the wrist to light intensity of <177 lux (lx). The intensity of ambient light the crew was exposed to while actively awake during the mission declined by 25.6% from a mean of 104.8 lx (SE = 4.9) to a mean of 78.0 lx (SE = 8.5) across MQs

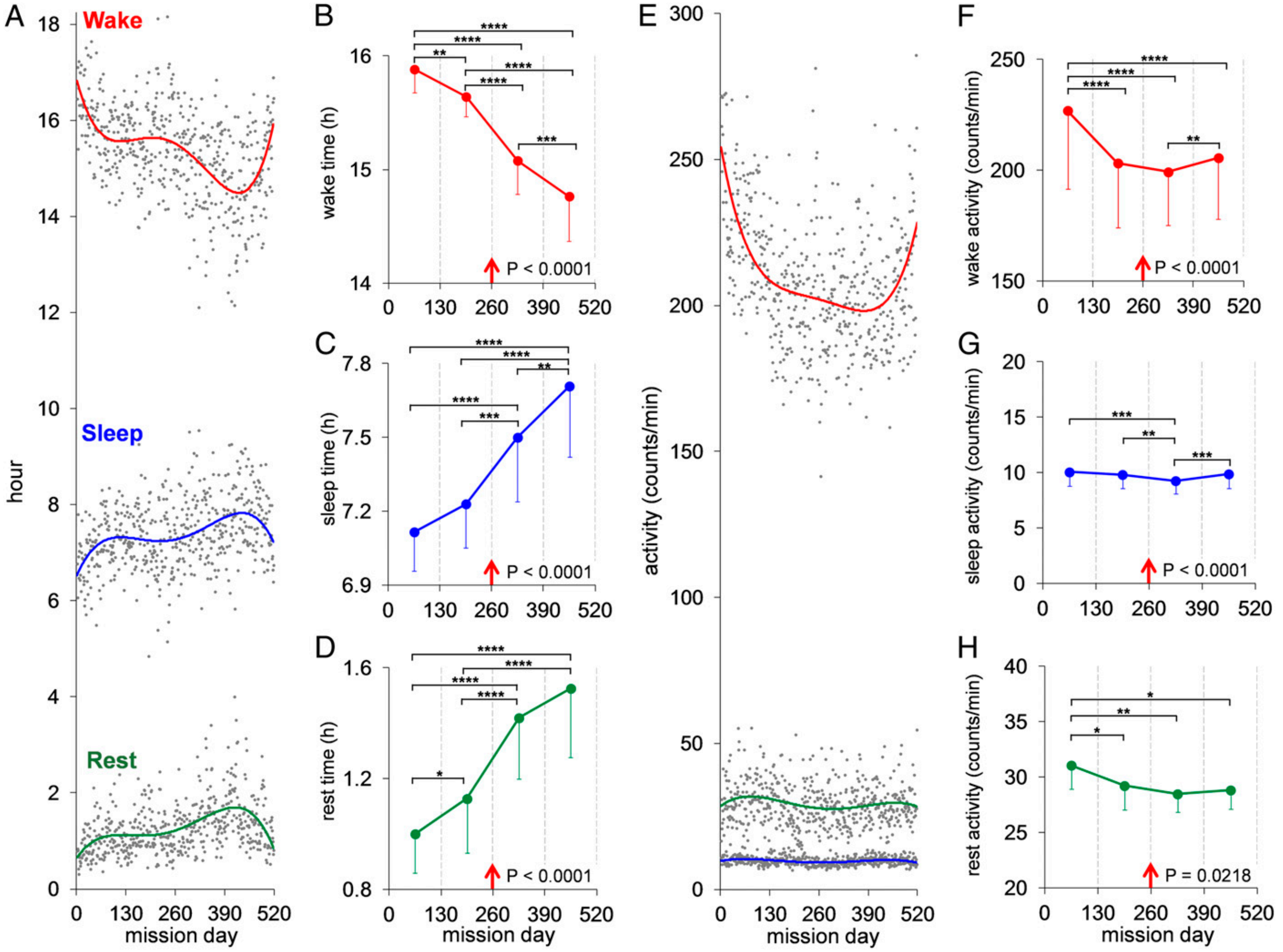

Fig. 1. Activity profiles of crewmembers measured by continuously worn wrist actigraphs throughout the 520-d simulated mission. (*A*) The crew's daily mean (gray points) time (hours) spent in active wakefulness (red trend line), sleeping (blue trend line), and resting (green trend line) across the mission (for information on statistical analyses, see *SI Appendix*, *SI Text*). (*B*–*D*) The crew's mean (SE) time in each behavioral state for each consecutive 130-d MQ (red arrow shows simulated midmission landing on Mars). There was a systematic decrease across MQs in active wakefulness (*B*) and systematic increases in both sleep time (*C*) and waking rest time (*D*). F test *P* values for these effects are shown in each graph; post hoc tests between MQs: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (*E*) The crew's daily mean (gray points) intensity of activity (counts per min) across the mission during active wakefulness (red trend line), sleep (blue trend line), and rest (green trend line). (*F*–*H*) The crew's mean (SE) intensity of activity in each state by MQ. Activity levels declined in MQ 2 and much of MQ 4 relative to the first MQ, but rose in the final 20 d of the last MQ (F test *P* values are shown in *F*–*H*). The sharp increases in active wake time (*A*) and intensity (*E*) in the final 20 mission days, and the commensurate sharp decreases in sleep and rest times (*A*), were significantly different from mean values for these variables relative to two sequential 60-d periods immediately before the final 20 d of the mission ($P < 0.002$).

($P < 0.0001$) and increased slightly during rest (*SI Appendix*, Fig. S3*C*).

Integrated peak acceleration change per second was used to evaluate movement intensity during each behavioral state. Active waking underwent a steep decline in movement intensity during the first MQ, followed by a more gradual decline for 1 y and a sharp rise in the final 20 mission days (Fig. 1*E*). Analyses of this hypokinesis by MQ confirmed reliability of the trend (Fig. 1*F*; $P < 0.0001$). The activity changes during the mission mirrored the crew's ratings of workload, which were highest in the first MQ and lower in subsequent MQs (*SI Appendix*, Fig. S4*A*; F test, $P < 0.0001$). Crew sleep durations averaged <6.78 h (SE = 0.19 h) per day during the first 40 d of the mission, which was lower than during all subsequent 40-d mission periods ($P < 0.05$). This value is close to actigraphically recorded sleep times of astronauts on US Space Shuttle missions (6) and International Space Station expeditions, where work tempos have historically been high. Sleep durations chronically at <7 h per day result in cumulative neurobehavioral performance deficits across days (7, 8), making chronic partial sleep loss a risk to optimal performance in space (3).

The work–rest schedule throughout the mission was 5 d on and 2 d off. The decrease in active wake and increase in sleep and rest times as the mission progressed occurred on both work and rest days (F tests, $P < 0.0001$). Other than the first 40 d of the mission, the only transient interruption of increasing sleep time with mission duration involved the 80-d period before and including the midmission simulated Mars surfacing, when mean sleep duration declined from 7.33 h (SE = 0.15) to 7.13 h (SE = 0.22) ($P = 0.0379$), and crew ratings of tiredness increased ($P = 0.0108$).

Collectively, the crew did not manifest cumulative deficits in PVT-B vigilance performance. Crew daily sleep time increased from a mean of 7.12 h in MQ 1 to 7.71 h in MQ 4 (Fig. 1*C*; F test, $P < 0.0001$), which included reliable increases in sleep duration from MQ 2 to 3 (post hoc test, $P < 0.001$) and from MQ 3 to 4 ($P < 0.01$), when workload ratings were not changing (*SI Appendix*, Fig. S4*A*). The cumulative effect of the increasing time spent asleep over the 1.42-y-long mission was substantial. In total, the crew obtained 673 h more sleep in the second half of the mission relative to the first half (*SI Appendix*, Table S5*B*). Consistent with the increased sleep time, their average normal PVT-B response speed further improved, and their already low rate of vigilance lapses further decreased during the second half of the mission (F tests, $P < 0.0001$; *SI Appendix*, Fig. S5 *A* and *B*). Thus, the added sleep in the latter half of the mission likely benefited behavioral alertness and psychomotor speed, and it may have occurred as a result of confinement, monotony, and habitat characteristics, which included relatively low light levels (<130 lx; *SI Appendix*, Fig. S3*C*) and privacy from monitoring cameras available only in areas for personal hygiene and sleeping quarters.

Variation Among Crewmembers in Sleep–Wake Activity. Cumulative functions were used to evaluate the degree to which changes in activity states across the mission reflected variation in crewmember adaptation to the mission. These functions revealed substantial differences among crewmembers that were unrelated to their roles, responsibilities, or workload ratings. For example, crewmember *d* maintained the highest wake activity level across the mission (Fig. 2*A*) but also one of the highest sleep amounts (Fig. 2*B*) and the most frequent ratings of good sleep quality (*SI Appendix*, Table S6*A*). In contrast to the rest of the crew, he had a very low rest time (Fig. 2*C*) and a PVT-B performance error rate that was low (Fig. 2*D*). His data illustrate that high wake activity levels and adequate sleep to maintain alertness are not incompatible in long-duration mission confinement.

Crewmember *f* had one of the lowest wake activity levels across the mission (Fig. 2*A*). He also had the lowest sleep amount (Fig. 2*B*) among the crew throughout the mission (mean = 6.54 h, SE = 0.04; *SI Appendix*, Table S5*A*) and the most frequent ratings of poor sleep quality (*SI Appendix*, Table S6*A*). He was comparable in rest time to most of the crew (Fig. 2*C*). He had a much higher PVT-B performance error rate than other crewmembers (Fig. 2*D*). He also had the majority of PVT-B facial videos displaying sleepiness and the most frequent ratings of difficulty performing the PVT-B (*SI Appendix*, *SI Text*). PVT-B performance is highly sensitive to acute and chronic sleep loss and is devoid of a learning curve (4, 9). The performance of crewmember *f* was consistent with his experiencing chronic partial sleep deprivation throughout the mission.

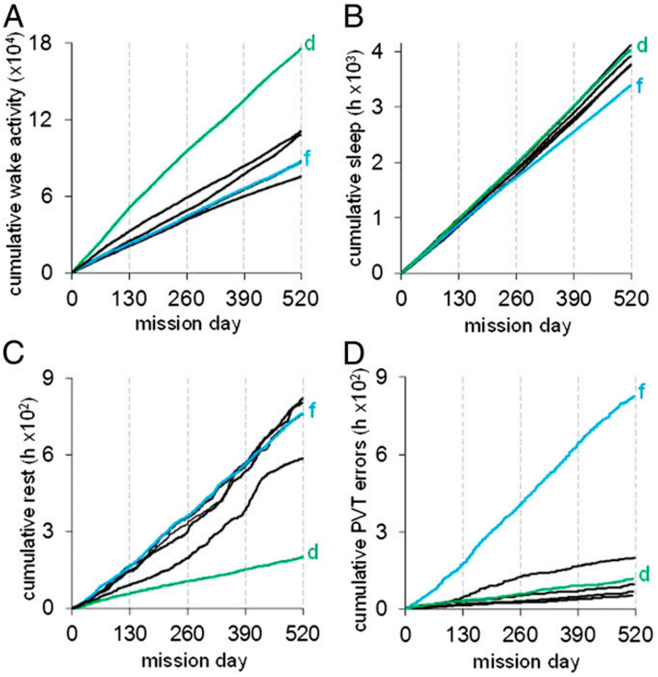

Fig. 2. Cumulative functions over 520 d of mission confinement for each crewmember's waking activity levels (*A*), time spent in sleep (*B*) and rest (*C*), and PVT-B error rate (*D*). Examination of data from crewmembers *d* and *f* illustrate the interindividual differences among the crew in reaction to the prolonged mission confinement.

There were also differences among crew in sleep–wake timing and periodicity. Operations were organized around 24-h clock time with a daily 8.5-h nocturnal sleep period (*SI Appendix*, Table S3). The crew had control over habitat lighting, food intake, physical exercise, and other factors that can promote circadian entrainment (10), but they were not exposed to Earth's geophysical light–dark cycle. The endogenous period of the human circadian pacemaker regulating sleep–wake timing averages 24.18 h (11), but it can be entrained to a 24.0-h period by certain synchronizers, the most important being ambient light. The appropriate phase, intensity, duration, and spectral characteristics of light can promote entrainment and thereby stabilize the timing of behavioral states relative to environmental time, ensuring daytime wakefulness and sleep at night (12). Measurements of light in the crew facility revealed a spectral power distribution consistent with fluorescent lighting (*SI Appendix*, Fig. S6) with low intensity in the 446- to 477-nm wavelength region of the photon spectrum, which is the most potent region for synchronizing or phase-shifting circadian rhythms of sleep and waking (13, 14) and for promoting sleep timing in polar darkness (15). A separate experiment sponsored by the European Space Agency involved adding blue-light exposure late (days 439–499) in the mission (*SI Appendix*, *SI Text*).

Spectrographic analyses of actigraphy data across the mission were conducted for each crewmember to evaluate the extent to which 24-h timing of sleep–wake cycles was maintained during the mission (Fig. 3). Four crewmembers had a monophasic nocturnal sleep pattern throughout the mission with a 24-h sleep–wake cycle (Fig. 3 *C*–*F*). Crewmember *a* manifested a split-sleep pattern (i.e., nocturnal anchor sleep plus a diurnal nap), which became more pronounced in the latter half of the mission as evidenced by a 50.8% increase in diurnal sleep (Fig. 3*A*). Despite this increasing displacement of sleep from the nocturnal to the diurnal portion of the day, a 24-h periodicity of sleep timing was evident for crewmember *a* because a major sleep episode always occurred nocturnally.

In contrast to other subjects, crewmember *b* had a sleep–wake cycle with a dominant period of 24.98 h, which lengthened across MQs from 24.72 to 25.06 h (Fig. 3*B*). This prolonged sleep–wake period is beyond the endogenous free-running circadian period found in healthy adults (11), but is very similar to periods observed in older, shorter-duration circadian isolation protocols (17) and polar studies (18), wherein subjects had access to room-light exposure before circadian temperature minimum. A number of factors may contribute to a prolonged sleep–wake cycle during confinement and isolation, including exercise (19) and light exposure at sensitive portions of the circadian-phase response curve for each zeitgeber (11). The circadian system is sensitive to even low levels of light before body temperature minimum (20), which can induce phase delays in molecular mechanisms of entrainment (21), suppress melatonin secretion (13), and induce longer sleep–wake periods (22). Examination of light-exposure data revealed that crewmember *b* was awake later at night and exposed to light at times that may have contributed to repeated phase delays of his sleep–wake cycle (23, 24). This nocturnal room-light exposure during a sensitive phase for circadian delays began in the first 30 d of the mission (*SI Appendix*,

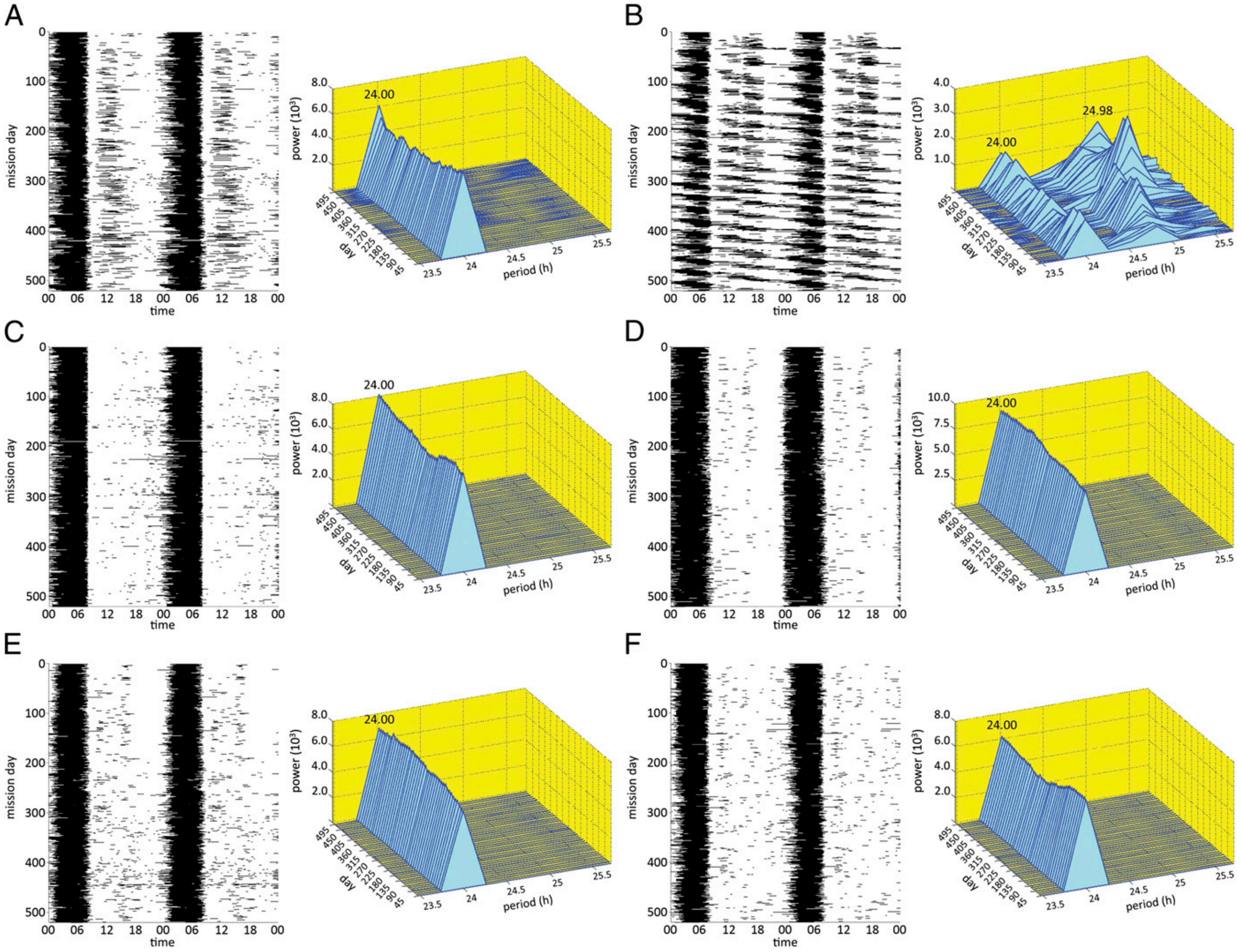

Fig. 3. Double raster plots of sleep (black) and wake (white) and spectral plots (blue and yellow) from actigraphically derived sleep and waking throughout the 520-d mission for crewmembers *a*, *b*, *c*, *d*, *e*, and *f* (*A*–*F*, respectively). Rest was classified as wake for these analyses. Spectral analyses to evaluate sleep–wake periodicity were performed on 1-min actigraphic epochs based on the power spectral density by using the periodogram method (16), multiplying the data with a 90-d rectangular window and taking the squared magnitude of the discrete time Fourier transform. The peak frequency was estimated by a 3-point quadratic interpolation based on the log-magnitudes of the periodogram at the frequency corresponding to the maxima in the periodogram and the two neighboring points. Spectrogram plots were derived from the 90-d window moved in increments of 10 d across the mission (*SI Appendix*, *SI Text*). As is evident in the double-raster and spectral plots, all crewmembers except *b* had a predominant 24-h sleep–wake periodicity. Crewmember *b* had a sleep–wake period that varied between 24.72 and 25.06 h across the mission, increased with time in mission, and averaged 24.98 h for the entire mission. The smaller 24-h peak seen in the spectrogram of crewmember *b* was due to his daily attendance at breakfast between 08:00 and 10:00 each morning (*SI Appendix*, Table S3).

Fig. S7*A*) and occurred periodically thereafter as the sleep–wake cycle lengthened to near 25 h (*SI Appendix*, Fig. S7*B*).

The near 25-h sleep–wake period of crewmember *b* (Fig. 3*B*) and the biphasic sleep pattern of crewmember *a* (Fig. 3*A*) were associated with more mission days in which their total sleep times was >10 h (12.6% and 12.2%, respectively) relative to all other crewmembers (mean = 1.7%). They were the only two crewmembers who showed average delayed sleep onset times of $\sim$2 h or more during the first month of the mission relative to their premission averages—3 h 22 min for crewmember *b* and 1 h 59 min for crewmember *a* (*SI Appendix*, *SI Text* and Table S4). This result is consistent with both crewmembers experiencing delayed sleep-phase syndrome or non-24-h sleep–wake syndrome (25). The longer-duration sleep episodes and increased temporal displacement of sleep resulted in these two crewmembers being asleep when other crewmembers were awake (or vice versa) a total of 2,498 h, or 20.1% of the mission (*SI Appendix*, Fig. S7*C*). Such unplanned temporal desynchrony among crewmembers has the potential to pose a challenge for effective crew coordination during long-duration spaceflight.

Discussion

A majority (four of six) of the crewmembers in this record-long, high-fidelity, simulated space mission confinement experienced one or more of the following problems: disrupted sleep–wake periodicity ($n = 1$), increased displacement of sleep into the diurnal period ($n = 2$), performance deficits associated with chronic partial sleep deprivation ($n = 1$), and frequent reductions in perceived sleep quality ($n = 2$). Spectral plots (Fig. 3) and cumulative functions (Fig. 2) indicate that these problems occurred early in the mission and persisted unabated. Such individual differences in disturbances of sleep–wake regulation are similar to those identified in winter-over polar expeditions (15, 26), which are often considered analogs for the study of behavioral reactions to the prolonged isolation of spaceflight (27–30). The fact that sleep–wake disruptions occur during confinement and isolation in some individuals more than others is consistent with increasing evidence that there are phenotypic and genotypic differences in vulnerability to both alterations of sleep and the resulting neurobehavioral consequences (31–37). This differential vulnerability has led to a search for predictive biomarkers of the effects of sleep loss (38), which would be useful for managing sleep–wake regulation during exploration spaceflight (39).

The progressive sedentariness of the crew that was evident through increased sleep and rest times and the decreased active wakefulness with time in mission support the view that ecological variables can be determinants of human sleep duration (40). It is suggestive of behavioral aspects of torpor, which historically refers to lethargy (41) but more recently has been used to define metabolic or body temperature changes characteristic of heterothermic mammals and birds (42). The concept of behavioral torpor as sedentariness is consistent with the increases in sleep reported in some migratory birds and other animals living in confinement or during winter photoperiods (43, 44).

The hypokinesis and behavioral torpor during the 520-d simulated exploration mission, the sleep loss induced by critical periods of high workload early in the mission, and the common and persistent disturbances of sleep–wake behaviors throughout the mission highlight the importance of preventing these conditions in exploration missions. There is a need for novel space exploration habitats and crew activity schedules that mimic the biological potency of Earth's geophysical cycle through both photic (22, 45) and nonphotic (10, 19, 46) synchronizers to promote circadian entrainment and the temporal optimization of behavioral states during prolonged spaceflight. These needs extend to circadian adjustment for work in near-circadian environments, such as the Martian solar day (i.e., 24.67 h) (47). A balance must be struck during human exploration of space between the critical need for adequate time for sleep and rest and the need to maintain activity levels for physical and physiological fitness. This balance is especially important given the deleterious effects of prolonged microgravity on the musculoskeletal, cardiovascular (48), and other systems (49) and the requirement to sustain fitness to work effectively, avoid injury, and successfully accomplish the mission.

Our findings also have implications for the increasing prevalence of sleep and circadian rhythm disorders among humans living on Earth in industrialized societies, with limited exposure to natural geophysical signals, widespread sedentary activities, and primarily artificial light exposure. There is considerable population evidence that work schedules (50), alarm clocks (51), television programming times (51), and cultural time shifts, such as school start times (52) and daylight savings time (53), contribute to sleep restriction and a discrepancy between circadian and social clocks (i.e., social jetlag), both of which have been linked to obesity (54). The essential need for humans to maintain sleep–wake activity cycles synchronized to the circadian biology that temporally coordinates human health and behavior appears to be as important on Earth as it will be en route to Mars.

Methods

The State Scientific Center of the Russian Federation–IBMP of the Russian Academy of Sciences (RAS) performed the 520-d simulated mission. Crewmembers signed informed consents approved by the Institutional Review Board of the University of Pennsylvania.

Wrist actigraphy (Actiwatch Spectrum; Philips/Respironics) for assessing sleep–wake activity (55) was worn by crewmembers throughout the 520 d. Both average light intensity and movement-induced accelerations at the wrist were recorded in 1-min epochs. Activity data were classified into active wake, sleep, or waking rest using Respironics Actiware (Version 5.59.0015). A separate validation study that we conducted of the Actiwatch state scoring algorithm established its detection sensitivity for sleep at 97.0%, its specificity for wakefulness at 96.2%, and overall accuracy of the Actiwatch algorithm at 96.4% (*SI Appendix*, *SI Text* and Fig. S2). A total of 4,396,333 min of activity was collected in the 520-d mission, which was 98.02% of the total possible. Actigraphy was also used to evaluate the intensity of activity for each 24-h period during the mission and in each of the three states.

Spectrographic analyses of actigraphy data were performed on 1-min epochs to determine the predominant periodicity of sleep–wake timing for each subject. Power spectra of the sleep–wake time series were estimated by using the periodogram method (16) of multiplying the data with a 90-d rectangular window and taking the squared magnitude of the discrete time Fourier transform.

Behavioral alertness was assessed by using psychomotor vigilance performance (refs. 4, 9, and 56; *SI Appendix*, *SI Text*) on a 3-min test (PVT-B) that was obtained weekly (once in the morning, once in the evening) by computer (4), with 100% complete data acquisition ($n = 888$ tests). Facial videos were recorded at 30 frames per second during each PVT-B and evaluated for slow eyelid closures indicative of sleepiness (57). Immediately before or after each PVT-B test, crewmembers completed computerized scales that included 100-mm visual analog scales with the following binary anchors: good/poor sleep quality (morning only), high/low workload (evening only), and high/low tiredness (evening only). Data acquisition for these subjective ratings was 100% ($n = 444$).

Mixed-model ANOVAs (Proc Mixed; Version 9.3; SAS Institute) with a random intercept for crewmembers and unstructured covariance were performed with 130-d MQs as the explanatory variable. If a type 3 test indicated a significant MQ effect ($P < 0.05$), two-sided post hoc *t* tests comparing individual MQs were performed. Fig. 1 and *SI Appendix*, Figs. S3–S5 graphically present these analyses. Significant findings of post hoc tests are indicated ($*P < 0.05$, $**P < 0.01$, $**P < 0.001$, $****P < 0.0001$). All statistical tests were two-tailed.

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Supporting Information Appendix

Mars 520-d mission simulation reveals protracted crew hypokinesis and alterations of sleep duration and timing

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Materials, Methods, Results
Figs. S1 to S7
Tables S1 to S7

ACCESS TO DATA

The data reported in this paper are deposited in a Microsoft Excel file. To access the data: i) Go to the http://www.med.upenn.edu/uep/user_documents/PNAS_Basner_et_al.xlsx. ii) When the “Save File” window appears, choose a destination location to save the file.

MATERIAL AND METHODS

Mars 520-day simulation.

The State Scientific Center of the Russian Federation – Institute for Biomedical Problems (IBMP) of the Russian Academy of Sciences (RAS) performed the Mars 500 project, which consisted of three isolation studies with six crewmembers each: a 14-day pilot study (completed in November 2007), a 105-day pilot study (completed in July 2009), and the main 520-day study simulating a mission to Mars (completed in November 2011). The high fidelity of the simulation to actual spaceflight was reflected in the following features of the experiment: (i) a multinational crew of N=6 healthy adult male volunteers selected by the Russian Federation (N=3), the European Space Agency (N=2), and the China National Space Administration (N=1), who were trained together and who were similar in age, careers, and education (e.g., engineers, physicians, military backgrounds) to astronauts/cosmonauts living on the International Space Station (ISS); (ii) 520 consecutive days of confinement (3 June 2010 to 4 November 2011) in a pressurized facility with a volume and configuration comparable to a spacecraft with interconnected habitable modules; (iii) facility modules equipped with life support systems and an artificial atmospheric environment at normal barometric pressure; (iv) activities that simulate aspects of the International Space Station with daily maintenance work, scientific experiments, and exercise; (v) isolation from Earth's daily environmental light-dark cycles, temperatures and seasonal conditions; (vi) a realistic Mars flight simulation based in orbital mechanics and under the direction of mission controllers; (vii) work throughout the 520-day mission included both routine and simulated emergency events; (viii) changes in communication modes and time delays that would occur in transit to and from Mars; (ix) limited consumable resources (food and water); and (x) the crew awareness of frequent publicity of the mission by media and the public. The crew lived on a 5-day work cycle, with two days off, except for simulation of special situations (e.g., emergencies). Fig. S1 displays the physical features of the mission facility.

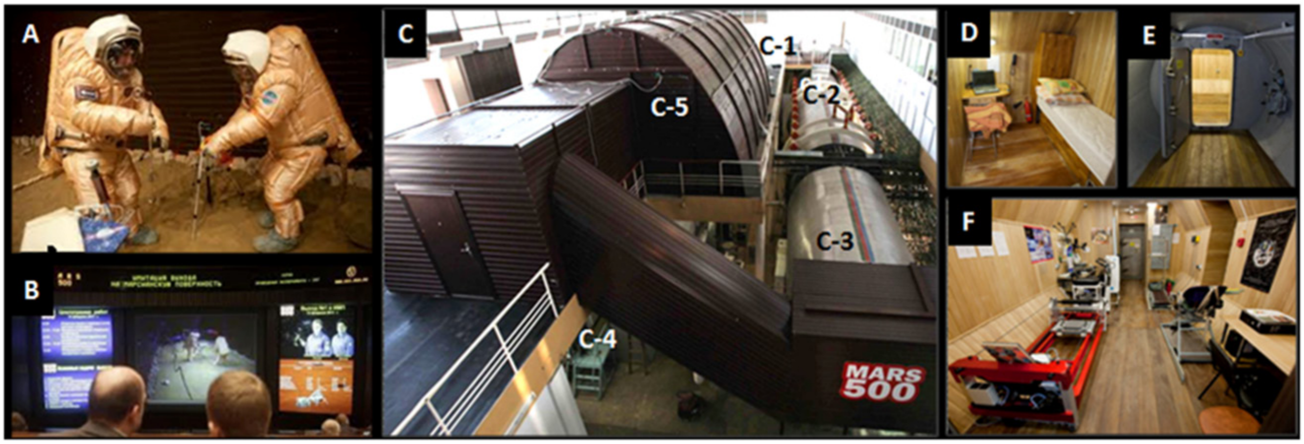

Fig. S1. The MARS 500 simulated spaceflight facility was developed by the Russian Federation – Institute for Biomedical Problems of the Russian Academy of Sciences (RAS) under the aegis of Roscosmos and RAS. The isolation facility was located on the IBMP site in Moscow in a building also containing the mission control operations room and technical facilities. The spaceflight habitat was a 550m^3 isolation facility composed of four hermetically-sealed, interconnected habitat modules and one external module, used to simulate the Martian surface (http://mars500.imbp.ru/en/index_e.html). The facility and mission control center outside the facility were designed to provide experimental data on crew health and working capacity while the crew lived in a confined environment during simulation of the main operational, environmental and behavioral features of a 520-day roundtrip mission to Mars. Midway through the 520-day mission, at the simulated arrival in Mars orbit (i.e., a 30-day Mars orbiting phase), three crewmembers landed on the Mars surface **(A)**, while the remaining three crewmembers in the MARS 500 chamber, as well as mission control **(B)**, observed and directed their activities. The exterior of the IBMP MARS 500 facility used for the simulated mission is shown in **(C)**. C-1 was the location of a medical module that housed a habitable compartment, areas for working with medical equipment, kitchen-dining room, and lavatory. C-2 was a habitable module consisting of six crew compartments for sleep and privacy **(D)**, community room, main console, kitchen, and lavatory. Module C-3 served as the simulator for the Mars landing. Module C-4 underneath the Mars surface housed an exercise facility **(F)**, a greenhouse for plants, storage for resources, refrigerator, thermal chamber, and a lavatory. The four habitable modules were interconnected by corridors **(E)**. The 50 m^3 Mars landing simulator module (C-3) was used only during the 30-day Mars orbiting phase (mission days 244-273). It accommodated three crewmembers and was equipped with a video control and communications system, gas analysis system, air-conditioning and ventilation systems, sewage system and water supply, fire alarm and suppression system, and transfer tunnels connected to the habitable module and into the chamber of the Martian surface simulator. The external Martian surface simulation module (C-5) was a 1200 m^3 unsealed chamber designed for crew exploration in space suits. It had storage for space suits, sealed stairs and a caisson separating it from the Mars landing simulator module. Photos **(A)** and **(C)** courtesy of European Space Agency (www.esa.int/specials/Mars500/). Photos **(B), (D), (E),** and **(F)** courtesy of IBMP (http://mars500.imbp.ru/en/gallery/html).

Table S1. Description of crewmembers by age at the start of the mission.

National self-identity	Mission role	Gender	Age	Professional experience
Russian	Crew Commander	Male	38	Naval engineer; prior space analog experience; trained cosmonauts on extra-vehicular activity (EVA) in conditions of simulated weightlessness
Russian / Tadzhikistan	Physician	Male	37	Military physician surgeon
Russian	Researcher	Male	32	Military physician and physiologist; research in aviation, space medicine, and military ergonomics
French	Flight Engineer	Male	31	Engineer
Italian / Colombian	Researcher	Male	27	Engineer; prior space analog experience
Chinese	Researcher	Male	27	Physician; Chinese astronaut trainer

Mission crew.

All crewmembers participating in the Mars 520-day simulation signed informed consents approved by the Institutional Review Board of the University of Pennsylvania, Philadelphia, Pennsylvania, USA. They were compensated for their participation in the study, and they were free to discontinue the study at any time. The volunteer crewmembers revealed their identities before the mission. To ensure the confidentiality of the crew relative to the data acquired and reported in this manuscript, results were de-identified and crewmembers were randomly assigned alphabetic letters (*a-f*). To further ensure crew confidentiality relative to the results, no data were reported relative to crewmembers' nationalities, ages, professions, or roles in the mission. The summary descriptions in Table S1 of each of the N=6 crewmembers show nationality; official role in mission; gender; age at time of mission initiation; and professional background. The information was derived from a publicly available IBMP document (http://mars500.imbp.ru/en/520_crew.html).

Table S2 lists some of the key milestones in the timeline of the 520-day simulation, which included the simulation of communication delays ranging from 8 s to 736 s between mission days 54 and 470, a Mars landing, and extra-vehicular activities (EVAs) on a simulated Mars surface between days 257 and 265. A total of 91 experiments were conducted throughout the mission, which included experiments in the areas of physiology (N=20), psychology (N=21), biochemistry, immunology, and biology (N=34), microbiology (N=8), and operations and technology (N=8). Not all of the experiments required the crew's active participation. Sampling frequency ranged between experiments from three times during the mission to continuously throughout the mission, with most of the studies sampling data on a regular but discontinuous basis (e.g., once every 30 days). The majority of the projects were of Russian origin, while 14% were supported by the European Space Agency (ESA), and 16% by other individual countries.

Experimental procedures and measurements.

Actigraphy. Wrist actigraphy is a reliable, non-invasive method to validly assess rest-activity cycles (1). Throughout the 520-day simulated mission to Mars, each crewmember continuously wore a watch-like, wristwatch size actigraph (Actiwatch Spectrum, Philips/Respironics) on the wrist of the non-dominant arm. The device measured both average white light intensity (illuminance in lux) and a calibrated activity level from movement-induced accelerations of the wrist. It also displayed clock time. Actigraphs were exchanged twice for each crewmember throughout the mission before the batteries in the currently used model dissipated. Each actigraph contained a piezo-electric sensor that generated voltage when the device underwent a change in acceleration. The voltage generated by the sensor was amplified and filtered by analog circuitry. This filtered and amplified voltage was then passed into an analog to digital (A/D) converter within a microprocessor to create a digital value. This A/D conversion and the following operations were repeated 32 times per second (32 Hz) or every 31.25 ms, the digital value is used to adjust a running baseline value. This makes it possible for the actigraph to effectively filter out constant accelerations, such as gravity. The current digital value is compared to the baseline value. The maximum deviation from baseline within 32 samples (1 s) is the activity value for that second. Hence the actigraph determines the peak or maximum acceleration change that occurred in each second. In the Mars 520-day study, actigraphs were set to record one activity value per minute (i.e., the resulting peak activity values from 60 consecutive seconds were added to an accumulated activity count). To minimize inter-sensor

Table S2. Timeline of the Mars 520-day study.*

Date	DiM[#]	Event
06/03/2010	1	Hatch closed, lift off
06/15/2010	13	Undocking from orbital assembly laboratory
06/23/2010	21	Transfer to heliocentric orbit towards Mars
07/26/2010	54	Start of communications delay
12/24/2010	205	Shifting to spiral orbit towards Mars
02/01/2011	244	Entering circular orbit around Mars, Mars Lander hatch opening
02/08/2011	251	Completion of loading, Lander hatch closure
02/12/2011	255	Undocking, landing on Mars
02/14 to 02/22/2011	257-265	Egresses on Martian surface
02/23/2011	266	Ascent, beginning of quarantine
02/24/2011	267	Docking with interplanetary craft
02/26/2011	269	End of quarantine
02/27/2011	270	Habitation module hatch opening, Crew transfer to Habitation module
03/01/2011	272	Hatch closure, Lander undocking
03/02/2011	273	Entering into spiral orbit away from Mars
04/07/2011	309	Transfer to heliocentric orbit towards Earth
04/25/2011	327	Start of 1-week crew autonomy drill
05/19/2011	351	Maximum communication delay of 12 min 16 sec
09/15/2011	470	End of communications delay, switchover to voice communications
10/13/2011	498	Shifting to spiral orbit towards Earth
11/04/2011	520	End of 520-day study, crew landing on Earth

*Source: http://www.esa.int/SPECIALS/Mars500/SEMGX9U889G_0.html); [#]days in mission

variation, each actigraph underwent an activity calibration procedure to normalize data compared between watches. This calibration procedure was carried out by the manufacturer and resulted in a calibration constant that was programmed into the actigraph device. This calibration constant was applied to the raw values to generate calibrated activity data.

Both mission control and the crew adhered to daylight savings times. In Moscow, time was delayed by 1 hour on the last Sunday of October 2010 at 03:00 local time and advanced by 1 hour on the last Sunday in March 2011 at 02:00 local time. The Russians changed their daylight savings rules and remained with summer time in 2011 (i.e., the time was not delayed in October 2011). Laptops provided by the University of Pennsylvania automatically applied the daylight savings time change at the time of the next data download. Therefore, some of the files required manual correction. We added or deleted 1 hour of data not at the time the clocks were advanced or delayed (i.e., 2 am or 3 am local time), but after subjects woke up in the morning on the same day. As there was no Internet connection inside the chamber, the laptops did not reflect the change in the new daylight savings policy adopted in Russia in October 2011 (i.e., time was automatically delayed by one hour). This was also corrected manually.

Table S3. Typical workday during the Mars 520-day simulation.*

Time Period	Activity
8:00-9:30	Personal hygiene, breakfast
9:30-10:00	Operative meeting
10:00-11:30	Facility inspection, familiarization with and preparation of scientific experiments
11:30-13:30	Operative work
13:30-14:30	Lunch
14:30-19:30	Implementation of scientific experiments and physical training
19:30-23:30	Supper and personal time
23:30-8:00	Sleep period

*Source: http://mars500.imbp.ru/en/520_one_year.html

Actigraphy scoring of active wake, rest, sleep. Actigraphy 1-min epochs were automatically classified into active wake, sleep, or waking rest based on a proprietary algorithm (Respironics Actiware, Version 5.59.0015, standard settings). Results of the algorithm scoring were visually examined. In rare cases in which routine visual inspection of data revealed obvious misclassification of state by the automated actigraph algorithm (e.g., sleep scored during active wakefulness), the automatic scoring was corrected. Less than 2.8% (range across subjects = 0.8%-5.2%) of the automatic scoring required correction in this manner. Epochs with off-wrist or missing data (due to data downloads or equipment failure) were classified accordingly. The six crewmembers started wearing the actiwatches on average at 13:46 (range 10:28-14:46) on the first mission day and stopped wearing them on average at 18:02 (range 17:57-18:08) on the last mission day. Mission days 1 and 520 were therefore excluded from actigraphy analyses because these were less than full days and the total time recorded on these two days varied among crewmembers. We also had to delete one hour of valid actigraphy data per crewmember to adjust for daylight savings times (see above). We thus expected 4,485,336 minutes (74,755.6 hours) of actigraphy data for all crewmembers while they lived in the facility (Fig. S1) throughout the 520-day study. We collected a total of 4,396,333 minutes (73,272.2 hours) of valid actigraphy, totaling 98.02% of the expected actigraphy data (i.e., 1.98% of the expected actiwatch data were off-wrist or missing, with a range of 0.5% to 6.0% across the six crewmembers). Analyses of the final actigraphy data set attributed 63.8% of the recorded data to active wakefulness, 31.0% to sleep, and 5.3% to waking rest. Actigraphy counts were also evaluated for the intensity of activity for each 24 h period during the mission and in each of the three states. For the descriptive and statistical analyses presented in Figures 1-3 and Figures S2-S5, off-wrist or missing actigraphy epochs were imputed with averages of non-missing epochs calculated for each crewmember, each mission quarter, and each of the 1440 minutes of the day.

Validation study of the Actiwatch algorithm for state classification. To validate the accuracy of the Actiware classification algorithm for active wake, rest and sleep we used an experiment involving N=22 healthy adults (mean age 35.1 ± 9.0 y [SD], which is not significantly different from the 520-day mission crew mean age 32.0 ± 4.7 y), who were monitored actigraphically while living in an environmentally isolated laboratory for 15 days each (total of 330 days). The laboratory confinement mimicked the mission confinement of the crew. We used a scheduled 16:8 wake:sleep ratio each day, which approximates the daily schedule of the 520-day mission crew (Table S3). Objective documentation (i.e., validation criteria) of wakefulness was accomplished by continuous behavioral monitoring of subjects, while physiological sleep and wakefulness were verified by polysomnography (PSG) during scheduled daily 8-hour sleep periods. The N=22 subjects had 115 days in the laboratory during which both behavioral monitoring and PSG were available for validation of the Actiwatch algorithm (i.e., permitting a 24-h validation). Actigraphy data were scored blind to validating criteria using the same algorithm scoring procedures used in the 520-day mission simulation (i.e., 1-min epochs from noon to noon each day).

The validation results confirmed the utility of the Actiwatch and its activity scoring algorithm. When wakefulness was objectively known to be present (by behavioral observation and/or PSG), the Actiwatch algorithm correctly classified wakefulness 96.2% of the time (i.e., 94.8% active wakefulness and 1.4% as waking rest). Similarly, when sleep was objectively verified to be present by PSG, the Actiwatch algorithm correctly classified sleep 97.0% of the time. The Actiwatch algorithm 3.0% misclassification error was divided between waking rest (1.4%) and active wakefulness (1.6%). Therefore the Actiwatch algorithm detection sensitivity (for sleep) was 97.0%, while specificity (for wakefulness) was 96.2%, and overall accuracy of the Actiwatch algorithm was 96.4%.

When validation analyses were confined to only 8-h time-in-bed periods for sleep, and PSG was used as the validation criterion, the algorithm had 97.0% sensitivity, 46.4% specificity, and 89.9% accuracy. The reduced specificity for sleep was due primarily to the algorithm overestimating sleep when subjects were resting but awake, including lying in bed awake before sleep onset or after awakening spontaneously from sleep before termination of the sleep period. Actiwatch sleep time overestimated PSG sleep time in the validation study by 26.4 min per sleep period (95% CI 18.0-34.8 min; $P < 0.0001$). Actigraphic overestimation of sleep time has been reported in another validation study of actigraphically-scored sleep in healthy adults (2).

Approximately half of the small classification error rate of the Actiwatch algorithm involved a misclassification between waking rest and sleep (Fig. S2), both of which are sedentary states with parallel profiles across the 520-day mission (Fig. 1). That is, increases in both waking rest and sleep time (as sedentary states) occurred within time in mission until the final month of the mission. The algorithm scoring error between these two sedentary states would be secondary (constant) error variance across the mission. Consequently, the validation study supports the acceptably high accuracy of the Actiwatch algorithm classifications of wake and sleep in confined healthy adults, and supports the validity of its use in the 520-day mission crew.

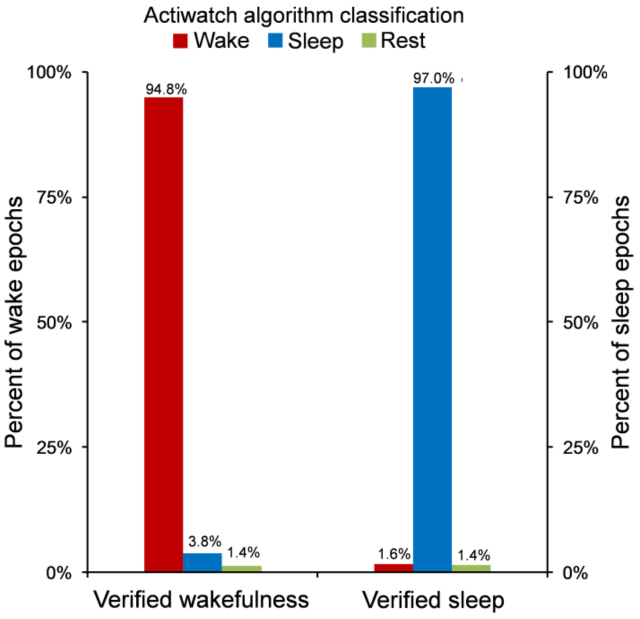

Fig. S2. Accuracy of the Actiwatch sleep-wake algorithm classification used in the 520-day simulated Mars mission was validated against continuous behavioral monitoring and polysomnography (PSG) during 24-h (noon to noon) periods in N=22 healthy adults. These subjects lived on a daily 16:8 h (wake:sleep) ratio, in a confined and environmentally isolated laboratory. They had a total of 115 days (165,600 min) during which both behavioral monitoring and PSG were available for validation of the Actiwatch algorithm state score. In a double-blind analysis of the validation data, the Actiwatch algorithm correctly identified wakefulness in 96.2% of epochs in which wakefulness (active or rest) was objectively verified. It correctly identified sleep in 97.0% of epochs in which sleep was objectively verified by PSG. Thus it misclassified only 3.0% of sleep as wake, and 3.8% of wake as sleep.

We do not consider this Actiwatch algorithm classification error rate to pose a significant confound in the data from the 520-day simulation, because it affects only a small portion of the data, and because it is primarily a misclassification between waking rest and sleep, both of which are sedentary states with parallel profiles across the 520-day mission (Fig. 1). That is, increases in both waking rest and sleep time (as sedentary states) occurred within time in mission until the final month of the mission. There is no evidence for or reason to expect that the Actiwatch misclassification error changed across time in mission (i.e., it is secondary error variance). Consequently, the validation study supports the accuracy of the Actiwatch algorithm classifications in the 520-day mission crew.

Spectrographic analyses of actigraphy data. These analyses were performed on 1-min epochs to determine the predominant periodicity of sleep-wake timing for each of the subjects. The period was estimated based on the power spectral density of each subject's actigraphy time series data. Sleep-wake time series data were assigned a value of one for all epochs marked as sleep and zero for all other times (i.e., active wake and wake rest). The power spectrum of the sleep-wake time series was estimated for each subject using the periodogram method (3) of multiplying the data with a 90-day rectangular window and taking the squared magnitude of the discrete time Fourier transform. The peak frequency was estimated by using a three-point quadratic interpolation based on the log-magnitudes of the periodogram at the frequency corresponding to the maxima in the periodogram and the two neighboring points.

Psychomotor Vigilance Test (PVT-B). The PVT measures sustained or vigilant attention by recording response times (RT) to visual stimuli that occur at random inter-stimulus intervals (ISI). Changes in vigilant attention as measured by the PVT are among the most sensitive indices of shifts in behavioral alertness (4), but this does not reflect all aspects of cognitive performance sensitive to sleep loss (5). The PVT has a number of advantages over other performance measures in that it has negligible aptitude and learning effects (4, 6). Acute total sleep deprivation, chronic sleep restriction, and time on task induce reliable changes in PVT performance, causing an overall slowing of response times, a steady increase in the number of errors of omission (i.e., lapses of attention), and an increase in errors of commission (i.e., responses without a stimulus, or premature responses). To increase the Mars mission crew acceptance and adherence to completing the PVT, we used a briefer, modified 3-minute version of the PVT (i.e., PVT-B), which was recently validated against the standard 10-minute PVT (7) and shown to predict performance on a simulated luggage screening task (8). Once per week, each crewmember performed two PVT-B performance tests (once in the morning after waking up and once in the evening) to assess the effects of potential changes in sleep-wake behavior. Each crewmember performed the PVT on a different day of the week. The tests were conducted using a calibrated laptop computer (Pulsar Informatics, Inc.). Subjects were instructed to monitor a red rectangular box on the computer screen, and press the space bar as soon as a yellow stimulus counter appeared, which stopped the counter and displayed the RT in milliseconds for a 1-s period. ISIs varied randomly from 2 to 5 s. Subjects were instructed to press the button as soon as each stimulus appeared, in order to keep the RT as low as possible, but not to press the button prematurely (which yielded a false start warning on the display). Performance outcome measures extracted from the task included mean response speed (i.e., reciprocal reaction time, 1/RT), the number of errors of omission (i.e., lapses of attention defined as $RT \geq 355$ ms threshold), the number of errors of commission (i.e., false starts defined as $RT < 130$ ms threshold), and the number of total errors (i.e., the sum of errors of omission and commission) (9). Subjects were provided with feedback on their performance after each test bout. Data acquisition for the PVT-B resulted in N=888 completed tests, which was 100% of the expected data (i.e., six crewmembers assessed two times [a.m. and p.m.] once in each of 74 mission weeks).

Subjective ratings. Immediately prior to and/or following each PVT-B test bout, the crewmembers filled out several computerized questionnaires and rating scales (all instructions and questionnaires were translated into Russian for the Russian crewmembers). Crewmembers indicated their current status on 100-mm visual analogue scales with the following binary anchors: good sleep quality, poor sleep quality (morning only); high workload, low workload (evening only); and high tiredness, low tiredness (evening only). Immediately after each PVT-B test bout, crewmembers indicated whether or not it was difficult to perform the PVT, and if so, for what reasons. Data acquisition for subjective ratings resulted in 100% completed tests (i.e., N = 444) for workload ratings and sleep quality ratings.

Video of the face. Facial videos were recorded at 30 frames per second from crewmembers during each 3-min PVT-B test using the integrated laptop camera. These videos were evaluated by human raters for slow eyelid closures (PERCLOS) indicative of sleepiness (10). Data acquisition for videos of the face resulted in 100% complete data.

Quality control of data acquisition. PVT-B and subjective rating data were time stamped, encrypted and saved on the hard drive of each crewmember's data acquisition computer. Weekly, following the evening PVT-B performance, each crewmember downloaded the actigraphy data of the past week together with the PVT-B performance file, subjective responses, and video data from their computers to an SD memory card. This download also included data from these measures for all previous weeks. The SD cards were jettisoned from the Mars 500 facility weekly. The cards were retrieved and the data downloaded to a secure eRoom and immediately checked by the programmed algorithms (Pulsar Informatics, Inc.) and by investigators at the University of Pennsylvania for data completeness and integrity.

Data Analyses.

Statistical analyses. Mixed model ANOVAs (Proc Mixed, SAS Institute, Version 9.3) with a random intercept for crewmembers and unstructured covariance were performed with 130-day mission quarters (MQ) as the only explanatory variable (MQ1, days 1-130; MQ2, days 131-260; MQ3, days 261-390; MQ4, days 391-520). Analyses by mission quarter are a conventional way in which changes during long-duration missions are evaluated. PVT-B lapses and false starts were transformed with a square root transform prior to analysis to better reflect a normal distribution. If a type 3 test indicated a significant MQ effect ($P<0.05$), post-hoc t-tests comparing individual mission quarters were performed. For variables that were sampled twice daily (e.g., PVT-B outcomes), the models were also controlled for administration time (morning or evening). Figures 1, S3, S4 and S5 graphically present the findings of these analyses. Significant ($P < 0.05$) post-hoc tests are indicated with asterisks (*$P < 0.05$, **$P < 0.01$, **$P < 0.001$, ****$P < 0.0001$). For figure S3, the day was divided into nocturnal (22:00 - 08:59) and diurnal (09:00 - 21:59) periods. All statistical tests were two-tailed.

For Figures 1A and 1C, we averaged the outcome variable across subjects for each mission day before plotting them. The red trend lines represent 4^{th} order polynomials. We displayed the 4^{th}-order polynomial fit to the data because there was no substantial increase in adjusted R^2 values (calculated with Proc REG in SAS) for higher-order polynomials.

To create the bar graph shown in Fig. S7C, we compared actigraphy measures across subjects on a minute per minute basis. One minute epochs that were classified as missing or off-wrist for at least one crewmember were excluded from the analysis for all crewmembers (86,068 minutes or 11.5% of the 520-day period had at least 1 minute of missing data in one of the crewmembers). For each crewmember, those minutes were counted where the crewmember was either the only crewmember sleeping (black bars in Fig. S7C) or the only crewmember awake (white bars in Fig. S7C). The ordinate in Fig. S7C shows cumulative time for both categories corrected for the amount of missing data. The percentage value indicated above each bar was also corrected for missing data and is therefore relative to the full 520-day mission.

Results

Diurnal and nocturnal sleep-wake states. Figure S3 presents analyses by mission quarter of the time the crew averaged in each active wakefulness, sleep and rest during nocturnal (22:00-08:59) and diurnal (09:00-21:59) segments of the day, as well as light exposure in discrete behavioral states. Nocturnal (Fig. S3A) and diurnal (Fig. S3B) graphs for wake, sleep and rest show profiles similar to those for 24-h periods (Fig. 1B-D and 1F-H).

Light exposure during diurnal and nocturnal periods. Light intensity as measured by the wrist actiwatch declined across mission quarters during the diurnal (09:00-21:59) segment of the day (F test, $P < 0.0001$) in a manner identical to the results (post-hoc t-tests) for active wakefulness depicted in Fig. S3C. Light intensity during the nocturnal (22:00-08:59) segment showed a nearly identical profile across mission quarters (F test, $P < 0.0001$, two-tailed). The maximum light exposures to which crewmembers were exposed to at the wrist were as follows: 10% of mission wake time they were exposed to a light intensity of at least 177 lux; 1% of mission wake time they were exposed to a light intensity of at least 412 lux; 0.1% of mission wake time they were exposed to a light intensity of at least 756 lux; and 0.05% of mission wake time they were exposed to a light intensity of at least 981 lux. Thus, 90% of mission wake time crewmembers were exposed to light intensity below 177 lux. Moreover, the facility lighting had a spectral power distribution consistent with fluorescent lighting (Fig. S6) with low irradiance in the 446-477 nm wavelength region of the photon spectrum, which is the most potent region for synchronizing or phase shifting circadian rhythms of sleep and waking.

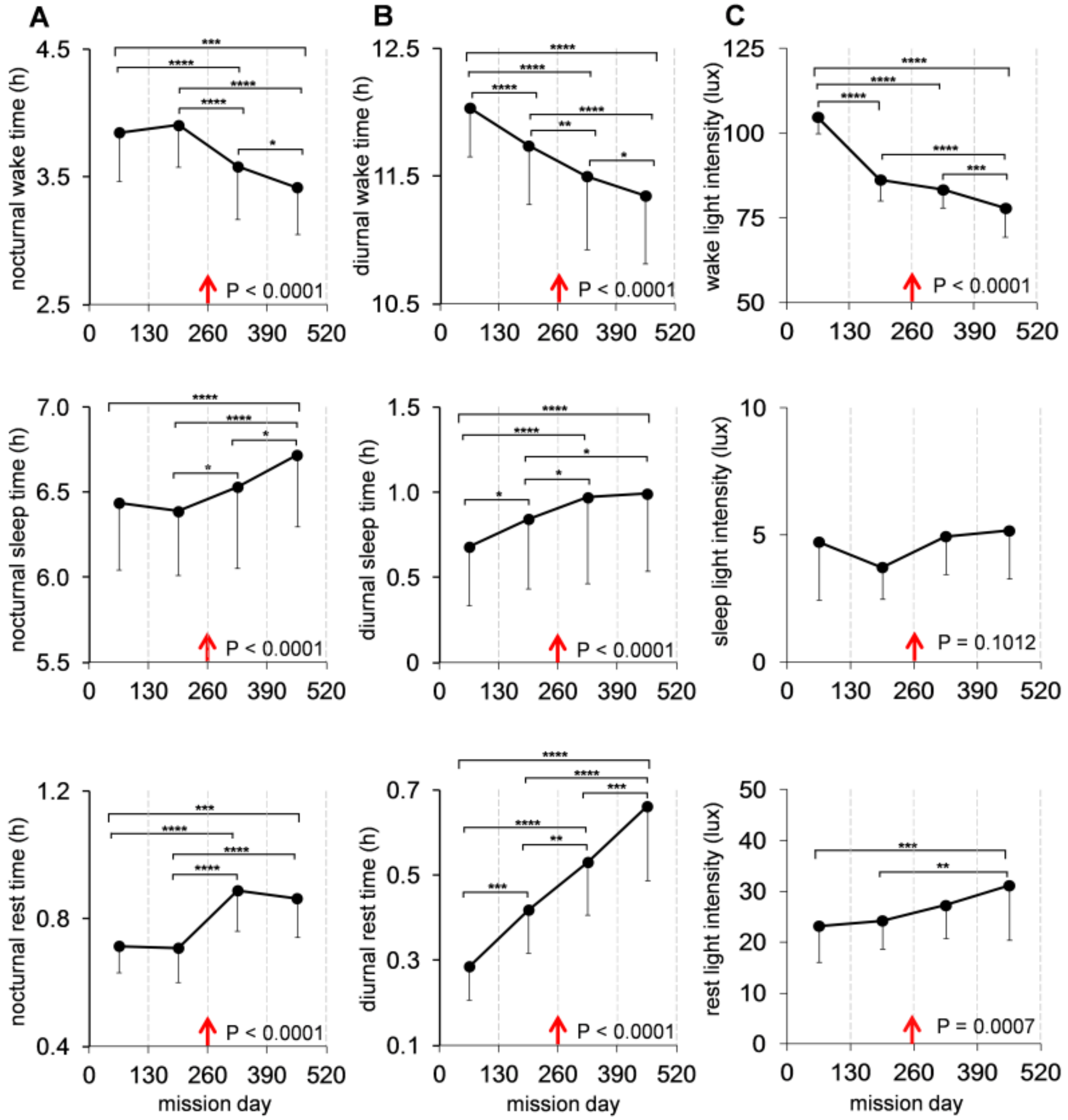

Fig. S3. Time (h) the crew averaged in each of three behavioral states during nocturnal (22:00-08:59) and diurnal (09:00-21:59) segments of the day, as well as light exposure in discrete behavioral states, as a function of mission quarter (mean, SE). The simulated mid-mission landing on Mars is indicated by a red arrow. **(A)** During the nocturnal period active wake time decreased, while sleep time and rest time both increased significantly across mission quarters (all F tests, $P < 0.0001$, post-hoc tests between quarters: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$). **(B)** During the diurnal period active wake time decreased, while sleep time and rest time both increased significantly across mission quarters (all F tests, $P < 0.0001$). **(C)** Light intensity (measured by actiwatch) during active wakefulness for the 24-h day decreased significantly across mission quarters (F test, $P < 0.0001$). Light intensity did not change during sleep across mission quarters, but it increased significantly in the final quarter during rest (F test, $P < 0.0007$).

A separate ESA-sponsored experiment that occurred late in the mission (days 439-499) involved scheduled exposure of crewmembers to blue light (daily from circa 08:00-19:00) and the use of red-tinted glasses. The results of the blue light experiment did not involve our assessments, and they will be reported separately by the ESA investigators who conducted that study. Agreements between the Russian IBMP (who developed and managed the 520-day simulation) and the other international partners supporting science in the simulated mission (e.g., ESA) precluded investigators from complete knowledge about each other's experiments. We do not know the extent to which crewmembers exposed themselves to blue light as scheduled by ESA investigators. To determine if the intended exposure to blue light affected our outcomes, mixed model analyses were used to compare the 60-day period of scheduled blue light exposure (days 439-499) to the 60-day period immediately preceding it (i.e., days 378-438). No statistically significant differences were found between the two 60-day periods in sleep or waking.

Additional information on crewmember's sleep-wake timing. Individual differences among crewmembers in the periodicity of their sleep-wake cycles across the mission (Fig. 3) were unrelated to their roles, responsibilities and perceived workload. We sought to determine if they were related to chronotype (i.e., preferred circadian phase for sleep). In particular, we sought evidence of sleep timing pre-mission to determine whether the near-25 h sleep-wake periodicity of crewmember *b* (Fig. 3B), and the biphasic sleep pattern of crewmember *a* (Fig. 3A), both of which developed during the mission, were consistent with an evening chronotype pre-mission (11). Logistical factors beyond our control prevented acquisition of endocrine or genotypic markers of crewmembers' chronotype prior to the mission.

Although subjective chronotype data (i.e., morningness-eveningness scale) were also unavailable, we acquired 2-3 days of behavioral (i.e., Actiwatch) data on crewmembers between 8 and 16 days before the mission. Table S4 displays these data and the average sleep onset times in each of the first 4 weeks of the mission for each crewmember. Crewmember *b* had a delay of sleep onset of 1 h and 10 min from the first (00:51 h:min) to the second (02:01) night in his pre-mission data, and he took daytime naps on both days. During the first week of mission confinement his sleep onset time averaged 2 h and 30 min—the latest among the crew. It continued to delay an average of 54 min each week during the first month of mission confinement, averaging a sleep onset time of 05:12 in the fourth mission week. These data are consistent with crewmember *b* having an evening chronotype, and a tendency to phase delay, which progressed systematically during the first mission month of environmental confinement (Fig. S7A). The pattern continued throughout the mission (Fig. 3B) and the final 30 days of the mission (Fig. S7B). It is noteworthy that among the crew, only crewmembers *b* and *a* averaged a much later sleep onset time (i.e., 3 h and 22 min, and 1 h and 59 min, respectively) during the first month of the mission than was evident pre-mission. The other four crewmembers had sleep onset times during the first mission month that were comparable to or earlier than their pre-mission sleep onset times (Table S4).

Additional confirmation of the evening chronotype of crewmember *b* was obtained from the post-mission debriefing. Although mission managers confirmed that the crew was selected based on extensive medical and psychological screenings to ensure he had no sleep disorders, in post-mission interviews, crewmember *b* indicated that he was "occasionally" prone to delayed sleep onset times. In response to questioning he indicated that he slept at times in the daytime to compensate for reduced sleep at night; and that he often "goes to bed very late", especially on holidays. He denied having a circadian rhythm disorder because he did not consider this pattern of sleeping to be unusual for him. We believe it is likely that the evening chronotype of crewmember

Table S4. Actigraphically derived sleep onsets of each crewmember on pre-mission days, and weekly average for each of the first 4 weeks of the 520-day mission confinement.

	Sleep onset times 8-16 days pre-mission*						**Sleep onset times during mission**			
Crew-member	-16 days	-15 days	-14 days	-10 days	-9 days	-8 days	week 1 mean	week 2 mean	week 3 mean	week 4 mean
a	23:00	22:49					00:42	00:11	01:24	01:16
b		0:51	2:01				02:30	02:59	04:23	05:12
c				1:31	2:36	1:57	00:31	00:31	01:22	01:18
d		0:14	22:24				00:01	23:47	00:13	00:07
e				0:07	0:05		01:46	01:19	01:53	01:21
f				1:15	1:43		01:24	00:57	00:46	01:09
Mean							01:14	00:33	01:43	01:49

*Logistical reasons prevented crewmembers from wearing Actiwatches more than 2-3 days pre-mission.

b, in combination with inadequate lighting in the 520-day facility for entrainment of his sleep-wake cycle to a 24-h periodicity, resulted in his continuing to phase delay and manifest a near 25-h sleep-wake periodicity during the mission. This phase-delay tendency of crewmember *b* reflects a vulnerability to inadequate entrainment that was likely present before the mission. The progressively biphasic sleep-wake pattern of crewmember *a* may also have reflected an entrainment problem.

Activity levels in the final 20 days of the mission. The crew was significantly more active during wakefulness in the final 20 days of the mission (days 500-520), relative to both the preceding 60-day blue-light exposure period (P = 0.0002), and relative to the 60 days before the blue light exposure period (P < 0.0001). The crew also spent more time awake and less time resting and sleeping relative to either of the two 60-day periods during or before the scheduled blue light (all P's < 0.002). While they increased their wake time and intensity of movement in the final 20 days (Fig. 1A and 1E), they did not demonstrate reliable changes in their sleep onset or offset times, or in their ratings of sleep quality during the final 20 days, relative to the two 60-day periods. Mission management indicated that the crew's increased waking activity in the final 20 days of the mission reflected no additional work, and the crew's own workload ratings did not increase during the 20 days. Instead, their increased activity in the final 20 days of the mission was associated with personal activities in anticipation of mission end and hatch opening.

Video evidence of sleepiness during PVT-B performance. Facial signs of sleepiness using PERCLOS were judged to be present by human scorers and OCR on a total of 51 of the 888 PVT-B 3-min performance tests. Crewmember *f* accounted for 76.4% (n=39) of the videos showing sleepiness during performance testing, while crewmember *e* accounted for 19.6% (n=10) of the videos. The remaining four crewmembers combined accounted for less than 4% (n=2) of the videos showing sleepiness.

Ratings of difficulty performing the PVT-B. Immediately after each PVT-B test bout, crewmembers indicated whether or not it was difficult to perform the task. Crewmember *f* indicated difficulties performing the PVT-B in 25.7% (38/148) of PVT-B test bouts. All other crewmembers indicated difficulty performing the PVT-B on average after only 1.6% (range 0.0% - 4.6%) of the test bouts.

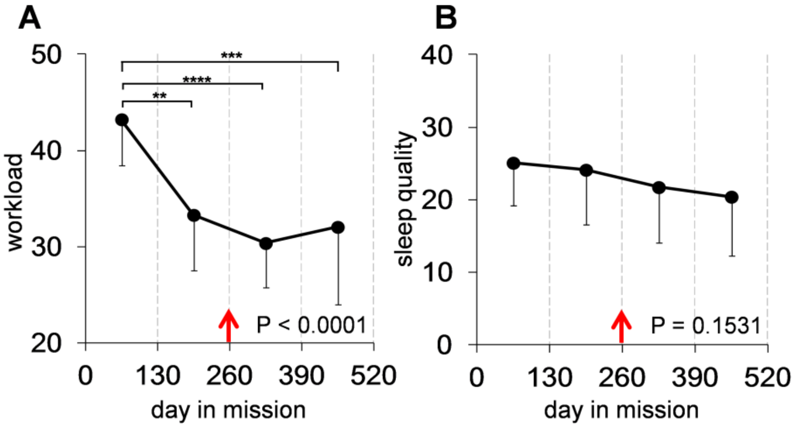

Fig. S4. Weekly crew workload and sleep quality visual analog ratings by mission quarter (mean, SE). The simulated mid-mission landing on Mars is indicated by a red arrow. **(A)** Workload ratings (0 = low workload, 100 = high workload) decreased after the first mission quarter (F test, P < 0.0001; post-hoc tests between quarters: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). **(B)** Sleep quality ratings (0 = good sleep, 100 = poor sleep) did not vary reliably across mission quarters.

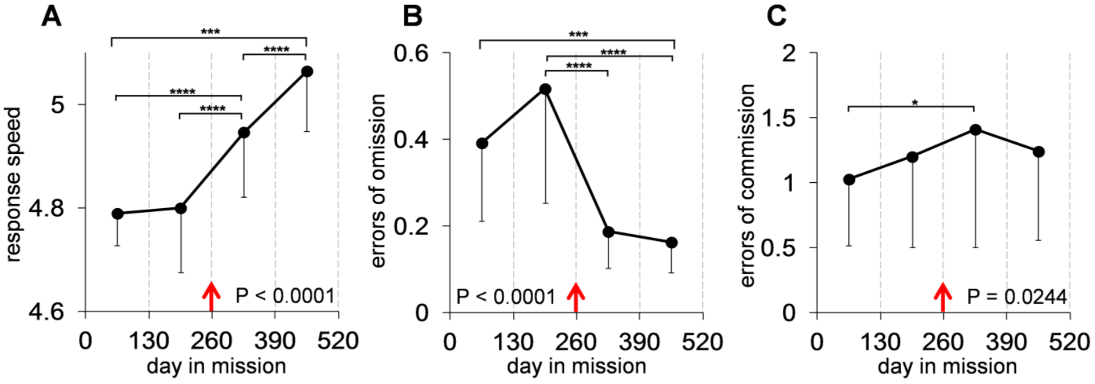

Fig. S5. Mean (SE) performance on the weekly Psychomotor Vigilance Test (PVT-B) by mission quarter. The simulated mid-mission landing on Mars is indicated by a red arrow. **(A)** PVT-B response speed (1/RT) increased (improved) across mission quarters, especially in the second half of the mission (F test, P < 0.0001; post-hoc tests between quarters: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). **(B)** The frequency of PVT-B errors of omission (i.e., lapses of attention) decreased (improved) across mission quarters, especially in the second half of the mission (F test, P < 0.0001, two-tailed; post-hoc tests between quarters same as in (A). **(C)** The frequency of PVT-B errors of commission (i.e., premature responses) increased (worsened) in the third quarter compared to the first quarter, largely due to crewmember *f*.

Table S5A. Mean (SE) total sleep time (h) per 24 h for each 130-day mission quarter (MQ)

Crew-member	MQ1	MQ2	MQ3	MQ4	Avg (MQ 1-4)
a	7.57 (0.13)	7.56 (0.14)	8.04 (0.15)	8.61 (0.18)	7.94 (0.08)
b	6.97 (0.18)	7.51 (0.2)	7.80 (0.19)	7.91 (0.19)	7.55 (0.10)
c	7.21 (0.08)	7.02 (0.12)	7.32 (0.12)	7.45 (0.10)	7.25 (0.05)
d	7.53 (0.09)	7.71 (0.08)	8.00 (0.06)	7.91 (0.08)	7.79 (0.04)
e	6.61 (0.09)	7.04 (0.09)	7.52 (0.10)	7.89 (0.13)	7.26 (0.06)
f	6.80 (0.08)	6.55 (0.09)	6.33 (0.09)	6.48 (0.09)	6.54 (0.04)
Avg (a-f)	7.12 (0.16)	7.23 (0.18)	7.50 (0.26)	7.71 (0.29)	

Table S5B. Cumulative total sleep time (h) for each 130-day mission quarter (MQ)

Crew-member	MQ1	MQ2	MQ3	MQ4	Sum (MQ 1-4)
a	984.2	983.0	1045.0	1119.0	4131.2
b	905.9	976.8	1014.2	1028.8	3925.7
c	937.5	912.1	951.7	968.4	3769.6
d	979.0	1001.8	1039.4	1028.1	4048.4
e	859.5	914.6	977.0	1025.6	3776.7
f	884.5	851.2	823.0	842.9	3401.6
Sum	5550.6	5639.5	5850.3	6012.8	
Avg (a-f) (SE)	925.4 (20.7)	939.9 (23.3)	975.1 (33.8)	1002.1 (37.4)	

Table S6A. Mean (SE) sleep quality* for each 130-day mission quarter (MQ)

Crew-member	MQ1	MQ2	MQ3	MQ4	Avg (MQ1-4)
a	20.5 (9.1)	9.4 (5.1)	5.8 (5.3)	0.6 (0.6)	9.2 (3.1)
b	14.2 (3.2)	13.2 (2.2)	12.2 (2.2)	9.4 (0.6)	12.3 (1.1)
c	17.9 (4.2)	23.9 (2.8)	24.2 (1.9)	22.8 (2.1)	22.2 (1.5)
d	12.6 (23.5)	5.0 (7.1)	2.1 (6.3)	1.7 (5.2)	5.4 (1.6)
e	37.2 (4.2)	45.3 (4.5)	35.0 (2.9)	46.3 (3.8)	41.1 (2.0)
f	48.3 (4.9)	47.9 (3.5)	51.1 (4.6)	41.7 (4.1)	47.3 (2.1)
Avg (a-f)	25.1 (5.9)	24.1 (7.6)	21.7 (7.7)	20.4 (8.2)	

*Visual analog scale ratings from 0 (good sleep) to 100 (poor sleep)

Table S6B. Cumulative sleep quality* for each 130-day mission quarter (MQ)

Crew-member	MQ1	MQ2	MQ3	MQ4	Sum (MQ1-4)
a	390	170	110	10	680
b	270	250	220	170	910
c	340	430	460	410	1640
d	240	90	40	30	400
e	670	860	630	880	3040
f	870	910	970	750	3500
Avg (a-f) (SE)	463 (103)	452 (145)	405 (145)	375 (152)	

*Visual analog scale ratings from 0 (good sleep) to 100 (poor sleep)

Table S7A. Mean (SE) total PVT-B error* rates per test for each 130-day mission quarter (MQ)

Crew-member	MQ1	MQ2	MQ3	MQ4	Avg (MQ1-4)
a	0.47 (0.10)	0.36 (0.09)	0.39 (0.10)	0.64 (0.13)	0.47 (0.05)
b	0.37 (0.10)	0.32 (0.09)	0.33 (0.10)	0.44 (0.09)	0.36 (0.05)
c	0.84 (0.12)	0.58 (0.15)	0.45 (0.10)	0.72 (0.15)	0.65 (0.07)
d	0.84 (0.14)	0.72 (0.12)	0.87 (0.14)	0.78 (0.14)	0.80 (0.07)
e	1.17 (0.18)	2.18 (0.28)	1.14 (0.19)	0.84 (0.12)	1.34 (0.11)
f	4.78 (0.35)	6.11 (0.35)	6.39 (0.25)	5.03 (0.35)	5.59 (0.17)
Avg (a-f)	1.41 (0.68)	1.71 (0.92)	1.60 (0.67)	1.41 (0.73)	

*Errors of omission (i.e., lapses) plus errors of commission.

Table S7B. Cumulative total PVT-B errors* for each 130-day mission quarter (MQ)

Crew-member	MQ1	MQ2	MQ3	MQ4	Sum (MQ1-4)
a	18	13	15	23	69
b	14	12	12	16	54
c	32	21	17	26	96
d	32	26	33	28	119
e	42	83	41	32	198
f	172	232	243	181	828
Avg (a-f) (SE)	51.7 (24.4)	64.5 (35.2)	60.2 (36.9)	51.0 (26.1)	

*Errors of omission (i.e., lapses) plus errors of commission.

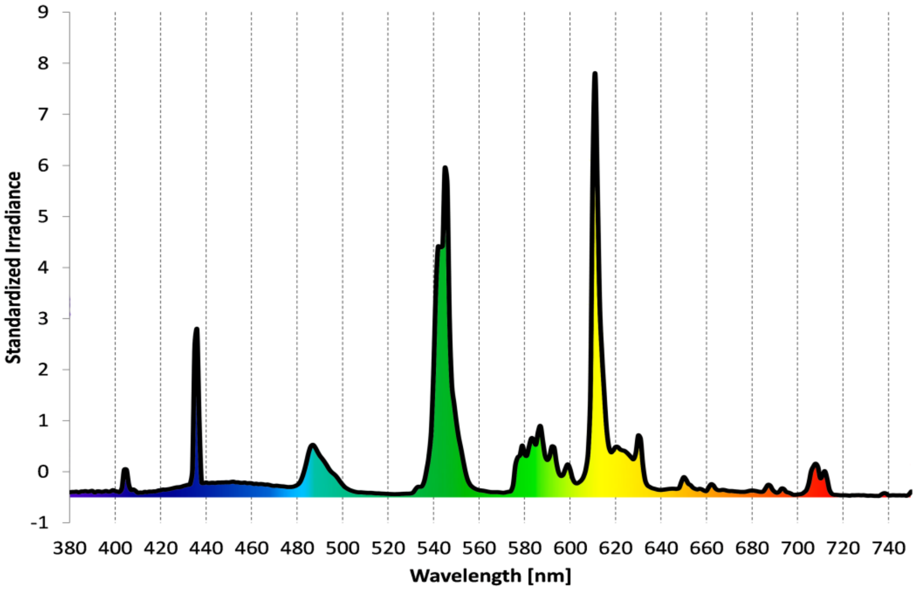

Fig. S6. Spectral power distribution of fluorescent lighting in the Mars mission crew facility. Identical distributions were found in all locations throughout the facility. Measurements were made with SpectraRad xpress-BWSpec 3.26 (Minolta).

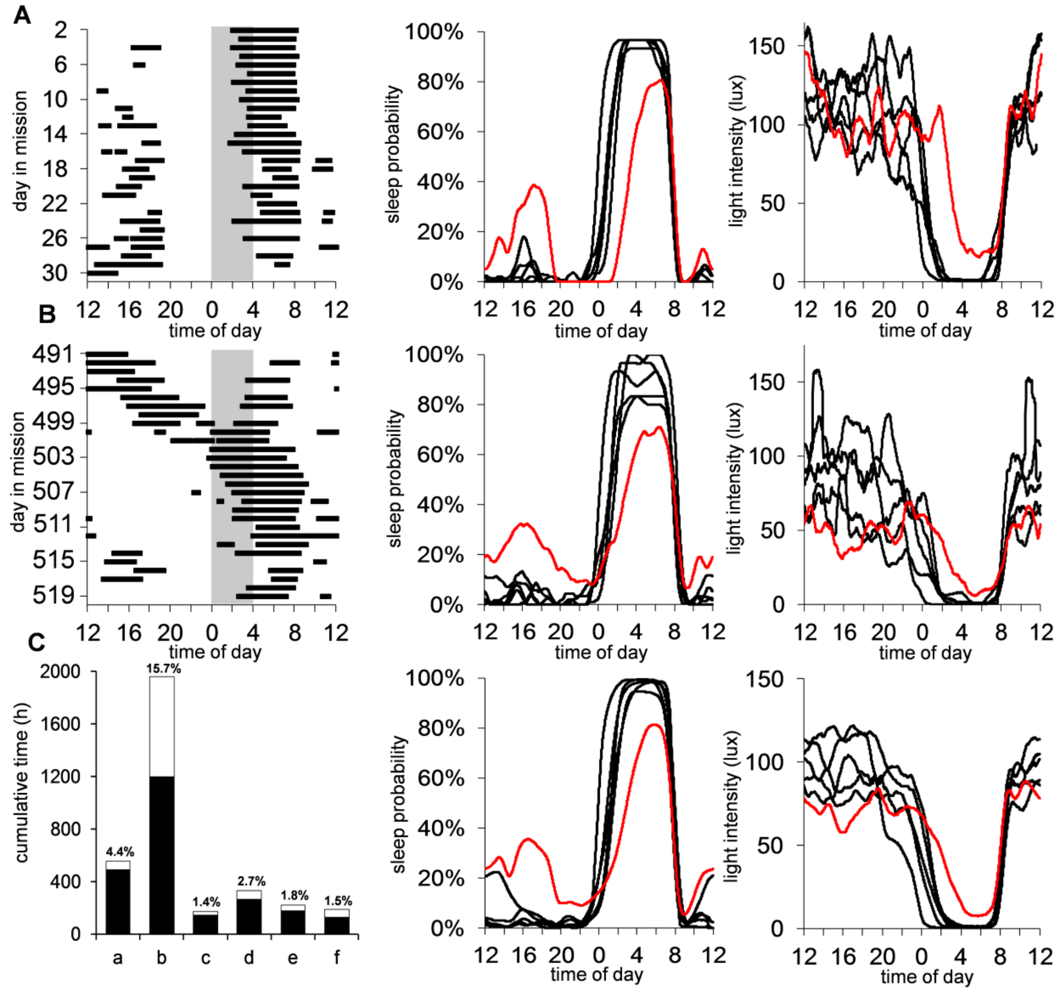

Fig. S7. Crewmember *b* sleep periods (black) and wake (active wake plus rest) periods (white), sleep probability and light exposure (measured by actiwatch) by time of day. **(A)** Sleep-wake timing of *b* during the first 30 days of the mission (gray bar for ease of viewing period from 00:00 to 04:00 when room light exposure would be predicted to induce phase delays). For the first 30 days, sleep probability and light exposure occur increasingly later into the night for crewmember *b* (red line) relative to other crewmembers (black lines). Nocturnal sleep onset for *b* drifted later that 04:00 after 16 days in mission. **(B)** Sleep-wake timing of *b* (red line) during the final 30 days of the mission. A near-25-h sleep-wake cycle that became dominant after the first mission quarter and was maintained thereafter is evident in the daily sleep-wake plot, as is at least one long sleep episode (~10 h on day 501). Sleep probability and light exposure by time of day remained different at key times relative to other crewmembers. **(C)** Plots of sleep probability and light exposure by time of day for the entire 520 mission reveal that crewmember *b* (red line) had a different profile from other crewmembers (e.g., sleeping more in the daytime). Bar graph shows the proportion of mission time that each crewmember was asleep when all other crewmembers were awake (black bars), or awake when all other crewmembers were asleep (white bars). The near-25-h sleep-wake cycle of crewmember *b* resulted in 15.7% of mission time (i.e., 1,959 h) when his sleep-wake activity was opposite to other crewmembers.

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