

Supplement 1

Supplementary Methods and Materials

Detailed Description of Polysomnography Methods

All digital records from polysomnography (PSG) and multiple sleep latency testing (MSLT) were manually scored by a single registered PSG technologist without knowledge of group assignment or clinical status. Regularly documented intra-scoring reliabilities for this single scorer exceeded 0.94. All records were reviewed by an American Board of Sleep Medicine specialist (DBR) to further ensure scoring accuracy, particularly in the determination of sleep onset and the quality and quantity of rapid eye movement (REM) sleep.

Nighttime Sleep Recordings

Sleep was recorded and analyzed using an Embla A10 device and compatible Somnologica Science software (Embla Systems; Broomfield, CO). PSG assessment included placement of electrodes for electroencephalography (EEG), electrooculography (EOG), and submental electromyography (EMG). A standard sleep montage of EEG (C3/A2-C4/A1) and (O2/C3-O1/C4), monopolar left and right EOG referenced to the opposite mastoid, and surface mentalis EMG was used. In addition, electrocardiography (ECG, modified lead II), respiratory airflow, snoring sounds, respiratory effort (rib cage and abdomen), and anterior tibialis surface EMG were recorded unilaterally. Sleep stages were manually scored by the same certified PSG technician following standard criteria (1). Nocturnal PSG sleep measures calculated included: sleep period time (minutes); total sleep time (TST, minutes); sleep efficiency ($SE = TST / \text{sleep period time} \times 100$); time spent (minutes) in Stages 1, 2, and 3/4 (referred to as slow wave sleep; SWS), REM sleep (minutes); and latency to the first epoch of REM sleep (REM latency, minutes). The number of spontaneous arousals (changes in EEG frequency lasting at least 3 seconds) was also measured according to American Academy of Sleep Medicine Guidelines (2). Subjects were connected to the PSG recorder at approximately 8:30 pm each evening of

sleep, and upon awakening following the first night of sleep, subjects underwent MSLT (see below). All recording was made with the Embla A10 System scored using Somnologica Science Software.

Sleep Period Time – total number of minutes from sleep onset (defined as the first minute of Stage 2 or REM sleep followed by at least 8 minutes of sleep in the next 9 minutes) until final awakening.

Total Sleep Time – total number of minutes in any stage of sleep, i.e. Stages 1, 2, and 3/4 or REM sleep, during the major nocturnal sleep period, as measured by PSG.

Wake After Sleep Onset – amount of wake time during the sleep period time characterized by relatively low voltage, mixed frequency EEG activity as measured by PSG and scored according to standard criteria (1).

Spontaneous Arousals - a change in EEG frequency lasting at least 3 seconds preceded by at least 10 seconds of normal sleep during the total sleep time (2).

Total Sleep Efficiency – total sleep time/sleep period time as measured by PSG. Nocturnal sleep efficiency is associated with sleep continuity and is a marker of sleep quality.

Non REM Sleep (NREM) Stages 1 and 2 – the total sleep time that is either Stage 1 or 2 sleep as measured by PSG and scored according to standard criteria (1).

NREM Stages 3/4 Sleep (SWS) - the total sleep time that is Stages 3/4 sleep collectively, as measured by PSG and scored according standard criteria (1). SWS is a marker of sleep quality.

REM Sleep - the total sleep time characterized by rapid eye movements, muscle atonia, and desynchronized EEG activity as measured by PSG and scored according to standard criteria (1).

REM Latency - the time between the first epoch of any stage of sleep and the first epoch of REM (defined by not less than 3 consecutive minutes of REM sleep) with intervening wake time excluded.

Multiple Sleep Latency Test

The morning following nocturnal PSG, approximately 2 hours after each patient's wake-up time, daytime sleepiness/alertness testing was initiated with the MSLT following the standard procedures outlined by Carskadon (3). Four nap opportunities were provided for all patients. Subjects were asked to rest quietly in their rooms for 20 minutes with lights out. Sleep records were visually scored, and if subjects entered sleep, they were allowed to sleep a total of 15 minutes before being awakened. The sleep latency on any given nap on the MSLT was defined as the time from lights out to the first 30-sec epoch scored as sleep. Each nap opportunity was terminated after 20 minutes or after a maximum of 15 minutes of sleep. A default latency of 20 minutes was recorded for each nap opportunity wherein the subject did not enter sleep. Subjects were monitored continuously by study staff to ensure that wake was maintained between nap opportunities.

Detailed Description of Behavioral Assessments

Montgomery-Asberg Depression Rating Scale (MADRS)

The MADRS is a 10-item, clinician-administered scale that assesses severity of depressive symptoms, including sadness, inner tension, concentration difficulties, inability to feel, pessimistic thoughts, suicidal thoughts, reduced sleep, reduced appetite and lassitude (4).

Multidimensional Fatigue Inventory (MFI)

Consistent with recent data regarding the structure of fatigue in medically ill patients (5), the MFI assesses 5 dimensions of fatigue, including general fatigue, physical fatigue, mental fatigue, reduced activity and reduced motivation. In addition to scores for each subscale, a total score can be derived by summing the 5-subscale scores (6).

Detailed Description of Neuroendocrine and Immune Assessments

Commercially available radioimmunoassay kits (DiaSorin, Stillwater, MN) were used for assessment of plasma cortisol. Intra- and inter-assay CVs respectively were 8.5% and 12.7%.

Interferon (IFN)-alpha and tumor necrosis factor (TNF)-alpha were measured by high sensitivity quantitative enzyme-linked immunosorbent assay (ELISA) (Amersham Biosciences Corporation, Piscataway, NJ and R&D Systems, Minneapolis, MN, respectively). Soluble TNF receptor 2 (sTNFR2) was determined by R&D Quantikine ELISA. Assays were performed according to manufacturer's specifications, and were run in duplicate. Inter- and intra-assay variability were reliably <12% for TNF-alpha and <10% for sTNFR2 and IFN-alpha.

Tables

The following tables provide a comprehensive listing of all the simple (non-adjusted) correlations among sleep variables that were found to change as a function of IFN-alpha treatment and relevant behavioral, neurocognitive, immune and neuroendocrine variables. In addition, Table S2 contains a composite sleep variable that was derived from factor analysis. Briefly, examination of the Scree plot including delta sleep parameters found to be altered by IFN-alpha administration revealed only 1 meaningful factor with an Eigen value of ~3, accounting for 47.1% of the variance. Two other factors were detected, each with Eigen values of ~1. Accordingly, an unrotated principal components analysis was used to extract a single factor for the composite sleep variable including all delta sleep variables. As noted above, correlations of this sleep variable with other behavioral, neurocognitive, immune and neuroendocrine measures are included in Table S2.

Table S1. Multivariable Correlation (R^2) Matrix Among delta (Visit 2- Visit1) Sleep Parameters Found to be Different between Groups

Sleep Parameter	delta WASO	delta SE	delta Stage 2	delta Stage 3/4	delta REM latency	delta mean SL (MSLT)
delta SPT	0.443**	-0.286**	0.154*	0.001	0.037	0.000
delta WASO	---	-0.952**	-0.000	-0.13*	0.13*	0.056
delta Sleep Efficiency	---	---	0.014	0.151*	-0.108	-0.053
delta Stage 2	---	---	---	-0.029	0.095	0.010
delta Stage 3/4	---	---	---	---	-0.109	-0.046
delta REM latency	---	---	---	---	---	0.147*

SPT, sleep period time; WASO, wake after sleep onset; SE, sleep efficiency; SL, sleep latency; REM, rapid eye movement sleep; MSLT, multiple sleep latency test.

* $p \leq 0.05$ level (2 tailed); ** $p \leq 0.01$ level (2 tailed); Of note, unlike correlations reported in the main text, correlations in this table are not controlled for age, sex, race, body mass index, history of substance abuse or history of depression.

Table S2. Multivariable Correlation (R^2) Matrix between delta (Visit 2- Visit1) Sleep Parameters and Relevant Behavioral, Cytokine, Neurocognitive and Cortisol Measures

Sleep Parameter	Behavioral, Cytokine, Neurocognitive And Cortisol Measures								
	delta MADRS	delta MFI	delta ESS	delta IFN	delta TNF	delta sTNFR2	delta cMT	delta pm cort	delta cort slope
delta SPT	-0.000	0.011	0.037	0.055	-0.002	0.038	0.001	0.059	0.114
delta WASO	0.011	0.125	0.176*	0.007	0.002	0.010	0.153	0.167*	0.139*
delta Sleep Efficiency	-0.024	-0.154*	-0.153	-0.005	-0.002	-0.013	-0.181*	-0.159*	-0.114
delta Stage 2	0.041	0.114	0.041	0.316**	-0.001	0.156*	0.006	0.015	0.062
delta Stage 3/4	-0.091	-0.226**	-0.059	-0.006	-0.013	-0.062	-0.132	-0.045	-0.023
delta REM latency	0.067	0.228**	0.012	0.070	0.024	0.070	0.036	0.066	0.180*
delta mean SL (MSLT)	0.043	0.099	0.052	0.011	-0.039	-0.011	0.024	0.102	0.142
Composite Sleep Factor	0.041	0.225**	0.143	0.048	0.005	0.064	0.158*	0.191*	0.212*

SPT, sleep period time; WASO, wake after sleep onset; REM, rapid eye movement sleep; SL, sleep latency; MSLT, multiple sleep latency test; MADRS, Montgomery-Asberg Depression Rating Scale; MFI, Multidimensional Fatigue Inventory; ESS, Epworth Sleepiness Scale; IFN, interferon-alpha; TNF, tumor necrosis factor alpha; sTNFR2, soluble TNF-alpha receptor 2; cMT, choice movement time; cort, cortisol.

* $p \leq 0.05$ (2 tailed); ** $p \leq 0.01$ (2 tailed); Of note, correlations in this table are not controlled for age, sex, race, body mass index, history of substance abuse or history of depression.

Table S3. Multivariable Correlation (R^2) Matrix Among delta Behavioral, Cytokine, Neurocognitive and Cortisol Measures

Variable	Behavioral, Cytokine, Neurocognitive And Cortisol Measures							
	delta MFI	delta ESS	delta IFN	delta TNF	delta sTNFR2	delta cMT	delta pm cort	delta cort slope
delta MADRS	0.483**	-0.059	0.268**	-0.001	0.182*	0.144	0.202*	0.186*
delta MFI	---	0.045	0.133	0.001	0.171*	0.256**	0.241**	0.186*
delta ESS	---	---	-0.002	0.012	-0.007	0.000	0.020	0.020
delta IFN	---	---	---	-0.005	0.336**	0.102	0.025	0.108
delta TNF	---	---	---	---	0.102	-0.083	-0.036	-0.069
delta sTNFR2	---	---	---	---	---	0.002	0.000	0.020
delta cMT	---	---	---	---	---	---	0.094	0.213*
delta pm cort	---	---	---	---	---	---	---	0.543**

MADRS, Montgomery-Asberg Depression Rating Scale; MFI, Multidimensional Fatigue Inventory; ESS, Epworth Sleepiness Scale; IFN, interferon-alpha; TNF, tumor necrosis factor alpha; sTNFR2, soluble TNF-alpha receptor 2; cMT, choice movement time; cort, cortisol.

* $p \leq 0.05$ (2 tailed); ** $p \leq 0.01$ (2 tailed); Of note, correlations in this table are not controlled for age, sex, race, body mass index, history of substance abuse or history of depression.

Supplemental References

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4. Montgomery SA, Asberg M (1979): A new depression scale designed to be sensitive to change. *Brit J Psychiatry*. 134:382-389.
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