

SLEEP FRAGMENTATION DIFFERENTIALLY MODIFIES EEG DELTA POWER DURING SLOW WAVE SLEEP IN SOCIALLY ISOLATED AND PAIRED MICE

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ABSTRACT

Background and objective: Sleep fragmentation (SF) is an important constituent of many sleep disorders. Sleep rebound following sleep disruption is regulated by homeostatic processes that also are influenced by stress and social isolation stress has not been studied in context of sleep disruption. We investigated interactions between social isolation and SF on sleep-wakefulness and delta EEG power during SWS in mice.

Methods: C57/BLJ adult male mice were exposed to 6 h SF using a custom-designed apparatus that elicits minimal stress, along with telemetric polygraphic recordings for 24h. In paired or isolated mice, baseline recordings were followed by SF (every 2 min), for 6h.

Results and conclusions: In contrast with other published methods that induce sleep disruption, SF procedures were void of increased serum corticosterone. SF in both paired and socially isolated mice elicited an increase in slow wave sleep (SWS) and REM, and a decrease in wake during the dark period. However, there was no change in total time (24 h) in wake or SWS in both the groups. SF also induced reduced sleep latencies following arousal. EEG delta power during SWS was significantly attenuated in isolated animals when compared to the paired group. Social interactions exert important effects on sleep structure and homeostasis, as evidenced by sleep latency and delta power of the EEG, the latter serving as a surrogate indicator of sleepiness. Social isolation may negatively affect the quality of sleep, even when total sleep time is unaffected, and experimental paradigms that induce sleep restriction should take into consideration the underlying effects of isolation on sleep.

Keywords: Sleep, Sleep fragmentation, Social isolation stress, Delta power, Sleep homeostasis, mice

INTRODUCTION

Sleep fragmentation (SF), unlike prolonged sleep deprivation, is a notable consequence of many diseases in adults and children, including obstructive sleep apnea (OSA) (1,2), narcolepsy (3,4), depression (5,6) and post-traumatic stress disorder (7,8). It has been postulated that uninterrupted sleep for a minimum length of time is required for optimal daytime vigilance and neurocognitive function (9-11). As a corollary to this assumption, experimentally-induced SF resulted in excessive daytime sleepiness and cognitive impairments in humans (9,11) and in animals (12). In sleep-disordered breathing, especially OSA, the neurocognitive impairments observed are most likely due to intermittent hypoxia (13,14) and to SF, rather than sleep deprivation, because in these patients total sleep time is not markedly compromised (9,11,15).

Although, there are many studies in animals that have examined the effects of sleep deprivation on sleep-wakefulness (16-18), there are only a selected few that have looked into the effects of SF (19). Multiple methodological approaches have been employed to restrict sleep, including the slow rotating wheel (20,21), disk over water (22), small platform (23), treadmill (19,24) and gentle handling (18,25). Even though the stress levels may alleviate after long-time adaptations to such methodologies, they do not simulate disease conditions, especially OSA. Moreover, the stress induced by the cable required for recording of electroencephalogram (EEG) and electromyogram (EMG) may persist. Indeed, recording cables introduce another set of stressors (limited climbing on water bottles and cage covers), especially in small animals, such as mice. A recent study concluded that cable weight and flexibility could affect amount and patterns of sleep in mice (26).

In this paper, we report on a newly designed and validated device to elicit SF in rodents. This approach entails relatively minimal stress, particularly when combined with telemetric recordings, thereby providing an improved and desirable methodological approach for the study of the effects of intermittent sleep disruption, which ideally mimic the SF that occurs in disease conditions, such as OSA. Thus, concurrent with the recent developments in transgenic technologies, the methods described herein should allow for examination of unaltered physiological responses to sleep disruptors, and their corresponding mechanisms. Furthermore, the absence of tethering in a telemetric sleep recording set-up provides the opportunity to study the effect of social interaction on sleep. Many recent studies have successfully demonstrated the efficacy of telemetric sleep recordings (11,27).

Multiple studies have conclusively identified social isolation as inducing behavioral abnormalities, such as increased aggressiveness, anxiety-related behaviors, cognitive deficits, and hyper locomotion (28,29). However, how social isolation affects sleep, and how it affects the response to sleep disruption has never been explored. We therefore examined whether intermittent sleep interruption leads to increases in 'sleep pressure', and also whether social isolation differentially modulates natural sleep patterns and the 'sleep pressure' responses to SF.

MATERIALS AND METHODS

Animals

Male C57BL/6J mice (20-25 g) were purchased from Jackson Laboratories, (Bar Harbor, Maine), were housed in a 12 hr light/dark cycle (light on 7:00 am to 7:00 pm) at a constant temperature ($26 \pm 1^\circ\text{C}$) and were allowed access to food and water ad libitum. The experimental protocols were approved by the Institutional Animal Use and Care Committee and are in close agreement with the National Institutes of Health Guide in the Care and Use of Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Surgical procedure and implantation of telemetric transmitter and electrodes

All surgical procedures were performed under sterile conditions and general anesthesia (i.p. injection of pentobarbital at a dose of 50 mg/kg body weight). First, the animals were positioned in sternal recumbency, and a dorsal neck incision of 2-3 cm was made through the skin along the dorsal midline, covered with a sterile bandage, after which, a 1.5 - 2 cm incision was performed through the skin and abdominal wall along the ventral midline. A telemetric transmitter weighing 3.5 g, F20-EET (DSI, Minnesota, USA), which allows simultaneous monitoring of two biopotential channels, temperature and locomotor activity was inserted, biopotential leads were exteriorized, and the abdominal wall was closed using 4-0 non-absorbable suture with a simple interrupted pattern. The 2 pairs of biopotential leads were then advanced subcutaneously from the ventral abdomen incision to the dorsal neck incision using a trocar. Animals were then fixed in a stereotaxic apparatus for implantation of EEG electrodes, with the first pair of biopotential leads being fixed to the skull above the frontal area (1mm anterior to bregma and 2mm lateral to mid sagittal suture for one of the leads, and 1mm anterior to lambda and 2.5 mm lateral to mid sagittal suture for the other lead). The other pair of biopotential leads was placed within the same bundle of dorsal neck muscles for the recording of nuchal EMG.

Design and fabrication of a novel sleep fragmenter device for sleep deprivation / sleep fragmentation

The sleep fragmenter device used to induce SF in rodents has been previously presented in abstract form (30) and employs intermittent tactile stimulation of freely behaving mice in a standard mouse laboratory cage, using a near-silent motorized mechanical device. However, mice can hear higher frequencies than humans, and this factor has to be taken into consideration. In brief, tactile stimulation is achieved with a horizontal bar sweeping just above the cage floor from one side to the other side of the mouse cage, the sweeper being powered by an electrical motor system in which the speed, torque, and interval of the intermittent functioning mode (2 min) are controlled (Fig. 1A), eliminating error induced by human intervention. On the other hand SD was performed by switching on the sweeper in the cage to continuous functioning mode. In this mode, the sweeper required around 9 sec to sweep the floor of the cage one way. When it reached to the end of the cage, a relay engaged the sweeper to move in the opposite direction.

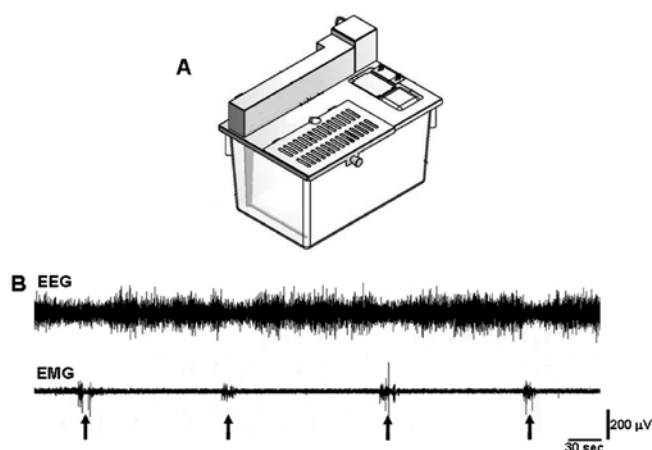


Fig. 1: A. Illustration of the new SF device. B. A representative recording of the polysomnogram during sleep fragmentation showing periodic arousal at 2 min intervals. Please note during each arousal event (arrows), there is a transitional desynchronized EEG waves corresponding to the EEG muscle artifacts. This methodology gently aroused the mice and did not appear to induce obvious stress. EEG, electroencephalogram; EMG, electromyogram.

Assay of corticosterone plasma levels

The fabrication of the sleep fragmenter device was designed to induce minimal stress to the animals, while effectively eliciting the desired frequency of sleep fragmentation. To verify this assumption, initial experiments were conducted to determine corticosterone (CT) plasma levels, as a surrogate indicator of stress. SF and sleep deprivation were carried out for 6 hours, starting at 7:00 am in C57BL/6J mice. Control mice were sacrificed at 1.00 pm (no intervention; $n=12$). SF using the novel sleep fragmenter device was conducted in 12 mice, sleep deprivation with the same device was completed in 11 animals, sleep deprivation using the disk over water method was completed in 7 mice, and REM sleep deprivation using the inverted flower pot technique was performed in 9 mice. Mice were rapidly decapitated immediately after their respective experimental procedure at 1.00 pm, and blood collected in EDTA-containing tubes, immediately centrifuged, and frozen at -80°C . Plasma levels for CT were then determined using a commercially available ELISA kit following the manufacturer recommendations (Immunodiagnostic Systems Ltd, Boldon, England, AC-14F1). This method has a detection level of 0.75 ng/ml, and exhibits linear behavior up to 200 ng/ml, with intra-assay and inter-assay coefficients of variability of 7.2% and 9.3%. The results were tabulated and statistics carried out by Student's *t* tests or ANOVA as appropriate.

Acclimatization, sleep recording and sleep fragmentation

After complete recovery from surgery, mice were transferred to the new sleep fragmenter device for habituation of the cage and the sweeper. The recording cages were mounted on a DSI telemetry receiver (RPC-1), which was in turn connected to an acquisi-

tion computer through a data exchange matrix. After at least one week of acclimatization in the cages, the magnetic switch of the transmitter was activated, and polygraphic recordings were begun at 7.00 am. Physiological data were continuously acquired for 24h using Dataquest ART acquisition software (DSI, Minnesota, USA; version 3.1), at a sampling rate of 500 Hz. Data were first scored automatically using Sleepsign software (Kissei Comtec, Japan), and records were visually confirmed or corrected as needed. Many researchers have adopted and successfully applied this software for sleep-wake analyses (31,32).

Behavior was classified into 3 different states: wake, slow wave sleep (SWS) and rapid eye movement (REM) sleep. EEG during W had low-amplitude, high-frequency (desynchronized) waves. During wake, EMG records showed gross body movement artifacts and behaviorally, animals had grooming, scratching and orienting activity. The SWS stage was characterized by low-frequency, high-amplitude (synchronized) EEG with a considerable reduction in EMG amplitude. The mice assumed a curled recumbent posture during this period. REM sleep was characterized by desynchronized EEG, and a drastic reduction in EMG (muscle atonia). Sleep-related low frequency (delta) activity was also derived from the records using bandpass filtering of 1– 4.0 Hz. Delta power was computed by using SleepSign software by Fast Fourier Transform (FFT), which was based on 512 points corresponding to 10 sec epochs, at a sampling rate of 250 Hz with Hanning as the window filter of FFT. Those SWS epoch which showed movement artifacts were excluded when computing delta power, since EEG signals are especially sensitive to movement, with the resulting artifact specifically enhancing signals in the delta band.

SF was performed by switching on the sweeper to a timer mode in the cage. In this mode, the sweeper required around 9 sec to sweep the floor of the cage one way. When it reached to the end of the cage, a relay engaged the timer which paused for 2 min before enabling the sweeper to move in the opposite direction. Between the 2 intervals, the animal remained undisturbed. During sweeper motion, animals would need to step over the sweeper, and continue with their unrestrained behavior. If the mouse was asleep, a brief tactile stimulation elicited intermittent brief arousal by the sweeper motion. This method prevents the need for human contact and intervention, and minimizes physical activity during the entire sleep disruption procedure, and closely mimicked the best methodological approach to study sleep disorders such as OSA. Since on average, 30 episodes of arousal per hour occur in patients with severe OSA (i.e, every 2 min), our aim was to mimic closely the severe disease condition, and thus, chose the interval of 2 min in our SF paradigm.

Experimental design

The various phases of the experimental paradigm are illustrated in Fig. 2.

Group 1: Social isolation

Part 1: During the 7-day acclimatization period and prior to recordings, implanted animals ($n=5$) were paired with another male mouse with which they had previously been housed. On day 8, baseline sleep recordings were carried out for 24h from 7.00 am

to 7.00 am next day (Fig. 2). The animals were left undisturbed on day 9. On day 10, animals were subjected to SF for 6 h during the light period from 7.00 am to 1.00 pm, and recovery sleep recordings were continued for the subsequent 18 h until 7.00 am next day.

Part 2: Following the above experiment, the companion mice were removed from the cages, and the experimental mice were placed in social isolation for 5 weeks. On day 45, baseline sleep recordings were conducted for 24h from 7.00 am to 7.00 am next day. The animals were left undisturbed on day 46. On day 47, the animals were subjected to SF for 6 h during the light period from 7.00 am to 1.00 pm, and recovery sleep recordings were continued for the subsequent 18 h until 7.00 am next day.

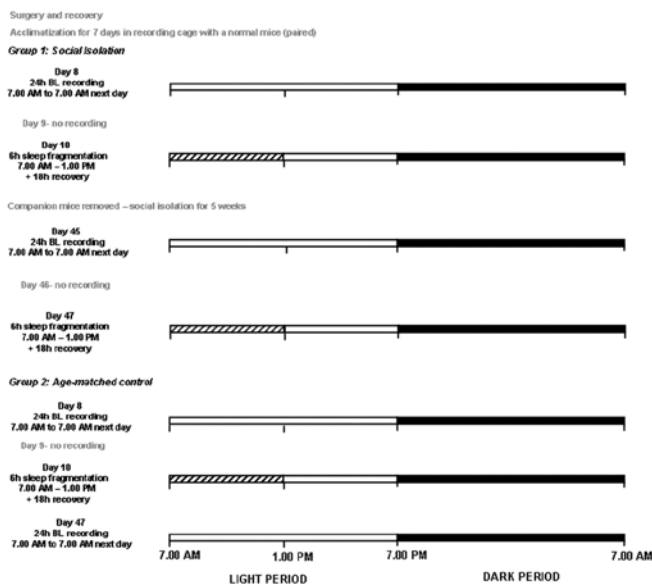


Fig. 2: Experimental protocol diagram. Open and dark portions of the bar represent light and dark periods of the 12:12-h light: dark cycle respectively. Hatched portion of the bar (within the light period) indicates the time of sleep fragmentation.

Group 2: Age-matched control

Part 1: During the 7-day acclimatization period and prior to recordings, implanted animals (n=5) were paired with another male mouse with which they had previously been housed. On day 8, baseline sleep recordings were carried out for 24h from 7.00 am to 7.00 am next day (Fig. 2). The animals were left undisturbed on day 9. On day 10, animals were subjected to SF for 6 h during the light period from 7.00 am to 1.00 pm, and recovery sleep recordings were continued for the subsequent 18 h until 7.00 am next day.

Part 2: Following the above experiment, the companion mice continued to stay in the cages. On day 47, baseline sleep recordings were conducted for 24h from 7.00 am to 7.00 am next day.

Sleep latency measurement:

To determine the time elapsed following a wake episode to initiation of SWS, the latency in seconds was calculated for each

arousal during the first two hours (7.00 am to 9.00 am) and the last two hours (11.00 am to 1.00 pm) during baseline conditions and during SF recordings in both paired and socially isolated conditions. The time was measured from the beginning of each wake episode to the beginning of the next SWS episode and the mean calculated.

Temperature and activity:

Body temperature and gross motor activity were acquired every 10 sec through out all experiments. To increase the precision of recording, the lower limit of temperature records was set at 34°C and the upper limit at 41°C, while in the activity record the lower limit was set at 0 counts (no gross activity) and upper limit was set at 3840 counts (a high level of activity) at the polling rate of 64 Hz. The transmitter underwent 3 point calibration at 35 °C, 37 °C and 39 °C.

Data analysis:

In all the experimental conditions, the sleep-wake data were divided into 10 sec epochs and scored. They were then divided into 2-h bins. EEG delta power (1–4 Hz) during SWS was calculated as percentage of each animal's baseline recording. We used multivariate MANOVA model (SPSS 11) to allow full assessment whether different conditions on three different behavioral states were present. The MANOVA model had: Two hr time bins as within factors (12 time points) and Two between factors: (1) Condition (four levels): BL (paired), SF (paired), BL (socially isolation) and SF (socially isolation) (2) State (three levels): wakefulness, SWS, and REM sleep. All F statistics are reported using Pillai's Trace. The interaction of three different factors, i.e., time, condition and state were determined using this mixed model repeated measures MANOVA.

To further elucidate the nature of identified interactions for the paired and socially isolated conditions, the data were analyzed by one way ANOVA. Firstly, overall statistical significance was determined for the 24-h period between the treatment groups (baseline and sleep fragmentation). In addition, statistical significance for 2 h bins for 24 h was assessed, followed by post-hoc Holm-Sidak analyses, as needed. Similar statistical approaches were used to compare delta power during SWS and the latency of SWS after each episode of wake. Repeated measures one-way ANOVA were used to analyze body temperature and gross activity in the paired and socially isolated conditions. For all comparisons, a p value <0.05 was considered to achieve statistical significance.

RESULTS

The main objectives of the present study were to assess changes in sleep architecture during and after SF under socially paired and isolated conditions. The novel technique used to fragment sleep was remarkably efficient in eliciting periodic arousals at the desired intervals (Fig. 1B), and did not appear to induce obvious stress in the animals. As shown in Fig. 3, animals subjected to SF spent more time in the awake state during the initial hours of SF, but subsequently manifested the same duration of wake as controls during the last 2 hours of SF.

State	Time of day	PAIRED		SOCIALLY ISOLATED	
		Significance	Percentage time spent	Significance	Percentage time spent
Wakefulness	7:00 AM - 9.00 AM	F=5.44, p<0.04	74.6 ± 6.3	F=64.68, p<0.001	75.5 ± 1.2
	9:00 AM - 11.00 AM	F=17.41, p<0.003	52.7 ± 3.3	NS	55.9 ± 4.8
	11:00 AM - 1.00 PM	NS	46.2 ± 5.2	NS	48.3 ± 3.9
	1:00 PM - 3.00 PM	NS	47.1 ± 7.1	F=8.08, p<0.04	36.9 ± 3.5
	3:00 PM - 5.00 PM	NS	33.9 ± 6.4	NS	26.1 ± 2.5
	5:00 PM - 7.00 PM	NS	37.8 ± 2.4	NS	56.3 ± 4.7
	7:00 PM - 9.00 PM	NS	78.3 ± 7.0	NS	84.4 ± 2.8
	9:00 PM - 11.00 PM	F=7.61, p<0.025	63.5 ± 6.7	NS	71.4 ± 2.0
	11:00 PM - 1.00 AM	F=5.34, p<0.05	60.4 ± 8.0	F=42.74, p<0.003	47.4 ± 7.4
	1:00 AM - 3.00 AM	F=18.90, p<0.002	45.4 ± 6.0	NS	50.9 ± 4.0
	3:00 AM - 5.00 AM	NS	60.7 ± 6.0	F=26.70, p<0.007	80.5 ± 1.6
	5:00 AM - 7.00 AM	NS	60.5 ± 3.7	F=9.83, p<0.03	54.5 ± 4.2
SWS	7:00 AM - 9.00 AM	NS	24.7 ± 6.2	F=35.04, p<0.004	24.5 ± 1.2
	9:00 AM - 11.00 AM	F=12.05, p<0.008	44.2 ± 3.2	NS	40.7 ± 4.1
	11:00 AM - 1.00 PM	NS	48.2 ± 4.4	NS	47.3 ± 2.6
	1:00 PM - 3.00 PM	NS	45.0 ± 5.8	NS	49.5 ± 1.7
	3:00 PM - 5.00 PM	NS	54.2 ± 4.1	NS	58.6 ± 2.6
	5:00 PM - 7.00 PM	NS	52.9 ± 2.2	NS	34.9 ± 3.7
	7:00 PM - 9.00 PM	NS	18.8 ± 5.9	F=8.71, p<0.042	14.6 ± 2.7
	9:00 PM - 11.00 PM	F=7.851, p<0.023	34.1 ± 5.9	NS	25.5 ± 1.8
	11:00 PM - 1.00 AM	F=5.258, p<0.051	35.1 ± 6.3	F=37.36, p<0.004	44.5 ± 5.8
	1:00 AM - 3.00 AM	F=16.69, p<0.004	47.9 ± 5.1	F=17.94, p<0.013	41.2 ± 3.6
	3:00 AM - 5.00 AM	NS	35.3 ± 5.1	F=277.60, p<0.001	18.4 ± 1.5
	5:00 AM - 7.00 AM	NS	36.8 ± 3.6	F=8.63, p<0.04	40.0 ± 3.2
REM sleep	7:00 AM - 9.00 AM	F=8.70, p<0.018	0.7 ± 0.5	F=149.06, p<0.001	0.0 ± 0.0
	9:00 AM - 11.00 AM	F=15.75, p<0.004	3.1 ± 0.6	F=30.04, p<0.005	3.5 ± 0.7
	11:00 AM - 1.00 PM	NS	5.6 ± 1.0	NS	4.4 ± 1.3
	1:00 PM - 3.00 PM	NS	7.9 ± 1.5	NS	13.7 ± 1.8
	3:00 PM - 5.00 PM	NS	11.9 ± 2.5	NS	15.3 ± 1.1
	5:00 PM - 7.00 PM	NS	9.3 ± 0.9	NS	8.8 ± 2.0
	7:00 PM - 9.00 PM	NS	3.0 ± 1.3	NS	1.1 ± 0.3
	9:00 PM - 11.00 PM	NS	2.4 ± 0.8	F=9.66, p<0.036	3.1 ± 0.3
	11:00 PM - 1.00 AM	NS	4.5 ± 1.7	F=24.45, p<0.008	8.2 ± 1.8
	1:00 AM - 3.00 AM	F=15.27, p<0.004	6.7 ± 1.4	F=11.40, p<0.028	7.9 ± 0.7
	3:00 AM - 5.00 AM	NS	4.0 ± 1.3	NS	1.1 ± 0.3
	5:00 AM - 7.00 AM	F=17.70, p<0.003	2.6 ± 0.3	F=11.56, p<0.027	5.5 ± 1.1

Table 1: The percentage time spent in wakefulness, slow wave sleep (SWS) and rapid eye movement (REM) sleep for paired and socially isolated groups. Data are expressed mean ± SEM.

Corticosterone plasma levels:

CT plasma levels did not increase in mice subjected to SF and sleep deprivation using the new SF technique reported herein, when compared to control animals. Indeed, control mice CT levels were 89.5 ± 7.3 ng/ml, while in SF mice CT concentrations were 92.5 ± 8.1 ng/ml (p-not significant), and were also similar to sleep deprivation mice (95.2 ± 8.3 ng/ml; p-not significant). However

in animals undergoing sleep deprivation using the disk over water technique, CT levels were significantly higher (198.5 ± 14.3 ng/ml; p<0.001 vs. controls, SF, and sleep deprivation). Similarly, animals exposed to the inverted flower pot approach also showed increased CT levels (178.9 ± 11.7 ng/ml; p<0.002 vs. controls, SF, and sleep deprivation).

MANOVA analysis:

Multivariate analysis showed that behavioral state was found to vary with time, state and condition, as reflected in a significant two-way interaction of time $\times$ state ($F=12.33$, $p<0.0001$) and condition $\times$ state ($F=3.02$, $p<0.0001$). Furthermore, the significant three-way interaction of time $\times$ condition $\times$ state showed that the experimental manipulations did have an influence on state and across 24 h recordings ($F=2.81$, $p<0.0001$).

Sleep-wakefulness and EEG delta power in socially paired mice:

Wakefulness: Overall analysis of the polygraphic data for a period of 24 h revealed significant changes between baseline and SF, ($p<0.001$) indicating that SF had influenced state. EEG monitoring during 6 h SF showed that the mice were awake 57.8 ± 8.6 % of the time, while the undisturbed control animals were awake 37.4 ± 6.6 % of the time. The SF group showed an initial increase in wake which was statistically significant compared to controls. However, SF-exposed animals showed decreased wake thereafter, indicating that they could easily resume sleep in the presence of the SF procedure from 7.00 am to 9.00 am, mice were awake 74.6 ± 6.3 % of the time ($F=5.44$, $p<0.04$), during 9.00 am to 11 am, they were awake 52.7 ± 3.3 % of the time ($F=17.41$, $p<0.003$), and during 11.00 am to 1.00 pm they were awake 46.2 ± 5.2 % of the time which were comparable to baseline (Fig. 3A). While there were no significant differences between controls and SF animals for the 6 hours of the light period immediately following cessation of SF, SF-exposed mice showed a significant decrease in wakefulness, 9.00 pm to 11.00 pm, ($F=7.61$, $p<0.025$), 11.00 pm to 1.00 am, ($F=5.34$, $p<0.05$) and 1.00 am to 3.00 am, ($F=18.90$, $p<0.002$) (Fig. 3A; Table 1).

Slow wave sleep: Overall analysis of the polygraphic data for a period of 24 h showed a significant change between baseline and SF, ($p<0.001$) indicating that SF influenced state. SF mice were in SWS 39.1 ± 7.3 % of the time while control animals were in SWS 55.3 ± 5.5 % of the time. The SF group showed an initial decrease in SWS during the first 2 h of the SF procedure (Fig. 3B), there were no significant differences thereafter till cessation of SF and even during the last 6 h of the light period. However, the SF group showed significant increases in SWS from 9.00 pm to 11.00 pm, ($F=7.85$, $p<0.023$), 11.00 pm to 1.00 am, ($F=5.25$, $p<0.05$) and 1.00 am to 3.00 am, ($F=16.69$, $p<0.004$) (Fig. 3B; Table 1).

REM sleep: As with other states, similar results were obtained with REM sleep for SF procedures ($p<0.001$). EEG monitoring during the 6 h of SF showed that the animals were in REM sleep 3.1 ± 1.4 % of the time and the undisturbed sleeping control animals were in REM sleep 7.2 ± 1.1 % of the time. There was a significant decrease in REM during SF, 7.00 am to 9.00 am ($F=8.70$, $p<0.018$) and 9.00 am to 11.00 am ($F=15.75$, $p<0.004$). However, as the SF progressed, animals showed a gradual increase in REM sleep towards control values (Fig. 3C). No significant differences were seen in REM sleep between controls and SF animals during the latter 6h of the light period. However, the SF group showed significant increases in REM sleep from 1.00 am to 3.00 am ($F=15.27$, $p<0.004$) and from 5.00 am to 7.00 am ($F=17.70$, $p<0.003$) (Fig. 3C; Table 1).

EEG delta power during SWS: Overall analysis of the data for a period of 24 h showed significant changes between baseline and SF ($p<0.001$), indicating the experimental condition had significant effects on global EEG delta power. SF animals showed a slight increase in delta power immediately after SF procedure, which was significantly greater throughout the dark period, 9.00 pm to 11.00 pm ($F=7.975$, $p<0.04$), 11.00 pm to 1.00 am ($F=7.984$, $p<0.04$), and 5.00 am to 7.00 am ($F=14.50$, $p<0.019$) (Fig. 3D).

Sleep-wakefulness and EEG delta power in socially isolated group:

Wakefulness: Overall analysis of the data for a period of 24h showed a significant change between treatments (control and SF) ($p<0.001$). EEG monitoring during 6 h SF showed that the mice were awake 59.9 ± 8.1 % and the undisturbed sleeping control animals were awake only 36.5 ± 2.3 % of time. SF animals exhibited a gradual decrease in wakefulness after the initial peak. The animals undergoing SF were awake (during 7.00 am to 9.00 am, 75.5 ± 1.2 %, during 9.00 am to 11 am, 55.9 ± 4.8 % and during 11.00 am to 1.00 pm, 48.3 ± 3.9 %) of the time (Fig. 4A). The SF group showed a significant increase in wakefulness only for the first 2 h during the SF period (7.00 am to 9.00 am ($F=64.688$, $p<0.001$) (Fig. 4A). Immediately after SF the SF group showed no significant changes in the percent time of wake. However during the dark period, the SF group showed a significant decrease in wake (11.00 pm to 1.00 am, ($F=42.74$, $p<0.003$)), (3.00 am to 5.00 am, ($F=26.70$, $p<0.007$)) and during (5.00 am to 7.00 am, ($F=9.83$, $p<0.035$)) (Fig. 4A; Table 1).

Slow wave sleep: The polygraphic data analysed for a period of 24h showed a significant change between treatments (control and SF) ($p<0.001$). EEG monitoring during 6 h SF showed that the mice were in SWS 37.5 ± 6.8 % and the undisturbed sleeping control animals were in SWS only 52.7 ± 1.8 % of time. Both SF animals exhibited a gradual increase in SWS after the initial dip. The animals undergoing SF were in SWS (during 7.00 am to 9.00 am, 24.4 ± 1.2 %, during 9.00 am to 11 am, 40.6 ± 4.1 % and during 11.00 am to 1.00 pm, 47.3 ± 2.6 %) of the time (Fig. 4B). The SF group showed a significant decrease in SWS only for the first 2 h ($F=35.04$, $p<0.004$) during the SF intervention respectively when compared to the sleeping controls (Fig. 4B). Immediately after SF the SF and SF-A groups showed no significant change in SWS. However, during the dark period, SF group showed a significant increase in SWS [7.00 pm to 9.00 pm, ($F=8.718$, $p<0.042$); 11.00 pm to 1.00 am, ($F=37.36$, $p<0.004$); 1.00 am to 3.00 am, ($F=17.94$, $p<0.013$) and 5.00 am to 7.00 am, ($F=8.63$, $p<0.042$)] and a significant decrease in SWS during [3.00 am to 5.00 am, ($F=277.60$, $p<0.001$) (Fig. 4B; Table 1)].

REM sleep: Similar results were obtained as with other states for a period. 24h data showed a significant change between treatments (control and SF) ($p<0.001$). EEG monitoring during 6 h SF showed that the mice were in REM sleep 2.6 ± 1.3 % and the undisturbed sleeping control animals were in REM sleep 10.7 ± 1.1 % of time. SF animals exhibited a gradual increase in REM sleep during the intervention, yet REM sleep remained significantly low throughout the 6 h SF period [7.00

am to 9.00 am, 0.03 ± 0.02 %, ($F=149.06$, $p<0.001$); 9.00 am to 11.00 am, 3.5 ± 0.7 %, ($F=30.04$, $p<0.005$) (Fig. 4C). There were no significant differences between controls and SF animals during the latter 6 h of the light period. However, SF group showed a significant increase in REM sleep during the dark period from 9.00 pm to 11.00 pm, ($F=9.66$, $p<0.036$), 11.00 pm to 1.00 am ($F=24.45$, $p<0.008$), 1.00 am to 3.00 am, ($F=11.40$, $p<0.028$) and from 5.00 am to 7.00 am ($F=11.56$, $p<0.027$) (Fig. 4C; Table 1).

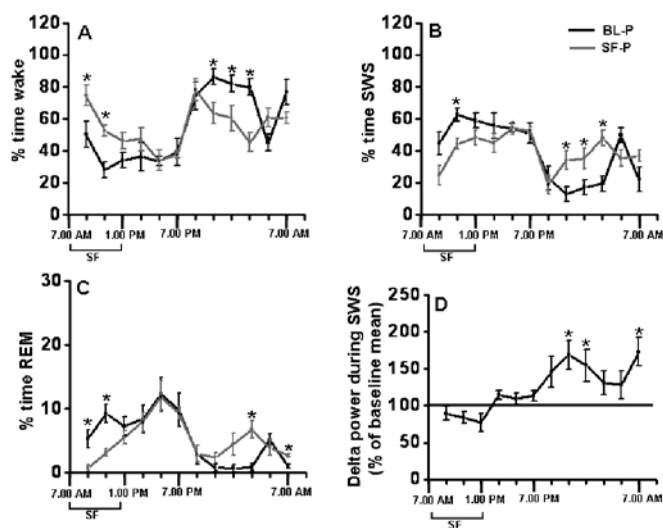


Fig. 3: Sleep-wakefulness and EEG delta power in paired mice. All graphs are plotted per 2 h for a 24 h period. A. Percent time waking during baseline (black line) and sleep fragmentation (SF; gray line). There was no significant difference in wake immediately following SF, but showed a significant decrease in wake during the dark period (9.00 pm to 3.00 am). B. Percent time in SWS during baseline (black line) and SF (gray line). There was no significant difference in SWS immediately following SF, but showed a significant increase in SWS during the dark period (9.00 pm to 3.00 am). C. Percent time in REM sleep during baseline (black line) and SF (gray line). There was no significant difference in REM sleep immediately following SF, but showed a significant increase during the latter part of the dark period. D. There was a significant increase in EEG delta power during the post-SF period. The black line indicates SF period (7.00 am to 1.00 pm). BL-P, baseline-paired; SF-P, sleep fragmentation-paired; SF, sleep fragmentation (7.00 am to 1.00 pm). * $p<0.05$. See text for more details.

EEG delta power during SWS: Overall analysis of the delta power for a period of 24h showed a significant change between treatments (control and SF) ($p<0.001$) (Fig. 4D). There were no significant changes in the EEG delta power in SF group during the 6 h of SF procedure (7.00 am to 1.00 pm). Immediately following SF, there was an increase in delta power only for the first 2 h (1.00 pm to 3.00 pm, 28.26%) (Fig. 4D). During the post fragmentation period there was a significant increase in delta power during 11.00 pm to 1.00 am (29.8%, ($F=11.31$, $p<0.028$)) (Fig. 4D).

Sleep-wakefulness and EEG delta power in age-matched control group:

Overall analysis and pair wise comparison showed there was no significant change in wake, SWS, REM and delta power in age matched control (Fig. 5 A-D).

Delta power during SWS is attenuated in socially isolated groups:

Comparison between baselines: Overall data for the period of 24 h showed a significant reduction in delta power between baseline (paired) and baseline (socially isolated) mice ($F=2.50$, $p<0.001$). In the light period, there was a significant decrease in delta power in socially isolated animals during 11.00 am to 3.00 pm, (11.00am to 1.00 pm, -31.3%, ($F=7.12$, $p<0.028$); 1.00 pm to 3.00 pm, -42.1% ($F=9.31$, $p<0.016$). During the dark period, significant decreases emerged from 11.00 pm to 1.00 am, -28.2%, ($F=6.08$, $p<0.039$); 1.00 am to 3.00 am, -14.6% ($F=5.46$, $p<0.048$) (Fig. 6A).

Comparisons between sleep fragmentation: The 24h period data showed no homeostatic increase in delta power between SF (paired) and SF (socially isolated) mice ($F=2.98$, $p<0.001$). There was no significant decrease in delta power in socially isolated animals during the light period, except during 5.00 pm to 7.00 pm, (-43.8%, ($F=6.88$, $p<0.030$)). However, the dark period showed a significant decrease throughout (7.00 pm to 9.00 pm, -49.1%, ($F=12.80$, $p<0.007$); 9.00 pm to 11.00 pm, -51.4%, ($F=8.72$, $p<0.018$); 11.00 pm to 1.00 am, -41.1%, ($F=5.47$, $p<0.047$); 1.00 am to 3.00 am, -45.7% ($F=7.26$, $p<0.027$); 5.00 am to 7.00 am, -46.3%, ($F=6.35$, $p<0.036$). No significant change during 3.00 am to 5.00 am was noted (Fig. 6B).

Comparisons between sleep fragmentation and age matched control: There was no significant change in delta power between SF and age matched control.

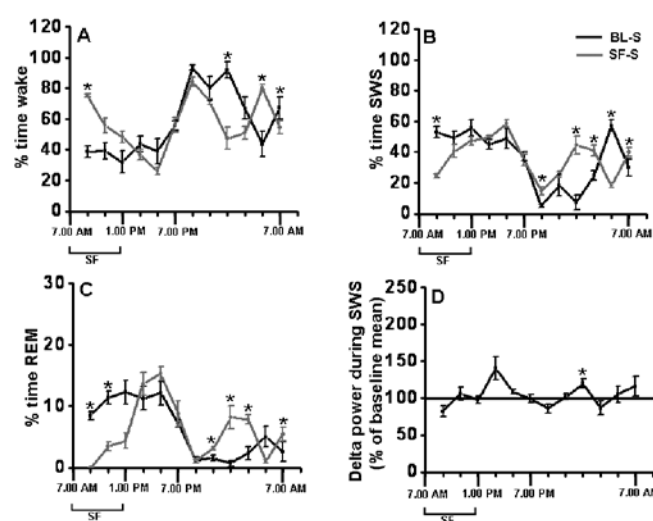


Fig. 4: Sleep-wakefulness and EEG delta power in socially isolated mice. All graphs are plotted per 2 h for a 24 h period. A. Percent time waking during baseline (black line) and sleep fragmentation (SF; gray line). There was no significant difference in wake immediately following SF,

but showed a significant decrease in wake during the latter half of the dark period (11.00 pm to 1.00 am). B. Percent time in SWS during baseline (black line) and SF (gray line). There was no significant difference in SWS immediately following SF, but showed a significant increase in SWS during the latter part of the dark period (11.00 pm to 1.00 am). C. Percent time in REM sleep during baseline (black line) and SF (gray line). There was no significant difference in REM sleep immediately following SF, but showed a significant increase in REM sleep during the latter part of the dark period. D. There was no significant increase in delta power after SF in isolated group). The black line indicates SF period (7.00 am to 1.00 pm). BL-S, baseline- single; SF-S, sleep fragmentation- single; SF, sleep fragmentation (7.00 am to 1.00 pm). * $p < 0.05$. See text for more details.

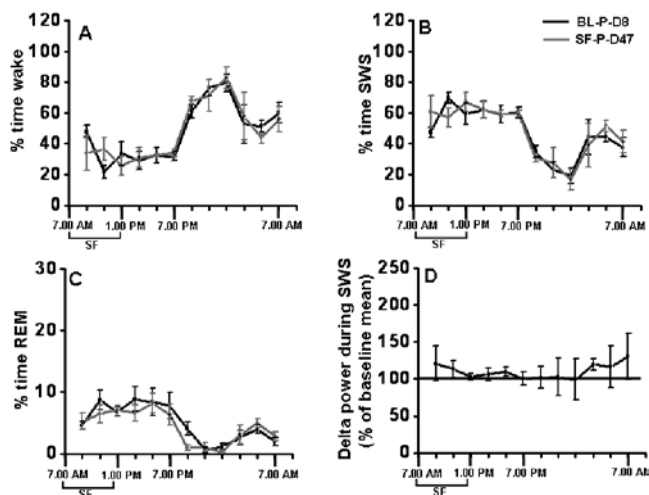


Fig. 5: Sleep-wakefulness and EEG delta power in age-matched control mice. All graphs are plotted per 2 h for a 24 h period. A. Percent time waking during baseline – paired Day 8 (black line) and baseline – paired Day 47 (gray line). There was no significant difference in wake (A), SWS (B), REM (C) and delta power (D). The black line indicates SF period (7.00 am to 1.00 pm). BL-P-D8, baseline- paired- day8; BL-P-D47, baseline- paired- day 47).

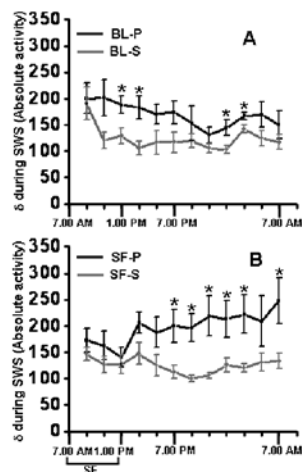


Fig. 6: EEG delta power during SWS is attenuated in socially isolated mice. A. Baseline recording showed a trend level decrease in EEG delta

power through out the 24 period in socially isolated mice (gray line) as compared to the paired mice (black line). B. After sleep fragmentation, the socially isolated mice showed a significant decrease in delta power through out the dark period (gray line). The black line indicates SF period (7.00 am to 1.00 pm). BL-P, baseline- paired; BL-S, baseline- single; SF-P, sleep fragmentation- paired; SF-S, sleep fragmentation- single; SF, sleep fragmentation (7.00 am to 1.00 pm); * $p < 0.05$.

Latency from wake to sleep is greatly reduced in SF animals:

The average latency of SWS after every episode of wake was calculated during the first 2 h (7.00 am to 9.00 am) and during the final 2 h (11.00 am to 1.00 pm) to determine the sleep propensity during sleep fragmentation. During the first 2 h, the mean latency to sleep was comparable to the corresponding baseline in both paired and socially isolated mice. However as the time progressed, the latency to sleep was significantly reduced in both the paired and socially isolated mice indicating development of sleep pressure. During the final 2 h, the paired mice had a latency of 25.0 ± 3.0 sec ($p < 0.05$) compared to the same circadian time of paired sleeping controls, 145.6 ± 45.9 sec (Fig 6). Similarly, during the final 2h of SF, the socially isolated mice had a latency of 14.5 ± 0.9 sec ($p < 0.04$) compared to the same circadian time of socially isolated sleeping controls, 115.7 ± 34.2 sec (Fig. 7).

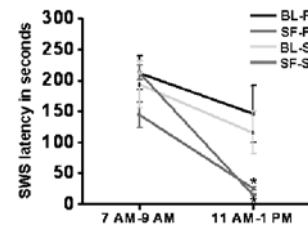


Fig. 7: Latency from wake to sleep is greatly reduced in sleep fragmented mice. The mean latency from wake to sleep was comparable to baseline recording during the first hour of recording. However during the last 2 hour of sleep fragmentation, both the paired and socially isolated mice showed a significant decrease in mean latency from wake to sleep indicating mounting sleep pressure. * $p < 0.05$. BL-P, baseline- paired; BL-S, baseline- single; SF-P, sleep fragmentation- paired; SF-S, sleep fragmentation- single; SF, sleep fragmentation (7.00 am to 1.00 pm); * $p < 0.05$.

Temperature and activity

To determine the overall statistical significance of the effect of baseline, SF (paired) and SF (isolated) body temperature and gross motor activity, repeated measures ANOVA was performed. There was a significant effect on body temperature across the 24 h period between the experimental groups ($F=14.26$, $p < 0.001$). Post hoc analyses comparing the baseline with SF (paired) and SF (isolated) for each 2 h bin showed a significant increase in body temperature in SF-isolated group during 11.00 am to 1.00 am ($F=5.68$, $p < 0.029$), and decrease during 3.00 pm to 5.00 pm ($F=5.88$, $p < 0.027$) and during 5.00 am to 7.00 am ($F=6.37$, $p < 0.022$) (Fig. 8A). Gross activity also showed a significant effect across the 24 h period between the

experimental groups ($F=7.97$, $p<0.001$). Further, post-hoc analysis showed a significant increase in activity in both SF-paired and SF-isolated during the initial 2 h of SF procedure ($F=6.37$, $p<0.022$) but gradually decreased for the latter part of the SF procedure. The SF-isolated animals showed a marked decrease in activity through most of the post-SF period, 3.00 pm to 5.00 pm ($F=5.30$, $p<0.034$) and 7.00 pm to 9.00 pm ($F=5.14$, $p<0.037$) (Fig. 8B).

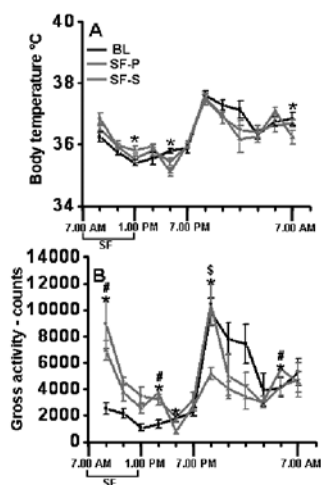


Fig. 8: Body temperature and gross activity in paired and single mice. A. 24 h recording showed no significant change in body temperature in socially isolated mice (red line) and paired mice (gray line) when compared to the baseline recording (black line) after SF. B. Similarly, 24 h recording showed no significant change in gross activity in socially isolated mice (red line) and paired mice (gray line) when compared to the baseline recording (black line) after SF, although the post-SF period showed a trend level decrease in activity in isolated animals. The black line indicates SF period (7.00 am to 1.00 pm). BL, baseline; SF-P, sleep fragmentation-paired; SF-S, sleep fragmentation-single; SF, sleep fragmentation (7.00 am to 1.00 pm). $p<0.05$. * = comparison between baseline and SF-S; \$ = comparison between SF-P and SF-S; # = comparison between baseline and SF-P. See text for more details.

DISCUSSION

We developed an animal model of SF in which mice were aroused periodically, to mimic the SF that occurs in many disease conditions, particularly in OSA. Using this model, we studied the effect of social isolation on sleep and EEG delta power during SWS. Six hours of SF in both socially paired and socially isolated group did not elicit an immediate change in sleep-wakefulness. There was an increase in SWS and REM and a decrease in wake during the dark period. However there were no significant differences in total time spent in wake or SWS. In fact, the most prominent changes emerged in the delta spectral power of the EEG, whereby socially isolated animals did not exhibit homeostatic increases in delta power as compared to the socially paired groups, both during baseline and also after SF. Furthermore, there was no increase in delta power during the post-SF period. Taken together, these

findings suggest that the sleep homeostatic responses seen in acute sleep deprivation (SD) cannot be generalized to the responses elicited by acute SF, in which sleep loss is markedly smaller.

Sleep in mammals is a complex phenomenon, generally alternating between two distinct states, SWS and REM sleep. The exact oscillatory mechanisms underlying the periodic cycling between these states are still largely unknown. Nevertheless, it is well documented that the duration of the prior wake period may directly influence the succeeding sleep bouts, especially with a sleep rebound and an increase in EEG delta power during SWS, a relationship that has now been firmly established in a variety of species (33-36), including humans (37,38). However, most of the observations indicate there is no consistent relationship between the duration of prior wakefulness and the duration of subsequent SWS, since the duration of the latter can be compensated by a shorter sleep period that exhibits higher slow-wave intensity. Notwithstanding these observations, how EEG delta power during SWS is affected by different kinds of stressors remains unknown. A previous study reported that rats showed a sharp increase in slow-wave activity during SWS after a social conflict with aggressive and dominant rats (39,40). This type of fear-awakening which elicits intense social stress will increase the ensuing slow-wave activity. However, the impact on recovery sleep following imposition of other stressors, such as those inherent to the disruption of sleep was never ascertained.

SF method is relatively stress-free

A plethora of studies supports the notion that acute sleep loss induces stress and the release of stress-related hormones. One of the major limitations of existing methods aiming to induce sleep loss is that they are stressors per se, and as such, the intrinsic stress effect of sleep loss can not be ascertained. Sleep deprivation induced either by the 'inverted flower pot' method, gentle manual handling, or by the forced locomotion method has been shown to increase levels of ACTH and corticosterone (41-43). Since our main interest was to mimic the patterns of activity seen in sleep disorders as closely as possible, these methods were not ideal for our experiments. In addition, these methods are either labor intensive (e.g., manual handling) or impose forced locomotion (12,19,41-43). In contrast, when mice were subjected to SF and SD using the novel procedure described herein, the resultant CT levels were comparable to the levels measured in control mice, indicating that this methodology does not appear to modify the circulating levels of stress hormones. Even though SF procedure did not result in increased CT levels at the time of euthanasia (i.e., the end of 6 h SF), this measurement does not conclusively demonstrate whether CT levels are increased earlier, during the initial phase of SF procedure. Future additional studies are needed to determine whether the absence of a systemic elevation in CT levels as seen in our novel SF/SD technique, when applied for a 6-hour period, is also confirmed with more extended exposures and not accompanied by changes in other hypothalamic-pituitary-adrenal response markers. Another surrogate indicator of stress is alterations in body temperature. Although restraint stress has shown to increase body temperature in mice (44), some studies have reported no change in body temperature in rats (45,46). Social stress in rats, on the other hand, has been associated with an increase in body temperature (47). In our study the SF procedure did not

cause significant changes in body temperature, suggesting a stress-free nature of the procedure itself. It is worth noting that the body temperature decreased after the initial 2 h SF period, indicating the mice could return to sleep in between arousals due to SF.

An additional comment pertaining to the social context in which SD or SF is applied deserves comment (see also below). The stressor response associated with the SD procedure may also be modulated by the social isolation that traditionally accompanies this type of experiments. Indeed, Suchecki and Tufik (2000) have shown that adrenocortical responses and eating behaviors were improved when rats exposed to SD using the multiple platform technique were allowed the presence of stable cagemates as opposed to unknown rats (48). Our ability to induce sleep disruption in either social isolation or socially paired conditions should permit improved delineation of the role played by the contextual social situation in the regulation of sleep homeostatic responses.

Further evidence attesting to the efficacy of our new SD/SF approach resides in the finding that the latency to enter SWS from a wake episode was significantly reduced over time in all the mice that underwent SF. Thus, the momentary arousal elicited by the device was not sufficiently stressful to maintain the vigilance of the animals for long periods of time.

Social isolation is accompanied by decreased EEG delta power during SWS compared to social pairing under natural sleep conditions and sleep fragmentation

Socially isolated mice showed a dramatic decrease in EEG delta power, even during the basal conditions, even though the total time spent in sleep-wakefulness did not change. Furthermore, socially isolated animals did not show a rebound increase in delta power when compared to the socially paired group. Recent studies have shown the role of NF- κ B and TNF- α in modulating SWA and EEG delta power. In particular, when NF- κ B activity is blocked by the inhibitor peptide, SN50, there was a significant reduction in the relative delta power (49). Such reduction in delta power, without gross change in sleep-wake activity was also reported when NF- κ B activity is blocked by the inhibitor peptide, SN50 in rats, particularly during the first 2 h of recovery sleep following 6h SD (18). TNF-2R KO mice also showed reduced delta power in response to viral challenge (50). In addition to sleep, social isolation dramatically affects many physiological functions, including level of aggressiveness, anxiety-related behaviors, cognitive deficits, and hyperlocomotion (51-53).

In the present study, SWS showed no immediate rebound in delta power in the socially paired group after SF, but instead showed a considerable increase in delta power in the dark period, which may have been partly due to enhanced SWS pressure, as reflected by such increased delta activity. Such a trend was not seen in socially isolated animals. However, these animals showed significantly reduced EEG delta activity when compared to the socially paired group.

The immediate increase in REM sleep following SF in the socially isolated group could be related to a compensatory mechanism for the reduced SWS activity seen in parallel with the light period homeostatic drive. If this is indeed the case, then we should expect increased REM sleep during the following light period. Stress may affect REM sleep but the effect is thus far controversial.

On the one hand, when restraint stress is used REM sleep was suppressed (54,55) and yet on the other hand, stress was associated with an increase in REM sleep in other studies (56,57). It is possible that different types of stressors and the underlying conditions in which the particular stressors are applied (i.e., time of day, intensity of stimulus applied and duration of stressor) may all account for the opposite effects of stress on REM sleep reported to date.

After SD, sleep rebound during recovery period is a well established phenomenon that is tightly regulated by homeostatic processes (58-62). The homeostatic drives that influence sleep rebound are in turn influenced by stress. However, this influence depends on the type and duration of the stressor and shows a dual-effect pattern. Acute stressors lead to a subsequent sleep rebound (54,63), while prolonged and/or chronic stress reduce the time spent in SWS, thereby affecting sleep quality (64,65). Previous studies have shown that short-lasting immobilization or restraint stress increase REM sleep duration (57), whereas chronic immobilization leads to a decrease in REM sleep rebound (54,55) and induces hippocampal atrophy (66), further confirming the hypothesis that stress actively modulates behavioral state. The absence of dramatic changes in sleep-wake patterns as observed in the mice subjected to SF, may therefore reflect the relatively stress-free nature of this novel intervention, and therefore allow conducting chronic SF procedures.

We should emphasize that the SF procedure used herein induced increases in sleep pressure but also acutely disrupted the amount of total sleep, particularly during the initial hours after onset of the device activity. While this is an undesired consequence of the procedure, the reduction in overall sleep progressively abated as the SF procedure is continued, such that global sleep duration returns to the pre-intervention baseline levels. Thus, the normalization of sleep duration using this new SF technique opens the way for long lasting studies on the effect of fragmented sleep independent from the effect of sleep restriction or deprivation.

The effect of SF in social isolation differs from the effect of SF in socially paired conditions

Social bonding in animals plays a pivotal role in modulating many physiological functions, including sleep-wake patterns. Previous studies have shown that REM sleep plays an important role in social bonding in mammals (67,68). In several species including humans, maternal deprivation is associated with disrupted and decreased REM sleep during separation followed by a REM sleep rebound after reunion (69,70). This "bonding hypothesis" suggests that, in addition to other physiological functions, REM sleep is fundamental to promote attachment between parent and siblings (and vice versa) and also between adult mating partners. In a recent study, rats subjected to 6h SD using a modified multiple platform method, who had their bonding renovated everyday for the remainder 18 h, showed marked rebounds in REM sleep compared to socially isolated rats (23). Thus, the social contextual setting needs to be controlled for in future experiments involving sleep manipulations and their subsequent recovery responses.

In summary, we present evidence supporting the use of a novel approach to induce either SD or SF in a murine model that is void of some of the major limitations of previous reported techniques, namely elevated stress responses or forced locomotion. Further-

more, we show that social interactions in the context of SF play an important role in modulating the quality of sleep and its recovery from SF, thereby emphasizing the need to incorporate the contextual social setting in future experiments aiming to determine the regulation of sleep homeostatic responses.

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REFERENCES

- Kimoff RJ. Sleep fragmentation in obstructive sleep apnea. *Sleep* 1996;19:S61-6.
- Montgomery-Downs HE, Crabtree VM, Gozal D. Cognition, sleep and respiration in at-risk children treated for obstructive sleep apnea. *Eur Respir J* 2005;25:336-42.
- Tafti M, Villemin E, Carlander B, Besset A, Billiard M. Sleep in human narcolepsy revisited with special reference to prior wakefulness duration. *Sleep* 1992;15:344-5.
- Zorick F, Roehrs T, Wittig R, Lamphere J, Sicklessteel J, Roth T. Sleep-wake abnormalities in narcolepsy. *Sleep* 1986;9:189-93.
- Jones D, Gershon S, Sitaram N, Keshavan M. Sleep and depression. *Psychopathology* 1987;20:20-31.
- Perlis ML, Giles DE, Buysse DJ, Tu X, Kupfer D. Self-reported sleep disturbance as a prodromal symptom in recurrent depression. *J Affect Disord* 1997;42:209-12.
- Mellman TA, Kulick-Bell R, Ashlock LE, Nolan B. Sleep events among veterans with combat-related posttraumatic stress disorder. *Am J Psychiatry* 1995;152:110-15.
- Ohayon MM, Shapiro CM. Sleep disturbances and psychiatric disorders associated with posttraumatic stress disorder in the general population. *Compr Psychiatry* 2000;41:469-78.
- Bonnet MH. Sleep restoration as a function of periodic awakening, movement, or electroencephalographic change. *Sleep* 1987;10:364-73.
- Franken P. Long-term vs. short-term processes regulating REM sleep. *J Sleep Res* 2002;11:17-28.
- Stepanski EJ. The effect of sleep fragmentation on daytime function. *Sleep* 2002;25:268-76.
- Tartar JL, Ward CP, McKenna JT, Thakkar M, Arrigoni E, McCarley RW, et al. Hippocampal synaptic plasticity and spatial learning are impaired in a rat model of sleep fragmentation. *Eur J Neurosci* 2006;23:2739-48.
- Gozal D, Daniel JM, Dohanich GP. Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat. *J Neurosci* 2001;21:2442-50.
- Row BW, Kheirandish L, Neville JJ, Gozal D. Impaired spatial learning and hyperactivity in developing rats exposed to intermittent hypoxia. *Pediatr Res* 2002;52:449-53.
- Coleman RM, Roffwarg HP, Kennedy SJ, Guilleminault C, Cinque J, Cohn MA, et al. Sleep-wake disorders based on a polysomnographic diagnosis. A national cooperative study. *JAMA* 1982;247:997-1003.
- Huber R, Deboer T, Tobler I. Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations. *Brain Res* 2000;857:8-19.
- Lydic R, Baghdoyan HA. Sleep, anesthesia, and the neurobiology of arousal state control. *Anesthesiology* 2005;103:1268-95.
- Ramesh V, Thattai HS, McCarley RW, Basheer R. Adenosine and sleep deprivation promote NF-kappaB nuclear translocation in cholinergic basal forebrain. *J Neurochem* 2007;100:1351-63.
- McKenna JT, Tartar JL, Ward CP, Thakkar MM, Cordeira JW, McCarley RW, et al. Sleep fragmentation elevates behavioral, electrographic and neurochemical measures of sleepiness. *Neuroscience* 2007;146:1462-73.
- Fenzl T, Romanowski CP, Flachskamm C, Honsberg K, Boll E, Hoehne A, et al. Fully automated sleep deprivation in mice as a tool in sleep research. *J Neurosci Methods* 2007;166:229-35.
- Ramesh V, Lakshmana MK, Shankaranarayana Rao BS, Raju TR, Kumar VM. Alterations in monoamine neurotransmitters and dendritic spine densities at the medial preoptic area after sleep deprivation. *Sleep Res Online* 1999;2:49-55.
- Rechtschaffen A, Bergmann BM. Sleep deprivation in the rat by the disk-over-water method. *Behav Brain Res* 1995;69:55-63.
- Machado RB, Suchecki D, Tufik S. Comparison of the sleep pattern throughout a protocol of chronic sleep restriction induced by two methods of paradoxical sleep deprivation. *Brain Res Bull* 2006;70:213-20.
- McCoy JG, Tartar JL, Bebis AC, Ward CP, McKenna JT, Baxter MG, et al. Experimental sleep fragmentation impairs attentional set-shifting in rats. *Sleep* 2007;30:52-60.
- Tobler I, Borbely AA. The effect of 3-h and 6-h sleep deprivation on sleep and EEG spectra of the rat. *Behav Brain Res* 1990;36:73-8.
- Tang X, Orchard SM, Liu X, Sanford LD. Effect of varying recording cable weight and flexibility on activity and sleep in mice. *Sleep* 2004;27:803-10.
- Morrow JD, Opp MR. Diurnal variation of lipopolysaccharide-induced alterations in sleep and body temperature of interleukin-6-deficient mice. *Brain Behav Immun* 2005;19:40-51.
- Chida Y, Sudo N, Mori J, Kubo C. Social isolation stress impairs passive avoidance learning in senescence-accelerated mouse (SAM). *Brain Res* 2006;1067:201-8.
- Oehler J, Jähkel M, Schmidt J. Altered neurobiological responses to acute immobilization in social-isolated mice. *Pharmacol Biochem Behav* 1986;25:41-4.
- DeMatteis M, Gozal E, Gozal D. A novel procedure for sleep deprivation and sleep fragmentation in rodents [abstract]. *Sleep* 2004;42(Suppl):27.
- Espana RA, McCormack SL, Mochizuki T, Scammell TE. Running promotes wakefulness and increases cataplexy in orexin knockout mice. *Sleep* 2007;30:1417-25.
- Qu WM, Huang ZL, Xu XH, Aritake K, Eguchi N, Nambu F, et al. Lipocalin-type prostaglandin D synthase produces prostaglandin D2 involved in regulation of physiological sleep. *Proc Natl Acad Sci USA* 2006;103:17949-54.
- Hu WP, Li JD, Zhang C, Boehmer L, Siegel JM, Zhou QY. Altered circadian and homeostatic sleep regulation in prokineticin 2-deficient mice. *Sleep* 2007;30:247-56.
- Jha SK, Coleman T, Frank MG. Sleep and sleep regulation in the ferret (*Mustela putorius furo*). *Behav Brain Res* 2006;172:106-13.
- Tobler I, Scherschlicht R. Sleep and EEG slow-wave activity in the domestic cat: effect of sleep deprivation. *Behav Brain Res* 1990;37:109-18.
- Vyazovskiy VV, Achermann P, Tobler I. Sleep homeostasis in the rat in the

- light and dark period. *Brain Res Bull* 2007;74:37–44.
37. Achermann P, Dijk DJ, Brunner DP, Borbely AA. A model of human sleep homeostasis based on EEG slow-wave activity: quantitative comparison of data and simulations. *Brain Res Bull* 1993;31:97–113.
 38. Gaudreau H, Moretini J, Lavoie HB, Carrier J. Effects of a 25-h sleep deprivation on daytime sleep in the middle-aged. *Neurobiol Aging* 2001;22:461–8.
 39. Meerlo P, de Bruin EA, Strijkstra AM, Daan S. A social conflict increases EEG slow-wave activity during subsequent sleep. *Physiol Behav* 2001;73:331–5.
 40. Meerlo P, Pragt BJ, Daan S. Social stress induces high intensity sleep in rats. *Neurosci Lett* 1997;225:1–4.
 41. Andersen ML, Bignotto M, Machado RB, Tufik S. Different stress modalities result in distinct steroid hormone responses by male rats. *Braz J Med Biol Res* 2004;37:791–7.
 42. Hairston IS, Ruby NF, Brooke S, Peyron C, Denning DP, Heller HC, et al. Sleep deprivation elevates plasma corticosterone levels in neonatal rats. *Neurosci Lett* 2001;315:29–32.
 43. Tobler I, Murison R, Ursin R, Ursin H, Borbely AA. The effect of sleep deprivation and recovery sleep on plasma corticosterone in the rat. *Neurosci Lett* 1983;35:297–300.
 44. Gilmore AJ, Billing RL, Einstein R. The effects on heart rate and temperature of mice and vas deferens responses to noradrenaline when their cage mates are subjected to daily restraint stress. *Lab Anim* 2008;42:140–8.
 45. Landeira-Fernandez J. Analysis of the cold-water restraint procedure in gastric ulceration and body temperature. *Physiol Behav* 2004;82:827–33.
 46. Wright BE, Katovich MJ. Effect of restraint on drug-induced changes in skin and core temperature in biotelemetered rats. *Pharmacol Biochem Behav* 1996;55:219–25.
 47. Bhatnagar S, Vining C, Iyer V, Kinni V. Changes in hypothalamic-pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats. *J Neuroendocrinol* 2006;18:13–24.
 48. Suchecki D, Tufik S. Social stability attenuates the stress in the modified multiple platform method for paradoxical sleep deprivation in the rat. *Physiol Behav* 2000;68:309–16.
 49. Kubota T, Kushikata T, Fang J, Krueger JM. Nuclear factor - κ B inhibitor peptide inhibits spontaneous and interleukin-1 β -induced sleep. *Am J Physiol Regulatory Integrative Comp Physiol* 2000;279:R404–R413.
 50. Kapas L, Bohnet SG, Traynor TR, Majde JA, Szentirmai E, Magrath P, et al. Spontaneous and influenza virus-induced sleep are altered in TNF- α double-receptor deficient mice. *J Appl Physiol* 2008;105:1187–98.
 51. Gentsch C, Lichtsteiner M, Frischknecht HR, Feer H, Siegfried B. Isolation-induced locomotor hyperactivity and hypoalgesia in rats are prevented by handling and reversed by resocialization. *Physiol Behav* 1988;43:13–6.
 52. Hatch A, Wiberg GS, Balazs T, Grice HC. Long-Term Isolation Stress in Rats. *Science* 1963;142:507–8.
 53. Jones GH, Marsden CA, Robbins TW. Behavioural rigidity and rule-learning deficits following isolation-rearing in the rat: neurochemical correlates. *Behav Brain Res* 1991;43:35–50.
 54. Marinesco S, Bonnet C, Cespuglio R. Influence of stress duration on the sleep rebound induced by immobilization in the rat: a possible role for corticosterone. *Neuroscience* 1999;92:921–33.
 55. Papale LA, Andersen ML, Antunes IB, Alvarenga TA, Tufik S. Sleep pattern in rats under different stress modalities. *Brain Res* 2005;1060:47–54.
 56. Bouyer JJ, Deminière JM, Mayo W, Le Moal M. Inter-individual differences in the effects of acute stress on the sleep-wakefulness cycle in the rat. *Neurosci Lett* 1997;225:193–6.
 57. Rampin C, Cespuglio R, Chastrette N, Jouvét M. Immobilisation stress induces a paradoxical sleep rebound in rat. *Neurosci Lett* 1991;126:113–8.
 58. Borbely AA. A two-process model of sleep regulation. *Hum Neurobiol* 1982;1:195–204.
 59. Dijk DJ, Brunner DP, Beersma DGM, Borbely AA. Slow wave sleep and electroencephalogram power density as a function of prior waking and circadian phase. *Sleep* 1990;13:430–40.
 60. Franken P, Tobler I, Borbely AA. Sleep homeostasis in the rat: simulation of the time course of EEG slow-wave activity. *Neurosci Lett* 1991;130:141–4. Erratum in: *Neurosci Lett* 1991;132:279.
 61. Friedman L, Bergmann BM, Rechtschaffen A. Effects of sleep deprivation on sleepiness, sleep intensity, and subsequent sleep in the rat. *Sleep* 1979;1:369–91.
 62. Machado RB, Suchecki D, Tufik S. Sleep homeostasis in rats assessed by a long-term intermittent paradoxical sleep deprivation protocol. *Behav Brain Res* 2005;160:356–64.
 63. Gonzalez MM, Debilly G, Valatx JL, Jouvét M. Sleep increase after immobilization stress: role of the noradrenergic locus coeruleus system in the rat. *Neurosci Lett* 1995;202:5–8.
 64. Sternthal HS, Webb WB. Sleep deprivation of rats by punitive and non punitive procedures. *Physiol Behav* 1986;37:249–52.
 65. Tobler I, Borbely AA. Sleep EEG in the rat as a function of prior waking. *Electroencephalogr Clin Neurophysiol* 1986;64:74–6.
 66. Govindarajan A, Rao BS, Nair D, Trinh M, Mawjee N, Tonegawa S, et al. Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. *Proc Natl Acad Sci USA* 2006;103:13208–13.
 67. McNamara P, Andresen J, Clark J, Zborowski M, Duffy CA. Impact of attachment styles on dream recall and dream content: a test of the attachment hypothesis of REM sleep. *J Sleep Res* 2001;10:117–27.
 68. McNamara P. REM sleep: a social bonding mechanism. *New Ideas Psychology* 1996;14:35–46.
 69. Hofer MA, Shair HN. Isolation distress in two-week-old rats: influence of home cage, social companions, and prior experience with littermates. *Dev Psychobiol* 1987;20:465–76.
 70. Reite M, Capitanio J. On the nature of social separation and social attachment. In: Reite M & Field T, editors. *The Psychobiology of Attachment and Separation*. Academic Press, New York, 1985.