Characterization of the Sleep-wake Patterns in Mice Lacking Fatty Acid Amide Hydrolase

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Study Objectives: Oleamide and anandamide are fatty acid amides implicated in the regulatory mechanisms of sleep processes. However, due to their prompt catabolism by fatty acid amide hydrolase (FAAH), their pharmacologic and behavioral effects, in vivo, disappear rapidly. To determine if, in the absence of FAAH, the hypnogenic fatty acid amides induce an increase of sleep, we characterized the sleep-wake patters in FAAH-knockout mice [FAAH (-/-)] before and after sleep deprivation.

Design: FAAH (-/-), FAAH (+/-), and FAAH (+/+) mice were implanted chronically for sleep, body temperature (Tb), and locomotor activity (LMA) recordings. Sleep-wake states were recorded during a 24-hour baseline session followed by 8 hours of sleep deprivation. Recovery recordings were done during the 16 hours following sleep deprivation. Total amount of wake, slow-wave sleep, and rapid eye movement sleep were calculated and compared between genotypes. The electroencephalographic spectral analysis was performed by fast Fourier transform analysis. Telemetry recordings of Tb and LMA were carried out continuously during 4 days under baseline conditions.

Setting: N/A.

Patients or Participants: FAAH (-/-) mice and their heterozygote (+/-) and control (+/+) littermates were used.

Interventions: Sleep deprivation.

Measurements and Results: FAAH (-/-) mice possess higher values of slow-wave sleep and more intense episodes of slow-wave sleep than do control littermates under baseline conditions that are not related to differences in Tb and LMA. A rebound of slow-wave sleep and rapid eye movement sleep as well an increase in the levels of slow-wave activity were observed after sleep deprivation in all genotypes.

Conclusion: These findings support the role of fatty acid amides as possible modulators of sleep and indicate that the homeostatic mechanisms of sleep in FAAH (-/-) mice are not disrupted.

Key Words: Fatty acid amide hydrolase, fatty acid amides, sleep, sleep deprivation, EEG spectral analysis, slow wave activity.

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INTRODUCTION

NATURAL LIPIDS HAVE RECENTLY BEEN PROPOSED TO BE INVOLVED IN THE REGULATION OF PHYSIOLOGIC FUNCTIONS IN THE CENTRAL NERVOUS SYSTEM. For example, N-arachidonoyl ethanolamine (anandamide), an endogenous ligand for cannabinoids receptors, induces hypothermia, analgesia, sleep, and locomotor and memory deficits in vivo.[1-4] Whereas 9-(Z)-octadecenamide (oleamide) promotes sleep, hypothermia, and alterations in motor activity in rodents.[5-7] Both anandamide and oleamide are members of the same class of endogenous lipids, the fatty acid amides (FAAs),[8] and are rapidly hydrolyzed, in vivo, by a brain membrane-bound enzyme, fatty acid amide hydrolase (FAAH).[9-13] Since its identification, FAAH has been cloned in numerous organs in mouse, rats, and humans, revealing a high degree of homology in its amino-acid sequence with a comparable affinity for its target substrates.[9,10,14] In the brain, FAAH is localized to pyramidal cortical neurons, hippocampal pyramidal cells, mitral cells in olfactory bulb, cerebellar Purkinje neurons, and the choroid plexus, while lowest concentrations have been detected in brainstem regions and the hypothalamus.[15-18]

Selective FAAH inhibitors have been developed in an effort to study, in detail, the chemical characteristics and sites of action of FAAs.[19-20] However, such studies have predominantly utilized in vitro preparations, whereas the in vivo properties of FAAH inhibitors are only beginning to be investigated.[21] In an attempt to study the role that FAAH plays in regulating FAAs levels and activity, in vivo, a mouse-model that possesses a target disruption of the FAAH gene [FAAH (-/-)] was generated.[22] FAAH (-/-) mice possess a 15-fold increase in brain levels of anandamide compared to normal mice, whereas the administration of anandamide or oleamide in FAAH (-/-) mice induces similar, but highly intensified behavioral responses, including hypomotility, analgesia, and hypothermia,[22,23] to those elicited by these FAAs in wild type rodents.[1,7]

The fact that FAAH (-/-) mice showed an increase in endogenous levels of anandamide,[22] as well as reduced hydrolytic rates for anandamide and oleamide,[23] raises a question concerning the sleep-wake cycle of these mice. Herein, we characterized the patterns of sleep and wakefulness in FAAH (-/-) mice that exhibited a significant increase in the amount of slow-wave sleep (SWS) during their rest phase compared to their heterozygote [FAAH (+/-)] and normal [FAAH (+/+)] littermates. In addition, because sleep deprivation is an experimental procedure for studying sleep homeostasis, we sleep deprived FAAH (-/-), (+/+), and (+/-) mice during 8 hours to investigate the role of FAAs in the homeostatic regulation of sleep on these mice.

MATERIALS AND METHODS

Subjects

Studies were performed on FAAH (-/-), FAAH (+/-), and FAAH (+/+) mice that were on a homogeneous genetic back-

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ground. Mice were created on a mixed 129/SvJ-C57Bl/6 background, as described previously,[22] and backcrossed onto the C57Bl/6 background for at least 5 generations prior to use in these studies. Mice represented littermates of crosses of FAAH (+/-) mice and were genotyped by Southern blotting as described previously. The detailed information on the origin and genotype of the mice is available in our original publication.[22] All animals were housed in individual Plexiglas recording cages located inside environmentally controlled chambers (Tech/Serv model EPC-010. BRS/LVE, Beltsville, Maryland, USA) maintained throughout the study at an ambient temperature of 25°C ± 1°C, and a 12:12-hour (6:00 AM-6:00 PM) light-dark cycle. Food and water were available ad libitum.

Surgery

Seventeen FAAH (-/-), 17 FAAH (+/-), and 15 FAAH (+/+) mice were implanted under halothane anesthesia (1%-2%), with a standard set of stainless-steel screw electrodes for chronic sleep recordings. The electroencephalogram (EEG) was recorded from electrodes placed in the frontal and parietal bone over the hippocampus (P = -2.06; L = 1.5). A third EEG electrode was placed in the skull over the cerebellum and served to ground the animal to reduce signal artifacts. Two wire electrodes inserted in the neck musculature were used to record postural tone through electromyography (EMG) activity. Insulated leads from the EEG and EMG electrodes were then soldered to a miniconnector that was cemented to the skull with dental acrylic. During anesthetic recovery, mice were monitored for recovery of normal righting capability and locomotion. All mice were allowed a 1-week recovery.

Separate groups of mice (n = 10 for each genotype) were implanted with radiotelemetry transmitters to record body temperature (Tb) and nonspecific locomotor activity (LMA), as described previously in detail.[7] Briefly, mice were anesthetized with halothane (1%-2%), the hair from the subxiphoid space to the pelvis was shaved, and the area was scrubbed with iodine. A midline abdominal incision was made to allow for the implantation of miniature transmitters (Data Sciences International, model TA10TA-F20. St. Paul, MN, USA) to monitor Tb and LMA. The incision was closed using sterile and absorbable suture. Tb and LMA were monitored by telemetry using Dataquest A.R.T., data-collection software (Data Science International, St. Paul, MN, USA). Data were recorded and averaged as discrete events during 10 seconds every 5 minutes, by receivers (Data Science International, model RPC-1, St. Paul, MN, USA) located beneath each cage. Animal care, maintenance, and experimental procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Scripps Research Institute Animal Care and the Committee Standards.

Experimental Protocol and Data Acquisition

For sleep sessions, groups of 8 mice selected randomly from the 3 genotypes were recorded simultaneously. Mice were connected to commutators with flexible cables allowing their unrestricted movement within the cage and were habituated to the recording cages for 96 hours. Without disturbing the animals, baseline recordings began after light onset (ZT-0; ZT = zeitgeber time), while the signals were continuously recorded from EEG and EMG electrodes during 24 hours. After baseline recording was completed, 7 mice of each genotype were subjected to 8 hours of total sleep deprivation and recorded for the remaining 16 hours. Sleep deprivation began at ZT-0 and was attained by gentle handling whenever the mice looked drowsy or attempted to engage in a sleeping posture. This method of sleep deprivation was applied in an effort to minimize stress and to exclude locomotion as a confounding variable observed in the rotating-cylinder procedure.[24] The EEG and EMG signals were amplified in a Grass Model 7D polygraph (Astro-Med, Icn Product Group. West Warwick, RI. USA) in a frequency range of 0.30 to 35 Hz and sampled at 256 Hz. The EEG and EMG were displayed on a computer monitor and stored with a resolution of 128 Hz in the hard drive of a computer for the off-line analysis of the sleep-wake states and spectral analysis using software supplied by Kissei-Comtec (Irvine, CA. USA). The recording chambers contained a mini video camera for continuously observing cage behavior during the recording session.

For Tb and LMA recordings, mice remained individually in their home cages, whereas radiotelemetry recordings were carried out simultaneously in all mice during 4 consecutive days under the same environmental conditions mentioned previously.

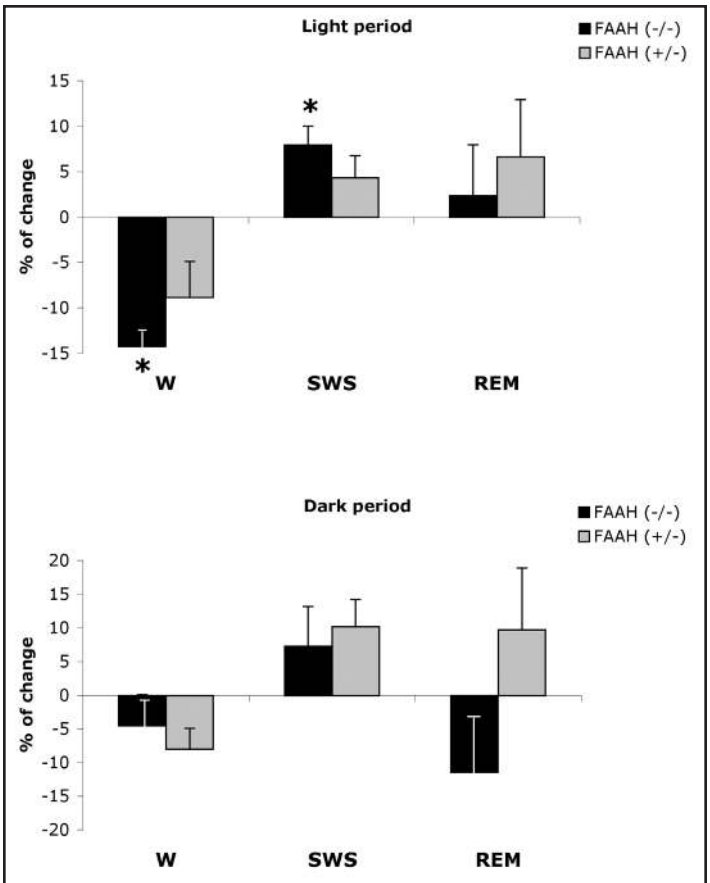

Figure 1—Percentage of change in the amount of W, SWS and REM sleep compared to FAAH (+/+) mice. The upper graphic shows that the amount of SWS during the light period in FAAH (-/-) mice was significantly higher ($p < 0.05$) than FAAH (+/+), whereas the amount of W was significantly lower than FAAH (+/+) mice ($p < 0.05$). No significant differences were observed between genotypes during the dark part of the cycle (bottom graphic). In FAAH (+/+) mice, n = 15 animals; FAAH (-/-) and (+/-), n = 17 mice. Significant differences are marked by asterisk. Bars represent mean values (± S.E.M.).

Data Analysis

The polygraphic results were analyzed semiautomatically by 15-second epochs and classified according to the following stages of vigilance: wakefulness (W), SWS, and rapid eye movement (REM) sleep. Electrophysiologic criteria were used to define the sleep-wake stages, as follows. W was characterized by desynchronization of the EEG and high theta activity and the presence of muscle tone; SWS by high-voltage waves, high delta activity, and decreased voltage in the EMG; and REM sleep by desynchronization of the EEG, high theta activity, and absence of voltage in the EMG. Percentage of total time spent in W, SWS, and REM sleep per hour and by periods of 12 hours was calculated. The frequency and duration of the individual SWS and REM-sleep episodes were evaluated, as well the sleep continuity, determined by the number of brief awakenings of 16 seconds (BA). In addition to standard sleep analysis, EEG spectral analyses in the different sleep-wake stages were performed by Fourier fast transformer analysis using 4-second epochs, giving 0.25-Hz bins from 0 to 30 Hz. Each bin was named after its lower limit. In addition, the slow-wave activity (SWA), an EEG frequency band in the range of 0.75 to 4.0 Hz, was calculated. Epochs with artifacts were excluded by software-visual review recognition of the polygraph records. In W, SWS, and REM sleep, only epochs that were both preceded and followed by the same stage were included in the analysis. Thus, epochs in between 2 stages were excluded. Likewise, the amount of LMA per hour and the average Tb across 4 days were calculated. Results were compared by a repeated-measure analysis of variance (ANOVA), with the Scheffé F test used for specific comparisons when indicated by ANOVA.

RESULTS

Baseline Vigilance States

The 3 genotypes studied showed a well-defined circadian rhythm, having during the diurnal part of the cycle, a preference for sleep and a preference to be awake during the night. However, the amount of wakefulness in the FAAH (-/-) mice (210 minutes) was significantly lower than that of the FAAH (+/+) mice (245 minutes) during the light period ($F_{2,46} = 3.17$; $P < .05$), whereas the amount of SWS in the FAAH (-/-) mice (459 minutes) was significantly higher than that of the FAAH (+/+) mice (425 minutes) during the same part of the cycle ($F_{2,46} = 3.19$; $P < .05$) (Figure 1). The increase in the amount of SWS observed in FAAH (-/-) mice was due to both a significant increase of 31% in the mean duration of the SWS episodes ($F_{2,46} = 5.76$; $P < .01$) and a significant reduction of 20% in the number of SWS episodes ($F_{2,46} = 4.55$; $P < .01$). There was also a significant reduction in the number of BA in FAAH (-/-) mice during the light period, compared to the other 2 genotypes ($F_{2,46} = 4.54$; $P < .01$) (Table 1). No significant changes in the amounts of BA, W, SWS, and REM sleep and sleep parameters were observed between genotypes during the dark part of the cycle.

Baseline EEG Power Spectrum

The frequency distribution of the EEG power density for W, SWS, and REM sleep in the 3 genotypes during the baseline recording is showed in Figure 2. The mean power density during sleep for all frequency ranges was used as 100% and the values were then expressed relative to this reference value.[25] The power density in sleep was employed because SWS is a particularly sen-

Table 1—Sleep parameters (mean ± SEM) during light and dark periods for baseline recordings.

12-h light	BA, no.	TT-W, min	TT-SWS, min	TT-REM, min	SWS-F, no.	SWS-D, min	REM-F, no.	REM-D, min
FAAH (+/+) mice	86.40 ± 7.33	245.57 ± 10.31	425.94 ± 8.16	48.48 ± 2.97	178.00 ± 11.06	2.39 ± 0.17	43.46 ± 3.32	1.10 ± 0.05
	**	*	*		**	**		
FAAH (-/-) mice	66.58 ± 5.35	210.67 ± 9.41	459.68 ± 8.93	49.63 ± 2.70	143.65 ± 10.78	3.13 ± 0.17	39.96 ± 2.74	1.14 ± 0.04
FAAH (+/-) mice	88.58 ± 4.64	223.90 ± 9.69	444.37 ± 10.45	51.72 ± 3.04	188.06 ± 8.56	2.28 ± 0.16	46.93 ± 3.85	1.06 ± 0.04
12-h dark	BA	TT-W	TT-SWS	TT-REM	SWS-F	SWS-D	REM-F	REM-D
FAAH (+/+) mice	59.86 ± 5.92	402.00 ± 18.71	291.55 ± 17.92	26.44 ± 1.90	123.33 ± 12.01	2.38 ± 0.14	19.40 ± 1.54	1.27 ± 0.05
FAAH (-/-) mice	50.23 ± 5.56	383.65 ± 17.89	312.91 ± 17.01	23.42 ± 2.47	112.50 ± 11.83	2.85 ± 0.19	16.82 ± 1.69	1.17 ± 0.07
FAAH (+/-) mice	59.00 ± 4.23	369.77 ± 12.50	321.21 ± 11.80	29.01 ± 2.42	128.80 ± 8.47	2.43 ± 0.19	20.33 ± 1.02	1.36 ± 0.14

Note that both brief awakenings (BA) and the number of slow-wave sleep (SWS) episodes are reduced in FAAH (-/-) mice, whereas the total time of SWS and duration of SWS episodes in these mice are significantly higher than the other 2 genotypes during the light period

*$P < .05$

**$P < .01$

FAAH refers to fatty acid amide hydrolase; TT-W, total time of wake; TT-SWS, total time of SWS; TT-REM, total time of rapid eye movement (REM) sleep; SWS-F, number of SWS episodes; REM-F, number of REM sleep episodes; SWS-D, duration of individual SWS episodes; REM-D, duration of individual REM sleep episodes. Latencies for SWS and REM sleep were not calculated because sleep recordings started without disturbing animals.

sitive indicator of sleep disruptions and because theta waves are more consistently present in REM sleep than in W.[25,26] Clear differences were observed for W, SWS, and REM sleep within FAAH (+/+), FAAH (-/-), and FAAH (+/-) mice. In all genotypes, the highest power peak was observed during SWS, and it was detected between the 0.5- and 4.5-Hz range, whereas for W and REM sleep, a peak was observed in the range between 5.5 and 8.0 Hz, being higher in REM sleep than in W (Figure 2, top). On the other hand, significant differences were detected between genotypes. Thus, FAAH (-/-) mice showed lower power spectra than did FAAH (+/+) mice during W at 7.5 Hz ($F_{2,40} = 3.79$; $P < .05$) and during REM sleep between 7.5 and 8.25 Hz and between 10.5 and 14.75 Hz ($F_{2,40} = 3.86$; $P < .05$). In contrast, FAAH (-/-) mice showed higher EEG power than did FAAH (+/+) and FAAH (+/-) mice during SWS at 0.75 to 4.5 Hz ($F_{2,40} = 3.91$; $P < .05$) (Figure 2, bottom).

In all genotypes, SWA during baseline recordings showed a marked trend having high initial values that decreased in the course of the main sleep period. However, the analysis of the level of SWA during the 12-hour light period showed that FAAH (-/-) mice have 6% and 4% more SWA than do FAAH (+/+) and (+/-) mice, respectively ($F_{2,18} = 11.86$, $P < .01$) (Figure 3). Due to the fact that SWA has been negatively correlated with BA and used as indicator of SWS intensity,[24,27] we calculated this value in our mice. A negative correlation was found between SWA and BA in FAAH (-/-) mice ($r = -0.11$, $P < .01$), whereas no significant changes were found in the r values for FAAH (+/+) and FAAH (+/-) mice ($r = 0.38$ and $r = -0.33$, respectively). During the 12-hour dark period, no trend was observed in the genotypes. However, FAAH (-/-) mice exhibited 7% lower values of SWA than did FAAH (+/+) mice ($F_{2,18} = 3.82$, $P < .05$) (Figure 3).

Tb and LMA Recordings

No significant differences in Tb and LMA were found between the 3 genotypes studied (data not shown). Continuous temperature recordings during 4 days revealed that all mice had a consistent circadian rhythm, with an average value (± SEM) of 37.50ºC ± 0.19ºC during the dark phase and an average value of 35.86ºC ± 0.27ºC in the light phase. LMA also showed a well-defined circadian rhythm, with a maximum activity during the dark phase and a minimum during the light period. Likewise, the 3 genotypes were largely indistinguishable in terms of general cage behavior.

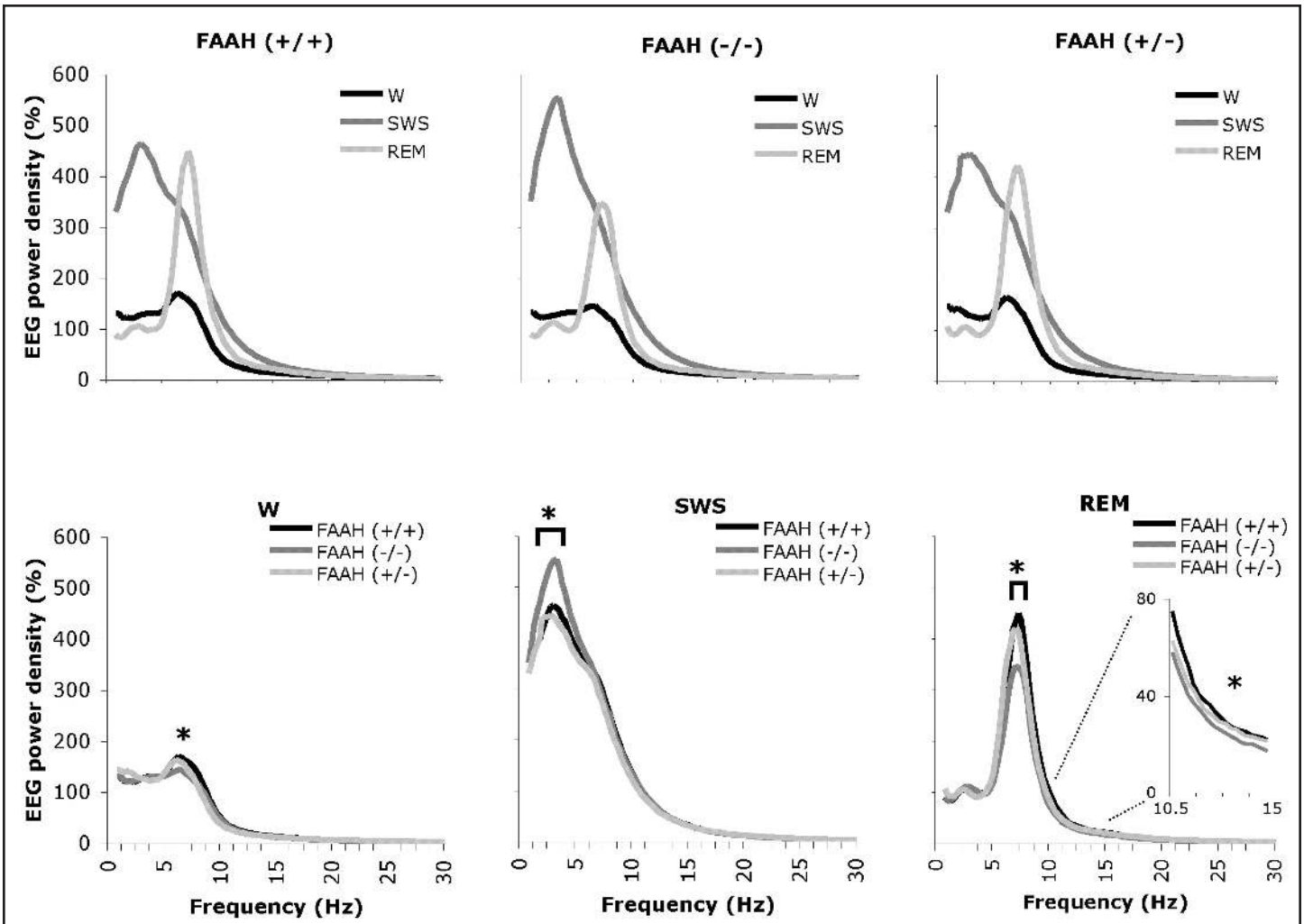

Figure 2—Top, EEG power spectra in the three genotypes studied here in W, SWS and REM sleep. The mean power density of 0.25 Hz bins during sleep for all frequency range was used as a 100% and then the values were expressed relatively to this reference value. Bottom graphics show that during W and REM sleep FAAH (-/-) had significant lower values than FAAH (+/+) in the EEG power in specific frequency bands, whereas during SWS the knockout mice showed higher EEG power values for the 2.75 – 4.5 Hz frequency band, than the other two genotypes ($p < 0.05$). Significant differences are indicated by asterisk. S.E.M. values were omitted to have a better view of the EEG power.

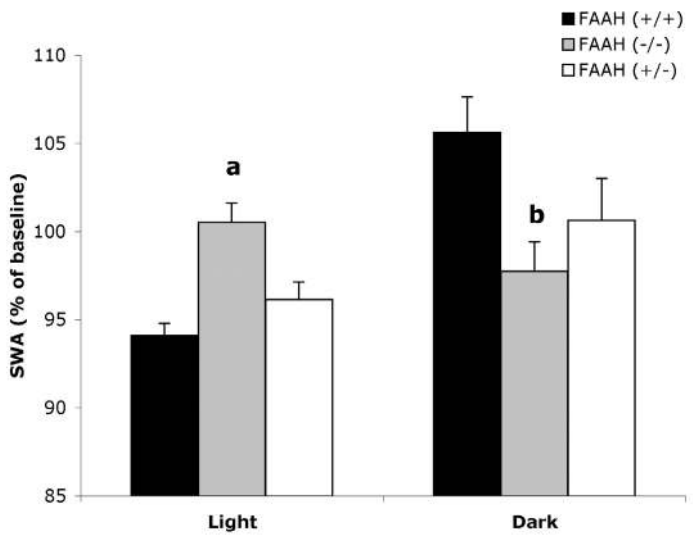

Figure 3—Slow wave activity (SWA) values obtained in FAAH (+/+), (-/-) and (+/-) mice during 12 hr light and 12 hr dark periods of baseline recordings. Values are expressed as a percentage of the 24 h baseline mean (=100%). Note that during the light period FAAH (-/-) mice showed bigger values of SWA than the other two genotypes ("a", $p < 0.01$), whereas during the dark part of the cycle their SWA values were lower than control littermates ("b", $p < 0.01$). Bars represent mean values (± S.E.M.). N = 7 mice by group.

Effects of Sleep Deprivation on Vigilance States

Our results indicate that sleep deprivation disrupted the vigilance states in all 3 genotypes studied here (Figure 4). Thus, FAAH (+/+) mice showed a significant rebound in SWS at the cost of W, during the first 9 hours following sleep deprivation ($F_{1,12} = 13.83$; $P < .01$), compared with their corresponding values obtained during the baseline recording. The increment in SWS was due to a significant increase both in the mean (± SEM) duration (baseline = 2.65 ± 0.08 minutes, after sleep deprivation = 3.38 ± 0.26 minutes; $F_{1,12} = 6.75$; $P < .05$) and number of SWS episodes (baseline = 112 ± 2.39, after sleep deprivation = 170 ± 12.8; $F_{1,12} = 19.47$; $P < .01$). FAAH (-/-) mice showed a compensatory rebound in SWS at the cost of W during the first 3 hours following sleep deprivation, as well as 9 and 10 hours after sleep deprivation ($F_{1,12} = 5.05$; $P < .05$), compared with their corresponding values obtained during the baseline recording. Such as rebound was due to a significant increase in the mean duration of SWS (baseline = 2.66 ± 0.29, after sleep deprivation = 3.64 ± 0.23 minutes; $F_{1,12} = 6.68$; $P < .05$). FAAH (+/-) mice showed a rebound in SWS at the cost of W during the first 5 hours and 9 hours after sleep deprivation ($F_{1,12} = 5.25$; $P < .05$), due to an increase in the mean duration of SWS (baseline = 2.39 ± 0.1, after sleep deprivation = 3.12 ± 0.2; $F_{1,12} = 10.47$; $P < .01$).

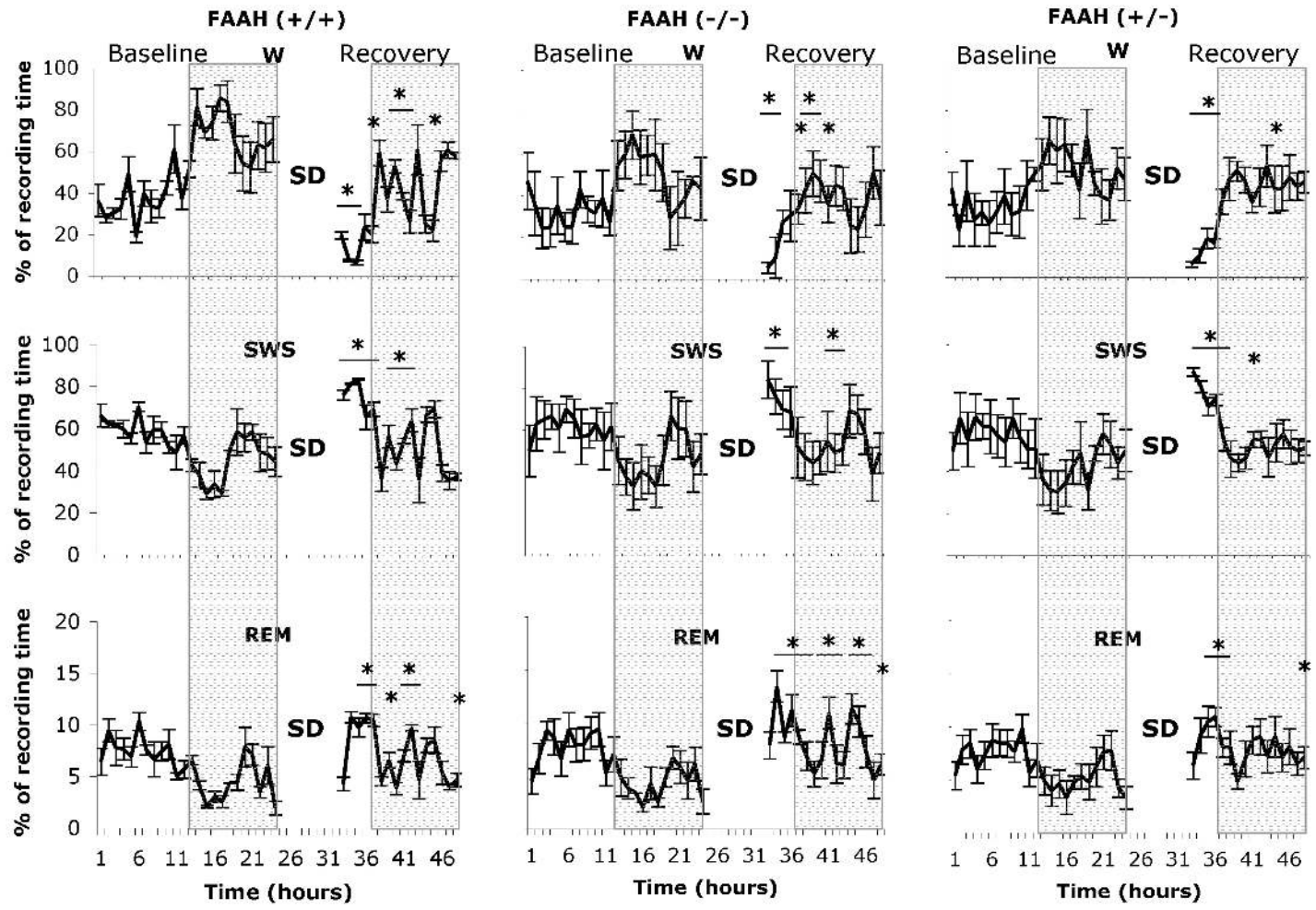

Figure 4—Time course of W, SWS and REM sleep in FAAH (+/+), (-/-) and (+/-) mice 24 hr before (baseline) and 16 hr after (recovery) SD. Lines connect 1 hr mean values (± S.E.M.). Note that in the three genotypes SWS and REM sleep rebounds are at cost of W. However, in FAAH (-/-) mice the SWS rebound is shorter than FAAH (+/+) and (+/-) mice, whereas REM sleep in this last genotype is not as robust as the other two groups of mice. N = 7 mice by group. Significant differences are indicated by asterisk (see results section for significant values).

On the other hand, in FAAH (+/+) mice, REM sleep was enhanced 3 to 5 hours after sleep deprivation, as well as 7, 9 to 10, and 16 hours after sleep deprivation ($F_{1,12} = 6.01$; $P < .05$), compared with their corresponding values obtained during the baseline recording. The changes in REM sleep were due to a significant increase both in the mean (± SEM) duration (baseline = 1.13 ± 0.01, after sleep deprivation = 1.38 ± 0.03 minutes; $F_{1,12} = 6.75$; $P < .05$) as well as in the number of REM sleep episodes (baseline = 29 ± 1.97, after sleep deprivation = 47 ± 0.94; $F_{1,12} = 65.24$; $P < .01$). FAAH (-/-) mice showed a rebound in REM sleep during almost all the recovery period following sleep deprivation, showing significant differences during 2 to 6, 8 to 10, 12 to 14, and 16 hours after sleep deprivation ($F_{1,12} = 11.28$; $P < .01$), compared with their corresponding values obtained during the baseline recording. The rebound in REM sleep was due to a significant increase in the number of its episodes (baseline = 30 ± 2.05, after sleep deprivation = 55 ± 3.31; $F_{1,12} = 42.45$; $P < .01$). In contrast, FAAH (+/-) mice showed a rebound in REM sleep only during 3 to 5 hours and 16 hours after sleep deprivation ($F_{1,12} = 8.63$; $P < .05$), as a consequence of a significant increase in the number of its episodes (baseline = 40.28 ± 2.62, after sleep deprivation = 59 ± 2.19; $F_{1,12} = 29.93$; $P < .01$). Comparisons of the effects of sleep deprivation on W, SWS, and REM sleep between genotypes were not significant (data not shown).

During the 4 hours of light following sleep deprivation, FAAH (+/+), FAAH (-/-), and FAAH (+/-) mice showed a reduced number of BA episodes (23.14 ± 1.29, 21.00 ± 1.27, and 22.57 ± 2.04, respectively) compared to their corresponding baseline values (27.28 ± 2.10, 24.14 ± 1.90, 30.28 ± 2.33, respectively), and no significant differences were found between groups. Likewise, no significant differences in BA were observed during the next 12 hours of dark following sleep deprivation (65.00 ± 6.02, 51.00 ± 6.14, and 64.28 ± 6.03, respectively).

Effects of Sleep Deprivation on the EEG Power Spectrum

All genotypes showed an increase in SWA during the recovery period, being high during the first hours after sleep deprivation and becoming low with the time course (Figure 5 and 6). Thus, FAAH (+/+) mice exhibited a significant increase in SWA during

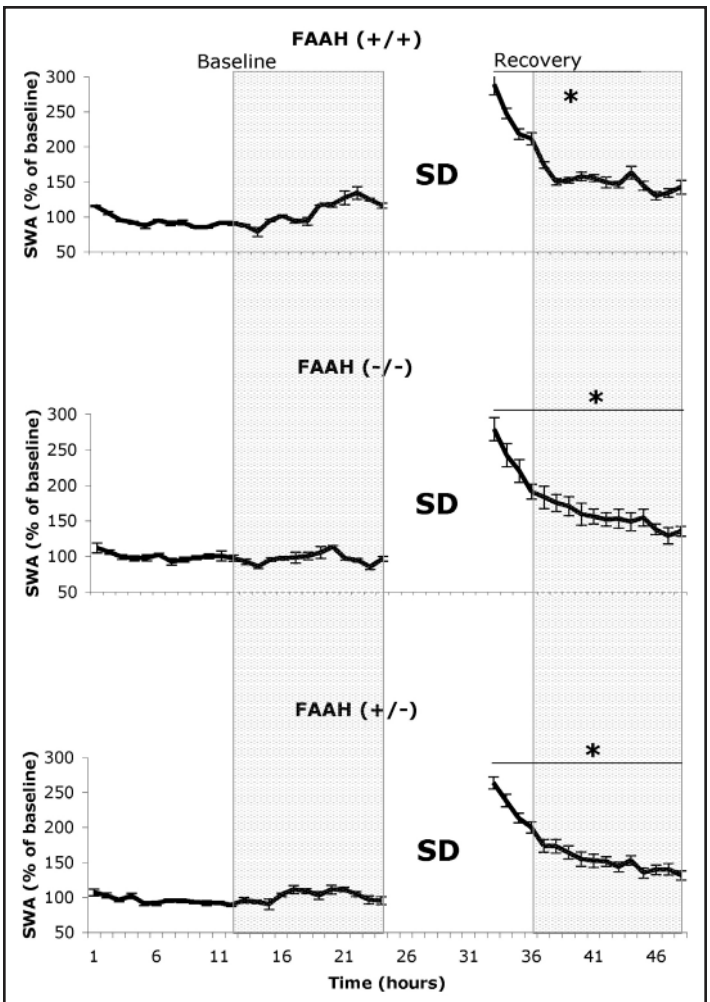

Figure 5—Time course of slow wave activity (SWA) of FAAH (+/+), (-/-) and (+/-) mice 24 hr before (baseline) and 16 hr after (recovery) SD. Lines connect 1 hr mean values (0.25 Hz bins ± S.E.M.). Note that in the three genotypes a significant increase in the levels of SWA is observed after SD, comparing to their corresponding control values. However, in FAAH (+/+) mice such as increase is shorter than FAAH (-/-) and (+/-) mice. N = 7 mice by group. Significant differences are indicated by asterisk (see results section for significant values).

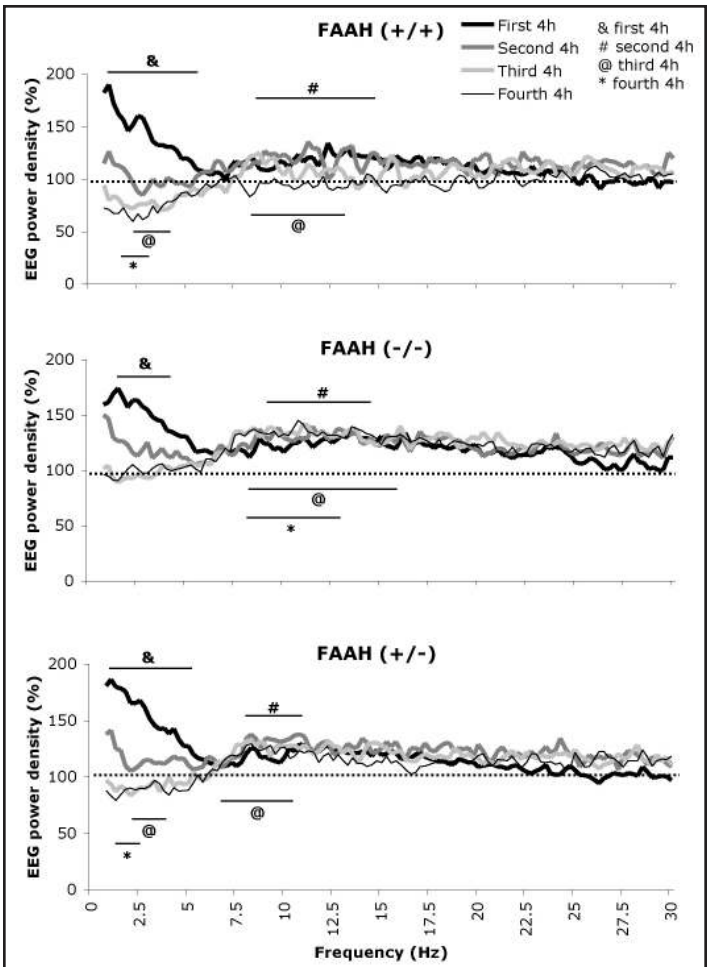

Figure 6—Effects of sleep deprivation (SD) on the EEG power spectra of FAAH (+/+), (-/-) and (+/-) mice during SWS. Lines represent the variation (mean values of 0.25 Hz bins) in the EEG power spectra after SD. This variation was expressed as a percent of the baseline recording during their corresponding time point values obtained after SD. N = 7 mice by group. Significant differences are indicated by "&", for the first period of 4 hr post SD; "#", for the second period of 4 hr post SD; "@", for the third period of 4 hr post SD; and "*", for the fourth period of 4 hr post SD (see results section for significant values). S.E.M. values were omitted to have a better view of the EEG power.

the 12-hour period following sleep deprivation ($F_{1,12} = 17.28$, $P < .01$), compared with its corresponding values obtained during the baseline recording, whereas FAAH (-/-) and FAAH (+/-) mice showed a significant increase in SWA during all the 16 hours of recovery recording ($F_{1,12} = 7.22$, $P < .05$ and $F_{1,12} = 7.47$, $P < .05$, respectively), compared to baseline. No significant differences in the SWA values were found between genotypes during the sleep-deprivation recovery period. A negative correlation was found between SWA and BA in FAAH (+/+), FAAH (-/-), and FAAH (+/-) mice during the 4 hours of light following sleep deprivation ($r = -0.68$, $r = -0.35$, and $r = -0.33$, respectively; $P < .01$), whereas during the dark period, FAAH (+/+) and FAAH (+/-) mice showed a negative correlation ($r = -0.53$ and $r = -0.08$, respectively; $P < .01$), and FAAH (-/-) showed a $r = 0.93$ ($P < .01$).

The analysis of the power spectrum by 0.25-Hz bins during the first 4 hours showed that, in SWS, FAAH (+/+), FAAH (+/-), and FAAH (-/-) mice exhibited a significant increase in the SWA values ($F_{1,12} = 5.55$; $P < .05$, $F_{1,12} = 5.74$; $P < .05$, and $F_{1,12} = 5.58$; $P < .05$, respectively), whereas FAAH (+/+) and FAAH (+/-) mice exhibited a significant decrease in the low-frequency range of 2.25 to 4.5 Hz during the third period of 4 hours ($F_{1,12} = 5.73$; $P < .05$ and $F_{1,12} = 7.50$; $P < .05$, respectively), as well as during the fourth period of 4 hours after sleep deprivation in the range of 1.25 to 3.75 Hz ($F_{1,12} = 5.36$; $P < .05$ and $F_{1,12} = 5.44$; $P < .05$, respectively). On the other hand, all genotypes showed a significant increase in the EEG power spectrum in the range of 8.0 to 16 Hz during the second ($F_{1,12} = 5.22$, $P < .05$), third ($F_{1,12} = 5.10$, $P < .05$), and fourth periods ($F_{1,12} = 5.44$, $P < .05$) of 4 hours followed sleep deprivation compared with their corresponding values obtained during baseline (Figure 6). No differences were observed in the power spectra of W and REM sleep during the recovery period in any of the genotypes (data not shown).

DISCUSSION

In this study, FAAH (-/-) mice had a significant increase in the amount of SWS during the light period compared to FAAH (+/+) mice that was not related to changes in Tb, LMA, or any other cage behavior. The increase of SWS was due to an increase in the duration and a decrease in the number of individual episodes of SWS. The mechanisms associated with these effects are unknown; however, recent evidence obtained from previous studies carried out in these knockout mice could explain, at least in part, our results. An increase over 15-fold in the levels of FAAs, including anandamide, N-oleoyl ethanolamine, and N-palimtoylethanolamine, and an exaggerated behavioral responses to both anandamide and oleamide reported in FAAH (-/-) mice[22,23,28] could be important factors. Quantifying the levels of oleamide in these mice also could be helpful in correlating the effects of FAAs on the sleep-wake cycle. However, we have had difficulty measuring oleamide levels in whole mouse brains due to contaminating signals on the mass spectrometer. Regarding the levels of FAAs in FAAH (+/-) mice, we have recently measured these values and found that they are approximately equal to the levels observed in FAAH (+/+) mice (data not shown), indicating that both copies of FAAH must be deleted to significantly raise endogenous brain levels of FAAs. Although we cannot determine for certain which, if any, of these FAAs is directly responsible for the altered sleep behavior of FAAH (-/-) mice, it is notable that anandamide has been implicated in sleep induction.[3]

Our results are consistent with preliminary studies in which the administration of FAAH inhibitors induced an increase in the amount of SWS.[29] However, we only observed an increase of 8% in the amount of SWS in FAAH (-/-) mice compared to control animals, during the light part of the cycle. Due to the fact that the amount of sleep recorded in FAAH (+/+), FAAH (-/-) and FAAH (+/-) mice across the light period is similar to the levels reported in other mice,[30,31] the small increase in SWS could be explained by the presence of an upper limit of SWS (a ceiling effect)[24] that precludes larger increases in sleep. Likewise, the levels of SWS in the 3 genotypes are similar to the level observed in other mice during the dark cycle,[30,31] whereby the lack of an increase in SWS in FAAH (-/-) mice in this period could be due to the fact that hypnogenic FAAs could, as many others sleep-inducing substances do, accumulate under wakefulness and exert their effect when animals are resting.[32] In fact, anandamide shows a diurnal variation in the pons of rats, with a maximum level during the dark phase,[33] whereas diurnal changes in the expression of the cannabinoid receptor CB1 has been reported, with maximum values during the light period.[34]

The time course of SWA during the light period in the baseline recordings of the 3 genotypes was in accordance with previous studies in rodents,[24,35] showing high SWA values at the beginning of the light period and declining thereafter. However, in contrast to FAAH (+/+) and FAAH (+/-) mice and other rodents,[36, 37] FAAH (-/-) mice showed lower values of SWA during the dark period, compared to light period. A similar phenomenon has been observed in the guinea pig,[38, 39] where the similar amount of W across 24 hours has been proposed as a one possible explanation. However, this does not apply to FAAH (-/-) mice because these animals showed a well-defined preference to be awake during the night. Thus, it is possible that, like in rats,[37] the low fragmentation of sleep during the dark cycle helped the FAAH (-/-) mice to dissipate the pressure for sleep, reducing the SWA values. Albeit, this hypothesis is supported by the small amount of BA in SWS observed in FAAH (-/-) mice in the dark cycle, other factors, including light-sensitivity differences, cannot be excluded as possible causes, and additional studies are needed to evaluate these factors.

The high values of waves of slow frequency and the high duration and low number of SWS episodes during the rest part of the cycle suggest that FAAH (-/-) mice have more intense episodes of SWS than do FAAH (+/+) and FAAH (+/-) mice, at least during the light part of the cycle. In support of this interpretation is the small amount of BA shown by the FAAH (-/-) mice compared to the other 2 genotypes, as well the inverse relationship observed between BA and SWA during sleep, and that has been used in other strains of mice and rodents,[24,35] as an indicator of sleep intensity. However, additional studies to test the response to arousing stimuli are needed to probe this hypothesis.

The mechanisms associated with the increase in power of slow-frequency waves in FAAH (-/-) mice are unknown. However, it is well known that such slow-frequency waves reflect synchronized burst firing patterns of hyperpolarized thalamocortical and corticothalamic neurons[40-42] and that neuronal activity in these networks is under the control of cholinergic, serotonergic, histaminergic, GABAergic, and noradrenergic modulatory systems.[40,43-46] Given that EEG activity reflects an extended variety of oscillations generated in the thalamus and cerebral cortex, the precise mechanisms related to the changes in

the EEG observed in FAAH (-/-) mice are too complex to resolve here. However, because some FAAs like oleamide and anandamide have been found to modulate cellular signaling at several serotonergic and GABAergic receptor subtypes[47-50] and because oleamide activates serotonin 5-HT_7 receptors in mouse thalamus[51] (whereas endocanabinoids disinhibit hippocampal neuronal activity by inhibiting GABAergic interneurons[52]), this suggests that the increase in the power spectrum in the slow range of 1.0 to 4.5 Hz that we observed in FAAH (-/-) mice could be due to the influence of some FAAs on the thalamocortical network involved in the generation of the slow waves of SWS. Basal forebrain, hypothalamic, and non-hypothalamic neuronal circuits involved in sleep regulation could also have complex roles in this phenomenon.[53,54]

Our results showed that the amount of SWS and REM sleep increased in the 3 genotypes in a way similar as to that reported by other studies that used gentle handling as a method for sleep deprivation,[55] even though slight differences were observed because of the duration of sleep deprivation. However, FAAH (-/-) mice showed a SWS rebound during the 3 hours following sleep deprivation, whereas FAAH (+/+) mice exhibited the rebound during the next 9 hours after sleep deprivation. The mechanisms that can account for these results are unknown. However, the fact that FAAH (-/-) mice showed SWS episodes that were more intense supports the proposal that SWS deficits may be recovered not only by the time spent in this state of sleep, but also by an increase in the intensity of SWS.[24]

On the other hand, the increase observed in the EEG power density of waves of low frequency in all genotypes studied here during the first 4 hours after sleep deprivation is in accordance with other studies carried out in rodents,[24,37] indicating that the homeostatic mechanisms of sleep regulation are not disrupted in FAAH (-/-) mice. Likewise, the reduction below baseline in specific bins observed in FAAH (+/+) and FAAH (+/-) mice after 12 hours of sleep deprivation has been reported previously.[24,26,56] Although the functional significance of this negative rebound in the EEG power density is still controversial, it has been proposed that such a reduction could be a consequence of the increase in the sleep amount after sleep deprivation.[24] In addition, an increasing trend in the middle- to high-frequency band (> 7.0 Hz and < 17.0 Hz) was observed 8, 12, and 16 hours after sleep deprivation in FAAH (+/+) and FAAH (-/-) mice, and to a in minor degree in FAAH (+/-) mice. Further studies are needed to understand these results.

In summary, we found that genetic modification of FAAH expression induced a significant increase in the amount of SWS, as well a significant increase in the intensity of SWS episodes. This is determined by the high values of SWA, the increase in the duration and decrease in the number of individual episodes of SWS and BA, and the inverse correlation between SWA and BA. Although the molecular mechanisms for the altered sleep behavior in FAAH (-/-) mice remains unknown, it is notable that these animals possess dramatically elevated brain levels of anandamide and other N-acyl ethanolamines[22] as well as reduced hydrolytic rates for these FAAs and the primary FAA oleamide,[23] suggesting that the prolonged sleep behavior of FAAH (-/-) mice may be due to enhanced signaling by 1 or more FAAs in the nervous system. In addition, similar rebounds of SWS and REM sleep in FAAH (+/+) and FAAH (-/-) mice were observed after sleep deprivation, indicating that the homeostatic mechanisms of sleep are not disrupted by the lack of FAAH. In conclusion, the studies described herein support the role of FAAH as a key regulator of the hypnogenic processes associated with FAAs in vivo. However, additional studies are needed to determine the precise participation of the different FAAs in the increase of SWS observed in this study.

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